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(2)

CONTRACT NO: DAMD17-91-C-1030

TITLE: CHEMOTHERAPY STUDIES USING COMPOUNDS TESTED AGAINST VIRUSES OF MILITARY IMPORTANCE

PRINCIPAL INVESTIGATOR: Robert W. Sidwell, Ph.D.

CONTRACTING ORGANIZATION: Utah State University
Logan, Utah 84322

REPORT DATE: March 19, 1992

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Distribution authorized to U.S. Government agencies, Proprietary Information, March 19, 1992. Other requests for this document shall be made to USAMRDC, Fort Detrick, Frederick, MD
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Utah State University; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Dept. ADVS, Logan, UT 84322-5600	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S. Army Medical Research and Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Contract No. DAMD17-91-C-1030
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, MD 21702-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 63002A; PROJECT NO. 3M2-63002D807; TASK NO. AD; WORK UNIT ACCESSION NO. WUDA335718

11. TITLE (Include Security Classification): Chemotherapy Studies using Compounds Tested Against Viruses of Military Importance

12. PERSONAL AUTHOR(S): Robert W. Sidwell

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 8 Jan 91 TO 31 Dec 91	1992 March 19	108

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
06	01		Punta Toro virus	bunyavirus	phlebovirus
06	15		Pichinde virus	animal infection	RAI
			immunomodulators	antiviral	

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Military Relevance: The viruses of military significance targeted by this research are sandfly fever virus and Rift Valley fever virus, both endemic to the Middle Eastern area and capable of severely hampering military operations if an outbreak occurs in susceptible populations; and Lassa fever, Junin, and Machupo viruses, all endemic to Africa or South America. The Punta Toro virus is a closely related virus which is safer to use in the laboratory and which, as target for antiviral agents, has been shown to be highly predictable of efficacy against sandfly and Rift Valley fever viruses. The Pichinde virus is a closely related virus to the Lassa fever, Junin and Machupo viruses and is highly predictable of efficacy against these viruses. **In Vivo Assessment of Lethal Toxicity:** Approximate LD50 values were obtained in mice for 19 AVS compounds and in hamsters for 4 AVS compounds. **Effect of AVS Compounds on Hepatotropic Infections in Mice Induced by the Adames Strain of Punta Toro Virus:** A total of 64 experiments were run in evaluating 29 AVS compounds against the hepatotropic PTV infection. Ribavirin (AVS01) and six chemical derivatives were considered markedly effective and acting specifically against the virus infection. A total of 23 immunomodulating substances also had strong anti-PTV effects. An apparent common immunological property among the latter PTV inhibitors was the induction of IFN by each compound. **Effect of AVS Compounds on Neurotropic Infections Induced by the Balliet Strain of Punta Toro Virus:** Two experiments were run with one AVS compound, AVS1018, evaluated against the neurotropic

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION	
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mrs. Virginia M. Miller	22b. TELEPHONE (Include Area Code): 301-619-7325	22c. OFFICE SYMBOL: SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

19. ABSTRACT

PTV infection, with moderate activity seen. **Effects of Drug Combinations on the Hepatropic Punta Toro Virus Infection in Mice:** A total of 4 drug combinations were evaluated against the hepatotropic PTV infection *in vivo*. These were AVS01 + 5079, AVS01 + 5311, AVS01 + 1761, and AVS2776 + 5079. An additional 2 combinations were studied to determine if murine toxicity of AVS01 could be reduced by treatment with AVS2149 or AVS2776. AVS01 + 5079 resulted in an increased therapeutic index. AVS01 + 5311 was possibly synergistic. AVS01 + 1761 was strongly antagonistic. AVS2776 + 5079 was also suggestive of synergy. The murine toxicity of AVS01 was moderately reduced by delayed AVS2149 or AVS2776 therapy. **Effect of AVS2149 Therapy on Toxicity Caused by Chronic AVS01 Therapy:** AVS01 (ribavirin) therapy, p.o. at 200 mg/kg/day, resulted in a significant initial decline in mouse blood hematocrit values, down to approximately 24% of H_2O-treated mice. AVS2149 (ampligen), when administered to these mice i.p. in doses of 0.005, 0.05, and 0.5 mg/kg, resulted in a general increase in hematocrit and accelerated weight gain. **Effect of AVS2776 Therapy on Toxicity Caused by Chronic AVS01 Therapy:** Chronic p.o. AVS01 (ribavirin) therapy with 200 mg/kg/day resulted in decreased hematocrit and less host weight gain in C57BL/6 mice. A single p.o. AVS2776 (bropirimine) treatment using 25 mg/kg of these mice resulted in a moderate increase in hematocrit and increased host weight gain. A higher dose of AVS2776 was essentially ineffective. Bropirimine induced IFN detectable in the serum 3 hr after treatment of all H_2O-treated mice, and the 50 mg/kg dose induced IFN in the animals receiving ribavirin. The 25 mg/kg dose of bropirimine did not induce detectable IFN in the ribavirin-treated animals, suggesting the latter therapy may have reduced the animals' ability to respond to weak IFN stimulation. **Interferon and Interleukin-2 Induction in Mice by a Series of AVS Compounds:** Compounds AVS581, 702, 709, 710, 712, 1644, 1841, 3362, 3547, 3935, 4156, 4277, 4611, 4923, 5065, 5067, 5075, and 5603 were evaluated for their ability to induce IFN and IL-2 in 3 week-old C57BL/6 mice. Compounds AVS581, 709, and 4611 were shown to induce detectable levels of serum IFN. Compounds AVS709, 710, 712, 1644, 1846, 3362, 4277, and 4611 were considered to be most effective in stimulating IL-2, with the induced levels of this cytokine more than twice those of normal controls and significantly different than the normal controls. **Susceptibility of Various Strains of Mice to Punta Toro Virus Infection:** DBA/2 and SASCO C57BL/6 mice were found moderately susceptible to infection with the hepatotropic Adames strain of PTV. The DBA/2 mice had a more pronounced insensitivity to high doses of virus. The SASCO mice were less sensitive than Simonsen animals. **Influence of Shipping Method on Sensitivity of C57BL/6 Mice to Punta Toro Virus:** C57BL/6 mice shipped by air were much more susceptible to lethal effects of i.p.-inoculated Adames strain PTV than were similar mice shipped via truck. **Determination of Potential Hepatic Toxicity of Human Interleukin-2 (AVS5079) in C57BL/6 Mice:** AVS5079, administered i.p. to mice qd x 5, did not cause significant increases in SGOT or SGPT when the serum was assayed 4 hr after the final treatment. **Failure of Pichinde Virus to Cause Lethal Infection in Genetically Immunosuppressed Mice:** The genetically immunodeficient NIH-III and SCID mice were not visibly susceptible to infection by PCV when the virus was inoculated s.c. **Studies on the Mechanisms of AVS01 Murine Toxicity: Immunologic Effects:** C57BL/6 mice treated i.p. twice daily for 3 days with 2000 or 1500 mg/kg/day of ribavirin exhibited weight loss and death within 2-3 days after treatment termination. The major gross pathologic finding was excessive intestinal hemorrhage. Hematocrit declined initially, but increased by day 3, perhaps due to a release of immature red blood cells. The ribavirin therapy caused significant T and B cell function and a depletion of splenic B cells. NK cell activity appeared to increase, but this may have been a reflection of more NK cells in the spleen in place of the decreased B cells. **Effects of BCH-523, 524, 525, 526, and 527 on Punta Toro Virus-Infected Mice:** The lipophilic desmuryl MDP analogs BCH-523, 524, 525, 526, and 527 were evaluated for efficacy against the hepatotropic PTV infection in C57BL/6 mice. Treatments were i.p. every other day for a total of 4 injections beginning 18 hr pre-virus inoculation. Only BCH-523 exerted an inhibitory effect; this was seen as decreased SGOT, SGPT, and liver virus titers in mice receiving the maximal dose. All the BCH compounds were well tolerated in the mice. Immunologic assays of splenocytes taken 24 hr after final treatment with the 50 mg/kg/day dose of each compound indicated the following: *Macrophage function:* Stimulation with BCH-523, 524, and 527; moderate suppression with BCH-525 and 526. *NK cell activity:* Stimulation with BCH-527, marginal suppression with BCH-524 and 525. *T and B cell enumeration:* B cell increase by BCH-526 and 527 with concomitant T cell suppression. T and B cell suppression by BCH 524. The time of assay as well as dosage of each compound used may markedly influence the outcome of these immunologic tests. **Treatment of Lethal Pichinde Virus Infections in Weanling LVG/Lak Hamsters with Ribavirin, Ribamidine, Selenazofurin, and Ampligen:** A lethal Pichinde (An 4763 strain) virus infection was produced in 3 week-old random-bred Golden Syrian (LVG/Lak strain) hamsters inoculated intraperitoneally with virus, causing mortality in 6-9 days. High virus titers ($\geq 10^{7.5}$ cell culture infectious doses/gram) were present in visceral organs, serum, brain and salivary glands near the time of death. Intraperitoneal treatments with ribavirin (10 and 32 mg/kg) and ribamidine (32, 100, and 320 mg/kg) for 10 days starting 24 h after virus challenge significantly decreased mortality and reduced virus titers by 100- to >10,000-fold in liver, spleen, brain, and serum. Serum alanine aminotransferase (an indicator of liver damage) was also reduced in animals treated with the two compounds (ribavirin at 32 mg/kg; ribamidine at 100 and 320 mg/kg). Intraperitoneal selenazofurin (1-100 mg/kg/day for 10 days) and ampligen (0.5 and 5 mg/kg every other day for 5 injections) treatments provided no protection from the lethal infection nor increased mean survival times. In fact, selenazofurin was overtly toxic causing death of uninfected hamsters at 32 and 100 mg/kg. The random-bred LVG/Lak hamster appears to be a viable and cost-effective model for evaluating new therapies for arenavirus infections.

SUMMARY

1. The viruses of military significance targeted by this research are sandfly fever virus, Rift Valley fever virus, and Crimean-Congo hemorrhagic fever virus all endemic to the Middle Eastern area and capable of severely hampering military operations if an outbreak occurs in susceptible populations; and Lassa fever, Junin, and Machupo viruses, all endemic to Africa or South America and capable of also causing serious problems to military personnel based in the area. The Punta Toro virus is a closely related virus which is safer to use in the laboratory and which, as target for antiviral agents, has been shown to be highly predictable of efficacy against sandfly and Rift Valley fever viruses. The Pichinde virus is a closely related virus to the Lassa, Junin and Machupo viruses and is highly predictable of efficacy against these viruses.

2. Approximate LD50 values were obtained in mice for 19 AVS compounds and in hamsters for 4 AVS compounds.

3. A total of 64 experiments were run in evaluating 29 AVS compounds against the hepatotropic PTV infection. The results were combined, for continuity, with the results of our previous 5 years' research. Ribavirin (AVS01) and six chemical derivatives were considered markedly effective and acting specifically against the virus infection. A total of 23 immunomodulating substances also had strong anti-PTV effects. An apparent common immunological property among the latter PTV inhibitors was the induction of IFN by each compound.

4. Two experiments were run with one AVS compound, AVS1018, evaluated against the neurotropic PTV infection, with moderate activity seen.

5. A total of 4 drug combinations were evaluated against the hepatotropic PTV infection *in vivo*. These were AVS01 + 5079, AVS01 + 5311, AVS01 + 1761, and AVS2776 + 5079. An additional 2 combinations were studied to determine if murine toxicity of AVS01 could be reduced by treatment with AVS2149 or AVS2776. AVS01 + 5079 resulted in an increased therapeutic index. AVS01 + 5311 was possibly synergistic. AVS01 + 1761 was strongly antagonistic. AVS2776 + 5079 was also suggestive of synergy. The murine toxicity of AVS01 was moderately reduced by delayed AVS2149 or AVS2776 therapy.

6. AVS01 (ribavirin) therapy, p.o. at 200 mg/kg/day, resulted in a significant initial decline in mouse blood hematocrit values, down to approximately 24% of H_2O-treated mice. AVS2149 (ampligen), when administered to these mice i.p. in doses of 0.005, 0.05, and 0.5 mg/kg, resulted in a general increase in hematocrit and accelerated weight gain.

7. Chronic p.o. AVS01 (ribavirin) therapy with 200 mg/kg/day resulted in decreased hematocrit and less host weight gain in C57BL/6 mice. A single p.o. AVS2776 (bropirimine) treatment using 25 mg/kg of these mice resulted in a moderate increase in hematocrit and increased host weight gain. A higher dose of AVS2776 was essentially ineffective. Bropirimine induced IFN detectable in the serum 3 hr after treatment of all H_2O-treated mice, and the 50 mg/kg dose induced IFN in the animals receiving ribavirin. The 25 mg/kg dose of bropirimine did not induce detectable IFN in the ribavirin-treated animals, suggesting the latter therapy may have reduced the animals' ability to respond to weak IFN stimulation.

8. Compounds AVS581, 702, 709, 710, 712, 1644, 1841, 3362, 3547, 3935, 4156, 4277, 4611, 4923, 5065, 5067, 5075, and 5603 were evaluated for their ability to induce IFN and IL-2 in 3 week-old C57BL/6 mice. Compounds AVS581, 709, and 4611 were shown to induce detectable levels of serum IFN. Compounds AVS709, 710, 712, 1644, 1846, 3362, 4277, and 4611 were considered to be most effective in stimulating IL-2, with the induced levels of this cytokine more than twice those of normal controls and significantly different than the normal controls.

