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TITLE: DETERMINATION OF THE *IN VITRO* AND *IN VIVO* ACTIVITY OF COMPOUNDS TESTED AGAINST PUNTA TORO VIRUS.

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Preliminary *In Vivo* Assessment of Toxicity: Approximate LD50 values were obtained in 3-4 week-old C57BL/6 mice or 4-5 week-old Swiss Webster mice for 37 AVS compounds. **Effect of AVS Compounds on Hepatotropic Infections in Mice Induced by the Adames Strain of Punta Toro Virus:** A total of 64 anti-PTV experiments were run with 25 AVS compounds using the Adames strain of PTV. Promising compounds included AVS65, 79, 111, 272, 347, 1761, 1968, 2276, 2285, 2812, and 5311. **Effect of AVS Compounds on Intracerebral Infections in Mice Induced by the Balliet Strain of Punta Toro Virus:** A total of 16 AVS compounds were evaluated against the CNS infection induced by the Balliet strain of PTV. Compounds AVS6080 and 6082 were considered moderately effective. **Comparison of the Effects of AVS01, AVS02, and AVS206 on Daily Disease Parameters in Punta Toro Virus-Infected Mice:** A single p.o. treatment given 24 hr post-virus inoculation with LD50/10 dosages of AVS01, 02, or 206 prevented the PTV-associated death of the mice and significantly lowered the already developing viral titers in the blood and all tissues. In this experiment, AVS01 was least effective in keeping the virus below detectable limits. AVS206 was consistently most effective. **Effect of a Combination of AVS01 and AVS5587 on *In Vivo* Punta Toro Virus Infections:** The combination of AVS01 and AVS5587 was used against PTV infections in mice. A definite synergy of antiviral effect was seen. In addition, use of AVS5587 with a usually lethal dose of AVS01 reduced the AVS01 toxicity. **Effect of a Combination of AVS01 and AVS1761 on *In Vivo* Punta Toro Virus Infections:** Treatment with the

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19. ABSTRACT

combination of AVS01 administered p.o. twice daily for 3 days beginning 24 hr after virus inoculation and AVS1761 administered i.p. 24 and 72 hr post-virus inoculation was not effective in either decreasing toxicity of AVS01 or in enhancing the therapeutic efficacy of either compound against PTV infections in mice. **Effect of a Combination of AVS01 and AVS2149 on *In Vivo* Punta Toro Virus Infections:** AVS01 administered p.o. to PTV-infected mice twice daily for 3 days starting 24 hr after virus inoculation was rendered less lethally toxic and its anti-PTV effects were increased in a synergistic fashion when the infected mice were also treated with AVS2149 in a single i.p. injection 1 hr prior to ribavirin treatment. **Studies on Interferon Induction by AVS2149 in Punta Toro Virus-Infected Mice:** Mice infected with PTV were treated i.p. with 2 concentrations of AVS2149 (5 and 0.6 mg/kg/day) using 4 treatment schedules: Once only 48 hr post-virus inoculation, once daily for 5 days beginning 4 hr pre-virus inoculation, twice daily for 5 days beginning 4 hr pre-virus inoculation, and every other day for 3 treatments beginning 4 hr post-virus inoculation. In the mice treated once only late in the infection, a significant IFN induction greater than seen in infected animals or in uninfected, treated mice, occurred 4 hr after treatment and persisted for 24 hr. In all other studies, less IFN was seen in the serum of PTV-infected, treated mice than occurred in uninfected, treated animals. In addition, a definite hyporeactive state occurred in the once and twice daily treated mice; spacing the treatment to every other day partially alleviated this hyporeactive condition. **A Comparison of Interferon Induction in C57BL/6 Mice by a Series of AVS1761 Derivatives:** A series of poly ICLC derivatives, AVS5588–AVS5596, were compared with regard to their ability to induce IFN in 3 week-old C57BL/6 mice. The IFN inducing activity generally coincided well with their in vivo anti-PTV effects, although some exceptions were noted. The known positive standard, AVS1761, exerted the strong IFN induction expected. **Interferon Induction by AVS5587:** Single or two i.p. injections of AVS5587 induced significant IFN titers as early as 1 hr after treatment of weanling C57BL/6 mice. The multiple injections resulted in a prolonged IFN titer in the serum, through 12 hr after final treatment. **Effects of AVS2276 and AVS2285 on Serum Interferon Induction in C57BL/6 Mice:** Neither AVS2276 nor AVS2285 induced significant quantities of detectable IFN within a 24 hr period after p.o. administration into 3 week-old C57BL/6 mice. **Reversal of the Anti-PTV Effects of AVS5587 by Treatment with Anti-Interferon Antibody:** The immunomodulator AVS5587 has therapeutic potential against PTV infections. The anti-PTV activity was eliminated by concomitant therapy with anti-IFN α/β antibody, indicating the rapid IFN induction by AVS5587 plays a major role in protecting the mice. **Effects of Human Interleukin-2 (AVS5079) on PTV Infections in C57BL/6 Mice:** Treatment of Adames PTV-infected mice with 12,500 and lower units of AVS5079 resulted in significant disease inhibition. Treatment was i.p. qd x 5 beginning 4 hr after PTV inoculation; assays for IFN production in the mice and for NK cell activation, done after termination of the final treatment, showed no IFN produced, and an increased NK cell activation in infected mice but a decreased NK cell activation in uninfected, treated mice. The latter data suggest a possible hyporeactive state induced in the animal. **Effect of Single i.p. Treatment with AVS5079 on Interferon Induction and Natural Killer Cell Activity in C57BL/6 Mice:** Single i.p. injections with AVS5079 significantly stimulated NK cell activity in C57BL/6 mice 15 min and 2 hr after treatment. Serum IFN was not detected in these animals at 15 min, 2 hr or 24 hr after treatment. **Effect of AVS1018 on Interferon and Interleukin-2 Induction in C57BL/6 Mice:** Splenocytes from mice treated with AVS1018 produced significant quantities of IL-2 when assayed 1 and 4 hrs after p.o. treatment. No serum IFN was detected in these animals. **Effect of AVS1761, 1968, 2933, and 4726 on Interferon and Interleukin-2 Induction in C57BL/6 Mice:** Splenocytes from mice treated with AVS1968 reduced significant quantities of IL-2 when assayed 1 hr after i.p. treatment. IFN was detected in serum at 24 hr. AVS1761 induced significant quantities of IL-2 at 1 and 4 hr. AVS2933 and AVS4726 were inactive. **A Measurement of AVS01 Toxicity Using Pulse Oximetry:** AVS01 administered i.p. twice a day for 5 days in doses of 800 and 1200 mg/kg/day was lethally toxic to 4 week-old BALB/c mice. As the animals approached the time of death, which was attributed to excessive hemorrhaging in the gut, their arterial oxygen saturation (SaO_2%) declined appreciably. **Effects of Punta Toro Virus on Macromolecular Synthesis of Cells:** Punta Toro virus infection appeared to significantly inhibit DNA, RNA and protein synthesis from 16-24 hours post-virus exposure. DNA synthesis, as reflected by deoxyadenosine uptake, remains perturbed throughout PTV infection from 8-48 hours post virus exposure. In addition, PTV seems to enhance macromolecular synthesis 1 hour post exposure to virus in log phase cells. Whether these effects are an actual stimulation or depression of macromolecular synthesis due to viral-induced stimulation or inhibition of cellular enzymes, to viral-induced enzymes, or to an increase or decrease in cell permeability is still to be determined. **Reduction of AVS01 Toxicity by Treatment with AVS5587 in Mice:** Treatment with AVS5587 of male C57BL/6 mice receiving lethal toxic doses of AVS01 prevented the usual deaths of the mice, particularly if the AVS5587 therapy was given 3 days after start of AVS01 therapy. Delaying AVS5587 therapy to 4 or 5 days reduced these toxicity reversal effects. Female mice treated in a similar manner responded in an erratic fashion to AVS01 therapy. **Reduction of AVS01 Toxicity by Treatment with AVS2776 in Mice:** Treatment of C57BL/6 mice with high dosages (800-1200 mg/kg/day) of AVS01 for a 5-day period, results in death of the mice, the mean day to death being less than 8 days. When AVS2776 is administered to these mice in a single oral treatment 3 days after start of ribavirin treatment, it may significantly prevent the usual ribavirin-associated death. If AVS2776 treatment is delayed to 4 or 5 days, this reversal of toxicity was less pronounced. **Comparison of AVS206 Toxicity in BALB/c and C57BL/6 Mice:** AVS206 was more toxic to weanling BALB/c mice than to weanling C57BL/6 mice when administered i.p. twice daily for 5 days. **Presentations and Publications:** A total of 7 presentations have been made and six papers have been submitted and accepted to scientific journals for publication this year.

SUMMARY

1. Approximate LD_{50} values were obtained in 3-4 week-old C57BL/6 mice or 4-5 week-old Swiss Webster mice for 37 AVS compounds.

2. Overview of In Vivo Anti-Punta Toro Virus Activity of AVS Compounds: Summary of Five Years' Testing.

3. A total of 64 anti-PTV experiments were run with 25 AVS compounds using the Adames strain of PTV. Promising compounds included AVS65, 79, 111, 272, 347, 1761, 1968, 2276 2285, 2812, and 5311.

4. A total of 16 AVS compounds were evaluated against the CNS infection induced by the Balliet strain of PTV. Compounds AVS6080 and 6082 were considered moderately effective. The majority of the compounds evaluated were Pharmatek-prepared compounds designed for delivery to the brain.

5. Mice infected with PTV rapidly developed a viremia with virus titers exceeding 10^6 being recovered from placebo-treated mice by 2 days after virus inoculation. Virus was similarly recovered from livers, lungs, spleens, kidneys, mesenteric lymph nodes, spinal cord, and brains from these same animals at about the same time periods as viral recovery from the serum. White blood cells declined in number with the development of the infection, and hepatic icterus increased concomitantly, together with SGOT and SGPT levels. A single p.o treatment given 24 hr post-virus inoculation with LD50/16 dosages of AVS01, 02, or 206 prevented the PTV-associated death of the mice and significantly lowered the already developing viral titers in the blood and all tissues. In this experiment, AVS01 was least effective in keeping the virus below detectable limits. AVS206 was consistently most effective.

6. The combination of AVS01 (ribavirin) and AVS5587 (7-thia-8-oxoguanosine) was used against PTV infections in mice. A definite synergy of antiviral effect was seen. In addition, use of AVS5587 with a usually lethal dose of ribavirin reduced the ribavirin toxicity.

7. Treatment with the combination of AVS01 administered p.o. twice daily for 3 days beginning 24 hr after virus inoculation and AVS1761 administered i.p. 24 and 72 hr post-virus inoculation was not effective in either decreasing toxicity of AVS01 or in enhancing the therapeutic efficacy of either compound against PTV infections in mice.

8. AVS01 (ribavirin) administered p.o. to PTV-infected mice twice daily for 3 days starting 24 hr after virus inoculation was rendered less lethally toxic and its anti-PTV effects were increased in a synergistic fashion when the infected mice were also treated with AVS2149 (ampligen) in a single i.p. injection 1 hr prior to ribavirin treatment.

9. Mice infected with PTV were treated i.p. with 2 concentrations of AVS2149 (5 and 0.6 mg/kg/day) using 4 treatment schedules: Once only 48 hr post-virus inoculation, once daily for 5 days beginning 4 hr pre-virus inoculation, twice daily for 5 days beginning 4 hr pre-virus inoculation, and every other day for 3 treatments beginning 4 hr post-virus inoculation. In the mice treated once only late in the infection, a significant IFN induction greater than seen in infected animals or in uninfected, treated mice, occurred 4 hr after treatment and persisted for 24 hr. In all other studies, less IFN was seen in the serum of PTV-infected, treated mice than occurred in uninfected, treated animals. In addition, a definite hyporeactive state occurred in the once and twice daily treated mice; spacing the treatment to every other day partially alleviated this hyporeactive condition.

10. A series of poly ICLC derivatives, AVS5588–AVS5596, were compared with regard to their ability to induce IFN in 3 week-old C57BL/6 mice. The IFN-inducing activity generally coincided well with their in vivo anti-PTV effects, although some exceptions were noted. The known positive standard, poly ICLC (AVS1761) exerted the strong IFN induction expected.

11. Single or two i.p. injections of AVS5587 induced significant IFN titers as early as 1 hr after treatment of weanling C57BL/6 mice. The multiple injections resulted in a prolonged IFN titer in the serum, through 12 hr after final treatment.

12. Neither AVS2276 (BL-002) nor AVS2285 (BL-012) induced significant quantities of detectable IFN within a 24 hr period after p.o. administration into 3 week-old C57BL/6 mice.

13. The immunomodulator AVS5587 (7-thia-8-oxoguanosine) has therapeutic potential against PTV infections. The anti-PTV activity was eliminated by concomitant therapy with anti-IFN α/β antibody, indicating the rapid IFN induction by AVS5587 plays a major role in protecting the mice.

14. Treatment of Adames PTV-infected mice with 12,500 and lower units of AVS5079 (recombinant human interleukin-2) resulted in significant disease inhibition. Treatment was i.p. qd x 5 beginning 4 hr after PTV inoculation; assays for IFN production in the mice and for NK cell activation, done after termination of the final treatment, showed no IFN produced, and an increased NK cell activation in infected mice but a decreased NK cell activation in uninfected, treated mice. The latter data suggest a possible hyporeactive state induced in the animal.

15. Single i.p. injections with AVS5079 (human IL-2) significantly stimulated NK cell activity in C57BL/6 mice 15 min and 2 hr after treatment. Serum IFN was not detected in these animals at 15 min, 2 hr or 24 hr after treatment.

16. Splenocytes from mice treated with AVS1018 produced significant quantities of IL-2 when assayed 1 and 4 hrs after p.o. treatment. No serum IFN was detected in these animals.

17. Splenocytes from mice treated with AVS1968 reduced significant quantities of IL-2 when assayed 1 hr after i.p. treatment. IFN was detected in serum at 24 hr. AVS1761 induced significant quantities of IL-2 at 1 and 4 hr. AVS2933 and AVS4726 were inactive.

18. Ribavirin administered i.p. twice a day for 5 days in doses of 800 and 1200 mg/kg/day was lethally toxic to 4 week-old BALB/c mice. As the animals approached the time of death, which was attributed to excessive hemorrhaging in the gut, their arterial oxygen saturation (SaO_2%) declined appreciably.

19. Punta Toro virus infection appeared to significantly inhibit DNA, RNA and protein synthesis from 16-24 hours post-virus exposure. DNA synthesis, as reflected by deoxyadenosine uptake, remains perturbed throughout PTV infection from 8-48 hours post virus exposure In addition, PTV seems to enhance macromolecular synthesis 1 hour post exposure to virus in log phase cells. Whether these effects are an actual stimulation or depression of macromolecular synthesis due to viral-induced stimulation or inhibition of cellular enzymes, to viral-induced enzymes, or to an increase or decrease in cell permeability is still to be determined.

20. Treatment with AVS5587 of male C57BL/6 mice receiving lethal toxic doses of AVS01 prevented the usual deaths of the mice, particularly if the AVS5587 therapy was given 3 days after start of AVS01 therapy Delaying AVS5587 therapy to 4 or 5 days reduced these toxicity reversal effects. Female mice treated in a similar manner responded in an erratic fashion to AVS01 therapy.

21. Treatment of C57BL/6 mice with high dosages (800-1200 mg/kg/day) of AVS01 (ribavirin) for a 5-day period, results in death of the mice, the mean day to death being less than 8 days. When bropirimine (AVS2776) is administered to these mice in a single oral treatment 3 days after start of ribavirin treatment, it may significantly prevent the usual ribavirin-associated death. If bropirimine treatment is delayed to 4 or 5 days, this reversal of toxicity was less pronounced.

22. Overall, AVS2776 at 50 mg/kg had a weak effect in reversing the toxicity of ribavirin (used at 800 and 1200 mg/kg) in mice. Since only one dose of bropirimine was used, one cannot rule out the possibility that higher doses or multiple treatments may enhance the activity of bropirimine in this setting as was seen in Section XXI.

23. Treatment of C57BL/6 mice with high (800-1200 mg/kg/day) of AVS01 (ribavirin) for a 5-day period caused an early lethal toxicity of the mice. When bropirimine (AVS2776) was administered to these mice in a single oral dose of 50 mg/kg, essentially no difference in toxicity was seen, which conflicts with earlier experiments (Sections XXI, XXII) where the lethal toxicity was alleviated. No significant effects on SGOT, SGPT, or liver discoloration were seen by AVS01 treatment, although some anemia exhibited as decline in hematocrit was observed by the 6th day of the study.

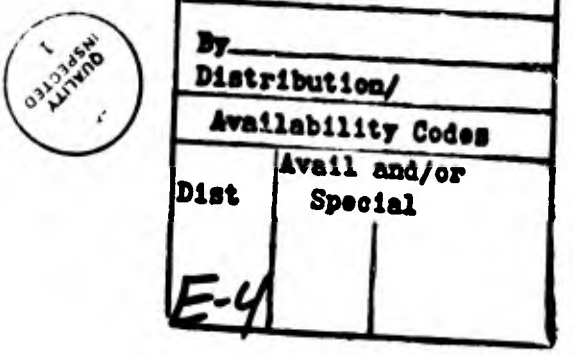
24. AVS206 was more toxic to weanling BALB/c mice than to weanling C57BL/6 mice when administered i.p. twice daily for 5 days.

25. NIH-III mice containing the *nu* mutation rendering them athymic, the *bg* mutation reducing their NK cells, and the *xid* mutation reducing the number of LAK cells were assayed for their sensitivity to PTV; only mice infected with the least concentrated virus dilution died, suggesting a greater resistance to defective interfering particles. The virus will be retitrated in these mice.

26. Swiss Webster mice were not satisfactorily sensitive to i.p. injection of Adames strain PTV, with an unacceptable number dying of the infection.

27. The Adames strain of PTV was lethal to 4 week-old mice when injected i.c. No virus could be isolated from the brains of the dying animals, but could be recovered from their livers, which also showed signs of icterus.

28 Presentations and publications: A total of 7 presentations have been made or submitted as abstracts for scientific meetings this year. Six papers have been submitted and accepted to scientific journals for publication. One additional paper has been submitted to a journal and is now undergoing review.



FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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I. PRELIMINARY *IN VIVO* ASSESSMENT OF TOXICITY

Introduction

Before compounds submitted to us can be evaluated for *in vivo* PTV activity, information is needed regarding the approximate LD50 of those compounds as determined using the same treatment schedule to be used in the antiviral experiments. This report summarizes the LD50 data generated either from preliminary toxicity assessment experiments or from data derived from use of concomitantly run toxicity controls in actual PTV experiments. Since some compounds submitted to us are immune modulating materials, their most effective dose is often remote from the maximum tolerated dose. In such cases, we are usually instructed by USAMRIID personnel on the doses to use in *in vivo* PTV experiments and we seldom have a need to determine an LD50 dose. Some data regarding these immunomodulating compounds are also included in this section, however, to provide information for others desiring to use such compounds.

Materials and Methods

Compounds: All compounds were submitted to us by Biological Research Faculty & Facility, Inc. (Rockville, MD). The compounds were weighed and dissolved or suspended in vehicles considered most appropriate for the compound. These vehicles were physiological saline, sterile water for injection, or 4% carboxy methylcellulose

Animals: C57BL/6 mice 3-4 weeks of age were obtained from Simonsen Laboratories (Gilroy, CA). All were quarantined at least 24 hr prior to use, and maintained on Wayne Lab Blox mouse chow and tap water *ad libitum*. They were caged in shoe box style polycarbonate cages with Sani-cell bedding used. All were housed 5 to a cage.

Toxicity Assessments: Mice were injected with varying 2-fold dilutions according to the indicated treatment regimens. All were weighed immediately prior to treatment and again 18 hr after the final treatment to determine if normal weight gain occurred. In preliminary toxicity studies, the mice were held a total of 14 days. When used as parallel toxicity controls in PTV studies, the animals were held a total of 21 days. Five mice were used at each dosage level. The volume administered was 0.01 ml/g of body weight. Parameters for evaluation included weight change, obvious signs of distress such as diarrhea, prostration, or tremors, and death, which was noted daily. The LD50 dose was calculated by the Reed-Muench method (1).

Results and Discussion

The toxicity determinations, expressed as LD50 values, are summarized in Table I-1. Data on 7 compounds are shown. In some cases ">" values are shown because we as yet have not achieved a lethal dose and we had insufficient compound to use higher dosages. Values shown as "~" were estimated based on the observation that slightly lower doses were lethal, but to less than 50% of the animals, or treatment with the lower dose caused marked weight loss in the animals, suggesting the MTD dose had essentially been reached.

Conclusions

Approximate LD50 values were obtained in 3-4 week-old C57BL/6 mice or 4-5 week-old Swiss Webster mice for 37 AVS compounds.

References

1. Reed, L.J. and H. Muench. (1938) A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27:493-497.

Table A-1. Preliminary Toxicity Evaluations of AVS Compounds[a]

Compound (AVS No.)	Name	Treatment Schedule	Treatment Route	Approximate LD50 (mg/kg/day)
65	Formycin B	bid x 5	s.c.	>500
		tid x 5	s.c.	~700
		tid x 5	i.p.	~700
79	9-ß-D-ribofuranosylpurine-6-thiocarboximide	tid x 5	i.p.	225
147	Enviroxime	tid x 5	s.c.	>500
		bid x 5	s.c.	>500
253	Selenazofurin	qd x 5	i.p.	~1200
		bid x 5	i.p.	~1200
272	3-Deazaguanine	bid x 5	s.c.	375
347	Phyllanthoside	bid x 5	s.c.	90
		bid x 5	s.c.	>100
1018	Phenyleneamine	once only	p.o.	>12.5
		e 4 days x 3	p.o.	>12.5
		once only	p.o.	~50[b]
1968	CL246,738	e 4 days x 3	p.o.	>100
		once only	p.o.	~200[b]
2563	Lycorine	bid x 5	s.c.	>75
2605	"Compound C"	bid x 5	s.c.	>220
2812	Narciclasine	bid x 5	s.c.	4.7
		bid x 5	i.p.	~5.0
		qd x 5	s.c.	6.5
		qd x 5	i.p.	~5.0
2885	3-T-butyl-1-adamantyl thiourea	bid x 5	i.p.	~800
		bid x 5	s.c.	>400
3679	Unidentified	bid x 5	s.c.	>400
4071	Ribavirin methylamidate	bid x 5	s.c.	>500
4206	3-Acetamido-7-amino-6-methyl-7H-S-triazolo[5,1-C]-S-triazole	bid x 5	s.c.	>400
		bid x 5	i.p.	>600
4272	Unidentified	bid x 5	i.p.	~16
		bid x 5	s.c.	~50
		bid x 5	p.o.	~150
		once only	i.p.	~15
4273	Unidentified	bid x 5	s.c.	~100
		once only	i.p.	~250
4588	1-Aminoadenosinium mesitylene-sulfonate	bid x 5	s.c.	>400
		bid x 5	i.p.	>600

Compound (AVS No.)	Name	Treatment Schedule	Treatment Route	Approximate LD50 (mg/kg/day)
4618	Unidentified	bid x 5	s.c.	>400
4796	Streptinidone	bid x 5	s.c.	>300[c]
4855	3-Amino-L-tyrosine	bid x 5	s.c.	>500
5058	Methyl carboxamide of AVS206	bid x 5	s.c.	>500
6081	Pharmatec compound	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	~33
6082	Pharmatec compound	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	~104
6083	Pharmatec compound	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	~33
6290	Pharmatec compound	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	158
6291	Pharmatec compound	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	~104
6292	Pharmatec compound	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	~104
6296	Pharmatec compound	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	104
6297	Pharmatec compound	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	32
6299	Pharmatec compound	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	158
6300	Pharmatec compound	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	104
6334	Unidentified	bid x 5	i.p.	>250
6337	Unidentified	bid x 5	i.p.	~350
6417	Unidentified	bid x 5	i.p.	188
6477	Unidentified	bid x 5	i.p.	>100
6501	Unidentified	bid x 5	i.p.	>250

[a]10-13 g C57BL/6 mice.

[b]15-17 g C57BL/6 mice.

[c]This compound did not readily go into solution, so the LD50 data are suspect. It became a taffy-like mass in saline; this partially dissolved in 0.6 ml MeOH. Addition of DMSO did not appear to help.

II. OVERVIEW OF *IN VIVO* ANTI-PUNTA TORO VIRUS ACTIVITY OF AVS COMPOUNDS: SUMMARY OF FIVE YEARS' TESTING

Introduction

It is appropriate to summarize in tabular fashion all the *in vivo* work run to date against this virus. This table is shown in this section. All *in vivo* experiments, including both Adames and Balliet virus strains, combination studies, and special intravenous therapy studies are seen in Table II-1.

The following explains the legend for each column in the table:

AVS #: Number assigned to the compound by Biological Research Faculty & Facility, Inc.

Compound Name: Often an abbreviated name for the compound as provided to us. The short version of the name is used in order to fit it into the space provided.

Expt. #: The USU experiment number (PtA—). Every PTV *in vivo* experiment is numbered consecutively.

Test Date: The date the experiment was begun.

Treatment Schedule: The schedule used for the animal treatments, indicated in abbreviated form:

bid: Twice daily, usually 8 am and 4 pm
qd: Once daily
tid: Three times daily
single: Once only
qid: Four times daily
eod: Every other day
beg: Beginning, with the hrs indicated pre or post-virus inoculation; if no time is shown, virus was not given to the animals.

Route: Treatment route:
ip: intraperitoneal
sc: subcutaneous
po: oral gavage
ic: intracerebral
iv: intravenous.

Dose Range: Range of doses of the compound used, in mg/kg/day (unless actually shown as µg/kg/day or units/mouse). Doses usually varied by two-fold dilution, although some immunomodulators were used in one-half $\log_{10}$ increments.

Tox. @: The lowest dose (in mg/kg/day or, if indicated, as µg/kg/day) of the compound at which toxicity (death of one or more toxicity control animals) was seen. If a ">" sign is indicated, no toxicity was seen. "All lost weight" indicates the toxicity control mice all lost weight between the time therapy was initiated and 18 hr after treatment was terminated. "ON TEST" indicates the study was not sufficiently complete to indicate actual data at the time the table was prepared.

Results: Our overall impression of the antiviral efficacy seen:
+: Significant ($P<0.05$ or $P<0.01$) increase in survivors.
±: Significant effect on one or more parameters other than survivors (i.e., mean survival time increase; decrease in liver score, SGOT, SGPT, serum virus or liver virus) without a significant survivor increase.
-: No significant effects by any parameter.
TI: Therapeutic index (minimum toxic dose ÷ minimum antivirally effective dose).
?: Designation of a test in which the results were compromised by a poor control result.
ON TEST: Experiment still underway at the time the table was prepared.

MIC: Minimum inhibitory dose, in mg/kg/day or, if indicated in Dose Range column, in µg/kg/day or units/mouse.

Remarks:

EXPANDED: An experiment in which the infection parameters were expanded from survivors/total and mean survival time to include other parameters such as liver score, SGOT, SGPT, serum virus, liver virus, etc.

EXPANDED ALL: An experiment in which the infection parameters were expanded from a regular expanded study to also include many other tissues, such as spleen, lungs, mesenteric, brains, etc.

BALLIET: An experiment run using the Balliet strain of PTV. All other experiments using the Adames strain of PTV.

TI: Therapeutic index determination study.

MMF: Mode modification study (determination of effect of varying virus challenge inoculum concentration).

COMBINATION: An experiment in which a combination of two compounds were evaluated.

REPEAT: An experiment run to repeat a previous unacceptable experiment.

IFN: An experiment run to determine if the compound induced interferon in the animals, and the kinetics of that induction.

IMMUNOLOGY: Experiments in which immunological parameters other than IFN are studied with an immunomodulating compound.

TERMINATED: Experiment which was stopped early because of some error in treatment or infection.

Table II-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
1	Ribavirin	1	7-28-86	bid x 5, beg 4 hr pre	sc	9.4-75	75	+	9.4	EXPANDED
1	Ribavirin	6	10-16-86	bid x 9, beg 30 hr pre	sc	9.4-75	9.4	-	>75	BALLIET
1	Ribavirin	7	10-16-86	bid x 9, beg 30 hr pre	sc	9.4-75	9.4	-	>75	BALLIET
1	Ribavirin	8	10-23-86	bid x 7, beg 4 hr pre	sc	0.6-75	>75	TI 16	4.7	TI, MMF
1	Ribavirin	9	10-23-86	bid x 7, beg 4 hr pre	sc	9.4-75	>75	+	9.4	MMF
1	Ribavirin	10	10-23-86	bid x 7, beg 4 hr pre	sc	9.4-75	>75	+	9.4	MMF
1	Ribavirin	11	10-23-86	bid x 7, beg 4 hr pre	sc	9.4-75	>75	+	18.8	MMF
1	Ribavirin	20	1-16-87	bid x 5, beg 24 hr post	sc	37.5-150	150	+	37.5	EXPANDED
1	Ribavirin	21	1-16-87	bid x 5, beg 36 hr post	sc	37.5-150	150	+	37.5	EXPANDED
1	Ribavirin	28	1-22-87	single, beg 4 hr pre	sc	175-700	>700	?		
1	Ribavirin	29	1-22-87	single, beg 8 hr pre	sc	175-700	>700	?		
1	Ribavirin	30	1-22-87	single, beg 24 hr pre	sc	175-700	>700	?		
1	Ribavirin	31	1-22-87	single, beg 48 hr pre	sc	175-700	>700	?		
1	Ribavirin	32	1-22-87	single, beg 72 hr pre	sc	175-700	>700	?		
1	Ribavirin	33	1-22-87	single, beg 96 hr pre	sc	175-700	>700	?		
1	Ribavirin	43	2-5-87	bid x 5, beg 4 hr pre	po	3.2-100	>100	+	12.5	EXPANDED
1	Ribavirin	44	2-5-87	bid x 5, beg 4 hr post	po	3.2-100	>100	+	6.3	EXPANDED
1	Ribavirin	45	2-5-87	bid x 5, beg 24 hr post	po	3.2-100	>100	+	6.3	EXPANDED
1	Ribavirin	46	3-6-87	single, beg 4 hr post	sc	175-700	>700	+	175	
1	Ribavirin	47	3-6-87	single, beg 8 hr post	sc	175-700	>700	+	175	
1	Ribavirin	48	3-6-87	single, beg 24 hr post	sc	175-700	>700	+	175	
1	Ribavirin	49	3-6-87	single, beg 48 hr post	sc	175-700	>700	+	175	
1	Ribavirin	50	3-6-87	single, beg 72 hr post	sc	175-700	>700	±	350	
1	Ribavirin	51	3-6-87	single, beg 96 hr post	sc	175-700	>700	-	>700	
1	Ribavirin	162	10-16-87	bid x 5, beg 24 hr post	po	0.32-150	>150	+	32	COMBINATION
1	Ribavirin	193	11-13-87	bid x 5, beg 24 hr post	po	0.32-150	>150	+	10	COMBINATION
1	Ribavirin	427	7-7-88	bid x 5, beg 24 hr post	po	1-200	>200	+	32	COMBINATION
1	Ribavirin	537	11-22-88	single, beg 24 hr post	ic	43.75-350	43.8	-	>350	BALLIET
1	Ribavirin	577	01-05-89	bid x 5, beg 24 hr post	po	1-300	>300	+	1	COMBINATION
1	Ribavirin	584	01-11-89	single, beg 4 hr pre	iv	62.5-500	>4500	±	500	BALLIET
1	Ribavirin	647	03-16-89	bid x 3, beg 24 hr post	po	3.13-1200	>1200	+	12.5	COMBINATION
1	Ribavirin	669	04-19-89	bid x 5, beg 24 hr post	sc	3.2-1000	1000	+	3.2	EXPANDED ALL
1	Ribavirin	687	05-17-89	bid x 5, beg 24 hr post	po	6.4-2000	2000	+	6.4	EXPANDED ALL
1	Ribavirin	690	05-25-89	qd x5, varying times	sc	140	>140	+	140	
1	Ribavirin	693	06-02-89	bid x 5, varying times	sc	140	>140	+	140	
1	Ribavirin	696	06-08-89	bid x 5, varying times	po	325	>325	+	48 post	
1	Ribavirin	701	07-14-89	qd x 5, varying times	po	325	>325	+	72 post	
1	Ribavirin	704	07-14-89	bid x 1-5, beg 24 hr post	po	325	~325	+	325	
1	Ribavirin	705	07-14-89	single, beg 24 hr post	po	325	~325	+	325	
1	Ribavirin	711	07-14-89	bid x 5, beg 4 hr post	sc	16	>16	?	?	BALLIET
1	Ribavirin	712	07-20-89	bid x 1-5, beg 24 hr post	sc	140	>140	+	140	
1	Ribavirin	713	07-20-89	single, beg 24 hr post	sc	140	>140	+	140	
1	Ribavirin	719	07-28-89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	720	07-28-89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	721	07-28-89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	722	07-28-89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	723	07-28-89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	736	08-10-89	bid x 1-5, beg 24 hr post	po	81	>81	+	81	

Table II-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Exp. #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
1	Ribavirin	737	08-10-89	single, beg 24 hr post	po	81	>81	+	81	
1	Ribavirin	761	09-15-89	bid x 5, beg 4 hr pre	ip	75-600	600	±	300	BALLIET
1	Ribavirin	765	09-21-89	bid x 1-5, beg 24 hr post	po	20	>20	+	20	
1	Ribavirin	766	09-21-89	single, beg 24 hr post	po	20	>20	+	20	
1	Ribavirin	771	09-27-89	single, beg 24 hr post	po	41	>41	+	41	EXPANDED ALL
1	Ribavirin	774	10-06-89	bid x 3, beg 24 hr post	po	6 25-1250	1250	+	25	COMBINATION
1	Ribavirin	788	11-03-89	bid x 5, beg 4 hr post	sc	16	>16	ON TEST	ON TEST	EXPANDED ALL
1	Ribavirin	813	02-22-90	bid x 3, beg 24 hr post	po	1 60-2000	2000	+	1 6	COMBINATION
1	Ribavirin	844	06-21-90	bid x 3, beg 24 hr post	po	146060	1200	±	10	COMBINATION
2	Ribavirin triacetate	106	8-14-87	bid x 5, beg 4 hr pre	sc	25-200	>200	+	25	
2	Ribavirin triacetate	112	8-21-87	bid x 5, beg 4 hr pre	sc	15.6-500	>500	TI 16	62 5	EXPANDED
2	Ribavirin triacetate	113	8-21-87	single, beg 4 hr post	sc	62 5-1000	>1000	+	62 5	
2	Ribavirin triacetate	114	8-21-87	single, beg 24 hr post	sc	62 5-1000	>1000	+	62 5	
2	Ribavirin triacetate	115	8-21-87	single, beg 48 hr post	sc	62 5-1000	>1000	+	62 5	
2	Ribavirin triacetate	116	8-21-87	single, beg 72 hr post	sc	62 5-1000	>1000	-	>1000	
2	Ribavirin triacetate	117	8-21-87	single, beg 96 hr post	sc	62 5-1000	>1000	-	>1000	
2	Ribavirin triacetate	134	9-18-87	bid x 5, beg 24 hr pre	po	9 4-600	600	TI 8	37 5	EXPANDED
2	Ribavirin triacetate	167	10-22-87	bid x 5, beg 4 hr pre	ip	12[illegible]1000	1000	+	250	BALLIET
2	Ribavirin triacetate	177	10-30-87	qd x 5, beg 4 hr pre	sc	62 5-500	>500	?		
2	Ribavirin triacetate	178	10-30-87	bid x 5, beg 4 hr pre	sc	62 5-500	>250	+	31 3	MMF
2	Ribavirin triacetate	179	10-30-87	bid x 5, beg 4 hr pre	sc	62 5-500	>250	+	62 5	MMF
2	Ribavirin triacetate	180	10-30-87	qd x 5, beg 4 hr pre	sc	62 5-500	>250	+	62 5	MMF
2	Ribavirin triacetate	181	10-30-87	qd x 5, beg 4 hr pre	sc	62 5-500	>250	+	62 5	MMF
2	Ribavirin triacetate	185	11-6-87	qd x 5, beg 4 hr pre	sc	31 3-1000	>1000	TI 16	62 5	
2	Ribavirin triacetate	339	4-15-88	single, beg 24 hr post	po	62 5-500	>500	+	62 5	EXPANDED
2	Ribavirin triacetate	340	4-15-88	single, beg 48 hr post	po	62 5-500	>500	+	250	EXPANDED
2	Ribavirin triacetate	377	5-20-88	bid x 5, beg 24 hr post	po	31 3-500	>500	+	31 3	EXPANDED
2	Ribavirin triacetate	378	5-20-88	bid x 5, beg 48 hr post	po	31 3-500	>500	+	31 3	EXPANDED
2	Ribavirin triacetate	671	04-19-89	bid x 5, beg 24 hr post	sc	9 6-3000	3000	+	9 6	EXPANDED ALL
2	Ribavirin triacetate	689	05-17-89	bid x 5, beg [illegible] hr post	po	12 8-4000	4000	+	12 8	EXPANDED ALL
2	Ribavirin triacetate	692	05-25-89	qd x 5, varying times	sc	425	>425	+	425	
2	Ribavirin triacetate	695	06-02-89	bid x 5, varying times	sc	425	>425	+	425	
2	Ribavirin triacetate	698	06-08-89	bid x 5, varying times	po	650	>563	+	96 post	
2	Ribavirin triacetate	702	07-14-89	qd x 5, varying times	po	563	>563	+	48 post	
2	Ribavirin triacetate	706	07-14-89	bid x 1-5, beg 24 hr post	po	563	>563	+	563	
2	Ribavirin triacetate	707	07-14-89	single, beg 24 hr post	po	563	>563	+	563	
2	Ribavirin triacetate	714	07-20-89	bid x 1-5, beg 24 hr post	sc	425	>425	+	425	
2	Ribavirin triacetate	715	07-20-89	single, beg 24 hr post	sc	425	>425	+	425	
2	Ribavirin triacetate	724	07-28-89	bid x 5, beg 24 hr post	po	11 3-1126	>1126	+	112 6	MMF
2	Ribavirin triacetate	725	07-28-89	bid x 5, beg 24 hr post	po	11 3-1126	>1126	+	112 6	MMF
2	Ribavirin triacetate	726	07-28-89	bid x 5, beg 24 hr post	po	11 3-1126	>1126	+	112 6	MMF
2	Ribavirin triacetate	727	07-28-89	bid x 5, beg 24 hr post	po	11 3-1126	>1126	+	112 6	MMF
2	Ribavirin triacetate	728	07-28-89	bid x 5, beg 24 hr post	po	11 3-1126	>1126	+	11 3	MMF
2	Ribavirin triacetate	738	08-10-89	bid x 1-5, beg 24 hr post	po	141	>141	+	141	
2	Ribavirin triacetate	739	08-10-89	single, beg 24 hr post	po	141	>141	+	141	
2	Ribavirin triacetate	762	09-15-89	bid x 5, beg 4 hr pre	ip	225-1800	900	-	>1800	BALLIET
2	Ribavirin triacetate	767	09-21-89	bid x 1-5, beg 24 hr post	po	35	>35	+	35	
2	Ribavirin triacetate	768	09-21-89	single, beg 24 hr post	po	35	>35	+	35	

Table II-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
2	Ribavirin triacetate	772	09-27-89	single, beg 24 hr post	po	71	>71	+	71	EXPANDED ALL
52	Thiolormycin B	2	10-10-86	bid x 5, beg 4 hr pre	sc	62.5-250	>250	-	>250	
52	Thiolormycin B	22	1-22-87	single, beg 4 hr post	sc	300-1200	>1200	-	>1200	
52	Thiolormycin B	23	1-22-87	single, beg 8 hr post	sc	300-1200	>1200	-	>1200	
52	Thiolormycin B	24	1-22-87	single, beg 24 hr post	sc	300-1200	>1200	-	>1200	
52	Thiolormycin B	153	10-9-87	tid x 5, beg 4 hr pre	sc	62.5-500	>500	+	250	
52	Thiolormycin B	231A	12-18-87	qid x 5, beg 4 hr pre	sc	25-400	>400	±	50	
52	Thiolormycin B	342	4-22-88	tid x 5, beg	po	50-400	>400	+	50	EXPANDED
65	Formycin B	52	3-12-87	bid x 5, beg 4 hr pre	sc	62.5-250	>250	-	>250	
65	Formycin B	551	12-01-88	tid x 5, beg 4 hr pre	sc	31.3-500	>500	+	125	
65	Formycin B	560	12-08-88	single, beg 4 hr pre	sc	31.3-500	>500	-	>500	
65	Formycin B	561	12-08-88	single, beg 24 hr post	sc	31.3-500	>500	+	62.5	
65	Formycin B	596	01-19-89	single, beg 24 hr post	sc	100-800	>800	-	>800	
65	Formycin B	597	01-19-89	single, beg 24 hr post	ip	100-800	>800	+	200	
65	Formycin B	806		bid x 5, beg 4 hr pre	sc	62.5-500	>500	+	62.5	EXPANDED
65	Formycin B	811	02-08-90	tid x 5, beg 4 hr pre	ip	62.5-500	>500	±	125	EXPANDED
65	Formycin B	818	03-01-90	tid x 5, beg 4 hr pre	ip	125-1000	1000	-	>1000	EXPANDED
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	3	10-10-86	bid x 5, beg 4 hr pre	sc	25-100	100	+	25	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	12	11-14-86	bid x 5, beg 4 hr pre	sc	6.25-50	>50	TI 2	6.25	EXPANDED
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	18	12-3-86	bid x 5, beg 24 hr pre	sc	9.4-75	>75	-	>75	BALLIET
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	25	1-22-87	single, beg 4 hr post	sc	175-700	700	?		
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	26	1-22-87	single, beg 8 hr post	sc	175-700	700	?		
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	27	1-22-87	single, beg 24 hr post	sc	175-700	700	?		
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	95	7-30-87	qd x 5, beg 4 hr pre	sc	25-200	200	?		
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	102	8-7-87	bid x 5, beg 4 hr pre	po	25-200	>200	±	>200	EXPANDED
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	107	8-14-87	bid x 5, beg 24 hr post	sc	18.8-150	>150	-	18.8	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	108	8-14-87	bid x 5, beg 36 hr post	sc	18.8-150	>150	-	37.5	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	109	8-14-87	bid x 5, beg 48 hr post	sc	18.8-150	>150	-	>150	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	133	9-11-87	qd x 5, beg 4 hr pre	sc	25-200	200	+	50	REPEAT #95
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	154	10-9-87	single, beg 4 hr post	sc	87.5-700	>700	±	350	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	155	10-9-87	single, beg 24 hr post	sc	87.5-700	>700	±	175	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	156	10-9-87	single, beg 48 hr post	sc	87.5-700	>700	+	87.5	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	157	10-9-87	single, beg 72 hr post	sc	87.5-700	>700	-	>700	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	158	10-9-87	single, beg 96 hr post	sc	87.5-700	>700	-	>700	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	187	11-6-87	bid x 5, beg 4 hr pre	ip	6.25-200	200	-	200	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	188	11-6-87	tid x 5, beg 4 hr pre	sc	6.25-200	200	+	6.25	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	336	4-15-88	single, beg 4 hr post	po	87.5-700	>700	±	175	EXPANDED
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	337	4-15-88	single, beg 24 hr post	po	87.5-700	>700	+	87.5	EXPANDED
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	338	4-15-88	single, beg 48 hr post	po	87.5-700	>700	+	87.5	EXPANDED
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	374	05-20-88	single, beg 4 hr post	po	87.5-700	>700	±	350	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	375	05-20-88	single, beg 24 hr post	po	87.5-700	>700	±	700	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	376	05-20-88	single, beg 48 hr post	po	87.5-700	>700	±	175	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	403	6-17-88	single, beg 60 hr post	po	43.8-700	>700	-	>700	
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	534	11-22-88	single, beg 24 hr post	ip	62.5-500	>500	-	>500	BALLIET
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	799	01-04-90	tid x 4, beg 4 hr pre	ip	125-1000	250			
79	9-8[-D-ribofuranosylpurine-6-thiocarboxamide	819	03-01-90	tid x 5, beg 4 hr pre	ip	7.8-62.5	>62.5	+	15.6	EXPANDED
111	Tiazofurin	53	3-12-87	bid x 5, beg 4 hr pre	sc	31.3-250	>250	+	62.5	
111	Tiazofurin	68	3-26-87	bid x 5, beg 4 hr pre	sc	31.3-250	>500	+	31.3	EXPANDED

Table II-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
111	Tiazofurin	110	8-14-87	bid x 5, beg 4 hr pre	sc	15.7-2000	2000	TI=8-16	125	TI
111	Tiazofurin	135	9-18-87	single, beg 4 hr post	sc	125-1000	250	+	250	
111	Tiazofurin	136	9-18-87	single, beg 24 hr post	sc	125-1000	250	+	1000	
111	Tiazofurin	137	9-18-87	single, beg 48 hr post	sc	125-1000	250	+	250	
111	Tiazofurin	138	9-18-87	single, beg 72 hr post	sc	125-1000	250	-	>1000	
111	Tiazofurin	139	9-18-87	single, beg 96 hr post	sc	125-1000	250	±	1000	
111	Tiazofurin	182	11-5-87	bid x 5, beg 24 hr pre	sc	62.5-500	>500	-	>500	BALLIET
111	Tiazofurin	365	5-6-88	bid x 5, beg 4 hr pre	po	93.8-750	>750	+	93.8	EXPANDED
111	Tiazofurin	832	04-19-90	bid x 5, beg 4 hr pre	sc	62.5-1000	>1000	+	125	EXPANDED
147	Enviroxime	15	11-19-86	bid x 5, beg 4 hr pre	sc	25-100	>100	-	>100	
147	Enviroxime	34	1-29-87	single, beg 4 hr post	sc	250-1000	>1000	+	1000	
147	Enviroxime	35	1-29-87	single, beg 12 hr post	sc	250-1000	>1000	±	>1000	
147	Enviroxime	36	1-29-87	single, beg 24 hr post	sc	250-1000	>1000	-	>1000	
147	Enviroxime	96	7-30-87	qd x 5, beg 4 hr pre	sc	62.5-500	>500	-	>500	
147	Enviroxime	371	5-13-88	single, beg 4 hr post	po	125-1000	>1000	-	BAD TEST	EXPANDED
147	Enviroxime	372	5-13-88	single, beg 24 hr post	po	125-1000	>1000	-	BAD TEST	EXPANDED
147	Enviroxime	373	5-13-88	single, beg 48 hr post	po	125-1000	>1000	-	BAD TEST	EXPANDED
147	Enviroxime	522	11-02-88	single, beg 24 hr pre	po	150-1200	1200	-	>1200	EXPANDED
147	Enviroxime	523	11-03-88	single, beg 4 hr post	po	150-1200	1200	±	300	EXPANDED
147	Enviroxime	524	11-03-88	single, beg 24 hr post	po	150-1200	1200	-	>1200	EXPANDED
147	Enviroxime	817	03-01-90	bid x 5, beg 4 hr pre	sc	75-500	>500	±	75	EXPANDED
147	Enviroxime	820	03-08-90	bid x 5, beg 4 hr pre	sc	75-500	>500	±	125	EXPANDED
167	Glycerrhetic Acid	54	3-12-87	bid x 5, beg 4 hr pre	sc	18.8-75	>75	-	>75	
167	Glycerrhetic Acid	87	4-24-87	bid x 5, beg 4 hr pre	sc	62.5-500	>500	-	500	REPEAT
167	Glycerrhetic Acid	304	3-3-88	bid x 5, beg 24 hr pre	ip	75-600	300	-	>600	
206	Ribamidine	4	10-10-86	bid x 5, beg 4 hr pre	sc	125-500	>500	+	125	
206	Ribamidine	13	11-14-86	bid x 5, beg 4 hr pre	sc	31.3-250	>250	+	31.3	
206	Ribamidine	71	4-3-87	bid x 5, beg 4 hr pre	sc	3.9-1000	>1000	TI >32	31.3	TI
206	Ribamidine	78	4-10-87	bid x 5, beg 24 hr post	sc	62.5-500	>500	+	62.5	EXPANDED
206	Ribamidine	79	4-10-87	bid x 5, beg 36 hr post	sc	62.5-500	>500	+	62.5	EXPANDED
206	Ribamidine	80	4-10-87	bid x 5, beg 48 hr post	sc	62.5-500	>500	+	62.5	EXPANDED
206	Ribamidine	81	4-10-87	bid x 5, beg 72 hr post	sc	62.5-500	>500	+	125	EXPANDED
206	Ribamidine	86	4-23-87	bid x 5, beg 24 hr pre	sc	125-500	>500	-	125	BALLIET
206	Ribamidine	92	7-28-87	bid x 5, beg 4 hr pre	po	7.8-1000	>1000	TI>564	31.3	TI
206	Ribamidine	169	10-23-87	single, beg 4 hr post	sc	15.7-1000	>1000	+	62.5	
206	Ribamidine	170	10-23-87	single, beg 24 hr post	sc	15.7-1000	>1000	+	500	
206	Ribamidine	171	10-23-87	single, beg 48 hr post	sc	15.7-1000	>1000	+	250	
206	Ribamidine	172	10-23-87	single, beg 72 hr post	sc	15.7-1000	>1000	-	>1000	
206	Ribamidine	173	10-23-87	single, beg 96 hr post	sc	15.7-1000	>1000	-	>1000	
206	Ribamidine	233	12-18-87	bid x 5, beg 4 hr pre	sc	7.8-2000	2000	+		
206	Ribamidine	234	12-3-87	bid x 5, beg 4 hr pre	po	7.8-2000	2000	+		
206	Ribamidine	287	2-19-88	bid x 5, beg 24 hr post	po	2.4-75	>75	+	2.4	COMBINATION
206	Ribamidine	363	5-6-88	bid x 5, beg 24 hr pre	ip	75-600	>600	±	600	BALLIET
206	Ribamidine	382	5-27-88	bid x 5, beg 18 hr post	po	2.4-75	>75	+	18.8	COMBINATION
206	Ribamidine	447	8-5-88	bid x 3, beg 24 hr post	po	1000	>1000	+	1000	
206	Ribamidine	535	11-22-88	single, beg 24 hr post	ip	250-2000	>2000	±	2000	BALLIET
206	Ribamidine	536	11-22-88	single, beg 24 hr post	ic	62.5-1000	500	-	>1000	BALLIET
206	Ribamidine	670	04-19-89	bid x 5, beg 24 hr post	sc	9.6-3000	3000	+	30	EXPANDED ALL

Table II-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
206	Ribamidine	688	05-17-89	bid x 5, beg 24 hr post	po	12.8-4000	4000	+	12.8	EXPANDED ALL
206	Ribamidine	691	05-25-89	qd x 5, varying times	sc	425	>425	+	425	
206	Ribamidine	694	06-02-89	bid x 5, varying times	sc	425	>425	+	425	
206	Ribamidine	697	06-08-89	bid x 5, varying times	po	650	>650	+	4[illegible] post	
206	Ribamidine	703	07-14-89	qd x 5, varying times	po	650	>650	+	48 post	
206	Ribamidine	708	07-14-89	bid x 1-5, beg 24 hr post	po	650	>650	+	650	
206	Ribamidine	709	07-14-89	single, beg 24 hr post	po	650	>650	+	650	
206	Ribamidine	716	07-20-89	bid x 1-5, beg 24 hr post	sc	425	>425	+	425	
206	Ribamidine	717	07-20-89	single, beg 24 hr post	sc	425	>425	+	425	
206	Ribamidine	729	07-28-89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	13	MMF
206	Ribamidine	730	07-28-89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	130	MMF
206	Ribamidine	731	07-28-89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	130	MMF
206	Ribamidine	732	07-28-89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	130	MMF
206	Ribamidine	733	07-28-89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	41.1	MMF
206	Ribamidine	740	08-10-89	bid x 1-5, beg 24 hr post	po	163	>163	+	163	
206	Ribamidine	741	08-10-89	single, beg 24 hr post	po	163	>163	+	163	
206	Ribamidine	763	09-15-89	bid x 5, beg 4 hr pre	ip	225-1800	1800	-	>1800	BALLIET
206	Ribamidine	769	09-21-89	bid x 1-5, beg 24 hr post	po	41	>41	+	41	
206	Ribamidine	770	09-21-89	single, beg 24 hr post	po	41	>41	+	41	
206	Ribamidine	773	09-27-89	single, beg 24 hr post	po	82	>82	+	82	EXPANDED ALL
212	Suramin	16	11-19-86	bid x 5, beg 4 hr pre	sc	18.8-75	>25	-	>75	
212	Suramin	37	1-29-87	single, beg 4 hr post	sc	250-1000	>600	-	>1000	
212	Suramin	38	1-29-87	single, beg 12 hr post	sc	250-1000	>600	-	>1000	
212	Suramin	39	1-29-87	single, beg 24 hr post	sc	250-1000	>600	-	>1000	
212	Suramin	103	8-7-87	bid x 5, beg 4 hr pre	po	75-200	>200	-	>200	EXPANDED
212	Suramin	159	10-9-87	tid x 5, beg 4 hr pre	sc	18.8-150	>150	-	>150	
215	3-Deazaguanosine	497	10-13-88	qd x 5, beg 4 hr pre	sc	18.8-300	150	±	37.5	
215	3-Deazaguanosine	557	12-08-88	tid x 5, beg 4 hr pre	sc	12.5-100	>100	-	>100	
215	3-Deazaguanosine	558	12-08-88	bid x 5, beg 4 hr pre	sc	12.5-100	>100	+	50	
215	3-Deazaguanosine	559	12-08-88	bid x 5, beg 4 hr pre	ip	12.5-100	>100	+	25	
215	3-Deazaguanosine	591	01-19-89	tid x 5, beg 4 hr pre	ip	12.5-100	>100	+	12.5	EXPANDED
215	3-Deazaguanosine	592	01-19-89	bid x 5, beg 4 hr pre	ip	12.5-100	>100	+	25	EXPANDED
215	3-Deazaguanosine	646	03-09-89	bid x 5, beg 24 hr pre	ip	3.13-50	>50	-	>50	BALLIET
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	55	3-12-87	bid x 5, beg 4 hr pre	sc	31.3-250	>250	-	>250	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	88	4-24-87	bid x 5, beg 4 hr pre	sc	31.3-250	>250	-	31.3	EXPANDED
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	302	3-3-88	qd x 5, beg 24 hr pre	sc	62.5-500	>500	-	>500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	366	5-6-88	bid x 5, beg 4 hr pre	sc	2000	2000	-	>2000	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	437	7-20-88	single, 24 hr pre	ip	187.5-1500	>1500	±	>1500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	438	7-21-88	single, 4 hr pre	ip	187.5-1500	>1500	-	>1500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	439	7-21-88	single, 24 hr post	ip	187.5-1500	>1500	-	>1500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	440	7-21-88	tid x 5, beg 4 hr pre	sc	62.5-500	>500	-	>500	
233	Formycin	17	11-19-86	bid x 5, beg 4 hr pre	sc	100-400	>400	-	>400	
233	Formycin	40	1-29-87	single, beg 12 hr post	sc	450-1800	900	±	450	
233	Formycin	41	1-29-87	single, beg 12 hr post	sc	450-1800	900	±	450	
253	Selenazofurin	5	10-10-86	bid x 5, beg 4 hr pre	sc	80-320	160	+	80	
253	Selenazofurin	14	11-14-86	bid x 5, beg 4 hr pre	sc	20-160	>160	+	80	
253	Selenazofurin	19	12-3-86	bid x 5, beg 24,4 hr pre	sc	18.8-150	>150	+	>150	BALLIET
253	Selenazofurin	97	7-30-87	qd x 5, beg 4 hr pre	sc	40-320	320	±	40	REPEAT

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AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
253	Selenazofurin	104	8-7-87	bid x 5, beg 4 hr pre	po	40-320	320	+	40	EXPANDED
253	Selenazofurin	538	11-22-88	single, beg 4 hr post	ip	93.75-750	>750	±	93.8	BALLIET
253	Selenazofurin	800	01-04-90	qd x 4, beg 4 hr pre	ip	125-1000	>1000	+		EXPANDED
253	Selenazofurin	801	01-04-90	bid x 5, beg 4 hr pre	ip	125-1000	1000	+		EXPANDED
257	Tiazofurin 5'-MP	445	7-21-88	bid x 5, beg 4 hr pre	ip	25-400	>400	+	400	
257	Tiazofurin 5'-MP	449	9-2-88	bid x 5, beg 4 hr pre	ip	50-400	>400	+	200	EXPANDED
272	3-Deazaguanine	186	11-6-87	bid x 5, beg 4 hr pre	sc	25-200	100	-	>200	
272	3-Deazaguanine	232	12-18-87	qd x 5, beg 4 hr pre	sc	25-200	>200	+	25	
272	3-Deazaguanine	280	2-11-88	bid x 5, beg 24 hr pre	ip	12.5-100	>100	?		
272	3-Deazaguanine	317	3-18-88	bid x 5, beg 24 hr pre	ip	12.5-100	100	-	>12.5	
272	3-Deazaguanine	343	4-22-88	qd x 5, beg	ip	25-200	200	-	>200	BALLIET
272	3-Deazaguanine	370	5-13-88	qd x 5, beg 4 hr pre	po	18.8-300	>300	+	18.8	EXPANDED
272	3-Deazaguanine	498	10-13-88	qd x 5, beg 4 hr pre	sc	18.8-300	>300	-	>300	EXPANDED
272	3-Deazaguanine	539	11-22-88	single, beg 4 hr post	ip	93.75-750	>750	-	>750	BALLIET
272	3-Deazaguanine	80?	01-12-90	bid x 5, beg 4 hr pre	sc	62.5-500	500	±	62.5	EXPANDED
347	UNIDENTIFIED	82	04-12-90	bid x 4, beg 4 hr pre	sc	15-120	120	±	30	EXPANDED
360	7-Deoxynarciclasin	42	1-29-87	bid x 5, beg 4 hr pre	sc	62.5-500	>500	±	7.5	
361	Pancratistatin	417	6-24-88	qd x 7, beg 24 hr pre	sc	.5-4	>4	-	>4	
1018	UNIDENTIFIED	791	11-16-89	single, beg 4 hr post	po	1.56-12.5	>12.5	±	12.5	EXPANDED
1018	UNIDENTIFIED	792	11-16-89	3 shots in 9 days, beg 24 hr post	po	1.56-12.5	>12.5	+	1.56	EXPANDED
1018	UNIDENTIFIED	830	04-12-90	single, beg 24 hr post	po	3.13-25	>25	+	6.25	EXPANDED
1018	UNIDENTIFIED	831	04-12-90	single, beg 36 hr post	po	3.13-25	>25	+	6.25	EXPANDED
1018	UNIDENTIFIED	838	05-31-90	4 hr pre, day 4	po	3.13-25	>25	±	3.13	BALLIET
1212	Uridine 2',3'-dialdehyde	550	12-01-88	bid x 5, beg 4 hr pre	sc	25-400	>400	-	>400	INITIAL
1212	Uridine 2',3'-dialdehyde	562	1 -08-88	single, beg 4 hr pre	sc	25-400	>400	±	25	
1212	Uridine 2',3'-dialdehyde	563	1.'-08-88	single, beg 24 hr post	sc	25-400	>40	+	25	
1212	Uridine 2',3'-dialdehyde	598	01-19-89	single, beg 24 hr post	sc	12.5-100	>100	-	>100	
1212	Uridine 2',3'-dialdehyde	599	01-19-89	single, beg 24 hr post	ip	12.5-100	>100	+	100	
1212	Uridine 2',3'-dialdehyde	661	04-06-89	single, beg 24 hr post	ip	100-800	800	±	100	EXPANDED
1754	MVE-2	58	3-19-87	single, beg 24 hr pre	ip	6.25-50	>50	+	12.5	
1754	MVE-2	89	4-23-87	single, beg 24 hr pre	ip	6.25-50	>50	+	6.25	EXPANDED
1754	MVE-2	98	7-30-87	single, beg 4 hr pre	ip	6.25-100	25	+	6.3	
1754	MVE-2	99	7-30-87	single, beg 4 hr post	ip	6.25-100	25	+	6.3	
1754	MVE-2	100	7-30-87	single, beg 24 hr post	ip	6.25-100	25	+	6.3	
1754	MVE-2	101	7-30-87	single, beg 48 hr post	ip	6.25-100	25	+	6.3	
1754	MVE-2	151	10-1-87	single, beg 24 hr pre	po	6.25-200	>200	-	>200	EXPANDED
1754	MVE-2	161	10-8-87	single, beg 4 hr pre	ip	12.5-100	100	±	50	BALLIET
1754	MVE-2	238	01-08-88	qd x 3, beg 4 hr pre	ip	3.13-50	50	+	6.25	
1754	MVE-2	240	01-08-88	single, beg 72 hr post	ip	6.25-100	>100	-	>100	
1754	MVE-2	241	01-08-88	single, beg 96 hr post	ip	6.25-100	>100	-	>100	
1754	MVE-2	249	01-15-88	single, beg 4 hr pre	sc	6.25-100	12.5	+	6.25	
1754	MVE-2	252	1-14-88	single	ip	6.25-100	>100	±	12.5	IFN
1754	MVE-2	311	3-11-88	bid x 5, beg 4 hr pre	ip	6.25-50	>50	+	6.25	
1754	MVE-2	431	7-7-88	single, beg 24 hr post	ip	0.05, 0.5, 5	>5	+	5	IFN, EXPANDED
1754	MVE-2	603	01-26-89	bid x 5, beg 24 hr post	ip	1.56-50	>50	+	12.5	EXPANDED
1754	MVE-2	624	02-24-89	single, beg 24 hr post	ip	0.75-25	>25	±	12.5	MMF
1754	MVE-2	625	02-24-89	single, beg 24 hr post	ip	0.75-25	>25	+	12.5	MMF
1754	MVE-2	626	02-24-89	single, beg 24 hr post	ip	0.75-25	>25	+	12.5	MMF