9. DBA/2 and SASCO C57BL/6 mice were found moderately susceptible to infection with the hepatotropic Adames strain of PTV. The DBA/2 mice had a more pronounced insensitivity to high doses of virus. The SASCO mice were less sensitive than Simonsen animals.

10. C57BL/6 mice shipped by air were much more susceptible to lethal effects of i.p.-inoculated Adames strain PTV than were similar mice shipped via truck.

11. AVS5079, administered i.p. to mice qd x 5, did not cause significant increases in SGOT or SGPT when the serum was assayed 4 hr after the final treatment.

12. The genetically immunodeficient NIH-III and SCID mice were not visibly susceptible to infection by PCV when the virus was inoculated s.c.

13. C57BL/6 mice treated i.p. twice daily for 3 days with 2000 or 1500 mg/kg/day of ribavirin exhibited weight loss and death within 2-3 days after treatment termination. The major gross pathologic finding was excessive intestinal hemorrhage. Hematocrit declined initially, but increased by day 3, perhaps due to a release of immature red blood cells. The ribavirin therapy caused significant T and B cell function and a depletion of splenic B cells. NK cell activity appeared to increase, but this may have been a reflection of more NK cells in the spleen in place of the decreased B cells.

14. The lipophilic desmuryl MDP analogs BCH-523, 524, 525, 526, and 527 were evaluated for efficacy against the hepatotropic PTV infection in C57BL/6 mice. Treatments were i.p. every other day for a total of 4 injections beginning 18 hr pre-virus inoculation. Only BCH-523 exerted an inhibitory effect; this was seen as decreased SGOT, SGPT, and liver virus titers in mice receiving the maximal dose. All the BCH compounds were well tolerated in the mice. Immunologic assays of splenocytes taken 24 hr after final treatment with the 50 mg/kg/day dose of each compound indicated the following: *Macrophage function:* Stimulation with BCH-523, 524, and 527; moderate suppression with BCH-525 and 526. *NK cell activity:* Stimulation with BCH-527, marginal suppression with BCH-524 and 525. *T and B cell enumeration:* B cell increase by BCH-526 and 527 with concomitant T cell suppression. T and B cell suppression by BCH 524. The time of assay as well as dosage of each compound used may markedly influence the outcome of these immunologic tests.

15. A lethal Pichinde (An 4763 strain) virus infection was produced in 3 week-old random-bred Golden Syrian (LVG/Lak strain) hamsters inoculated intraperitoneally with virus, causing mortality in 6-9 days. High virus titers ($\geq 10^{7.5}$ cell culture infectious doses/gram) were present in visceral organs, serum, brain and salivary glands near the time of death. Intraperitoneal treatments with ribavirin (10 and 32 mg/kg) and ribamidine (32, 100, and 320 mg/kg) for 10 days starting 24 h after virus challenge significantly decreased mortality and reduced virus titers by 100- to >10,000-fold in liver, spleen, brain, and serum. Serum alanine aminotransferase (an indicator of liver damage) was also reduced in animals treated with the two compounds (ribavirin at 32 mg/kg; ribamidine at 100 and 320 mg/kg). Intraperitoneal selenazofurin (1-100 mg/kg/day for 10 days) and ampligen (0.5 and 5 mg/kg every other day for 5 injections) treatments provided no protection from the lethal infection nor increased mean survival times. In fact, selenazofurin was overtly toxic causing death of uninfected hamsters at 32 and 100 mg/kg. The random-bred LVG/Lak hamster appears to be a viable and cost-effective model for evaluating new therapies for arenavirus infections.

16. Overview of *In Vivo* Anti-Punta Toro Virus Activity of AVS Compounds: Summary of Six Years' Testing

17. Presentations and publications: A total of 18 presentations were made to various scientific meetings during this contract. Nineteen papers have been published or submitted to scientific journals.

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FOREWORD

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☐ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

PI Signature

March 19, 1992
Date

TABLE OF CONTENTS

I. GENERAL INTRODUCTION AND EXPLANATION

This report, although designated a "Final Report" describes only an overview of one year's experiments run on this contract, since the contract only began in January, 1991, and was prematurely terminated December 31, 1991. This contract was a renewal of a previous contract (DAMD17-86-C-6028), which was described in our Final Report dated January 28, 1991. This report also briefly summarizes the findings of that earlier contract to provide a continuity to this work.

We feel impelled to state that it was with much regret that this work has terminated. The objective: To discover and develop new substances which could be used as drugs for treating virus diseases of military importance, was most significant, and still is. The diseases targeted, to be described in Section II of this report, are still a major problem militarily, and without continuation of this work are essentially no closer to being reduced in their impact. The work accomplished to date was also most significant: 29 compounds were identified in that previous contract to have very strong activity against the *Phlebovirus* being studied, and 5 more substances discovered and verified to have similar potential during this one-year contract period. In addition, one new compound was identified as having strong potential as a drug for treatment of *Arenavirus* infections. Without followup of these active leads, many having the potential to become effective drugs to add to our military's armentarium, the massive efforts of six years' research will have been wasted.

We must also state that those individuals designated as the Contracting Officer's Technical Representatives (COTRs) to this project were most helpful and provided keen, in-depth advice which markedly accelerated the progress made in this contract. The COTRs were, for the present project, Drs. John Huggins, B.J. Gabrielsen, Meir Kende, and Thomas Monath. Drs. Dominique Pifat and Peter Canonico worked with us for the early stages of our first contract.

II. MILITARY RELEVANCE

The Punta Toro virus (PTV) is a *Phlebovirus* in the Bunyaviridae family of viruses, and is closely related to sandfly fever (SF) virus and Rift Valley fever (RVF) virus and is somewhat related to Crimean-Congo hemorrhagic fever, which is also in the Bunyaviridae family of viruses. The Pichinde virus is an *Arenavirus* in the Arenaviridae family of viruses, and is closely related to Lassa fever, Junin, and Machupoviruses. All of these related viruses are considered important viruses militarily, as will be described below.

Sandfly fever: During World War II, approximately 19,000 members of the Allied armed forces in the Middle Eastern area were afflicted with SF infections, with most requiring hospitalization (1, 2). From 3% to 10% of all troops were afflicted with the disease at that time, with some units reporting attack rates of over 50% (3). These rates were especially high in the Persian Gulf command, reaching a peak of 235 cases/1000 men (1).

Oldfield et al. (3), in a recent review indicating the potential importance of SF in the current Iraqi conflict, stated the following concerning the further military significance of this virus:

> "The military significance of sandfly fever is magnified because of its short incubation period, which can render large numbers of nonimmune troops ineffective early in an operation, while the endemic forces would be largely immune and unaffected."

The disease has a sudden onset and intense symptoms of fever, severe frontal headache with retro-orbital pain associated with severe myalgias, and often nausea, vomiting, abdominal pain and diarrhea (4, 5). These disease manifestations persist 2-4 days. The disease is transmitted by *Phlebotumus papatasi*, a nocturnal biting midge which is especially abundant in the Middle East from June to August (6).

Rift Valley fever: Severe epidemics of RVF have been reported since 1930 throughout much of the African area. An outbreak occurred in Sudan in 1976 (7), presumably with the disease spreading to Egypt in 1977-78 which resulted in an estimated 200,000 human cases and at least 600 deaths (8, 9). In the epidemic areas, the human infection rates were as high as 35% (8). In the last 10 years, there have been several outbreaks in the sub-Saharan Africa, the most recent being an ongoing epidemic in Mauritania (10).

The RVF disease often resembles human influenza, with abrupt onset of fever and associated symptoms lasting 2-5 days. Some cases may be more serious or fatal, resulting from liver necrosis with hemorrhagic phenomena, retinitis with visual impairment, and meningoencephalitis (11, 12).

The RVF virus can be transmitted by a variety of mosquito species (13), and infects many domestic animals. Because of this insect transmission and the movement of vertebrates potentially carrying the virus, it has the potential to be spread to distant geographic sites. In view of the close proximity of Egypt and Sudan to Saudi Arabia, the potential for Allied forces stationed in the Gulf War area contracting this significant virus disease appeared very real.

Crimean-Congo hemorrhagic fever (CCHF): This virus is becoming recognized as an important zoonotic disease of humans in the Middle East as well as in Eastern Europe and Asia. The infection caused by this virus was first reported in World War II among Soviet military personnel in Crimea (14), and subsequent outbreaks have been reported in Bulgaria, Pakistan, Iraq, Southern USSR, Dubai, Kuwait, and the United Arab Emirates (15–18). The virus is primarily transmitted by ticks (14), but many cases, and usually the more severe, occur nosocomially in hospitals and similar facilities (19). The disease in man is characterized by sudden onset with a long-lasting (7–9 days) fever with rigors and chills which subsides and then remanifests itself. Intense myalgia, nausea and vomiting frequently also occur and the patients often develop a number of other symptoms, including diarrhea, facial hyperemia, hepatomegaly, and petechial rash. The disease is often lethal to the patient, with fatality rates of 13–50% reported (20).

Punta Toro virus: This virus, as pointed out at the beginning of this section, is particularly closely related to both SF and RVF viruses, and like those viruses, is also transmitted by biting insects. The virus is of particular value because is induces a disease very similar to that induced by RVF in mice, but causes a less severe disease in man and is not readily transmitted in the

laboratory. PTV, RVF, and SF viruses all appear quite similarly sensitive to the same antiviral compounds (21–27, unpublished findings reported by Drs. J. Huggins and M. Kende of the U.S. Army Medical Research Institute for Infectious Diseases [USAMRIID]).

Lassa fever: This disease is a serious, often fatal infection which has primarily occurred in Western and Central Africa (28, 29). The disease is characterized by diverse clinical manifestations which include a major, long-lasting fever, headache, malaise, joint and back aches, cough, sore throat, and severe nausea. Surviving patients often display acute loss of hearing, uveitis, pericarditis, orchitis, pleural effusion and ascites. Pregnant women often spontaneously abort (22). The virus is spread from the urine, feces, and saliva of infected rodents (30).

Junin virus: This virus is the cause of Argentine hemorrhagic fever, which was first recognized in the 1950's. From that time until the mid-1970's, approximately 21,000 cases have been reported from Argentina (31). The disease is characterized by a high fever, malaise, general myalgia, skin rash and petechiae, with ulcerations occurring in the digestive tract. Pneumonia is often observed, as is splenic hemorrhage. Severe nausea often occurs. The patient often develops intractable shock which leads to their death (32, 33). Like Lassa fever, this disease is also transmitted by rodents (31).

Machupo virus: This virus causes Bolivian hemorrhagic fever, which was first recognized in 1964 (34). The disease is very similar to Argentine hemorrhagic fever, and is also transmitted by rodents (35).

Pichinde virus: This virus is closely related to Lassa fever, Junin and Machupo viruses. It was selected for use in these chemotherapy studies because it induces a disease in hamsters and guinea pigs that is similar to the hemorrhagic fevers (14, 15) but causes a less severe disease in man and is not as readily transmitted in the laboratory. All these *Arenaviruses* appear to have similar sensitivity to antiviral compounds (14, 15, unpublished findings reported by Dr. Kende of USAMRIID).

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III. IN VIVO ASSESSMENT OF LETHAL 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 describes the results of all toxicity experiments in which death was used as an endpoint. In some cases, due to lack of sufficient compound, an *in vivo* antiviral experiment was run without preliminary toxicity data. In all *in vivo* antiviral experiments, toxicity controls were run in parallel, so those data were also included in this section. The results will hopefully provide sufficient information on the murine toxicity of the AVS compounds that other investigators will be able to run antiviral studies with appropriate dosages of the compounds.

Materials and Methods

Compounds: All compounds were submitted to us by our USAMRIID COTR during this contract period. 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). Syrian golden hamsters 3 weeks of age were obtained from SASCO, Inc. (Omaha, NE). All were quarantined at least 24 hr prior to use, and maintained on Wayne Lab Blox mouse chow or hamster 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 or hamsters 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 or PCV studies, the animals were held a total of 21 days. Five animals 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 III-1 and III-2. A total of 19 compounds were evaluated in mice and 4 compounds in hamsters over the period of this contract. In some cases ">" values are shown because we had not achieved a lethal dose and no further studies were run due to inadequate compound. 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 lower doses caused marked weight loss in the animals, suggesting the maximum tolerated dose (MTD) had essentially been reached.

Conclusions

Approximate LD50 values were obtained in mice for 19 AVS compounds and in hamsters for 4 AVS compounds.

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Table III-1. Preliminary Toxicity Evaluations of AVS Compounds in Mice[a]

Compound (AVS No.)	Name	Treatment Schedule	Treatment Route	Approximate LD50 (mg/kg/day)
148	Pyrazofurin	bid x 5	i.p.	8
1018	Phenyleneamine	once only	i.p.	>25
		once only	s.c.	>25
2318	6-Azauridine	bid x 5	i.p.	~550
3679	Unidentified	bid x 5	i.p.	~500
4273	Uniroyal Compound	bid x 5	i.p.	>12.5
		bid x 5	s.c.	>12.5
		once only	i.p.	~300
4617	Glycine analog of ribamidine	bid x 3	s.c.	>1200
4785	Actidione	bid x 5	s.c.	~10
		bid x 5	p.o.	4
5058	N-methyl ribamidine	bid x 4	s.c.	>1200
5601	Dimethylribamidine	bid x 4	s.c.	>1200
6724	2-Thia-6-azauridine	bid x 5	i.p.	>2000
8361	Carrisyn	eod x 6	i.p.	>10
11717	2-Thia-6-azauridine triacetate	bid x 4	s.c.	>1200
11941	SRI 7959	bid x 5	i.p.	>1100
—	BCH-523	eod x 3	i.p.	>50
—	BCH-524	eod x 3	i.p.	>50
—	BCH-525	eod x 3	i.p.	>50
—	BCH-526	eod x 3	i.p.	>50
—	BCH-527	eod x 3	i.p.	>50
—	gmCSF	qd x 5	i.p.	>3
		eod x 3	i.p.	>3

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

Table III-2. Preliminary Toxicity Evaluations of AVS Compounds in Hamsters[a]

Compound (AVS No.)	Name	Treatment Schedule	Treatment Route	Approximate LD50 (mg/kg/day)
01	Ribavirin	bid x 10	i.p.	>40
206	Ribamidine	bid x 7	i.p.	>500
253	Selenazofurin	bid x 10	i.p.	~16
2149	Ampligen	eod x 5	i.p.	>5

[a]45-50 g Syrian golden hamsters.

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

Introduction

The primary thrust of this research contract is to discover and develop drugs for the treatment of experimentally induced Punta Toro virus (PTV) infections. The PTV is a *Phlebovirus* in the Bunyaviridae family which is closely related to sandfly fever (SF) and Rift Valley fever (RVF) viruses, inducers of diseases which had had a major impact in Europe, the Middle East, and Africa (1, 2), and are yet uncontrolled by antiviral drugs. PTV induces in inbred strains of parenterally inoculated mice a hepatocellular necrotic disease, leukopenia and lymphopenia which resembles the disease in man induced by SF and RVF viruses (3, and see last year's Annual Report).

This section summarizes our results over the 1-year span of this contract in evaluating test substances against the PTV infection in mice. Unless otherwise directed by our Contract Officer's Technical Representative (COTR), we generally follow a relatively standard protocol in which new substances are initially tested for general toxicity in range-finding studies in mice (Section II of this report). They are then used at the maximum tolerated dose (MTD) and 2 to 3 2-fold dilutions below the MTD against a lethal infection induced by the virus. Unless otherwise instructed, our treatment regimen is subcutaneous (s.c.) treatment twice daily (bid) for 5 days beginning 4 hr pre-virus inoculation. Active compounds are then retested using expanded parameters which include death, mean survival time, liver discoloration score, serum glutamic oxalic acid and pyruvic acid transaminases (SGOT, SGPT) as indicators of liver damage, and virus titer determinations in liver homogenates and in serum. Further testing will involve determining if the compound is active orally against the infection and how long after initiation of infection can treatment be started and still render a therapeutic effect. Further followup studies may include experiments in which the efficacy of the drug is tested against increasing viral challenge. Included in all studies was the testing of a single dose of a positive control, which was ribavirin (AVS01), which has been shown to have strong anti-PTV effects (4-6).

In the preparation of this summary report, the overall activity of each active compound has been considered and the compound then categorized regarding its concluded efficacy. In many cases, insufficient compound was available for adequate follow-up studies and sometimes, due to the relative shortages of compounds, preliminary range-finding toxicity was not determined. Hence, some compounds may be categorized as having slight or no anti-PTV activity when the MTD's of the compounds had not yet been achieved. Often, certain compounds are highly dependent on the treatment protocol used; we attempt to illustrate the best means to achieve strong efficacy, but again, insufficient compound may prevent such follow-up experiments from being run.

We felt it appropriate to also indicate the positive materials found in our previous contract, since much of the present work was a direct continuation of the earlier contract. The previously discovered materials are indicated in italics.

Materials and Methods

Virus: The Adames strain of PTV was provided by Dr. Dominique Pifat of USAMRIID. It was identified by Dr. Pifat as virus pool #215588, and had been safety tested by Dr. Pifat prior to being sent to us. The PTV was first isolated from the serum of A. Adames, an entomologist in the Darien Province of Panama in 1972. It was passaged twice in Vero cells prior to being sent to us. When received by us, virus was passaged 2 times through LLC-MK_2 cells, plaques isolated each time from these cells, and a large pool made from the second plaque isolate in these cells following confirmation of virus identify by serum neutralization.

Animals: Three week-old C57BL/6 mice were obtained from Simonsen Laboratories (Gilroy, CA). All weighed 10-2 g when used; heavier or lighter mice were rejected since our previous studies as well as those of Pifat and Smith (3) showed a strong difference in susceptibility with age of mouse. 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 for 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 our COTR from USAMRIID. 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 Evaluations: 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

A total of 64 experiments were run during this 1-year report period, with 29 AVS compounds being evaluated against the PTV infection.

AVS compounds considered to be non-immunomodulators which were significantly inhibitory to the PTV infection are summarized in Table IV-1. Included among these compounds was ribavirin as well as 6 compounds chemically related to ribavirin. All were either found to be orally effective or had not yet been tested orally.

Compounds thought to be acting through immunomodulation mechanisms which were highly active vs PTV in vivo are seen in Table IV-2. These 23 compounds appear to have one common immunological property: They all induce interferon (IFN), which is known to have a profound effect on PTV (3, 7, 1990 Annual Report). With one compound, AVS5587, which both induces IFN and activates natural killer cells, pretreatment with anti-IFN antibody completely eliminated the usual anti-PTV effects of this compound (7, 1990 Annual Report). Probably most effective of all these immunomodulators were poly IC•LC (AVS1761), ampligen (AVS2149), and a poorly defined poly IC•LC derivative (AVS5593). Only bropirimine (AVS2776), CL246,738 (AVS1968) and AM-5 (AVS4282) had efficacy when given orally (by gavage) to the infected

animals. All active substances exerted their antiviral effects therapeutically, i.e., after virus inoculation. None were effective if treatment began later then 48 hr after the virus, however, which is not surprising, for by this time the disease has progressed rapidly in the animals (see Section VIII of the Report for a full description of the disease).

Non-immunomodulating AVS compounds considered slightly or moderately inhibitory to the PTV infection are seen in Table IV-3. These compounds represent a rather broad range of chemical substances, of which only a few are ribavirin derivatives. In some cases, relatively high therapeutic indices (TI) are noted, but often this was seen at a single dose; higher doses, while tolerated in the mouse, yielded no antiviral effect.

Table IV-4 summarizes the immunomodulating substances having slight to moderate inhibition to the PTV disease. Again, if an erratic dose response was seen, it was so noted, but in our view reduced the potential usefulness of the compound.

Those AVS compounds not shown to inhibit the hepatotropic PTV infection are seen in Table IV-5. In many cases, only one or two tests were run, with the compound nontoxic at all doses run, suggesting they may need to be resynthesized and further tests performed.

Conclusions

A total of 64 experiments were run in evaluating 29 AVS compounds against the hepatotropic PTV infection. The results were combined, for continuity, with the results of our previous 5 years' research. Ribavirin (AVS01) and six chemical derivatives were considered markedly effective and acting specifically against the virus infection. A total of 23 immunomodulating substances also had strong anti-PTV effects. An apparent common immunological property among the latter PTV inhibitors was the induction of IFN by each compound.

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Table IV-1. AVS Non-Immunomodulating Compounds Considered Significantly Inhibitory to Hepatotropic Punta Toro Virus Infections in Mice.

Compound AVSNo.	Compound Name[b]	Maximum Therapeutic Indices[a]						Orally Active?
		Survivor Increase	Reduction in Liver Score	Reduction in SGOT	Reduction in SGPT	Liver Virus Inhibition	Serum Virus Inhibition	
01	*Ribavirin*	65	65	200	200	200	200	yes
02	*Ribavirin triacetate*	57	65	65	200	65	65	yes
111	*Tiazofurin*	16	65	65	65	8	8	yes
148	Pyrazofurin	≥8[c]	≥8	≥4	≥4	≥8	≥8	yes
206	*Ribamidine*	65	65	200	200	65	200	yes
253	*Selenazofurin*	6	12	6	6	3	3	yes
257	*Tiazofurin 5'-MP*	≥5	≥5	≥5	≥5	≥5	≥5	?[d]
3706	*Tiazofurin triacetate*	≥9	≥3	≥18	≥36	≥18	≥18	?
4617	Glycine analog of ribamidine	≥4	≥4	≥4	≥4	≥2	≥2	?
4785	Actidione	≥4	≥4	≥4	≥4	≥4	≥4	?

[a]LD50 dose ÷ minimum effective dose.

[b]Italicized compounds were tested in the previous contract (DAMD17-86-C-6028).

[c]"≥" for therapeutic indices indicates an LD50 dose was not achieved.

[d]"?" indicates no oral test was run.

Table IV-2. AVS Immunomodulating Compounds Considered Significantly Inhibitory to Hepatotropic Punta Toro Virus Infections in Mice.

Compound AVSNo.[b]	Compound Name[b]	Maximum Therapeutic Indices[a] Survivor Increase	Reduction in Liver Score	Reduction in SGOT	Reduction in SGPT	Liver Virus Inhibition	Serum Virus Inhibition	Orally Active?	Active Therapeut?[c]
1018	Phenyleneamine	≥16[d]	≥8	≥8	≥8	≥8	≥8	Yes	36 hr post
1754	*MVE-2*	≥16	≥16	≥16	≥16	≥8	≥16	No	48 hr post
1761	*Poly IC•LC*	1000	100	1000	1000	3125	3125	No	48 hr post
1767	*AM-3*	125	1	≥40	≥40	≥40	≥40	No	48 hr post
1778	*Mannozym*	≥129	≥65	≥129	≥129	≥1	≥1	No	48 hr post
1968	*CL246,968*	≥32	≥4	≥8	≥8	≥8	≥8	Yes	24 hr post
2149	*Ampligen*	1000	100	100	100	100	100	No	48 hr post
2776	*Bropirimine*	20	20	40	40	40	20	Yes	48 hr post
2779	*MVE-1*	≥275	≥138	≥138	≥138	≥275	≥138	No	24 hr post
3588	*Meta fluorobropirimine*	16	nd	nd	nd	nd	nd	?[e]	24 hr post
4282	*AM-5*	≥10	≥10	≥10	≥10	≥10	≥10	Yes	24 hr post
4286	*P-136*	≥65	nd	nd	nd	nd	nd	?	≥24 hr post
4287	*P-117*	190	190	190	190	190	190	?	≥24 hr post
5311	*Human rIFN*	≥100	≥32	≥320	≥320	≥320	≥320	?	24 hr post
5587	*7-thia-8-oxoguanosine*	26	26	26	52	26	13	?	36 hr post
5588	*"ICLC"*	100	313	100	100	100	100	?	≥4 hr post
5589	*"ICL-CMA"*	40	40	40	40	40	40	?	≥4 hr post
5590	*"ICL-CMD"*	313	313	100	100	313	313	?	≥4 hr post
5591	*"ICL-CMB-C-dextrin"*	313	1000	313	313	3125	3125	?	≥4 hr post
5592	*"ICL-GEL"*	313	313	313	313	313	313	?	≥4 hr post
5593	*"ICL-sulfated gel"*	1000	1000	1000	313	313	313	?	≥4 hr post
5594	*"ICL-(PLL-dextran)"*	100	1000	100	100	40	40	?	≥4 hr post
5595	*"IC-(PLL-dextran)"*	100	100	100	100	100	100	?	≥4 hr post

[a]LD50 dose ÷ minimum effective dose.

[b]Italicized compounds were tested in the previous contract (DAMD17-86-C-6028).

[c]Times shown are the latest that treatment could be initiated and significant antiviral activity achieved. "≥" indicates that time was the last time initiated; it is possible the material would still be active if therapy was started later.

[d]"≥" for therapeutic indices indicates an LD50 dose was not achieved.

[e]"?" indicates no oral test was run.

Table IV-3. AVS Non-Immunomodulating Compounds Considered Slightly or Moderately Inhibitory to Hepatotropic Punta Toro Virus Infections in Mice.

Compound AVS No.	Compound Name	Maximum Therapeutic Index—Any Parameter	Active Orally?	Comments
52	*Thioformycin B*	≥24 (MST)	Yes	
65	*Formycin B*	16 (Survivors only)	?	
79	*9-ß-D-ribofuranosylpurine-6-thiocarboxamide*	24 (MST)	Yes	Erratic–best when given 48 hr post
215	*3-Deazaguanosine*	16 (MST)	?	Best when given i.p.
272	*3-Deazaguanine*	27 (liver score only)	Yes	
347	*Phyllanthoside*	3 (survivors, SGOT)	?	
1212	*Uridine 2',3'-dialdehyde*	8 (MST, SGOT, SGPT)	?	Erratic, not always dose-responsive
1976	*Thymineriboside 2',3'-dialdehyde*	6 (MST only)	?	
2318	6-Azauridine	4 (survivors only)	?	
2700	*6-Ethylthiopurine riboside*	~12 (survivors, MST, SGOT, SGPT)	Yes	Erratic, not always dose-responsive
2811	*7-Deoxynarciclasine*	≥4 (survivors)	?	Active only at one dose, insuff. to retest
2812	*Narciclasine*	8 (MST, liver score, SGOT, SGPT, virus titers)	?	Highly treatment schedule dependent
2885	*3-T-Butyl-1-adamantylthiourea*	~32 (liver score, SGOT, SGPT, liver virus)	?	Erratic—not dose dependent
3425	*8-Bromoguanosine*	~4.5 (survivors, MST)	No	Erratic—not dose dependent, active given in 1 shot only
3580	*Unidentified*	~16 (MST only)	?	Active in 1 shot 24 hr pre only
4272	*Unidentified*	~16 (all parameters)	?	Erratic—not dose responsive, active s.c. only
4617	*Glycine derivative of ribamidine*	~10 (MST)	?	Treatment with higher doses needed
5058	N-methyl analog of ribamidine	≥2 (all parameters but survivors)	?	
8361	Carrisyn	8 (SGOT, SGPT)	No	Erratic—possibly due to deteriorated drug—expired date
11941	SRI 7959	4 (survivors only)	?	