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AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
1754	MVE-2	627	02-24-89	single, beg 24 hr post	ip	0.75-25	>25	+	12.5	MMF
1757	Isoprinosine	76	4-10-87	bid x 5, beg 24 hr post	po	250-1000	>1000	-	>1000	
1761	Poly IC•LC	307	3-3-88	qd x 8, beg 24 hr pre	ip	0.0195-5	5	+	0.0195	
1761	Poly IC•LC	324	3-24-88	qd x 8, beg 24 hr pre	sc	0.031-1	>1	+	0.031	EXPANDED
1761	Poly IC•LC	325	3-24-88	qd x 8, beg 24 hr pre	po	0.031-1	>1	-	1	EXPANDED
1761	Poly IC•LC	326	3-25-88	single, beg 4 hr pre	ip	0.31-2.5	>2.5	+	0.31	
1761	Poly IC•LC	327	3-25-88	single, beg 4 hr post	ip	0.31-2.5	>2.5	+	0.31	
1761	Poly IC•LC	328	3-25-88	single, beg 24 hr post	ip	0.31-2.5	>2.5	+	0.31	
1761	Poly IC•LC	329	3-25-88	single, beg 48 hr post	ip	0.31-2.5	>2.5	+	0.625	
1761	Poly IC•LC	330	3-25-88	single, beg 72 hr post	ip	0.31-2.5	>2.5	-	>2.5	
1761	Poly IC•LC	331	3-25-88	single, beg 96 hr post	ip	0.31-2.5	>2.5	-	>2.5	
1761	Poly IC•LC	361	4-29-88	qd x 5, beg 4 hr pre	sc	0.0625-0.5	>0.5	-	>0.5	BALLIET
1761	Poly IC•LC	672	04-27-89	3 in 7 days, beg 4 hr post	ip	0.125-1	>1	+	0.125	EXPANDED
1761	Poly IC•LC	734	08-04-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.0032	EXPANDED
1761	Poly IC•LC	742	08-10-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.01	EXPANDED
1761	Poly IC•LC	745		eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.01	EXPANDED
1761	Poly IC•LC	749	08-24-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.01	EXPANDED
1761	Poly IC•LC	814	02-22-90	eod x 3, beg 24 hr post	ip	0.32	>0.32	+	0.32	COMBINATION
1761	Poly IC•LC	821	03-08-90	eod x 3, beg 24 hr post	ip	0.001-0.01	>0.01	+	0.001	COMBINATION
1767	AM-3	72	4-3-87	bid x 5, beg 4 hr pre	sc	112.5-450	>450	+	112.5	
1767	AM-3	73	4-3-87	bid x 5, beg 4 hr pre	po	112.5-450	>450	-	>450	
1767	AM-3	111	8-14-87	bid x 5, beg 4 hr pre	sc	62.5-2000	2000	+	62.5	EXPANDED
1767	AM-3	168	10-22-87	bid x 5, beg 24 hr pre	ip	62.5-500	500	-	>500	BALLIET
1767	AM-3	243	01-15-88	single, beg 4 hr pre	sc	25-400	>400	+	50	
1767	AM-3	244	01-15-88	single, beg 4 hr post	sc	25-400	>400	+	25	
1767	AM-3	245	01-15-88	single, beg 24 hr post	sc	25-400	>400	+	25	
1767	AM-3	246	01-15-88	single, beg 48 hr post	sc	25-400	>400	+	25	
1767	AM-3	247	01-15-88	single, beg 72 hr post	sc	25-400	>400	-	>400	
1767	AM-3	248	01-15-88	single, beg 96 hr post	sc	25-400	>400	-	>400	
1767	AM-3	251	1-14-88	single	sc	25-400	>400	-	>400	IFN
1767	AM-3	259	1-29-88	qd x 5, beg 4 hr pre	sc	31.3-250	>250	+	62.5	
1767	AM-3	260	1-29-88	single, beg 4 hr pre	sc	15.6-1000	1000	+	15.6	
1767	AM-3	261	1-29-88	single, beg 4 hr post	sc	15.6-1000	1000	+	62.5	
1767	AM-3	262	1-29-88	single, beg 24 hr post	sc	15.6-1000	1000	±	62.5	
1767	AM-3	263	1-29-88	single, beg 48 hr post	sc	15.6-1000	1000	+	15.6	
1767	AM-3	264	1-29-88	single, beg 72 hr post	sc	15.6-1000	1000	±	500	
1767	AM-3	265	1-29-88	single, beg 96 hr post	sc	15.6-1000	1000	±	15.6	
1767	AM-3	267	1-29-88	single	sc	31.3-250	>250	-	>250	IFN
1767	AM-3	308	3-11-88	bid x 5, beg 4 hr pre	ip	15.7-250	>250	+	15.7	
1767	AM-3	386	5-27-88	single, beg 48 hr post	sc	5, 16, 50	>50	-	50	COMBINATION
1767	AM-3	540	11-22-88	single, beg 4 hr post	sc	62.5-500	>500	-	>500	BALLIET
1777	Streptonigrin	77	4-10-87	qd x 5, beg 4 hr pre	sc	0.125-1	0.5	-	>1	
1777	Streptonigrin	566	12-14-88	single, beg 24 hr pre	ip	0.31-5	1.25	-	>5	
1777	Streptonigrin	567	12-14-88	single, beg 4 hr post	ip	0.31-5	1.25	-	>5	
1777	Streptonigrin	568	12-14-88	single, beg 24 hr post	ip	0.31-5	1.25	-	>5	
1777	Streptonigrin	569	12-15-88	bid x 5, beg 4 hr pre	ip	0.125-1	0.5	-	>1	
1777	Streptonigrin	570	12-15-88	bid x 5, beg 4 hr pre	ip	0.125-1	0.5	-	>1	
1778	Mannozym	74	4-3-87	single, beg 4 hr pre	sc	12.5-50	50	+	25	

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AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
1778	Mannozym	75	4-3-87	bid x 5, beg 4 hr pre	sc	3.1-50	>50	+	3.13	
1778	Mannozym	93	7-28-87	bid x 5, beg 4 hr pre	po	9.4-150	>150	-	>150	
1778	Mannozym	118	8-28-87	bid x 5, beg 4 hr pre	sc	1.6-100	>100	+	3.1	EXPANDED
1778	Mannozym	119	8-28-87	bid x 5, beg 4 hr pre	po	1.6-100	>100	-	>100	EXPANDED
1778	Mannozym	152	10-2-87	bid x 5, beg 4 hr pre	sc	6.25-100	>100	-	>100	BALLIET
1778	Mannozym	198	11-19-87	single, beg 24 hr pre	sc	6.3-50	>50	-	>50	
1778	Mannozym	199	11-19-87	single, beg 4 hr pre	sc	6.3-50	>50	-	>50	
1778	Mannozym	200	11-19-87	single, beg 4 hr post	sc	6.3-50	>50	-	>50	
1778	Mannozym	201	11-19-87	single, beg 24 hr post	sc	6.3-50	>50	-	25	
1778	Mannozym	202	11-19-87	single, beg 48 hr post	sc	6.3-50	>50	-	>50	
1778	Mannozym	203	11-19-87	single, beg 72 hr post	sc	6.3-50	>50	-	>50	
1778	Mannozym	204	11-19-87	single, beg 96 hr post	sc	6.3-50	>50	-	>50	
1778	Mannozym	216	12-4-87	qd x 5, beg 4 hr pre	sc	3.13-100	>100	±	3.13	
1778	Mannozym	217	12-4-87	bid x 5, beg 4 hr pre	ip	0.78-400	200	-	>100	
1778	Mannozym	239	01-08-88	qd x 5, beg 4 hr pre	sc	9.4-150	>150	?		
1778	Mannozym	250	01-15-88	single, beg 4 hr pre	sc	6.25-100	12.5	±	50	
1778	Mannozym	253	1-14-88	single	sc	6.75-100	>100	-	>100	IFN
1778	Mannozym	293	2-26-88	qd x 5, beg 4 hr pre	sc	9.4-150	>150	+	9.4	
1778	Mannozym	294	2-26-88	bid x 5, beg 4 hr pre	sc	1.6-50	>50	+	1.6	
1778	Mannozym	295	2-26-88	bid x 5, beg 24 hr post	sc	9.4-150	>150	+	1.6	
1778	Mannozym	296	2-26-88	bid x 5, beg 48 hr post	sc	9.4-150	>150	+	3.2	
1968	UNIDENTIFIED	797	12-14-89	single, 4 hr pre	po	12.5-100	>100	+	12.5	EXPANDED
1968	UNIDENTIFIED	798	12-14-89	3 shots, beg 24 hr post	po	12.5-100	>100	+	12.5	EXPANDED
1968	UNIDENTIFIED	839	05-31-90	single, 4 hr pre	po	12.5-100	>100	-	>100	BALLIET
1969	CL259763	356	4-29-88	single, beg 24 hr pre	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	357	4-29-88	single, beg 4 hr pre	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	358	4-29-88	single, beg 24 hr post	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	359	4-29-88	single, beg 48 hr post	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	360	4-29-88	single, beg 72 hr post	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	391	6-9-88	single, beg 24 hr pre	ip	2, 20, 200	>200	±	20	
1969	CL259763	392	6-9-88	single, beg 4 hr pre	ip	2, 20, 200	>200	-	>200	
1969	CL259763	393	6-9-88	single, beg 48 hr post	ip	2, 20, 200	>200	-	>200	
1969	CL259763	394	6-9-88	single, beg 24 hr post	ip	2, 20, 200	>200	-	>200	
1969	CL259763	395	6-9-88	bid x 5, beg 4 hr pre	ip	6.25-100	>100	±	25	
1969	CL259763	425	7-1-88	bid x 5, beg 4 hr pre	po	2, 20, 200	>200	-	>200	
1969	CL259763	434	7-13-88	single, beg 24 hr pre	ip	5-80	>80	±	5	EXPANDED
1969	CL259763	436	7-13-88	eod x 3, beg 24 hr pre	ip	2-200	>200	-	>200	IFN
1969	CL259763	541	11-22-88	single, beg 4 hr post	ip	50-400	>400	-	>400	BALLIET
1976	Thymine riboside 2',3'-dialdehyde	446	7-21-88	bid x 5, beg 4 hr pre	ip	6.25-100	>100	-	>100	
1976	Thymine riboside 2',3'-dialdehyde	452	9-2-88	single, beg 24 hr pre	ip	62.5-500	500	±	62.5	
1976	Thymine riboside 2',3'-dialdehyde	453	9-2-88	single, beg 4 hr pre	ip	62.5-500	500	±	62.5	
1976	Thymine riboside 2',3'-dialdehyde	454	9-2-88	single, beg 24 hr post	ip	62.5-500	500	-	>500	
1976	Thymine riboside 2',3'-dialdehyde	481	9-30-88	bid x 5, beg 4 hr pre	ip	50-400	400	-	>400	
2149	Ampligen	56	3-12-87	qd x 8, beg 24 hr pre	ip	0.6-5	>5	+	0.625	
2149	Ampligen	57	3-12-87	eod x 8, beg 24 hr pre	sc	0.6-5	>5	+	0.625	
2149	Ampligen	69	3-26-87	qd x 8, beg 24 hr pre	sc	0.6-5	>5	+	0.313	EXPANDED
2149	Ampligen	128	9-10-87	qd x 5, beg 24 hr pre	ip	0.6-5	>5	+	0.625	
2149	Ampligen	129	9-10-87	qd x 5, beg 4 hr pre	ip	0.6-5	>5	+	0.625	

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AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
2149	Ampligen	130	9-10-87	qd x 5, beg 4 hr post	ip	0.6-5	>5	+	0.625	
2149	Ampligen	131	9-10-87	qd x 5, beg 24 hr post	ip	0.6-5	>5	+	0.625	
2149	Ampligen	132	9-10-87	qd x 5, beg 48 hr post	ip	0.6-5	>5	+	0.625	
2149	Ampligen	142	9-25-87	qd x 5, beg 4 hr pre	po	0.04-5	>5	±	0.039	EXPANDED
2149	Ampligen	160	10-8-87	qd x 5, beg 4 hr pre	ip	0.625-5	>5	+	0.63	BALLIET
2149	Ampligen	166	10-16-87	qd x 5, beg 24 hr post	ip	0.05-5	>5	+	0.05	COMBINATION
2149	Ampligen	195	11-13-87	qd x 5, beg 24 hr post	ip	0.005	>0.005	±	0.005	COMBINATION
2149	Ampligen	205	11-20-87	bid x 5, beg 4 hr pre	ip	0.31-5	>5	+	0.625	
2149	Ampligen	207	12-4-87	qd x 5, beg 4 hr pre	ip	3.13-25	>25	+	3.13	TI
2149	Ampligen	208	12-4-87	single, beg 4 hr pre	ip	1.25-10	>10	+	1.25	
2149	Ampligen	209	12-3-87	single, beg 24 hr pre	ip	1.25-10	>10	+	1.25	
2149	Ampligen	210	12-4-87	single, beg 4 hr post	ip	1.25-10	>10	+	1.25	
2149	Ampligen	211	12-4-87	single, beg 24 hr post	ip	1.25-10	>10	+	1.25	
2149	Ampligen	212	12-4-87	single, beg 48 hr post	ip	1.25-10	>10	+	1.25	
2149	Ampligen	213	12-4-87	single, beg 72 hr post	ip	1.25-10	>10	-	>10	
2149	Ampligen	214	12-4-87	single, beg 96 hr post	ip	1.25-10	>10	-	>10	
2149	Ampligen	215	12-3-87	bid x 5, beg 24 hr pre	ip	0.6-5	>5	+	0.6	
2149	Ampligen	242	1-7-88	single	ip	0.05, 0.5, 5	>5	-		IFN
2149	Ampligen	257	01-22-88	qd x 5, beg 4 hr pre	ip	0.31-5	>5	+	0.31	
2149	Ampligen	309	3-11-88	qd x 5, beg 72 hr post	ip	0.625-5	>5	-	>5	
2149	Ampligen	310	3-11-88	qd x 5, beg 96 hr post	ip	0.625-5	>5	-	>5	
2149	Ampligen	362	5-6-88	bid x 5, beg 4 hr pre	ip	0.625-5	>5	-	.5	BALLIET
2149	Ampligen	407	6-17-88	qd x 5, beg 4 hr pre	ip	0.6-5	>5	-	>5	IFN
2149	Ampligen	408	6-17-88	single, beg 48 hr post	ip	0.6-5	>5	-	>5	IFN
2149	Ampligen	409	6-17-88	bid x 5, beg 4 hr pre	ip	0.6-5	>5	-	>5	IFN
2149	Ampligen	575	12-22-88	single, beg 4 hr pre	ip	0.63-5	>5	-	>5	BALLIET
2149	Ampligen	576	12-22-88	single, beg 4 hr post	ip	0.63-5	>5	±	0.63	BALLIET
2149	Ampligen	653	03-23-89	qd x 5, beg 4 hr pre	ip	0.05-5	>5	+	0.05	MMF
2149	Ampligen	654	03-23-89	qd x 5, beg 4 hr pre	ip	0.05-5	>5	+	0.05	MMF
2149	Ampligen	655	03-23-89	qd x 5, beg 4 hr pre	ip	0.05-5	>5	+	0.05	MMF
2149	Ampligen	656	03-23-89	qd x 5, beg 4 hr pre	ip	0.05-5	>5	+	0.05	MMF
2149	Ampligen	668	04-12-89	single, beg 48 hr post	ip	2.5	>2.5	ON TEST	ON TEST	IMMUNOLOGY
2149	Ampligen	673	04-27-89	3 in 7 days, beg 4 hr post	ip	0.125-1	>1	+	0.125	EXPANDED
2149	Ampligen	782	10-19-89	bid x 5, beg 4 hr pre	ip	0.6,5	>5	+	0.6	IFN
2149	Ampligen	783	10-19-89	eod x 3, beg 4 hr post	ip	0.6,5	>5	+	0.6	IFN
2149	Ampligen	784	10-19-89	single, beg 48 hr post	ip	0.6,5	>5	+	0.6	IFN
2149	Ampligen	786	10-19-89	qd x 5, beg 4 hr pre	ip	0.6,5	>5	+	0.6	IFN
2149	Ampligen	849	06-21-90	single, beg 23 hr post	ip	0.005-5	>5	+	0.005	COMBINATION
2276	UNIDENTIFIED	867	09-13-90	qd x 5, beg 4 hr post	po	10-500	>500	±	32	EXPANDED
2276	UNIDENTIFIED	879	10-17-90	qd x 5, beg 24 hr pre	po	125-2000	>2000	+	250	EXPANDED
2276	UNIDENTIFIED	881	10-22-90	qd x 1	po	125 2000	ON TEST	ON TEST	ON TEST	IFN
2285	UNIDENTIFIED	866	09-13-90	qd x 5, beg 4 hr post	po	10-500	>500	±	32	EXPANDED
2285	UNIDENTIFIED	880	10-17-90	qd x 5, beg 24 hr pre	po	125-2000	>2000	+	125	EXPANDED
2285	UNIDENTIFIED	882	10-22-90	qd x 1	po	125-2000	ON TEST	ON TEST	ON TEST	IFN
2700	6-Ethyl thiopurine riboside	432	7-14-88	bid x 5, beg 4 hr pre	ip	25-400	400	+	25	
2700	6-Ethyl thiopurine riboside	450	9-2-88	bid x 5, beg 4 hr pre	ip	3.13-100	>100	±	~50	EXPANDED
2700	6-Ethyl thiopurine riboside	473	9 22-88	bid x 5, beg 4 hr pre	ip	1.56-50	>50	±	~50	EXPANDED
2700	6-Ethyl thiopurine riboside	593	01-19-89	bid x 5, beg 4 hr pre	ip	12.5-100	>100	+	12.5	EXPANDED

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AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
2700	6-Ethyl thiopurine riboside	594	01-19-89	single, beg 4 hr pre	ip	31.3-500	>500	-	>500	
2700	6-Ethyl thiopurine riboside	595	01-19-89	single, beg 24 hr post	ip	31.3-500	>500	+	62.5	
2700	6-Ethyl thiopurine riboside	600	01-26-89	single, beg 4 hr pre	ip	31.3-500	250	-	>500	
2700	6-Ethyl thiopurine riboside	601	01-26-89	single, beg 24 hr post	ip	31.3-500	250	±	31.3	
2700	6-Ethyl thiopurine riboside	602	01-26-89	bid x 5, beg 4 hr pre	ip	12.5-100	>100	+	50	EXPANDED
2700	6-Ethyl thiopurine riboside	645	03-09-89	bid x 5, beg 24 hr pre	ip	9.4-150	>150	-	>150	BALLIET
2700	6-Ethyl thiopurine riboside	657	03-30-89	single, beg 24 hr post	po	31.3-500	>500	+	31.3	EXPANDED
2712	Bryostatin 1	305	3-4-88	qd x 5, beg 4 hr pre	ip	4.5-36	>36	±	18	
2712	Bryostatin 1	379	05-20-88	bid x 5, beg 4 hr pre	ip	6.25-50	>50	±	12.5	
2712	Bryostatin 1	426	7-1-88	qd x 5, beg 4 hr pre	ip	2.25-18 µg/ml	>18	-	>18	
2712	Bryostatin 1	503	10-20-88	qd x 5, beg 4 hr pre	ip	4.5-144 µg/ml	>144	-	>144	
2712	Bryostatin 1	509	10-26-88	single, beg 24 hr pre	ip	6.25-200 µg/ml	>200	+	12.5	
2712	Bryostatin 1	510	10-27-88	single, beg 4 hr post	ip	6.25-200 µg/ml	>200	+	12.5	
2712	Bryostatin 1	556	12-08-88	bid x 5, beg 4 hr pre	ip	1.13-18 µg/ml	>18	+	2.3	
2712	Bryostatin 1	565	12-15-88	single, beg 4 hr post	ip	6.25-100 µg/ml	>100	-	>100	EXPANDED
2713	Bryostatin 2	306	3-4-88	qd x 5, beg 4 hr pre	ip	4.5-36	>36	-	>36	
2713	Bryostatin 2	380	05-20-88	bid x 5, beg 4 hr pre	ip	5-40	>40	-	>40	
2716	UNIDENTIFIED	666	04-13-89	bid x 5, beg 4 hr pre	sc	18.8-300	>300	-	>300	
2741	Ribavirin tetrahydropyrimidine	149	10-2-87	bid x 5, beg 4 hr pre	sc	31.3-500	>500	-	>500	
2741	Ribavirin tetrahydropyrimidine	297	2-26-88	bid x 5, beg 4 hr pre	sc	75-600	>600	-	>600	
2742	Ribavirin 5-OH tetrahydropyrimidine	150	10-2-87	bid x 5, beg 4 hr pre	sc	31.3-500	>500	±	500	
2742	Ribavirin 5-OH tetrahydropyrimidine	607	02-09-89	single, beg 4 hr post	sc	31.3-500	>500	±	250	
2742	Ribavirin 5-OH tetrahydropyrimidine	608	02-09-89	single, beg 24 hr post	sc	31.3-500	>500	-	>500	
2776	Bropirimine	59	3-19-87	qd x 3, beg 24 hr pre	ip	50-400	400	+	100	
2776	Bropirimine	60	3-19-87	single, beg 24 hr pre	ip	50-400	400	+	100	
2776	Bropirimine	61	3-19-87	e 3 days x 3, beg 24 hr pre	ip	50-400	400	+	100	
2776	Bropirimine	90	4-23-87	single, beg 24 hr pre	ip	100-400	400	+	100	EXPANDED
2776	Bropirimine	143	9-25-87	single, beg 4 hr pre	ip	100-400	>400	+	100	
2776	Bropirimine	144	9-25-87	single, beg 4 hr post	ip	100-400	>400	+	200	
2776	Bropirimine	145	9-25-87	single, beg 24 hr post	ip	100-400	>400	+	200	
2776	Bropirimine	146	9-25-87	single, beg 48 hr post	ip	100-400	>400	+	400	
2776	Bropirimine	147	9-25-87	single, beg 72 hr post	ip	100-400	>400	-	>400	
2776	Bropirimine	148	9-25-87	single, beg 96 hr post	ip	100-400	>400	-	400	
2776	Bropirimine	254	1-21-88	qd x 3, beg 24 hr pre	po	25-400	400	+	25	EXPANDED
2776	Bropirimine	255	1-21-88	single, beg 24 hr pre	po	25-400	>400	+	25	EXPANDED
2776	Bropirimine	256	01-21-88	single, beg 24 hr pre	sc	50-400	200	+	200	
2776	Bropirimine	291	2-19-88	single, beg 24 hr post	po	25-100	>100	+	50	COMBINATION
2776	Bropirimine	312	3-11-88	qd x 3, beg 24 hr post	po	12.5-200	>200	+	12.5	EXPANDED
2776	Bropirimine	364	5-6-88	single, beg 4 hr pre	ip	50-400	>400	+	100	BALLIET
2776	Bropirimine	413	6-24-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	414	6-24-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	415	6-24-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	416	6-24-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	448	8-5-88	single, beg 24 hr post	po	400	>400	+	400	IFN
2776	Bropirimine	474	9-22-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	475	9-22-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	476	9-22-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	477	9-22-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF

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AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
2776	Bropirimine	549	11-30-88	single, beg 48 hr post	ip	200	ON TEST	ON TEST	ON TEST	IMMUNOLOGY
2776	Bropirimine	573	12-22-88	single, beg 4 hr pre	ip	50-400	>400	+	50	BALLIET
2776	Bropirimine	574	12-22-88	single, beg4 hr post	ip	50-400	>400	-	>400	BALLIET
2776	Bropirimine	631	03-01-89	qd x 3, beg 24 hr pre	po	25-400	>400	+	25	EXPANDED
2776	Bropirimine	632	03-02-89	qd x 3, beg 4 hr post	po	25-400	>400	+	50	EXPANDED
2776	Bropirimine	633	03-01-89	qd x 3, beg 24 hr pre	ip	25-400	>400	+	25	
2776	Bropirimine	634	03-02-89	qd x 3, beg 4 hr post	ip	25-400	>400	+	25	
2776	Bropirimine	635	03-02-89	qd x 3, beg 24 hr post	ip	25-400	>400	+	50	
2776	Bropirimine	636	03-02-89	qd x 3, beg 24 hr pre	ip	62.5-1000	1000	+	62.5	BALLIET
2776	Bropirimine	637	03-08-89	single, beg 24 hr pre	po	25-800	>800	+	50	
2776	Bropirimine	638	03-09-89	single, beg 24 hr post	po	25-800	>800	+	100	
2776	Bropirimine	639	03-09-89	single, beg 48 hr post	po	25-800	>800	±	400	
2776	Bropirimine	640	03-09-89	single, beg 72 hr post	po	25-800	>800	-	>800	
2776	Bropirimine	641	03-08-89	eod x 3, beg 24 hr pre	po	25-400	>400	+	100	
2776	Bropirimine	642	03-08-89	e2d x 3, beg 24 hr pre	po	25-400	>400	+	50	
2776	Bropirimine	643	03-08-89	single, beg 24 hr pre	sc	25-400	>400	+	50	
2776	Bropirimine	644	03-08-89	bid x 3, beg 24 hr pre	ip	25-400	>400	+	200	
2776	Bropirimine	648	03-16-89	qd x 3, beg 24 hr post	po	25-100	>100	+	25	COMBINATION
2776	Bropirimine	658	03-29-89	single, beg 24 hr post	ip	200	ON TEST	ON TEST	ON TEST	IMMUNOLOGY
2776	Bropirimine	662	04-06-89	single, beg 4 hr pre	sc	25-400	>400	+	25	
2776	Bropirimine	663	04-05-89	eod x 3, beg 24 hr pre	po	50-400	>400	+	50	
2776	Bropirimine	664	04-05-89	etd x 3, beg 24 hr pre	po	50-400	>400	+	100	
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	62	3-19-87	qd x 3, beg 24 hr pre	ip	50-400	400	+	200	
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	63	3-19-87	single, beg 24 hr pre	ip	50-400	400	+	>400	
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	64	3-19-87	e 3 days x 3, beg 24 hr pre	ip	50-400	>400	+	400	
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	91	5-23-87	single, beg 24 hr pre	ip	100-400	200	±	100	EXPANDED
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	174	10-29-87	qd x 3, beg 24 hr pre	ip	37.5-300	>300	-	>300	BALLIET
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	231	12-10-87	qd x 3, beg 24 hr pre	po	50-400	200	+	50	EXPANDED
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	313	03-11-88	single, beg 4 hr pre	po	25-200	>200	±	25	
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	65	3-26-87	qd x 3, beg 24 hr pre	ip	50-400	>400	±	50	
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	66	3-26-87	single, beg 24 hr pre	ip	50-400	>400	+	50	
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	67	3-26-87	e 3 days x 3, beg 24 hr pre	ip	50-400	>400	+	50	
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	235	1-7-88	single, beg 24 hr pre	ip	50-800	>800	+	50	EXPANDED
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	274	2-4-88	single, beg 24 hr pre	po	50-400	200	+	50	EXPANDED
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	333	3-31-88	qd x 3, beg 24 hr pre	po	12.5-400	200	+	12.5	EXPANDED
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	665	04-13-89	single, beg 24 hr post	po	12.5-200	>200	±	100	EXPANDED
2779	MVE-1	500	10-19-88	single, beg 24 hr pre	ip	6.25-100	>100	+	6.25	
2779	MVE-1	501	10-20-88	single, beg 4 hr pre	ip	6.25-100	>100	+	6.25	
2779	MVE-1	502	10-20-88	single, beg 24 hr post	ip	6.25-100	>100	+	12.5	
2779	MVE-1	543	11-22-88	single, beg 24 hr post	ip	12.5-100	>100	-	>100	BALLIET
2779	MVE-1	554	12-01-88	single, beg 4 hr pre	ip	0.78-100	>100	+	3.13	EXPANDED
2779	MVE-1	581	01-05-89	single, beg 24 hr post	ip	3.13-12.5	>12.5	±	6.25	COMBINATION
2779	MVE-1	585	01-13-89	qd x 3, beg 24 hr post	ip	1.56-50	>50	+	1.56	
2779	MVE-1	586	01-13-89	bid x 3, beg 24 hr post	ip	1.56-50	>50	-	>50	
2779	MVE-1	587	01-13-89	single, beg 24 hr post	sc	6.25-100	>100	+	6.25	
2779	MVE-1	588	01-13-89	single , beg 36 hr post	ip	6.25-100	>100	-	>100	
2779	MVE-1	589	01-13-89	single , beg 48 hr post	ip	6.25-100	>100	+	12.5	
2779	MVE-1	590	01-13-89	single, beg 4 hr pre	po	6.25-200	>200	-	>200	EXPANDED

Table II-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
2779	MVE-1	604	01-26-89	eod x 5, beg 4 hr pre	ip	3.13-100	>100	+	3.13	
2779	MVE-1	652	03-23-89	single, beg 4 hr pre	po	6.25-200	>200	±	25	EXPANDED
2779	MVE-1	660	04-06-89	single, beg 4 hr pre	po	9.4-150	>150	±	9.4	EXPANDED
2786	UNIDENTIFIED	667	04-13-89	bid x 5, beg 4 hr pre	sc	18.8-300	>300	-	>300	
2811	7-Deoxynarciclasine	236	01-08-88	qd x 5, beg 4 hr pre	ip	3.13-25	>25	-	>25	
2811	7-Deoxynarciclasine	369	05-13-88	bid x 5, beg 4 hr pre	ip	1-8	>8	±	4	
2812	Narciclasine	237	01-08-88	qd x 5, beg 4 hr pre	ip	0.75-6	>6	+	6	
2812	Narciclasine	292	2-26-88	qd x 5, beg 4 hr pre	ip	0.75-12	>12	+	0.75	EXPANDED
2812	Narciclasine	807	01-25-90	bid x 5, beg 4 hr pre	sc	0.75-25	6.25	±	3.13	EXPANDED
2812	Narciclasine	808	01-25-90	qd x 5, beg 4 hr pre	sc	0.75-25	6.25	+	0.75	EXPANDED
2812	Narciclasine	809	02-01-90	bid x 5, beg 4 hr pre	ip	0.195-3.13	3.13	-	>3.13	EXPANDED
2812	Narciclasine	810	02-01-90	qd x 5, beg 4 hr pre	ip	0.195-3.13	>3.13	+	0.78	EXPANDED
2880	Oxamisole	82	4-16-87	qd x 3, beg 24 hr pre	ip	1.6-25	>25	±	1.6	
2880	Oxamisole	83	4-16-87	qd x 3, beg 24 hr post	ip	1.6-25	>25	-	>25	
2880	Oxamisole	84	4-17-87	single, beg 24 hr post	ip	1.6-50	>50	±	25	
2880	Oxamisole	105	8-6-87	bid x 3, beg 24 hr pre	po	1.6-25	>25	±	1.56	EXPANDED
2880	Oxamisole	183	11-5-87	qd x 3, beg 24 hr pre	ip	1.55-25	>25	±	1.55	BALLIET
2880	Oxamisole	184	11-5-87	single, beg 24 hr pre	ip	3.13-50	50	±	25	BALLIET
2880	Oxamisole	206	11-19-87	bid x 3, beg 24 hr pre	ip	0.78-25	>25	±	1.56	
2880	Oxamisole	258	01-21-88	qd x 2, beg 24 hr pre	ip	0.78-50	50	±	0.78	
2880	Oxamisole	268	2-5-88	qd x 2, beg 4 hr pre	ip	0.78-50	>50	-	>50	
2880	Oxamisole	269	2-5-88	qd x 2, beg 4 hr post	ip	0.78-50	>50	±	25	
2880	Oxamisole	270	2-5-88	qd x 2, beg 24 hr post	ip	0.78-50	>50	-	>50	
2880	Oxamisole	271	2-5-88	qd x 2, beg 48 hr post	ip	0.78-50	>50	±	1.56	
2880	Oxamisole	272	2-4-88	e 3 day x 3, beg 24 hr pre	ip	0.78-50	>50	-	>50	
2880	Oxamisole	273	2-4-88	single	ip	3.13-50	>50	-	>50	IFN
2880	Oxamisole	334	4-1-88	qd x 3, beg 4 hr post	po	0.78-50	>50	±	0.76	
2880	Oxamisole	335	4-6-88	qd x 3, beg 24 hr pre	ip	1.5-25	>25	-	>25	
2885	3-T-butyl-1-adamantylthiourea	835	04-19-90	bid x 5, beg 4 hr pre	sc	25-400	>400	±	100	INITIAL
2885	3-T-butyl-1-adamantylthiourea	841	06-07-90	bid x 5, beg 4 hr pre	sc	25-100	>100	±	25	EXPANDED
2885	3-T-butyl-1-adamantylthiourea	850	06-28-90	bid x 5, beg 4 hr pre	ip	75-600	>600	-	>600	
2933	CGP 19835 A Lipid	350	4-29-88	single, beg 48 hr pre	ip	10,100,1000µ	>1000 µg	±	1000	
2933	CGP 19835 A Lipid	351	4-29-88	single, beg 24 hr pre	ip	10,100,1000µ	>1000 µg	±	10	
2933	CGP 19835 A Lipid	352	4-29-88	single, beg 4 hr pre	ip	10,100,1000µ	>1000 µg	-	>1000	
2933	CGP 19835 A Lipid	353	4-29-88	single, beg 24 hr post	ip	10,100,1000µ	>1000 µg	+	100	
2933	CGP 19835 A Lipid	354	4-29-88	single, beg 48 hr post	ip	10,100,1000µ	>1000 µg	-	>1000	
2933	CGP 19835 A Lipid	355	4-29-88	single, beg 72 hr post	ip	10,100,1000µ	>1000 µg	±	1000	
2933	CGP 19835 A Lipid	402	6-9-88	eod x 3, beg 24 hr pre	ip	1,10,100,1000µ	>1000 µg	-	>1000	
2933	CGP 19835 A Lipid	410	6-17-88	single, beg 24 hr post	ip	313-10000µ	>10,000	+	313	EXPANDED
2933	CGP 19835 A Lipid	455	9-9-88	single, beg 24 hr post	sc	600-4800µ	>4800 µg	-	>4800	BALLIET
2933	CGP 19835 A Lipid	609	02-08-89	qd x 3, beg 24 hr pre	ip	1-1000µ	>1000 µg	+	100	
2933	CGP 19835 A Lipid	610	02-09-89	single, beg 24 hr post	po	1,10,100,1000µ	>1000 µg	-	>1000	EXPANDED
2933	CGP 19835 A Lipid	859	08-09-90	single, beg 4 hr post	ip	1250-10000 µ	>10000µg	-	>10000	BALLIET
2933	CGP 19835 A Lipid	860	08-09-90	single, beg 24 hr post	ip	1250-10000 µ	>10000µg	-	>10000	BALLIET
2978	Tetraacetate ester of 2980	298	2-26-88	bid x 5, beg 4 hr pre	sc	25-200	>200	±	50	
2978	Tetraacetate ester of 2980	332	4-1-88	bid x 5, 4 hr pre	ip	25-400	>400	-	>400	
2980	Tetrahydroxy analog of Pancratistatin	266	1-29-88	bid x 5, beg 4 hr pre	sc	31.3-500	31.3	-	>500	
2980	Tetrahydroxy analog of Pancratistatin	396	6-10-88	single, beg 4 hr pre	ip	6.25-50	>50	-	>50	

Table II-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
2980	Tetrahydroxy analog of Pancratistatin	397	6-10-88	single, beg 4 hr post	ip	6.25-50	>50	-	>50	
2980	Tetrahydroxy analog of Pancratistatin	398	6-10-88	single, beg 24 hr post	ip	6.25-50	>50	-	>50	
3425	8-Bromoguanosine	451	9-2-88	bid x 5, beg 4 hr pre	ip	15.6-500	500	-	>500	
3425	8-Bromoguanosine	491	10-12-88	single, beg 24 hr pre	sc	15.6-250	~250	-	>250	
3425	8-Bromoguanosine	492	10-12-88	single, beg 4 hr post	sc	15.6-250	~250	-	>250	
3425	8-Bromoguanosine	493	10-12-88	single, beg 24 hr post	sc	15.6-250	~250	-	>250	
3425	8-Bromoguanosine	505	10-27-88	qd x 5, beg 4 hr pre	sc	25-200	>200	±	50	
3425	8-Bromoguanosine	506	10-26-88	single, beg 24 hr pre	sc	50-400	400	-	>400	
3425	8-Bromoguanosine	507	10-27-88	single, beg 4 hr post	sc	50-400	400	-	>400	
3425	8-Bromoguanosine	508	10-27-88	single, beg 24 hr post	sc	50-400	400	-	>400	
3425	8-Bromoguanosine	525	11-02-88	bid x 5, beg 24 hr pre	po	15.6-250	>250	-	>250	
3425	8-Bromoguanosine	526	11-09-88	single, beg 4 hr pre	sc	100-800	800	+	100	
3425	8-Bromoguanosine	527	11-10-88	single, beg 4 hr post	sc	100-800	800	+	100	
3425	8-Bromoguanosine	528	11-10-88	single, beg 24 hr post	sc	100-800	800	±	800	
3425	8-Bromoguanosine	564	12-08-88	qd x 5, beg 4 hr pre	sc	15.7-250	>250	-	>250	
3580	UNIDENTIFIED	404	6-17-88	bid x 5, beg 4 hr pre	ip	6.25-100	>100	±	25	
3580	UNIDENTIFIED	532	11-09-88	single, beg 24 hr pre	sc	37.5-300	>300	±	37.5	
3580	UNIDENTIFIED	533	11-10-88	single, beg 4 hr post	sc	37.5-300	>300	-	>300	
3585	Neurotropin	126	9-3-87	twice 3 days sep., beg 24 pre	ip	3-24	>24	-	>400	
3585	Neurotropin	127	9-3-87	single, beg 24 hr pre	ip	3-24	>24	-	>400	
3585	Neurotropin	140	9-24-87	qd x 3, beg 24 hr pre	ip	3-24	>24	-	>24	
3585	Neurotropin	141	9-24-87	eod x 3, beg 24 hr pre	ip	3-24	>24	-	>24	
3585	Neurotropin	278	2-11-88	single, beg 24 hr pre	po	3-24		?		
3585	Neurotropin	316	03-17-88	single, beg 24 hr pre	po	3-24	>24	-	>24	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	120	9-3-87	qd x 3, beg 24 hr pre	ip	50-400	400	-	>400	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	121	9-3-87	single, beg 24 hr pre	ip	50-400	400	+	100	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	399	6-10-88	single, beg 4 hr pre	ip	50-400	>400	-	>400	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	400	6-10-88	single, beg 4 hr post	ip	50-400	>400	+	100	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	401	6-10-88	single, beg 24 hr post	ip	50-400	>400	+	50	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	435	7-14-88	single, beg 4 hr post	ip	31.3-500	>500	±	31.3	EXPANDED
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	457	9-8-88	single, beg 4 hr post	po	31.3-500	500	±	125	EXPANDED
3588	Meta Fluoro ABPP	122	9-3-87	qd x 3, beg 24 hr pre	ip	50-400	200	+	100	
3588	Meta Fluoro ABPP	123	9-3-87	single, beg 24 hr pre	ip	50-400	100	+	100	
3588	Meta Fluoro ABPP	175	10-29-87	qd x 3, beg 24 hr pre	ip	50-400	>400	±	50	BALLIET
3588	Meta Fluoro ABPP	281	2-12-88	single, beg 4 hr pre	ip	50-400	400	?		
3588	Meta Fluoro ABPP	282	2-12-88	single, beg 4 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	283	2-12-88	single, beg 24 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	284	2-12-88	single, beg 48 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	285	2-12-88	single, beg 72 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	286	2-12-88	single, beg 96 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	318	3-18-88	single, beg 4 hr pre	ip	50-400	400	±	<50	
3588	Meta Fluoro ABPP	319	3-18-88	single, beg 4 hr post	ip	50-400	400	+	100	
3588	Meta Fluoro ABPP	320	3-18-88	single, beg 24 hr post	ip	50-400	400	+	50	
3588	Meta Fluoro ABPP	321	3-18-88	single, beg 48 hr post	ip	50-400	400	-	<50	
3588	Meta Fluoro ABPP	322	3-18-88	single, beg 72 hr post	ip	50-400	400	-	<50	
3588	Meta Fluoro ABPP	323	3-18-88	single, beg 96 hr post	ip	50-400	400	-	<50	
3588	Meta Fluoro ABPP	344	4-22-88	single, beg 4 hr pre	ip	37.5-300	300	±	75	
3588	Meta Fluoro ABPP	345	4-22-88	single, beg 4 hr post	ip	37.5-300	300	±	75	

Table II-1. Punta Toro In vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
3588	Meta Fluoro ABPP	346	4-22-88	single, beg 24 hr post	ip	37.5-300	300	+	37.5	
3588	Meta Fluoro ABPP	347	4-22-88	single, beg 48 hr post	ip	37.5-300	300	-	>300	
3588	Meta Fluoro ABPP	348	4-22-88	single, beg 72 hr post	ip	37.5-300	300	-	>300	
3589	5-Chloro-2,3-difluorophenyl ABPP	124	9-3-87	qd x 3, beg 24 hr pre	ip	50-400	>400	+	200	
3589	5-Chloro-2,3-difluorophenyl ABPP	125	9-3-87	single, beg 24 hr pre	ip	50-400	400	-	>400	
3589	5-Chloro-2,3-difluorophenyl ABPP	176	10-29-87	qd x 3, beg 24 hr pre	ip	50-400	>400	±	400	BALLIET
3589	5-Chloro-2,3-difluorophenyl ABPP	458	9-7-88	qd x 3, beg 24 hr pre	po	31.3-500	>500	±	250	EXPANDED
3593	Ly 253,963	389	6-2-88	tid x 6, beg 24 hr pre	ip	1.2-150	>150	-	>150	
3593	Ly 253,963	390	6-2-88	bid x 6, beg 24 hr pre	ip	1.2-150	>150	-	>150	
3593	Ly 253,963	459	9-8-88	single, 24 hr pre	ip	31.3-500	>500	±	31.3	
3593	Ly 253,963	460	9-8-88	single, 4 hr post	ip	31.3-500	>500	±	31.3	
3593	Ly 253,963	461	9-9-88	single, 24 hr post	ip	31.3-500	>500	±	31.3	
3593	Ly 253,963	499	10-19-88	ad lib x 7, beg 4 hr pre drink	po	0.96-93	>93	±	0.96	EXPANDED
3679	UNIDENTIFIED	836	05-10-90	bid x 5, beg 4 hr pre	sc	25-400	>400	-	>400	INITIAL
3706	Tiazofurin triacetate	301	3-4-88	bid x 5, beg 4 hr pre	sc	56.3-450	>450	+	225	
3706	Tiazofurin triacetate	405	6-17-88	bid x 5, beg 4 hr pre	sc	75-600	>600	+	75	EXPANDED
3706	Tiazofurin triacetate	456	9-8-88	bid x 5, beg 24 hr pre	ip	100-800	~800	-	>800	BALLIET
3706	Tiazofurin triacetate	529	11-10-88	bid x 5, beg 4 hr pre	po	43.8-700	>700	+	175	EXPANDED
3925	du Pont A2222-1	189	11-12-87	single, beg 24 hr pre	ip	25-200	50	-	100	
3925	du Pont A2222-1	219	12-11-87	single, beg 4 hr pre	ip	25-200	50	-	>200	
3925	du Pont A2222-1	220	12-11-87	single, beg 4 hr post	ip	25-200	50	±	25	
3925	du Pont A2222-1	221	12-11-87	single, beg 24 hr post	ip	25-200	50	-	>200	
3925	du Pont A2222-1	222	12-11-87	single, beg 48 hr post	ip	25-200	50	-	>200	
3925	du Pont A2222-1	275	2-10-88	qd x 5, beg 36 hr pre	ip	3.13-25	25	?		
3925	du Pont A2222-1	300	3-4-88	single, beg 4 hr pre	ip	3.13-25	>25	-	>25	
3925	du Pont A2222-1	406	6-15-88	qd x 5, beg 36 hr pre	ip	3.13-25	>25	±	6.25	
3925	du Pont A2222-1	441	7-20-88	3 times, beg 24 hr pre	ip	2.5-40	>40	-	>40	
3925	du Pont A2222-1	442	7-20-88	bid x 5, beg 24 hr pre	ip	2.5-40	>40	-	>40	
3925	du Pont A2222-1	530	11-09-88	single, beg 4 hr pre	ip	6.25-200	>200	+	6.25	EXPANDED
3925	du Pont A2222-1	531	11-10-89	single, beg 4 hr pre	ip	6.25-200	>200	-	>200	EXPANDED
3926	du Pont A2227-1	190	11-12-87	single, beg 24 hr pre	ip	25-200	25	-	25	
3926	du Pont A2227-1	223	12-11-87	single, beg 4 hr pre	ip	25-200	25	-	>200	
3926	du Pont A2227-1	224	12-11-87	single, beg 4 hr post	ip	25-200	25	-	>200	
3926	du Pont A2227-1	225	12-11-87	single, beg 24 hr post	ip	25-200	25	-	>200	
3926	du Pont A2227-1	226	12-11-87	single, beg 48 hr post	ip	25-200	25	-	>200	
3926	du Pont A2227-1	276	2-10-88	qd x 5, beg 36 hr pre	ip	3.13-25	>25	?		
3926	du Pont A2227-1	421	6-30-88	single, beg 24 hr pre	ip	12.5-100	>100	±	25	
3926	du Pont A2227-1	422	6-30-88	single, beg 4 hr pre	ip	12.5-100	>100	+	25	
3926	du Pont A2227-1	443	7-20-88	bid x 5, beg 24 hr pre	ip	2.5-40	>40	-	>40	
3926	du Pont A2227-1	619	02-16-89	single, beg 4 hr pre	ip	3.2-50	>50	-	>50	EXPANDED
3927	du Pont A754-1	191	11-12-87	single, beg 24 hr pre	ip	25-200	100	-	100	
3927	du Pont A754-1	227	12-11-87	single, beg 4 hr pre	ip	25-200	200	-	>200	
3927	du Pont A754-1	228	12-11-87	single, beg 4 hr post	ip	25-200	200	-	>200	
3927	du Pont A754-1	229	12-11-87	single, beg 24 hr post	ip	25-200	200	-	>200	
3927	du Pont A754-1	230	12-11-87	single, beg 48 hr post	ip	25-200	200	-	>200	
3927	du Pont A754-1	277	2-10-88	qd x 5, beg 36 hr pre	ip	3.13-25	>25	?		
3927	du Pont A754-1	315	3-16-88	qd x 5, beg 36 hr pre	ip	3.13-25	>25	?		
3927	du Pont A754-1	341	4-22-88	qd x 5, beg 24 hr pre	ip	3.13-25	>25	-	>25	

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AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
3927	du Pont A754-1	411	6-24-88	bid x 5, beg 24 hr pre	ip	3.13-25	>25	-	>25	
3927	du Pont A754-1	423	6-30-88	single, beg 24 hr pre	ip	25-200	>200	-	>200	
3927	du Pont A754-1	424	6-30-88	single, beg 4 hr pre	ip	25-200	>200	±	200	
3927	du Pont A754-1	444	7-20-88	bid x 5, beg 24 hr pre	ip	2.5-40	>40	-	>40	
3933	Ge 089	303	3-3-88	qd x 5, beg 24 hr pre	ip	31.3-250	>250	-	>250	
3934	Ge 132, Germanium	192	11-12-87	qd x 7, beg 24 hr pre	po	9.4-300	>300	±	9.4	
3934	Ge 132, Germanium	218	12-10-87	qd x 7, beg 24 hr pre	ip	18.8-300	300	±	300	
3934	Ge 132, Germanium	367	5-6-88	bid x 7, beg 24 hr pre	ip	37.5-300	>300	+	37.5	
3934	Ge 132, Germanium	368	5-6-88	bid x 7, beg 4 hr pre	ip	37.5-300	>300	+	37.5	
3934	Ge 132, Germanium	387	6-3-88	bid x 5, beg 4 hr pre	ip	4.7-300	>300	±	4.7	EXPANDED
3934	Ge 132, Germanium	388	6-3-88	bid x 7, beg 4 hr pre	po	4.7-300	>300	±	18.8	EXPANDED
3934	Ge 132, Germanium	485	10-05-88	bid x 7, beg 24 hr pre	ip	18.8-600	>600	-	>600	EXPANDED
3934	Ge 132, Germanium	486	10-5-88	bid x 7, beg 24 hr pre	po	18.8-600	>600	-	>600	EXPANDED
3934	Ge 132, Germanium	487	10-5-88	bid x 7, beg 48 hr pre	po	18.8-600	>600	±	75	EXPANDED
3934	Ge 132, Germanium	515	10-26-88	single, beg 24 hr pre	ip	18.8-300	>300	-	>300	
3934	Ge 132, Germanium	516	10-27-88	single, beg 4 hr post	ip	18.8-300	>300	-	>300	
3934	Ge 132, Germanium	517	10-27-88	single, beg 24 hr post	ip	18.8-300	>300	-	>300	
3934	Ge 132, Germanium	542	11-22-88	single, beg 4 hr post	ip	100-800	>800	±	100	BALLIET
3934	Ge 132, Germanium	555	12-06-88	tid x 7, beg 48 hr pre	po	4.7-600	>600	±	37.5	EXPANDED
3934	Ge 132, Germanium	611	02-08-89	tid x 5, beg 24 hr pre	ip	37.5-600	>600	+	37.5	
3960	DMG	196	11-19-87	bid x 7, beg 36 hr pre	po	6.3-800	>800	-	>100	
3960	DMG	197	11-19-87	bid x 7, beg 36 hr pre	sc	6.3-800	>800	-	>100	
3960	DMG	279	2-11-88	bid x 7, beg 24 hr pre	ip	9.4-600	>600	?		
3960	DMG	349	4-22-88	bid x 5, beg 24 hr pre	sc	112.5-900	>900	-	>900	
4113	Pseudolycorine HCl	433	7-14-88	qd x 5, beg 4 hr pre	sc	0.75-12	>12	±	0.75	
4206	3-Acetamido-7-amino-6-methyl-7H-S-triazolo[5,1-C]-S-triazole	833	04-19-90	bid x 5, beg 4 hr pre	sc	25-400	>400	±	100	INITIAL
4206	3-Acetamido-7-amino-6-methyl-7H-S-triazolo[5,1-C]-S-triazole	842	06-07-90	bid x 5, beg 4 hr pre	sc	25-100	>100	±	50	EXPANDED
4206	3-Acetamido-7-amino-6-methyl-7H-S-triazolo[5,1-C]-S-triazole	851	06-28-90	bid x 5, beg 4 hr pre	ip	75-600	>600	-	>600	
4272	Unidentified	862	09-06-90	bid x 5, beg 4 hr pre	sc	3.13 - 50	>50	-	>50	INITIAL
4272	Unidentified	863	09-06-90	bid x 5, beg 4 hr pre	ip	3.13 - 50	25	+	3.13	INITIAL
4272	Unidentified	868	09-20-90	single, beg 4 hr post	ip	12.5 - 200	25	±	12.5	
4272	Unidentified	870	09-20-90	single, beg 24 hr post	ip	12.5 - 200	25	-	>200	
4272	Unidentified	873	10-05-90	bid x 5, beg 4 hr pre	sc	03-13-50	50	+	3.13	
4272	Unidentified	874	10-05-90	bid x 5, beg 4 hr pre	ip	3.13 - 50	25	-	12.5	EXPANDED
4272	Unidentified	875	10-05-90	bid x 5, beg 4 hr pre	po	3.13 - 50	>50	-	>50	
4272	Unidentified	888	11-01-90	single, 4 hr post	ip	1.56-12.5	>12.5	±	3.13	
4272	Unidentified	889	11-01-90	bid x 5, beg 4 hr pre	ip	0.8-6.25	>6.25	±	0.8	
4272	Unidentified	890	11-08-90	bid x 5, beg 4 hr pre	sc	06-25-50	>50	-	25	
4273	Unidentified	864	09-06-90	bid x 5, beg 4 hr pre	sc	6.25 - 100	>100	-	>100	INITIAL
4273	Unidentified	865	09-06-90	bid x 5, beg 4 hr pre	ip	6.25 - 100	>100	+	6.25	INITIAL
4273	Unidentified	869	09-20-90	single, beg 4 hr post	ip	12.5 - 200	>200	-	>200	
4273	Unidentified	871	09-20-90	single, beg 24 hr post	ip	12.5 - 200	>200	-	>200	
4273	Unidentified	876	10-11-90	bid x 5, beg 4 hr pre	sc	6.25 - 100	100	-	>100	EXPANDED
4273	Unidentified	877	10-11-90	bid x 5, beg 4 hr pre	ip	6.25 - 100	100	-	>100	EXPANDED
4273	Unidentified	878	10-11-90	bid x 5, beg 4 hr pre	po	6.25 - 100	>100	-	>100	
4282	AM-5	463	9-14-88	single, beg 24 hr pre	ip	12.5-200	12.5	-	<12.5	
4282	AM-5	464	9-14-88	single, beg 4 hr post	ip	3.125-50	3.125	-	3.125	
4282	AM-5	465	9-14-88	single, beg 24 hr post	ip	3.125-50	3.125	-	3.125	

Table II-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
4282	AM-5	494	10-12-88	single, beg 24 hr pre	ip	0.025-0.8	all lost wt	-	0.05	
4282	AM-5	495	10-12-88	single, beg 4 hr post	ip	0.025-0.8	all lost wt	±	0.025	
4282	AM-5	496	10-12-88	single, beg 24 hr post	ip	0.025-0.8	all lost wt	+	0.025	
4282	AM-5	552	12-01-88	single, beg 24 hr post	ip	0.025-0.8	0.4	+	0.025	EXPANDED
4282	AM-5	553	12-01-88	single, beg 48 hr post	ip	0.025-0.8	0.4	±	0.2	EXPANDED
4282	AM-5	571	12-14-88	eod x 3, beg 24 hr pre	ip	0.19-3	0.75	+	0.19	
4282	AM-5	572	12-14-88	qd x 3, beg 24 hr pre	ip	0.09-1.5	0.33	-	>1.5	
4282	AM-5	605	02-02-89	single, beg 4 hr pre	po	0.05-0.8	>0.8	+	0.05	EXPANDED
4282	AM-5	606	02-02-89	single, beg 24 hr post	po	0.05-0.8	>0.8	+	0.05	EXPANDED
4282	AM-5	616	02-17-89	single, beg 4 hr pre	ip	0.025-0.2	>0.2	-	>0.2	BALLIET
4282	AM-5	617	02-17-89	single, beg 4 hr post	ip	0.025-0.2	>0.2	-	>0.2	BALLIET
4282	AM-5	618	02-17-89	single, beg 24 hr post	ip	0.025-0.2	>0.2	-	>0.2	BALLIET
4282	AM-5	630	02-23-89	eod x 3, beg 24 hr pre	ip	0.025-0.8	0.8	+	0.025	EXPANDED
4282	AM-5	659	04-06-89	single, beg 24 hr post	ip	0.0031-0.05	>0.05	±	0.0031	
4283	AM-6	466	9-14-88	single, beg 24 hr pre	ip	12.5-200	all lost wt	+	12.5	
4283	AM-6	467	9-14-88	single, beg 4 hr post	ip	12.5-200	all lost wt	±	25	
4283	AM-6	468	9-14-88	single, beg 24 hr post	ip	12.5-200	all lost wt	+	12.5	
4284	AM-7	469	9-14-88	single, beg 24 hr pre	ip	11.25-80	>180	+	11.25	
4284	AM-7	470	9-14-88	single, beg 4 hr post	ip	11.25-80	>180	-	>180	
4284	AM-7	471	9-14-88	single, beg 24 hr post	ip	11.25-80	>180	±	22.5	
4285	AM-8	472	9-14-88	single, beg 24 hr pre	ip	6.25-100	>100	-	>100	
4285	AM-8	620	02-16-89	single, beg 24 hr post	ip	6.3-50	>50	Terminate	Terminate	TERMINATED
4285	AM-8	628	02-24-89	single, beg 24 hr post	ip	6.3-50	>50	+	25	
4286	P-136	488	10-5-88	single, beg 24 hr pre	ip	12.5-200	>200	+	12.5	
4286	P-136	489	10-5-88	single, beg 4 hr post	ip	12.5-200	>200	+	25	
4286	P-136	490	10-5-88	single, beg 24 hr post	ip	12.5-200	>200	+	12.5	
4287	P-117	478	9-21-88	single, beg 24 hr pre	ip	12.5-200	all lost wt	+	12.5	
4287	P-117	479	9-21-88	single, beg 4 hr post	ip	12.5-200	all lost wt	+	25	
4287	P-117	480	9-21-88	single, beg 24 hr post	ip	12.5-200	all lost wt	+	12.5	
4287	P-117	504	10-27-88	single, beg 24 hr post	ip	0.78-50	>50	+	0.78	EXPANDED
4588	1-aminoadenosinium mesitylenesulfonate	834	04-19-90	bid x 5, beg 4 hr pre	sc	25-400	>400	±	100	INITIAL
4588	1-aminoadenosinium mesitylenesulfonate	843	06-07-90	bid x 5, beg 4 hr pre	sc	25-100	>100	-	>100	EXPANDED
4588	1-aminoadenosinium mesitylenesulfonate	852	06-28-90	bid x 5, beg 4 hr pre	ip	75-600	>600	-	>600	
4593	P-188	482	9-29-88	single, beg 24 hr pre	ip	12.5-200	>200	+	12.5	INITIAL
4593	P-188	483	9-29-88	single, beg 4 hr post	ip	12.5-200	>200	±	12.5	INITIAL
4593	P-188	484	9-29-88	single, beg 24 hr post	ip	12.5-200	>200	±	12.5	INITIAL
4616	Noxymethyl pennicillinic acid	412	6-24-88	bid x 5, beg 4 hr pre	sc	18.8-150	>150	-	>150	INITIAL
4616	Noxymethyl pennicillinic acid	621	02-16-89	qd x 5, beg 4 hr pre	sc	25-200	>200	-	>200	
4616	Noxymethyl pennicillinic acid	622	02-16-89	single, beg 4 hr pre	sc	62.5-500	>500	Terminate	Terminate	TERMINATED
4616	Noxymethyl pennicillinic acid	623	02-16-89	single, beg 24 hr post	sc	62.5-500	>500	Terminate	Terminate	TERMINATED
4616	Noxymethyl pennicillinic acid	629	02-24-89	single, beg 24 hr post	sc	62.5-500	>500	-	>500	
4617	206-glycine	718	07-20-89	bid x 5, beg 4 hr pre	sc	50-800	>800	+	200	
4618	UNIDENTIFIED	837	05-10-90	bid x 5, beg 4 hr pre	sc	25-400	>400	±	50	INITIAL
4618	UNIDENTIFIED	853	06-28-90	bid x 5, beg 4 hr pre	sc	18439	>50	-	>50	EXPANDED
4726	CPG 19835 A Lipid - Placebo	462	9-8-88	single, beg 24 hr post	ip	undilute	no	-	>undilute	EXPANDED
5027	Imexon	699	07-07-89	qd x 5, beg 4 hr pre	ip	18.8-150	>150	-	>150	
5027	Imexon	700	07-07-89	qd x 5, beg 24 hr post	ip	18.8-150	>150	-	>150	
5054	UNIDENTIFIED	612	02-15-89	single, beg 4 hr post	iv	4.3-34	34	-	>34	BALLIET