Table IV-4. AVS Immunomodulating Compounds Considered Slightly or Moderately Inhibitory to Hepatotropic Punta Toro Virus Infections in Mice.

Compound AVS No.	Compound Name	Maximum Therapeutic Index—Paramter Used For TI Determination	Active Orally?	Comments
1969	*CL 259,763*	100 (liver score, SGOT, SGPT)	Yes	
2276	*Theracel #BL-002*	~5 (SGOT, SGPT, virus titers)	Yes	Active only at a single mid-range dose
2285	*Theracel #BL-012*	~31(SGOT, SGPT, virus titers only)	Yes	Pretreatment most effective
2777	*AIPP*	4 (liver score, SGOT, SGPT, virus titers)	Yes	Higher doses prevented death
2778	*ABMP*	8 (liver score, SGOT, SGPT, virus titers)	Yes	Somewhat erratic dose response
2880	*Oxamisole*	8 (MST)	Yes	Higher doses prevented death; somewhat erratic dose response
2933	CGP19835 A lipid	10 (survivors)	No	Somewhat erratic
3587	*ACPP*	15 (SGOT, SGPT)	Yes	Higher doses prevented death
3589	*CFABPP*	2 (liver score, SGOT, SGPT, virus titers)	Yes	
3593	*LY253,963*	52 (MST)	Yes	Highly erratic dose response
3925	*duPont A2222-1*	≥8 (survivors)	?	Active at low doses only
3926	*duPont A2227-1*	~4 (survivors)	?	Highly erratic dose response
3927	*duPont A754-1*	1 (survivors)	?	Active prophylactically only
3934	*Germanium, Ge132*	~16 (survivors, liver score, SGOT, SGPT, virus titers)	Yes	Highly erratic dose response
4283	*AM-6*	≥16 (MST)	?	Higher doses prevented death
4284	*AM-7*	≥16 (MST)	?	
4285	*AM-8*	~4 (survivors)	?	Most active therapeutically, insuff. for retesting
4593	*P-188*	~16-32 (survivors)	?	Most active therapeutically, insuff. for retesting
5079	*hu Recomb. IL-2*	~16 (SGOT, SGPT, virus titers)	?	Higher doses prevented death
5596	*Heat-cycled ICLC*	≥1 (liver score, SGOT, SGPT, virus titers)	?	
—	gmCSF	≥8 (MST)	?	

Table IV-5. AVS Compounds Considered Inactive Against Hepatotropic Punta Toro Virus Infections in Mice.

Compound AVS No.	Compound Name	Comments
147	*Enviroxime*	Single i.p. treatment 4 hr post prevented death at high dose; this has not been confirmed
167	*Glycerrhetic Acid*	Only s.c. bid, tid x 5 regimens used.
212	*Suramin*	6 separate treatment regimens studied
222	*3-Bromo-4-chloropyrazolo-[3,4-d]pyrimidine*	6 separate treatment regimens studied
233	*Formycin*	1 dose caused significant increases in MST
360	*7-Deoxynarciclasin*	1 mid-range dose caused significant increase in MST in only 1 experiment run—nontoxic at all doses
361	*Pancratistatin*	Only 1 expt. run—nontoxic at all doses
1757	*Isoprinosine*	Only 1 expt. run, using manufacturer's recommended regimen
1777	*Streptonigrin*	6 separate treatment regimens studied
1976	*Thymine riboside 2',3'-dialdehyde*	Singe i.p. treatments caused occasoinal MST increases
2713	*Bryostatin 2*	2 Treatment regimens studied—nontoxic at all doses
2716	*Unidentified*	Only 1 test run—nontoxic at all doses
2741	*Ribavirin tetrahydropyrimidine*	Only 2 tests run—nontoxic at all doses
2742	*Ribavirin-5-OH-tetrahydropyrimidine*	3 separate treatment regimens used—nontoxic all all doses
2786	*Unidentified*	Only 1 test run—nontoxic at all doses
2978	*Tetraacetate ester of 2980*	1 mid-range dose caused survivor increase. Not yet confirmed
2980	*Tetrahydroxy analog of Pancratistatin*	4 separate treatment regimens studied
3585	*Neurotropin*	4 separate treatment regimens studied
3679	*1-(4-Methoxybenzyloxy)adenosine*	Only 1 expt. run—all doses nontoxic
3679	1-(4-methoxybenzyloxy)adenosine perchloric acid salt	2 expts. run—all doses nontoxic
3933	*Ge089*	Only 1 expt. run—all doses nontoxic
3960	*DMG*	4 separate treatment regimens studied
4113	*Pseudolycorine HCl*	MST increase at lowest dose only. Only 1 expt. run to date
4206	*3-Acetamido-7-amino-6-methyl-7H-5-triazolo[5,1-C]-S-triazole*	Marginal effects seen at highest dose, which was nontoxic
4273	2,3-Dihydro-5-iodiothiphene-1,1-dioxide	Multiple expts. run—toxic doses reached

4273	*2,3-Dihydro-5-iodothiophene-1,1-dione*	5 separate treatment regimens studied
4273	Uniroyal compound	Only 1 expt. run—high doses were toxic
4588	*1-Aminoadenosinium mesitylenesulfonate*	Nontoxic at all doses used
4616	*Noxymethylpennicillinic acid*	3 separate treatment regimens studied—nontoxic at all doses
4618	*5'-N,N-diethylthiocarbamate-5'-deoxy-5'-thioadenosine*	
4618	5'-N,N-diethylthiocarbamate-5'-deoxy-5'-thioadenosine	Only 1 expt. run—all doses nontoxic
5027	*Imexon*	2 regimens which showed efficacy against a retrovirus infection were used
5601	Dimethyl ribamidine	Only 1 expt. run—all doses nontoxic
6334	Unidentified	Only 1 expt. run—all doses nontoxic; one low dose was moderately active
6337	Unidentified	Only 1 expt. run—all doses nontoxic; one low dose was moderately active
6417	Unidentifed	Only 1 expt. run—toxicity achieved
6477	Unidentifed	Only 1 expt. run—all doses nontoxic
6501	Unidentified	Only 1 expt. run—all doses nontoxic; two low doses were moderately active
6724	2-Thia-6-azauridine	Only 1 expt. run—all doses nontoxic
11717	2-Thia-6-azauridine triacetate	Only 1 expt. run—all doses nontoxic
—	BCH-523	Only 1 expt. run—all doses nontoxic
—	BCH-524	Only 1 expt. run—all doses nontoxic
—	BCH-525	Only 1 expt. run—all doses nontoxic
—	BCH-526	Only 1 expt. run—all doses nontoxic
—	BCH-527	Only 1 expt. run—all doses nontoxic

V. EFFECT OF AVS COMPOUNDS ON NEUROTROPIC INFECTIONS 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.

Materials and Methods

Virus: The Balliet strain of PTV was obtained from the American Type Culture Collection (ATCC, Rockville, MD). This virus was originally isolated from a young adult male in Panama in 1966. The virus was twice plaque purified through LLC-MK_2 cells, and a pool subsequently made in these cells. Virus identity was confirmed by serum neutralization.

Animals: Balb/c 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*.

Compounds: All compounds were provided by our USAMRIID COTR.

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 earlier. 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 Yates' correction. Increases in mean survival time and decreases in mean brain virus titers were analyzed using *t* test.

Results and Discussion

Only a single AVS compound was evaluated in two experiments against the Balliet strain of PTV in mice. This compound, which was highly active vs the hepatropic infection, rendered a moderately significant positive effect when given i.p. in single injections 24 and 48 hr post-virus inoculation.

A summary of all AVS compounds considered inhibitory to this infection, as seen in the combined periods of our last contract and this one, is seen in Table V-1.

Conclusions

Two experiments were run with one AVS compound, AVS1018, evaluated against the neurotropic PTV infection, with moderate activity seen.

Table V-1. AVS Compounds Considered Inhibitory[a] to Neurotropic Punta Toro Virus Infections in Mice

Compound AVS No.	Name[b]	Therapeutic Index: Survivor Increase	Therapeutic Index: MST Increase	Therapeutic Index: Brain Virus Decrease	Comments
01	*Ribavirin*	0	2	0	Treatment i.p. or i.v.
02	*Ribavirin triacetate*	0	4	2	
206	*Ribamidine*	0	1	2	Once only i.p. therapy
253	*Selenazofurin*	0	8	0	Not dose-responsive
1018	Phenyleneamine	≥2	0	≥8	Erratic dose response
1754	*MVE-2*	0	2	0	
2149	*Ampligen*	8	4	16	4 tests run with differing regimens active in 2 tests
2776	*Bropirimine*	0	8	4	Brain virus reduction study done in intranasally inoculated virus, repeated with i.c. inoculated virus
3588	*Metafluoro ABPP*	0	0	1,8	Erratic dose response
3589	*5-Chloro-2,3-difluorophenyl ABPP*	0	0	1	
3934	*Ge132*	0	0	1	
5896	*Pharmatec 01 derivative*	0	2	0	i.v. + i.p. therapy
6080	*Pharmatec 01 derivative*	0	≥4	0	erratic dose response
6082	*Pharmatec 01 derivative*	≥2	≥2	≥2	

[a]Rendered a statistically significant improvement in any parameter.

[b]Italized compounds reported in 1991 Final Report.

VI. EFFECTS OF DRUG COMBINATIONS ON THE HEPATOTROPIC PUNTA TORO VIRUS INFECTION IN MICE

Introduction

It is a recognized concept that the prudent use of two or more drugs in combination will often result in an improved effect against certain diseases when compared to using the drugs by themselves. An objective in this contract research work was to examine certain PTV disease inhibitors in various combinations in an attempt to ascertain those which may have clinical potential.

Two approaches were generally made in these experiments; the first utilized an experiment design oriented to determine if the drug combination had an increased therapeutic index (TI) compared to using either drug alone. Such combinations would conceivably reduce the risk of toxicity when treating the disease because less drug would be required to yield a positive therapeutic effect. The second approach was to determine if the use of one drug, such as a recognized immunomodulator, would significantly reduce the toxicity of high dosages of another, more standard, antiviral drug. Thus an "antidote" for the better drug could potentially be developed. In the latter approach, we concentrated our efforts primarily in attempting to reduce the toxicity of ribavirin (AVS01).

To orient these experiments to apply as much as possible to clinical situations, oral therapy was used where feasible, and initiation of treatments was after virus inoculation.

Materials and Methods

Virus: The Adames strain of PTV as described earlier was used. The virus concentration was selected to be approximately 95% lethal to the mice used.

Animals: Female 3 week-old C57BL/6 mice weighing 10-13 g were obtained from Simonsen Laboratories (Gilroy, CA). Quarantine, caging, and feeding of these mice was as described in Section IV.

Compounds: All compounds were provided by USAMRIID. The following drug combinations were studied:

AVS01 (ribavirin) + AVS5079 (human recombinant interleukin 2 [IL-2])

AVS01 + AVS5311 (human recombinant interferon [IFN])

AVS01 + AVS1761 (poly IC•LC)

AVS2776 (bropirimine) + AVS5079

AVS01 + AVS2149 (ampligen)

AVS01 + AVS2776

Each drug was prepared in the vehicle considered most appropriate; for AVS01 this was sterile water for injection. AVS5079 was prepared in 5% sterile dextrose solution. AVS5311 was dissolved in sterile physiological saline with 10% bovein serum albumin. AVS2149 was first annealed according to manufacturer's directions, then diluted in physiological saline for these studies. AVS1761 was dissolved in physiological saline. AVS2776, which is quite water-insoluble, utilized 0.4% carboxymethylcellulose (CMC).

Experiment Design: Treatment regimens for each drug combination are summarized in Table VI-1. In these studies, 5 to 6 experiments were run in parallel, according to the following general scheme:

#1: Compound A (AVS01 or 2776) at 4 or 5 dosages. These dosages in some experiments included a usually lethally toxic dose and 3 or 4 usually marginally PTV-active or -inactive dosages.

#2: Compound B (always an immune modulator) at three doses ranging from active to inactive against PTV.

#3: Compound A at all dosages used in #1 + Compound B used at the highest dose only.

#4: Compound A at all dosages used in #1 + Compound B used at the mid dose only.

#5: Compound A at all dosages used in #1 + Compound B used at the lowest dose only.

An expanded parameter anti-PTV experiment as described in Section IV was run in each study, the disease parameters being survivors, mean survival time, liver score, SGOT, SGPT, liver virus and serum virus titer, with 20 infected mice used in each treatment group, 40 mice used as placebo-treated controls, 10 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.