Table II-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
5055	UNIDENTIFIED	613	02-15-89	single, beg 4 hr post	iv	21.9-175	>175	-	>175	BALLIET
5056	UNIDENTIFIED	614	02-15-89	single, beg 4 hr post	iv	1.9-15	>15	-	>15	BALLIET
5057	UNIDENTIFIED	615	02-15-89	single, beg 4 hr post	iv	3.13-25	>25	-	>25	BALLIET
5079	Human Recombinant Interleukin II	758	09-14-89	qd x 5, beg 4 hr post	ip	1,563-25,000 curr	>25,000	+	1563	EXPND. IMMUN
5079	Human Recombinant Interleukin II	812	02-08-90	qd x 5, beg 4 hr post	ip	1,563-12,500 curr	>12,500	+	1563	EXPND. IMMUN
5221	Ribavirin 2'-3'-acetonide	582	01-11-89	single, beg 4 hr pre	iv	62.5-500	>500	-	>500	BALLIET
5222	2',3',N'-triisobutyrate-5',1,4-dihydrotri. of AVS01	583	01-11-89	single, beg 4 hr pre	iv	1.95-15.6	>15.6	-	>15.6	BALLIET
5311	rIFN	789	11-09-89	single, beg 4 hr post	ip	10^3.5-10^5 upm	>10^5	+	4	EXPANDED
5311	rIFN	790	11-09-89	qd x 9, beg 4 hr post	ip	10^3.5-10^5 upm	>10^5	+	3.5	EXPANDED
5311	rIFN	826	04-05-90	qd x 5, beg 24 hr post	ip	10^3.5-10^5 upm	>10^5	+	5	EXPANDED
5311	rIFN	827	04-05-90	qd x 5, beg 36 hr post	ip	10^3.5-10^5 upm	>10^5	-	>10^5	EXPANDED
5311	rIFN	828	04-05-90	qd x 5, beg 48 hr post	ip	10^3.5-10^5 upm	>10^5	±	10^5	EXPANDED
5311	rIFN	840	05-31-90	qd x 8, beg 4 hr pre	ip	10^3.5-10^5 upm	>10^5	-	>10^5	BALLIET
5311	rIFN	858	07-26-90	qd x 5, beg 4 hr post	ip	10^3-10^5 upm	ON TEST	ON TEST	ON TEST	COMBINATION
5581	UNIDENTIFIED	779	10-09-89	qd x 5, beg 4 hr post	iv,ip	31.3-125	>125	-	>125	BALLIET
5582	UNIDENTIFIED	780	10-09-89	qd x 5, beg 4 hr post	iv,ip	125-500	>500	-	>500	BALLIET
5587	7-Thia-8-oxoguanosine	674	05-03-89	2 times, 24 hr pre	ip	6.25-100	>100	+	50	EXPANDED
5587	7-Thia-8-oxoguanosine	675	05-04-89	2 times, 4 hr pre	ip	6.25-100	>100	+	6.25	EXPANDED
5587	7-Thia-8-oxoguanosine	676	05-04-89	2 times, 24 hr post	ip	6.25-100	>100	+	6.25	EXPANDED
5587	7-Thia-8-oxoguanosine	677	05-04-89	single, beg 24 hr post	ip	6.25-100	>100	+	25	EXPANDED
5587	7-Thia-8-oxoguanosine	757	09-08-89	2 shots, beg 36 hr post	ip	12.5-100	ON TEST	ON TEST	ON TEST	EXPANDED
5587	7-Thia-8-oxoguanosine	775	10-06-89	2 shots, 24, 31 hr post	ip	6.25-25	>25	+	12.5	COMBINATION
5587	7-Thia-8-oxoguanosine	872	09-20-90	2 shots, 24, 31 hr post	ip	25-50	>50	+	25	
5588	ICLC	679	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	0.25	EXPANDED
5588	ICLC	750	08-24-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.032	EXPANDED
5589	ICL-CMA	680	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	0.25	EXPANDED
5589	ICL-CMA	735	08-04-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1		>0.1	EXPANDED
5590	ICL-CMD	681	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	0.25	EXPANDED
5590	ICL-CMD	743	08-10-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.032	EXPANDED
5591	ICL-CM-Beta-C-Dextrin	682	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5591	ICL-CM-Beta-C-Dextrin	744	08-10-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.0032	EXPANDED
5592	ICL-GEL	683	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5592	ICL-GEL	751	08-24-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.032	EXPANDED
5593	ICL-Sulfated Gel	684	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5593	ICL-Sulfated Gel	746	08-10-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.01	EXPANDED
5594	IC-(PLL-Dextran)	685	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5594	IC-(PLL-Dextran)	752	08-24-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.1	EXPANDED
5595	IC-(PLL-Dextran)	686	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5595	IC-(PLL-Dextran)	747	08-18-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	±	0.1	EXPANDED
5596	ICLC (heat cycled)	678	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	1	EXPANDED
5596	ICLC (heat cycled)	748	08-18-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	-	>0.1	EXPANDED
5786	UNIDENTIFIED	759	09-11-89	qd x 5, beg 4 hr post	iv,ip	4.0-16	>16	-	>16	BALLIET
5896	UNIDENTIFIED	760	09-11-89	qd x 5, beg 4 hr post	iv,ip	12.5-50	>50	±	50	BALLIET
5897	UNIDENTIFIED	781	10-16-89	qd x 5, beg 4 hr post	iv,ip	50-200	~100	-	>200	BALLIET
5898	UNIDENTIFIED	764	09-18-89	qd x 5, beg 4 hr post	iv,ip	12.5-50	>50	-	>50	BALLIET
6080	UNIDENTIFIED	795	12-11-89	qd x 5, beg 4 hr post	iv,ip	25-100	>100	±	50	BALLIET
6081	UNIDENTIFIED	796	12-11-89	qd x 5, beg 4 hr post	iv,ip	8 - 32	>32	-	>32	BALLIET
6082	UNIDENTIFIED	793	12-04-89	qd x 5, beg 4 hr post	iv,ip	18.8-75	>75	+	75	BALLIET

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AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
6083	UNIDENTIFIED	794	12-04-89	qd x 5, beg 4 hr post	iv,ip	8 0 32	>32	-	>32	BALLIET
6290	UNIDENTIFIED	805	01-22-90	qd x 5, beg 4 hr post	iv,ip	39 5-158	>158	-	>158	BALLIET
6291	UNIDENTIFIED	803	01-22-90	qd x 5, beg 4 hr post	iv ip	12 5 50	>50	-	>50	BALLIET
6292	UNIDENTIFIED	804	01-22-90	qd x 5, beg 4 hr post	iv ip	12 5 50	>50	-	>50	BALLIET
6297	UNIDENTIFIED	824	03-26-90	qd x 5, beg 4 hr post	iv,ip	6 25-25	>25	-	>25	BALLIET
6300	UNIDENTIFIED	825	03-26-90	qd x 5, beg 4 hr post	iv,ip	6 25-25	>25	-	>25	BALLIET
6334	UNIDENTIFIED	893	11-15-90	bid x 5, beg 4 hr pre	ip	7.8–250	>250	+	15 6	
6337	UNIDENTIFIED	894	11-15-90	bid x 5, beg 4 hr pre	ip	7 8–250	250	±	7 8	
6417	UNIDENTIFIED	895	11-15-90	bid x 5, beg 4 hr pre	ip	7.8–250	250	-	>250	
6477	UNIDENTIFIED	896	11-15-90	bid x 5, beg 4 hr pre	ip	3.2–100	>100	-	>100	
6501	UNIDENTIFIED	897	11-15-90	bid x 5, beg 4 hr pre	ip	7.8–250	>250	+	7 8	
01 + 2149	Ribavirin + Ampligen	163	10-16-87	01 bid 2149 qd x 5, 24 hr post	po, ip		>150 + 5	+	0 32 + 5	COMBINATION
01 + 2149	Ribavirin + Ampligen	164	10-16-87	01 bid 2149 qd x 5, 24 hr post	po, ip		>150 + 0 5	+	0 32 + 0 5	COMBINATION
01 + 2149	Ribavirin + Ampligen	165	10-16-87	01 bid 2149 qd x 5, 24 hr post	po, ip		>150 + 0 05	+	0 32 + 0 05	COMBINATION
01 + 2149	Ribavirin + Ampligen	194	11-13-87	01 bid 2149 qd x 5, 24 hr post	po, ip		>150+0 005	+	0 32 + 0 005	COMBINATION
206 + 2776	Ribamidine + Bropirimine	288	2-19-88	206 bid x 5 2776 single, 24 post	po	2 4-75, 100	>75 + 100	+	2 4 + 100	COMBINATION
206 + 2776	Ribamidine + Bropirimine	289	2-19-88	206 bid x 5 2776 single, 24 post	po	2 4-75, 50	>75 + 50	+	2 4 + 50	COMBINATION
206 + 2776	Ribamidine + Bropirimine	290	2-19-88	206 bid x 5 2776 single, 24 post	po	2 4-75, 25	>75 + 25	+	2 4 + 25	COMBINATION
206 + 1767	Ribamidine + AM-3	383	5-27-88	206 bid x 5 1767 single, 48 post	po, sc	2 4-75, 50	>75 + 50	+	4 7 + 50	COMBINATION
206 + 1767	Ribamidine + AM-3	384	5-27-88	206 bid x 5 1767 single, 48 post	po, sc	2 4-75, 16	>75 + 16	+	4 7 + 16	COMBINATION
206 + 1767	Ribamidine + AM-3	385	5-27-88	206 bid x 5 1767 single, 48 post	po, sc	2 4-75, 5	>75 + 5	+	37 5 + 5	COMBINATION
01 + 1754	Ribavirin + MVE-2	428	7-7-88	01 bid x 5, 1754 single, 24 post	po, ip	1-200 + 5	>200 + 5	+	1 0 + 5	COMBINATION
01 + 1754	Ribavirin + MVE-2	429	7-7-88	01 bid x 5, 1754 single, 24 post	po, ip	1-200 + 0 5	>200 + 0 5	+	1 0 + 0 5	COMBINATION
01 + 1754	Ribavirin + MVE-2	430	7-7-88	01 bid x 5, 1754 single, 24 post	po, ip	1-200 + 0 05	>200 + 0 05	+	32 + 0 05	COMBINATION
01 + 2779	Ribavirin + MVE-1	578	01-05-89	01 bid x 5, 2779 single, 24 hr post	po,ip	1-300 + 12 5	>300+12 5	+	1 + 12 5	COMBINATION
01 + 2779	Ribavirin + MVE-1	579	01-05-89	01 bid x 5, 2779 single, 24 hr post	po,ip	1-300 + 6 25	>300+6 25	+	1 + 6 25	COMBINATION
01 + 2779	Ribavirin + MVE-1	580	01-05-89	01 bid x 5, 2779 single, 24 hr post	po,ip	1-300 + 3 13	>300+3 13	+	1 + 3 13	COMBINATION
01 + 2776	Ribavirin + Bropirimine	649	03-16-89	01 bid x 3, 2776 qd x 3, 24 hr post	po	3 13-1200+100	>1200+100	+	3 13 + 100	COMBINATION
01 + 2776	Ribavirin + Bropirimine	650	03-16-89	01 bid x 3, 2776 qd x 3, 24 hr post	po	3 13-1200+50	>1200+50	+	3 13 + 50	COMBINATION
01 + 2776	Ribavirin + Bropirimine	651	03-16-89	01 bid x 3, 2776 qd x 3, 24 hr post	po	3 13-1200+25	>1200+25	+	3 13 + 25	COMBINATION
01 + 5587	Ribavirin + 7-thia-8-oxoguanosine	776	10-06-89	01 bid x 3, 5587 2 shots, 24 hr post	po,ip	6 25-1250+25	1250+25	+	6 25+25	COMBINATION
01 + 5587	Ribavirin + 7-thia-8-oxoguanosine	777	10-06-89	01 bid x 3, 5587 2 shots, 24 hr post	po,ip	6 25-1250+12 5	1250+12 5	+	6 25+12 5	COMBINATION
01 + 5587	Ribavirin + 7-thia-8-oxoguanosine	778	10-06-89	01 bid x 3, 5587 2 shots, 24 hr post	po,ip	6 25-1250+6 25	1250+6 25	+	12 5+6 25	COMBINATION
01 + 1761	Ribavirin + Poly ICLC	815	02-22-90	01 bid x 3, 1761 eod x 3, 24 hr post	po,ip	1 6-2000+0 32	2000+0 32	+	1 6+0 32	COMBINATION
01 + 1761	Ribavirin + Poly ICLC	816	02-22-90	01 bid x 3, 1761 eod x 3, 24 hr post	po,ip	1 6-2000+0 01	2000+0 01	+	1 6+0 01	COMBINATION
01 + 1761	Ribavirin + Poly ICLC	822	03-08-90	01 bid x 3, 1761 eod x 3, 24 hr post	po,ip	1 6-2000+0 0032	2000+0 0032	+	1 6+0 0032	COMBINATION
01 + 1761	Ribavirin + Poly ICLC	823	03-08-90	01 bid x 3, 1761 eod x 3, 24 hr post	po,ip	1 6-2000+0 001	2000+0 001	+	16+0 001	COMBINATION
01 + 2149	Ribavirin + Ampligen	845	06-21-90	01 bid x 3, 2149 single 23 hr post	po,ip	2 5-1500+5	1500+5	+	2 5+5	COMBINATION
01 + 2149	Ribavirin + Ampligen	846	06-21-90	01 bid x 3, 2149 single 23 hr post	po,ip	2 5-1500+0 5	1200+0 5	+	2 5+0 5	COMBINATION
01 + 2149	Ribavirin + Ampligen	847	06-21-90	01 bid x 3, 2149 single 23 hr post	po,ip	2 5-1500+0 05	1500+0 05	+	2 5+0 05	COMBINATION
01 + 2149	Ribavirin + Ampligen	848	06-21-90	01 bid x 3, 2149 single 23 hr post	po,ip	2 5-1500+0 005	>1500+0 005	+	2 5+0 005	COMBINATION
5587+antiIFN	7-Thia-8-oxoguanosine + anti-IFN	861	08-30-90	2 shots, 24 hr post, 24 5 hr post	ip	25-50 + 2000	>50+2000	+	25	COMBINATION
01 + 5311	Ribavirin + rHuIFN	856	07-26-90	01 bidx3 24 post, 5311 qdx5 4 post	po,ip	6 25-1500+10^4	ON TEST	ON TEST	ON TEST	COMBINATION
01 + 5311	Ribavirin + rHuIFN	857	07-26-90	01 bidx3 24 post, 5311 qdx5 4 post	po,ip	6 25-1500+10^3	ON TEST	ON TEST	ON TEST	COMBINATION

III. EFFECT OF AVS COMPOUNDS ON HEPATOTROPIC INFECTIONS IN MICE INDUCED BY THE ADAMES STRAIN OF PUNTA TORO VIRUS

Introduction

This report describes initial experiments run to determine if new AVS compounds submitted to us or compounds previously tested with need for further testing were active vs the hepatotropic PTV. The initial evaluation of potential anti-PTV compounds is usually performed using death only as endpoint unless directed otherwise by our COTR. Compounds found positive in this initial evaluation are then retested using expanded evaluation parameters. If the compound is negative after the initial evaluation, further tests using other treatment regimens may be run in consultation with our COTR.

Materials and Methods

Virus: The Adames strain of PTV was used. This was identified as virus pool #215588 by Dr. D. Pifat of the USAMRIID, and had been safety tested by Dr. Pifat prior to being sent to us. This was a twice-plaque isolated virus prepared in LLC-MK_2 cells. The experiments run in this fifth year of the project used a new, more lethally potent PTV obtained by using low multiplicity of infection coupled with late harvest of infected supernate as described in Section I of our Annual Report No. 2.

Animals: Three week-old C57BL/6 mice were obtained from Simonsen Laboratories (Gilroy, CA). All were quarantined 24 to 48 hr prior to use and maintained on Wayne Lab Blox mouse chow and tap water ad libitum. Female mice were used in all antiviral experiments and caged 10 to a cage; males were used for toxicity controls and held 5 to a cage.

Compounds: All compounds were submitted to us by Biological Research Faculty & Facility, Inc. Compounds were usually prepared one day prior to being used for the first time in an experiment, using the vehicle considered most appropriate. Insoluble compounds were subjected to 15-30 min. treatment in a sonifying water bath, warmed to 45°C, vortexed, and used as a suspension if a full solution was not achieved. Each was distributed to sterile injection bottles, sealed and stored at 4°C until used. During use, each was stored at room temperature unless we were advised to the contrary. 1-ß-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin, AVS01) was included in each series of experiments as a known positive control.

Experiment Design: A total of 10 s.c.-infected mice were treated with each drug dosage, and 20 infected mice were treated with placebo (drug vehicle) as virus controls. Five sham-infected mice were used in each drug dosage as toxicity controls, and 5 or 10 additional mice were used as normal controls. The toxicity and normal controls were held in a room separate from the infected area. Treatments were s.c., b.i.d. x 5 beginning 4 hr pre-virus inoculation unless another treatment schedule was recommended to us by the COTR or other individual acquainted with the material to be tested. Because of the pretreatments, the animals could not be randomized after virus infection, but the infection was given to each cage on a random, scattered basis in an attempt to randomize between cages. The animals were examined daily for death through day 21. Toxicity and normal controls were weighed on day 0 and again 18 hr after final drug treatment to ascertain weight loss or failure to gain weight. Dosages ranged in 2-fold dilutions, the number of dosages depending on the compound and what was initially known about it. A single dose of ribavirin was run in parallel as a positive control. The anti-PTV activity of this compound was described previously by us (1).

In follow-up studies to confirm initial antiviral activity seen, or when oral therapy was employed, the infection parameters were extended to include reduction in hepatic icterus (liver score assigned a reading of 0, or normal, to 4, or maximum discoloration), serum glutamic oxaloacetic and pyruvic acid transaminases (SGOT, SGPT), recoverable virus from liver and from serum of infected animals 3 or 4 days after virus inoculation. Titration of SGOT and SGPT was accomplished by using colorimetric kits from Sigma Chemical Co. (St. Louis, MO). Spectrophotometric readings for these colorimetric assays were performed in duplicate by using a microplate autoreader (EL309, Bio-Tek Instruments, Inc., Winooski, UT). Livers were homogenized to a 10% (wt/vol) suspension prepared in minimum essential medium (MEM); liver homogenates and serum samples were assayed for PTV by diluting each 10-fold to a titer of 10^{-5}; 0.2 ml of each dilution were added to triplicate cups of LLC-MK_2 cell monolayers in 96-well

microplates. Viral CPE was determined after 5 days incubation at 37°C, and 50% endpoints determined.

Statistical Analysis: Increases in survivors were analyzed using chi-square analysis with Yates' correction. Increases in mean survival times of mice that died on or before day 21 and reductions in SGOT, SGPT and PTV levels in liver or serum were evaluated using Student's *t* test. Ranked sum analysis (Wilcoxon test) was used to compare inhibition of mean liver scores.

Results and Discussion

Tables III-1 through III-14 show the individual experiments for all compounds evaluated during this report period.

AVS65 (Formycin B) (Table III-1-3): A previous experiment (PtA 551) showed that AVS065, when used thrice daily for 5 days, was effective in preventing death of PTV-infected mice. This experiment was repeated during this report period, using expanded parameters, but the compound was only moderately effective against the infection. In earlier reports, this material was also shown to have moderate efficacy using other treatment regimens. Using a twice daily s.c. treatment regimen, significant survivor increases were seen using 3 dosages of this compound; however, effects against other disease manifestations were not seen, suggesting a weak overall efficacy. The thrice daily treatment study was repeated, with higher dosages used to attain maximum tolerated levels. At the highest, 1000 mg/kg/day, dosage, slight anti-PTV activity was seen as manifested by decreased SGOT and SGPT negatives and reduced serum virus titers. We conclude this compound's activity vs PTV is unacceptable.

AVS79 (9-β-D-ribofuranosyl-6-thiocarboxamide) (Table III-4): This compound has exhibited moderate activity against PTV infections in previous studies. In the present experiment, thrice daily i.p. treatments were administered to infected mice, with significant activity seen at the two highest dosages, this efficacy displayed using all evaluation parameters.

AVS111 (Tiazofurin) (Table III-5): This compound was retested vs PTV per the request of our COTR, using twice daily s.c. treatments, and strong activity was again seen. We have previously shown 2000 mg/kg/day to be the approximate MTD for this compound, so the therapeutic index, considering 125 mg/kg/day as the minimum effective dose, is 16.

AVS147 (Enviroxime) (Tables III-6-7): Treatments with relatively high, but well-tolerated doses of this compound given twice or three times daily were ineffective vs PTV infections.

AVS272 (3-Deazaguanine) (Table III-8): We previously found (PtA 186) that twice daily s.c. treatment with this compound was essentially inactive vs PTV in mice using death as endpoint, although once daily treatments were relatively effective in preventing death in the mice. We were asked to repeat the initial twice daily s.c. experiment using expanded disease parameters to determine if antiviral effects exerted by the drug were more subtle. As seen in Table III-2, moderate inhibition of liver score, SGOT, SGPT, and liver and serum virus were seen at the high dose of the compound and also at the lowest dose. This lack of the usual dose response cannot be explained at this point.

AVS347 (Phyllanthoside) (Table III-9): This compound was moderately active vs the PTV infection in this initial evaluation, with significant efficacy seen at the highest two non-toxic dosage levels.

AVS1018 (Phenyleneamine) (Tables III-10-13): Single oral treatment with this Riker immunomodulator was effective only in slightly reducing virus titers in the PTV-infected mice. When treatments were repeated every 4 days, however, marked anti-PTV activity was seen. This is especially important since few immunomodulators are active orally. It was found that delaying the single treatment to 24 and preferably 36 hr post-PTV inoculation, greatly enhanced the antiviral activity of this compound.

AVS1761 (Poly ICLC) (Tables III-14-15): This compound was run in parallel with a series of related derivatives (reported in our last Quarterly Report) to serve as a positive control. As expected, it was highly active using all disease parameters, with essentially the same activity seen in both experiments.

AVS1968 (CL246,768) (Tables III-16-17): This immunomodulator was evaluated using oral therapy given a single time 4 hr pre-virus inoculation and given every 4 days for a total of 3

treatments beginning 24 hr post-virus inoculation. Both regimens worked well, with anti-PTV efficacy seen using all disease evaluation parameters.

AVS2276 (Theracel no. BL-002) (Tables III-18-19): This compound exhibited marginal activity vs PTV when given p.o. beginning 24 hr pre- or 4 hr post-virus inoculation.

AVS2285 (Theracel no. BL-012) (Tables III-20-21): This compound was moderately effective vs PTV if therapy began prior to virus inoculation, suggesting a possible immunomodulator effect.

AVS2812 (Narciclasine) (Tables III-22-25): In two previous experiments, this compound had exhibited moderately positive anti-PTV effects using i.p. treatments once daily for five days. In these studies, the oral inoculum was relatively low, resulting in a less than acceptable number of animals dying in virus controls. The compound has been subsequently further evaluated using s.c. and i.p. treatment given once or twice daily for 5 days. The data indicate this compound has a moderate efficacy against PTV, with s.c. treatments being more effective than i.p. treatments and once daily therapy better than the twice daily treatments.

AVS2885 (3-T-butyl-1-adamantyl thiourea) (Table III-26-28): This compound was marginally active vs the PTV infection at the mid-dose used. The experiment is being repeated using expanded parameters to confirm this initial observation. When the experiment was repeated using expanded parameters, weak activity was again seen, but at a lower (25 mg/kg/day) dose. In a second experiment, the i.p. treatment route was used, with higher dosage levels. No activity was seen, however.

AVS3679 (Unidentified) (Table III-29): No effect vs PTV was seen in this initial experiment; since all dosages used were well tolerated, there is a need to repeat the experiment using higher dosage levels.

AVS4206 (3-Acetamido-7-amino-6-methyl-7H-S-triazolo[5,1-C]-S-triazole) (Table III-30-32): Moderate efficacy vs PTV was seen using this compound at 2 dosage levels. The experiment is being repeated using expanded parameters in an attempt to confirm these data. When an expanded experiment was run, weak activity was again seen at a mid-dose. A second study used i.p. treatments on the same schedule and higher doses, but no activity was seen. All doses used were again nontoxic.

AVS4272 (unidentified) (Table III-33-42): Experiments with this compound yielded erratic results using various treatment regimens. We conclude the anti-PTV activity was marginal at best.

AVS4273 (unidentified) (Tables III-43-49): A series of experiments run with this compound indicated it to have marginal or no anti-PTV activity.

AVS4588 (1-Aminoadenosinium mesitylene-sulfonate) (Table III-50-52): Marginal activity was seen using this compound at the mid-dose utilized. When the experiment was repeated, however, no efficacy was seen. Intraperitoneal therapy with higher doses was similarly ineffective but also nontoxic.

AVS4618 (Unidentified) (Table III-53-54): Marginal activity was seen using this compound at its 2 lowest dosages. An attempt to confirm the results was not successful, however.

AVS5311 (Recombinant human interferon) (Tables III-55-59): Recombinant IFN was evaluated vs the PTV infection using a single i.p. treatment 4 hr post-virus inoculation and 9 daily i.p. treatments starting the same time. While both regimens were effective, the multiple treatments resulted in significant disease inhibition at all dosages used, whereas only the highest dose was active when given a single time. Once daily IFN treatments beginning 24 hr after virus inoculation were significantly effective at the highest dosage used. Delaying these treatments to 36 or 48 hr after virus inoculation greatly reduced the anti-PTV effects of this cytokine.

AVS6334 (unidentified) (Table III-60): A single low dose of this compound significantly prevented deaths of PTV-infected mice. The experiment will be repeated.

AVS6337 (unidentified) (Table III-61): This compound was considered essentially ineffective vs PTV in the single experiment run with it.

AVS6417 (unidentified) (Table III-62): This compound was ineffective vs PTV in the single experiment run with it.

AVS6477 (unidentified) (Table III-63): This compound was ineffective vs PTV, but so well tolerated that further experiments using higher dosages may need to be considered with it.

AVS6501 (unidentified) (Table III-64): This compound was marginally effective vs PTV, but at two low dosages making the data questionable. The experiment will be repeated.

Conclusions

A total of 64 anti-PTV experiments were run with 25 AVS compounds using the Adames strain of PTV. Promising compounds included AVS65, 79, 111, 272, 347, 1761, 1968, 2276, 2285, 2812, and 5311.

References

1. Sidwell, R. W., J. H. Huffman, B. B. Barnett, and D. Y. Pifat. 1988. *In vitro* and *in vivo phlebovirus* inhibition by ribavirin. Antimicrob. Ag. Chemother. 32:331-336.

Table III-1. Expt. PtA806. Effect of Twice Daily s.c. Treatments with AVS65 on Punta Toro Virus Infections in Mice.

Animals: 10.3-11.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected-Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS65^	500	5/5	2.4	5/10**	4.6	3.0	1/10(7780)	1/10(7797)	6.1	6.0
	250	5/5	3.1	2/10*	4.8	2.6	2/10(5805)	2/10(5929)	5.6	4.9
	125	5/5	2.8	0/10	4.5	3.0	0/9(9097)	0/9(9613)	6.3	6.5
	62.5	5/5	2.6	4/10**	4.8	2.4	3/10(7457)	3/10(8000)	5.1	5.3
Ribavirin	75	5/5	2.1	10/10**	>21.0**	0.3**	10/10**(99**)	9/10**(55**)	0.0**	1.7**
Saline	-	-	-	0/20	4.6	2.9	2/19(7378)	3/19(8178)	6.1	5.7
Normals	-	5/5	2.3	-	-	0.2	5/5(92)	5/5(37)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^Compound is insoluble, drug is in suspension.

Conclusions: AVS65 (Formycin B) was moderately active vs PTV using twice daily s.c. treatments.

*P<0.05 **P<0.01

Table III-2. Expt. PtA818. Effect of Thrice Daily i.p. Treatments with AVS65 on Punta Toro Virus Infections in Mice.

Animals: 11.3-12.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: tid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS65	1000	4/5	-1.9	0/10	4.3	4.0	2/10*(7111)	3/10*(6382)	4.7	5.4*
	500	5/5	2.2	0/10	4.7	3.3	1/10(10,009)	1/10(9332)	6.4	6.0
	250	5/5	3.5	0/10	4.2	3.2	1/10(10,148)	1/10(9233)	5.7	6.0
	125	5/5	3.2	1/10	5.0	3.5	1/10(10,795)	1/10(9972)	6.2	6.5
Ribavirin	75	5/5	3.5	10/10**	>21.0**	0.4**	10/10**(95**)	10/10**(31**)	0.5**	1.6**
Saline	-	-	-	0/20	4.9	3.4	0/10(8729)	0/20(8248)	5.6	6.5
Normals	-	5/5	5.4	-	-	0.3	4/5(177)	5/5(36)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS65 (Formycin B) was weakly effective vs PTV using a thrice daily i.p. treatment schedule.

*P<0.05 **P<0.01

Table III-3. Expt. PtA811. Effect of Thrice Daily i.p. Treatments with AVS65 on Punta Toro Virus Infections in Mice.

Animals: 8.7-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 5% EtOH in saline.

Treatment Schedule: tid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS65	500	5/5	0.8	1/10	4.9	3.2	0/10(5237)	0/10(7225)	5.3*	6.3
	250	5/5	2.3	0/10	4.8	3.4	0/10(6630)	0/10(9725)	6.4	6.5
	125	5/5	2.0	1/10	5.1	2.5*	0/9(3281)	0/9(4636)	4.4**	5.8
	62.5	5/5	2.3	0/10	5.3	3.4	0/10(4793)	0/10(6740)	5.5	6.1
Ribavirin	75	5/5	2.0	10/10**	>21.0**	0.3**	10/10**(102**)	10/10**(47**)	0.3**	2.2**
Saline	-	-	-	2/20	5.9	3.5	0/19(5262)	0/19(6832)	6.2	6.2
Normals	-	5/5	2.9	-	-	0.3	4/5(130)	5/5(48)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: AVS65 (Formycin B) was ineffective vs PTV using death endpoint, but moderate reductions in liver virus titer and liver score were seen. Since at the high (500 mg/kg/day) dose, the animals gained little weight, we presume the MTD was reached with this material.

*P<0.05 **P<0.01

Table III-4. Expt. PtA819. Effect of Thrice Daily i.p. Treatments with AVS79 on Punta Toro Virus Infections in Mice.

Animals: 11.3-12.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: tid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS79	62.5	5/5	3.1	8/10**	9.0	1.4**	1/10(3676*)	1/10(3309*)	4.0*	5.1**
	31.3	5/5	3.2	7/10**	6.7	2.8	0/10(4868)	0/10(4772)	3.4**	4.4**
	15.6	5/5	3.4	2/10*	6.0	3.0	0/10(8111)	1/10(8204)	5.0	5.9*
	7.8	5/5	4.1	0/10	5.9	3.3	0/10(8995)	0/10(9135)	6.1	6.4
Ribavirin	75	5/5	3.5	10/10**	>21.0**	0.4**	10/10**(95**)	10/10**(31**)	0.5**	1.6**
Saline	-	-	-	0/20	4.9	3.4	0/10(8729)	0/20(8248)	5.6	6.5
Normals	-	5/5	5.4	-	-	0.3	4/5(177)	5/5(36)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS79 (9-ß-D-ribofuranosyl-6-thiocarboxamide) was significantly effective vs PTV when administered i.p. thrice daily.

*P<0.05 **P<0.01

Table III-5. Expt. PtA832. Effect of Twice Daily s.c. Treatment with AVS111 on Punta Toro Virus Infections in Mice.

Animals: 11.4-12.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS111	1000	5/5	-0.5	9/10**	6.7	0.2**	9/10**(145**)	10/10**(42**)	4.1	5.8
	500	5/5	0.4	10/10**	>21.0**	0.6**	8/10**(224**)	10/10**(58**)	2.1**	4.3**
	250	5/5	1.5	10/10**	>21.0**	0.6**	3/10*(551**)	2/10*(495**)	2.1**	4.6**
	125	5/5	1.5	9/10**	9.0	1.2**	0/10(1190**)	0/10(823**)	3.4	4.3**
	62.5	5/5	2.5	1/10	5.1	2.2	0/10(3082)	0/10(2312)	4.6	4.9
Ribavirin	75	5/5	1.8	9/9**	>21.0**	0.4**	9/9**(125**)	9/9**(35**)	0.0**	1.7**
Saline	-	-	-	0/20	4.8	2.9	0/20(4546)	0/20(3168)	4.6	5.7
Normals	-	5/5	3.7	-	-	0.0	5/5(115)	5/5(36)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS111 (tiazofurin) was highly active vs PTV in this experiment.

*P<0.05 **P<0.01

Table III-6. Expt. PtA817. Effect of Thrice Daily s.c. Treatments with AVS147 on Punta Toro Virus Infections in Mice.

Animals: 11.3-12.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: tid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS147	500	5/5	2.7	0/10	3.5	3.7	0/10(6510)	0/10(5150)	6.2	6.3
	250	5/5	3.5	0/10	4.2	4.0	0/10(7500)	0/10(5550)	6.5	6.5
	125	5/5	3.6	1/10	4.3	4.0	0/10(7500)	0/10(5550)	6.5	6.5
	75	5/5	4.1	1/10	4.8	3.2	2/10*(5494)	2/10*(4148)	5.1	5.2
Ribavirin	75	5/5	3.5	10/10**	>21.0**	0.4**	10/10**(95**)	10/10** (31**)	0.5**	1.6**
CMC	-	-	-	0/20	4.4	3.6	0/20(6713)	0/20(4985)	6.3	6.3
Normals	-	5/5	5.4	-	-	0.3	4/5(177)	5/5(36)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: AVS147 (Enviroxime) was essentially inactive vs PTV using this thrice daily treatment schedule.

*P<0.05 **P<0.01

Table III-7. Expt. PtA820. Effect of Twice Daily s.c. Treatments with AVS147 on Punta Toro Virus Infections in Mice.

Animals: 11.3-12.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS147	500	5/5	2.5	0/10	4.6	3.5	0/10(9420)	0/10(7430)	6.7	6.3
	250	5/5	2.6	0/10	4.5	3.9	0/10(10,975)	0/10(8785)	7.2	6.4
	125	5/5	2.2	0/10	4.6	2.8	2/10*(8653)	2/10*(6963)	5.7	5.4
	75	5/5	2.1	0/10	4.2	3.6	0/9(10,622)	0/9(8356)	6.8	6.3
Ribavirin	75	5/5	1.9	10/10**	>21.0**	0.5**	9/10**(111**)	10/10**(32**)	0.0**	1.0**
Saline	-	-	-	0/20	4.7	3.7	0/19(10,047)	0/19(8303)	6.9	6.3
Normals	-	5/5	3.8	-	-	0.2	4/5(137)	5/5(33)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: AVS147 (Enviroxime) was essentially inactive vs PTV using this twice daily treatment schedule.

*P<0.05 **P<0.01

Table III-8. Expt. PtA802. Effect of Twice Daily s.c. Treatments with AVS272 on Punta Toro Virus Infections in Mice.

Animals: 10.1-12.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS272	500	0/5	-2.5	1/10	6.9	2.4**	0/10(4910**)	0/10(3972**)	4.7**	5.2**
	250	5/5	-0.1	1/10	6.2	3.3	0/10(8387**)	1/10(6921)	6.4*	6.2
	125	5/5	0.5	0/10	5.4	3.6	0/10(9577)	0/10(7945)	7.3	6.3
	62.5	5/5	1.1	1/10	7.7	0.4**	6/10**(173**)	8/10**(63**)	3.3**	5.2**
Ribavirin	75	5/5	3.3	10/10**	>21.0**	0.0**	8/10**(163**)	8/10**(66**)	1.2**	1.9**
CMC	-	-	-	0/20	5.1	4.0	0/20(9768)	0/20(8133)	7.3	6.5
Normals	-	5/5	4.0	-	-	0.0	5/5(103)	5/5(48)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS272 (3-deazaguanine) was moderately effective vs PTV using reduction in liver and serum virus titer, liver score, and mean SGOT and SGPT. No effect was seen using the survivors parameter. Ribavirin exhibited the positive activity expected.

*P<0.05 **P<0.01

Table III-9. Expt. PtA829. Effect of Once Daily s.c. Treatment with AVS347 on Punta Toro Virus Infections in Mice.

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS347	120	0/5	—	0/10	3.6	—	—	—	—	—
	60	5/5	0.1	4/10*	5.3	2.1**	0/10(5042**)	0/10(3784**)	5.0	4.8
	30	5/5	1.1	4/10*	6.2*	3.2	0/10(6221*)	0/10(4642)	5.0	5.7
	15	5/5	2.5	0/10	4.9	3.5	0/10(7406)	0/10(5114)	5.5	5.8
Ribavirin	75	5/5	2.3	10/10**	>21.0**	0.4**	9/10**(147**)	10/10**(30**)	0.3**	1.8**
CMC	-	-	-	1/20	4.7	3.2	1/19(9971)	1/19(6441)	6.2	6.1
Normals	-	5/5	3.5	-	-	0.0	5/5(106)	5/5(24)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS347 (phyllanthoside) was significantly effective vs PTV in this initial evaluation.

*P<0.05 **P<0.01

Table III-10. Expt. PtA791. Effect of Single p.o. Treatment with AVS1018 on Punta Toro Virus Infections in Mice.

Animals: 9.7-11.0 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: once only, 4 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1018	12.5	5/5	0.6	2/10	4.9	3.4	0/9(6531)	0/9(4978)	5.4*	6.1*
	6.25	5/5	0.2	0/8	4.3	3.8	0/10(7325)	0/10(5645)	6.0	6.5
	3.13	5/5	0.4	0/10	3.9	4.0	0/10(7550)	0/10(5750)	6.0	6.5
	1.56	5/5	0.8	0/10	4.2	4.0	0/10(7550)	0/10(5750)	6.0	6.5
Ribavirin	350	5/5	0.0	6/10**	5.3*	1.3**	0/9(2657**)	0/9(1654**)	5.0**	5.9**
Saline	-	-	-	1/20	4.2	3.9	0/20(7012)	0/20(5360)	5.9	6.5
Normals	-	5/5	0.5	-	-	0.5	5/5(99)	5/5(31)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS1018 (phenyleneamine) was active vs PTV in this experiment only in moderately reducing liver and serum virus titers.

*P<0.05 **P<0.01

Table III-11. Expt. PtA792. Effect of Every Four Day p.o. Treatment with AVS1018 on Punta Toro Virus Infections in Mice.

Animals: 9.7-11.0 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Days +1, +5, +9.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1018	12.5	5/5	5.2	6/10**	3.5	1.1**	5/10**(1085**)	5/10**(1702**)	1.6**	1.5**
	6.25	5/5	5.9	2/10*	5.4**	3.8	0/10(3503**)	0/10(4865*)	5.4*	6.4
	3.13	5/5	7.3	3/10*	5.0*	3.8	0/9(4494)	0/9(7000)	6.3	6.4
	1.56	5/5	6.8	1/10	5.2**	4.0	0/10(4575)	0/10(8000)	6.6	6.5
Ribavirin^	350	5/5	4.6	6/10**	5.3**	1.3**	0/9(2657**)	0/9(1654**)	5.0**	5.9**
Saline	-	-	-	0/20	4.2	3.7	0/20(4390)	0/20(6965)	5.9	6.5
Normals	-	5/5	7.8	-	-	0.5	5/5(99)	5/5(31)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^Administered once only, 4 hr post-virus inoculation.

Conclusions: AVS1018 (phenyleneamine) was significantly active vs PTV in this experiment using all disease parameters. The activity was dose responsive, and since the animals gained weight at the highest dosage used, we could presumably increase the dosage and achieve possibly greater anti-PTV effects. This anti-PTV effect was much greater than seen using a single p.o. treatment (PtA 791).

*P<0.05 **P<0.01

Table III-12. Expt. PtA830. Effect of Single Delayed (24 hr) p.o. Treatment with AVS1018 on Punta Toro Virus Infections in Mice.

Animals: 11.4-12.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1018	25	5/5	0.5	4/10**	6.0**	2.3**	3/10*(1709**)	3/10*(1355**)	3.6**	4.3**
	12.5	5/5	0.5	0/10	5.4**	3.4	0/10(9133)	0/10(7716)	6.4	6.4
	6.25	5/5	0.5	2/10*	5.6**	3.7	0/10(10,725)	0/10(9136)	6.6	6.2
	3.13	5/5	0.2	0/10	4.2	3.3	0/10(13,000)	0/10(11,055)	7.0	6.5
Ribavirin	350	5/5	0.9	10/10**	>21.0**	0.6**	6/10**(230**)	5/10**(120**)	3.5**	5.0**
H_2O	-	-	-	0/20	4.4	3.8	0/20(11,408)	0/20(9462)	6.6	6.4
Normals	-	5/5	0.8	-	-	0.0	3/5(164)	5/5(35)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS1018 (phenyleneamine) was moderately effective vs PTV when administered in a single p.o. treatment 24 hr post-virus inoculation (compare with Table C-9).

*P<0.05 **P<0.01

Table III-13. Expt. PtA831. Effect of Single Delayed (36 hr) p.o. Treatment with AVS1018 on Punta Toro Virus Infections in Mice.

Animals: 11.4-12.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, 36 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1018	25	5/5	0.5	7/10**	6.7**	2.1**	2/10*(2535**)	2/10*(2288**)	3.6**	4.5**
	12.5	5/5	0.5	5/10**	5.4**	3.5	0/10(8397)	0/10(6757)	5.6*	5.7*
	6.25	5/5	0.5	0/10	5.5**	2.6**	0/10(5649**)	0/10(4097**)	5.1**	5.6**
	3.13	5/5	0.2	0/10	4.4	3.6	0/10(13,665)	0/10(10,620)	6.3	6.5
Ribavirin	350	5/5	0.9	10/10**	>21.0**	0.6**	6/10**(230**)	5/10**(120**)	3.5**	5.0**
H_2O	-	-	-	0/20	4.4	3.8	0/20(11,408)	0/20(9462)	6.6	6.4
Normals	-	5/5	0.8	-	-	0.0	3/5(164)	5/5(35)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS1018 (phenyleneamine) was significantly effective vs PTV when administered in a single p.o. treatment 36 hr post-virus inoculation (compare with Table C-8).

*P<0.05 **P<0.01

Table III-14. Expt. PtA745. Effect of Every Other Day i.p. Treatment with AVS1761 on Punta Toro Virus Infections in Mice.

Animals: 11.7-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: Every other day x 3, 4 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1761	0.1	5/5	2.9	9/10**	4.0	1.0**	6/10**(617**)	8/10**(539**)	1.1**	1.4**
	0.032	4/5	2.0	9/10**	5.0	0.3**	9/10**(144**)	9/10*(57**)	1.1 *	1.6**
	0.01	4/4	2.8	5/10**	4.8	1.3**	8/10**(323**)	8/10**(189**)	1.5**	2.1**
	0.0032	5/5	3.8	2/10	4.5	3.9	0/7(4050)	0/7(4200)	6.1	6.5
Saline	-	-	-	1/20	4.5	4.0	0/20(3954)	0/20(4093)	6.1	6.5
Normals	-	5/5	3.5	-	-	0.2	3/5(180)	5/5(44)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: Every other day i.p. treatment with AVS1761 (Poly ICLC) was highly active vs the PTV infection using all disease parameters.

*P<0.05 **P<0.01

Table III-15. Expt. PtA749. Effect of Every Other Day i.p. Treatment with AVS1761 on Punta Toro Virus Infections in Mice.

Animals: 11.6-13.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: Every other day x 3, 4 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1761	0.1	5/5	2.6	9/10**	5.0	0.4**	7/10**(165**)	10/10**(43**)	1.0**	1.6**
	0.032	5/5	2.3	10/10**	>21.0**	0.4**	4/9*(285**)	6/9**(89**)	5.4**	5.5
	0.01	5/5	2.9	8/10**	8.0	0.7**	2/10(1587**)	2/10(1301**)	4.4**	4.0*
	0.0032	5/5	2.7	0/10	5.1	3.6	0/9(7828)	0/9(5472)	6.8	6.5
Saline	-	-	-	3/19	5.0	3.7	1/19(8899)	1/19(6150)	6.9	6.0
Normals	-	5/5	3.3	-	-	0.3	5/5(138)	5/5(34)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: Every other day i.p. treatment with AVS1761 (poly ICLC) was highly active vs the PTV infection using all disease parameters.

*P<0.05 **P<0.01

Table III-16. Expt. PtA797. Effect of Single p.o. Treatment with AVS1968 on Punta Toro Virus Infections in Mice.

Animals: 10.1-12.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: once only, 4 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1968	100	5/5	0.2	10/10**	>21.0**	1.3**	3/10*(1155**)	3/10*(913**)	5.5	6.2
	50	5/5	0.3	7/10**	6.7	1.9**	2/9*(1748**)	2/9*(1268**)	4.3*	5.3*
	25	5/5	0.5	5/10**	6.6	2.3	0/10(2073**)	0/10(1456**)	3.3**	4.8**
	12.5	5/5	0.4	7/10**	8.3	3.1	0/10(4612)	0/10(4132)	4.9	5.7
Ribavirin^	350	5/5	0.1	10/10**	>21.0**	0.6**	2/10*(266**)	4/10*(91**)	4.5	5.7
H_2O	-	-	-	2/20	6.2	3.2	0/19(6822)	0/19(5797)	5.5	6.1
Normals	-	5/5	0.8	-	-	0.4	4/5(178)	5/5(26)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

^ Administered 24 hr post-virus inoculation

Conclusions: A single oral treatment with AVS1968 (CL246,968) was highly active against the PTV infection in this experiment using all disease parameters.

*P<0.05 **P<0.01

Table III-17. Expt. PtA798. Effect of Three p.o. Treatments with AVS1968 on Punta Toro Virus Infections in Mice.

Animals: 10.1-12.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Days +1, +5, +9.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1968	100	5/5	1.6	5/10**	5.2	1.6**	2/10*(500**)	2/10*(342**)	2.3**	2.9**
	50	5/5	3.7	10/10**	>21.0**	1.5**	2/9*(1048**)	1/9(911**)	2.4**	2.6**
	25	5/5	4.2	3/10*	5.9	2.6	0/10(6100)	0/10(5305)	5.0	5.3
	12.5	5/5	4.4	2/10*	5.5	3.3	0/10(8880)	0/10(8367)	5.3	6.1
Ribavirin^	350	5/5	0.1	10/10**	>21.0**	0.6**	2/10*(266**)	4/10*(91**)	4.5	5.7
H_2O	-	-	-	0/20	4.6	3.5	0/20(9455)	0/20(8308)	5.3	6.1
Normals	-	5/5	4.0	-	-	0.4	4/5(178)	5/5(26)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^Single, p.o., 24 hr post-virus inoculation.

Conclusions: Oral treatment with AVS1968 (CL246,738) was highly active vs the PTV infection, with efficacy seen using all evaluation parameters at the two highest drug dosages.

*P<0.05 **P<0.01

Table III-18. Expt. PtA867. Effect of Once Daily p.o. Treatment with AVS2276 on Punta Toro Virus Infections in Mice.

Animals: 11.2-12.9 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: qd x 5, beginning 4 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2276	500	5/5	2.3	4/10	4.3	3.8	0/10(8471)	0/10(10,435)	5.1	6.3
	320	5/5	2.4	4/10	4.5	3.8	0/10(7151)	0/10(8158)	4.9	5.9
	100	5/5	2.9	1/10	5.4	3.1	0/9(3652**)	1/9(4156*)	4.1*	4.4*
	32	5/5	2.6	0/10	5.3	3.5	0/10(6144*)	0/10(7280)	4.9	5.6
	10	5/5	2.9	1/10	5.6	3.4	0/8(6735)	0/8(8144)	4.7	5.5
Ribavirin	75	5/5	2.3	10/10**	>21.0**	0.8**	9/10**(137**)	5/10**(92**)	1.3**	1.1**
H_2O	-	-	-	2/20	5.1	3.9	0/20(9430)	0/20(7445)	5.5	5.9
Normals	-	5/5	3.7	-	-	0.8	4/5(152)	5/5(61)	1.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Conclusions: AVS2276 (Theracel no. BL-002) was marginally effective when given p.o. beginning 4 hr post-virus inoculation in this experiments, with greatest efficacy seen at a dose in the mid-range of those used.

Table III-19. Expt. PtA879. Effect of Once Daily p.o. Treatment with AVS2276 on Punta Toro Virus Infections in Mice.

Animals: 10.2-11.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: qd x 5, beginning 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2276	2000	5/5	0.8	0/10	5.1	2.9	0/10(5574*)	0/10(4807*)	5.2**	5.1**
	1000	5/5	1.9	2/10*	5.9	3.8	0/10(6750)	0/10(5979)	5.3	5.8*
	500	5/5	1.1	1/10	5.4	3.9	0/10(7515)	0/10(6815)	6.0	6.4
	250	5/5	1.6	2/10*	5.4	3.6	0/10(5762)	0/10(5399*)	5.7	5.9*
	125	5/5	2.5	0/10	5.2	4.0	0/10(7780)	0/10(7350)	6.5	6.4
Ribavirin	75	5/5	1.9	10/10**	>21.0**	0.9**	4/10**(262**	6/10**(108**)	2.9**	3.5**
H_2O	-	-	-	0/20	5.5	4.0	0/20(7786)	0/20(7148)	6.5	6.5
Normals	-	5/5	2.5	-	-	0.6	3/5(288)	4/5(84)	0.7	0.4

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Conclusions: AVS2276 (Theracel no. BL-002) was marginally effective vs PTV when given p.o. beginning 24 hr pre-virus inoculation. This activity was somewhat better than that seen when the material was given later in the infection (Table C-1).

Table III-20. Expt. PtA866. Effect of Once Daily p.o. Treatment with AVS2285 on Punta Toro Virus Infections in Mice.

Animals: 11.2-12.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: qd x 5, beginning 4 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2285	500	5/5	1.5	0/10	5.1	3.3	0/9(5741*)	0/9(5872)	4.5	5.7
	320	5/5	1.5	0/10	4.8	3.7	0/10(8451)	0/10(7445)	5.6	5.9
	100	5/5	1.1	1/10	4.7	3.2	0/10(6750)	0/10(5859)	4.7	5.3
	32	5/5	2.2	1/10	4.9	2.8	0/9(3607**)	0/9(3312**)	3.9*	4.6*
	10	5/5	2.7	0/10	5.6	3.6	0/10(8187)	0/10(6825)	5.7	6.2
Ribavirin	75	5/5	2.3	10/10**	>21.0**	0.8**	9/10**(137**)	5/10**(92**)	1.3**	1.1**
H_2O	-	-	-	2/20	5.1	3.9	0/20(9430)	0/20(7445)	5.5	5.9
Normals	-	5/5	3.7	-	-	0.8	4/5(152)	5/5(61)	1.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Conclusions: AVS2285 (Theracel no. BL-012) was marginally effective vs PTV when p.o. therapy began 4 hr post-virus inoculation (compare with Table C-4).

Table III-21. Expt. PtA880. Effect of Once Daily p.o. Treatment with AVS2285 on Punta Toro Virus Infections in Mice.

Animals: 10.2-11.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: qd x 5, beginning 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2285	2000	5/5	3.1	0/10	5.5	3.8	0/10(3549**)	0/10(3435**)	5.2**	5.5**
	1000	5/5	1.1	0/10	5.3	3.6	0/10(5080**)	0/10(4729**)	5.3**	5.8**
	500	5/5	2.3	0/10	5.3	3.8	0/10(4569**)	0/10(4224**)	5.3**	5.8**
	250	5/5	2.1	0/10	5.3	3.8	0/10(5482**)	0/10(5101**)	5.4**	5.9**
	125	5/5	3.2	0/10	5.6	3.9	0/10(6110*)	0/10(5400**)	5.5**	6.1**
Ribavirin	75	5/5	1.9	10/10**	>21.0**	0.9**	4/10**(262**)	6/10**(108**)	2.9**	3.5**
H_2O	-	-	-	0/20	5.5	4.0	0/20(7786)	0/20(7148)	6.5	6.5
Normals	-	5/5	2.5	-	-	0.6	3/5(288)	4/5(84)	0.7	0.4

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Conclusions: AVS2285 (Theracel no. BL-012) was significantly effective in reducing liver and serum virus titers and in lowering SGOT and SGPT values when p.o. treatment began 24 hr pre-virus inoculation (compare with Table C-3).

Table III-22. Expt. PtA808. Effect of Once Daily s.c. Treatments with AVS2812 on Punta Toro Virus Infections in Mice.

Animals: 10.3-11.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 5% EtOH in saline.

Treatment Schedule: qd x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2812^	25	0/5	—	0/10	1.6					
	12.5	0/5	—	0/10	2.7					
	6.25	3/5	-1.2	1/10	6.1**	0.8**	2/10*(2248**)	2/10*(1728**)	6.1**	6.5
	3.13	5/5	1.2	2/10	6.4**	1.9**	3/9*(1308**)	3/9*(1258**)	3.2**	4.0**
	1.5	5/5	1.6	1/10	4.4	2.7	3/10*(9114)	3/10*(7474*)	4.4**	4.8*
	0.75	5/5	2.0	0/10	4.1	3.6	0/10(14,440)	0/10(11,615)	6.4**	6.5
Ribavirin	75	5/5	2.1	10/10**	>21.0**	0.3**	10/10**(99**)	9/10**(55**)	0.0**	1.7**
Saline	-	5/5	3.0	2/20	4.4	3.7	0/19(7897)	0/19(11,355)	7.1	6.5
Normals	-	5/5	2.3	-	-	0.2	5/5(92)	5/5(37)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^Compound is insoluble, drug is in suspension.

Conclusions: AVS2812 (narciclasine) was weakly effective vs PTV using death as endpoint, but a moderate degree of activity was seen using the other disease parameters.

*P<0.05 **P<0.01

Table III-23. Expt. PtA810. Effect of Once Daily i.p. Treatments with AVS2812 on Punta Toro Virus Infections in Mice.

Animals: 8.7-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 5% EtOH in saline.

Treatment Schedule: qd x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2812	3.13	5/5	0.8	0/10	5.2	1.9**	1/10(1203**)	1/10(1171**)	4.4	5.3
	1.56	5/5	2.0	0/10	4.8	2.1*	3/9(2558*)	3/9(2727*)	4.3	4.5
	0.78	5/5	2.5	5/10**	5.0	3.2	0/9(6082)	0/9(6139)	6.9	6.5
	0.39	5/5	3.0	1/10	4.6	3.4	2/10(5245)	2/10(5191)	5.7	5.4
	0.195	5/5	2.5	0/10	4.5	3.1	1/10(5677)	1/10(5376)	6.5	6.0
Ribavirin	75	5/5	1.1	10/10**	>21.0**	0.1**	10/10**(102**)	10/10**(45**)	1.4**	2.7**
Saline	-	-	-	2/20	4.2	3.3	4/20(6449)	3/20(5247)	6.0	5.9
Normals	-	5/5	3.4	-	-	0.1	5/5(75)	5/5(34)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: AVS2812 (narciclacine), using a once daily treatment, was effective in preventing death at a single mid-range dose only. Higher doses were also effective in reducing liver score, SGOT and SGPT levels.

*P<0.05 **P<0.01

Table III-24. Expt. PtA807. Effect of Twice Daily s.c. Treatments with AVS2812 on Punta Toro Virus Infections in Mice.

Animals: 10.3-11.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 5% EtOH in saline.

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2812^	25	0/5	—	0/10	<1.0					
	12.5	0/5	—	0/10	2.3					
	6.25	0/5	—	0/10	4.0					
	3.13	5/5	0.0	0/10	4.0	2.1*	5/10**(4259**)	5/10**(6141**)	5.5**	3.9**
	1.5	5/5	1.3	0/10	5.4	3.8	0/10(8015)	0/10(11,505)	7.2	6.5
	0.75	5/5	1.1	0/10	4.1	3.7	0/10(6995)	0/10(10,042)	6.7	6.0
Ribavirin	75	5/5	2.1	10/10**	>21.0**	0.3**	10/10**(99**)	9/10**(55**)	0.0**	1.7**
Saline	-	5/5	3.0	2/20	4.4	3.7	0/19(7897)	0/19(11,355)	7.1	6.5
Normals	-	5/5	2.3	-	-	0.2	5/5(92)	5/5(37)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^Compound is insoluble, drug is in suspension.

Conclusions: AVS2812 (narciclasine) was inactive vs PTV using death endpoint, but a moderate degree of activity was seen at the highest nontoxic dose using the other disease parameters.

*P<0.05 **P<0.01

Table III-25. Expt. PtA809. Effect of Twice Daily i.p. Treatments with AVS2812 on Punta Toro Virus Infections in Mice.

Animals: 8.7-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 5% EtOH in saline.

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route. i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2812	3.13	4/5	1.6	0/10	4.1	2.2	3/10(4630)	3/10(3792)	5.4	4.6
	1.56	5/5	1.3	0/10	4.2	3.7	1/10(7403)	1/10(5937)	6.6	5.8
	0.78	5/5	2.1	0/10	4.7	3.5	1/10(7826)	1/10(6254)	6.4	5.9
	0.39	5/5	1.3	0/10	4.4	3.6	0/10(8095)	0/10(6280)	7.0	6.5
	0.195	5/5	2.5	0/10	4.1	2.6	4/10(4880)	4/10(4213)	4.2	4.7
Ribavirin	75	5/5	1.1	10/10**	>21.0**	0.1**	10/10**(102**)	10/10**(45**)	1.4**	2.7**
Saline	-	-	-	2/20	4.2	3.3	4/20(6449)	3/20(5247)	6.0	5.9
Normals	-	5/5	3.4	-	-	0.1	5/5(75)	5/5(34)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS2812 (narciclacine) was not effective vs PTV using any disease parameter using this twice daily treatment regimen.

*P<0.05 **P<0.01

Table III-26. Expt. PtA835. Effect of Twice Daily s.c. Treatment With AVS2885 on Punta Toro Virus Infections in Mice.

Animals: 11.2-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2885	400	5/5	2.8	0/10	4.7
	200	5/5	3.5	0/10	4.3
	100	5/5	2.8	3/10*	6.0**
	50	5/5	3.0	0/10	4.7
	25	5/5	3.3	0/10	4.3
Ribavirin	75	5/5	1.8	9/9**	>21.0**
CMC	-	-	-	0/20	4.5
Normals	-	5/5	3.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS2885 (3-T-butyl-1-adamantylthiourea) was marginally active vs PTV at the mid-dose utilized. The compound was well tolerated at all dosage levels, suggesting a higher dosage may wish to be considered.

Table III-27. Expt. PtA841. Effect of Twice Daily s.c. Treatment with AVS2885 on Punta Toro Virus Infections in Mice.

Animals: 8.6-10.6 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: bid x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2885	100	5/5	3.0	0/10	4.4	3.6	0/10(12,434)	0/10(9222)	5.9	6.3
	50	4/5^	3.2	0/10	4.2	3.7	0/10(15,730)	0/10(11,785)	6.5	6.5
	25	5/5	3.4	0/10	4.0	2.6**	2/10*(10,773*)	2/10*(7226**)	5.2*	5.2
Ribavirin	75	4/4	2.9	10/10**	>21.0**	0.4**	10/10**(121**)	10/10**(39**)	0.9**	2.8**
Saline	-	-	-	0/20	4.6	3.9	0/19(15,384)	0/19(11,495)	6.4	6.4
Normals	-	5/5	3.7	-	-	0.3	5/5(106)	5/5(45)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^Mouse died on day 20 of experiment.

Conclusions: AVS2885 (3-T-butyl-1-adamantylthiourea) exhibited marginal anti-PTV activity only at the lowest dosage used. These data are suspect unless this material is exerting some type of immunomodulatory effect.

*P<0.05 **P<0.01

Table III-28. Expt. PtA850. Effect of Twice Daily i.p. Treatment With AVS2885 on Punta Toro Virus Infections in Mice.

Animals: 11.2-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2885	600	5/5	0.3	0/10	3.3
	300	5/5	0.4	0/10	4.4
	150	5/5	0.6	0/10	4.5
	75	5/5	1.2	1/10	4.9
Ribavirin	75	5/5	1.9	10/10**	>21.0**
CMC	-	-	-	4/20	5.4
Normals	-	5/5	4.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS2885 (3-T-butyl-1-adamantyl thiourea) was not active vs PTV in this experiment. The compound was nontoxic at all doses used, but at the higher doses the animals gained less weight suggesting an MTD was being approached.

Table III-29. Expt. PtA836. Effect of Twice Daily s.c. Treatment With AVS3679 on Punta Toro Virus Infections in Mice.

Animals: 11.2-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3679	400	5/5	3.1	0/10	4.3
	200	5/5	1.7	0/10	4.4
	100	5/5	3.1	0/10	4.6
	50	5/5	2.6	0/10	4.9
	25	5/5	2.4	0/10	4.5
Ribavirin	75	5/5	2.3	10/10**	>21.0**
CMC	-	-	-	0/20	4.4
Normals	-	5/5	4.2	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS3679 (unidentified) was inactive at all dosages used in this initial experiment. Since the material appeared to be well-tolerated at the highest dosage used, the experiment may need repeating using higher dosages.

Table III-30. Expt. PtA842. Effect of Twice Daily s.c. Treatment with AVS4206 on Punta Toro Virus Infections in Mice.

Animals: 9.8-10.6 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: bid x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS4206	100	5/5	3.9	0/10	4.3	3.8	0/10(9975)	0/10(9145)	5.8	6.5
	50	5/5	3.8	1/10	4.1	3.7	0/7(3509**)	0/7(3509**)	4.7	5.7**
	25	5/5	4.2	0/10	4.4	3.7	0/9(11,000)	0/9(10,072)	5.1	6.5
Ribavirin	75	4/4	2.9	10/10**	>21.0**	0.4**	10/10**(121**)	10/10**(39**)	0.9**	2.8**
Saline	-	-	-	0/20	4.2	3.8	0/16(10,497)	0/16(9306)	5.5	6.4
Normals	-	5/5	3.7	-	-	0.3	5/5(106)	5/5(45)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: AVS4206 (3-acetamido-7-amino-6-methyl-7H-S-triazolo[5,1-C]-S-triazole) was only sporadically effective vs PTV in this expanded experiment. Since the material was well tolerated at all doses used, it was retested using higher dosages and i.p. treatment route (see Table C-4)

*P<0.05 **P<0.01

Table III-31. Expt. PtA851. Effect of Twice Daily i.p. Treatment With AVS4206 on Punta Toro Virus Infections in Mice.

Animals: 10.5-11.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline

Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4206	600	5/5	3.1	0/10	5.1
	300	5/5	3.6	1/10	5.0
	150	5/5	2.9	0/10	5.2
	75	5/5	3.7	0/10	5.7
Ribavirin	75	5/5	1.9	10/10**	>21.0**
Saline	-	-	-	2/20	4.9
Normals	-	5/5	4.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4206 (3-acetamido-7-amino-6-methyl-7H-S-triazolo[5,1-C]-S-triazole) was not active vs PTV in this study. The material was well tolerated at all doses used, however, suggesting the MTD was still not yet attained.

Table III-32. Expt. PtA833. Effect of Twice Daily s.c. Treatment With AVS4206 on Punta Toro Virus Infections in Mice.

Animals: 11.2-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4206	400	5/5	3.4	2/10*	4.9
	200	5/5	2.4	1/10	4.8
	100	5/5	2.9	3/10*	5.0
	50	5/5	3.2	0/10	4.9
	25	5/5	2.7	0/10	4.4
Ribavirin	75	5/5	1.8	9/9**	>21.0**
Saline	-	-	-	0/20	4.8
Normals	-	5/5	3.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4206 (3-acetamido-7-amino-5-methyl-7H-S-triazolo[5,1-C]-S-triazole) was marginally active vs PTV at the high (400 mg/kg/day) and mid (100 mg/kg/day) dosages. The compound was well tolerated at all dosage levels, suggesting a higher dosage may wish to be considered.

Table III-33. Expt. PtA862. Effect of Twice Daily s.c. Treatment With AVS4272 on Punta Toro Virus Infections in Mice.

Animals: 9.9-12.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: bid x 5, beginning, 4 hr pre-virus inoculation.
Treatment Route: s.c.

Experiment Duration: 21 days

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4272	50	5/5	2.6	7/10	4.0
	25	5/5	3.1	4/10	5.2
	12.5	5/5	3.4	1/10	4.9
	6.25	5/5	3.2	4/10	5.4
	3.13	4/4	4.0	6/10	4.5
Ribavirin	75	3/3	3.2	8/8**	>21.0**
CMC	-	-	-	6/20	5.4
Normals	-	3/3	4.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4272 was ineffective vs PTV when administered by the treatment regimen indicated. The virus controls died at a less than satisfactory rate, so the experiment will be repeated.

Table III-34. Expt. PtA863. Effect of Twice Daily i.p. Treatment With AVS4272 on Punta Toro Virus Infections in Mice.

Animals: 9.9-12.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: bid x 5, beginning, 4 hr pre-virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4272	50	0/5	—	0/10	1.9
	25	2/5	-0.6	0/10	3.9
	12.5	5/5	3.3	0/10	4.3
	6.25	4/4	3.3	6/10**	5.5
	3.13	4/4	3.4	5/10**	4.4
Ribavirin	75	3/3	3.2	8/8**	>21.0**
CMC	-	-	-	2/20	5.0
Normals	-	3/3	4.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4272 was considered effective vs PTV when administered i.p. in this study.

Table III-35. Expt. PtA868. Effect of Single i.p. Treatment With AVS4272 on Punta Toro Virus Infections in Mice.

Animals: 11.6-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: Once only, 4 hr post-virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4272	200	1/5	-1.7	0/10	—
	100	1/5	-2.2	0/10	1.6
	50	1/5	1.3	0/10	2.1
	25	3/5	-0.1	2/10	4.8
	12.5	5/5	-0.1	5/10	5.0
Ribavirin	350	5/5	0.1	6/9	6.3
CMC	-	-	-	5/20	4.8
Normals	-	5/5	0.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4272 was moderately effective vs PTV when used at the only non-toxic dose in this experiment.

Table III-36. Expt. PtA870. Effect of Single i.p. Treatment With AVS4272 on Punta Toro Virus Infections in Mice.

Animals: 11.6-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4272	200	1/5	-1.7	0/9	3.0
	100	1/5	-2.2	0/10	2.7
	50	1/5	1.3	0/10	2.8
	25	3/5	-0.1	2/9	4.9
	12.5	5/5	-0.1	1/10	5.3
Ribavirin	350	5/5	0.1	9/9**	>21.0**
CMC	-	-	-	6/20	4.8
Normals	-	5/5	0.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4272 was not considered effective vs PTV when single i.p. therapy began 24 hr after virus inoculation.

Table III-37. Expt. PtA873. Effect of Twice Daily s.c. Treatment with AVS4272 on Punta Toro Virus Infections in Mice.

Animals: 9.9-10.8 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS4272	50	3/5	-0.4	0/10	4.1	3.0	0/2(2910**)	0/2(2888**)	4.*	5.4
	25	5/5	1.6	0/10	5.0	3.7	0/10(7844*0	0/10(7442*)	5.8	5.8
	12.5	5/5	2.2	1/10	5.0	3.2	0/9(7285*)	0/9(7714)	5.0**	6.1
	6.25	5/5	2.3	0/10	4.8	2.7	0/9(7652*)	0/9(7094*)	6.0	6.5
	3.13	5/5	2.6	5/10**	5.4	1.2**	3/10*(2762**)	4/10*(2448**)	3.5**	3.9**
Ribavirin	75	5/5	2.1	10/10**	>21.0**	0.0**	8/9**(160**)	9/9**(45**)	0.6**	1.0**
CMC	-	-	-	0/20	4.3	3.5	1/17(12,495)	1/17(11,144)	6.9	6.3
Normals	-	5/5	3.3	-	-	0.0	5/5(79)	2/5(144)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Conclusions: AVS4272 was moderately effective vs PTV in this expanded study.

Table III-38. Expt. PtA874. Effect of Twice Daily i.p. Treatment with AVS4272 on Punta Toro Virus Infections in Mice.

Animals: 9.8-11.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS4272	50	0/5	-	0/10	2.5					
	25	1/5	0.5	0/10	3.7					
	12.5	5/5	2.2	0/10	4.5	3.7	2/10*(11,282)	2/10*(9250)	6.3	5.9
	6.25	5/5	1.7	1/10	4.2	3.6	0/9(10,223)	0/9(9567)	6.1	5.7
	3.13	5/5	0.5	3/10	4.4	3.7	0/10(12,078)	0/10(10,335)	6.1	5.8
Ribavirin	75	5/5	2.1	8/10**	8.5	1.3**	7/10**(1695**)	7/10**(1333**)	1.4**	2.7**
CMC	-	-	-	1/20	5.2	3.4	0/20(11,065)	0/20(9311)	6.0	5.5
Normals	-	5/5	3.3	-	-	0.0	5/5(79)	2/5(144)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4)

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

*P<0.05 **P<0.01

Conclusions: AVS4272 was considered significantly effective in the initial experiment using this treatment regimen (see Table C-6). That activity was not confirmed in the present expanded study.

Table III-39. Expt. PtA875. Effect of Twice Daily p.o. Treatment With AVS4272 on Punta Toro Virus Infections in Mice.

Animals: 11.6-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: p.o.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4272	50	5/5	1.5	0/10	4.2
	25	5/5	2.5	0/10	4.1
	12.5	5/5	3.5	0/10	4.0
	6.25	5/5	2.3	0/10	4.4
	3.13	5/5	3.2	0/10	4.1
Ribavirin	75	5/5	2.9	10/10**	>21.0**
CMC	-	-	-	0/20	4.3
Normals	-	5/5	3.3	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4272 was ineffective vs PTV when given orally.

Table III-40. Expt. PtA888. Effect of Single i.p. Treatment with AVS4272 on Punta Toro Virus Infections in Mice.

Animals: 9.8-10.8 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: once only, 4 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS4272	12.5	5/5	-0.5	3/10	4.7	3.0	1/10(6323)	0/10(5843)	4.3	5.2
	6.25	5/5	0.2	3/10	5.3	2.7	0/9(3373)	0/9(3282)	3.4*	4.1*
	3.13	5/5	1.3	6/10*	5.5	3.1	0/10(3840)	0/10(4117)	3.6*	5.0
	1.56	5/5	0.7	2/10	5.3	2.8	0/10(4872)	0/10(4682)	4.1	5.4
Ribavirin	350	5/5	0.5	10/10**	>21.0**	0.7**	3/10*(549**)	4/10*(651**)	3.7*	4.6
CMC	-	-	-	3/20	5.1	3.3	1/19(5412)	0/19(4771)	5.0	5.5
Normals	-	5/5	0.0	-	-	0.2	5/5(100)	5/5(48)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Conclusions: AVS4272 was marginally effective vs PTV when given i.p. in a single early injection. Compare with the data of Table C-7.

Table III-41. Expt. PtA889. Effect of Twice Daily i.p. Treatment with AVS4272 on Punta Toro Virus Infections in Mice.

Animals: 9.8-10.8 g (3 wk.; C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS4272	6.25	5/5	3.7	5/10**	5.4	3.5	2/10(6569)	2/10(5956)	4.6	4.7
	3.13	5/5	3.2	0/10	5.0	3.8	1/10(6234)	0/10(6471)	6.1	5.8
	1.56	5/5	2.6	0/10	4.9	3.8	1/10(8180)	0/10(7707)	6.3	6.5
	0.8	5/5	2.7	1/10	4.4	2.7	4/10*(5176)	2/10(5518)	4.8	4.7
Ribavirin	75	5/5	2.6	10/10**	>21.0**	0.1**	7/9**(158**)	8/9**(52**)	0.7**	1.3**
CMC	-	-	-	1/20	4.8	3.5	1/19(6164)	1/19(5369)	5.1	5.6
Normals	-	5/5	0.0	-	-	0.2	5/5(100)	5/5(48)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

*P<0.05 **P<0.01

Conclusions: AVS4272 was again not significantly effective vs PTV when given i.p. on a chronic therapy schedule. Compare with the data of Tables C-10 and C-6.

Table III-42. Expt. PtA890. Effect of Twice Daily s.c. Treatment with AVS4272 on Punta Toro Virus Infections in Mice.

Animals: 9.8-10.8 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS4272	50	5/5	1.8	1/10	3.9	3.4	0/9(7439)	0/9(5044)	5.0	6.1
	25	5/5	2.7	0/10	4.1	3.0	1/10(5083*)	1/10(3781*)	4.8	5.7
	12.5	5/5	1.9	0/10	4.7	3.8	0/10(10,630)	0/10(7750)	5.6	6.3
	6.25	5/5	2.0	4/10	5.3	3.2	2/10(7647)	2/10(5959)	4.9	5.4
Ribavirin	75	5/5	3.3	8/8**	>21.0**	0.4**	7/8**(97**)	8/8**(30**)	0.4**	1.6**
CMC	-	-	-	3/20	5.0	3.5	1/10(9158)	1/10(7034)	4.8	5.6
Normals	-	5/5	3.8	-	-	0.3	4/5(155)	5/5(44)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Conclusions: AVS4272 was effective vs PTV when administered s.c. on a chronic treatment schedule.

Table III-43. Expt. PtA8t4. Effect of Twice Daily s.c. Treatment With AVS4273 on Punta Toro Virus Infections in Mice.

Animals: 9.9-12.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: bid x 5, beginning, 4 hr pre-virus inoculation.
Treatment Route: s.c.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4273	100	5/5	2.0	0/10	4.0
	50	5/5	3.7	1/10	4.1
	25	5/5	3.0	0/10	5.7
	12.5	4/4	3.1	4/10	5.2
	6.25	4/4	3.5	3/10	5.1
Ribavirin	75	3/3	3.2	8/8**	>21.0**
CMC	-	-	-	6/20	5.4
Normals	-	3/3	4.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4273 was ineffective vs PTV in this experiment.

Table III-44. Expt. PtA865. Effect of Twice Daily i.p. Treatment With AVS4273 on Punta Toro Virus Infections in Mice.

Animals: 9.9-12.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: bid x 5, beginning, 4 hr pre-virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4273	100	5/5	3.7	2/10	4.4
	50	5/5	2.9	4/10	4.3
	25	5/5	3.6	1/10	5.1
	12.5	4/4	3.0	3/10	6.3*
	6.25	4/4	3.5	7/10**	5.0
Ribavirin	75	3/3	3.2	8/8**	>21.0**
CMC	-	-	-	2/20	5.0
Normals	-	3/3	4.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4273 was significantly effective vs PTV at the lowest dosage used in this experiment.

Table III-45. Expt. PtA869. Effect of Single i.p. Treatment With AVS4273 on Punta Toro Virus Infections in Mice.

Animals: 11.6-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: Once only, 4 hr post-virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4273	200	5/5	-1.0	1/8	4.0
	100	5/5	0.0	0/8	4.1
	50	5/5	0.7	0/8	4.5
	25	5/5	0.8	1/8	4.4
	12.5	5/5	0.5	0/8	4.8
Ribavirin	350	5/5	0.1	6/9	6.3
CMC	-	-	-	5/20	4.8
Normals	-	5/5	0.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4273 was ineffective vs PTV when administered in a single early i.p. injection.

Table III-46. Expt. PtA871. Effect of Single i.p. Treatment With AVS4273 on Punta Toro Virus Infections in Mice.

Animals: 11.6-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4273	200	5/5	-1.0	2/8	4.2
	100	5/5	0.0	1/8	3.6
	50	5/5	0.7	0/8	4.8
	25	5/5	0.8	0/8	4.5
	12.5	5/5	0.5	0/8	4.6
Ribavirin	350	5/5	0.1	9/9**	>21.0**
CMC	-	-	-	6/20	4.8
Normals	-	5/5	0.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4273 was ineffective vs PTV when administered in a single late i.p. injection.

Table III-47. Expt. PtA876. Effect of Twice Daily s.c. Treatment with AVS4273 on Punta Toro Virus Infections in Mice.

Animals: 9.8-10.8 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS4273	100	2/5	0.3	0/10	3.9	2.5	0/1(5600)	0/1(4700	6.5	6.5
	50	5/5	2.0	0/10	4.1	3.0	2/10(11,642)	1/10(8766)	4.9	5.2
	25	5/5	2.1	4/10	4.5	3.5	0/10(10,025	0/10(10,040	6.5	6.4
	12.5	5/5	2.3	2/10	4.3	3.1	2/10(11,468)	2/10(9427)	6.0	6.0
	6.25	5/5	2.5	1/10	4.3	3.2	2/10(12,247)	2/10(8664)	5.8	5.3
Ribavirin	75	5/5	1.3	10/10**	>21.0**	0.5**	10/10**(76**)	10/10**(43**)	0.0**	0.6**
CMC	-	-	-	5/20	4.4	3.4	2/19(11,960)	2/19(8752)	5.6	5.7
Normals	-	5/5	3.5	-	-	0.6	5/5(70)	5/5(28)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Conclusions: The somewhat erratic activity seen in PtA864 (Table C-15) prompted the present study, in which no anti-PTV activity was demonstrated.

Table III-48. Expt. PtA877. Effect of Twice Daily i.p. Treatment with AVS4273 on Punta Toro Virus Infections in Mice.

Animals: 9.7-11.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS4273	100	4/5	1.2	0/10	4.1	3.7	0/10(9675)	0/10(9609)	5.5	5.4
	50	5/5	2.9	1/10	3.7	3.8	0/10(9592)	0/10(9543)	5.5	5.5
	25	5/5	1.8	3/10	3.4	3.4	1/10(8504)	2/10(8452)	4.6	4.8
	12.5	5/5	1.0	1/10	4.4	3.6	0/10(8984)	0/10(8644)	5.2	5.1
	6.25	5/5	2.5	0/10	3.9	3.3	0/10(8148)	1/10(8093)	4.6	5.0
Ribavirin	75	5/5	2.5	9/10**	4.0	1.1**	6/10**(242**)	10/10**(64**)	0.0**	1.0**
CMC	-	-	-	2/20	4.3	3.1	1/20(5276)	1/20(5071)	4.1	4.4
Normals	-	5/5	3.5	-	-	0.6	5/5(70)	5/5(28)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Conclusions: AVS4273 was not effective vs PTV in this study; these data did not confirm the possible activity seen in Table C-16.

Table III-49. Expt. PtA878. Effect of Twice Daily p.o. Treatment With AVS4273 on Punta Toro Virus Infections in Mice.

Animals: 10.4-11.2 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: p.o.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4273	100	5/5	2.7	0/10	3.9
	50	5/5	2.9	0/10	4.0
	25	5/5	2.2	0/10	3.9
	12.5	5/5	2.8	0/10	4.0
	6.25	5/5	2.4	0/10	4.0
Ribavirin	75	5/5	3.3	10/10**	>21.0**
CMC	-	-	-	1/20	4.3
Normals	-	5/5	3.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4273 was ineffective vs PTV when administered orally in this experiment.

Table III-50. Expt. PtA843. Effect of Twice Daily s.c. Treatment with AVS4588 on Punta Toro Virus Infections in Mice.

Animals: 9.2-10.9 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: bid x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS4588	100	5/5	2.8	0/10	4.2	2.8	1/9(12,114)	1/9(10,026)	6.8	6.0
	50	5/5	3.9	0/10	4.5	4.0	0/10(12,598)	0/10(11,500)	7.3	6.4
	25	5/5	3.9	0/10	4.8	3.8	0/10(13,170)	0/10(11,170)	7.3	6.5
Ribavirin	75	4/4	2.9	10/10**	>21.0**	0.4**	10/10**(121**)	10/10**(39**)	0.9**	2.8**
Saline	-	-	-	0/20	4.2	3.8	0/16(10,497)	0/16(9306)	5.5	6.4
Normals	-	5/5	3.7	-	-	0.3	5/5(106)	5/5(45)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: AVS4588 (1-aminoadenosinium mesitylenesulfonate) was not active vs PTV in this experiment. Since the material was well tolerated at all doses used, a second study was run using higher doses (see Table C-6).

*P<0.05 **P<0.01

Table III-51. Expt. PtA852. Effect of Twice Daily i.p. Treatment With AVS4588 on Punta Toro Virus Infections in Mice.

Animals: 10.5-11.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4588	600	5/5	2.0	0/10	4.2
	300	5/5	2.8	2/10	4.8
	150	5/5	3.0	0/10	5.3
	75	5/5	3.2	0/10	5.3
Ribavirin	75	5/5	1.9	10/10**	>21.0**
Saline	-	-	-	2/20	4.9
Normals	-	5/5	4.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4588 (1-aminoadenosinium mesitylene sulfonate) was not active vs PTV in this study. The compound was again well tolerated at all doses used.

Table III-52. Expt. PtA834. Effect of Twice Daily s.c. Treatment With AVS4588 on Punta Toro Virus Infections in Mice.

Animals: 11.2-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4588	400	5/5	2.6	0/10	4.1
	200	5/5	2.4	0/10	4.2
	100	5/5	3.0	2/10*	4.9
	50	5/5	3.1	0/10	4.3
	25	5/5	3.1	0/10	4.9
Ribavirin	75	5/5	1.8	9/9**	>21.0**
Saline	-	-	-	0/20	4.8
Normals	-	5/5	3.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4588 (1-aminoadenosinium mesitylenesulfonate) was considered marginally active vs PTV in this experiment. The compound was well tolerated at all dosage levels, suggesting a higher dosage may wish to be considered.

Table III-53. Expt. PtA837. Effect of Twice Daily s.c. Treatment With AVS4618 on Punta Toro Virus Infections in Mice.

Animals: 11.2-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4618	400	5/5	3.2	0/10	4.6
	200	5/5	3.0	0/10	4.8
	100	5/5	2.4	0/10	4.9
	50	5/5	3.7	2/10*	5.5*
	25	5/5	3.2	2/10*	4.6
Ribavirin	75	5/5	2.3	10/10**	>21.0**
CMC	-	-	-	0/20	4.4
Normals	-	5/5	4.2	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4618 (unidentified) was marginally active vs PTV at the 2 lowest dosages used.

Table III-54. Expt. PtA853. Effect of Twice Daily s.c. Treatment with AVS4618 on Punta Toro Virus Infections in Mice.

Animals: 8.6-10.6 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: bid x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS4618	50	5/5	4.0	0/10	4.4	3.8	0/10(9248)	0/10(6655)	5.6	6.3
	25	5/5	2.8	0/10	4.8	3.8	0/10(14,190)	0/10(10,485)	6.2	6.4
	12.5	5/5	3.6	1/10	4.2	3.5	0/10(14,125)	0/10(9765)	5.9	6.5
	6.25	5/5	3.3	0/10	5.2	3.5	0/10(11,229)	0/10(7600)	5.6	6.1
Ribavirin	75	4/4	2.8	10/10**	>21.0**	0.5**	8/10**(243**)	10/10**(55**)	0.3**	1.5**)
CMC	-	-	-	2/20	4.4	3.4	0/20(12,123)	0/20(8973)	5.9	6.3
Normals	-	5/5	4.2	-	-	0.3	4/5(188)	5/5(50)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS4618 (unidentified) was not effective vs PTV in this expanded study. The material was also well tolerated at all doses used, suggesting higher dosages be considered in further experiments.

*P<0.05 **P<0.01

Table III-55. Expt. PtA789. Effect of Single i.p. Treatment with AVS5311 on Punta Toro Virus Infections in Mice.

Animals: 11.7-13.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: once only 4 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (units/mouse)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[i] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS5311	$10^{5.0}$	5/5	0.4	7/10**	5.3	2.7	2/10(1588**)	1/10(1329**)	1.8**	2.8**
	$10^{4.5}$	5/5	0.4	1/10	5.1	3.3	1/10(5440**)	1/10(4733**)	5.0	5.5*
	$10^{4.0}$	5/5	0.0	1/9	5.0	3.7	0/9(6304**)	0/9(5667**)	5.4	6.0
	$10^{3.5}$	5/5	0.1	1/10	4.1	4.0	1/10(7997)	0/10(7069)	6.0	6.3
Ribavirin	350^		0.2	9/10**	9.0	0.5**	4/10**(592**)	5/10**(625**)	2.7**	3.0**
Saline	-	-	-	3/20	4.8	3.9	0/14(8700)	0/14(7750)	5.3	6.5
Normals	-	5/5	0.9	-	-	0.2	—	—	0.0	—

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

^mg/kg

Conclusions: A single i.p. treatment with AVS5311 (recombinant IFN) was inhibitory to PTV infections using all disease parameters at the highest dosage used.

*P<0.05 **P<0.01

Table III-56. Expt. PtA790. Effect of Once Daily i.p. Treatments with AVS5311 on Punta Toro Virus Infections in Mice.

Animals: 10.5-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: qd x 9, beginning 4 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (units/mouse)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS5311	$10^{5.0}$	4/5§	6.6	10/10**	>21.0**	1.5**	6/10**(1204**)	6/10**(933**)	1.1**	1.1**
	$10^{4.5}$	5/5	7.4	8/10**	4.0	1.8**	0/10(3469**)	0/10(2848**)	2.2**	2.8**
	$10^{4.0}$	5/5	7.3	5/10**	5.2	3.3	0/9(5650**)	0/9(4645**)	4.7*	5.3**
	$10^{3.5}$	5/5	7.7	1/10	4.9	3.2	0/10(3921**)	0/10(3657**)	4.5*	4.9**
Ribavirin	350^		0.2	9/10**	9.0	0.5**	4/10**(592**)	5/10**(625**)	2.7**	3.0**
Saline	-	-	-	0/20	4.4	3.9	0/16(9100)	0/16(7600)	5.9	6.5
Normals	-	5/5	6.9	-	-	0.2	—	—	0.0	—

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

§Animal died on day 20 of experiment.

^mg/kg, given once only 4 hr post-virus inoculation.

Conclusions: Multiple i.p. treatments with AVS5311 (recombinant IFN) were highly active vs the PTV infection using all disease parameters. This activity was greater than when the material was given only a single time (PtA 789).

*P<0.05 **P<0.01

Table III-57. Expt. PtA826. Effect of Once Daily i.p. Treatment with AVS5311 on Punta Toro Virus Infections in Mice.
Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: qd x 5, beginning 24 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (units/mouse)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Live; Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS5311	$10^{5.0}$	5/5	2.4	9/10**	7.0	3.1	1/10(4947*)	0/10(4020*)	4.5*	5.2*
	$10^{4.5}$	5/5	2.6	0/10	4.2	3.5	0/10(7830)	0/10(6535)	5.4	6.4
	$10^{4.0}$	5/5	2.3	0/10	4.3	3.5	0/10(7440)	0/10(6045)	5.2	6.4
	$10^{3.5}$	5/5	1.8	0/10	3.7	3.7	0/10(8674)	0/10(7002)	5.2	6.1
Ribavirin	75^	5/5	2.3	10/10**	>21.0**	0.3	9/10**(117**)	10/10**(45**)	0.3**	0.6**
Saline	-	-	-	0/20	4.8	3.6	0/20(8389)	0/20(6823)	5.5	6.3
Normals	-	5/5	2.5	-	-	0.4	5/5(89)	5/5(24)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^mg/kg/day

Conclusions: AVS5311 (rec. IFN) was moderately active vs PTV when treatment was begun 24 hr post-virus inoculation (compare with Tables C-16, 17).

*P<0.05 **P<0.01

Table III-58. Expt. PtA827. Effect of Once Daily i.p. Treatment with AVS5311 on Punta Toro Virus Infections in Mice.

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: qd x 5, beginning 36 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (units/mouse)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS5311	$10^{5.0}$	5/5	2.4	1/10	4.1	4.0	0/10(7960)	0/10(6575)	5.7	6.5
	$10^{4.5}$	5/5	2.6	0/10	3.8	3.8	0/10(7365)	0/10(5985)	5.6	6.4
	$10^{4.0}$	5/5	2.3	2/10*	4.3	3.4	0/10(6369)	0/10(5232)	5.4	6.3
	$10^{3.5}$	5/5	1.8	0/10	4.5	3.8	0/10(6968)	0/10(5595)	5.4	6.3
Ribavirin	75^	5/5	2.3	10/10**	>21.0**	0.3**	9/10**(117**)	10/10**(45**)	0.3**	0.6**
Saline	-	-	-	0/20	4.8	3.6	0/20(8389)	0/20(6823)	5.5	6.3
Normals	-	5/5	2.5	-	-	0.4	5/5(89)	5/5(24)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^mg/kg/day

Conclusions: AVS5311 (rec. IFN) was essentially inactive vs PTV when treatment was delayed until 36 hr post-virus inoculation (compare with Tables C-15, 17).

*P<0.05 **P<0.01

Table III-59. Expt. PtA828. Effect of Once Daily i.p. Treatment with AVS5311 on Punta Toro Virus Infections in Mice.

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: qd x 5, beginning 48 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (units/mouse)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS5311	$10^{5.0}$	5/5	2.4	3/10*	6.3**	3.5	0/10(9080)	0/10(7130)	6.2	6.2
	$10^{4.5}$	5/5	2.6	0/10	5.2	3.9	0/10(11,390)	0/10(9600)	6.4	6.5
	$10^{4.0}$	5/5	2	0/10	4.1	3.8	0/10(10,630)	0/10(8825)	6.3	6.3
	$10^{3.5}$	5/5	1.8	0/10	4.5	3.5	0/10(8980)	0/10(8265)	5.7	6.4
Ribavirin	75^	5/5	2.3	10/10**	>21.0**	0.3**	9/10**(117**)	10/10**(45**)	0.3**	0.6**
Saline	-	-	-	0/20	4.8	3.6	0/20(8389)	0/20(6823)	5.5	6.3
Normals	-	5/5	2.5	-	-	0.4	5/5(89)	5/5(24)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^mg/kg/day

Conclusions: AVS5311 (rec. IFN) was marginally effective vs PTV when treatment was delayed until 48 hr after virus inoculation (compare with Tables C-15, 16).

*P<0.05 **P<0.01

Table III-60. Expt. PtA893. Effect of Twice Daily i.p. Treatment With AVS6334 on Punta Toro Virus Infections in Mice.

Animals: 10.4-11.2 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS6334	250	3/3	1.8	0/10	4.1
	125	3/3	3.4	0/10	4.3
	62.5	3/3	3.4	1/10	4.4
	31.3	3/3	1.8	0/10	4.4
	15.6	3/3	3.2	4/10**	5.8**
	7.8	3/3	3.0	0/10	4.3
Ribavirin	75	3/3	2.1	10/10**	>21.0**
CMC	-	-	-	0/20	4.4
Normals	-	3/3	3.4	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS6334 was effective vs PTV only at a single, low dose in this experiment.

Table III-61. Expt. PtA894. Effect of Twice Daily i.p. Treatment With AVS6337 on Punta Toro Virus Infections in Mice.

Animals: 10.9-12.2 g (3 4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS6337	250	2/3	0.8	0/10	3.7
	125	3/3	1.3	0/10	3.9
	62.5	3/3	2.2	0/10	4.2
	31.3	3/3	2.5	1/10	4.9
	15.6	3/3	3.7	0/10	4.7
	7.8	3/3	4.0	2/10*	4.8
Ribavirin	75	3/3	2.1	10/10**	>21.0**
CMC	-	-	-	0/20	4.4
Normals	-	3/3	3.4	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS6337 was marginally effective vs PTV at the lowest dose used in this experiment.

Table III-62. Expt. PtA895. Effect of Twice Daily i.p. Treatment With AVS6417 on Punta Toro Virus Infections in Mice.

Animals: 10.9-12.2 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS6417	250	0/3	-1.0	0/10	4.0
	125	3/3	0.5	1/10	4.1
	62.5	3/3	2.5	0/10	4.8
	31.3	3/3	3.4	1/10	5.9
	15.6	3/3	2.8	0/10	5.3
	7.8	3/3	3.0	0/10	4.8
Ribavirin	75	3/3	2.1	10/10**	>21.0**
CMC	-	-	-	0/20	4.4
Normals	-	3/3	3.4	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS6417 was ineffective vs PTV in this experiment.

Table III-63. Expt. PtA896. Effect of Twice Daily i.p. Treatment With AVS6477 on Punta Toro Virus Infections in Mice.

Animals: 10.9-13.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS6477	100	3/3	3.3	0/10	4.4
	50	3/3	3.0	0/10	4.5
	25	3/3	3.0	0/10	4.4
	12.5	3/3	3.5	0/10	4.7
	6.3	3/3	3.8	0/10	4.5
	3.2	3/3	2.9	0/10	4.5
Ribavirin	75	3/3	2.1	10/10**	>21.0**
CMC	-	-	-	0/20	4.4
Normals	-	3/3	3.4	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS6477 was ineffective vs PTV in this experiment. All doses used were well-tolerated, however, so it may need to be further studied with higher dosages.

Table III-64. Expt. PtA897. Effect of Twice Daily i.p. Treatment With AVS6501 on Punta Toro Virus Infections in Mice.

Animals: 10.9-12.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC

Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS6501	250	3/3	2.6	1/10	4.3
	125	3/3	2.6	0/10	4.0
	62.5	3/3	3.9	0/10	4.5
	31.3	3/3	3.0	2/10*	5.4*
	15.6	3/3	1.7	0/10	4.3
	7.8	3/3	2.0	4/10**	4.3
Ribavirin	75	3/3	2.1	10/10**	>21.0**
CMC	-	-	-	0/20	4.4
Normals	-	3/3	3.4	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS6501 was marginally effective vs PTV at two low dosages used.

IV. EFFECT OF AVS COMPOUNDS ON INTRACEREBRAL INFECTIONS IN MICE INDUCED BY THE BALLIET STRAIN OF PUNTA TORO VIRUS

Introduction

It has been stressed from the inception of this project that the PTV infection in mice is being used as a model for Rift Valley fever and sandfly fever infections in man. A late and often fatal form of Rift Valley fever involves encephalitis, and patients with sandfly fever also develop certain symptoms suggestive of central nervous system (CNS) infection. We therefore felt it was important to determine if AVS compounds active against the hepatotropic Adames PTV infection would also have an effect on an encephalitic disease induced in mice by the neurotropic (Balliet) strain of PTV. As described earlier, our protocol for *in vivo* evaluations of anti-PTV compounds includes follow-up testing of PTV-inhibitory compounds against the CNS disease in mice. The results of these follow-up investigations are described in this section. Studies with i.v.-administered compounds prepared by Pharmatek, Inc. for specific delivery to the brain are also described in this section.

Materials and Methods

Virus: The Balliet strain of PTV as described in Sections I and III of our Annual Report No. 1 was used. A mouse brain-prepared virus pool was used in the present studies. The virus, suspended in Pucks balanced salt solution (PBSS) was used at dilutions of 10^{-3} or 10^{-4} (10 and 1LD50), coinciding with 10^4 and 10^3 Vero cell CCID50 of virus. The latter dose was used in most studies in an attempt to increase the sensitivity of the test.

Animals: Four week-old female Balb/c or Swiss Webster mice were obtained from Simonsen Laboratories. The animals were quarantined 48 hr prior to use and were maintained on standard mouse chow and water *ad libitum*. The Swiss Webster mice were used when i.v. administration of drugs was given.

Compounds: All compounds were provided by Biological Research Faculty & Facility, Inc. Many were AVS01 derivatives prepared by Pharmatek, Inc.

Experiment Design: Ether-anesthetized mice were infected by inoculating 0.05 ml of PTV i.c. into the right hemisphere of the brain. Twenty infected mice were used with each drug level, with 5 infected mice used as virus controls which received drug diluent only. Treatment and schedule varied depending upon the compound being evaluated, with those regimens considered highly effective against the hepatotropic virus infection selected for treatment of this CNS disease. Five toxicity control mice were used at each drug dose level, and 10 mice were used as normal controls. The latter two groups of controls were weighed before and after treatment as described in Section III. On infection day 6, one-half (one or two pre-designated cages) of each group of infected animals were killed and their brains removed. Ten percent homogenates of each brain were diluted through a series of 10-fold dilutions and each was assayed for virus using CPE production in triplicate cups of LLC-MK_2 cells. The remaining animals were observed daily for death through infection day 21, which was the termination of the experiment.

Increases in survivor number were evaluated using chi square analysis with Yate's correction. Increases in mean survival time and decreases in mean brain virus titers were analyzed using *t* test.

Results and Discussion

The results with each AVS compound tested are summarized in Tables IV-1-17. The following summarizes the activity of each.

AVS1018 (Table IV-1): Inactive vs PTV at all dosages.

AVS1968 (CL246,768) (Table IV-2): Inactive vs PTV at all dosages.

AVS2933 (CGP 19835 A lipid) (Table IV-3-4): Inactive vs PTV at all dosages.

AVS5311 (human recombinant interferon) (Table IV-5): Inactive vs PTV at all dosages.

AVS5581 (Table IV-6): Inactive vs PTV at all dosages.

AVS5582 (Table IV-7): Inactive vs PTV at all dosages.

AVS5597 (Table IV-8): Inactive vs PTV at all dosages.

AVS6080 (Table IV-9): This compound was possibly weakly active vs this PTV infection as seen by increased mean survival times.

AVS6081 (Table IV-10): Inactive vs PTV at all dosages.

AVS6082 (Table IV-11): This compound was significantly inhibitory to the Balliet PTV infection at the highest dosage used. This activity was seen as increased survivors, mean survival time, and decreased brain virus titers.

AVS6083 (Table IV-12): Inactive vs PTV at all dosages.

AVS6290 (Table IV-13): Inactive vs PTV at all dosages.

AVS6291 (Table IV-14): Inactive vs PTV at all dosages.

AVS6292 (Table IV-15): Inactive vs PTV at all dosages.

AVS6297 (Table IV-16): Inactive vs PTV at all dosages.

AVS6300 (Table IV-17): Inactive vs PTV at all dosages.

Conclusions

A total of 16 AVS compounds were evaluated against the CNS infection induced by the Balliet strain of PTV. Compounds AVS6080 and 6082 were considered moderately effective. The majority of the compounds evaluated were Pharmatek-prepared compounds designed for delivery to the brain.

Table IV-1. Expt. PtA838. Effect of Two p.o. Treatments with AVS1018 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 15.0 - 17.0 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: Sterile H_2O.
Treatment Schedule: Two shots, 4 hr pre-virus inoculation and on day 4.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS1018	25	5/5	0.4	0/10	8.3	7.2
	12.5	5/5	1.1	0/10	8.1	7.4
	6.25	5/5	1.2	0/10	9.2	7.5
	3.13	5/5	1.2	0/10	8.9	6.7**
H_2O	-	-	-	1/20	8.5	7.5
Normals	-	5/5	1.5	-	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS1018 (unidentified) was essentially inactive vs the infection induced by the Balliet strain of PTV in this study.

Table IV-2. Expt. PtA839. Effect of Single p.o. Treatment with AVS1968 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 15.0 - 17.0 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, 4 hr pre-virus inoculation.
Treatment Route: p.o.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS1968	100	5/5	-0.5	0/10	8.3	7.5
	50	5/5	-0.7	0/10	8.2	7.2
	25	5/5	-0.3	1/10	8.3	7.4
	12.5	5/5	0.1	0/9	7.9	7.5
H_2O	-	-	-	1/20	8.5	7.5
Normals	-	5/5	0.3	-	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS1968 (unidentified) was inactive vs the infectio induced by the Balliet strain of PTV in this study.

Table IV-3. Expt. PtA859. Effect of Single i.p. Treatment with AVS2933 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 13.7 - 15.6 g (4 wk) C57BL/6 mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: Ca^{++}, Mg^{++} free saline.
Treatment Schedule: Once only, 4 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (µg/kg)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS6297	10,000	5/5	0.2	0/10	7.9	7.1
	5,000	5/5	0.3	0/10	7.5	6.9
	2,500	5/5	0.0	0/10	8.7	7.3
	1,250	5/5	0.0	0/10	8.1	6.6
Saline	-	-	-	0/20	7.6	7.5
Normals	-	5/5	1.1	-	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 7. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS2933 (CPG 19835A lipid) was ineffective vs PTV-induced encephalitis in this experiment.

Table IV-4. Expt. PtA860. Effect of Single i.p. Treatment with AVS2933 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 13.7 - 15.6 g (4 wk) C57BL/6 mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: Ca^H, Mg^H free saline.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Injected, Treated		
Compound	Dosage (µg/kg)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS6297	10,000	5/5	0.2	0/10	8.1	7.3
	5,000	5/5	0.3	0/10	7.3	7.3
	2,500	5/5	0.0	1/10	8.4	7.4
	1,250	5/5	0.0	1/10	7.6	7.4
Saline	-	-	-	0/20	7.6	7.5
Normals	-	5/5	1.1	-	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 7. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS2933 (CPG 19835A lipid) was ineffective vs PTV-induced encephalitis in this experiment.

Table IV-5. Expt. PtA840. Effect of Once Daily i.p. Treatment with AVS5311 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 15.0 - 17.0 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Once daily x 8, beg. 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (units/mouse)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS5311	10^5	5/5	2.0	0/10	8.4	7.3
	$10^{4.5}$	5/5	2.0	0/9	8.3	7.5
	10^4	5/5	1.8	0/10	8.0	7.2
	$10^{3.5}$	5/5	2.1	0/10	7.5	7.5
Saline	-	-	-	0/19	8.6	7.4
Normals	-	5/5	2.0	-	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS5311 (human recombinant interferon) was inactive vs the infection induced by the Balliet strain of PTV in this study.

Table IV-6. Expt. PtA779. Effect of Once Daily i.v., i.p. Treatment with AVS5581 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 21.9 - 23.2 g (4 wk) Swiss Webster Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO, neat.

Treatment Schedule: Once daily x 5, beginning 4 hr post-virus inoculation.
Treatment Route: i.v., i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS5581	125	5/5	1.9	0/10	9.4	7.5
	62.5	4/4	1.7	0/10	8.5	7.2
	31.3	3/3	2.1	0/9	9.0	7.1
DMSO	-	-	-	0/20	8.7	7.3
Normals	-	5/5	2.3	-	-	

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 7. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS5581, a Pharmatec preparation, was inactive vs the Balliet PTV infection.

Table IV-7. Expt. PtA780. Effect of Once Daily i.v., i.p. Treatment with AVS5582 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 21.9 - 23.2 g (4 wk) Swiss Webster Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO, neat.

Treatment Schedule: Once daily x 5, beginning 4 hr post-virus inoculation.
Treatment Route: i.v., i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS5582	500	5/5	1.0	0/10	9.7	7.5
	250	5/5	1.8	0/10	7.9	7.3
	125	5/5	1.9	0/9	8.9	7.3
DMSO	-	-	-	0/20	8.7	7.3
Normals	-	5/5	2.3	-	-	

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 7. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS5582, a Pharmatec preparation, was inactive vs the Balliet PTV infection.

Table IV-8. Expt. PtA781. Effect of Once Daily i.v., i.p. Treatment with AVS5897 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 21.9 - 23.2 g (4 wk) Swiss Webster Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO, neat.

Treatment Schedule: Once daily x 5, beginning 4 hr post-virus inoculation.
Treatment Route: i.v., i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS5897	200	2/2	-2.2	0/2	7.0	7.4
	100	5/5	-1.8	0/8	8.1	7.2
	50	5/5	0.6	0/10	8.2	6.9
DMSO	-	-	-	1/19	8.4	7.3
Normals	-	5/5	1.2	-	-	

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 7. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS5897, a Pharmatec preparation, was inactive vs the Balliet PTV infection.

Table IV-9. Expt. PtA795. Effect of Once Daily i.v., i.p. Treatment with AVS6080 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 21.9 - 23.2 g (4 wk) Swiss Webster Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO, neat.

Treatment Schedule: Once daily x 5, beginning 4 hr post-virus inoculation.
Treatment Route: i.v., i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS6080	100	5/5	0.8	0/6	8.7	7.5
	50	5/5	0.5	1/10	9.6*	6.9
	25	5/5	0.6	0/8	8.0	7.1
DMSO	-	-	-	0/16	7.8	7.1
Normals	-	5/5	2.1	-	-	0.6

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 7. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS6080, a Pharmatec preparation, was possibly weakly active vs the Balliet PTV infection as seen by increased mean survival times.

Table IV-10. Expt. PtA796. Effect of Once Daily i.v. + i.p. Treatment with AVS6081 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 14.2 - 14.8 g (4 wk) Swiss Webster Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO, neat.
Treatment Schedule: Once daily x 5, beginning 4 hr post-virus inoculation.
Treatment Route: i.v., i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS6081	32	5/5	0.6	0/7	8.6	7.2
	16	5/5	1.4	0/9	7.8	7.3
	8	5/5	2.3	0/9	8.9	7.0
DMSO	-	-	-	0/16	7.8	7.1
Normals	-	5/5	2.1	-	-	0.6

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS6081 (Pharmatec compound) was inactive vs i.c. PTV infections in this experiment.

Table IV-11. Expt. PtA793. Effect of Once Daily i.v., i.p. Treatment with AVS6082 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 21.9 - 23.2 g (4 wk) Swiss Webster Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO, neat.

Treatment Schedule: Once daily x 5, beginning 4 hr post-virus inoculation.
Treatment Route: i.v., i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS6082	75	3/3	0.2	3/7**	8.8	6.1*
	37.5	5/5	0.1	0/10	8.8	6.6
	18.8	5/5	0.9	0/10	7.6	7.3
DMSO	-	-	-	0/20	7.9	7.1
Normals	-	5/5	1.7	-	-	0.6

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 7. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS6082, a Pharmatec preparation, was signficantly inhibitory to the Balliet PTV infection at the highest dosage used. This activity was seen as increased survivors and decreased brain virus titers.

Table IV-12. Expt. PtA794. Effect of Once Daily i.v., i.p. Treatment with AVS6083 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 21.9 - 23.2 g (4 wk) Swiss Webster Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO, neat.

Treatment Schedule: Once daily x 5, beginning 4 hr post-virus inoculation.
Treatment Route: i.v., i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS6083	32	4/4	1.3	0/7	8.3	7.5
	16	5/5	1.0	0/10	8.0	6.9
	8	5/5	0.5	0/10	7.1	7.1
DMSO	-	-	-	0/20	7.9	7.1
Normals	-	5/5	1.7	-	-	0.6

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 7. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS6083, a Pharmatec preparation, was inactive vs the Balliet PTV infection.

Table IV-13. Expt. PtA805. Effect of Once Daily i.v., i.p. Treatment with AVS6290 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 21.9 - 23.2 g (4 wk) Swiss Webster Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO, neat.
Treatment Schedule: Once daily x 5, beginning 4 hr post-virus inoculation.
Treatment Route: i.v., i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS6290	158	3/4	1.3	0/5	8.8	7.1
	79	5/5	1.6	0/9	8.4	7.4
	39.5	5/5	2.0	0/10	8.4	7.1
DMSO	-	-	-	0/18	8.1	7.1
Normals	-	5/5	1.6	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 7. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS6290, a Phamatec preparation, was ineffective vs the Balliet PTV infection.

Table IV-14. Expt. PtA803. Effect of Once Daily i.v., i.p. Treatment with AVS6291 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 21.9 - 23.2 g (4 wk) Swiss Webster Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO, neat.

Treatment Schedule: Once daily x 5, beginning 4 hr post-virus inoculation.
Treatment Route: i.v., i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS6291	50	5/5	-0.2	0/7	8.0	7.0
	25	5/5	0.8	0/9	8.9	7.2
	12.5	5/5	1.6	0/10	7.9	7.0
DMSO	-	-	-	0/18	8.1	7.1
Normals	-	5/5	1.6	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 7. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS6291, a Pharmatec preparation, was ineffective vs the Baliiet PTV infection.

Table IV-15. Expt. PtA804. Effect of Once Daily i.v., i.p. Treatment with AVS6292 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 21.9 - 23.2 g (4 wk) Swiss Webster Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO, neat.

Treatment Schedule: Once daily x 5, beginning 4 hr post-virus inoculation.
Treatment Route: i.v., i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS6292	50	4/4	1.4	0/4	7.8	7.3
	25	5/5	2.5	0/9	7.9	7.1
	12.5	5/5	1.7	0/10	8.6	7.2
DMSO	-	-	-	0/18	8.1	7.1
Normals	-	5/5	1.6	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 7. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS6292, a Pharmatec preparation, was ineffective vs the Balliet PTV infection.

Table IV-16. Expt. PtA824. Effect of Once Daily i.v. + i.p. Treatment with AVS6297 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 19.1 - 22.2 g (4 wk) Swiss Webster Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO, neat.

Treatment Schedule: Once daily x 5, beginning 4 hr post-virus inoculation.
Treatment Route: i.v., i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS6297	25	5/5	1.3	0/9	7.4	6.9
	12.5	5/5	0.2	0/10	7.5	7.3
	6.25	5/5	0.5	0/7	7.1	7.4
DMSO	-	-	-	0/18	7.1	7.2
Normals	-	5/5	1.0	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS6297 (Pharmatec compound) was inactive vs i.c. PTV infections in this experiment.

Table IV-17. Expt. PtA825. Effect of Once Daily i.v. + i.p. Treatment with AVS6300 on Intracerebrally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 19.1 - 22.2 g (4 wk) Swiss Webster Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO, neat.

Treatment Schedule: Once daily x 5, beginning 4 hr post-virus inoculation.
Treatment Route: i.v., i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS6300	25	5/5	0.3	0/10	7.1	7.2
	12.5	5/5	1.3	0/10	7.7	7.5
	6.25	5/5	-0.2	0/9	7.4	7.5
DMSO	-	-	-	0/18	7.1	7.2
Normals	-	5/5	1.0	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS6300 (Pharmatec compound) was inactive vs i.c. PTV infections in this experiment.

V. COMPARISON OF THE EFFECTS OF AVS01, AVS02, AND AVS206 ON DAILY DISEASE PARAMETERS IN PUNTA TORO VIRUS-INFECTED MICE

Introduction

We have previously described in the last Annual Report the relative anti-PTV efficacies of the related compounds AVS01 (ribavirin), AVS02 (ribavirin triacetate) and AVS206 (ribamidine). Since that time, at the request of our COTR, one additional set of experiments were run in which a low dose of each compound, each dose selected to be approximately the LD50/10, was evaluated to determine their influence on daily development of hepatic icterus and virus titers in various tissues as well as on the usual PTV-associated decline in white blood cells. In this experiment, all treatments were administered orally in a single administration 24 hr after virus inoculation.

Materials and Methods

Animals: Three week-old C57BL/6 mice were obtained from Simonsen Laboratories (Gilroy, CA). All were quarantined and fed Wayne Mouse Chow and tap water as has been described previously.

Virus: Adames strain PTV as described earlier was used.

Compounds: AVS01, 02, and 206 were provided by Biological Research Faculty & Facility, Inc. All were dissolved in sterile water for this study.

Disease Parameter Assays: The disease parameters studied included hepatic icterus, scored from 0 (normal) to 4 (maximal discoloration); SGOT and SGPT, assayed by colorimetric test using kits from Sigma (St. Louis, MO); total white blood cells in heparinized blood using a hemocytometer; and viral titers in serum, liver, mesenteric lymph node, spleen, kidney, lung, spinal cord, and brain assayed using cytopathic endpoint in LLC-MK2 cells exposed to serial dilutions of homogenates of each tissue. Mice were perfused with sterile saline prior to tissues being taken for viral assay.

Experiment Design: Groups of PTV-infected were treated with AVS01 (41 mg/kg), AVS02 (71 mg/kg), AVS206 (82 mg/kg) or sterile water by gavage 24 hr post-virus inoculation. A total of 100 mice were used in each treatment group. Six animals (or less, if too many had died later in the experiment) in each group were killed on days 1-10, 13, 16, 19, and 22, and their blood and tissues described above in *Disease Parameters Assays* taken. One normal mouse was killed and similarly handled at each assay time as a control. All the organs from one of the six animals killed each day were placed in formalin for later histopathological examination. Ten infected, treated mice in each group were held the duration of the study and deaths recorded daily. Five sham-infected mice were treated with each drug to serve as toxicity controls. These mice, as well as untreated normal mice, were weighed prior to and 18 hr after treatment.

Results and Discussion

Effect on Prevention of Death: The results of treatment with these compounds on PTV-associated death in the mice are summarized in Table and Figure V-1. The placebo-treated virus control animals began dying on day 4 and continued dying until day 8, with a mean survival time of 5.2 days. All three compounds were essentially equally effective in preventing death. At the dosage used, all appeared well tolerated, although the toxicity control mice treated with AVS01 gained the least weight in this experiment.

Effect on Hepatic Icterus: (Table and Figure V-2). Liver scores developed rapidly in the virus control mice to reach peaks by 4 to 6 days after virus inoculation. Of the 3 compounds, treatment with AVS02 inhibited the development of liver scores to the greatest extent, with AVS01 being least effective.

Effect on SGOT and SGPT: (Tables and Figures V-3, 4). The initial development of both transaminase enzyme levels in the serum coincided well with the hepatic icterus seen, with both enzymes achieving maximal levels by day 3. The levels declined immediately thereafter for 3 days before rising again to high levels by day 7, after which all the mice had died. Treatment with all three drugs was effective in preventing these levels from elevating significantly, with AVS02 again performing best in keeping the enzymes at essentially normal levels.

Effects on White Blood Cell Counts: (Table and Figure V-5). Total white blood cells were assayed on days 1, 2, 4, 6, 8, 10, 13, 16, 19, and 22. As has been observed previously, these cells decline by day 2 of infection in virus control mice, presumably due to destruction of the cells by the PTV, since we have previously isolated virus from these cells. This decline was lessened by all drugs, although AVS01-treated animals exhibited the most steady increase. It should be noted that the single normal control killed and assayed at each sampling time tended to exhibit considerable fluctuation, presumably due to the method of cell counting which can tend to be somewhat inaccurate.

Effects on Serum Virus Titers: (Table and Figure V-6). Viremia was seen in these s.c.-inoculated mice by the first day after virus inoculation, increasing to maximal levels exceeding 6 $\log_{10}$ by day 2 and maintaining at high levels until death of the animal. A similar, rapidly developing viremia occurred in each treated group of mice, with the titers reaching the same high level, but then rapidly declining to low or undetectable levels by day 5. Only AVS01-treated mice still exhibited approximately one $\log_{10}$ of virus on days 5 and 6. It should be pointed out that the single treatments given were done orally on day 1, after virus titers were already high in the serum.

Effects on Liver Virus Titers: (Table and Figure V-7). The virus titers in the livers of virus control mice increased dramatically by infection day 2 and maintained at high levels until the animals had all died. The virus titers also increased in the livers despite treatment with all three drugs but after 2 days these declined rapidly to low or below detectable levels by day 5. Only AVS01-treated mice showed relatively high virus titers (1.2 $\log_{10}$) by days 5 and 6.

Effects on Spleen Virus Titers: (Table and Figure V-8). The spleen virus titers developed in a pattern similar to that seen in the liver in the control mice. In the drug-treated group, the virus also developed, but to a somewhat lower maximal level and then they declined rather slowly to undetectable levels by about day 10. In this study, AVS206 was somewhat more effective than the other compounds used. In mice treated with AVS01, some virus was still seen at less than 1 $\log_{10}$ titers at the 16, 19, and 22 day sampling periods.

Effects on Kidney Virus Titers: (Table and Figure V-9). Kidney virus was initially seen in the control mice on day 2 of the infection. The mean virus titers at this time, which were maximal then, were approximately 4 $\log_{10}$. The virus titers in all drug-treated groups were initially one-half to over 1 $\log_{10}$ lower than those in the control mice, and, after the initial peaks seen on days 2 or 3, declined to low or undetectable levels by day 5. AVS01-treated mice continued to have detectable virus in their kidneys at 4 different assay times after the titers in mice treated with the other two drugs were below the limits of detection.

Effects on Lung Virus Titers: (Table and Figure V-10). Lung virus titers developed in the virus control mice essentially as in the other tissues, although did not achieve the same high titers. Therapy with the 3 drugs again had a similar effect as above, with AVS01 still having slightly less efficacy.

Effects on Mesenteric Lymph Node Virus Titers: (Table and Figure V-11). The virus titers in the lymph node tissue developed at the same rate as other tissues but to lower titers not exceeding 2.5 $\log_{10}$. Drug treatment lowered the titers to limits of detection, although only AVS206 kept the titers below detectable limits.

Effects on Spinal Cord Virus Titers: (Table and Figure V-12). We have previously found the hepatotropic PTV to penetrate brain tissues when inoculated s.c., as described in the 1989 4th Quarterly Report. It was interesting to find that the virus was also seen in the spinal cord beginning by one day after infection. This very early occurrence of virus in this tissue suggests to us that the spinal cord itself may not have been infected, but the spinal fluid surrounding the cord would be infected since the viremia in the animal occurs very early in the infection. Perfusion of the tissue would not affect the virus in the spinal fluid. Treatment with each compound reduced the recoverable virus titers, but only AVS206 kept the virus below detectable limits.

Effects on Brain Virus Titers: (Table and Figure V-13). Brain virus titers were seen by day 2 of the infection; this was quite surprising, since it has been assumed that if the virus developed in the brain it would probably do so rather late in the infection. These data suggest that, although the animals were perfused, there was either virus from the spinal fluid contaminating the brain tissues, or virus in the blood remaining in smaller capillaries in the brain. Treatment with all three

drugs eliminated all detectable virus by day 7; again, in view of the poor penetration of neuronal tissues by these drugs, this would suggest an effect of eliminating the viremia rather than virus in the brain tissues.

Conclusions

Mice infected with PTV rapidly developed a viremia with virus titers exceeding 10^6 being recovered from placebo-treated mice by 2 days after virus inoculation. Virus was similarly recovered from livers, lungs, spleens, kidneys, mesenteric lymph nodes, spinal cord, and brains from these same animals at about the same time periods as viral recovery from the serum. White blood cells declined in number with the development of the infection, and hepatic icterus increased concomitantly, together with SGOT and SGPT levels. A single p.o. treatment given 24 hr post-virus inoculation with LD50/16 dosages of AVS01, 02, or 206 prevented the PTV-associated death of the mice and significantly lowered the already developing viral titers in the blood and all tissues. In this experiment, AVS01 was least effective in keeping the virus below detectable limits. AVS206 was consistently most effective.

Table and Figure V-1. Expt. PtA771-773. Effect of Single p.o. Treatment with AVS01, AVS02, or AVS206 on Punta Toro Virus Infections in Mice (Death and Weight Loss Parameters).

Animals: 10.0-11.4 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: p.o.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS01	41	5/5	0.2	9/10**	7.0
AVS02	71	5/5	0.6	9/10**	5.0
AVS206	82	5/5	0.5	10/10**	>21.0**
H_2O	-	-	-	2/20	5.2
Normals	-	5/5	0.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

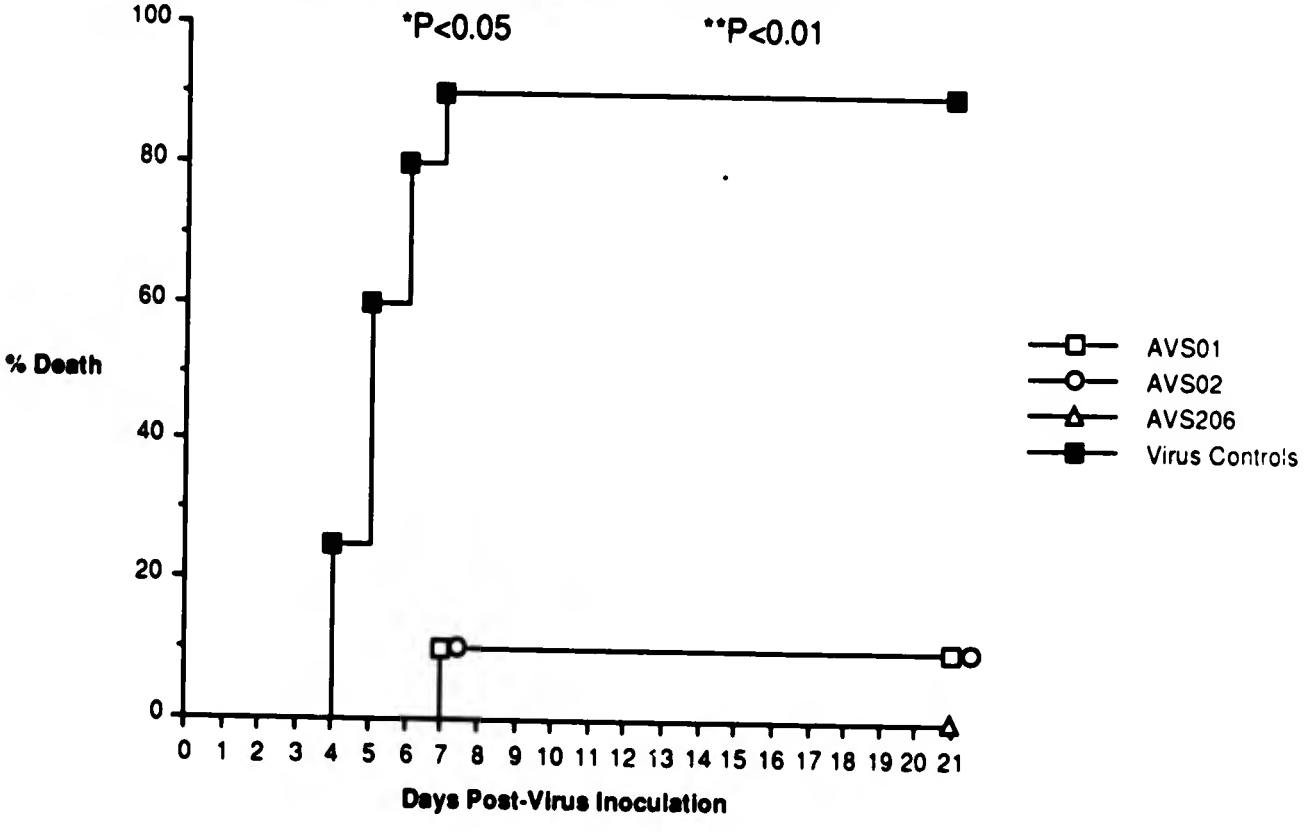


Table and Figure V-2. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Hepatic Icterus in Punta Toro Virus-Infected Mice.

Animals: 10.0-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Mean Liver Score[a] (Geometric mean of 5 animals for each treatment)

Compound	Dosage (mg/kg/day)	Day Post-Infection 1	2	3	4	5	6	7	8	9	10	13	16	19	22
AVS01	41	0.2	0.0	0.5	2.0	1.0	1.0	0.1	0.3	0.7	0.4	0.5	0.5	0.5	0.3
AVS02	71	0.6	0.8	0.7	1.0	0.5	0.3	0.4	0.4	0.5	1.0	0.0**	0.0**	0.2	0.8
AVS206	82	0.4	0.0	0.7	1.6	1.2	0.6	0.4	0.3	0.4	0.9	0.3	0.3	0.8	0.5
H_2O	-	0.5	0.8	2.0	2.9	2.1	3.2	2.7	AD[b]	—	—	—	—	—	—
Normals	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on specified day (animals dying prior to that day assigned a liver score of 4).

[b]All dead. All animals had died in this group by this time. Statistics after day 7 for treated group were compared to the day 7 virus titer of H_2O controls.

*P<0.05 **P<0.01 as compared to the corresponding virus control data.

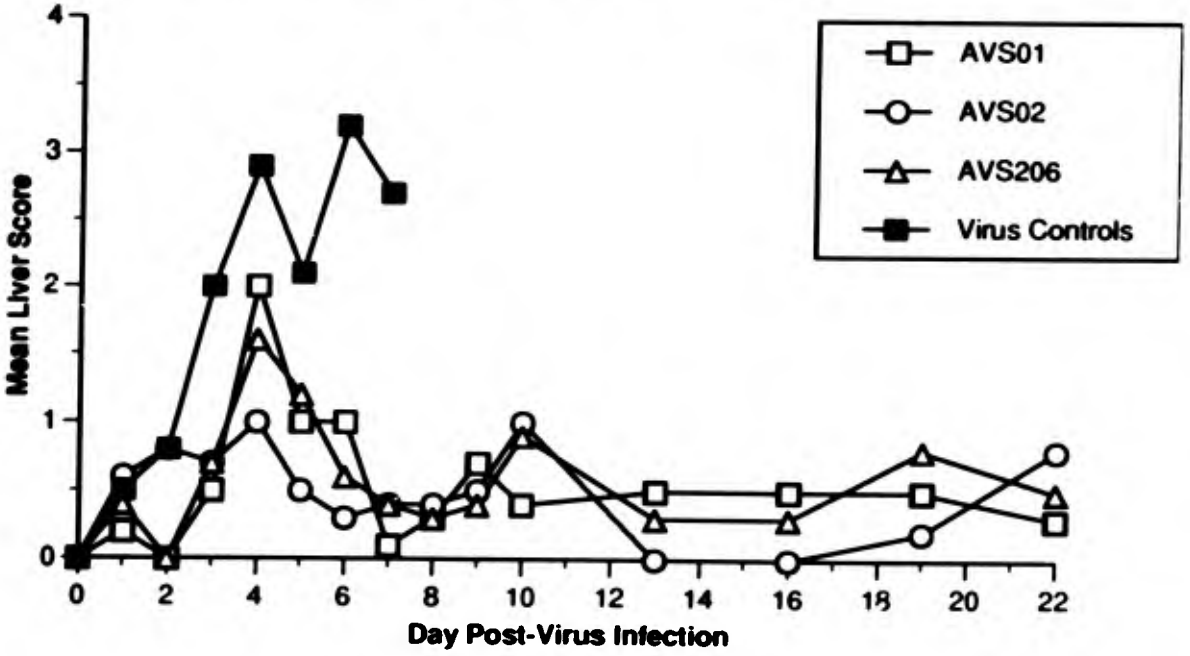


Table and Figure V-3. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on SGOT in Punta Toro Virus-Infected Mice.