Statistical Evaluations: Alterations in the various virus parameters were analyzed by the standard statistical tests described in Section IV. Determinations of antagonistic, additive, or synergistic drug interactions were made by calculating fractional inhibitory concentration (FIC) indices, as was described by Berenbaum (1). In this method, the FIC was determined using the modified formula:

$$FIC = \frac{\text{MIC of Drug A in Combination}}{\text{MIC of Drug A alone}} + \frac{\text{MIC of Drug B in Combination}}{\text{MIC of Drug B alone}}$$

This FIC has been used by Huggins et al. (2) and Allen et al. (3) in their combination studies. Allen et al. (3) has interpreted the FIC values as:

FIC < 0.5: Significant synergism

FIC 0.5 - 0.9: Suggestive of synergism

FIC ~1: Effects are additive

FIC 1.1-1.9: Indifference or partial antagonism

FIC ≥2: Antagonism

Results and Discussion

A summary of all combination experiments run is seen in Table VI-2. Of the six drug combinations studied, two were considered synergistic, one additive to indifferent, one was antagonistic, and two were run only to attempt to reduce AVS01 toxicity. Each individual combination will be discussed in the following:

Combination #1: AVS01 + AVS5079: The results of this experiment indicated an additive or indifferent combination effect using the FIC index method. However, as seen in Table VI-3, the lethal toxicity of AVS01 was eliminated by i.p. therapy using AVS5079. Thus this decreased toxicity would result in a markedly increased therapeutic index in the combination therapy group.

Combination #2: AVS01 and AVS5311: This drug combination was considered possibly synergistic based on increased antiviral activity. The FIC index method cannot differentiate degree of inhibition at a particular drug level, only the minimum dose at which any significance was seen. As seen in Figures VI-1–4, the combination caused considered enhancement of antiviral effect.

Combination #3: AVS01 + AVS1761: In our last Final Report, we reported that the combination of AVS01 and AVS1761 was strongly antagonistic when used against *in vivo* PTV infections. In that study, AVS1761 was administered i.p. on an every other day treatment regimen. We have retested this drug combination, using AVS1761 given i.p. once only 1 hr prior to initiation of AVS01 therapy. This latter regimen was found to be markedly synergistic for the combination of AVS01 and AVS2149 (ampligen, a poly nucleotide closely related to the poly IC•LC used in the present study). The altered treatment regimen of AVS1761 in this latest combination experiment did not improve the performance of the drug combination; decreased anti-PTV activity and increased toxicity of AVS01 were evident, and the effects were considered antagonistic.

Combination #4: AVS2776 + AVS5079: This is an unusual combination of an interferon inducer and a cytokine which we have previously demonstrated to have significant anti-PTV

effects *in vivo*. The results indicated a synergistic effect with this drug combination. See also Figures VI-5–7 which better illustrate this effect.

Combination #5: AVS01 + AVS2149: This was a specially run experiment to determine if AVS2149 treatment late in AVS01 therapy, at a time when hematocrit has declined, would reverse this anemia-inducing property. Some positive effect was seen, which is reviewed separately in Section VII of this report.

Combination #6: AVS01 + AVS2776: This combination was previously reported by us to be synergistic (last Final Report). The present study was run to determine if late AVS2776 therapy to mice treated chronically with AVS01 would reverse the decline in hematocrit or enhance host weight gain. This study showed some positive effects, which are reviewed in detail in Section VIII of this report.

Conclusions

A total of 4 drug combinations were evaluated against the hepatotropic PTV infection *in vivo*. These were AVS01 + 5079, AVS01 + 5311, AVS01 + 1761, and AVS2776 + 5079. An additional 2 combinations were studied to determine if murine toxicity of AVS01 could be reduced by treatment with AVS2149 or AVS2776. AVS01 + 5079 resulted in an increased therapeutic index. AVS01 + 5311 was possibly synergistic. AVS01 + 1761 was strongly antagonistic. AVS2776 + 5079 was also suggetive of synergy. The murine toxicity of AVS01 was moderately reduced by delayed AVS2149 or AVS2776 therapy.

Literature Cited

1. Berenbaum, M.C. 1978. A method for testing synergy with any number of agents. J. Infect. Dis. 137:122-130.
2. Huggins, J.W., R.K. Robins, and P. Canonico. 1984. Synergistic antiviral effects of ribavirin and the C-nucleoside analogs tiazofurin and selenazofurin against togaviruses, bunyaviruses and arenaviruses. Antimicrob. Ag. Chemother. 26:476-480.
3. Allen, L.B., L.K. Vanderslice, C.M. Fingal, F.H. McCright, E.F. Harris, and P.D. Cook. 1982. Evaluation of the anti-herpesvirus drug combinations: Virazole plus arabinofuranosylhypoxanthine and Virazole plus arabinofuranosyladenine. Antiviral Res. 2:203-216.

Table VI-1. Drug Combinations Studied in the Hepatotropic Punta Toro Virus Model.

Combination #	Compound AVS No.	Treatment Route	Beginning of Treatment	Treatment Schedule
1	01 +	p.o.	+24 hr	bid x 3
	5079	i.p.	+4 hr	qd x 5
2	01 +	p.o.	+24 hr	bid x 3
	5311	i.p.	+4 hr	qd x 5
3	01 +	p.o.	+24 hr	bid x 3
	1761	i.p.	+23 hr	once only
4	2776 +	p.o.	+24 hr	once only
	5079	i.p.	+4 hr	qd x 5
5	01 +	p.o.	(no infection)	bid x 60
	2149	i.p.	6 or 11 days after 01 initiation	once only
6	01 +	p.o.	(no infection)	bid x 60
	2776	p.o.	20 days after 01 initiation	once only

Table VI-2. Values for the Various Drug Combinations Evaluated Against the Hepatotropic Punta Toro Infection or to Reduce Toxicity.

Drug Combination No. (AVS Nos.)	Evaluation Parameter	FIC Index	Mean FIC	Interpretation
1 (01+5079)	Death	~1.90		Additive to indifference, but reduced AVS01 toxicity (see Table VI-3)
	Liver Score	0.65		
	SGOT	1.33		
	SGPT	1.50		
	Liver Virus	0.83		
	Serum Virus	0.83		
			1.17	
2 (01+5311)	Death	1.5		Suggestive of synergism
	Liver Score	0.6		
	SGOT	0.6		
	SGPT	0.6		
	Liver Virus	0.6		
	Serum Virus	1.5		
			0.9	
3 (01+1761)	Death	nc		Increase toxicity and reduced efficacy supports antagonism
	Liver Score	nc		
	SGOT	nc		
	SGPT	nc		
	Liver Virus	nc		
	Serum Virus	nc		
			nc	
4 (2776+5079)	Death	0.31		Suggestive of synergism
	Liver Score	0.56		
	SGOT	1.06		
	SGPT	1.06		
	Liver Virus	0.31		
	Serum Virus	0.6		
			0.6	
5 (01+2149)	Toxicity only			Suggestive of decreased toxicity
6 (01+2776)	Toxicity only			Suggestive of decreased toxicity

Table VI-3. Reduction of AVS01-Induced Murine Lethal Toxicity by AVS5079 Therapy[a].

Compound (AVS No.)	Dose	% Survivors
01	1,500 mg/kg/day	0
5079	12,000 units/mouse/day	100
	6,000 units/mouse/day	100
	3,000 units/mouse/day	100
01+5079	1,500 + 12,000	100**
	1,500 + 6,000	100**
	1,500 + 3,000	60**

[a]AVS01: p.o. bid x 3; AVS5079: i.p. qd x 5 20 hr pre-AVS01.

**P<0.01 compared to AVS01 used alone.

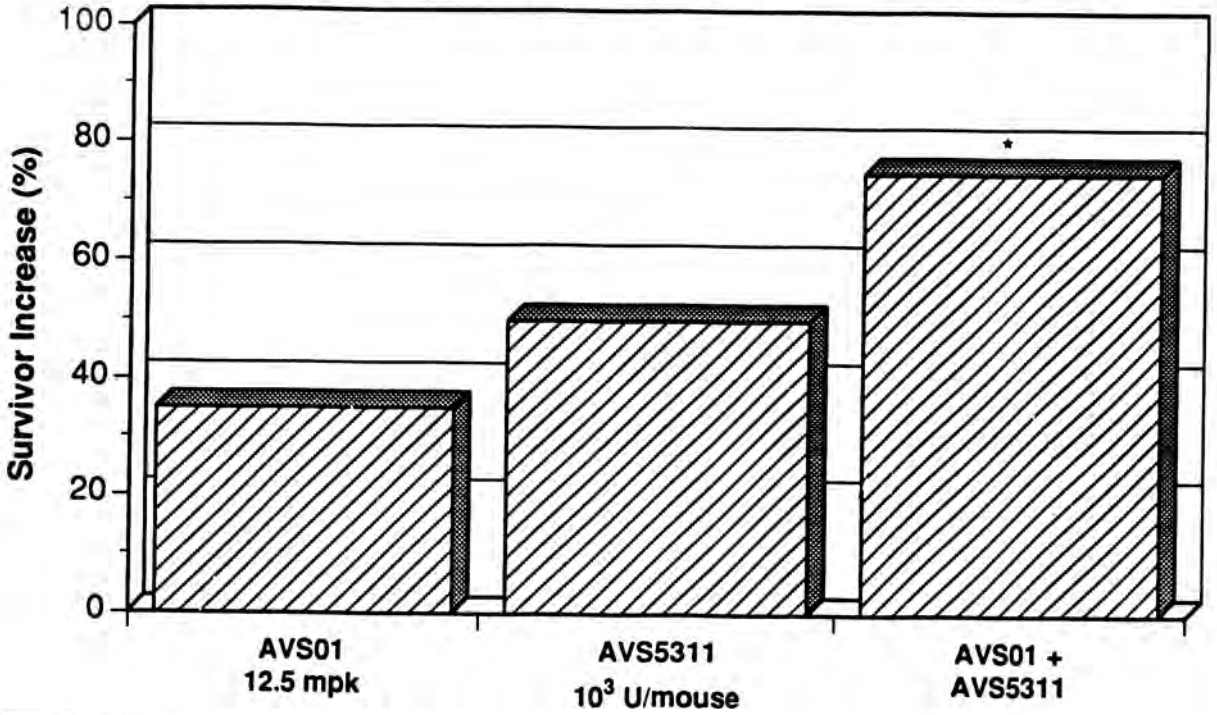
Table VI-4. Enhancement of AVS-01-Induced Murine Lethal Toxicity by AVS1761 Therapy[a].

Compound (AVS No.)	Dose (mg/kg/day)	% Survivors
01	1,500	40
	1,200	100
1761	0.005	100
	0.05	100
	0.5	100
	5	100
01+1761	1,500 + 0.005	75
	1,500 + 0.0524	
25		
	1,500 + 0.5	25
	1,500 + 5	0
	1,200 + 0.005	100
	1,200 + 0.05	75
	1,200 + 0.5	25*
	1,200 + 5	25*

[a]AVS01: p.o. bid x 3; AVS1761: i.p. once only 1 hr pre-AVS01.

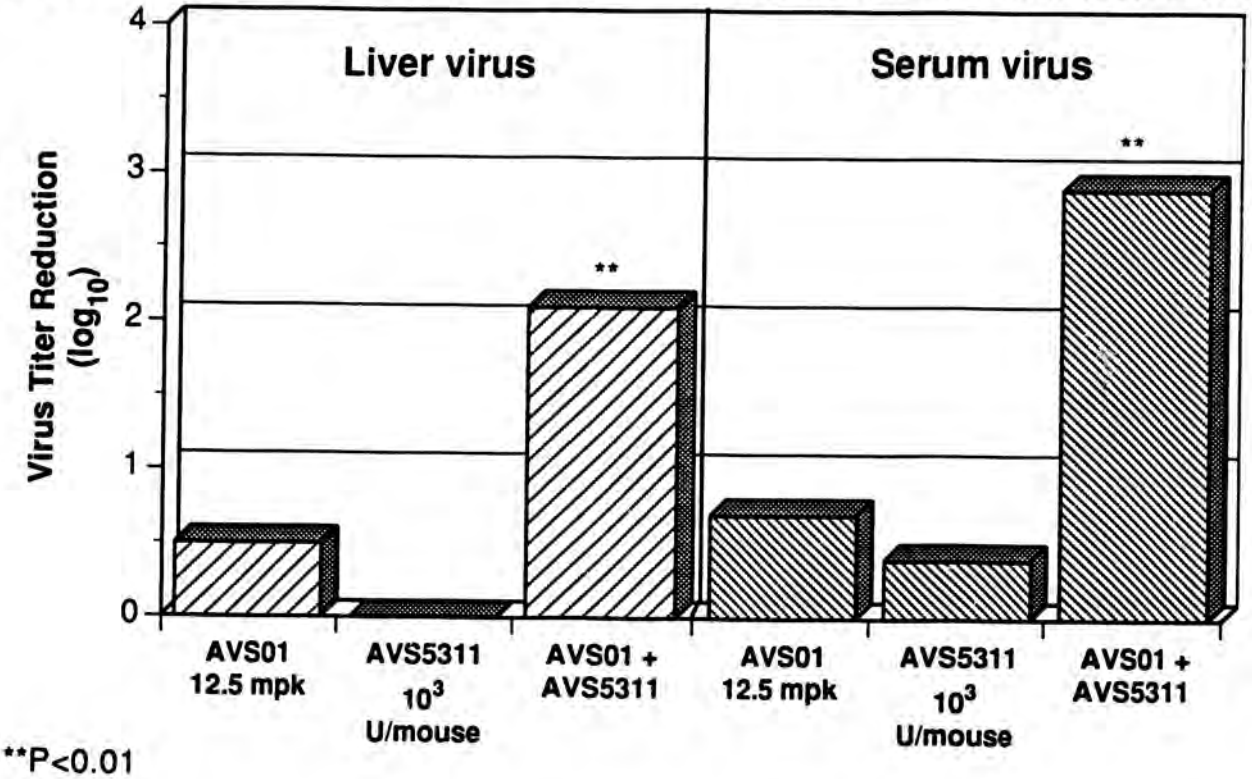
*P<0.01 compared to the same dose of AVS01 used alone.