Animals: 10.0-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Mean SGOT[a] (Geometric mean of 6 animals for each treatment)

Compound	Dosage (mg/kg/day)	Day Post-Infection 1	2	3	4	5	6	7	8	9	10	13	16	19	22
AVS01	41	132	138	2607*	430**	491	359*	117*	394*	125*	100*	86*	122*	205*	196*
AVS02	71	104	38*	1000**	228**	183**	134**	166*	144*	101*	114*	88*	128*	121*	30*
AVS206	82	226	35*	2360	555**	228**	289*	164*	118*	133*	108*	112*	121*	116*	133*
H_2O	-	160	230	6525	1562	905	1590	5429	AD[b]	—	—	—	—	—	—
Normals	-	52	—	452	110	120	66	146	212	131	120	338	82	—	129

[a]Sigma-Frankel units/ml.

[b]All dead. All animals had died in this group by this time. Statistics after day 7 for treated group were compared to the day 7 virus titer of H_2O controls.

*P<0.05 **P<0.01 as compared to the corresponding virus control data.

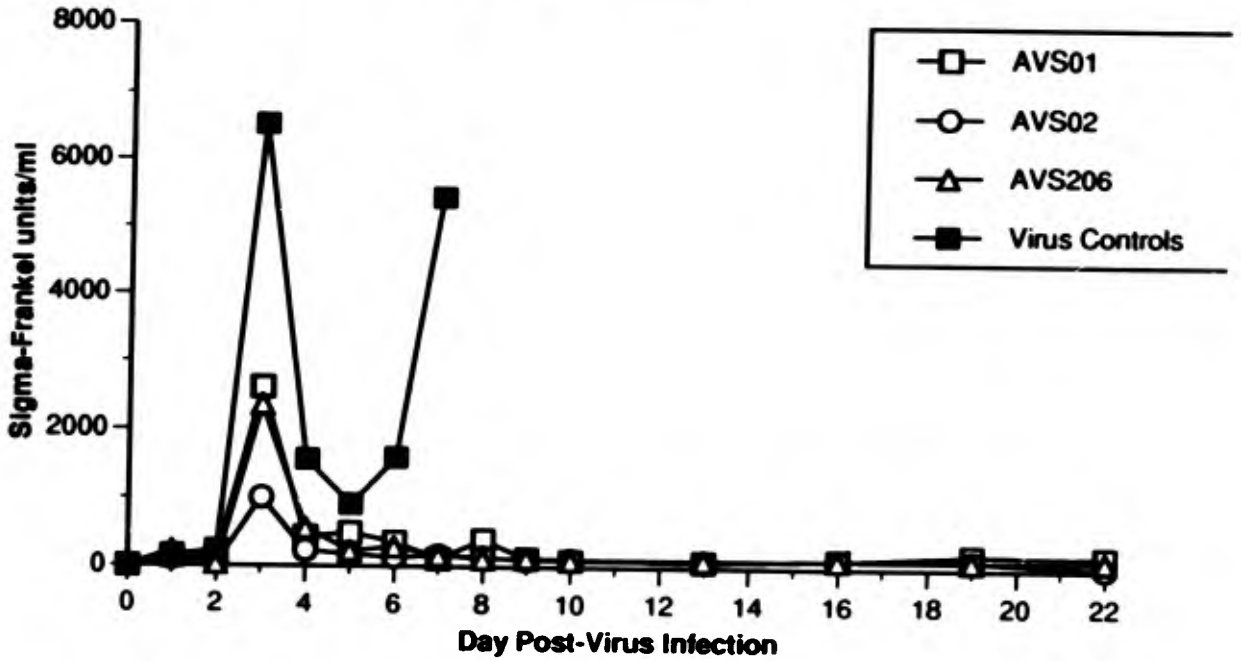


Table and Figure V-4. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on SGPT in Punta Toro Virus-Infected Mice.

Animals: 10.0-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Mean SGPT[a] (Geometric mean of 6 animals for each treatment)

Compound	Dosage (mg/kg/day)	Day Post-Infection 1	2	3	4	5	6	7	8	9	10	13	16	19	22
AVS01	41	23	46	1918*	511**	414	257*	37*	261	35*	22*	26*	27*	34*	35*
AVS02	71	28	38*	1000**	219**	80**	55**	86*	32*	26*	30*	24*	33*	36*	30*
AVS206	82	56	35*	2360	685*	120**	137**	45*	32*	34*	30*	29*	24*	32*	31*
H_2O	-	33	82	5050	1825	799	1020	3441	AD[b]	—	—	—	—	—	—
Normals	-	12	—	110	21	16	10	36	49	44	22	56	20	—	31

[a]Sigma-Frankel units/ml.

[b]All dead. All animals had died in this group by this time. Statistics after day 7 for treated group were compared to the day 7 virus titer of H_2O controls.

*P<0.05 **P<0.01 as compared to the corresponding virus control data.

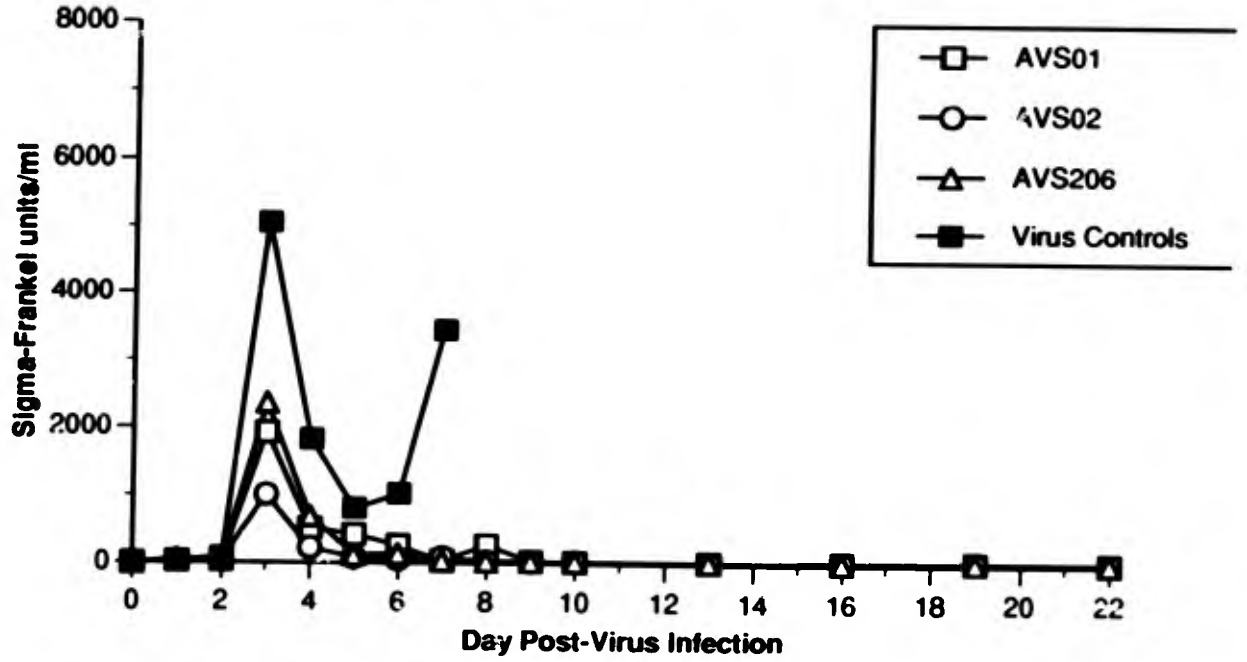


Table and Figure V-5. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on White Blood Cell Counts in Punta Toro Virus-Infected Mice.

Animals: 10.0-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Mean White Blood Cell Count[a] (Geometric mean of 3 animals for each treatment)

Compound	Dosage (mg/kg/day)	Day Post-Infection 1	2	4	6	8	10	13	16	19	22
AVS01	41	127 (330)	124 (53)	184 (188)	216 (120)	215**(185)	232**(196)	237**(277)	321**(50)	282*(250)	216**(227)
AVS02	71	138 (141)	186 (55)	133 (149)	108**(89)	250*(266)	304**(338)	214**(230)	392**(85)	173 (135)	248**(485)
AVS206	82	178 (138)	78*(64)	130 (145)	259**(60)	210**(108)	341**(162)	134*(184)	316*(209)	258**(172)	348**(176)
H_2O	-	154	36	136	32	AD[a]	—	—	—	—	—
Normals	-	242	90	179	77	156	346	267	55	395	360

[a]Number in () is count for toxicity controls, mean of 2 animals.

[b]All dead. All animals had died in this group by this time. Statistics after day 6 for treated group were compared to the day 6 count of H_2O controls.

*P<0.05 **P<0.01 as compared to the corresponding virus control data.

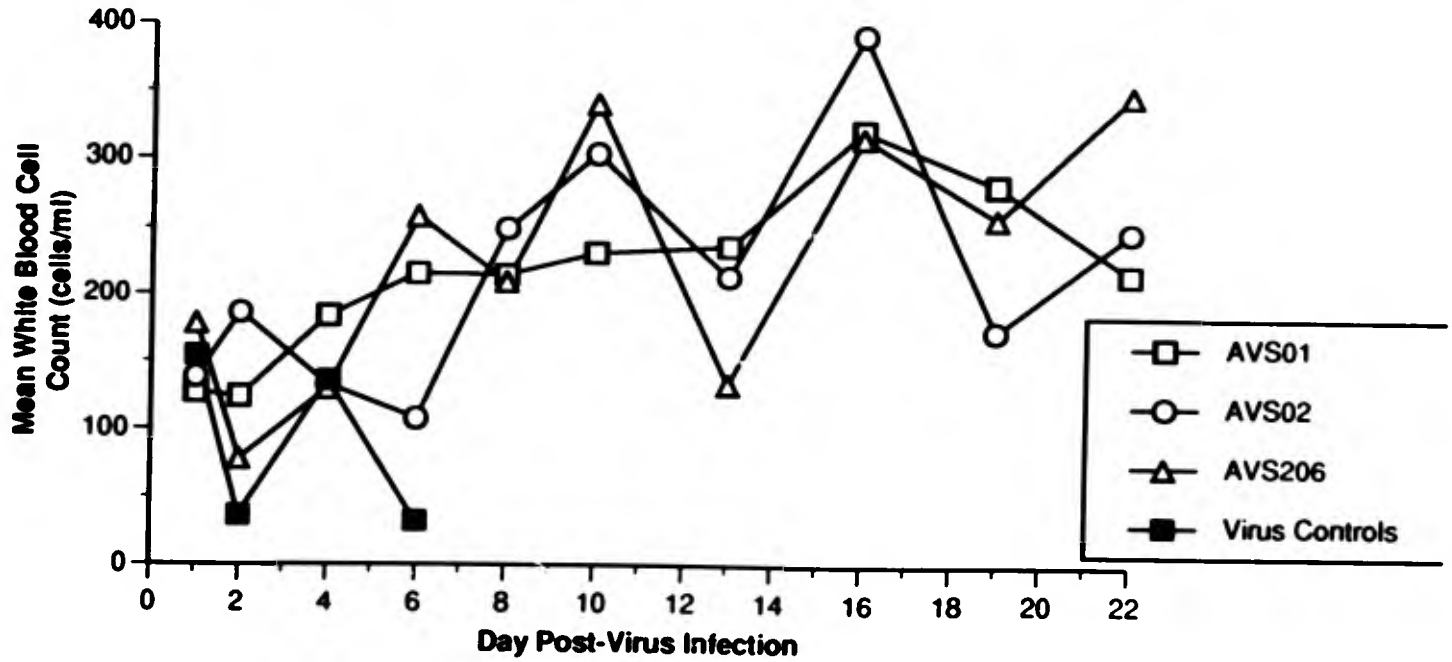


Table and Figure V-6. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Serum Virus Titer in Punta Toro Virus-Infected Mice.

Animals: 10.0-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Mean Serum Virus Titer (log_{10}) (Geometric mean of 5 animals for each treatment)

Compound	Dosage (mg/kg/day)	Day Post-Infection 1	2	3	4	5	6	7	8	9	10	13	16	19	22
AVS01	41	2.4	6.3	5.7*	0.8**	0.9**	1.1**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**
AVS02	71	4.7	6.4	5.5**	1.3**	0.0**	0.0**	0.0**	0.0**	0.0**	0.3**	0.0**	0.0**	0.0**	0.0**
AVS206	82	4.7	6.5	5.1**	0.9**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**
H_2O	-	3.6	6.5	6.5	5.9	5.4	6.0	4.4	AD[a]	—	—	—	—	—	—
Normals	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a]All dead. All animals had died in this group by this time. Statistics after day 7 for treated group were compared to the day 7 virus titer of H_2O controls.

*P<0.05 **P<0.01 as compared to the corresponding virus control data.

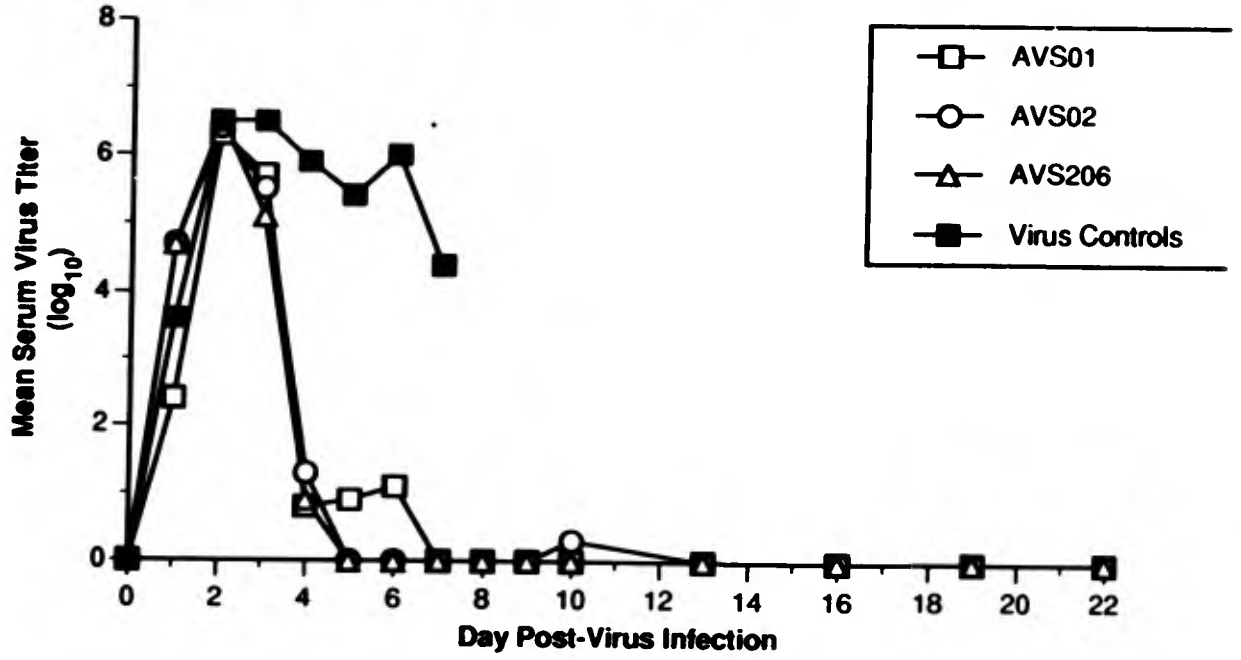


Table and Figure V-7. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Liver Virus Titer in Punta Toro Virus-Infected Mice.

Animals: 10.0-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Mean Liver Virus Titer (log_{10}) (Geometric mean of 5 animals for each treatment)

Compound	Dosage (mg/kg/day)	Day Post-Infection 1	2	3	4	5	6	7	8	9	10	13	16	19	22
AVS01	41	0.7	5.7	3.9	1.4**	1.6**	2.5**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**
AVS02	71	0.0	4.5	4.8	2.3**	0.0**	0.0**	0.5*	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**
AVS206	82	0.5	5.0*	4.4	1.6**	0.0**	0.0**	0.0**	0.5*	0.0**	0.0**	0.5*	0.0**	0.0**	0.7*
H_2O	-	0.5	5.8	5.1	5.4	5.8	6.6	5.5	AD[a]	—	—	—	—	—	—
Normals	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a]All dead. All animals had died in this group by this time. Statistics after day 7 for treated group were compared to the day 7 virus titer of H_2O controls.

*P<0.05 **P<0.01 as compared to the corresponding virus control data.

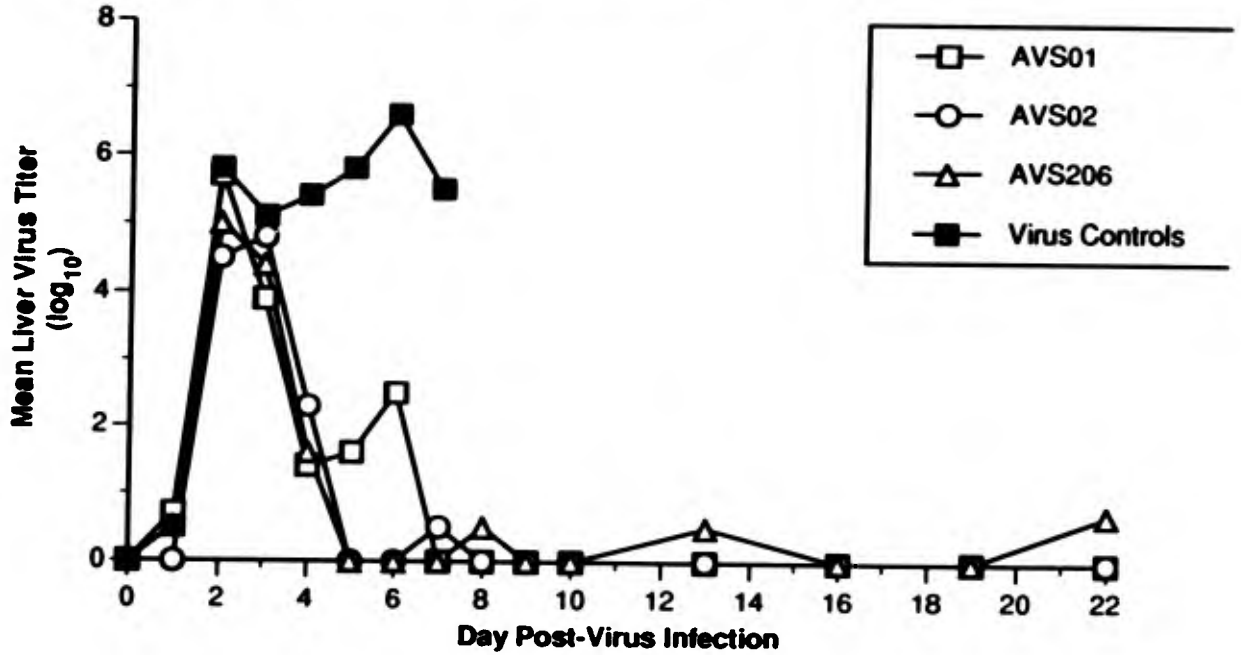


Table and Figure V-8. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Spleen Virus Titer in Punta Toro Virus-Infected Mice.

Animals: 10.0-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Mean Spleen Virus Titer (log_{10}) (Geometric mean of 5 animals for each treatment)

Compound	Dosage (mg/kg/day)	Day Post-Infection 1	2	3	4	5	6	7	8	9	10	13	16	19	22
AVS01	41	0.0	4.8*	4.6	4.0	4.0	2.0	2.4**	2.4**	0.7**	0.0**	0.0**	0.0**	0.5**	0.3**
AVS02	71	1.4	4.8	3.8**	4.2	3.2	1.7*	2.1**	1.7**	1.0**	0.3**	0.7**	0.0**	0.0**	0.0**
AVS206	82	2.7	3.4**	4.1**	3.1	3.1**	1.2*	1.7**	1.2**	0.0**	0.3**	0.0**	0.0**	0.0**	0.0**
H_2O	-	0.5	5.7	5.2	4.5	4.6	4.2	5.2	AD[a]	—	—	—	—	—	—
Normals	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a]All dead. All animals had died in this group by this time. Statistics after day 7 for treated group were compared to the day 7 virus titer of H_2O controls.

*P<0.05 **P<0.01 as compared to the corresponding virus control data.

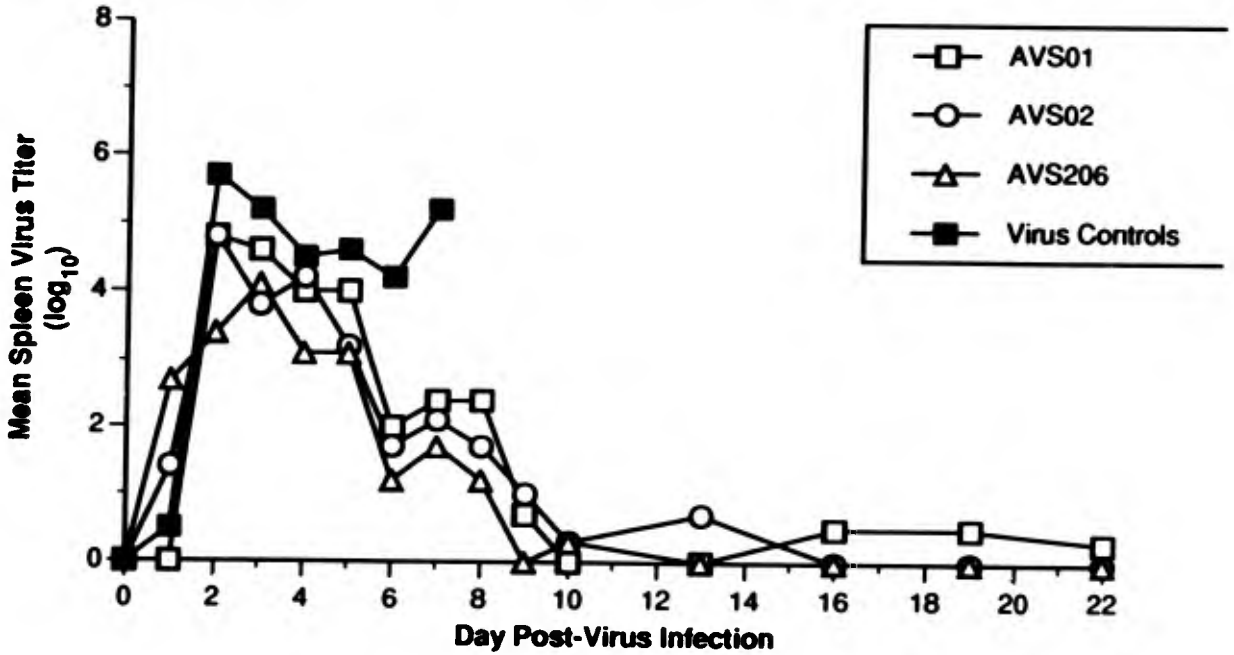


Table and Figure V-9. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Kidney Virus Titer in Punta Toro Virus-Infected Mice.

Animals: 10.0-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Mean Kidney Virus Titer (log_{10}) (Geometric mean of 5 animals for each treatment)

Compound	Dosage (mg/kg/day)	Day Post-Infection 1	2	3	4	5	6	7	8	9	10	13	16	19	22
AVS01	41	0.6	3.3**	2.4*	0.8**	0.3**	0.5**	0.4**	0.4**	0.0**	0.0**	1.5*	0.0**	0.0**	0.0**
AVS02	71	0.0	3.6	2.6*	1.5*	0.3**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**
AVS206	82	0.0	2.4*	2.6*	2.0	0.3**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**
H_2O	-	0.0	4.4	3.9	3.3	3.4	3.1	3.5	AD[a]	—	—	—	—	—	—
Normals	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a]All dead. All animals had died in this group by this time. Statistics after day 7 for treated group were compared to the day 7 virus titer of H_2O controls.

*P<0.05 **P<0.01 as compared to the corresponding virus control data.

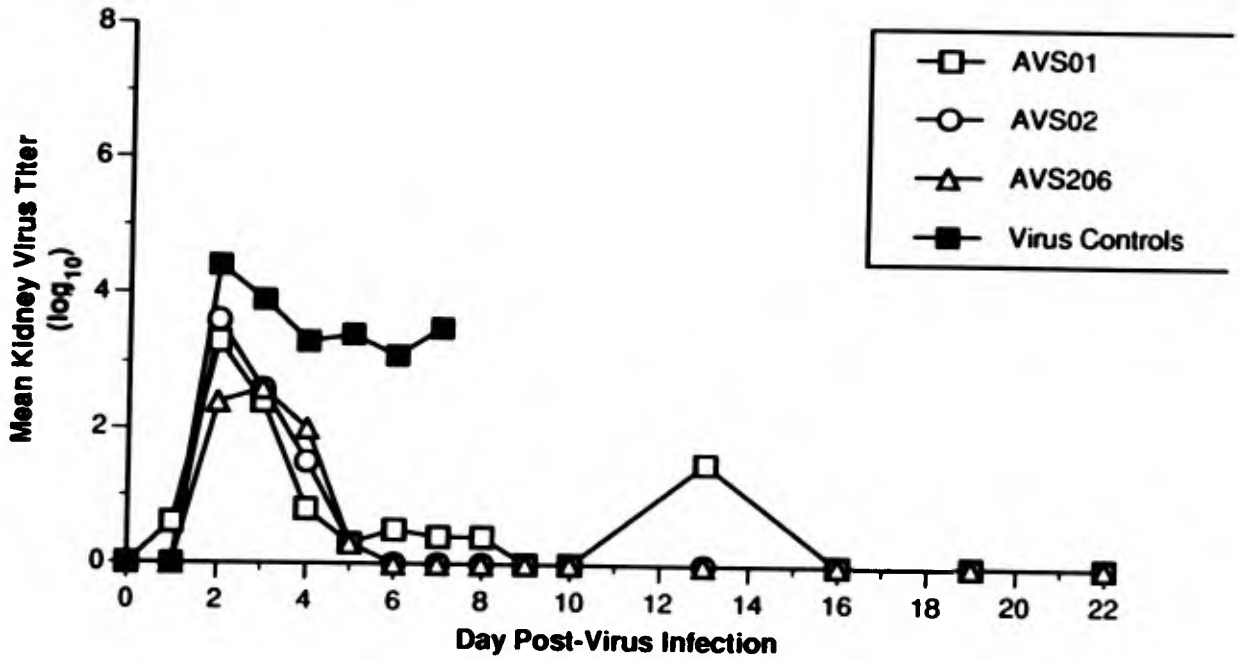


Table and Figure V-10. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Lung Virus Titer in Punta Toro Virus-Infected Mice.

Animals: 10.0-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Mean Lung Virus Titer (log_{10}) (Geometric mean of 5 animals for each treatment)

Compound	Dosage (mg/kg/day)	Day Post-Infection 1	2	3	4	5	6	7	8	9	10	13	16	19	22
AVS01	41	1.1	3.8	2.9*	0.8	0.3**	0.6**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVS02	71	0.0	3.5**	1.4**	1.2	0.0**	0.0**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVS206	82	0.0	2.9	0.8**	1.5	0.0**	0.0**	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
H_2O	-	0.0	4.6	3.6	2.6	3.7	2.9	2.7	AD[a]	—	—	—	—	—	—
Normals	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a]All dead. All animals had died in this group by this time. Statistics after day 7 for treated group were compared to the day 7 virus titer of H_2O controls.

*P<0.05 **P<0.01 as compared to the corresponding virus control data.

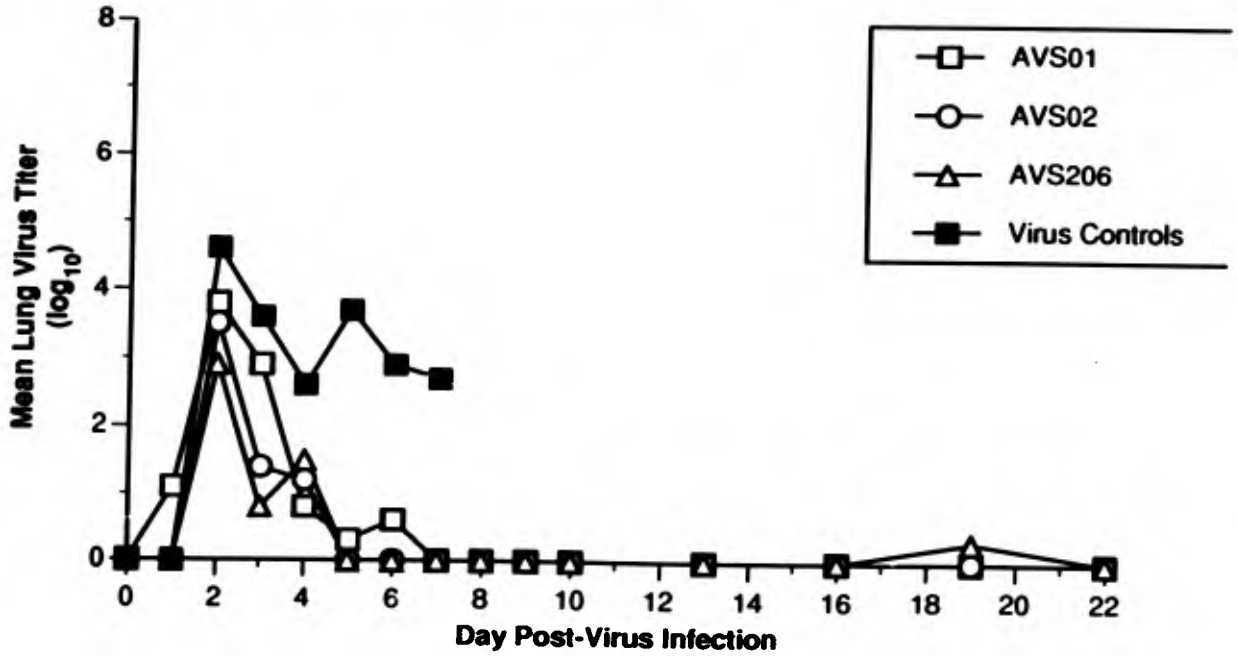


Table and Figure V-11. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Mesenteric Lymph Node Virus Titer in Punta Toro Virus-Infected Mice.

Animals: 10.0-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Mean Mesenteric Lymph Node Virus Titer (log_{10}) (Geometric mean of 5 animals for each treatment)

Compound	Dosage (mg/kg/day)	Day Post-Infection 1	2	3	4	5	6	7	8	9	10	13	16	19	22
AVS01	41	0.5	2.3	1.6	0.6	0.0**	0.3**	0.6	0.6	0.2*	0.0*	0.6	0.8	0.9	0.9
AVS02	71	1.2	1.2	1.9	0.8	0.9	0.3**	0.0*	0.2*	0.0*	0.6	1.6	0.3	0.0*	0.0*
AVS206	82	0.8	2.4	1.9**	1.2	0.0**	0.0**	0.9	0.3	0.0*	0.0*	0.0*	0.0*	0.0*	0.0*
H_2O	-	0.6	2.9	2.7	0.9	2.7	2.1	2.4	AD[a]	—	—	—	—	—	—
Normals	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a]All dead. All animals had died in this group by this time. Statistics after day 7 for treated group were compared to the day 7 virus titer of H_2O controls.

*P<0.05 **P<0.01 as compared to the corresponding virus control data.

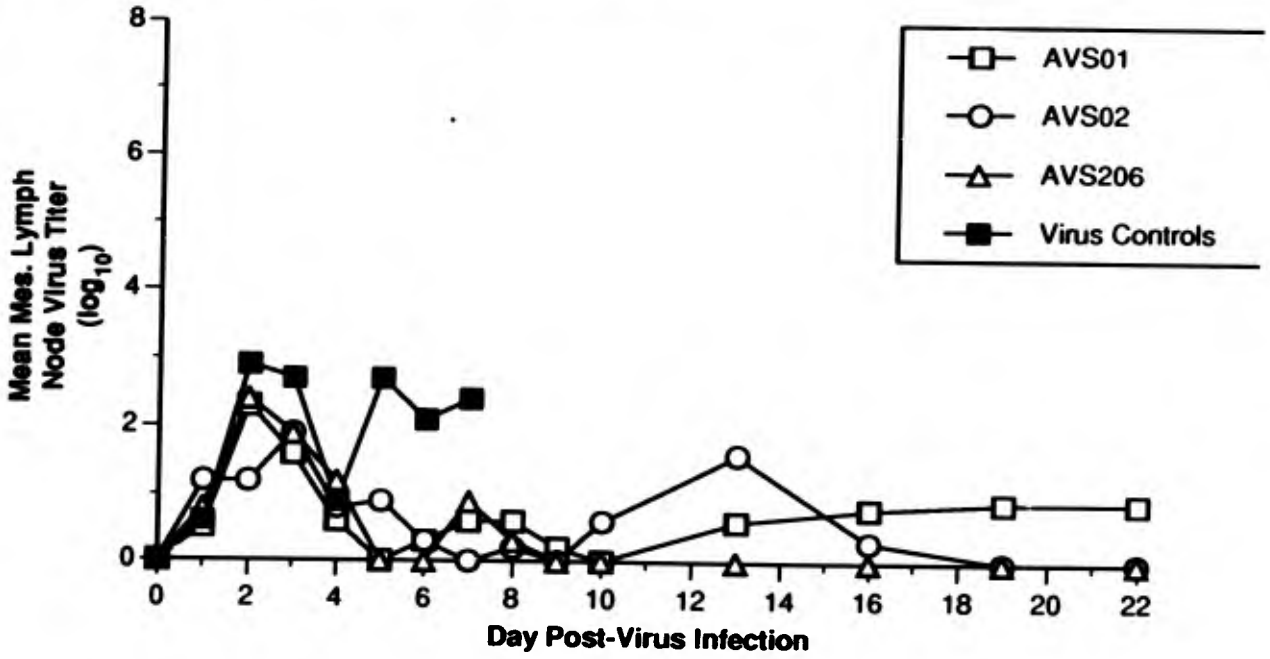


Table and Figure V-12. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Spinal Cord Virus Titer in Punta Toro Virus-Infected Mice.

Animals: 10.0-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: Once only, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Mean Spinal Cord Virus Titer (log_{10}) (Geometric mean of 5 animals for each treatment)

Compound	Dosage (mg/kg/day)	Day Post-Infection 1	2	3	4	5	6	7	8	9	10	13	16	19	22
AVS01	41	0.7*	3.3	2.2*	0.0	0.7**	1.2*	0.4*	0.0*	0.8	0.0*	0.0*	0.0*	0.0*	0.9
AVS02	71	0.5*	3.4	0.4**	0.0	0.3**	0.6**	0.4*	0.6	0.9	0.9	1.0	1.7	1.6	0.0*
AVS206	82	0.4*	2.3*	0.3**	0.4	0.0**	0.8**	0.0*	0.0*	0.0*	0.0*	0.0*	0.0*	0.0*	0.0*
H_2O	-	1.9	4.2	4.4	0.8	4.3	3.3	3.6	AD[a]	—	—	—	—	—	—
Normals	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a]All dead. All animals had died in this group by this time. Statistics after day 7 for treated group were compared to the day 7 virus titer of H_2O controls.

*P<0.05 **P<0.01 as compared to the corresponding virus control data.

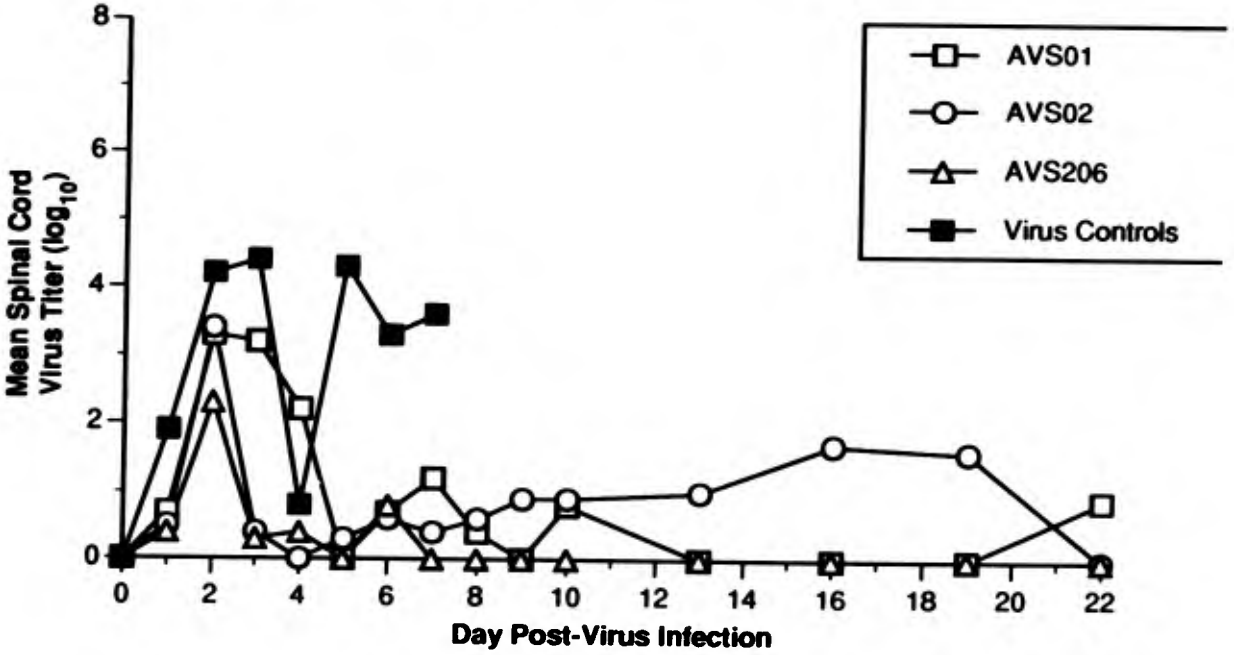


Table and Figure V-13. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Brain Virus Titer in Punta Toro Virus-Infected Mice.

Animals: 10.0-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

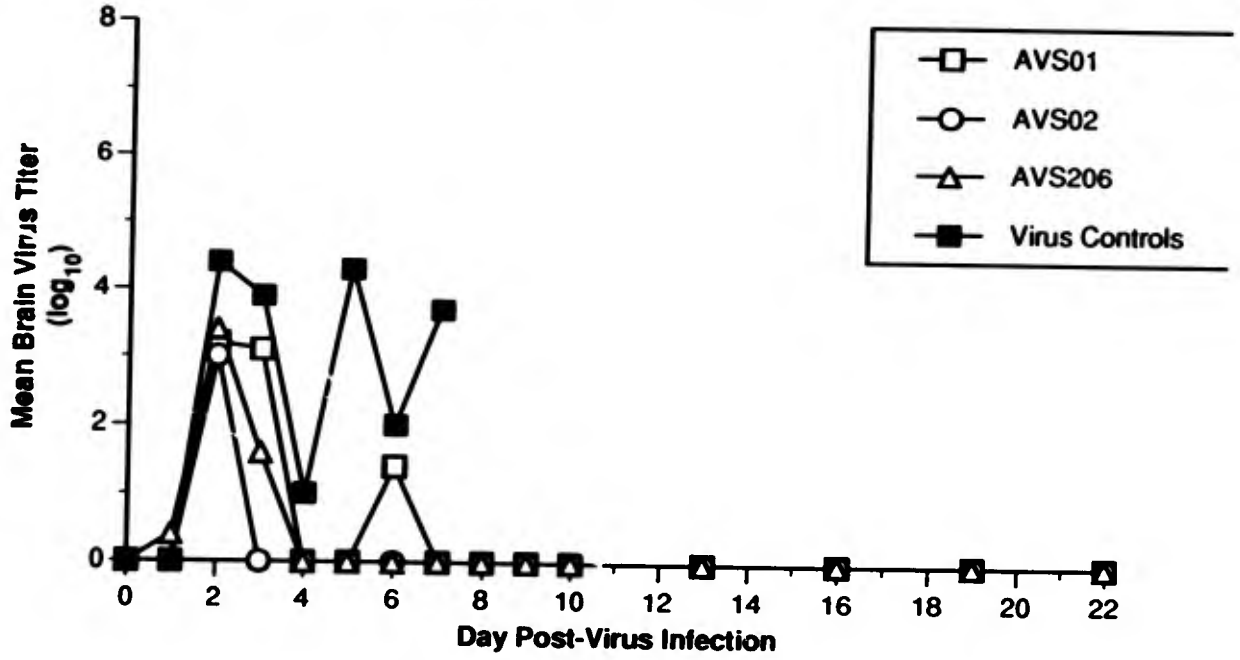
Treatment Schedule: Once only, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Mean Brain Virus Titer (log_{10}) (Geometric mean of 5 animals for each treatment)

Compound	Dosage (mg/kg/day)	Day Post-Infection 1	2	3	4	5	6	7	8	9	10	13	16	19	22
AVS01	41	0.0	3.2	3.1	0.0	0.0**	1.4	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**
AVS02	71	0.0	3.0	0.0**	0.0	0.0**	0.0*	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**
AVS206	82	0.4	3.4	1.6*	0.0	0.0**	0.0*	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**
H_2O	-	0.0	4.4	3.9	1.0	4.3	2.0	3.7	AD[a]	—	—	—	—	—	—
Normals	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a]All dead. All animals had died in this group by this time. Statistics after day 7 for treated group were compared to the day 7 virus titer of H_2O controls.

*P<0.05 **P<0.01 as compared to the corresponding virus control data.



VI. EFFECT OF A COMBINATION OF AVS01 AND AVS5587 ON *IN VIVO* PUNTA TORO VIRUS INFECTIONS

Introduction

In the 1989 Annual Report, we described the strong anti-PTV effects of AVS5587 (7-thia-8-oxoguanosine), an immune modulating compound with antiviral activity also against other virus infections (1). It was found the compound was effective when given i.p. to PTV infected mice in a divided dose as late as 36 and 43 hr after virus inoculation. In view of this activity, this compound appeared to be a likely candidate to be used in combination with AVS01 (ribavirin) against the PTV infection.

Two approaches were used in the design of the experiment: 1) To attempt to reduce the toxicity of a high, usually lethal dose of ribavirin using AVS5587, and 2) to enhance ribavirin's antiviral activity *in vivo* using AVS5587.

This report describes our experiment investigating this drug combination.

Materials and Methods

Virus: Adames strain PTV as described earlier was used.

Animals: Female 3 week old C57BL/6 mice weighing 10-13 g were obtained from Simonsen. Quarantine, caging, and feeding of these mice was as described in previous sections.

Compounds: AVS01 and AVS5587 were provided by Biological Research Faculty and Facility, Inc. AVS01 was dissolved in water and AVS5587 was dissolved in a 2% bicarbonate solution at pH 8.6-8.9 (1).

Experiment Design: AVS01 was administered p.o. twice daily for 3 days beginning 24 hr post-virus inoculation. AVS5587 was injected i.p. 24 and 31 hr post-virus inoculation. Both treatment regimens have been previously shown by us to be highly effective vs PTV infections in mice. A total of 5 experiments were run in parallel, as follows:

#1 (PtA 774): AVS01 only, at dosages of 1250, 25, 12.5 and 6.25 mg/kg/day. These dosages were selected because the high dose is known to be somewhat lethally toxic to mice and the lower doses were generally below the acceptable active dose of the compound.

#2 (PtA 775): AVS5587 only, at dosages of 25, 12.5, and 6.25 mg/kg/day. these dosages were previously found by us to be marginally effective or inactive against the virus infection.

#3 (PtA 776): AVS01 at dosages used in #1 + AVS5587 at 25 mg/kg/day.

#4 (PtA 777): AVS01 at dosages used in #1 + AVS5587 used at 12.5 mg/kg/day.

#5 (PtA 778): AVS01 at doses used in #1 + AVS5587 at 6.25 mg/kg/day.

An expanded parameter anti-PTV experiment was run in each study, the disease parameters being survivors, mean survival time, liver score, SGOT, SGPT, liver virus and serum virus titers, with 20 infected mice used in each treatment group, 40 mice used as placebo-treated controls, 20 mice as normal controls and 5 animals in each treatment group as toxicity controls. One-half of each treatment group, virus controls, and normal controls were killed 4 days after virus inoculation, bled, and their livers removed. Livers were scored from 0 to 4, homogenates prepared from each, and the homogenates tested for virus titer. Serum was assayed for SGOT, SGPT, and PTV titers. The remainder of the mice were held 21 days post-virus inoculation with deaths noted daily

Results and Discussion

The overall results are summarized in Tables VI-1 to VI-5.

AVS01 at 1250 mg/kg/day was lethally toxic to 80% of the toxicity controls mice (Table VI-1). Anti-PTV activity was seen at the 25 mg/kg/day dosage only, reflected in essentially all evaluation parameters.

AVS5587 used alone (Table VI-2) was effective vs the disease using all parameters at the high dosage level; the 12.5 mg/kg/day dosage treatment prevented death of the mice but activity was surprisingly not manifested by other disease parameters. The low dose used of this immune modulator had no anti-PTV activity.

The use of 25 mg/kg/day of AVS5587 with the 1250 mg/kg/day dose of AVS01 (Table VI-3) resulted in an apparent decrease in lethal toxicity of the latter compound, with only 20%, as opposed to the 80% deaths occurring. This combination then resulted in a maximal antiviral effect in the mice, with literally no virus isolated from the animals, and liver scores, SGOT and SGPT levels reduced to normal values. At the lower levels of ribavirin, the drug combination could not be evaluated effectively since the AVS5587 at the 25 mg/kg/day dose was so active by itself.

The lower doses of AVS5587 had no apparent effect on reducing the toxicity of the high dose of AVS01, with all the toxicity control mice in this group dying. An unexpected observation, however, was the 100% survival of the infected mice treated with this drug combination. No virus could be isolated from the mice in this combination treatment group. At the lower AVS01 dosages, use of the 12.5 mg/kg/day dose of AVS5587 was apparently synergistic.

Using the 6.25 mg/kg/day dosage of AVS5587 with the high dosage of ribavirin (Table VI-5) again did not alleviate ribavirin's lethal toxicity, but again the PTV-infected mice treated with this combination all survived the infection and essentially al disease parameters were returned to normal values. The anti-PTV activity of the lower dosages of AVS01 were again enhanced by the combination of this lowest dosage of AVS5587.

These data are presented in a different fashion in Table VI-6, and the near synergistic to synergistic interactions of the two compounds are also presented in Figures VI-1 and VI-2.

Determinations of antagonistic, additive, or synergistic drug interaction were made by calculating fractional inhibitory concentration (FIC) indices, as was described by Allen et al. (2) and Berenbaum (3) and used in evaluating our previous combination experiments (see 1989 Annual Report, Sections XI and XII). Using this method, the interpretation of FIC indices in terms of drug interactions is as follows:

<0.5 = synergistic
0.5–0.89 = suggestive of synergy
0.8–1.2 = additive
1.2–2.0 = indifference to partial antagonism
>2.0 = antagonism

This is a slight modification of that given previously (2), in which additive drug interaction was defined with an FIC index of ~1.0 with indications of range around that value.

The FIC indices for this study are seen in the bar graphs VI-1 and VI-2. All indicate this drug combination to be synergistic or near-synergistic.

Conclusions

The combination of AVS01 (ribavirin) and AVS5587 (7-thia-8-oxoguanosine) was used against PTV infections in mice. A definite synergy of antiviral effect was seen. In addition, use of AVS5587 with a usually lethal dose of ribavirin reduced the ribavirin toxicity.

Table VI-1. Expt. PtA774. Effect of Twice Daily p.o. Treatments with AVS01 on Punta Torc Virus Infections in Mice (Part 1 of a 5 Part Combination).

Animals: 11.7-13.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: bid x 3, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01	1250	1/5	-1.5	2/10	8.8**	0.1**	8/10**(140**)	10/10**(25**)	0.0**	0.0**
	25	5/5	1.4	9/10**	7.0	1.4*	4/10(741)	2/10*(734)	2.4	2.0**
	12.5	4/5	1.5	0/10	4.8	3.2	0/10(4872)	0/10(5531)	5.3	6.1
	6.25	5/5	1.9	1/10	5.0	2.8	1/10(4887)	0/10(5810)	5.2	5.9
H_2O	-	-	-	5/20	4.8	2.4	4/20(753)	0/20(863)	3.8	4.1
Normals	-	5/5	2.4	-	-	0.0	5/5(81)	5/5(26)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

*P<0.05 **P<0.01

Table VI-2. Expt. PtA775. Effect of Two i.p. Treatments with AVS5587 on Punta Toro Virus Infections in Mice (Part 2 of a 5 Part Combination).

Animals: 11.7-13.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 2% $NaHCO_3$ in H_2O.

Treatment Schedule: Twice, 24 and 31 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS5587	25	5/5	0.5	8/10**	5.0	0.0**	10/10**(114**)	9/10**(73**)	0.0**	0.0**
	12.5	5/5	0.3	8/10**	7.5	2.0	1/10(742)	0/10(825)	2.9	3.1
	6.25	5/5	0.1	2/10	5.3	2.9	0/10(1196)	0/10(1373)	3.4	4.1
H_2O	-	-	-	5/20	4.8	2.4	4/20(753)	0/20(863)	3.8	4.1
Normals	-	5/5	2.4	-	-	0.0	5/5(81)	5/5(26)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

*P<0.05 **P<0.01

Table VI-3. Expt. PtA776. Effect of Combination Treatments with AVS01 and AVS5587 on Punta Toro Virus Infections in Mice (Part 3 of a 5 Part Combination).

Animals: 11.7-13.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 2% $NaHCO_3$ in H_2O.

Treatment Schedule: bid x 3 and twice, 24 and 31 hr post-virus inoculation.
Treatment Route: 01: p.o. 5587: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01 +	1250 + 25	4/5*[g]	-0.7	10/10**	>21.0**	0.2**	8/10**(169**)	10/10**(29**)	0.0**	0.2**
AVS5587	25 + 25	5/5	1.5	10/10**	>21.0**	0.2**	10/10**(112**)	9/10**(51**)	0.0**	0.0**
	12.5 + 25	5/5	1.0	10/10**	>21.0**	0.1**	7/10**(199**)	5/10**(153**)	1.2**	0.0**
	6.25 + 25	5/5	2.1	9/9**	>21.0**	0.7**	7/10**(201**)	7/10**(136**)	0.3**	0.4**
H_2O	-	-	-	5/20	4.8	2.4	4/20(753)	0/20(863)	3.8	4.1
Normals	-	5/5	2.4	-	-	0.0	5/5(81)	5/5(26)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

[g] As compared to AVS01 at 1250 mg/kg/day used alone.

*P<0.05 **P<0.01

Table VI-4. Expt. PtA777. Effect of Combination Treatments with AVS01 and AVS5587 on Punta Toro Virus Infections in Mice (Part 4 of a 5 Part Combination).

Animals: 11.7-13.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 2% $NaHCO_3$ in H_2O.

Treatment Schedule: bid x 3 and twice, 24 and 31 hr post-virus inoculation.
Treatment Route: 01: p.o. 5587: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01 +	1250 + 12.5	0/5	-2.2	9/9**	>21.0**	0.0**	6/10*(260**)	9/10**(51**)	0.0**	0.0**
AVS5587	25 + 12.5	5/5	2.0	10/10**	>21.0**	0.5**	5/9(231**)	6/9**(159**)	0.3**	0.0**
	12.5 + 12.5	5/5	1.1	7/9**	6.0	2.7	3/8(708)	3/8*(609)	2.7	3.2
	6.25 + 12.5	5/5	2.2	9/10**	5.0	1.7	2/9(488)	2/9*(417*)	1.7*	2.4*
H_2O	-	-	-	5/20	4.8	2.4	4/20(753)	0/20(863)	3.8	4.1
Normals	-	5/5	2.4	-	-	0.0	5/5(81)	5/5(26)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Table VI-5. Expt. PtA778. Effect of Combination Treatments with AVS01 and AVS5587 on Punta Toro Virus Infections in Mice (Part 5 of a 5 Part Combination).

Animals: 11.7-13.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 2% $NaHCO_3$ in H_2O.

Treatment Schedule: bid x 3 and twice, 24 and 31 hr post-virus inoculation.
Treatment Route: 01: p.o. 5587: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01 +	1250 + 6.25	0/5	-1.8	10/10**	>21.0**	0.2**	6/8**(215**)	8/8**(51**)	0.0**	0.0**
AVS5587	25 + 6.25	5/5	2.6	10/10**	>21.0**	0.5**	4/10(334**)	3/10*(274**)	0.8**	0.2**
	12.5 + 6.25	5/5	1.4	5/10	6.4**	2.5	1/9(3270)	0/9(3925)	4.3	5.6
	6.25 + 6.25	5/5	2.1	0/10	4.9	2.3	0/10(1135)	0/10(1149)	2.9	3.4
H_2O	-	-		5/20	4.8	2.4	4/20(753)	0/20(863)	3.8	4.1
Normals	-	5/5	2.4	-	-	0.0	5/5(81)	5/5(26)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Table VI-6. Expts. PtA774-778. Combination Chemotherapy of Punta Toro Virus Infection in Mice.

AVS01[a] (mg/kg/day)	AVS5587 (mg/kg/day)[b] 0	6.25	12.5	25	0	6.25	12.5	25
	Survivors/Total				*Mean Liver Score*[c]			
0	5/20	2/10	8/10**	8/10**	2.4±1.4[d]	2.9±1.4	2.0±1.7	0.0±0.0**
6.25	1/10	0/10	9/10**	9/9**	2.8±1.4	2.3±1.3	1.7±1.5	0.7±1.0**
12.5	0/10	5/10	7/9**	10/10**	3.2±0.7	2.5±1.0	2.7±1.5	0.1±0.2**
25	9/10**	10/10**	10/10**	10/10**	1.4±1.6	0.5±0.5**	0.5±0.0**	0.2±0.2**
1250	2/10	10/10**	9/9**	10/10**	0.1±0.2**	0.2±0.3**	0.0±0.0**	0.2±0.2**
	Mean Liver Virus Titer[c]				*Mean Serum Virus Titer*[c]			
0	3.8±3.0	3.4±2.5	2.9±2.2	0.0±0.0**	4.1±2.6	4.1±2.7	3.1±2.6	0.0±0.0**
6.25	5.2±0.7	2.9±2.2	1.7±2.2	0.3±0.9**	5.9±1.5	3.4±2.5	2.4±1.7	0.4±1.1**
12.5	5.3±0.6	4.3±0.8	2.7±2.7	0.6±1.2**	6.1±0.5	5.6±1.1	3.2±2.6	0.0±0.0**
25	2.4±2.2	0.8±1.3**	0.3±0.9**	0.0±0.0**	2.0±2.0*	0.2±0.6**	0.0±0.0**	0.0±0.0**
1250	0.0±0.0**	0.0±0.0**	0.0±0.0**	0.0±0.0**	0.0±0.0**	0.0±0.0**	0.0±0.0**	0.2±0.5**
	Mean SGOT Activity[c]				*Mean SGPT Activity*[c]			
0	753±459	1092±561	742±579	114±26**	863±685	1303±723	825±735	73±53**
6.25	2832±2761	1135±1364	507±378	201±128**	3165±2976	1149±1551	491±536	136±117**
12.5	2132±1256	2503±1607	727±589	199±152**	2271±916	3158±1917	774±769	153±147**
25	741±902	334±260**	231±106**	112±43**	734±837	274±205**	159±150**	51±29*
1250	140±73**	215±193**	260±165**	169±182**	25±8**	51±26**	51±26**	29±16**

[a]Oral treatments were twice a day for 3 days starting 24 h after virus inoculation.

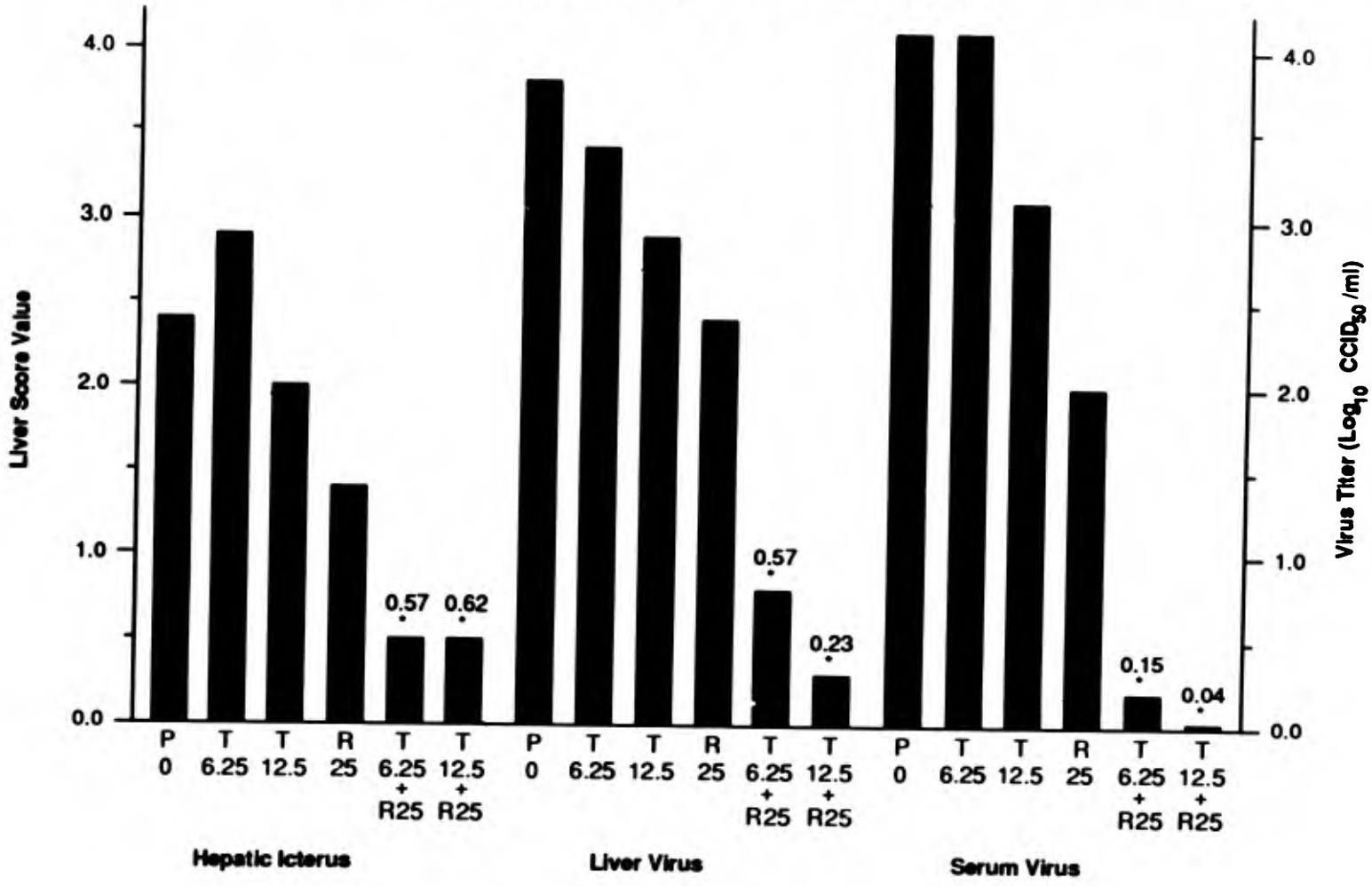
[b]Intraperitoneal treatments were given 24 and 31 h after virus challenge.

[c]Determined 4 days after virus inoculation.

[d]Standard deviation.

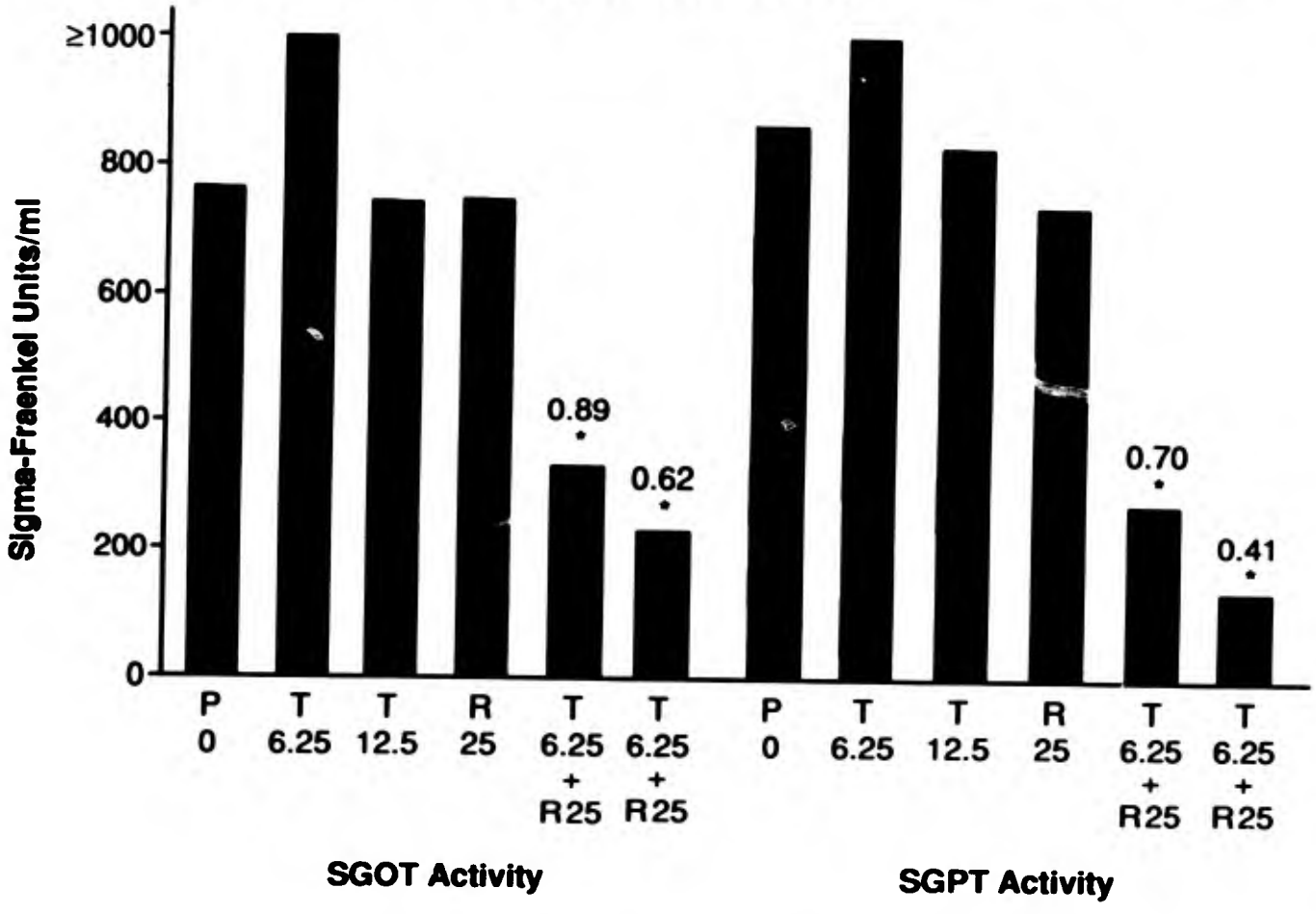
*P<0.05 **P<0.01

Figure VI-1. Near-synergistic to Synergistic Interactions of AVS01 and AVS5587 on Hepatic Icterus Scores, and on Liver and Serum Virus Titers in Punta Toro Virus-Infected Mice. Infection Parameters were Assessed 4 Days After Virus Inoculation. P = Placebo (0 mg/kg), T = AVS5587 (6.25 and 12.5 mg/kg), R = AVS01 (25 mg/kg),



*P<0.01. Values above each are fractional inhibitory concentration (FIC) indices

Figure VI-2. Additive to Synergistic Interactions of AVS01 and AVS5587 on Serum Glutamic Oxalacetate Transaminase (SGOT) and Serum Glutamic Pyruvate Transaminase (SGPT) Activities in Punta Toro Virus-Infected Mice.



*P<0.01. Values above each are fractional inhibitory concentration (FIC) indices

VII. EFFECT OF A COMBINATION OF AVS01 AND AVS1761 ON *IN VIVO* PUNTA TORO VIRUS INFECTIONS

Introduction

In previous reports, we have described the striking anti-PTV effects of AVS1761 (poly ICLC), a known IFN inducer. In view of this activity, this compound appeared to be a likely candidate to be used in combination with AVS01 (ribavirin) against the PTV infection.

Two approaches were used in the design of the experiment: 1) To attempt to reduce the toxicity of a high, usually lethal dose of ribavirin using AVS1761, and 2) to enhance ribavirin's antiviral activity at low doses using AVS1761 in combination.

This report describes our experiments investigating this drug combination.

Materials and Methods

Virus: Adames strain PTV as described earlier was used.

Animals: Female 3 week old C57BL/6 mice weighing 10-13 g were obtained from Simonsen. Quarantine, caging, and feeding of these mice was as described in previous sections.

Compounds: AVS01 and AVS1761 were provided by Biological Research Faculty and Facility, Inc. AVS01 was dissolved in water and AVS1761 was dissolved in physiological saline.

Experiment Design: AVS01 was administered p.o. twice daily for 3 days beginning 24 hr post-virus inoculation. AVS1761 was injected i.p. 24 and 72 hr post-virus inoculation. Both treatment regimens have been previously shown by us to be highly effective vs PTV infections in mice. A total of 6 experiments were run in parallel, as follows:

#1 (PtA 813): AVS01 only, at dosages of 2000, 16, 5 and 1.6 mg/kg/day. These dosages were selected because ribavirin is known to be lethally toxic at the high dose and to have minimal anti-PTV activity at the low doses.

#2 (PtA 814, 821): AVS1761 only, at doses of 0.32, 0.01, 0.0032 and 0.001 mg/kg/day, selected to bracket active and sub-active doses of this compound.

#3 (PtA 815): AVS01 at dosages used in #1 + AVS1761 at 0.32 mg/kg/day.

#4 (PtA 816): AVS01 at dosages used in #1 + AVS1761 at 0.01 mg/kg/day.

#5 (PtA 817): AVS01 at dosages used in #1 + AVS1761 at 0.032 mg/kg/day.

#6 (PtA 818): AVS01 at dosages used in #1 + AVS1761 at 0.001 mg/kg/day.

An expanded parameter anti-PTV experiment was run in each study, the disease parameters being survivors, mean survival time, liver score, SGOT, SGPT, liver virus and serum virus titers, with 20 infected mice used in each treatment group, 40 mice used as placebo-treated controls, 20 mice as normal controls and 5 animals in each treatment group as toxicity controls. One-half of each treatment group, virus controls, and normal controls were killed 4 days after virus inoculation, bled, and their livers removed. Livers were scored from 0 to 4, homogenates prepared from each, and the homogenates tested for virus titer. Serum was assayed for SGOT, SGPT, and PTV titers The remainder of the mice were held 21 days post-virus inoculation with deaths noted daily.

Results and Discussion

The overall results are summarized in Tables VII-1 to VII-6.

AVS01 at 2000 mg/kg/day was lethally toxic to 40% of the mice, with the remainder losing weight through the treatment period (Table VII-1). The 16 and 5 mg/kg/day dosages were surprisingly active, preventing death in 50 to 60% of the infected mice, although at 5 mg/kg/day activity was only seen using survival as endpoint. The lowest dosage, 1.6 mg/kg/day, resulted in significant virus titer reductions in liver and serum.

AVS1761 (Table VII-2) was highly effective at all but the 0.001 mg/kg/day dosage. This efficacy was seen using all evaluation parameters. Treatment with the 0.001 mg/kg/day dosage resulted in lower virus levels in liver and serum. All dosages were well tolerated.

The combination of AVS01 with the 0.32 mg/kg/day dose of AVS1761 (Table VII-3) was apparently more toxic than using AVS01 by itself, since all toxicity controls treated with the high

dose of AVS01 died during treatment. Because AVS1761 was markedly effective at the doses 0.0032 through 0.32 mg/kg/day, enhanced efficacy could not be seen when using these dosages in combination with any dose of ribavirin (Tables VII-3-5). No reduction in toxicity of the high dose of ribavirin was achieved using any dose of AVS1761 (Tables VII-3-6).

The lowest marginally PTV-inhibitory dose of AVS1761 did not appear to enhance the anti-PTV efficacy of any dose of AVS01 (Table VII-6).

Conclusions

Treatment with the combination of AVS01 administered p.o. twice daily for 3 days beginning 24 hr after virus inoculation and AVS1761 administered i.p. 24 and 72 hr post-virus inoculation was not effective in either decreasing toxicity of AVS01 or in enhancing the therapeutic efficacy of either compound against PTV infections in mice.

Table VII-1. Expt. PtA813. Effect of Twice Daily p.o. Treatment with AVS01 on Punta Toro Virus Infections in Mice (Part 1 of a 6 Part Combination Experiment).

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.

Treatment Schedule: bid x 3, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01	2000	2/5	-1.1	0/10	5.2	1.7**	0/6(803)	0/6(436)	0.0**	0.4**
	16	5/5	0.9	5/10**	4.6	1.9**	4/10**(2672)	2/10*(2947)	2.5**	2.2**
	5	5/5	0.8	6/10**	9.3	3.7	0/10(5181)	0/10(5933)	5.6	6.2
	1.6	5/5	1.6	0/10	5.1	2.1	1/10(739*)	1/10(1114)	2.2**	3.3**
H_2O	-	-	-	0/20	4.8	3.4	0/20(3056)	0/20(3530)	4.9	5.3
Normals	-	5/5	1.3	-	-	0.1	5/5(102)	5/5(55)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Table VII-2. Expt. PtA814, 821. Effect of Every Other Day i.p. Treatment with AVS1761 on Punta Toro Virus Infections in Mice (Part 2 of a 6 Part Combination Experiment).

Animals: 11.8-13.0 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.

Treatment Schedule: eod x 2, beginning 24 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1761	0.32	5/5	1.8	9/10**	8.0	0.5**	10/10**(111**)	10/10**(50**)	0.0**	0.0**
	0.01	5/5	1.6	7/10**	6.0	1.7**	5/9**(1058**)	2/10*(924**)	2.2**	2.0**
	0.0032	5/5	1.5	9/10**	5.0	1.9**	3/9*(1184**)	0/10(1124**)	1.3**	1.9**
	0.001	5/5	2.0	0/10	5.2	2.9	0/10(3033*)	1/10(2525*)	3.6**	4.2**
Saline	-	-	-	0/20	4.8	3.5	0/20(4955)	0/20(4457)	5.7	6.3
Normals	-	5/5	1.6	-	-	0.1	5/5(74)	5/5(28)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Table VII-3. Expt. PtA815. Effect of Combination Treatment with AVS01 and AVS1761 on Punta Toro Virus Infections in Mice (Part 3 of a 6 Part Combination Experiment).

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O + Saline.

Treatment Schedule: bid x 3 and eod x 2, beginning 24 hr post-virus inoculation.
Treatment Route: p.o., i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01 +	2000 + 0.32	0/5	-2.3	0/10	5.6	2.2	0/7(1504)	0/7(923)	0.0**	0.0**
AVS1761	16 + 0.32	5/5	1.2	1/10	6.7	0.0**	7/10**(142**)	9/10**(56**)	0.4**	0.6**
	5 + 0.32	5/5	1.1	9/9**	19.0	0.6**	8/10**(196*)	8/10**(148*)	0.5**	0.5**
	1.6 + 0.32	5/5	0.8	9/10**	17.0	0.6**	8/10**(121**)	9/10**(76**)	0.3**	0.4**
H_2O + Saline	-	-	-	2/20	5.3	2.9	1/20(3398)	0/20(3801)	4.2	5.0
Normals	-	5/5	1.3	-	-	0.1	5/5(102)	5/5(55)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Table VII-4. Expt. PtA816. Effect of Combination Treatment with AVS01 and AVS1761 on Punta Toro Virus Infections in Mice (Part 4 of a 6 Part Combination Experiment).

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O + Saline.

Treatment Schedule: bid x 3 and eod x 2, beginning 24 hr post-virus inoculation.
Treatment Route: p.o., i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01 +	2000 + 0.01	2/5	-1.5	0/10	7.0	0.9**	7/10**(405*)	7/10**(214*)	0.0**	0.0**
AVS1761	16 + 0.01	5/5	1.5	10/10**	>21.0**	0.4**	9/10**(134**)	9/10**(72**)	0.3**	0.0**
	5 + 0.01	5/5	1.8	10/10**	>21.0**	1.0**	7/10**(248*)	7/10**(234*)	0.7**	0.8**
	1.6 + 0.01	5/5	1.8	10/10**	>21.0**	1.2**	8/10**(302*)	5/10**(264*)	1.2**	1.5**
H_2O + Saline	-	-	-	2/20	5.3	2.9	1/20(3398)	0/20(3801)	4.2	5.0
Normals	-	5/5	1.3	-	-	0.1	5/5(102)	5/5(55)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Table VII-5. Expt. PtA822. Effect of Combination Treatment with AVS01 and AVS1761 on Punta Toro Virus Infections in Mice (Part 5 of a 6 Part Combination Experiment).

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O + Saline.

Treatment Schedule: bid x 3 and eod x 2, beginning 24 hr post-virus inoculation.
Treatment Route: p.o., i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01 + AVS1761	2000 + 0.0032	1/5	-0.9	0/10	7.1	1.2**	5/10**(259**)	7/10**(90**)	0.0**	0.0**
	16 + 0.0032	5/5	1.2	10/10**	>21.0**	0.9**	4/10**(425**)	2/10*(397**)	1.5**	1.5**
	5 + 0.0032	5/5	2.0	8/10**	4.5	1.8	1/10(1222**)	1/10(1328**)	3.0**	3.3**
	1.6 + 0.0032	5/5	1.3	5/10**	6.2	2.7	0/9(2709)	0/9(2536*)	4.0	4.5
H_2O + Saline	-	-	-	0/20	5.0	3.3	0/18(6535)	0/18(6859)	5.7	5.7
Normals	-	5/5	1.6	-	-	0.1	5/5(74)	5/5(28)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Table VII-6. Expt. PtAS23. Effect of Combination Treatment with AVS01 and AVS1761 on Punta Toro Virus Infections in Mice (Part 6 of a 6 Part Combination Experiment).

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O + Saline.

Treatment Schedule: bid x 3 and eod x 2, beginning 24 hr post-virus inoculation.
Treatment Route: p.o., i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01 +	2000 + 0.001	1/5	-1.1	2/10*	8.3	1.9	3/10*(302**)	4/10**(130**)	0.0**	0.0**
AVS1761	16 + 0.001	5/5	1.4	8/10**	7.0	1.6	1/10(903**)	1/10(854**)	2.7**	2.8**
	5 + 0.001	5/5	0.7	1/10	5.4	3.1	0/10(5408)	0/10(5616)	5.7	5.9
	1.6 + 0.001	5/5	1.7	1/10	5.2	2.2	0/10(4487)	0/10(5045)	5.4	5.3
H_2O + Saline	-	-	-	0/20	5.0	3.3	0/18(6535)	0/18(6859)	5.7	5.7
Normals	-	5/5	1.6	-	-	0.1	5/5(74)	5/5(28)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

VIII. EFFECT OF A COMBINATION OF AVS01 AND AVS2149 ON *IN VIVO* PUNTA TORO VIRUS INFECTIONS

Introduction

A previous series of experiments (PtA163-166) indicated that the combination of AVS01 (ribavirin) and AVS2149 (ampligen) was synergistic when used against *in vivo* PTV infections. In that earlier study, doses of p.o.-administered ribavirin ranged from 0.32 to 150 mg/kg/day. Treatment was twice daily for 5 days beginning 24 hr post-virus inoculation. Ampligen was given i.p. in once daily doses for 5 days beginning concomitantly with ribavirin. We have since demonstrated that multiple ampligen treatments, spaced one day apart, resulted in interferon hyporesponsiveness. We therefore repeated the above study, reducing the total number of ribavirin treatments to 3 days and ampligen treatment to a single injection given 1 hr prior to ribavirin therapy. Ribavirin dosages were extended to include 2 dosages which were lethally toxic to mice to determine if ampligen treatment would reduce the ribavirin toxicity when used in combination.

Materials and Methods

Virus: Adames strain of PTV was used.

Animals: Three week-old female C57BL/6 mice provided by Simonsen Laboratories (Gilroy, CA) were used.

Compounds: Ribavirin (AVS01) and ampligen (AVS2149) were provided by Biological Research Faculty and Facility, Inc. The ampligen was annealed as described in PtA 162-166.

Experiment Design: An expanded parameter anti-PTV experiment was run, the parameters being survivors, mean survival time, liver score, SGOT, SGPT, liver virus titer, and serum virus titer. Six separate experiments were run in parallel, as follows:

#1 (PtA 844): AVS01 only given p.o. twice daily for 3 days beginning 24 hr post-virus inoculation, at dosages of 1500, 1200, 10, 5, and 2.5 mg/kg/day.

#2 (PtA 849): AVS2149 only given i.p. once only 23 hr post-virus inoculation, at dosages of 5, 0.5, 0.05, and 0.005 mg/kg.

#3 (PtA 845): AVS01 at dosages used in #1 + AVS2149 at 5 mg/kg.

#4 (PtA 846): AVS01 at dosages used in #1 + AVS2149 at 0.5 mg/kg.

#5 (PtA 847): AVS01 at dosages used in #1 + AVS2149 at 0.05 mg/kg.

#6 (PtA 848): AVS01 at dosages used in #1 + AVS2149 at 0.005 mg/kg.

One-half of each treatment group, virus controls, and normal controls were killed 4 days after virus inoculation, bled, and their livers removed. Livers were scored from 0 to 4, homogenates prepared from each, and the homogenates tested for virus titer using cytopathic effect production in LLC-MK_2 cells. Serum was assayed for PTV titers and for SGOT and SGPT using Sigma colorimetric kits. The remainder of the groups were held for a total of 21 days with deaths noted daily.

Toxicity and normal controls were weighed immediately prior to treatment and again 18 hr after final treatment.

Results and Discussion

The overall results are summarized in Tables VIII-1 to VIII-6.

AVS01 at 1500 mg/kg/day was lethally toxic to all mice. At 1200 mg/kg/day, one toxicity control animal died. AVS2149 was reasonably well tolerated at all dosages, with only the maximal 5 mg/kg dose causing host weight loss. When AVS2149 was used at all doses in combination with 1500 mg/kg/day of AVS01, the lethal toxicity of the latter was reduced from 40 to 100%, with the greatest elimination of toxicity occurring at the lowest AVS2149 dose (0.005 mg/kg). These data are most interesting, and suggest that ampligen has the potential to reduce ribavirin's acute toxicity effects.

Ribavirin used alone (Table VIII-1) was marginally active vs the PTV infection at all the doses utilized. This was expected, since the low dosages were below the previously reported minimum PTV-inhibitory concentration (1). At the high dosages, the antiviral activity seen was manifested as reduced liver scores, hepatic icterus, and virus titers in the liver and serum. The animals died, however, before the end of the experiment because of the toxic effects of the drug at these high dosages.

Ampligen used alone (Table VIII-2) was highly effective in preventing death and reducing liver scores in the infected mice at the 3 highest dosages used. Virus titers were also significantly reduced at these dosage levels The lowest, 0.005 mg/kg, dosage was essentially not effective in preventing PTV infection.

Combination therapies with ribavirin and the 3 highest dosages of ampligen (Tables VIII-3-5) were not too definitive in view of the activity seen using ampligen alone at these dosages. The combination of ribavirin and the 0.005 mg/kg dosage of ampligen, however, yielded highly significant results indicative of a synergistic antiviral effect (Table VIII-6). These effects are portrayed in more detail in Figures VIII-1 to VIII-3.

Analyzing these data from a therapeutic index (TI) standpoint, in which TI = maximum tolerated dose (MTD) divided by minimum effective dose (MED), the results were as follows:

Compound	TI
AVS01 used alone:	~120
AVS2149 used alone:	~100
AVS01 + AVS2149 (0.005 mg/kg):	600

These indicate the drug combination was more effective than either drug used alone. It should be pointed out that an exact MED of ribavirin was not obtained in this study; 10 mg/kg/day showed a marginal antiviral effect using one parameter, so this dose was assumed to be the MED. Also, an MTD was not reached using ampligen. For the purposes of calculating the data shown above, 5 mg/kg was used, which, while tolerated, caused some weight loss.

A more useful method for measuring synergy utilizes the fractional inhibitory concentration (FIC) described by Berenbaum (2) and used by others (3, 4). The FIC is determined using the following formula:

$$FIC = \frac{\text{MIC of Drug A in Combination}}{\text{MIC of Drug A alone}} + \frac{\text{MIC of Drug B in Combination}}{\text{MIC of Drug B alone}}$$

The FIC index is interpreted as follows:

FIC < 0.5: Significant synergism
FIC 0.5-0.9: Suggestive of synergism
FIC ~1: Additive
FIC 1.1-1.9: Indifference or partial antagonism
FIC > 2: Antagonism

The FIC values for the combination of ribavirin + ampligen using each parameter is seen in Table VIII-7. The effects were considered to be synergistic, or, using reductions of hepatic icterus, additive.

These data confirm our previous findings that ampligen used in combination with ribavirin results in a synergistic anti-PTV effect.

While it is known that ribavirin at high doses is immunosuppressive (review, 5), it is not known whether ampligen, a recognized interferon inducer (6) which we have found to have other immunostimulatory effects, acts to reverse that immunosuppression to reduce ribavirin's toxicity or if some other mechanism is involved.

Conclusions

AVS01 (ribavirin) administered p.o. to PTV-infected mice twice daily for 3 days starting 24 hr after virus inoculation was rendered less lethally toxic and its anti-PTV effects were increased in a synergistic fashion when the infected mice were also treated with AVS2149 (ampligen) in a single i.p. injection 1 hr prior to ribavirin treatment.

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Table VIII-7. Expt. PtA844. Effect of p.o. Treatment with AVS01 on Punta Toro Virus Infections in Mice (Part 1 of a 6 Part Combination Experiment).

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O

Treatment Schedule: bid x 3, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01	1500	0/5	-1.2	0/10	6.8**	0.8**	5/5**(120**)	5/5**(50**)	0.0**	0.0**
	1200	4/5	-1.0	0/10	6.1**	0.6**	6/6**(102**)	6/6**(33**)	0.0**	0.0**
	10	5/5	2.1	1/10	4.6	3.4	0/6(4733*)	0/6(4608*)	5.4	6.3
	5	5/5	2.1	0/10	4.0	3.8	0/6(6192)	0/6(6267)	5.6	6.5
	2.5	5/5	1.4	0/10	4.3	4.0	0/6(6450)	0/6(6650)	5.7	6.5
H_2O	-	-	-	0/20	4.1	3.9	0/11(6450)	0/11(6650)	5.6	6.4
Normals	-	4/4	2.8	-	-	0.1	5/5(73)	5/5(15)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Table VIII-2. Expt. PtA849. Effect of i.p. Treatment with AVS2149 on Punta Toro Virus Infections in Mice (Part 2 of a 6 Part Combination Experiment).

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: once only, 23 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2149	5	5/5	-0.3	10/10**	>21.0**	0.1**	1/6(330**)	6/6**(78**)	1.3**	2.2**
	0.5	5/5	0.6	10/10**	>21.0**	0.0**	3/6*(269**)	6/6**(66**)	2.0**	2.8**
	0.05	5/5	0.7	10/10**	>21.0**	0.6**	1/6(278**)	5/6**(92**)	0.6**	2.4**
	0.005	5/5	0.3	3/10*	5.3	3.8	1/6(7776)	1/6(5218)	5.4	6.0
Saline	-	-	-	0/20	4.1	3.9	0/11(6450)	0/11(6650)	5.6	6.4
Normals	-	4/4	2.8	-	-	0.1	5/5(73)	5/5(15)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Table VIII-3. Expt. PtA845. Effect of Combination Treatment with AVS01 and AVS2149 on Punta Toro Virus Infections in Mice (Part 3 of a 6 Part Combination Experiment).

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O + Saline.

Treatment Schedule: 01: bid x 3, 24 hr post-virus inoculation.
2149: once only, 23 hr post-virus inoculation.
Treatment Route: 01: p.o.; 2149: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01 +	1500 + 5	2/5	-1.3	1/10	8.0**	1.1**	5/6**(153**)	6/6**(40**)	0.0**	0.0**
AVS2149	1200 + 5	5/5	-0.7	2/10	10.4**	0.8**	5/6**(111**)	6/6**(30**)	0.6**	0.0**
	10 + 5	5/5	1.6	10/10**	>21.0**	0.6**	6/6**(141**)	6/6**(55**)	1.7**	4.2**
	5 + 5	5/5	2.1	10/10**	>21.0**	0.6**	6/6**(138**)	5/6**(61**)	0.5**	0.0**
	2.5 + 5	5/5	0.8	10/10**	>21.0**	0.7**	3/6**(179**)	6/6**(40**)	3.1**	4.1**
H_2O + Saline	-	-	-	2/20	4.2	3.8	0/12(758)	0/12(4825)	5.4	6.4
Normals	-	4/4	2.8	-	-	0.1	5/5(73)	5/5(15)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Table VIII-4. Expt. PtA846. Effect of Combination Treatment with AVS01 and AVS2149 on Punta Toro Virus Infections in Mice (Part 4 of a 6 Part Combination Experiment).

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O + Saline.

Treatment Schedule: 01: bid x 3, 24 hr post-virus inoculation.
2149: once only, 23 hr post-virus inoculation.
Treatment Route: 01: p.o.; 2149: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01 +	1500 + 0.5	2/5	-1.7	0/10	6.1**	0.7**	3/6**(198**)	6/6**(36**)	0.0**	0.3**
AVS2149	1200 + 0.5	0/5	-2.2	0/10	6.1**	1.0**	1/6(424**)	6/6**(48**)	0.0**	0.5**
	10 + 0.5	5/5	1.5	10/10**	>21.0**	0.6**	6/6**(152**)	6/6**(59**)	3.2**	3.7**
	5 + 0.5	5/5	1.8	10/10**	>21.0**	0.6**	4/6**(150**)	6/6**(50**)	0.0**	0.8**
	2.5 + 0.5	5/5	0.9	9/10**	5.0	1.3**	4/6**(401**)	5/6**(253**)	1.7**	2.7**
H_2O + Saline	-	-	-	2/20	4.2	3.8	0/12(7588)	0/12(4825)	5.4	6.4
Normals	-	4/4	2.8	-	-	0.1	5/5(73)	5/5(15)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Table VIII-5. Expt. PtA847. Effect of Combination Treatment with AVS01 and AVS2149 on Punta Toro Virus Infections in Mice (Part 5 of a 6 Part Combination Experiment).

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O + Saline.

Treatment Schedule: 01: bid x 3, 24 hr post-virus inoculation.
2149: once only, 23 hr post-virus inoculation.
Treatment Route: 01: p.o.; 2149: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01 +	1500 + 0.05	3/5	-0.5	9/10**	11.0	0.3**	4/6**(142**)	6/6**(36**)	0.0**	0.4**
AVS2149	1200 + 0.05	5/5	0.0	10/10**	>21.0**	0.6**	4/6**(161**)	6/6**(18**)	0.0**	0.0**
	10 + 0.05	5/5	1.8	10/10**	>21.0**	0.4**	5/6**(143**)	6/6**(59**)	1.2**	1.7**
	5 + 0.05	5/5	1.1	10/10**	>21.0**	1.1**	6/6**(87**)	5/6**(365**)	0.8**	0.9**
	2.5 + 0.05	5/5	2.1	10/10**	>21.0**	0.8**	4/6**(492**)	3/6**(455**)	2.6**	3.4**
H_2O + Saline	-	-	-	2/20	4.2	3.8	0/12(7588)	0/12(4825)	5.4	6.4
Normals	-	4/4	2.8	-	-	0.1	5/5(73)	5/5(15)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

*P<0.05 **P<0.01

Table VIII-6. Expt. PtA848. Effect of Combination Treatment with AVS01 and AVS2149 on Punta Toro Virus Infections in Mice (Part 6 of a 6 Part Combination Experiment).

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O + Saline.

Treatment Schedule: 01: bid x 3, 24 hr post-virus inoculation.
2149: once only, 23 hr post-virus inoculation.
Treatment Route: 01: p.o.; 2149: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS01 +	1500 + 0.005	5/5	-0.1	7/10**	8.7	1.2**	3/5**(278**)	4/5**(88**)	0.5**	0.0**
AVS2149	1200 + 0.005	5/5	0.3	10/10**	>21.0**	0.8**	5/5**(106**)	5/5**(36**)	0.0**	0.0**
	10 + 0.005	5/5	2.1	8/10**	6.5	1.7**	3/6**(458**)	2/6*(563**)	2.1**	3.0**
	5 + 0.005	5/5	2.0	8/10**	4.5	1.7**	0/6(3326*)	1/6(1792**)	1.5**	3.1**
	2.5 + 0.005	5/5	2.4	8/10**	5.5	0.5**	1/5(516**)	3/5**(303**)	0.6**	0.8**
H_2O + Saline	-	-	-	2/20	4.2	3.8	0/12(7588)	0/12(4825)	5.4	6.4
Normals	-	4/4	2.8	-	-	0.1	5/5(73)	5/5(15)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

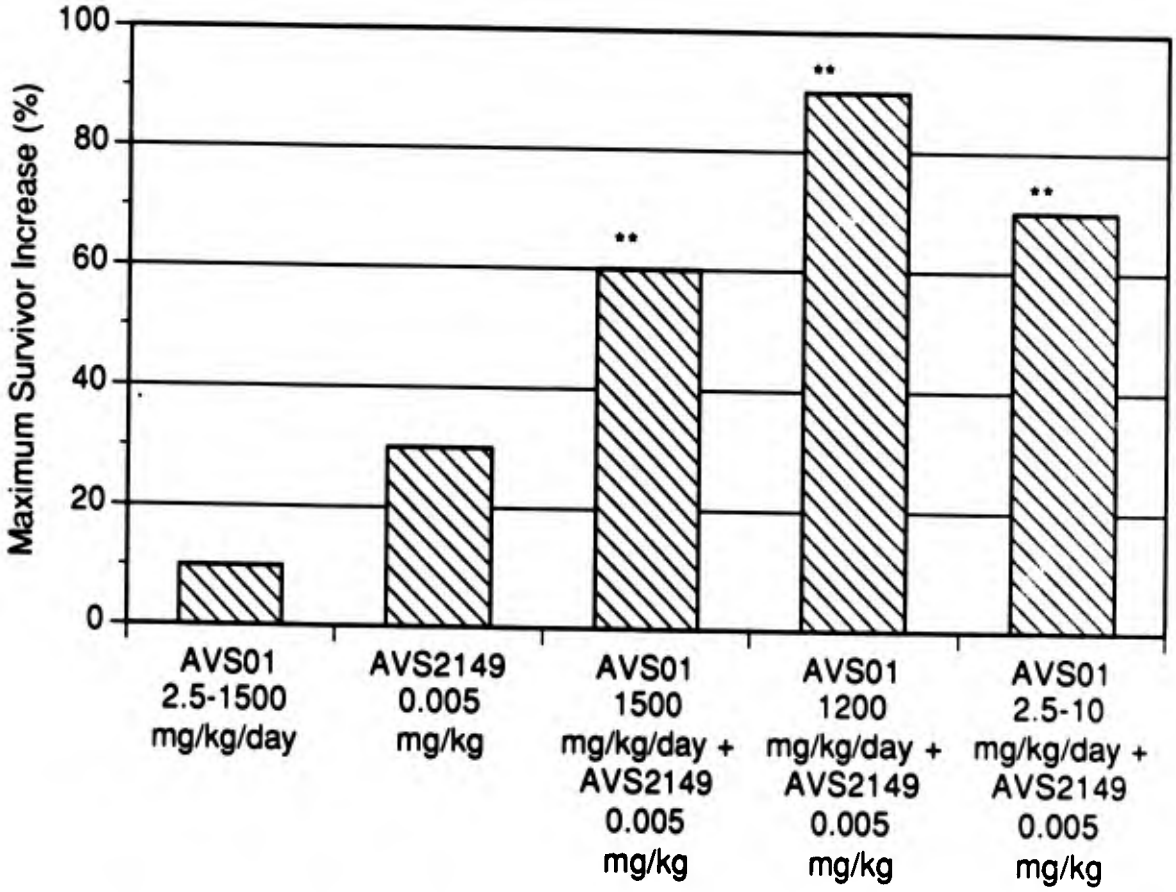
[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

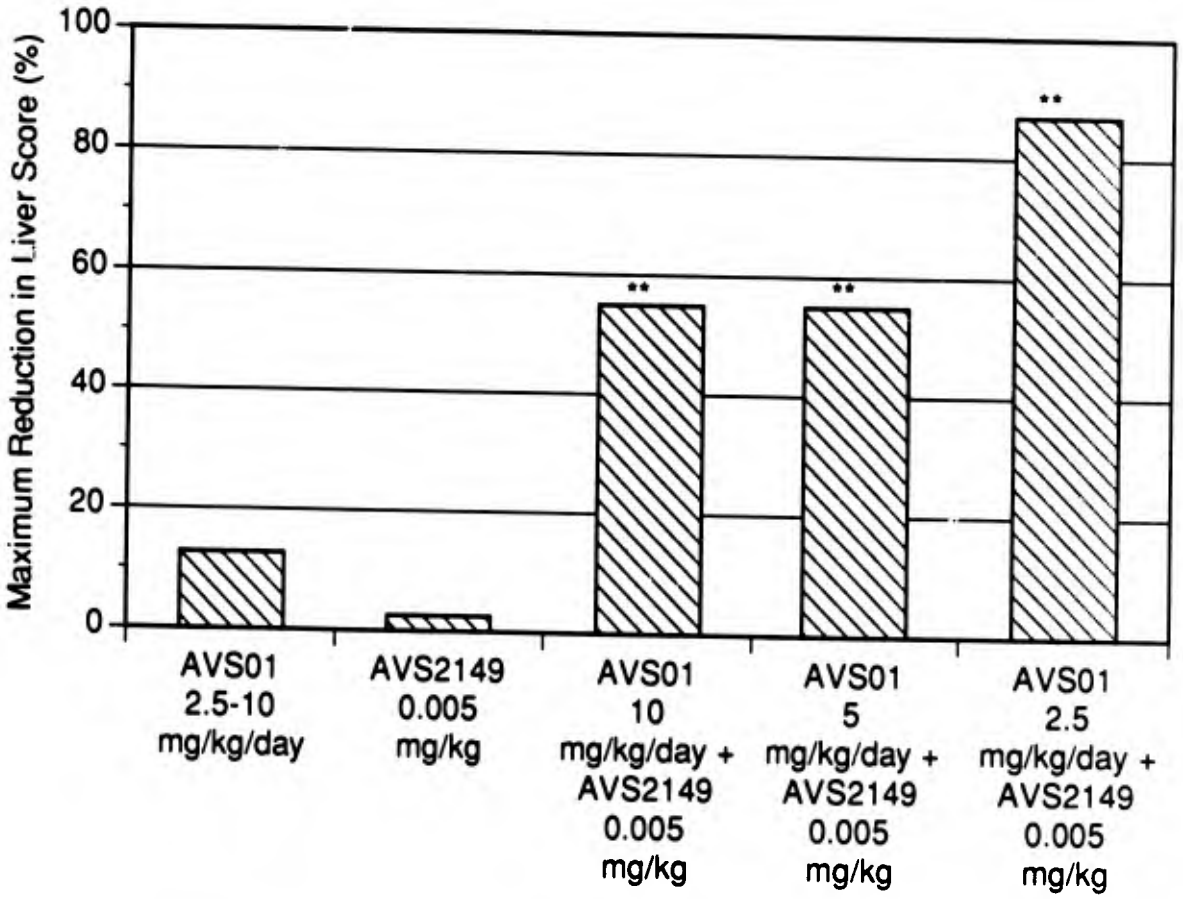
*P<0.05 **P<0.01

Figure VIII-1. Effect of the Combination of AVS01 + AVS2149 on Survivor Increase in PTV Infected Mice.



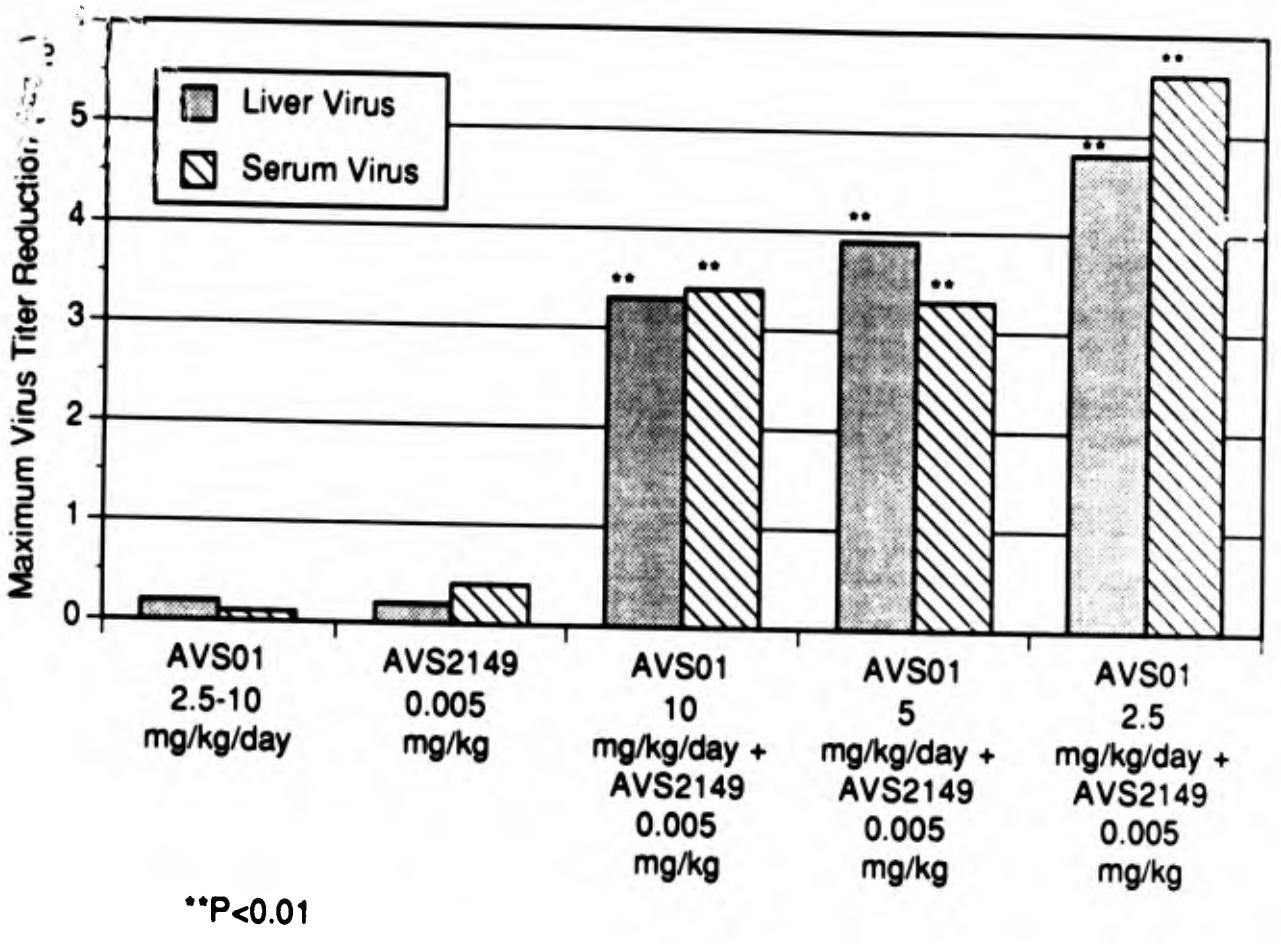
**P<0.01

Figure VIII-2. Effect of the Combination of AVS01 + AVS2149 on Hepatic Icterus Reduction in PTV Infected Mice.



**P<0.01

Figure VIII-3. Effect of the Combination of AVS01 + AVS2149 on Liver and Serum Virus Titer Reduction in PTV Infected Mice.



**P<0.01

IX. STUDIES ON INTERFERON INDUCTION BY AVS2149 IN PUNTA TORO VIRUS-INFECTED MICE

Introduction

We have previously reported on the dramatic inhibitory effect AVS2149 (ampligen) induces on Adames strain PTV infections in mice. We also confirmed the reports of others of ampligen's striking interferon (IFN)-inducing properties in the 3 week-old C57BL/6 mice used in our experiments. Since this compound has exhibited anti-PTV effects using a variety of treatment schedules, it was of interest to determine the relative IFN induction by this material using these various treatment schedules in the PTV-infected animal. We felt this information would be of particular importance in view of evidence showing that multiple treatments with IFN inducers tend to result in a hyporeactive state in the animal when the treatments are spaced adequately apart

This section describes these IFN induction studies.

Materials and Methods

Virus: Adames strain PTV as described earlier was used.

Animals: Three week old C57BL/6 mice were obtained from Simonsen (Gilroy, CA) All were quarantined for 24 hr. They were maintained on standard mouse chow and tap water *ad libitum*.

Compound: AVS2149 was provided by Biological Research Faculty and Facility. It was annealed by adding 20 ml of sterile pyrogen-free water to a vial, which was then placed in a 65°C water both for 30-40 minutes, then allowed to sit at room temperature for 1 hr. The contents were then refrigerated until used. It was diluted in sterile phosphate-buffered saline for use in these studies.

IFN Assay: Samples to be assayed for IFN were placed in 0.1 ml volume on an 18-24 hr monolayer of L cells in 96 well microplates and allowed to incubate for 24 hr at 37°C. After incubation, the cells were drained and 0.1 ml of a 10^3 CCID50/0.1 ml of vesicular stomatitis virus (VSV) strain Indiana was added to each and incubated for 6 days at 37°C. Viral CPE was read microscopically after this incubation. The IFN titer was expressed as units/0.1 ml based on the maximum dilution of serum sample that inhibited VSV CPE by 50% or greater. No attempt was made to separate out IFN α, β, or γ, so we must presume all types were present in the samples assayed. Controls in the study were virus controls, which were cells exposed to test medium (MEM with 2% FBS, 0.18% $NaHCO_3$ and 50 µg gentamicin/ml) and then to VSV, and cell controls which were exposed to test medium only. Serum from infected mice was treated with 1N HCl to bring the pH to ~2 for an overnight period in order to inactivate any PTV present in the serum.

Experiment Design: Four treatment schedules were studied using this compound in PTV-infected mice: Once only, 48 hr post-virus inoculation; once daily for 5 days beginning 4 hr pre-virus inoculation; twice daily for 5 days beginning 4 hr pre-virus inoculation; and every other day x 3 beginning 4 hr post-virus inoculation. Two dosages of AVS2149 were used: 5 and 0.6 mg/kg/day. At varying times after each treatment (or, in the case of twice daily treatments, after 2, 4, 6, 8, and 10 treatments) 5 infected and uninfected mice were killed and their serum assayed for IFN titer.

Results and Discussion

Single i.p. treatment (Table IX-1): Treatment with both doses in uninfected mice induced detectable IFN at the 4 hr sampling time; IFN was still detectable at low levels by 12 hr, but not at any time interval thereafter. Untreated virus controls did not have detectable IFN until 48 hr after virus inoculation. This IFN persisted for 1 more day, then was not detectable. In the PTV-infected mice treated with ampligen 48 hr post-virus inoculation, the IFN titers reached much higher levels than seen in the uninfected mice and these titer persisted through 24 hr. Both dosages of the compound induced essentially the same IFN titers. This 48 hr post-virus inoculation time of ampligen treatment was selected because ampligen has exhibited significant protection to PTV-infected mice in previously reported studies.

qd x 5 i.p. treatment (Table IX-2): In this experiment, IFN sampling was done 2, 4, and 12 hr after ampligen treatment. At these relatively early sampling times, the placebo-treated virus controls had not yet exhibited detectable IFN. After the initial treatment, IFN was detectable at all 3

sampling times in the toxicity control animals, coordinating reasonably well with the single treatment study. Note that since ampligen treatment was given 4 hr pre-virus inoculation, the 2 and 4 hr post-therapy sampling times would be at times before virus was introduced, so the IFN titers at these times would be as the toxicity controls run in parallel. It is interesting that no IFN was detectable at the 12 hr sampling time, whereas in the single treatment study, where ampligen was given 48 hr post-virus inoculation, high IFN titers were seen. This suggests the time of ampligen treatment relative to virus inoculation is quite critical to the amount and rate at which IFN is induced.

As the daily therapies continued beyond 2 days, little or no IFN was seen in the toxicity control mice, indicating the anticipated hyporeactive state indeed occurred in the animal. It was interesting to note that at almost no time in this experiment was IFN detected in the infected, treated animals, again indicating the critical nature of time of therapy relative to virus inoculation as well as the probable development of hyporeactive state.

bid x 5 i.p. treatment (Table IX-3): In this experiment, uninfected mice treated twice with ampligen had high IFN titers at all 3 sampling times. It should be pointed out that the sampling times were actually 10, 14, and 22 hr after the first treatment, and thus the IFN titers seen may have been a result of the first ampligen injection, and not the second. If true, these data indicate that the 12 hr sampling period used in the above-described qd x 5 experiment, which was the latest time samples were taken, may have been too early for the infected, treated animals. Not surprisingly, little IFN could be detected following subsequent treatments.

eod x 3 i.p. treatment (Table IX-4): These results again indicate that when ampligen was administered early in the infection, this time 4 hr post-virus inoculation, the IFN production is less in the infected animals than in the toxicity controls run in parallel. In this study, a hyporeactiveness was again seen, but spacing the treatments further apart enabled some further activation of IFN so that in the toxicity controls treated 3 times with ampligen a relatively strong IFN induction was still occurring.

These data, especially with the multiple treatments beginning early in the infection, incite a question: If the ampligen treatment in the infected animal does not induce IFN as early or in as high a titer as in uninfected mice, why do such treatments still protect the PTV-infected mice? It is probably that our sampling times in these experiments were too early, and later times may have shown a major rise in IFN titer.

As mentioned in the Materials and Methods, in order to cope with the almost certain presence of infectious PTV in the serum which would possibly affect our IFN assays, the serum was treated with acid to inactivate the virus. Could the acid have affected the IFN titers in the serum? To determine this, we exposed IFN induced by poly I:C to the acid treatment, and compared the IFN titer to the same sample which had not been exposed. No significant differences were seen in titer. In addition, the data in Table IX-1 showing higher IFN titers in acid-exposed serum from PTV-infected mice treated with ampligen than in non-acid-exposed serum from ampligen-treated toxicity control mice. Thus we conclude the acid was not affecting the IFN titers in this study.

Conclusions

Mice infected with PTV were treated i.p. with 2 concentrations of AVS2149 (5 and 0.6 mg/kg/day) using 4 treatment schedules: Once only 48 hr post-virus inoculation, once daily for 5 days beginning 4 hr pre-virus inoculation, twice daily for 5 days beginning 4 hr pre-virus inoculation, and every other day for 3 treatments beginning 4 hr post-virus inoculation. In the mice treated once only late in the infection, a significant IFN induction greater than seen in infected animals or in uninfected, treated mice, occurred 4 hr after treatment and persisted for 24 hr. In all other studies, less IFN was seen in the serum of PTV-infected, treated mice than occurred in uninfected, treated animals. In addition, a definite hyporeactive state occurred in the once and twice daily treated mice; spacing the treatment to every other day partially alleviated this hyporeactive condition.

Table IX-1. PtA784. Effects of a Single i.p. Treatment with AVS2149 on Interferon Production in PTV-Infected Mice.

Animals: 3 week-old C57BL/6 Mice

Virus: Adames strain Punta Toro virus, s.c. injected.

Drug Diluent: Sterile Saline

Treatment Schedule: Once only, 48 hr post-virus inoculation.

Treatment Route: i.p.

Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Mean Interferon Titer (log_{10} units/0.1 ml) Time Post-Treatment							
		-36 hr	-24 hr	0 hr	4 hr	12 hr	24 hr	36 hr	48 hr
AVS2149									
Infected	5	nr	nr	nr	4.5	2.3	3.4	0.0	0.0
	0.6	nr	nr	nr	4.4	2.2	3.5	0.0	0.0
Toxicity	5	nr	nr	nr	2.3	0.9	0.0	0.0	0.0
	0.6	nr	nr	nr	1.0	0.6	0.0	0.0	0.0
Virus Controls	—	0.0	0.0	3.0	nr	nr	1.8	0.0	0.0
Normals	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table IX-2. PtA786. Effects of Once Daily i.p. Treatment with AVS2149 on Interferon Production in PTV-Infected Mice.

Animals: 3 week-old C57BL/6 Mice

Virus: Adames strain Punta Toro virus, s.c. injected.

Drug Diluent: Sterile Saline

Treatment Schedule: qd x 5, beginning 4 hr pre-virus inoculation.

Treatment Route: i.p.

Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Mean Interferon Titer (log_{10} units/0.1 ml) Time Post-Treatment 2 hr	4 hr	12 hr
AVS2149				
Treatment 1 Infected	5	1.8[a]	1.2[a]	0.0
	0.6	1.3[a]	2.1[a]	0.0
Treatment 1 Toxicity	5	1.8	1.2	2.5
	0.6	1.3	2.1	1.3
Treatment 2 Infected	5	0.0	0.0	0.0
	0.6	0.0	0.0	0.0
Treatment 2 Toxicity	5	1.1	2.0	0.4
	0.6	0.0	0.3	0.0
Treatment 3 Infected	5	0.0	0.0	0.0
	0.6	0.0	0.0	0.0
Treatment 3 Toxicity	5	0.0	0.0	0.0
	0.6	0.0	0.0	0.0
Treatment 4 Infected	5	0.0	0.0	0.0
	0.6	0.0	0.4	0.0
Treatment 4 Toxicity	5	0.0	0.0	0.0
	0.6	0.0	0.0	0.0
Treatment 5 Infected	5	0.0	0.0	0.0
	0.6	0.0	0.0	0.0
Treatment 5 Toxicity	5	0.0	0.4	0.0
	0.6	0.0	0.6	0.0
Virus Controls	—	—	0.0	0.0
Normals	—	—	0.0	0.0

[a]Unifected animals at these assay times, so IFN titers are the same as toxicity controls.

Table IX-3. PtA782. Effects of Twice Daily i.p. Treatment with AVS2149 on Interferon Production in PTV-Infected Mice.

Animals: 3 week-old C57BL/6 Mice
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline
Treatment Schedule: bid x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Mean Interferon Titer (log_{10} units/0.1 ml) Time Post-Treatment 2 hr	4 hr	12 hr
AVS2149				
Treatment 2 Infected	5	0.5	1.8	0.0
	0.6	0.0	0.0	0.0
Treatment 2 Toxicity	5	3.3	3.0	2.2
	0.6	2.1	2.8	0.7
Treatment 4 Infected	5	0.0	0.0	0.0
	0.6	0.0	0.0	0.0
Treatment 4 Toxicity	5	0.0	0.2	0.0
	0.6	0.0	0.0	0.0
Treatment 6 Infected	5	0.0	0.0	0.0
	0.6	0.0	0.0	0.0
Treatment 6 Toxicity	5	0.0	0.0	0.0
	0.6	0.0	0.0	0.0
Treatment 8 Infected	5	0.0	0.0	0.0
	0.6	0.0	0.0	0.0
Treatment 8 Toxicity	5	0.0	0.8	0.0
	0.6	0.0	0.0	0.0
Treatment 10 Infected	5	0.0	0.0	0.0
	0.6	0.0	0.0	0.0
Treatment 10 Toxicity	5	0.0	0.4	0.0
	0.6	0.0	0.3	0.0
Virus Controls	—	—	0.0	0.0
Normals	—	—	0.0	0.0

Table IX-4. PtA783. Effects of Every Other Day i.p. Treatment with AVS2149 on Interferon Production in PTV-Infected Mice.

Animals: 3 week-old C57BL/6 Mice

Virus: Adames strain Punta Toro virus, s.c. injected.

Drug Diluent: Sterile Saline

Treatment Schedule: Every other day x 3, beginning 4 hr post-virus inoculation.

Treatment Route: i.p.

Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Mean Interferon Titer (log_{10} units/0.1 ml) Time Post-Treatment 2 hr	4 hr	12 hr	24 hr
AVS2149					
Treatment 1 Infected	5	0.0	1.1	0.0	0.0
	0.6	0.0	0.0	0.0	0.0
Treatment 1 Toxicity	5	2.7	3.0	2.5	0.2
	0.6	2.1	1.5	1.6	0.0
Treatment 2 Infected	5	0.0	0.0	0.0	0 0
	0.6	0.0	0.7	0.4	1.7
Treatment 2 Toxicity	5	0.0	1.7	0.7	0.0
	0.6	0.0	0.0	0.0	0.0
Treatment 3 Infected	5	0.0	0.0	0.4	0.7
	0.6	0.0	0.0	0.5	0.0
Treatment 3 Toxicity	5	0.0	2.3	2.2	0.0
	0.6	0.0	2.0	1.0	0.0
Virus Controls	—	—	0.0	0.0	0.0
Normals	—	—	0.0	0.0	0.0

X. A COMPARISON OF INTERFERON INDUCTION IN C57BL/6 MICE BY A SERIES OF AVS1761 DERIVATIVES

Introduction

We have previously reported in the 1989 Annual Report on the relative *in vivo* anti-PTV efficacy of a series of AVS1761 (poly ICLC) derivatives. As a follow-up of those observations, the relative ability of a single concentration of each compound to induce interferon (IFN) in mice was determined.

Materials and Methods

Animals: Three week old C57BL/6 mice were obtained from Simonsen (Gilroy, CA). All were quarantined 24 or 48 hr prior to use and maintained on Wayne Lab Blox and tap water *ad libitum*.

Compounds: All compounds were submitted to us by Biological Research Faculty and Facility, Inc. These were: AVS5588, 5589, 5590, 5591, 5592, 5593, 5594, 5595, and 5596.

IFN Assay: Samples to be assayed for IFN were placed in 0.1 ml volume on an 18-24 hr monolayer of L cells in 96 well microplates and allowed to incubate for 24 hr at 37°C. After incubation, the cells were drained and 0.1 ml of a 10^3 CCID50/0.1 ml of vesicular stomatitis virus (VSV) strain Indiana was added to each and incubated for 6 days at 37°C. Viral CPE was read microscopically after this incubation. The IFN titer was expressed as units/0.1 ml based on the maximum dilution of serum sample that inhibited VSV CPE by 50% or greater. No attempt was made to separate out IFN α, β, or γ, so we must presume all types were present in the samples assayed. Controls in the study were virus controls, which were cells exposed to test medium (MEM with 2% FBS, 0.18% $NaHCO_3$ and 50 µg gentamicin/ml) and then to VSV, and cell controls which were exposed to test medium only.

Experiment Design: Twenty mice were injected i.p. with 0.1, and, in some cases if adequate material was available, 0.25 mg/kg of the respective compounds. Five mice were killed and their sera assayed for IFN 2, 6, 12, and 24 hr later. As a positive control, AVS1761 (poly ICLC) was run in parallel.

Results and Discussion

AVS5588 ("ICLC") (Figure X-1): This material induced IFN in a relatively weak manner, with titers initially seen 6 hr after treatment and raising slightly to a peak of approximately 1 $\log_{10}$ units/0.1 ml by 12 hrs.

AVS5589 ("ICL-CMA") (Figure X-2): This compound caused a rapid and marked IFN induction, with maximal titer seen using both 0.1 and 0.25 mg/kg/day dosages by the 2 hr sampling period.

AVS5590 ("ICL-CMD") (Figure X-3): A relatively slow and only moderate IFN induction was seen using this compound.

AVS5591 ("ILC-CM-ß-C-dextrin") (Figure X-4): This compound induced a rapid and marked IFN induction, with maximal titers exceeding 3.5 $\log_{10}$ units/0.1 ml seen by 6 hr after treatment. The IFN persisted over 24 hrs.

AVS5592 ("ICL-GEL") (Figure X-5): The IFN response induced by this compound was also quite rapid and strong, with peak IFN titers seen at the 12 hr assay period.

AVS5593 ("ICL-Sulfated Gel") (Figure X-6): This material induced a rapid, relatively high IFN response with peak titers seen by the 2 hr assay time.

AVS5594 ("ICL-(PLL-dextran)") (Figure X-7): A good IFN induction approximately 3 $\log_{10}$ units/0.1 ml was seen using this compound. Peak titers were seen 6 hr after treatment.

AVS5595 ("IC-(PLL-dextran)") (Figure X-8): This compound induced a moderate IFN response of approximately 2 $\log_{10}$ units/0.1 ml by 6 hr after injection of 0.1 mg/kg.

AVS5596 ("ICLC (heat cycled)") (Figure X-9): This heat cycled material, which had very weak antiviral activity, was also a poor IFN inducer, with a mean IFN titer of approximately 1 $\log_{10}$ units/0.1 ml seen at 12 hr after treatment. This was the only time IFN was detected following treatment with this compound.

AVS1761 (Poly ICLC) (Figure X-10): This compound, run as the positive control standard, induced a rapid and high IFN response, with peak titers seen by 2 hr and persisting at high levels through 12 hr. Approximately 1 $\log_{10}$ units/ml of IFN was still present by 24 hr after treatment.

General Commentary

It was interesting that IFN induction often, but not always, matched the relative antiviral efficacy. For example, the known positive standard, poly ICLC, which was highly active vs PTV, was also a rapid and marked inducer of IFN. Similarly, the antiviral activity of AVS5591, AVS5592, and AVS5593 closely compared to their IFN induction. Contrasting with this was the weak anti-PTV activity of AVS5589, which was one of the best IFN inducers seen in our experiments. Also, AVS5588 and AVS5590, which were relatively effective vs PTV< were only weak to moderate in their IFN induction.

It is possible that the type of IFN may be important; we have not yet determined which type of IFN was being induced in these studies.

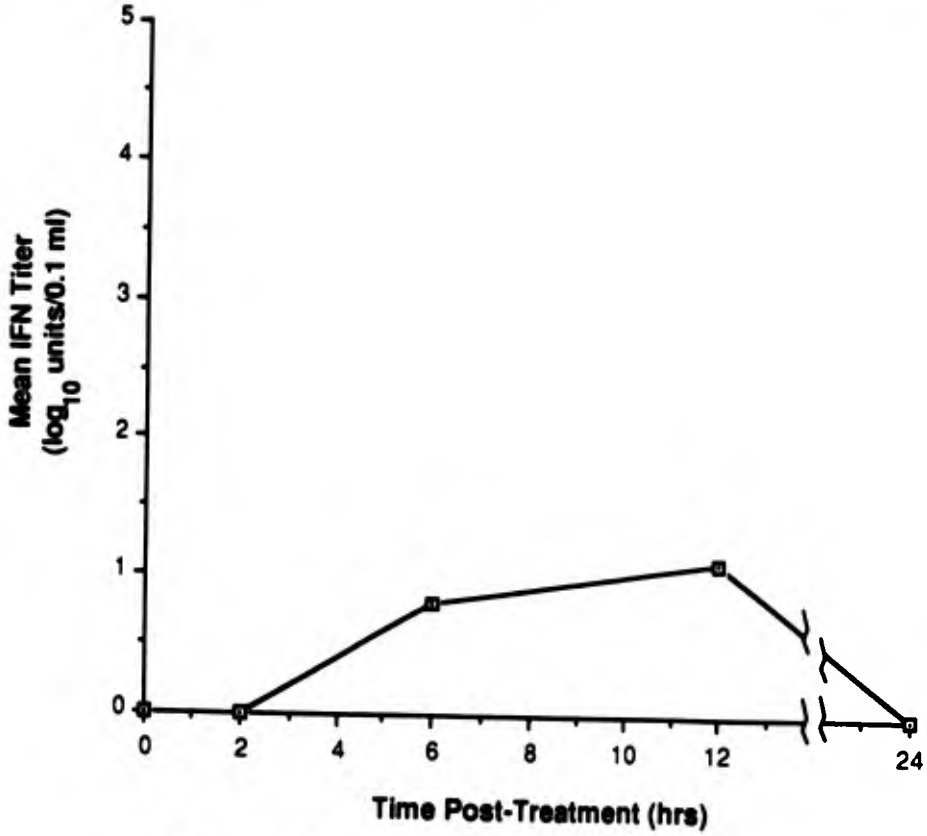
We must generally conclude, that, as has been reported previously, PTV infections are highly sensitive to the effects of IFN and thus IFN inducers are good candidates as potential anti-PTV compounds.

We were not fully informed as to the exact constituents of these poly ICLC derivatives, so can make no firm conclusions regarding the relation of their chemical structure to their antiviral activity.