Figure VI-1. PtA 854-858. Effect of the Combination of AVS01 + AVS5311 on Survivor Increase in PTV-Infected Mice.



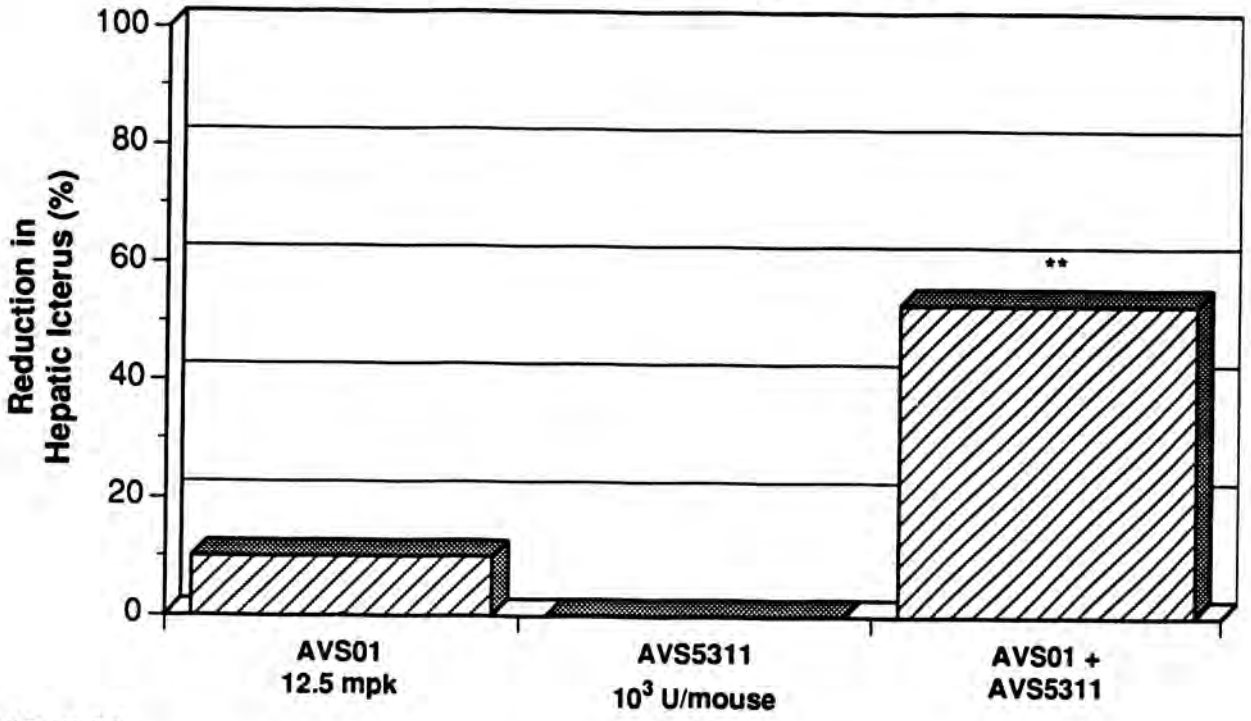
*P<0.05

Figure VI-2. PtA 854-858. Effect of the Combination of AVS01 + AVS5311 on Liver and Serum Virus Titer Reduction in PTV-Infected Mice.



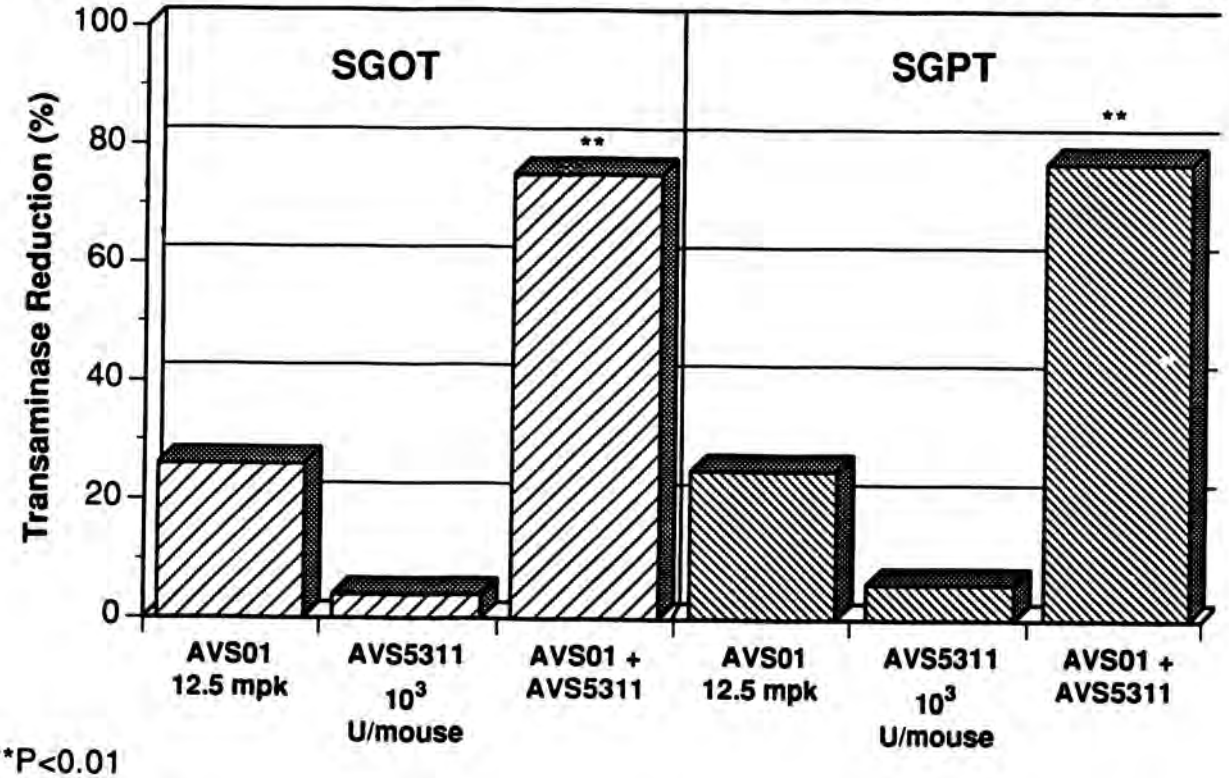
**P<0.01

Figure VI-3. PtA 854-858. Effect of the Combination of AVS01 + AVS5311 on Reduction of Hepatic Icterus in PTV-Infected Mice.



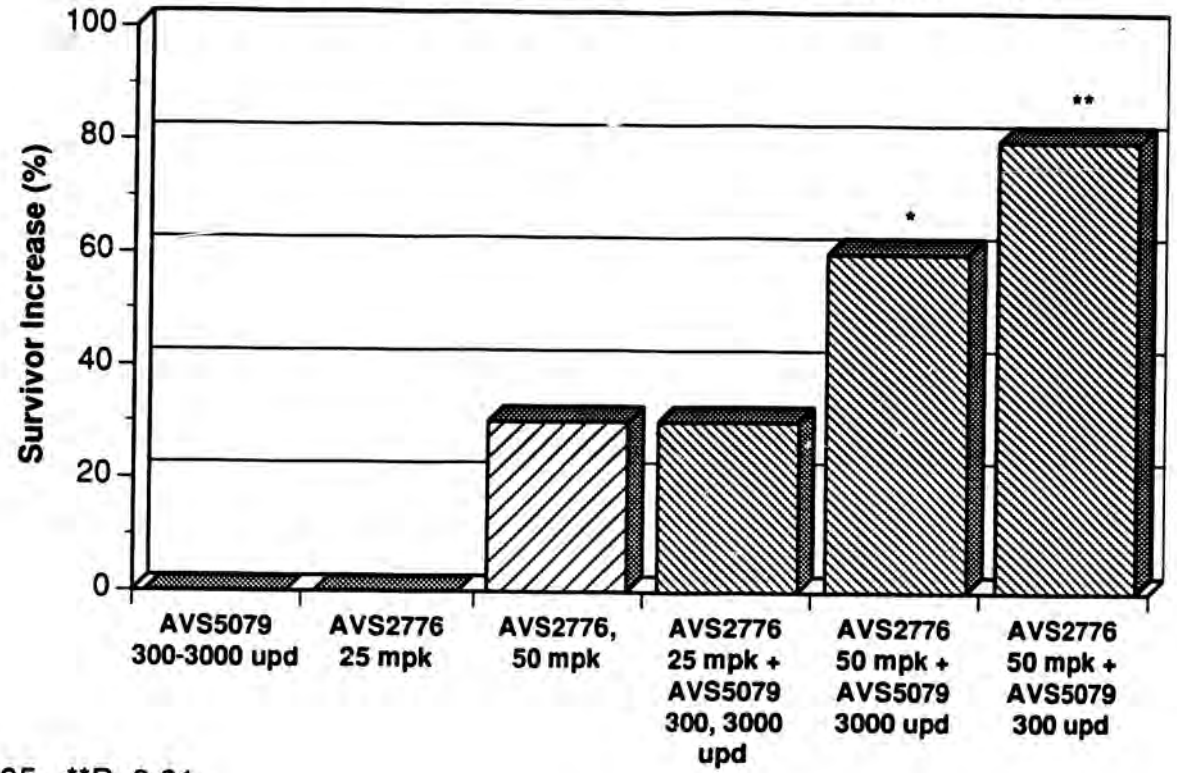
**P<0.01

Figure VI-4. PtA 854-858. Effect of the Combination of AVS01 + AVS5311 on Reduction of SGOT and SGPT Values in PTV-Infected Mice.



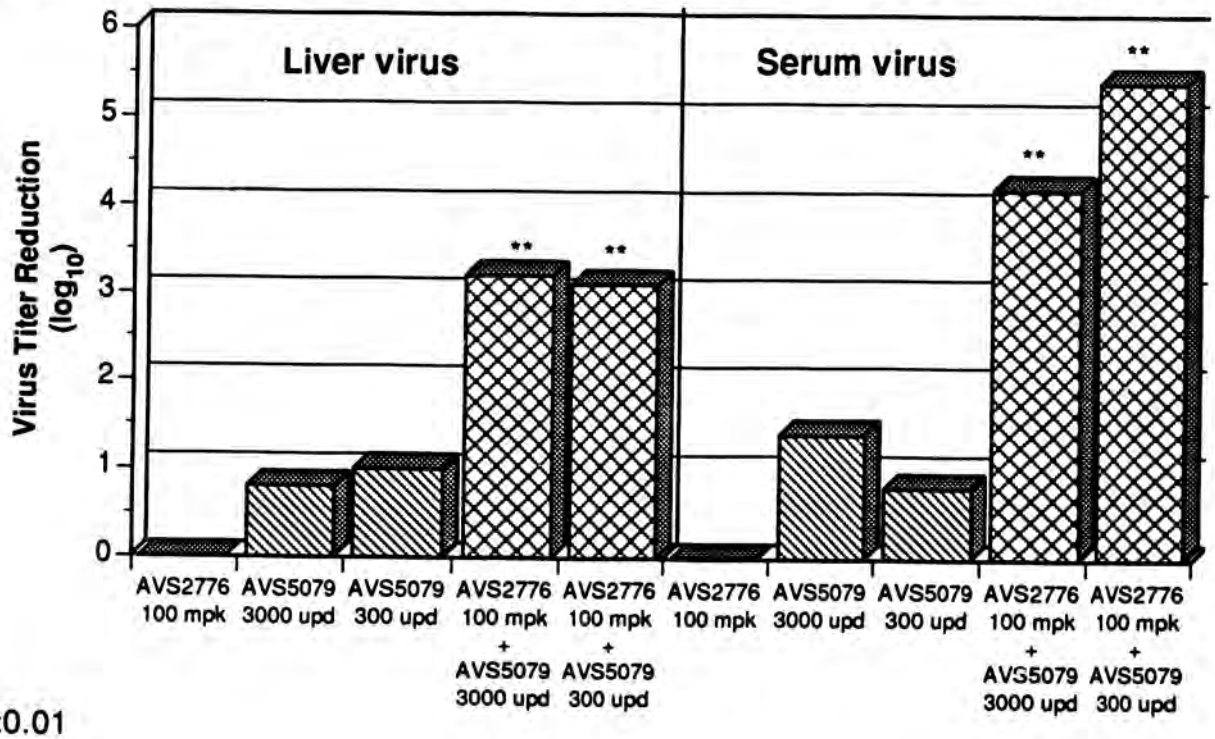
**P<0.01

Figure VI-5. PtA 883-886. Effect of the Combination of AVS2776 + AVS5079 on Survivor Increase in PTV-Infected Mice.