Conclusions

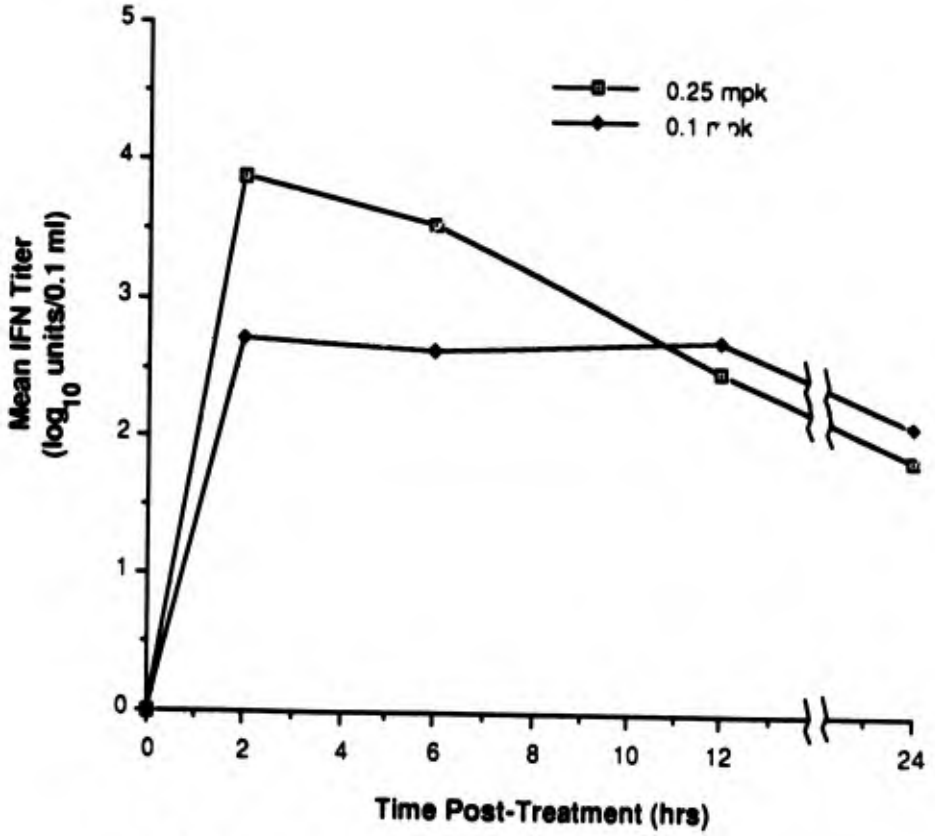
A series of poly ICLC derivatives, AVS5588–AVS5596, were compared with regard to their ability to induce IFN in 3 week-old C57BL/6 mice. The IFN-inducing activity generally coincided well with their in vivo anti-PTV effects, although some exceptions were noted. The known positive standard, poly IC..C (AVS1761) exerted the strong IFN induction expected.

Figure X-1. PT 205. Interferon Induction in 3 Week-Old C57BL/6 Mice Treated i.p. with AVS5588.[a]



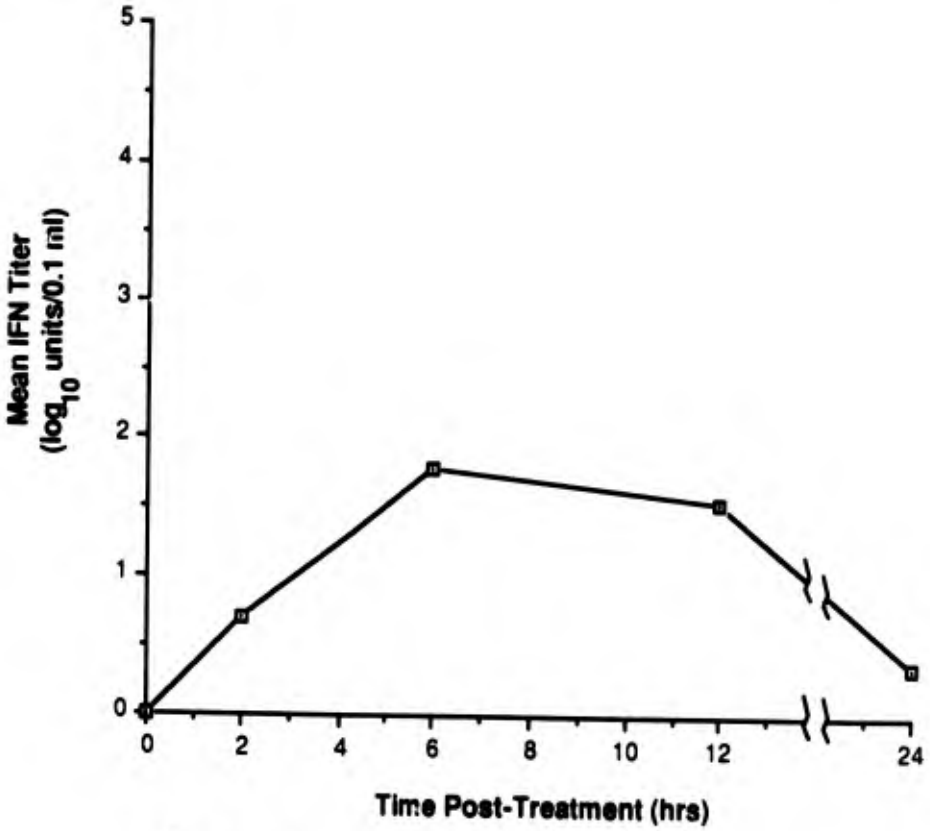
[a]Once only, 0.1 mg/kg

Figure F-2. PT 206. Interferon Induction in 3 Week-Old C57BL/6 Mice Treated i.p. with AVS5589.[a]



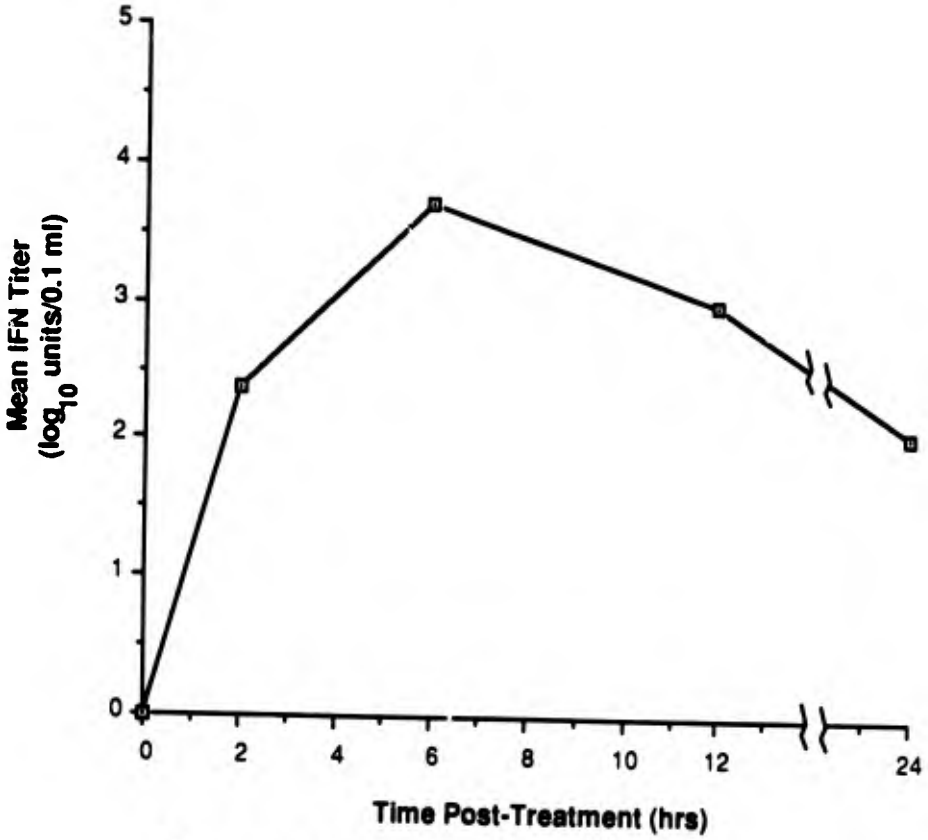
[a]Once only

Figure X-3. PT 207. Interferon Induction in 3 Week-Old C57BL/6 Mice Treated i.p. with AVS5590.[a]



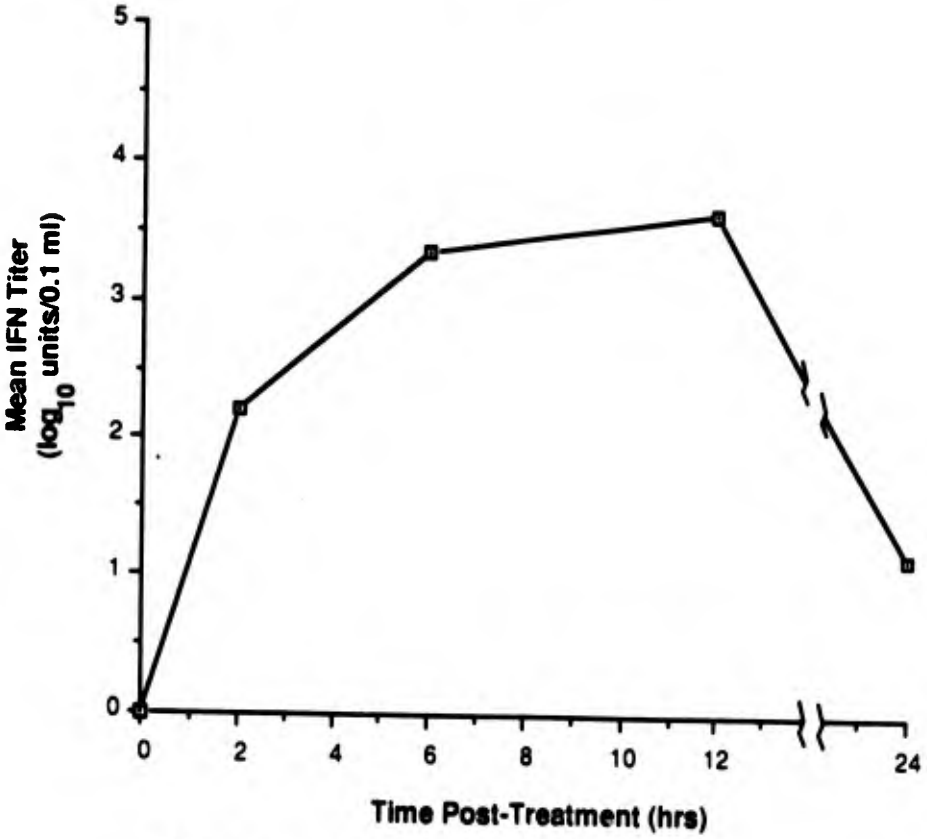
[a]Once only, 0.1 mg/kg

Figure X-4. PT 208. Interferon Induction in 3 Week-Old C57BL/6 Mice Treated i.p. with AVS5591[a]



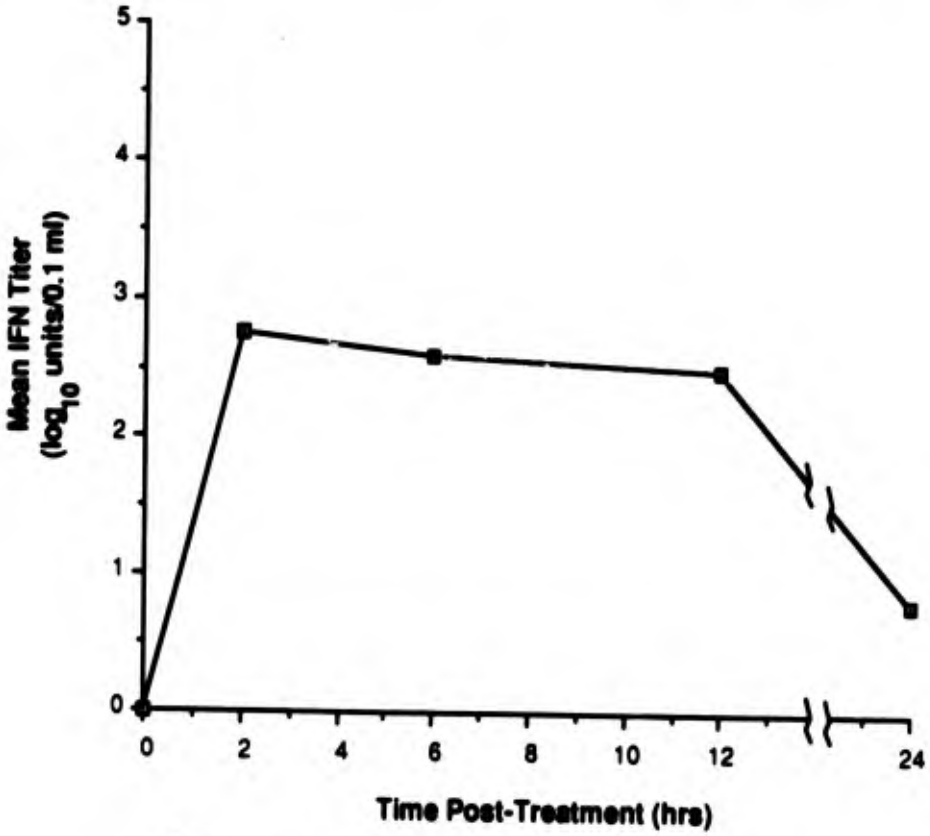
[a]Once only, 0.1 mg/kg

Figure X-5. PT 209. Interferon Induction in 3 Week-Old C57BL/6 Mice Treated i.p. with AVS5592.[a]



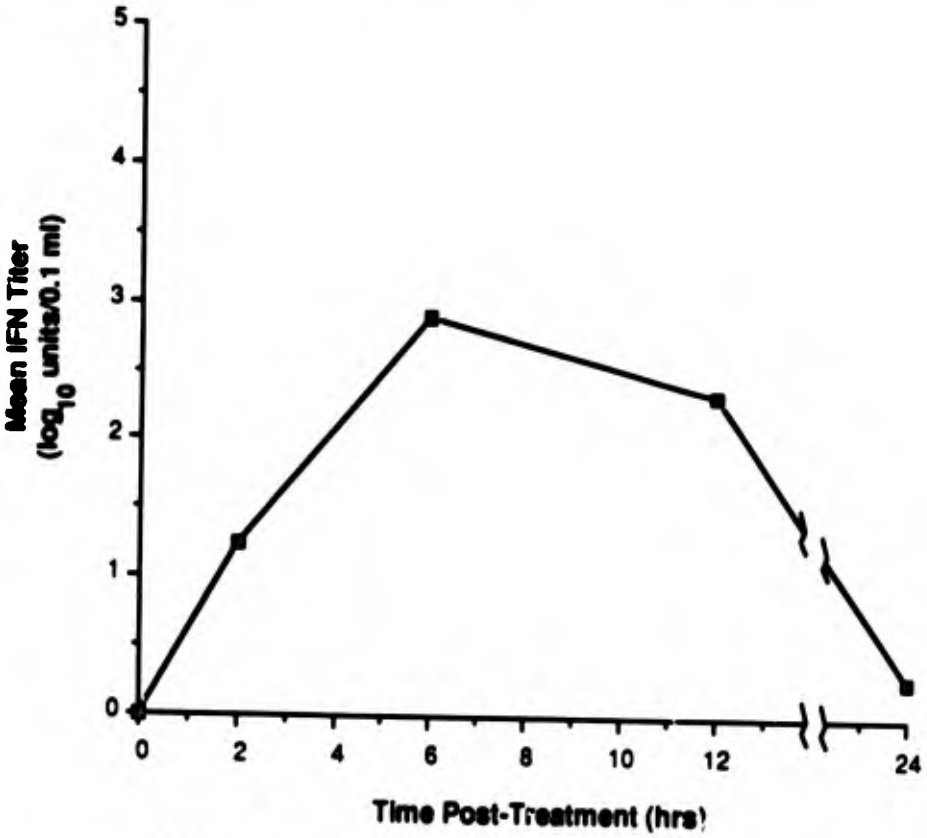
[a] Once only, 0.1 mg/kg

Figure X-6. PT 210. Interferon Induction in 3 Week-Old C57BL/6 Mice Treated i.p. with AVS5593.[a]



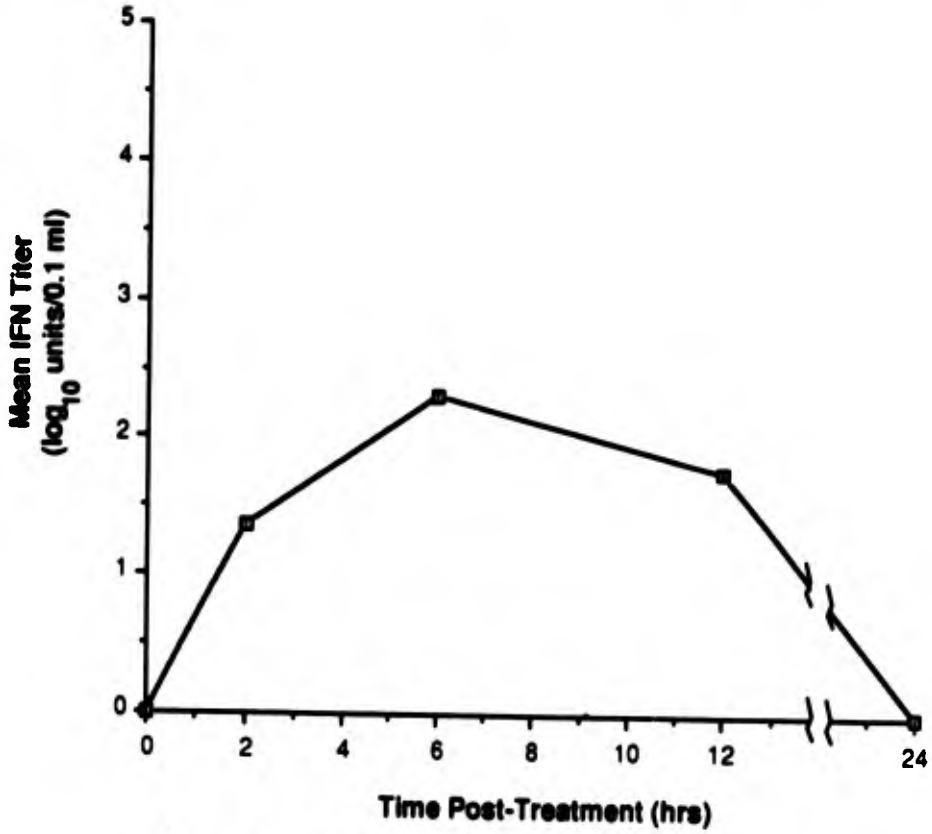
[a]Once only, 0.1 mg/kg

Figure X-7. PT 211. Interferon Induction in 3 Week-Old C57BL/6 Mice Treated i.p. with AVS5594.[a]



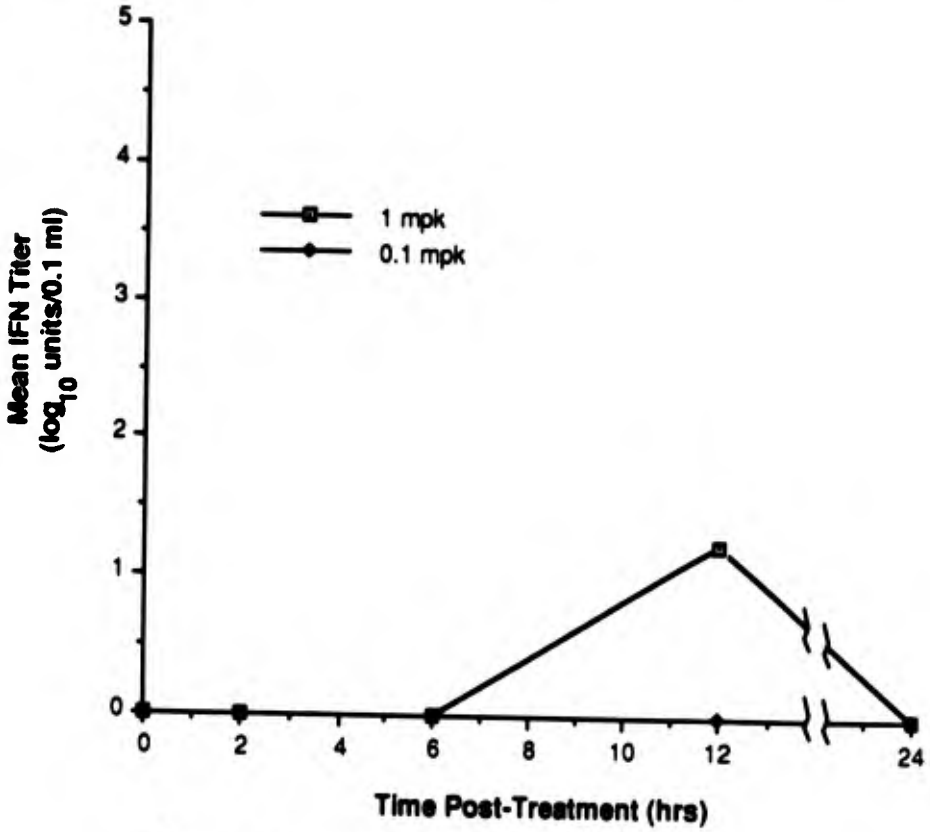
[a]Once only, 0.1 mg/kg

Figure X-8. PT 212. Interferon Induction in 3 Week-Old C57BL/6 Mice Treated i.p. with AVS5595.[a]



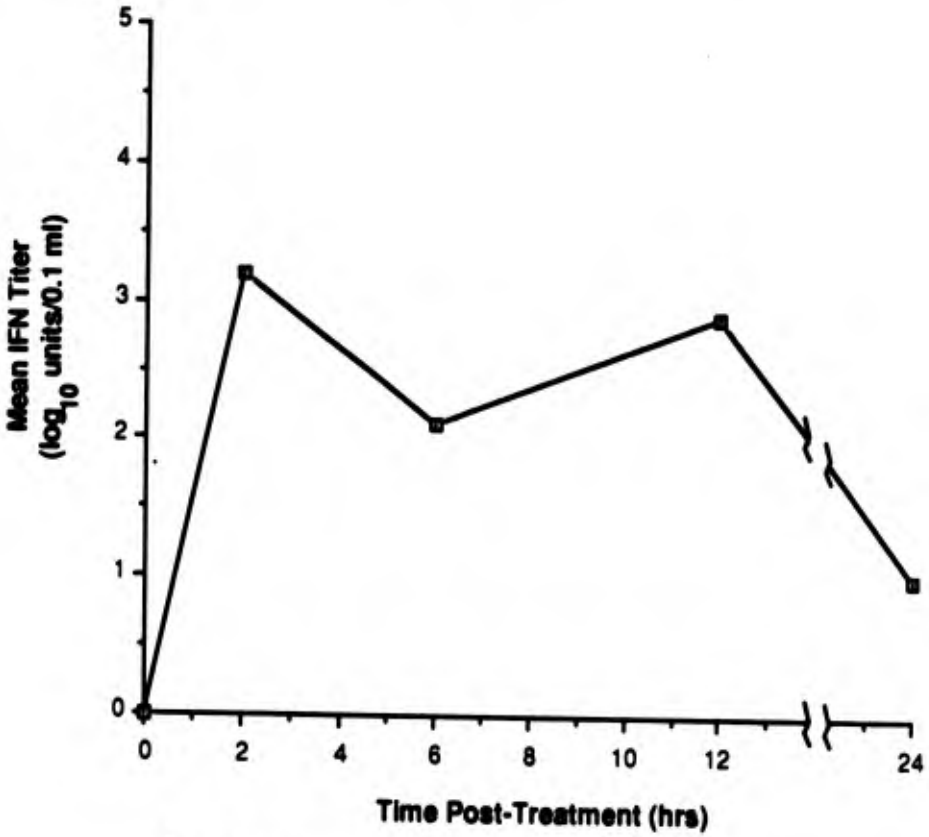
[a]Once only, 0.1 mg/kg

Figure X-9. PT 213. Interferon Induction in 3 Week-Old C57BL/6 Mice Treated i.p. with AVS5596.[a]



[a] Once only

Figure X-10. PT 214. Interferon Induction in 3 Week-Old C57BL/6 Mice Treated i.p. with AVS1761.[a]



[a]Once only

XI. INTERFERON INDUCTION BY AVS5587

Introduction

We have previously reported the striking *in vivo* anti-PTV effects of AVS5587 (7-thia-8-oxoguanosine) (1). This material is known to be an immunomodulator, having several immunologic effects in mice (2). This report describes the interferon (IFN)-inducing effects of this material in the weanling C57BL/6 mice used in our PTV experiments.

Materials and Methods

Compound: AVS5587 was provided by Biological Research Faculty and Facility, Inc. The material was dissolved in 2% $NaHCO_3$ in H_2O for this experiment.

Mice: Weanling female C57BL/6 mice (Simonsen) were used. All were maintained on Wayne mouse chow and tap water *ad libitum*.

IFN Assay: Samples to be assayed for IFN were placed in 0.1 ml volume on an 18-24 hr monolayer of L cells in 96 well microplates and allowed to incubate for 24 hr at 37°C. After incubation, the cells were drained and 0.1 ml of a 10^3 CCID50/0.1 ml of vesicular stomatitis virus (VSV) strain Indiana was added to each and incubated for 6 days at 37°C. Viral CPE was read microscopically after this incubation. The IFN titer was expressed as units/0.1 ml based on the maximum dilution of serum sample that inhibited VSV CPE by 50% or greater. No attempt was made to separate out IFN α, β, or γ, so we must presume all types were present in the samples assayed. Controls in the study were virus controls, which were cells exposed to test medium (MEM with 2% FBS, 0.18% $NaHCO_3$ and 50 µg gentamicin/ml) and then to VSV, and cell controls which were exposed to test medium only. Serum from infected mice was treated with 1N HCl to bring the pH to ~2 for an overnight period in order to inactivate any PTV present in the serum.

Experiment Design: Mice were treated i.p. once or twice 7 hr apart with AVS5587 at dosages of 100, 50, or 25 mg/kg/injection. Five mice in each group were killed 1, 3, 5, and 12 hr later and their serum assayed for IFN titer.

Results and Discussion

The results of this study are summarized in Figures XI-1 and 2. AVS5587 was a significant and rapid inducer of IFN, with high titers seen by 1 hr after injection. When the drug was administered a single time (Figure XI-1), the titers were not as high as when given twice 7 hr apart (Figure XI-2). In addition, the two treatments resulted in a prolongation of IFN titer through 12 hr. The IFN induction was dose responsive, with IFN seen when 100 or 50 mg/kg were injected, but not 25 mg/kg.

These results correlate well with our previously described anti-PTV data (1), where two i.p. treatments were more effective than a single treatment. Also, the rapid IFN induction may explain why this compound was still effective when treatments were begun as late as 36 hr post-virus inoculation.

Conclusions

Single or two i.p. injections of AVS5587 induced significant IFN titers as early as 1 hr after treatment of weanling C57BL/6 mice. The multiple injections resulted in a prolonged IFN titer in the serum, through 12 hr after final treatment.

References

1. Smee, D.F., J.H. Huffman, J. Coombs, J.W. Huggins, and R.W. Sidwell. 1990. Prophylactic and therapeutic activities of 7-thia-8-oxoguanosine against Punta Toro virus infections in mice. Antiviral Res. (in press).
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Figure XI-1. Induction of IFN by a Single i.p. Injection of AVS5587 in C57BL/6 Mice.

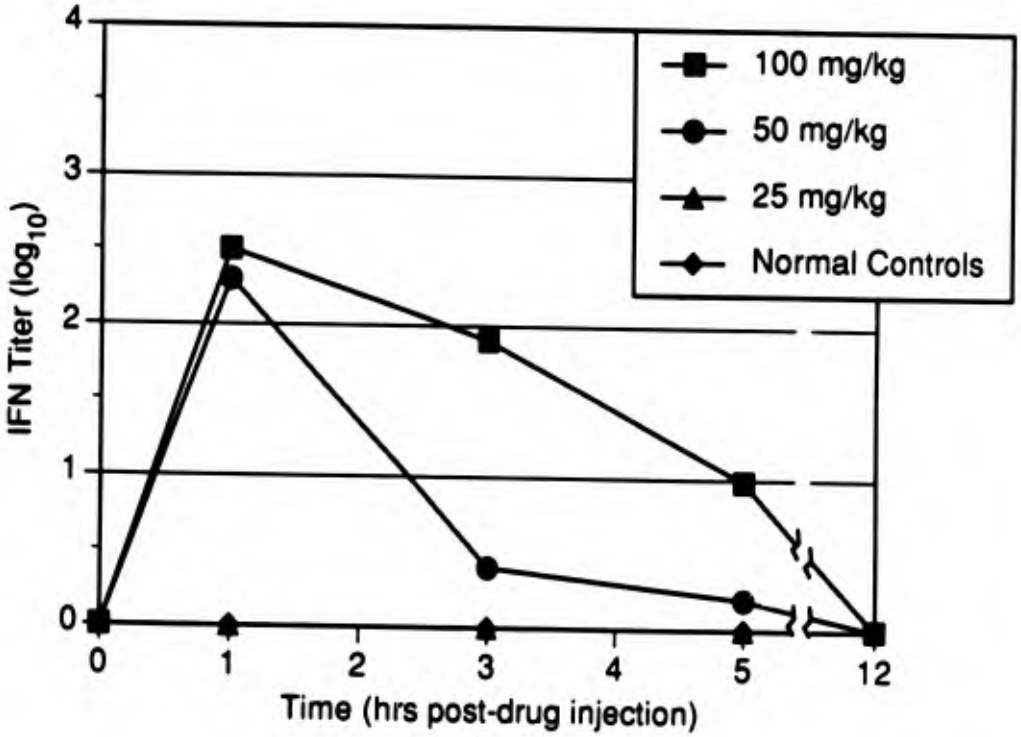
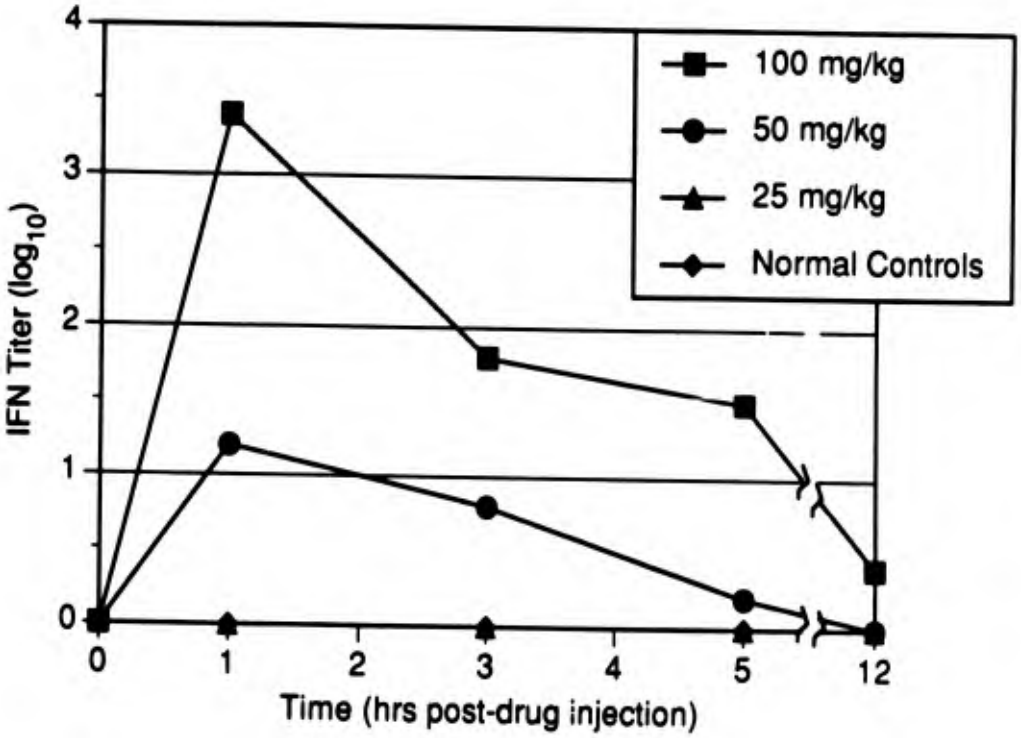


Figure XI-2. Induction of IFN by Two i.p. Injections of AVS5587 in C57BL/6 Mice.



XII. EFFECTS OF AVS2276 AND AVS2285 ON SERUM INTERFERON INDUCTION IN C57BL/6 MICE

Introduction

We have previously found that p.o. treatment with Theracel compounds AVS2276 and AVS2285 were moderately effective against PTV infections in mice (PtA 866, 867, 879, 880). Communication with the company indicated there was some indication that interferon (IFN) induction by these substances may be a mechanism of their antiviral activity. An experiment was therefore run to determine if IFN could be detected in mice used in PTV experiments after treatment with either compound.

Materials and Methods

Compound: AVS2276 (Theracel no. BL-002) and AVS2285 (Theracel no. BL-012) were initially provided by Biological Research Faculty and Facility, Inc., and in subsequent studies were sent directly to us by Theracel Corp. (Rockville, MD) in order to allow us to utilize freshly prepared material. Each was stored at -20°C with desiccant until used. Each was prepared in sterile water daily.

Mice: Three week-old female C57BL/6 mice were obtained from Simonsen (Gilroy, CA) and used after a 24 hr quarantine.

IFN Assay: Samples to be assayed for IFN were placed in 0.1 ml volume on an 18-24 hr monolayer of L cells in 96 well microplates and allowed to incubate for 24 hr at 37°C. After incubation, the cells were drained and 0.1 ml of a 10^3 CCID50/0.1 ml of vesicular stomatitis virus (VSV) strain Indiana was added to each and incubated for 6 days at 37°C. Viral CPE was read microscopically after this incubation. The IFN titer was expressed as units/0.1 ml based on the maximum dilution of serum sample that inhibited VSV CPE by 50% or greater. No attempt was made to separate out IFN α, β, or γ, so we must presume all types were present in the samples assayed. Controls in the study were virus controls, which were cells exposed to test medium (MEM with 2% FBS, 0.18% $NaHCO_3$ and 50 µg gentamicin/ml) and then to VSV, and cell controls which were exposed to test medium only. Serum from infected mice was treated with 1N HCl to bring the pH to ~2 for an overnight period in order to inactivate any PTV present in the serum.

Experiment Design: Twenty mice were treated p.o. a single time with either compound using dosages of 125, 250, 500, 1000, and 2000 mg/kg. Three mice from each group were killed and serum taken at 1, 2, 6, 12, and 24 hr after treatment. The serum was immediately frozen at -80°C and later thawed and assayed for IFN.

Results and Discussion

No detectable IFN was detected in the serum of mice treated with AVS2285. In animals receiving AVS2276, IFN (2.8 $\log_{10}$ units/0.1 ml) was detected in a single mouse killed at 6 hr; at 12 hr one additional mouse had an IFN titer of 1.9 $\log_{10}$ units/0.1 ml. Both mice received 125 mg/kg of the compound. The known positive IFN standard run in parallel had the IFN titer expected.

These data suggest that either these compounds do not induce IFN when administered by oral gavage, or the IFN is produced very late (>24 hr) after treatment.

Oral treatment was used at the suggestion of Dr. Esposito of Theracel, who assured us the substances were very well adsorbed when given by that route, and that was the route used in our previous anti-PTV experiments.

Conclusions

Neither AVS2276 (BL-002) nor AVS2285 (BL-012) induced significant quantities of detectable IFN within a 24 hr period after p.o. administration into 3 week-old C57BL/6 mice.

XIII. REVERSAL OF THE ANTI-PTV EFFECTS OF AVS5587 BY TREATMENT WITH ANTI-INTERFERON ANTIBODY

Introduction

The nucleoside analog 7-thia-8-oxoguanosine (AVS5587) has been previously reported by us to have marked anti-PTV activity in mice (1). The compound is known to be a biological response modifier, with strong IFN-inducing properties as well as being an NK cell activator and an activator of macrophages (2, 3). The compound does not have a direct anti-PTV effect *in vitro*. This study was run to determine if the IFN induction was the primary mechanism whereby this material exerts its *in vivo* anti-PTV activity.

Materials and Methods

Compounds: AVS5587 and AVS01 were provided by Biological Research Faculty and Facility, Inc.; AVS5587 was dissolved in water containing 2% biocarbonate and AVS01 in sterile saline. Antibody to interferon α/β was purchased from Lee Biomolecular (San Diego, CA). It was diluted in sterile water for injection.

Animals: Female 3 week-old C57BL/6 mice were obtained from Simonsen (Gilroy, CA) for these studies and used after a 24 hr quarantine.

Virus: The Adames strain of PTV was propagated as previously described by Sidwell et al. (4).

Experiment Design: Mice were inoculated s.c. with 10 50% lethal doses of PTV, then treated i.p. with AVS5587 or AVS01 24 and 31 hr later. Dosages of AVS5587 were 50 and 25 mg/kg/day; ribavirin was used at 350 mg/kg/day. Ten infected mice were used at each dosage level, and 20 animals were treated in parallel with H_2O. Toxicity controls using 5 animals/dose were run in parallel. In a concurrent study, PTV-infected mice treated with AVS5587 were also treated 30 min after the initial AVS5587 treatment with anti-IFN antibody at a dosage of 2000 units/mouse. As controls, infected and non-infected mice were also treated with the antibody. All mice were held 21 days with deaths noted daily.

Results and Discussion

Used alone, AVS5587 again was found to be significantly inhibitory to PTV infection, as summarized in Table XIII-1. These data confirm our previous results (1). The single anti-IFN antibody treatment totally reversed this antiviral effect, with all infected mice receiving both AVS5587 and antibody dying more rapidly than the placebo-treated controls (Table XIII-2). The latter mice would be expected to have a normal IFN-producing capability, which would serve to at least slow the time to death of the animals.

These data indicate that the IFN induction of AVS5587 appears to be a key factor for anti-PTV activity. We have previously described the rapid and high level of IFN induced in C57BL/6 mice by this compound. This reversal of this effect by anti-IFN antibody is similar to work described by Smee et al. previously (5) using Semliki Forest virus. Although natural killer cell activation is elicited by this compound, it is apparent that immunological response is not significant in protecting mice from PTV.

Conclusions

The immunomodulator AVS5587 (7-thia-8-oxoguanosine) has therapeutic potential against PTV infections. The anti-PTV activity was eliminated by concomitant therapy with anti-IFN α/β antibody, indicating the rapid IFN induction by AVS5587 plays a major role in protecting the mice.

References

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2. Smee, D. F., H. A. Alaghamandan, H. B. Cottam, W. B. Jolley and R. K. Robins. 1991. Antiviral activity of the novel immune modulator 7-thia-8-oxoguanosine. J. Biol. Resp. Mod. (in press).

3. Smee, D. F., H. A. Alaghamandan, H. B. Cottam, B. S. Sharma, W. B. Jolley, and R. K. Robins. 1989. Broad-spectrum in vivo antiviral activity of 7-thia-8-oxoguanosine, a novel immunopotentiating agent. Antimicrob. Ag. Chemother. 33:1487-1492.

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5. Smee, D. F., H. A. Alaghamandan, A. Jin, B. S. Sharma, and W. B. Jolley. 1990. Role of interferon and natural killer cells in the antiviral activity of 7-thia-8-oxoguanosine against Semliki Forest virus infections in mice. Antiviral Res. (in press).

Table XIII-1. Expt. PtA872. Effect of Multiple i.p. Treatment With AVS5587 on Punta Toro Virus Infections in Mice.

Animals: 11.6-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 2% $NaHCO_3$ in H_2O
Treatment Schedule: 24 and 31 hr, post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS5587	50	5/5	0.0	10/10**	>21.0**
	25	5/5	0.4	8/10**	8.5**
Ribavirin	350	5/5	0.1	6/9	6.3
H_2O	-	-	-	1/10	3.8
Normals	-	5/5	0.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS5587 was markedly inhibitory to PTV in this experiment. Compare with Table G-2.

Table XIII-2. Expt. PtA861. Reversal of PTV Inhibitory Effects of AVS5587 by Treatment with Anti-IFN Antibody.

Animals: 11.7-12.6 g (3-4 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.

Drug Diluent: 2% $NaHCO_3$ in H_2O

Treatment Schedule: 5587: 24 & 31 hr post-inoculation.
Anti-IFN: 24.5 & 31.5 hr post-virus

Treatment Route: i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS5587 + Anti-IFN[c]	50	5/5	0.8	0/10	3.6
	25	5/5	0.6	0/10	3.3
Anti-IFN	4000[d]	3/3	0.9	0/7	3.3
Ribavirin	350	5/5	1.0	10/10**	>21.0**
H_2O	-	-	-	1/10	5.8
Normals	-	5/5	1.4	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]2000 units/mouse/treatment.

[d]units/mouse.

*P<0.05 **P<0.01

Conclusions: Treatment with anti-IFN antibody reversed the anti-PTV effects of AVS5587. Compare with Table G-1.

XIV. EFFECTS OF HUMAN INTERLEUKIN-2 (AVS5079) ON PUNTA TORO VIRUS INFECTIONS IN C57BL/6 MICE

Introduction

We have previously reported (1989 Annual Report) that administration of human IL-2 (AVS5079) had a significant inhibitory effect on PTV infections in mice. In that study, murine IL-2 production, usually reduced by PTV infection, was restored by IL-2 treatment, and natural killer (NK) cell activity depressed by the PTV infection appeared to be stimulated by IL-2 treatment.

The present report describes a study designed to confirm the previous results and to determine also the effects of IL-2 on induction of interferon (IFN) and on activity of NK cells in both infected and uninfected mice. We felt it was important to use the same lot of IL-2 in this confirming study; because of a shortage of the material, we were unable to use the maximal (25,000 units/mouse) used previously, but were able to use lower dosages.

Materials and Methods

Virus: Adames strain PTV as described earlier was used.

Animals: Three week-old C57BL/6 mice (Simonsen) as described previously were used after a 24 hr quarantine. They were maintained on standard mouse chow and tap water *ad libitum*.

Compounds: AVS5079 (human rIL-2) and AVS01 (ribavirin) were provided by Biological Research Faculty & Facility, Inc. for these studies. The rIL-2 was used in 5% dextrose in water for injection.

Murine IL-2 Production Assay: Splenic lymphocytes from infected animals were tested for their ability to produce IL-2 by incubating them (2×10^6 cells) in 2 ml of RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% phytohemagglutinin (PHA), and 2-mercapto ethanol. After 48 hr at 37°C, the supernatant was harvested, centrifuged at 500 x g for 5 minutes to remove cells, and assayed for IL-2. The IL-2 assay was done by adding 0.1 ml of serial 2-fold dilutions of the supernate to triplicate wells in 96-well flat-bottomed microplates, after which 4×10^4 HT-2 cells in 0.1 ml medium were added to each well. The HT-2 cells, a murine Balb/c cloned cell line, is IL-2 dependent for its growth. The cell-supernate mixture was incubated at 37°C for 20 hr, pulsed with [^{3}H]thymidine, incubated 4 more hr, and the radiolabel uptake determined.

Assay for Human IL-2 in Serum: Serum taken from rIL-2 treated mice was assayed for the presence of human IL-2 using the Intertest-2 human IL-2 ELISA kit produced by Genzyme Corp. (Boston, MA). The assay was run according to the kit instructions. As controls, a known concentration of human IL-2 and a known negative sample was tested in parallel.

Natural Killer Cell Assay: Spleen cells were assayed for their ability to lyse YAC-1 tumor cells in a conventional chromium release assay (5). YAC cells were labeled with sodium chromate-51, washed and incubated in 96-well round-bottom plates with spleen cells in a ratio of 100:1 effector to target cells. An aliquot of supernatant was removed from each well and the counts per minute (CPM) of radioactivity was determined with a Packard scintillation analyzer. The % chromium release was expressed as:

$$\frac{\text{Experimental CPM - Background CPM}}{\text{Maximum CPM - Background CPM}}$$

Background CPM was determined by incubating a sample of target cells in RPMI-1640 medium and maximum CPM was obtained by incubating target cells in saponin.

IFN Assay: Samples to be assayed for IFN were placed in 0.1 ml volume on an 18-24 hr monolayer of L cells in 96 well microplates and allowed to incubate for 24 hr at 37°C. After incubation, the cells were drained and 0.1 ml of a 10^3 CCID50/0.1 ml of vesicular stomatitis virus (VSV) strain Indiana was added to each and incubated for 6 days at 37°C. Viral CPE was read microscopically after this incubation. The IFN titer was expressed as units/0.1 ml based on the maximum dilution of serum sample that inhibited VSV CPE by 50% or greater. No attempt was made to separate out IFN α, β, or γ, so we must presume all types were present in the samples assayed. Controls in the study were virus controls, which were cells exposed to test medium (MEM with 2% FBS, 0.18% $NaHCO_3$ and 50 µg gentamicin/ml) and then to VSV, and cell controls which were exposed to test medium only.

Experiment Design: Mice were infected s.c. with our standard concentration of PTV and treated i.p. with rIL-2 once daily for 5 days beginning 4 hr post-virus inoculation. Four dosage levels of rIL-2 were used: 12,500; 6,250; 3,125; and 1,563 units/mouse. As a known positive control, AVS01 at a dosage of 75 mg/kg/day was tested in parallel using the same treatment schedule. A standard expanded parameter anti-PTV experiment was run, but in addition, 5 mice in each IL-2 and placebo-treated, infected and treated, uninfected (toxicity control) group were killed 15 min and 2 hr after the final treatment. The sera and spleens from these animals were removed and the spleens suspended in RPMI-1640 medium, then disassociated by use of a tissue homogenizer. The spleen cell preparations were pipetted onto nylon wool columns and incubated 1 hr at 37°C. Non-adherant cells were eluted from the column with warm RPMI-1640 medium and further treated by hypotonic lysis to eliminate red blood cells. They were then assayed for ability to produce IL-2 and for NK cell function. The serum was assayed for IFN titer at the two times indicated.

Results and Discussion

The anti-PTV portion of this study is summarized in Table XIV-1. In the experiment, rIL-2 was again inhibitory to the PTV infection at all dosage levels, as seen particularly by increased numbers of survivors. Liver scores were not reduced at the rIL-2 levels used—a similar observation as seen in the previous experiment. Liver virus titers were reduced particularly at the highest rIL-2 dose used. Serum virus was not assayed since the serum was used for IFN testing. AVS01 exerted the positive activity expected. The toxicity controls all survived therapy with both rIL-2 and AVS01.

No IFN could be detected from the serum of uninfected, rIL-2-treated mice at the times indicated. Relatively low titers of IFN were seen in the infected, treated mice, but we attribute this to the induction by the virus, which we have previously seen at this time.

The effects of treatment on NK cell activity are summarized in Table XIV-2. Infection by PTV again caused a significant depression in NK cell activity, seen at both assay times. At the 15 min post-treatment assay time, a modest increase of NK cell activity was seen in the mice receiving the 2 highest doses; by 2 hr post-treatment, this increase was ore pronounced, and seen at all dosage levels. The results are confused, however, by an apparent erratic NK cell activity seen in the uninfected, treated mice which were run in parallel. We have concern that this repeated rIL-2 treatment, with assay after the termination of the fifth treatment, may have caused a hyporeactive state in the animal, both for IFN induction and NK cell activation. Such a hyporeactive state may account for the lower than normal NK cell activation in the uninfected animals.

An experiment is planned to determine the rIL-2 effects after a single treatment in uninfected animals.

The splenocyte IL-2 production was still in process of determination at the time this report was prepared.

Conclusions

Treatment of Adames PTV-infected mice with 12,500 and lower units of AVS5079 (recombinant human interleukin-2) resulted in significant disease inhibition. Treatment was i.p. qd x 5 beginning 4 hr after PTV inoculation; assays for IFN production in the mice and for NK cell activation, done after termination of the final treatment, showed no IFN produced, and an increased NK cell activation in infected mice but a decreased NK cell activation in uninfected, treated mice. The latter data suggest a possible hyporeactive state induced in the animal.

Table XIV-1. Expt. PtA812. Effect of Once Daily i.p. Treatments with AVS5079 on Punta Toro Virus Infections in Mice.

Animals: 8.7-11.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 5% Dextrose in H_2O.

Treatment Schedule: qd x 5, beginning 4 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity Controls				Infected Treated					
			Mean IFN Titer					Mean	Mean Liver	Mean IFN Titer	
Compound	Dosage (Units/day)	Surv/ Total	15 min post[a]	2 hr post	24 hr post	Surv/ Total	MST[b] (days)	Liver Score[c] 15 min post	Virus Titer[d] (log_{10}) 15 min post	15 min post	2 hr post
AVS5079	12,500	5/5	0.0	0.0	0.0	9/10**	6.0	2.9	2.4	0.0	0.0
	6,250	5/5	0.0	0.0	0.0	7/10*	6.3	2.9	3.1	0.0	0.3
	3,125	5/5	0.0	0.0	0.0	8/10**	5.5	2.8	4.3	0.0	0.4
	1,563	5/5	0.0	0.0	0.0	8/10**	5.0	2.3	3.9	0.5	1.1
Ribavirin	75^	5/5	0.2	0.0	0.0	10/10**	>21.0**	0.4**	1.1*	0.0	0.0
Dextrose in H_2O	-	-	-	-	-	6/20	6.5	2.7	4.0	0.6	0.5
Normals	-	5/5	0.0	0.0	0.0	-	-	0.0	0.0	0.0	

[a]Serum samples from animals killed at the times indicated after the final treatment.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 5.

[d]Geometric mean of five livers taken on day 5.

^mg/kg/day.

*P<0.05 **P<0.01

Table XIV-2. Expt. PTA812. Effect of Human IL-2 (AVS5079) on Splenocyte Natural Killer Cell Activity in PTV-Infected and Uninfected C57BL/6 Mice

Treatment	Dosage (units/mouse)	Mean Natural Killer Cell Activity (% Cr Release) 15 min Post-Treatment[a]		2 hr Post-Treatment	
		PTV-infected	Uninfected	PTV-infected	Uninfected
Hu IL-2	12,500	62.1	62.5	65.8	76.9
	6,250	65.9	77.7	63.0	69.1
	3,125	56.1	41.8	57.6	69.1
	1,563	52.1	41.8	75.0	57.7
5% Dextrose in water	0	60.0	—	55.2	—
Normals	—	—	79.3	—	79.3

[a]Spleens taken from animals killed at the times indicated after the final treatment.

XV. EFFECT OF SINGLE I.P. TREATMENT WITH AVS5079 ON INTERFERON INDUCTION AND NATURAL KILLER CELL ACTIVITY IN C57BL/6 MICE

Introduction

We have previously reported that multiple i.p. treatments with AVS5079 (human recombinant IL-2) was significantly effective in preventing PTV-induced infections in mice. In those previous studies, we showed that the material stimulated the production of murine IL-2 by splenocytes and enhanced NK cell activity in the mice. After the last treatment had been administered, IFN production was also sought but not found. We were concerned that the somewhat low NK cell activity and lack of IFN response may have been a result of a hyporesponsiveness by the host due to the multiple treatments received. We have subsequently repeated the study and measured the NK cell activity and IFN response after a single i.p. treatment.

Materials and Methods

Compound: AVS5079 (human rIL-2) was provided by Biological Research Faculty and Facility, Inc. for this study. The material was used in 5% dextrose in water for injection.

Animals: Three week old C57BL/6 mice (Simonsen) as described previously were used.

Natural Killer Cell Assay: Spleen cells were assayed for their ability to lyse YAC-1 tumor cells in a conventional chromium release assay (5). YAC cells were labeled with sodium chromate-51, washed and incubated in 96-well round-bottom plates with spleen cells in a ratio of 100:1 effector to target cells. An aliquot of supernatant was removed from each well and the counts per minute (CPM) of radioactivity was determined with a Packard scintillation analyzer. The % chromium release was expressed as:

$$\frac{\text{Experimental CPM - Background CPM}}{\text{Maximum CPM - Background CPM}}$$

Background CPM was determined by incubating a sample of target cells in RPMI-1640 medium and maximum CPM was obtained by incubating target cells in saponin.

IFN Assay: Samples to be assayed for IFN were placed in 0.1 ml volume on an 18-24 hr monolayer of L cells in 96 well microplates and allowed to incubate for 24 hr at 37°C. After incubation, the cells were drained and 0.1 ml of a 10^3 CCID50/0.1 ml of vesicular stomatitis virus (VSV) strain Indiana was added to each and incubated for 6 days at 37°C. Viral CPE was read microscopically after this incubation. The IFN titer was expressed as units/0.1 ml based on the maximum dilution of serum sample that inhibited VSV CPE by 50% or greater. No attempt was made to separate out IFN α, β, or γ, so we must presume all types were present in the samples assayed. Controls in the study were virus controls, which were cells exposed to test medium (MEM with 2% FBS, 0.18% $NaHCO_3$ and 50 µg gentamicin/ml) and then to VSV, and cell controls which were exposed to test medium only. Serum from infected mice was treated with 1N HCl to bring the pH to ~2 for an overnight period in order to inactivate any PTV present in the serum.

Experiment Design: Mice were treated i.p. with AVS5079 in doses of 1,562; 3,125; 6,250; 12,500; and 25,000 Cetus units/mouse. At 15 minutes, 2 hr and 24 hr post-treatment, 5 mice were killed and their serum assayed for IFN. At the 15 min and 2 hr times, their spleens were also taken, processed as described in E, and assayed for NK cell activity.

Results and Discussion

This experiment is summarized in Table XV-1. All doses of AVS5079 stimulated KN cell activity significantly as seen both 15 min and 2 hr post-treatment. No circulating IFN was seen at any time period.

The NK cell activity seen after this single injection of human IL-2 was greater than that seen in our previously reported experiment where 5 daily treatments were administered prior to NK cell activity was determined. These data suggest that a hyporesponsiveness indeed was seen because of the multiple treatments. The lack of detectable serum IFN after a single treatment indicates to us that IL-2 is not a good IFN inducer and that the anti-PTV activity seen previously was a result of immunological factors other that IFN.

Conclusions

Single i.p. injections with AVS5079 (human IL-2) significantly stimulated NK cell activity in C57BL/6 mice 15 min and 2 hr after treatment. Serum IFN was not detected in these animals at 15 min, 2 hr or 24 hr after treatment.

Literature Cited

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Table XV-1. Effect of a Single i.p. Treatment with AVS5079 on Interferon Production and Natural Killer Cell Activity in C57BL/6 Mice.

Dosage (units/mouse)	15 Min. Post-Treatment Mean IFN Titer[a]	15 Min. Post-Treatment Mean NK Cell Activity[b]	2 Hr Post-Treatment Mean IFN Titer[a]	2 Hr Post-Treatment Mean NK Cell Activity[b]	24 Hr Post-Treatment Mean IFN Titer
25,000	<1.0	25++±3	<1.0	29**±3	<1.0
12,500	<1.0	23**±7	<1.0	27**±9	<1.0
6,250	<1.0	18**±6	<1.0	25**±8	<1.0
3,125	<1.0	19**±8	<1.0	23**±6	<1.0
1,562	<1.0	17**±7	<1.0	21**±6	<1.0
0	<1.0	15±3	nd	nd	nd

[a]Units/ml.

[b]% Cr Release ± 2 SE.

*P<0.05 **P<0.01, compared to untreated controls.

XVI. EFFECT OF AVS1018 ON INTERFERON AND INTERLEUKIN-2 INDUCTION IN C57BL/6 MICE

Introduction

We have reported previously the efficacy of AVS1018 (phenyleneamine) on in vivo PTV infections. We were asked by Dr. Kende of USAMRIID to also determine if this material would induce interleukin-2 (IL-2) production in the mouse. This report describes our experiment to determine this, as well as the potential for this compound to induce interferon (IFN) in mice.

Materials and Methods

Compound: AVS1018 was provided by Biological Research Faculty and Facility for this study. The material was dissolved in sterile water and held at 4°C until used.

Mice: Female 3-week-old C57BL/6 mice were provided by Simonsen Laboratories. All were housed under normal, previously defined, conditions.

IFN Assay: Samples to be assayed for IFN were placed in 0.1 ml volume on an 18-24 hr monolayer of L cells in 96 well microplates and allowed to incubate for 24 hr at 37°C. After incubation, the cells were drained and 0.1 ml of a 10^3 CCID50/0.1 ml of vesicular stomatitis virus (VSV) strain Indiana was added to each and incubated for 6 days at 37°C. Viral CPE was read microscopically after this incubation. The IFN titer was expressed as units/0.1 ml based on the maximum dilution of serum sample that inhibited VSV CPE by 50% or greater. No attempt was made to separate out IFN α, β, or γ, so we must presume all types were present in the samples assayed. Controls in the study were virus controls, which were cells exposed to test medium (MEM with 2% FBS, 0.18% $NaHCO_3$ and 50 µg gentamicin/ml) and then to VSV, and cell controls which were exposed to test medium only. Serum from infected mice was treated with 1N HCl to bring the pH to ~2 for an overnight period in order to inactivate any PTV present in the serum.

Murine IL-2 Production Assay: Splenic lymphocytes from infected animals were tested for their ability to produce IL-2 by incubating them (2×10^6 cells) in 2 ml of RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% phyhtohemagglutinin (PHA), and 2-mercapto ethanol. After 48 hr at 37°C, the supernatant was harvested, centrifuged at 500 x g for 5 minutes to remove cells, and assayed for IL-2. The IL-2 assay was done by adding 0.1 ml of serial 2-fold dilutions of the supernate to triplicate wells in 96-well flat-bottomed microplates, after which 4×10^4 HT-2 cells in 0.1 ml medium were added to each well. The HT-2 cells, a murine Balb/c cloned cell line, is IL-2 dependent for its growth. The cell-supernate mixture was incubated at 37°C for 20 hr, pulsed with [^{3}H]thymidine, incubated 4 more hr, and the radiolabel uptake determined.

Experiment Design: AVS1018 in dosages of 1.56 3.13, 6.25, and 12.5 mg/kg was administered p.o. to mice. One and 4 hr later, 5 mice in each group were killed, bled and the spleens removed. Five normal, untreated mice were similarly killed and processed at 1 hr only. The serum was assayed for IFN and the spleens suspended in RPMI-1640 medium, then disassociated by use of a tissue homogenizer. The spleen cell preparations were pipetted into nylon wool columns and incubated 1 hr at 37°C. Non-adherent cells were eluted from the column with warm RPMI-1640 medium and further treated by hypotomic lysis to eliminate red blood cells, then assayed for IL-2 production.

Results and Discussion

The results of this study are summarized in Table XVI-1. No serum IFN was detected at either time point in either AVS1018-treated or normal mice although a standard IFN sample with known titer run in parallel had the IFN titer expected. AVS1018 appeared to markedly induce the production of IL-2 in the mice, with this induction especially manifested at the 4 hr post-treatment sampling period. This IL-2 induction amounted to a 30% to 250% increase in titer. This increase was not too dependent on dosage of AVS1018, since maximal IL-2 production occurred at the lowest and the highest drug concentrations used.

We have previously shown that AVS1018, when administered p.o. on days +1, +5, and +9 significantly prevented the death of PTV-infected mice. Virus titers were reduced and signs of hepatic icterus were also significantly reduced. It is apparent that this efficacy was either a result of specific PTV inhibition or due to modulation of some host immune or other biological response. Since IFN was not induced in this study, whereas IL-2 was produced in high titer by the treated

mice, we presume the IL-2 may have had a significant influence on the infection. It is notable that human recombinant IL-2 also has an effect on PTV infections, with the immunological responses stimulated being IL-2 and natural killer cell activity (see 1989 Annual Report, section XXVI). As described in the previous section of this report, that human IL-2 did not induce detectable quantities of serum IFN. Thus IL-2 induction may be an important means of defense against PTV infections.

Summary

Splenocytes from mice treated with AVS1018 produced significant quantities of IL-2 when assayed 1 and 4 hrs after p.o. treatment. No serum IFN was detected in these animals.

Table XVI-1. Effect of a Single Oral Treatment with AVS1018 on Interferon and Interleukin-2 Induction in C57BL/6 Mice.

Dosage (mg/kg/day)	1 Hour After Treatment		4 Hour After Treatment	
	Mean IFN Titer (units/ml)	Mean IL-2 Production (CPM)	Mean IFN Titer (units/ml)	Mean IL-2 Production (CPM)
12.50	<1.0	17,768**	<1.0	43,924**
6.25	<1.0	16,041**	<1.0	16,019*
3.13	<1.0	16,702**	<1.0	20,779**
1.56	<1.0	26,049**	<1.0	21,258**
0	<1.0	12,747	—	—

*P<0.05 **P<0.01, compared to untreated controls.

XVII. EFFECT OF AVS1761, 1968, 2933, AND 4726 ON INTERFERON AND INTERLEUKIN-2 INDUCTION IN C57BL/6 MICE

Introduction

We have reported previously (Section XVI) the efficacy of AVS1018 to induce interleukin-2 (IL-2) *in vivo*. We were asked by Dr. Kende of USAMRIID to also determine if other materials would induce IL-2 production in the mouse. This report describes our experiment to determine this, as well as the potential for these compounds to induce interferon (IFN) in mice.

Materials and Methods

Compound: All compounds was provided by Biological Research Faculty and Facility for this study. The materials were dissolved in sterile water and held at 4°C until used.

Mice: Female 3-week-old C57BL/6 mice were provided by Simonsen Laboratories. All were housed under normal, previously defined, conditions.

IFN Assay: Samples to be assayed for IFN were placed in 0.1 ml volume on an 18-24 hr monolayer of L cells in 96 well microplates and allowed to incubate for 24 hr at 37°C. After incubation, the cells were drained and 0.1 ml of a 10^3 CCID50/0.1 ml of vesicular stomatitis virus (VSV) strain Indiana was added to each and incubated for 6 days at 37°C. Viral CPE was read microscopically after this incubation. The IFN titer was expressed as units/0.1 ml based on the maximum dilution of serum sample that inhibited VSV CPE by 50% or greater. No attempt was made to separate out IFN α, β, or γ, so we must presume all types were present in the samples assayed. Controls in the study were virus controls, which were cells exposed to test medium (MEM with 2% FBS, 0.18% $NaHCO_3$ and 50 µg gentamicin/ml) and then to VSV, and cell controls which were exposed to test medium only. Serum from infected mice was treated with 1N HCl to bring the pH to ~2 for an overnight period in order to inactivate any PTV present in the serum.