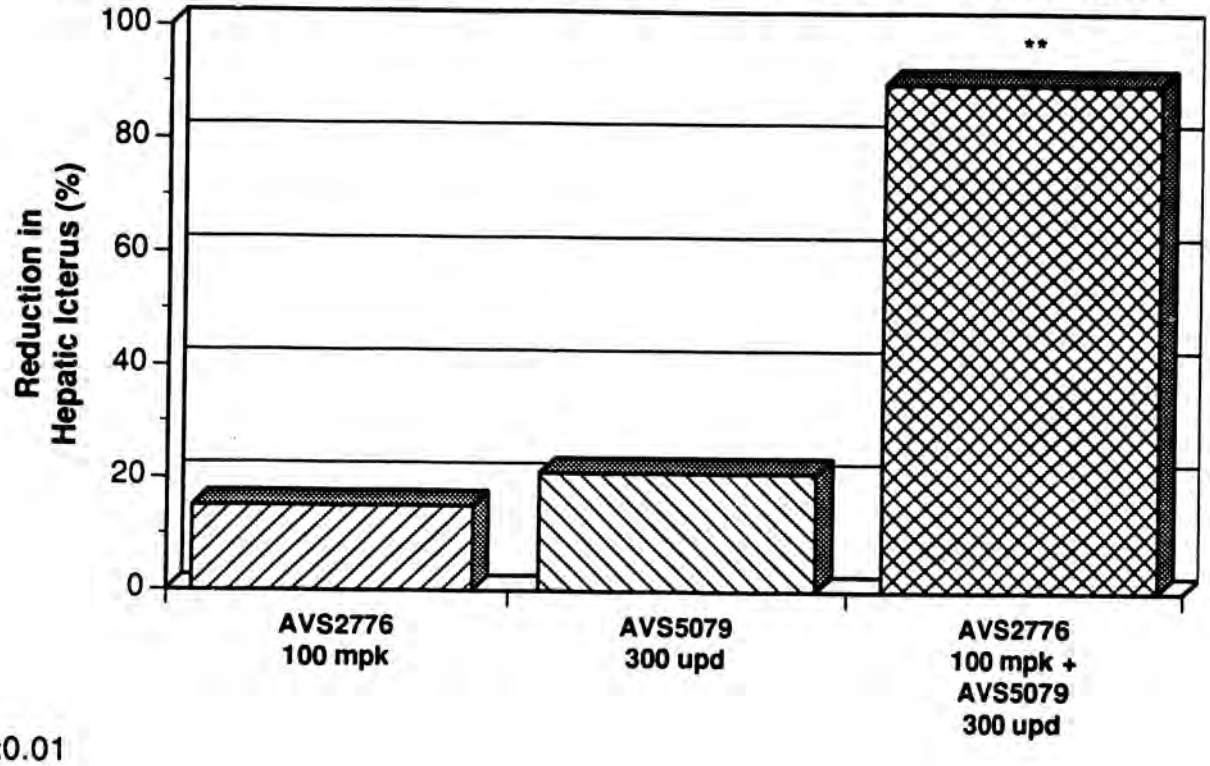
*P<0.05 **P<0.01

Figure VI-6. PtA 883-886. Effect of the Combination of AVS2776 + AVS5079 on Liver and Serum Virus Titer Reduction in PTV-Infected Mice.



**P<0.01

Figure VI-7. PtA 883-886. Effect of the Combination of AVS2776 + AVS5079 on Reduction of Hepatic Icterus in PTV-Infected Mice.



**P<0.01

VII. EFFECT OF AVS2149 THERAPY ON TOXICITY CAUSED BY CHRONIC AVS01 THERAPY

Introduction

As summarized in our Final Report on Contract DAMD17-86-C-6028, a single injection with varying dosages of ampligen (AVS2149) appeared to reverse the lethal toxicity effects of ribavirin (AVS01). In the experiment, ampligen was given i.p. 1 hr prior to initiation of high dose oral ribavirin treatment. The present experiment was run to follow up on this study, with ampligen now given after chronic p.o. ribavirin therapy had resulted in significant hematocrit decline.

Materials and Methods

Compounds: All compounds were provided by U.S. Army Medical Research Institute for Infectious Diseases via Biological Research Faculty and Facility, Inc. (Rockville, MD). Ampligen was annealed by adding 20 ml of sterile pyrogen-free water to a vial of the compound, which was then placed in a 65°C water bath for 30-40 minutes, then held 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. Ribavirin was dissolved in sterile water for this study.

Animals: Three week-old female C57BL/6 mice were purchased from Simonsen Labs (Gilroy, CA). They were quarantined 24 hr before use, and maintained on Wayne Lab Blox and tap water *ad libitum.*

Experiment Design: A total of 300 mice were treated p.o. with 200 mg/kg/day of ribavirin twice daily for up to 60 days. The mice were weighed twice weekly, and every 7 days 5 mice were exanguinated and their blood hematocrit determined. When the hematocrit values had fallen by 20% or more, the animals (20/dose) were then also treated with 0.5, 0.05, or 0.005 mg/kg of ampligen. This drug was given i.p. once only. As controls, mice receiving water only instead of ribavirin were similarly treated with ampligen. Five mice in each group were subsequently bled 24, 48, and 72 hr after the ampligen therapy and hematocrits again determined. Ribavirin therapy was discontinued in one experiment when ampligen treatment was given, but continued in a second experiment.

Results and Discussion

The experiment where ribavirin therapy ceased at the time ampligen was given is summarized in Figure VII-1. Ribavirin therapy caused a significant decline in hematocrit, down to approximately 24% less then normal, at which point these blood values leveled off through the remainder of treatment. This suggests the animals adapted in some manner to treatment with this relatively high ribavirin dose. When ampligen was administered 6 days after initiation of therapy, an initial further decline in hematocrit was seen in mice receiving 0.5 and 0.005 mg/kg; the 0.05 mg/kg dose caused an immediate rise in hematocrit by 24 hr after treatment. All values returned to the approximate level at the time of ampligen treatment by 48 hr; on day 7 after ampligen therapy, all values had risen to near normal levels. We consider this later rise a result of termination of ribavirin therapy, however, since it is known that ribavirin's toxicity is essentially reversed by treatment termination (1).

Host weight increases occurred in both H_2O- and ribavirin-treated mice (Figure VII-2). The ribavirin treated group had an initial lower mean weight than the mice receiving H_2O, and this difference was maintained throughout the study. Upon ampligen therapy and concomitant cessation of ribavirin therapy, the animals receiving the two higher doses of ampligen rapidly gained weight so that by 7 days after treatment, they weighed 0.8-1.3 g more than H_2O-treated controls (Figure VII-2). These data suggest a possible positive effect due to the ampligen therapy.

When ampligen was given to the ribavirin-treated mice without stopping ribavirin therapy, an increase in hematocrit was seen in the mice by 7 days after the injection of the two highest doses of ampligen (Figure VII-3). It was of interest that the ribavirin-treated mice which did not receive ampligen also began increasing in their hematocrit values 14 days into ribavirin therapy, although this increase was not back to normal levels. Host weight gain was accelerated by two ampligen dosages (Figure VII-4). These data again suggest the potential ribavirin toxicity-reducing effects of ampligen therapy.

Summary

AVS01 (ribavirin) therapy, p.o. at 200 mg/kg/day, resulted in a significant initial decline in mouse blood hematocrit values, down to approximately 24% of H_2O-treated mice. AVS2149 (ampligen), when administered to these mice i.p. in doses of 0.005, 0.05, and 0.5 mg/kg, resulted in a general increase in hematocrit and accelerated weight gain.

Literature Cited

1. Hillyard, I.W. 1980. The preclinical toxicology and safety of ribavirin. *In:* Ribavirin: A Broad Spectrum Antiviral Agent (R.A. Smith and W. Kirkpatrick, eds.) Academic Press, New York, pp. 59-71.

Figure VII-1. PT327. Effect of a Single Ampligen Treatment on Hematocrit Values in C57BL/6 Mice Treated with Ribavirin (ribavirin therapy ceased in ampligen-treated groups with ampligen therapy).

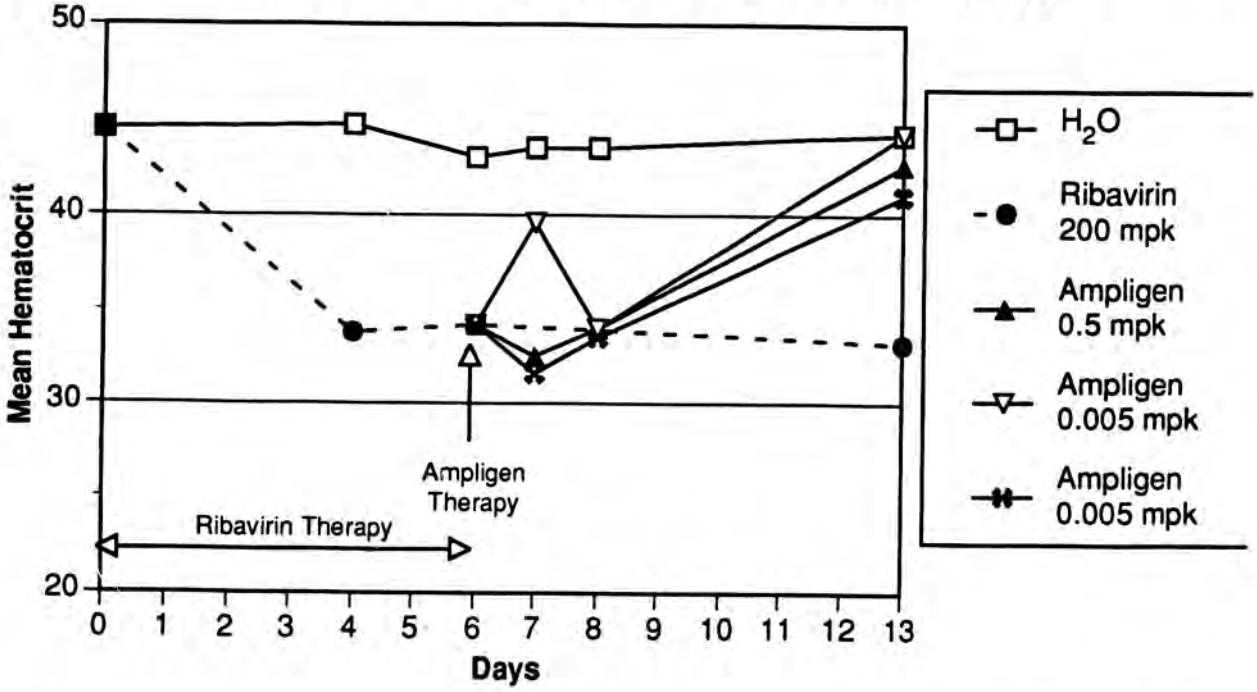


Figure VII-2. PT327. Effect of a Single Ampligen Treatment on Host Weight Change in C57BL/6 Mice Treated with Ribavirin (ribavirin therapy ceased in ampligen-treated groups with ampligen therapy).

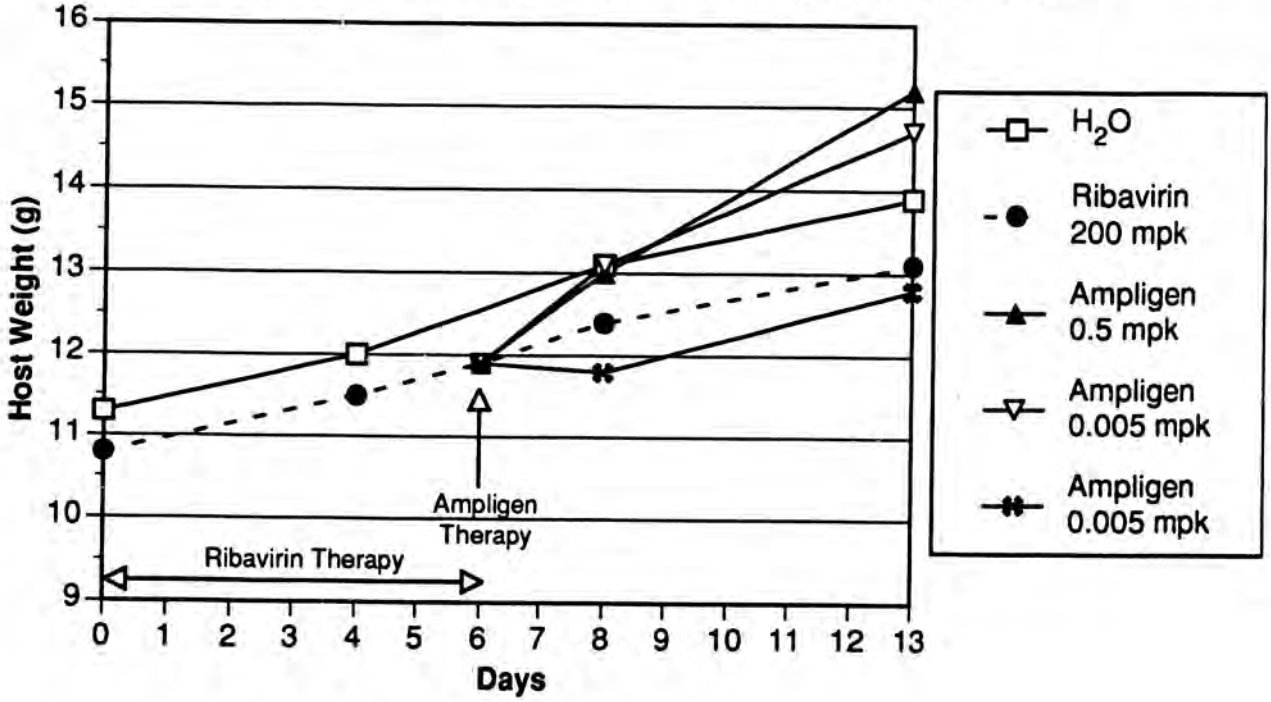


Figure VII-3. PT330. Effect of a Single Ampligen Treatment on Hematocrit Values in C57BL/6 Mice Treated with Ribavirin (ribavirin therapy continued with ampligen therapy).