Murine IL-2 Production Assay: Splenic lymphocytes from infected animals were tested for their ability to produce IL-2 by incubating them (2×10^6 cells) in 2 ml of RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% phyhtohemagglutinin (PHA), and 2-mercapto ethanol. After 48 hr at 37°C, the supernatant was harvested, centrifuged at 500 x g for 5 minutes to remove cells, and assayed for IL-2. The IL-2 assay was done by adding 0.1 ml of serial 2-fold dilutions of the supernate to triplicate wells in 96-well flat-bottomed microplates, after which 4×10^4 HT-2 cells in 0.1 ml medium were added to each well. The HT-2 cells, a murine Balb/c cloned cell line, is IL-2 dependent for its growth. The cell-supernate mixture was incubated at 37°C for 20 hr, pulsed with [^{3}H]thymidine, incubated 4 more hr, and the radiolabel uptake determined.

Experiment Design: Compounds at various doses were administered i.p. to mice. One and 4 hr later, 5 mice in each group were killed, bled and the spleens removed. Other mice were bled at 24 hr. Five normal, untreated mice were similarly killed and processed at 1 hr only. The serum was assayed for IFN and the spleens suspended in RPMI-1640 medium, then disassociated by use of a tissue homogenizer. The spleen cell preparations were pipetted into nylon wool columns and incubated 1 hr at 37°C. Non-adherent cells were eluted from the column with warm RPMI-1640 medium and further treated by hypotomic lysis to eliminate red blood cells, then assayed for IL-2 production.

Results and Discussion

The results of these studies are summarized in Table XVII-1-4. AVS1968 (Lederle IFN-inducer) induced IL-2 by 1 hr after i.p. treatment (50 and 100 mg/kg) but no significant levels were detected at 4 hr. IFN was detected at 24 hr in sera of mice receiving AVS1968 at 50 and 100 mg/kg. AVS1761 (poly IC•LC) induced significant levels of IL-2 only at 2.5 mg/kg. IFN was not measured in that assay since it has been determined in detail previously. AVS2933 (CGP 19835 A lipid) and AVS4726 (placebo for CGP 19835 A lipid) induced no IL-2 or IFN at the doses tested. It will be of interest to study the role of IL-2 in protection against PTV infection. Since AVS1968 induced IFN, it should be further evaluated in vivo in the PTV disease model. Previous anti-PTV experiments revealed this material to have significant activity against the hepatotropic PTV infection.

Summary

Splenocytes from mice treated with AVS1968 reduced significant quantities of IL-2 when assayed 1 hr after i.p. treatment. IFN was detected in serum at 24 hr. AVS1761 induced significant quantities of IL-2 at 1 and 4 hr. AVS2933 and AVS4726 were inactive.

Table XVII-1. Effect of a Single i.p. Treatment with AVS1761 on Interleukin-2 Induction in C57BL/6 Mice.

Dosage (mg/kg/day)	1 Hour After Treatment Mean IL-2 Production (CPM)	4 Hour After Treatment Mean IL-2 Production (CPM)
2.5	884*	992*
1.25	709	754
0.625	620	758
0.31	583	673
0	515	—

*P<0.05, compared to untreated controls.

Table XVII-2. Effect of a Single I.p. Treatment with AVS1968 on Interferon and Interleukin-2 Induction in C57BL/6 Mice.

Dosage (mg/kg/day)	1 Hour After Treatment		4 Hour After Treatment		24 Hour After Treatment
	Mean IFN Titer (units/ml)	Mean IL-2 Prod. (CPM)	Mean IFN Titer (units/ml)	Mean IL-2 Prod. (CPM)	Mean IFN Titer (units/ml)
100	<1.0	1,174**	<1.0	779	2.1**
50	<1.0	1,064**	<1.0	577	1.5
25	<1.0	679	<1.0	316	<1.0
12.5	<1.0	732	<1.0	302	<1.0
0	<1.0	671	—	—	—

*P<0.05 **P<0.01, compared to untreated controls.

Table XVII-3. Effect of a Single i.p. Treatment with AVS2933 on Interferon and Interleukin-2 Induction in C57BL/6 Mice.

Dosage (mg/kg/day)	1 Hour After Treatment		4 Hour After Treatment		24 Hour After Treatment
	Mean IFN Titer (units/ml)	Mean IL-2 Prod. (CPM)	Mean IFN Titer (units/ml)	Mean IL-2 Prod. (CPM)	Mean IFN Titer (units/ml)
10	<1.0	938	<1.0	684	<1.0
5	<1.0	939	<1.0	573	<1.0
2.5	<1.0	794	<1.0	640	<1.0
1.25	<1.0	921	<1.0	679	<1.0
0.625	<1.0	866	<1.0	—	<1.0
0	<1.0	876	—	—	—
0.313	<1.0	871	<1.0	793	<1.0
0.157	<1.0	764	<1.0	905	<1.0
0.078	<1.0	842	<1.0	791	<1.0
0	<1.0	739	—	—	—

*P<0.05 **P<0.01, compared to untreated controls.

Table XVII-4. Effect of a Single I.p. Treatment with AVS4726 on Interferon and Interleukin-2 Induction in C57BL/6 Mice.

Dosage (mg/kg/day)	1 Hour After Treatment		4 Hour After Treatment		24 Hour After Treatment
	Mean IFN Titer (units/ml)	Mean IL-2 Prod. (CPM)	Mean IFN Titer (units/ml)	Mean IL-2 Prod. (CPM)	Mean IFN Titer (units/ml)
Undilute	<1.0	749	<1.0	862	<1.0
0	<1.0	739	—	—	—

XVIII. A MEASUREMENT OF AVS01 TOXICITY USING PULSE OXIMETRY

Introduction

We have recently found that arterial oxygen saturation (SaO_2%), measured by pulsatile absorbance of light, can be readily determined in albino mice using a pulse oximeter (Figure XVIII-1). An experiment was run to determine if this parameter could be used to measure ribavirin (AVS01) toxicity in mice.

Materials and Methods

Compounds: AVS01 was supplied by Biological Research Faculty and Facility, Inc. It was dissolved in sterile saline for use in this study.

Animals: Four-week-old female BALB/c mice were obtained from Simonsen Laboratories (Gilroy, CA). Following a 24 hr quarantine, the animals were used in this study. They were maintained on Wayne mouse chow and tap water *ad libitum*.

Pulse Oximeter: An Ohmeda Biox 3740 pulse oximeter (Ohmeda, Louisville, OH) was used. The Ohmeda Finger Probe clip, which sends a light beam through the mouse, was used.

Experiment Design: Ten mice were treated i.p. with 800 or 1200 mg/kg/day of ribavirin twice daily for 5 days. Pulse oximeter readings were taken each morning on days 1 through 4 of the experiment, and the animals were observed daily for occurrence of death. Pulse oximeter readings were expressed as SaO_2%, read directly from the instrument approximately 10 seconds after each mouse was placed in the finger probe clip. Hematocrit readings were taken from 5 mice in each group killed each day of the experiment.

Results and Discussion

This study is summarized in Figure XVIII-2. SaO_2% began to decline by day 3 of treatment, continuing by day 4. By day 5, all the mice had died. Hematocrit readings (not shown in the figure) did not decline appreciably during this early phase of treatment. In our experience, anemia begins to appear with prolonged therapy.

By days 3 and 4, excessive hemorrhaging in the intestinal area was observed in sacrificed mice. This loss of blood, which would reduce the amount available for oxygen transport, would cause a significant lowering of the SaO_2%, which was observed in this study.

The pulse oximeter transmits light through the combination of blood and non-blood components of the finger, and, in our case, the entire mouse. The light transmitted through this pulsating vascular bed will be attenuated by the blood and non-blood components, but because the attenuation is pulsatile and assumed to result solely from the arterial component, the oximeter is calibrated to separate, literally by subtraction, the effects of the two components and can thus measure the SaO_2% of arterial blood in the mass. In the oximeter, two light-emitting diodes (660 nm and 940 nm) are mounted on one side of the vascular bed (in our case, the mouse), and a photodiode which converts light intensity into electrical current, is mounted on the opposite side. The diodes pulse at regular intervals and the photodiode measures the varying light intensities, which are changed to digital information processed by the algorithm in the oximeter. The Ohmeda Biox 3240 pulse oximeter (Ohmeda, Louisville, OH) used by us calculates SaO_2% as $K1(V)^2 + K2(V) + K3$, in which V is the change in the voltage in the red channel divided by the change in voltage in the infrared channel. K1, K2, and K3 are constants that are functions of the optical characteristics of hemoglobin as well as other variables.

These data indicate the pulse oximeter measurement of SaO_2% is an efficient means of measuring certain forms of toxicity in mice.

Conclusions

Ribavirin administered i.p. twice a day for 5 days in doses of 800 and 1200 mg/kg/day was lethally toxic to 4 week-old BALB/c mice. As the animals approached the time of death, which was attributed to excessive hemorrhaging in the gut, their arterial oxygen saturation (SaO_2%) declined appreciably.

Figure XVIII-1. Use of the Ohmeda Biox 3740 Pulse Oximeter with Finger Probe for Monitoring SaO_2% in Mice.

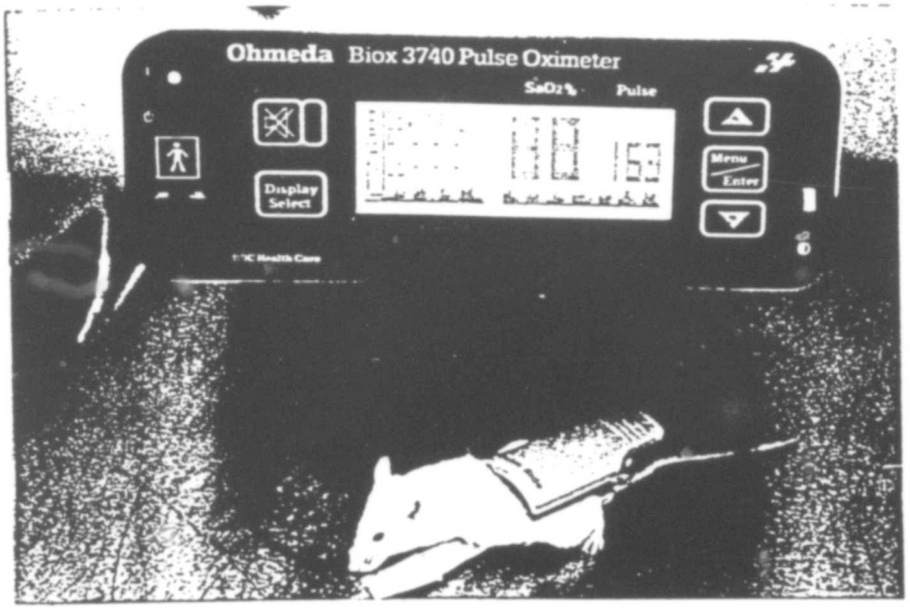
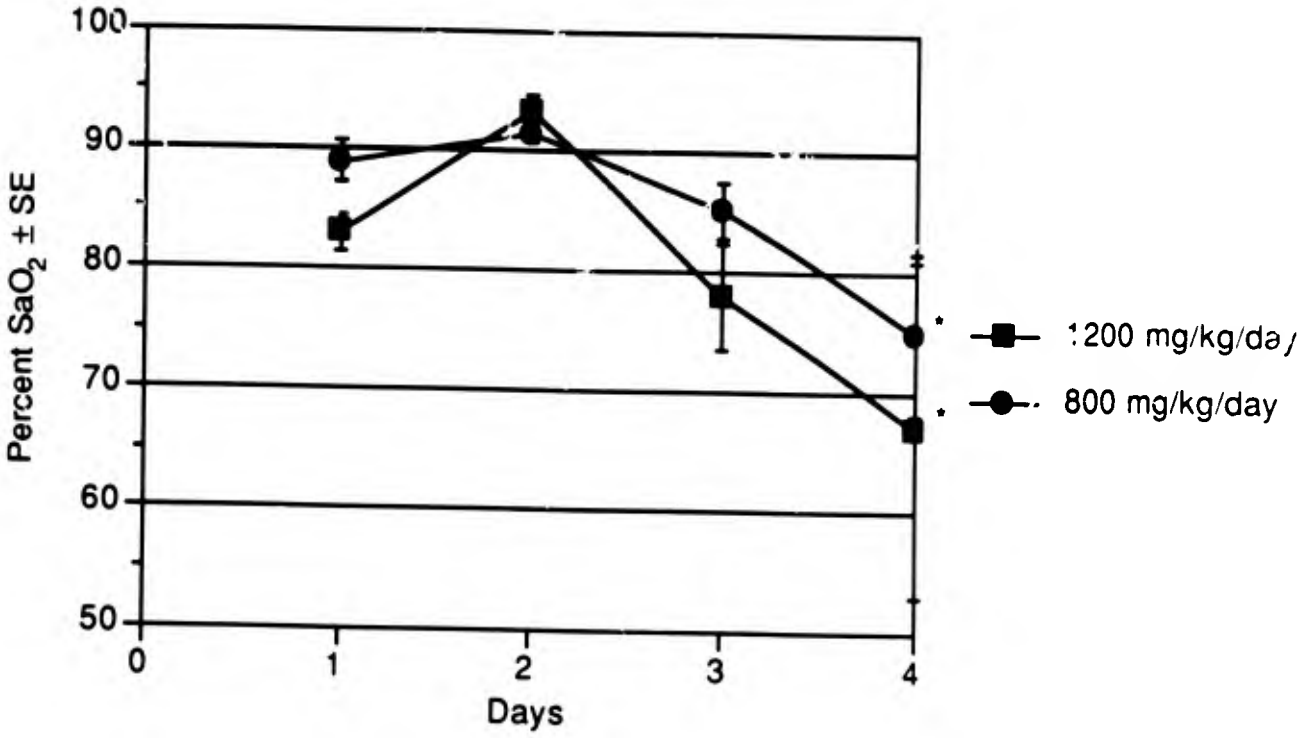


Figure XVIII-2. Effect of Intraperitoneal AVS01[a] Treatment on SaO_2% in Uninfected Balb/c Mice.



[a]bid x 5.

*All animals died after these times.

Conclusions: High-dose AVS01 (ribavirin) treatment resulted in significant declines in SaO_2% prior to death of the animal. This decline correlated with the appearance of severe hemorrhaging in the intestinal tract of the animals.

XIX. EFFECTS OF PUNTA TORO VIRUS ON MACROMOLECULAR SYNTHESIS OF CELLS

Introduction

Little has been published about the effects of Punta Toro virus (PTV) on the macromolecular synthesis in cells infected by the virus and its relationship to virus infection. Smith and Pifat (1) have shown that 12 hours after infection assembly of PTV virions can be seen occurring in membranes of the Golgi cisternae. At 24 hours post-infection distinct cytopathic effects were also observed in culture, and cell lysis did not occur until 36 hours after infection. In La Crosse virus infections, the major nucleocapsed protein is first detected at 1-2 hr after infection and remains detectable at 12-15 hr after infection (2). In contrast, the G_1 large glycoprotein was not detected until 4 hr after infection and the G_2 glycoprotein is not detectable until 6 hr after infection. The kinetics is also similar for Rift Valley Fever virus (3). In addition, the members of the *Phlebovirus* group are all thought to inhibit cellular RNA and protein synthesis (4).

The object of this study was to examine the effects of Punta Toro virus on macromolecular synthesis of infected host cells.

Materials and Methods

Virus: The Adames strain of Punta Toro virus (PTV) was propagated as previously described by Sidwell et al. (5).

Cells: A derivative strain of continuously passaged monkey kidney cells (LLC-MK_2), maintained in minimum essential medium (MEM, Grand Island Biological, Grand Island, N.Y.) containing 5% fetal bovine serum (FBS, HyClone Labs, Logan UT) and 0.1% $NaHCO_3$ without antibiotics was used. The cells were determined to be free of mycoplasma.

Effect of PTV infection on log phase and stationary phase LLC-MK_2 cells: LLC-MK_2 cells were seeded in 12 well plates at 1×10^5 cells well and allowed to reach confluence by incubation at 37°C for two days. In another set of experiments cells were seeded at 5×10^4 cells and incubated overnight at 37°C, to obtain log phase cells. Cells were then washed with MEM without serum and PTV virus stock absorbed for 1 hr in each well. Mock infected wells were incubated with MEM without serum. Virus or medium was removed and MEM + 2% serum was added to each well. At various times after virus exposure or mock infection (1, 2, 4, 8, 16, 24, 48 hours for log phase cells and 8, 12, 16, 20 hours for stationary phase cells) media was aspirated and appropriate isotope, diluted in MEM without serum was added to each well. [^{3}H]Leucine was diluted in leucine-deficient MEM. An equal volume of MEM with serum was added for a final concentration of 2% serum. To wells with [^{3}H]leucine, MEM leucine deficient medium + 4% fetal bovine serum was added. Medium was also removed at time 0 from log phase cells, directly after application of virus and cells treated as above. Isotope was incubated for 1 hour at 37°C, removed, and cells fixed with 10% TCA and harvested as previously described by Sidwell, et al. (5). Acid-insoluble CPM were determined in a Packard Scintillation Counter.

Test Statistics: Analysis of variance was used to determine significant differences between log phase and stationary cell experiments. To determine significant differences between time periods for each type of cell, Fisher's LSD test was employed.

Results and Discussion

PTV infection significantly ($P<0.01$) stimulates macromolecular synthesis in log phase cells at 1 hr post-exposure. This time period was not assayed in stationary cells. In contrast virus infection reduced the uptake of [^{3}H]deoxyadenosine into the acid insoluble portions of both stationary phase and log phase cells (Figures XIX-1, 2) at 8-24 hours, although more drastically in stationary phase cells. The effects on protein and RNA synthesis appeared to be similar for both PTV infected log- and stationary-phase cells. However, the decrease in uptake of label was significantly more dramatic from 8-24 hours ($P<0.01$) in PTV-infected stationary phase cells.

Interestingly, the inhibition effects of PTV infection on RNA and protein synthesis in LLC-MK_2 cells seemed to be abrogated by 48 hours, although DNA synthesis was still significantly inhibited ($P<0.01$). The depression of macromolecular synthesis at the 16-hour time period in the log phase cell experiment probably represents an aberrant set of wells in which the cells were

not growing very well, since the uptake of a nucleoside of precursors into control cells was 2-5 fold less than that into control cells from other time periods (the level of uptake in control cells remained rather constant for all other time periods, data not shown).

If PTV infection resembles other bunyavirus infections, then from 1-6 hours post-virus exposure, viral proteins and transcripts are being made at optimal amounts (4). The data of this study show that during this time period, the cellular macromolecular processes were initially enhanced and then went back down to normal levels in log phase cells. At 12-24 hours post-virus exposure, other studies have shown that PTV virion assembly begins and cell surface expression of PTV antigens can be detected during this time period (1). The data presented here suggest that during virus assembly the cellular macromolecular synthesis decreased to levels below normal in stationary and log phase cells, in agreement with other studies which show that the *Phlebovirus* group inhibits cellular RNA and protein synthesis. Pifat et al. (1) have also shown that cytopathic effects occur at 36-48 hours post infection in PTV-infected cells. In our study, cellular DNA synthesis became inhibited at that time, while RNA and protein synthesis levels were returned to near normal levels, perhaps reflecting the beginning of cellular death due to viral infection.

Whether the effects described above are an actual stimulation or depression of macromolecular synthesis due to viral induced stimulation or inhibition of cellular enzymes, or viral induced enzymes, or to an increase or decrease in cell permeability to the radiolabeled nucleotide precursors has not been determined.

To better understand these data, they should be correlated with time course studies on the appearance and abundance of viral transcripts and viral proteins within infected cells. It would be useful to determine virus yields from each time period in log phase and stationary cells since the data here indicates that active macromolecular synthesis of the cell may not be necessary for virus production. We should monitor the effects of PTV infection on macromolecular synthesis in stationary phase 0-48 hours to determine if these types of cells are also stimulated during early PTV infection. In addition, studies need to be done to determine the permeability of PTV-infected cells at various times post exposure, to see if the apparent effects on cellular macromolecular synthesis are due to perturbation of those processes or if the uptake is merely a reflection of permeability changes of the cell membrane.

Summary

Punta Toro virus infection appeared to significantly inhibit DNA, RNA and protein synthesis from 16-24 hours post-virus exposure. DNA synthesis, as reflected by deoxyadenosine uptake, remains perturbed throughout PTV infection from 8-48 hours post virus exposure. In addition, PTV seems to enhance macromolecular synthesis 1 hour post exposure to virus in log phase cells. Whether these effects are an actual stimulation or depression of macromolecular synthesis due to viral-induced stimulation or inhibition of cellular enzymes, to viral-induced enzymes, or to an increase or decrease in cell permeability is still to be determined.

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Figure XIX-1. Effect of Punta Toro Virus Infection on the Uptake of Radiolabeled Precursors of Macromolecular Synthesis into Stationary Phase LLC-MK$_2$ Cells.

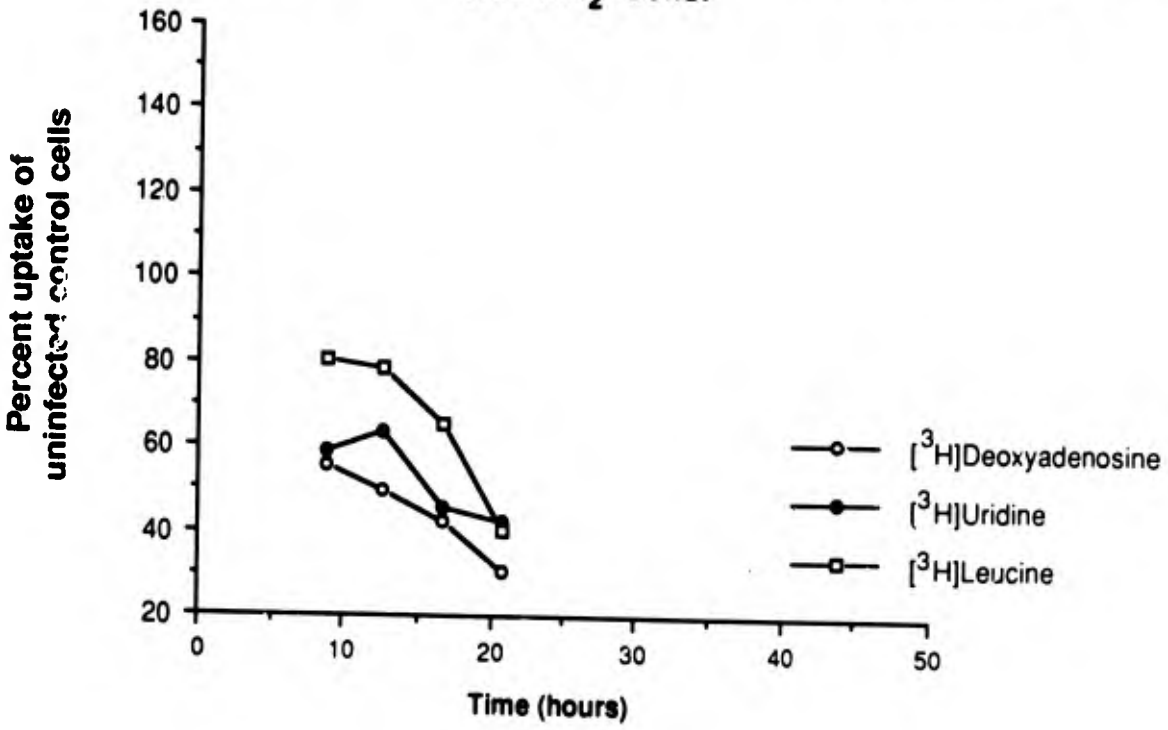
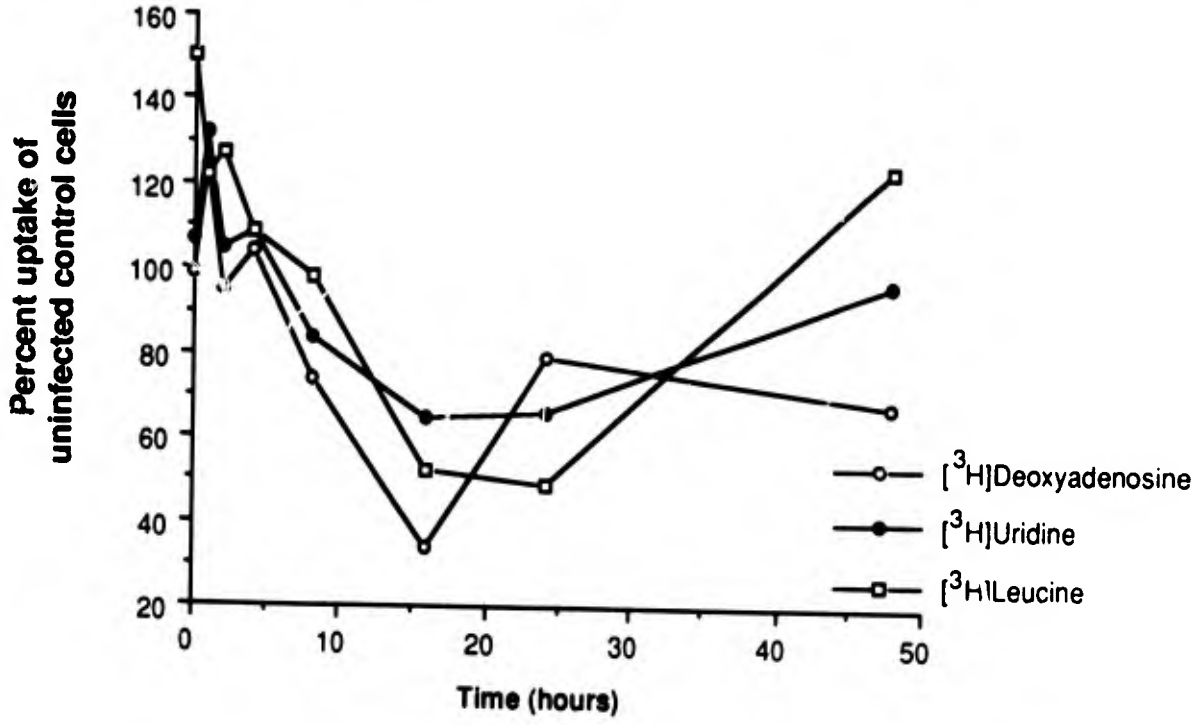


Figure XIX-2. Effect of Punta Toro Virus Infection on the Uptake of Radiolabeled Precursors of Macromolecular Synthesis into Log Phase LLC-MK_2 Cells.



XX. REDUCTION OF AVS01 TOXICITY BY TREATMENT WITH AVS5587 IN MICE

Introduction

The synergistic effects against PTV infections of the combination treatment of AVS01 (ribavirin) and AVS5587 (7-thia-8-oxoguanosine, TOGuo) were described in our last Quarterly Report. Of particular interest in that combination chemotherapy study was the apparent reversal of ribavirin's high dose-induced toxicity by treatment with TOGuo. This report describes an additional study to confirm this toxicity reversal.

Materials and Methods

Compounds: AVS01 and AVS5587 were supplied by Biological Research Faculty and Facility, Inc. AVS01 was dissolved in sterile water and AVS5587 was dissolved in a 2% bicarbonate solution at pH 8.6-8.9.

Animals: Three to four week-old C57BL/6 mice (Simonsen) as described previously were used after a 24 hr quarantine.

Experiment Design: Groups of 10 mice (5 females, 5 males) each were treated as follows:

1: AVS01 at dosages of 1250, 1500, and 1750 mg/kg/day, p.o. twice daily for 5 days.

2: AVS5587 at a dosage of 25 mg/kg/day, i.p. in a single injection.

3: AVS01 as described in #1, with AVS5587 as described in #2 given 3 days after initial AVS01 treatment.

4: AVS01 as described in #1, with AVS5587 as described in #2 given 4 days after initial AVS01 treatment.

5: AVS01 as described in #1, with AVS5587 as described in #2 given 5 days after initial AVS01 treatment.

The mice were weighed prior to initial treatment and again 18 hr after final treatment with each compound. They were observed daily for death for 21 days.

Results and Discussion

This study is summarized in Tables XX-1 and 2. In the male mice (Table XX-1), ribavirin at all 3 dosages used was lethally toxic to the majority of the mice, the mean day to death varying from 7.3 to 10.5 days. The mice lost over 1.5 g over the duration of the treatment. The results were not consistent in the female mice (Table XX-2), which we usually do not use in toxicity studies. In these animals, much erraticism occurred in the mice receiving AVS01 only, with only the middle dose lethal to all the mice. Major host weight loss was seen at all dosages, and mean day to death varied from 6.8 to 10.7 days.

AVS5587 was reasonably well-tolerated with all male mice treated only with this compound surviving through 21 days, but a mild (0.2 g) host weight loss was seen (Table XX-1). The female mice also all survived treatment, and gained weight during therapy (Table XX-2).

The male mice treated with ribavirin and then with AVS5587 on day 3 did not die as readily from ribavirin toxicity, with only 2 mice dying during the experiment and less weight loss occurring (Table XX-1). As the TOGuo therapy was delayed to 4 or 5 days after initiation of ribavirin treatments, this protective effect generally decreased.

Again, considerable erraticism was seen in the female mice (Table XX-2), with little protection from lethal toxicity seen using TOGuo. Considerably less weight loss was seen in the mice receiving the drug combinations.

We cannot offer an explanation for the erraticism seen with the female mice in this study. We ordinarily use male mice for toxicity controls in our PTV chemotherapy experiments, and the results seen here with the male mice appear to confirm our earlier observation that treatment with TOGuo of mice receiving a usually lethally toxic dose of ribavirin will prevent the deaths from occurring. Other, previous studies using i.p. ribavirin treatment of female mice yielded more uniform results.

Conclusions

Treatment with AVS5587 of male C57BL/6 mice receiving lethal toxic doses of AVS01 prevented the usual deaths of the mice, particularly if the AVS5587 therapy was given 3 days after start of AVS01 therapy. Delaying AVS5587 therapy to 4 or 5 days reduced these toxicity reversal effects. Female mice treated in a similar manner responded in an erratic fashion to AVS01 therapy.

Table XX-1. Expt. Pt263-267. Effect of Treatment With AVS01 and AVS5587 on Death and Weight Loss in Uninfected Mice.

Animals: 7.1-15.1 g (3-4 wk) C57BL/6 Male Mice.
Virus: None
Drug Diluent: Sterile H_2O, 2% $NaHCO_3$
Treatment Schedule: 01: Twice daily x 5
5587: single, days 3, 4, or 5
Treatment Route: p.o., i.p.
Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	MST[b] (days)
AVS01	1750	2/5	-1.5	8.7
	1500	1/5	-1.6	10.5
	1250	1/5	-1.7	7.3
AVS5587[c]	25	5/5	-0.2	>21.0
AVS01 + AVS5587[c]	1750 + 25	4/5	-0.5	6.0
	1500 + 25	4/5*	-0.5	7.0
	1250 + 25	5/5**	-0.1	>21.0
AVS01 + AVS5587[d]	1750 + 25	3/5	-1.3	8.5
	1500 + 25	3/5	-1.2	6.5
	1250 + 25	2/5	-1.2	9.3
AVS01 + AVS5587[e]	1750 + 25	1/5	-1.5	7.0
	1500 + 25	0/5	-2.2	8.8
	1250 + 25	4/5*	-0.9	6.0
Normals	-	5/5	2.3	>21.0

[a]Difference between initial weight at start of treatment and weight 18 hr following last treatment.

[b]Mean survival time of mice dying on or before day 21.

[c]Administered on day 3.

[d]Administered on day 4.

[e]Administered on day 5.

Table XX-2. Expt. Pt263-267. Effect of Treatment With AVS01 and AVS5587 on Death and Weight Loss in Uninfected Mice.

Animals: 7.5-12.2 g (3-4 wk) C57BL/6 Female Mice.
Virus: None
Drug Diluent: Sterile H_2O, 2% $NaHCO_3$
Treatment Schedule: 01: Twice daily x 5
5587: single, days 3, 4, or 5
Treatment Route: p.o., i.p.
Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	MST[b] (days)
AVS01	1750	2/5	-2.3	10.7
	1500	0/5	-3.3	6.8
	1250	4/5	-1.7	8.0
AVS5587[c]	25	5/5	0.7	>21.0
AVS01 + AVS5587[c]	1750 + 25	2/5	-1.0	8.0
	1500 + 25	1/5	-0.5	7.3
	1250 + 25	4/5	-0.4	8.0
AVS01 + AVS5587[d]	1750 + 25	1/5	-2.3	6.8
	1500 + 25	4/5**	-0.3	8.0
	1250 + 25	2/5	-1.6	8.0
AVS01 + AVS5587[e]	1750 + 25	2/5	-1.8	8.3
	1500 + 25	5/5**	-0.2	>21.0
	1250 + 25	4/5	-0.4	11.0
Normals	-	5/5	1.5	>21.0

[a]Difference between initial weight at start of treatment and weight 18 hr following last treatment.

[b]Mean survival time of mice dying on or before day 21.

[c]Administered on day 3.

[d]Administered on day 4.

[e]Administered on day 5.

XXI. REDUCTION OF AVS01 TOXICITY BY TREATMENT WITH AVS2776 IN MICE

Introduction

We have previously reported that the combination therapy of AVS01 (ribavirin) and AVS2776 (bropirimine) was synergistic against PTV infections in mice. An important second observation was that treatment with AVS2776 appeared to lessen the toxicity of ribavirin as seen by weight gain of mice treated with both drugs in contrast to loss of weight in the animals treated with ribavirin only.

This latter observation was followed up in the present study, in which the relative toxicities were determined in mice treated with usually lethally toxic doses of ribavirin were also treated with bropirimine at various times relative to ribavirin's treatments.

Materials and Methods

Compounds: AVS01 and AVS2776 were provided by Biological Research Faculty and Facility, Inc. AVS01 was dissolved in sterile water and AVS2776 was suspended in 0.4% carboxymethylcellulose.

Animals: Three to four week-old male C57BL/6 mice (Simonsen) were used after a 24 hr quarantine.

Experiment Design: Groups of 10 mice each were treated as follows:

1: AVS01 at dosages of 800, 1000, and 1200 mg/kg/day, p.o. twice daily for 5 days.

2: AVS2776 at dosages of 25 and 50 mg/kg/day, p.o. in a single treatment.

3: AVS01 as described in #1, with AVS2776 as described in #2, the latter given 3 days after initial AVS01 treatment.

4: AVS01 as described in #1, with AVS2776 as described in #2, the latter given 4 days after initial AVS01 treatment.

5: AVS01 as described in #1, with AVS2776 as described in #2, the latter given 5 days after initial AVS01 treatment.

The mice were weighed prior to initial treatment and again 18 hr following final treatment with each compound. They were observed daily for 21 days for occurrence of death.

Results and Discussion

The results are summarized in Table XXI-1. All doses of AVS01 were lethally toxic to the mice, the animals dying 7-8 days after initiation of therapy. Major host weight loss occurred prior to death in all AVS01-treated groups.

AVS2776 was reasonably well tolerated at both dosages used, all mice surviving the duration of the study and no host weight loss seen.

Treatment of AVS01-treated mice with the 50 mg/kg/day dose of AVS2776 given on day 3 resulted in highly significant increases in survivors in the mice treated with the 1000 and 800 mg/kg/day doses of AVS01. At these dosages, weight loss was also lessened when the mice were also treated with this dose of AVS2776. The 25 mg/kg/day dose of AVS2776 given at this same time period prevented AVS01 lethal toxicity at the 800 mg/kg/day dose of this latter compound.

When the AVS2776 treatments were given later after initiation of ribavirin therapy, the prevention of lethal toxicity was lessened but was still apparent, especially in mice receiving the lower dose of bropirimine and the lower dose of ribavirin.

The mean survival times of the mice dying from AVS01 toxicity were usually increased throughout the study when AVS2776 was also administered.

These data indicate that a single oral treatment with the immunomodulator bropirimine can indeed prevent the usually ribavirin-induced lethal toxicity, particularly if the immunomodulator treatment is given during the time of ribavirin therapy.

Ribavirin is known to be immunosuppressive when used at high dosage levels. This has included suppression of the primary immune response in mice to sheep red blood cells (1), reduction of serum antibodies to various virus challenges (2), moderate inhibition of cellular immune response to EL-4 tumor cells (3), reduced guinea pig contact hypersensitivity to dinitrochlorobenzene (3), and inhibition of adjuvant-induced arthritis in rats (4). In contrast, bropirimine has a broad spectrum of immune stimulatory effects, including macrophage activation (5), augmentation of NK-cell cytotoxicity (7), interleukin-1 and interleukin-2 stimulation (8), enhancement of antigen-mediated antibody formation (6), and stimulation of bone marrow proliferation (6). It would seem apparent that treatment with bropirimine would tend to reverse the adverse immunological effects of the high dose of ribavirin.

Ribavirin is a competitive inhibitor of IMP dehydrogenase (9) leading to inhibition of DNA synthesis. This presumably results in development of anemia (10) which may be the cause of death of the animals. This anemia is characterized by a decrease in hematocrit, hemoglobin concentration, and erythrocyte count (10). We have not yet determined the actual cause of the ribavirin-associated death in the mice treated in our studies; this will be the subject of future investigations. The actual role of bropirimine in preventing the death is still to be elucidated.

Conclusions

Treatment of C57BL/6 mice with high dosages (800-1200 mg/kg/day) of AVS01 (ribavirin) for a 5-day period, results in death of the mice, the mean day to death being less than 8 days. When bropirimine (AVS2776) is administered to these mice in a single oral treatment 3 days after start of ribavirin treatment, it may significantly prevent the usual ribavirin-associated death. If bropirimine treatment is delayed to 4 or 5 days, this reversal of toxicity was less pronounced.

References

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Table XXI-1. Expt. Pt255-262. Effect of Treatment With AVS01 and AVS2776 on Death and Weight Loss in Uninfected Mice.

Animals: 10.4-12.0 g (3-4 wk) C57BL/6 Mice.
Virus: None
Drug Diluent: Sterile H_2O, 0.4% CMC
Treatment Schedule: 01: Twice daily x 5
2776: single, days 3, 4, or 5
Treatment Route: p.o.
Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	MST[b] (days)
AVS01	1200	2/10	-1.4	7.9
	1000	0/10	-1.7	7.2
	800	0/10	-1.3	7.9
AVS2776[c]	50	10/10	—	>21.0
	25	10/10	—	>21.0
AVS01 + AVS2776[c]	1200 + 50	2/10	-1.9	8.5
	1000 + 50	9/10**	0.0	6.0
	800 + 50	9/10**	-0.6	9.0
	1200 + 25	0/10	-2.8	7.4
	1000 + 25	0/10	-1.7	8.7**
	800 + 25	6/10**	-1.3	8.5
AVS01 + AVS2776[d]	1200 + 50	0/10	-1.6	7.8
	1000 + 50	2/11	-1.5	7.6
	800 + 50	0/9	-1.7	7.9
	1200 + 25	0/10	-2.0	8.6
	1000 + 25	0/10	-1.8	8.8**
	800 + 25	6/10**	-1.2	9.5*
AVS01 + AVS2776[e]	1200 + 50	1/10	-2.1	7.9
	1000 + 50	0/10	-1.6	9.4*
	800 + 50	0/10	-2.6	7.0
	1200 + 25	0/10	-2.0	7.7
	1000 + 25	6/10**	-1.0	8.5
	800 + 25	0/10	-1.6	9.3*
Normals	-	10/10	1.4	>21.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of mice (day 6 of experiment).

[b]Mean survival time of mice dying on or before day 21.

[c]Administered on day 3.

[d]Administered on day 4.

[e]Administered on day 5.

XXII. EFFECT OF AVS2776 IN THE REDUCTION OF AVS01-INDUCED TOXICITY IN MICE

Introduction

This report describes a combination experiment run to determine if AVS2776 (bropirimine) can reverse the lethal toxicity of ribavirin in mice. Previously, we showed that AVS5587 (7-thia-8-oxoguanosine), a BRM similar in action to bropirimine, had this potential.

Materials and Methods

Compounds: AVS01 and AVS2776 were provided by Biological Research Faculty and Facility, Inc. AVS01 was dissolved in saline and AVS2776 suspended in 0.4% carboxymethylcellulose for these studies.

Animals: Female 3 week old C57BL/6 mice were obtained from Simonsen (Gilroy, CA) for these studies and used after a 24 hr quarantine.

Experiment Design: Twenty mice per group were held for death through 21 days. Other mice treated similarly were sacrificed on days 1 and 3 after initiation of ribavirin treatment in order to evaluate liver toxicity and hematocrits. Ribavirin was inoculated i.p. by itself, bropirimine was given p.o. by itself, or the two were combined. Ribavirin treatments were twice daily for 5 days, whereas bropirimine was given once shortly after the first dose of ribavirin was administered. Normal (untreated) mice were run in parallel.

Statistical Analysis: Increases in survivors were analyzed using chi-square analysis with Yates' correction. Increases in mean survival times of mice that died on or before day 21 and reductions in SGOT, SGPT and hematocrit were evaluated using Student's *t* test. Ranked sum analysis (Wilcoxon test) was used to compare inhibition of mean liver scores.

Results and Discussion

Table XXII-1 shows the results obtained for this combination study. With reference to the toxicity of ribavirin by itself, doses of 800 and 1200 mg/kg/day were lethal to mice. On day 3, SaO_2% levels in these mice had declined to 76-77%. We had a lower than usual reading in the normal controls, however, which obscures the significance of the above results. Liver scores were high in the 1200 mg/kg group, and SGOT/SGPT values were significantly elevated.

Bropirimine by itself did not alter the parameters measured. The combination of bropirimine and ribavirin did not protect mice from death at 1200 mg/kg ribavirin, but may have had a slight effect in the 800 mg/kg ribavirin group. SGOT and SGPT values were lower in the groups receiving both compounds compared to those receiving ribavirin alone, suggesting a decrease in toxicity.

Conclusions

Overall, AVS2776 at 50 mg/kg had a weak effect in reversing the toxicity of ribavirin (used at 800 and 1200 mg/kg) in mice. Since only one dose of bropirimine was used, one cannot rule out the possibility that higher doses or multiple treatments may enhance the activity of bropirimine in this setting as was seen in Section XXI.

Table XXII-1. Expt. PT277-279. Effect of Combination Treatment with AVS01 and AVS2776 to Reverse Toxicity.

Animals: 8-10 g BALB/c Mice.
Virus: None.
Drug Diluent: Sterile saline + 0.4% CMC.

Treatment Schedule: 01: bid x 5; 2776: once only.
Treatment Route: 01: i.p.; 2776: p.o.
Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Surv/ Total	MST[a] (days)	% SaO_2 Day 1	% SaO_2 Day 3	Mean Liver Score[b] Day 1	Mean Liver Score[b] Day 3	Hematocrit Day 1	Hematocrit Day 3	SGOT Day 1	SGOT Day 3	SGPT Day 1	SGPT Day 3
AVS01 +	1200 + 0	0/20	4.2	91	76	0.7	2.3	42	54	466	746	184	289
AVS2776	800 + 0	0/20	4.1	92	77	0.4	0.6	43	55	174	339	71	161
	1200 + 50	0/20	3.8	92	—	0.7	2.1	43	54	266	135	86	164
	800 + 50	4/20	3.9	91	85	0.7	0.9	43	50	184	216	49	208
	0 + 50	20/20	>21.0	—	82	0.2	0.0	47	44	132	122	52	48
CMC	-	20/20	>21.0	90	84	0.0	0.3	46	44	142	122	35	37

[a]Mean survival time of mice dying on or before day 21.

[b]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver.

*P<0.05 **P<0.01

XXIII. EXPANDED PARAMETER EFFECTS OF ORAL TREATMENT WITH AVS2776 ON AVS01 TOXICITY

Introduction

We have previously reported (Sections XXI, XXII) that oral treatment with the immunomodulator AVS2776 (bropirimine) significantly prevented the usual lethal toxicity associated with i.p. treatment using high dosages of AVS01 ribavirin). This experiment was run to confirm those earlier findings, and to expand our evaluation parameters to examine the effects of the combination treatment on hepatic icterus, SGOT, SGPT and hematocrit levels. The latter was thought to be a particularly important parameter since high dosage of ribavirin are known to cause pronounced anemia in mice.

Materials and Methods

Compounds: AVS01 and AVS2776 were provided by Biological Research Faculty and Facility, Inc. AVS01 was dissolved in sterile water, AVS2776 was suspended in 0.4% carboxymethylcellulose.

Animals: Three to four week-old C57BL/6 mice (Simonsen) were used after a 24 hr quarantine.

Experiment Design: Groups of 5 mice were treated as follows:

1. AVS01 at dosages of 800 and 1200 mg/kg/day, p.o. twice daily for 5 days.
2. AVS2776 at a dosage of 50 mg/kg p.o. once only.
3. AVS01 as described in #1, with AVS2776 as described in #2 administered 3 days after initiation of ribavirin therapy.

One group of 10 mice in each group were weighed prior to initial treatment and again 18 hr following final treatment. Deaths were recorded daily. Five mice in each group were designated to be killed on days 2, 4, 6, 8, 10, 12, and 21 or until the animals died from apparent toxicity. At each time of sacrifice, the animals were bled and liver discoloration scored. The livers and spleens were then preserved in formalin for possible later evaluation. The heparinized blood was tested for hematocrit value and the plasma separated and assayed for SGOT and SGPT levels as we have previously described.

Results and Discussion

Treatment with 800 and 1200 mg/kg/day of AVS01 was lethally toxic to mice, with all animals dying at both dosage levels by day 6, 24 hr after termination of treatment. AVS2776, used at 50 mg/kg, was well tolerated by the mice. In this study, the AVS2776 treatment, given 3 days after start of AVS01 therapy, did not prevent the toxicity-associated deaths (Table XXIII-1).

Ribavirin therapy resulted in a decline of hematocrit (Figure XXIII-1), but this decline was not considered precipitous. The mice dying often displayed a hemorrhaging in the intestinal area, which presumably was the cause of death. AVS2776 did not appreciably change this hemocrit decline, seen also in the same figure.

No increases in SGOT, SGPT or liver score were seen in any animals in the approximately one week observation time of this experiment.

The lethality data of this experiment contrast strongly with our previous data in which 50 mg/kg of AVS2776 significantly prevented death of the mice receiving a usually lethal dose of ribavirin. At this point, we can offer no explanation for this difference in result.

Conclusions

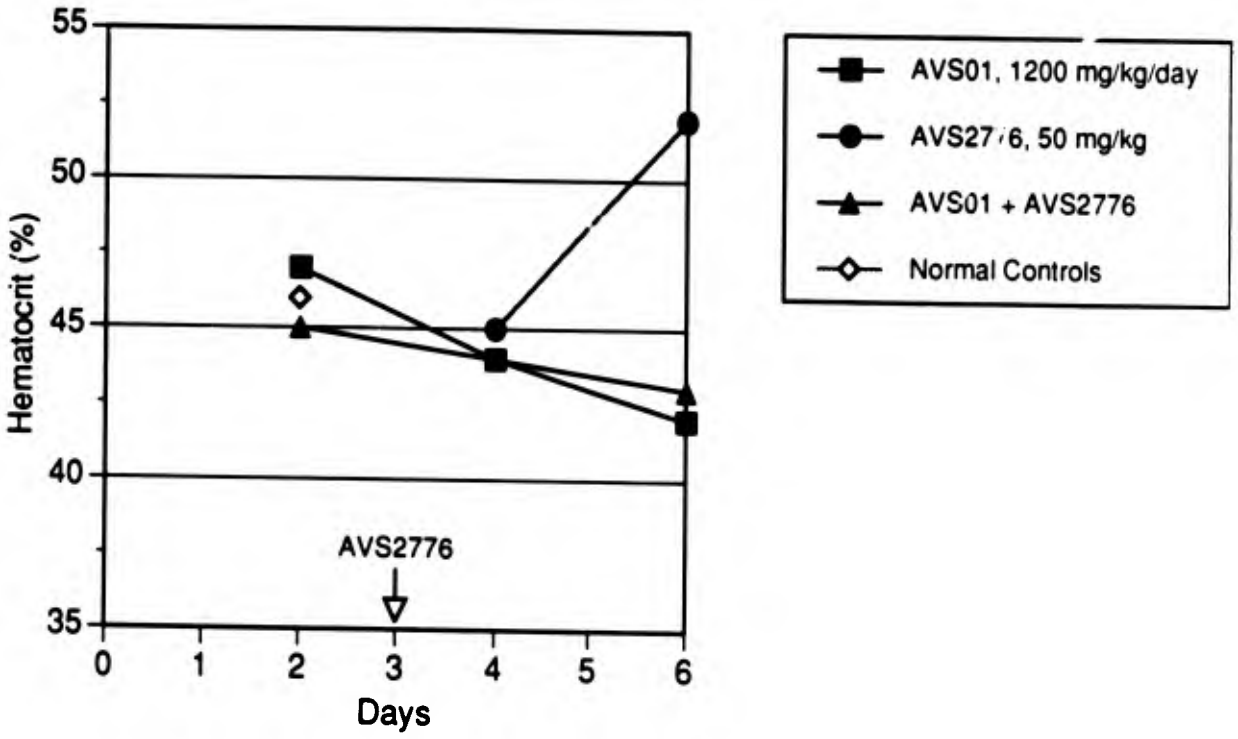
Treatment of C57BL/6 mice with high (800-1200 mg/kg/day) of AVS01 (ribavirin) for a 5-day period caused an early lethal toxicity of the mice. When bropirimine (AVS2776) was administered to these mice in a single oral dose of 50 mg/kg, essentially no difference in toxicity was seen, which conflicts with earlier experiments (Sections XXI, XXII) where the lethal toxicity was alleviated. No significant effects on SGOT, SGPT, or liver discoloration were seen by AVS01 treatment, although some anemia exhibited as decline in hematocrit was observed by the 6th day of the study.

Table XXIII-1. PT274-276. Effect of Single p.o. AVS2776 Treatment on the Usually Lethal Toxic Effects of AVS01 in C57BL/6 Mice[a].

Compound	Dosage	% Survival
AVS01	1200	0
	800	0
AVS2776	50	100
AVS01 + AVS2776	1200 + 50	0
	800 + 50	0

[a]AVS01 administered p.o. bid x 5; AVS2776 administered p.o. once only on day 3.

Figure XXIII-1. PT274-276. Effect of p.o. Treatment with AVS2776 on AVS01-Induced Anemia in C57BL/6 Mice[a].



[a]AVS01 administered p.o. bid x 5 starting on day 0.

XXIV. COMPARISON OF AVS206 TOXICITY IN BALB/C AND C57BL/6 MICE

Introduction

In our last Annual Report, many experiments were reported on the use of AVS206 (ribamidine). All of those studies were run in C57BL/6 mice, and a rather accurate 50% lethal dose (LD50) was determined. In separate studies run in BALB/c mice, this compound appeared to be significantly more toxic than in C57BL/6 mice. An experiment was therefore run to compare the toxicity of AVS206 in both strains of mice, the results of which are here described.

Materials and Methods

Compounds: AVS206 was provided by Biological Research Faculty and Facility, Inc. The same lot was used in the entire study. The compound was dissolved in sterile saline for use in the study.

Animals: Three to four week-old female BALB/c and C57BL/6 mice, both strains weighing 8-10 g., were obtained from Simonsen Laboratories (Gilroy, CA). The animals were maintained on Wayne mouse chow and tap water *ad libitum*.

Experiment Design: Dosages of 1000, 500, 250, and 125 mg/kg/day of AVS206 were administered to groups of 3 mice of each strain and age i.p. twice daily for 5 days. The mice, together with normal controls, were weighed prior to initial treatment and again 18 hr after the final treatment. All were observed daily for death for 21 days.

Results and Discussion

The results are summarized in Tables XXIV-1 and 2. In the BALB/c mice of both ages, the 1000 mg/kg/day dose of AVS206 killed all the mice, whereas in the C57BL/6 mice, all survived with moderate weight loss. The LD50 in BALB/c mice was thus determined to be approximately 700 mg/kg/day; it was >1000 mg/kg/day in C57BL/6 mice.

These data confirm our earlier, preliminary findings that the toxicity of AVS206 varied according to mouse strain. This is not a unique finding; one of the Utah State University toxicologists, Dr. Rhagubir Sharma, has made a similar observation that C57BL/6 mice were 2 to 4 times more resistant to aflatoxin than BALB/c or CD-1 mice. He attributed this difference to different liver enzyme profiles in the various mouse strains. Pifat and Smith (1) have reported that C57BL/6 mice were more sensitive to PTV infection than other mouse strains; PTV is known to be hepatotropic, thus the differences in viral sensitivity also probably are dependent on differences in some aspect of the liver in each mouse strain.

These results suggest caution in interpreting antiviral and toxicity data when a single strain of mice is used.

Conclusions

AVS206 was more toxic to weanling BALB/c mice than to weanling C57BL/6 mice when administered i.p. twice daily for 5 days.

References

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Table XXIV-1. Expt. Pt272-273. Effect of Twice Daily i.p. Treatment With AVS206 on Death and Weight Loss in Uninfected Mice: Comparison of Toxicity in BALB/c and C57BL/6 Mice.

Animals: 8.0-10.0 g female mice.
Virus: None
Drug Diluent: Sterile saline

Treatment Schedule: bid x 5
Treatment Route: i.p.
Experiment Duration: 21 days.

		BALB/c Mice			C57BL/6 Mice		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	MST[b] (days)	Surv/ Total	Host Wt. Change (g)[a]	MST[b] (days)
AVS206	1000	0/3	-2.1	7.7	3/3	-0.7	>21.0
	500	3/3	0.6	>21.0	3/3	0.3	>21.0
	250	3/3	1.4	>21.0	3/3	-0.4	>21.0
	125	3/3	1.0	>21.0	3/3	1.0	>21.0
Normals	-	3/3	1.3	>21.0	3/3	2.1	>21.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of mice.

[b]Mean survival time of mice dying on or before day 21.

Conclusions: AVS206 (ribamidine) was more toxic in BALB/c mice than in equal aged C57BL/6 mice.

Table XXIV-2. Expt. Pt270-271. Effect of Twice Daily i.p. Treatment With AVS206 on Death and Weight Loss in Uninfected Mice: Comparison of Toxicity in BALB/c and C57BL/6 Mice.

Animals: 16.0-18.0 g female mice.
Virus: None
Drug Diluent: Sterile saline

Treatment Schedule: bid x 5
Treatment Route: i.p.
Experiment Duration: 21 days.

		BALB/c Mice			C57BL/6 Mice		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	MST[b] (days)	Surv/ Total	Host Wt. Change (g)[a]	MST[b] (days)
AVS206	1000	0/3	-4.2	6.7	3/3	-1.4	>21.0
	500	3/3	-3.5	>21.0	3/3	0.3	>21.0
	250	3/3	-2.7	>21.0	3/3	-0.5	>21.0
	125	3/3	-0.5	>21.0	3/3	0.2	>21.0
Normals	-	3/3	-0.6	>21.0	3/3	0.6	>21.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of mice.

[b]Mean survival time of mice dying on or before day 21.

Conclusions: AVS206 (ribamidine) was more toxic in BALB/c mice than in equal aged C57BL/6 mice.

XXV. DETERMINATION OF THE SUSCEPTIBILITY OF NIH-III MICE TO PTV

Introduction

It was of interest to determine the relative susceptibilities of immunocompromised mice to PTV infection. A strain of mice having genetically-induced immune deficiencies is being raised in our laboratory and was evaluated. These were the NIH-III mouse. The NIH-III mice combine the nude (*nu*) mutation rendering them athymic, the beige (*bg*) mutation reducing the number of NK cells, and the *xid* mutation reducing the number of LAK cells (1).

Materials and Methods

Animals: The NIH-III mice were reared under special aseptic conditions in the Utah State University Laboratory Animal Research Center. They were maintained on sterile food and water *ad libitum*.

Virus: The Adames strain of PTV as used in all our standard PTV studies was employed.

Experiment Design: Four or five mice were injected s.c. with each of 5 dilutions of PTV, then held and observed for death over a 21-day period.

Results and Discussion

The results are summarized in Table XXV-1. The high concentrations of PTV did not cause death in the mice. However, the 10^{-3} concentration apparently killed one animal, and the 10^{-4} concentration killed all mice injected. We have encountered a "window" of sensitivity in the past using this virus in other mouse strains. In such a situation, the higher concentration (usually 10^{0} or 10^{-1}) were less lethal to the mice than were lower concentrations, suggesting the presence of defective interfering particles. These results with NIH-III mice, however, indicate a much exaggerated "window", if indeed the deaths were due to the virus at all. This could be due to some immunological defect in the mice. We intend to repeat this experiment using lower virus concentrations.

Conclusions

NIH-III mice containing the *nu* mutation rendering them athymic, the *bg* mutation reducing their NK cells, and the *xid* mutation reducing the number of LAK cells were assayed for their sensitivity to PTV; only mice infected with the least concentrated virus dilution died, suggesting a greater resistance to defective interfering particles. The virus will be retitrated in these mice.

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Table XXV-1. Susceptibility of NIH-III Mice[a] to s.c. PTV Inoculation.

Virus Dilution	Surv/ Total	Mean Surv. Time
10^{0}	4/4	>21
10^{-1}	4/4	>21
10^{-2}	4/4	>21
10^{-3}	3/4	8.0
10^{-4}	0/5	4.4

[a]5-8 month-old females.

XXVI. TITRATION OF PUNTA TORO VIRUS IN THREE AND FOUR WEEK-OLD SWISS WEBSTER MICE

Introduction

In the initial work studying PTV in mice, Pifat and Smith (1) reported Swiss Webster mice to be moderately sensitive to the infection. Since these mice are considerably lower in price than the C57BL/6 mice currently used, a titration was performed to assess their sensitivity to the Adames strain of PTV now in our laboratory.

Materials and Methods

Virus: The Adames strain of PTV was propagated as previously described by Sidwell et al. (1).

Animals: Female Swiss Webster mice weighing 9-11 g (3 week-old) or 14-17 g (4 week-old) were obtained from Simonsen Laboratories (Gilroy, CA) for this study. They were used after a 24 hr quarantine.

Experiment Design: Ten mice in each age group of mice were injected i.p. with 0.2 ml of PTV in dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, or 10^{-6}. Deaths were monitored over a 21-day period.

Results and Discussion

The results of these titrations are summarized in Table XXVI-1. As expected, 3 week-old mice were more susceptible to the infection than were 4 week-old animals, but in neither group did sufficient animals die to warrant changing to this strain of mice from the currently used C57BL/6.

Conclusions

Swiss Webster mice were not satisfactorily sensitive to i.p. injection of Adames strain PTV, with an unacceptable number dying of the infection.

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Table XXVI-1. Infectivity of PTV in Swiss Webster Mice.

Three week-old mice

Virus Dilution	Survivors/Total	Mean Surv. Time (days)
10^{-2}	7/10	5.3
10^{-3}	9/10	6.0
10^{-4}	8/10	5.0
10^{-5}	4/10	4.5
10^{-6}	4/10	4.3

Four week-old mice

Virus Dilution	Survivors/Total	Mean Surv. Time (days)
10^{-2}	10/10	>21.0
10^{-3}	9/10	6.0
10^{-4}	9/10	5.0
10^{-5}	9/10	4.0
10^{-6}	9/10	5.0

XXVII. TITRATION OF ADAMES STRAIN PTV IN INTRACEREBRALLY INJECTED MICE

Introduction

It has generally been assumed that the Adames strain of PTV is an hepatotropic, and not neurotropic virus. Thus the known neurotropic Balliet strain of PTV has been routinely used for evaluating compounds against PTV-induced encephalitis. An experiment was run to determine if the Adames strain of the virus was also capable of causing encephalitis in mice.

Materials and Methods

Virus: The Adames strain of PTV was propagated as previously described by Sidwell et al. (1).

Animals: Four week-old male C57BL/6 mice were obtained from Simonsen Labs (Gilroy, CA), and used after a 24 hr quarantine.

Experiment Design: Groups of 5 mice each were inoculated i.c. with varying 10-fold dilutions of PTV. The animals were observed through 21 days and deaths recorded daily. As mice died, livers and brains were removed, frozen at -80°C and later assayed for infectious virus titer in LLC-MK_2 cells.

Results and Discussion

The results are summarized in Table XXVII-1. The virus was lethally infective when administered i.c., with an LD50 determined to be a 10^{-5} dilution of the virus stock. The most concentrated virus inoculum, 10^{-1}, was not lethal to the mice, an observation also seen using the hepatic infection and due presumably to an accumulation of defective interfering particles at that high concentration.

There is some question whether the deaths occurring were due to encephalitis. Of 5 brains taken from dying mice which were assayed for virus, none yielded infectious virus. Three out of 5 livers did yield virus, however, at titers ranging from $10^{1.7}$ to $10^{4.5}$ 50% cell culture infectious units/0.1 ml. These livers had discoloration scores of 1 to 3. This suggests to us that this virus was truly hepatotropic, and although inoculated i.c., still gravitated to the liver to induce a lethal infection.

Conclusions

The Adames strain of PTV was lethal to 4 week-old mice when injected i.c. No virus could be isolated from the brains of the dying animals, but could be recovered from their livers, which also showed signs of icterus.

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Table XXVII-1. Titration of Adames Strain PTV in i.c.-Inoculated C57BL/6 Mice.

Virus Dilution	Survivors/Total	Mean Surv. Time (days)
10^{-1}	5/5	>21.0
10^{-2}	0/5	4.0
10^{-3}	0/5	4.0
10^{-4}	1/5	4.5
10^{-5}	2/5	6.0
10^{-6}	5/5	>21.0
10^{-7}	5/5	>21.0

LD50 = 10^{-5}

XXVIII. PRESENTATIONS AND PUBLICATIONS

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