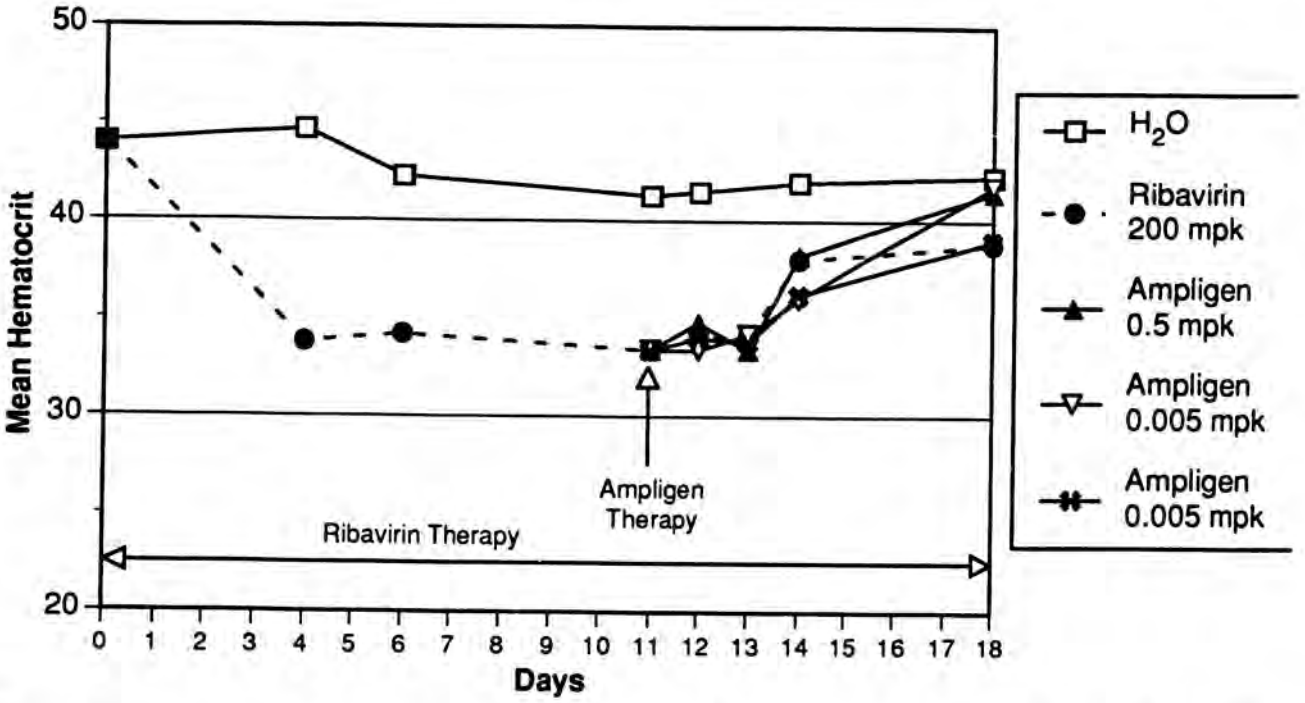
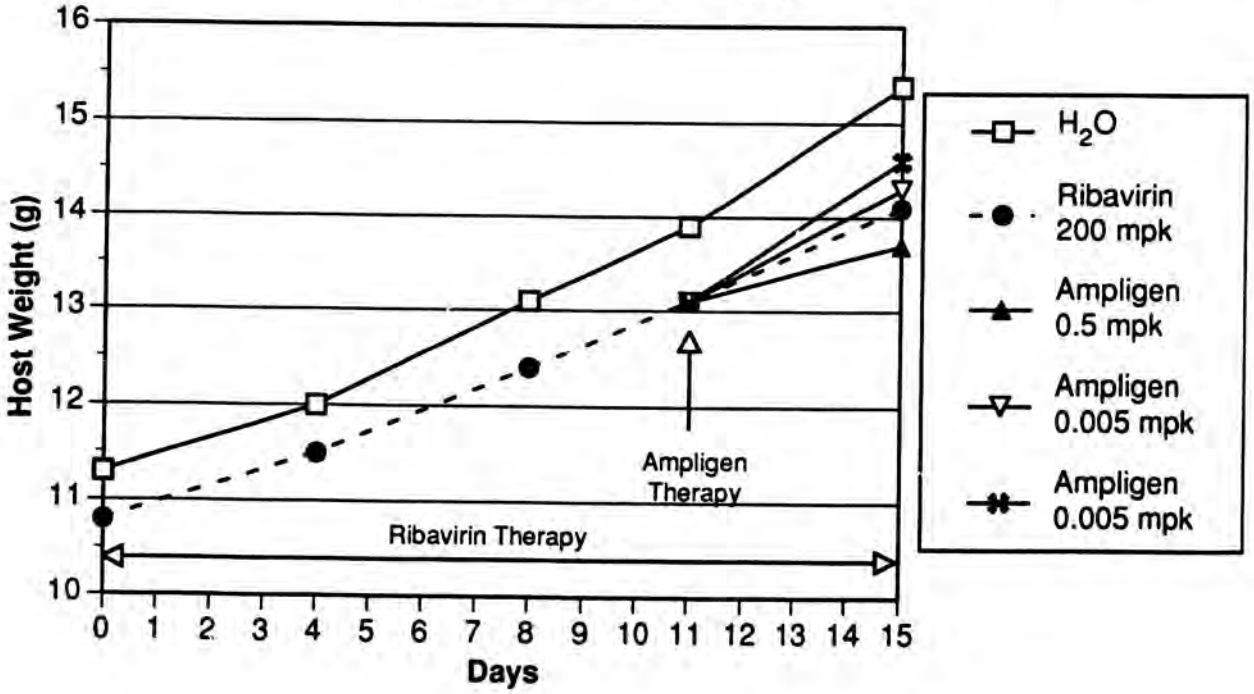


Figure VII-4. PT327. Effect of a Single Ampligen Treatment on Host Weight Change in C57BL/6 Mice Treated with Ribavirin (ribavirin therapy continued with ampligen therapy).



VIII. EFFECT OF AVS2776 THERAPY ON TOXICITY CAUSED BY CHRONIC AVS01 THERAPY

Introduction

As summarized in our Final Report on Contract DAMD17-86-C-6028, oral treatment with varying dosages of bropirimine (AVS2776) appeared to cause a possible reversal of ribavirin (AVS01)-induced lethal toxicity in mice. Our results of those earlier studies have been somewhat erratic, however. The present experiment was run to determine if a single p.o. bropirimine treatment would have a significant influence on lowered hematocrit values of mice receiving chronic ribavirin therapy.

This experiment is a followup of the previous study described with ampligen (Expt. PT 328, 330).

Materials and Methods

Compounds: All were provided by the U.S. Army Medical Research Institute for Infectious Diseases via Biological Research Faculty and Facility, Inc. (Rockville, MD). Bropirimine was suspended in 0.4% carboxymethylcellulose for this study. Ribavirin was dissolved in sterile water.

Animals: Three week-old female C57BL/6 mice were purchased from Simonsen Labs (Gilroy, CA). They were quarantined 24 hr before use, and maintained on Wayne Lab Blox and tap water *ad libitum*.

Experiment Design: Mice were treated p.o. with 200 mg/kg/day of ribavirin twice daily for up to 60 days. Twenty days after initiation of this chronic treatment, bropirimine in doses of 25 and 50 mg/kg was administered p.o. to these mice and to H_2O-treated mice. Upon initiation of bropirimine therapy, the mice were weighed daily for 2 days. Three to 5 mice from both ribavirin and H_2O-treated groups were killed 3, 24, and 48 hr after bropirimine treatment and the blood hematocrits determined. Their sera was assayed for IFN titer at 3 and 24 hr after bropirimine treatment as has been described earlier. Ribavirin therapy continued during and after the bropirimine treatment.

Results and Discussion

The ribavirin-treated mice had a mean hematocrit value of 40 as compared to 44.3 for H_2O-treated animals (Figure VIII-1). Upon bropirimine treatment (50 mg/kg), ribavirin-treated mice showed an initial rise in hematocrit to 41.6 by 3 hr; this declined to slightly below the values of mice receiving ribavirin only by 24 and 48 hr. The lower (25 mg/kg) bropirimine dose caused a moderate increase in hematocrit in the ribavirin-treated mice by 24 and 48 hrs (Figure VIII-1). The lower bropirimine dose also resulted in an accelerated host weight gain in the mice receiving the chronic ribavirin therapy (Figure VIII-2).

In control mice treated chronically with H_2O, neither bropirimine treatment caused significant alterations in either hematocrit or host weight compared to mice receiving H_2O only.

The IFN data are summarized in Table VIII-1. By 3 hrs after treatment, bropirimine at 50 mg/kg induced a significant amount of serum IFN in both the ribavirin-treated and H_2O-treated mice. The mean IFN titers were essentially the same in each group. The 25 mg/kg dose of bropirimine, which we have previously shown to be a weak IFN inducer, stimulated detectable IFN in H_2O-treated mice, but not in those chronically treated with ribavirin. This suggests the ribavirin treatment may have reduced the animals' ability to respond to weak IFN stimulation. No IFN was detected by 24 hr after bropirimine treatment, as we have previously described. Also, no IFN was seen in the serum of H_2O- or ribavirin-treated mice not treated with bropirimine.

These data suggest bropirimine to have a marginal influence on reversing ribavirin's toxicity in chronically treated mice.

Summary

Chronic p.o. AVS01 (ribavirin) therapy with 200 mg/kg/day resulted in decreased hematocrit and less host weight gain in C57BL/6 mice. A single p.o. AVS2776 (bropirimine) treatment using 25 mg/kg of these mice resulted in a moderate increase in hematocrit and increased host

weight gain. A higher dose of AVS2776 was essentially ineffective. Bropirimine induced IFN detectable in the serum 3 hr after treatment of all H_2O-treated mice, and the 50 mg/kg dose induced IFN in the animals receiving ribavirin. The 25 mg/kg dose of bropirimine did not induce detectable IFN in the ribavirin-treated animals, suggesting the latter therapy may have reduced the animals' ability to respond to weak IFN stimulation.

Figure VIII-1. PT331. Effect of a Single Bropirimine Treatment on Hematocrit Values in C57BL/6 Mice Treated with Ribavirin (ribavirin therapy continued after bropirimine therapy).

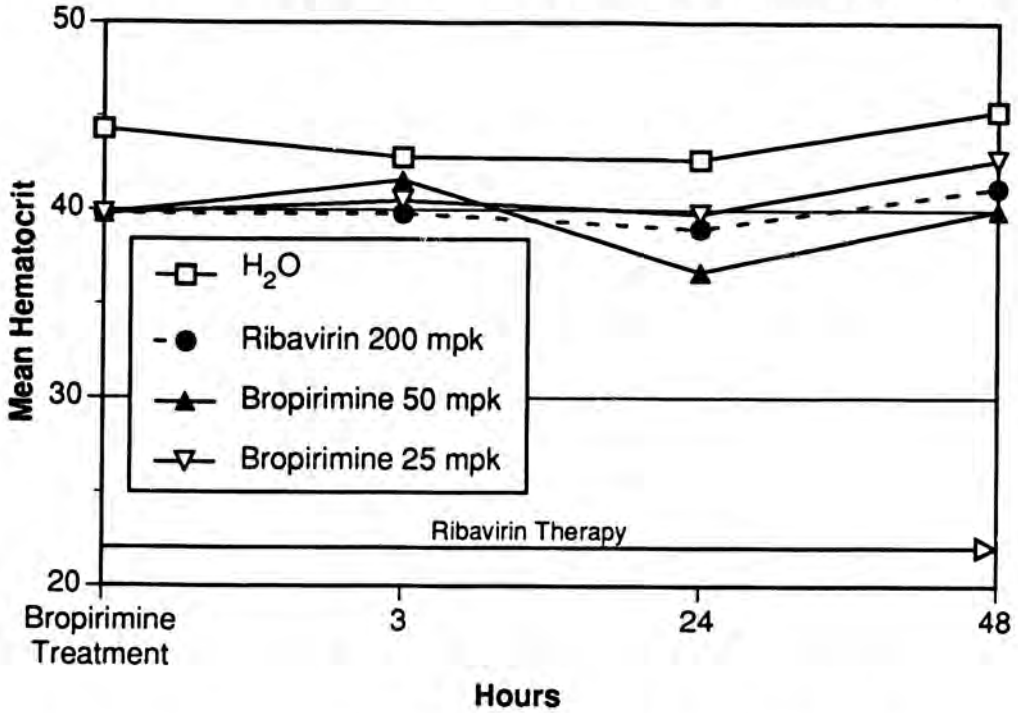


Figure VIII-2. PT331. Effect of a Single Bropirimine Treatment on Host Weight Change in C57BL/6 Mice Treated with Ribavirin (ribavirin therapy continued after bropirimine therapy).

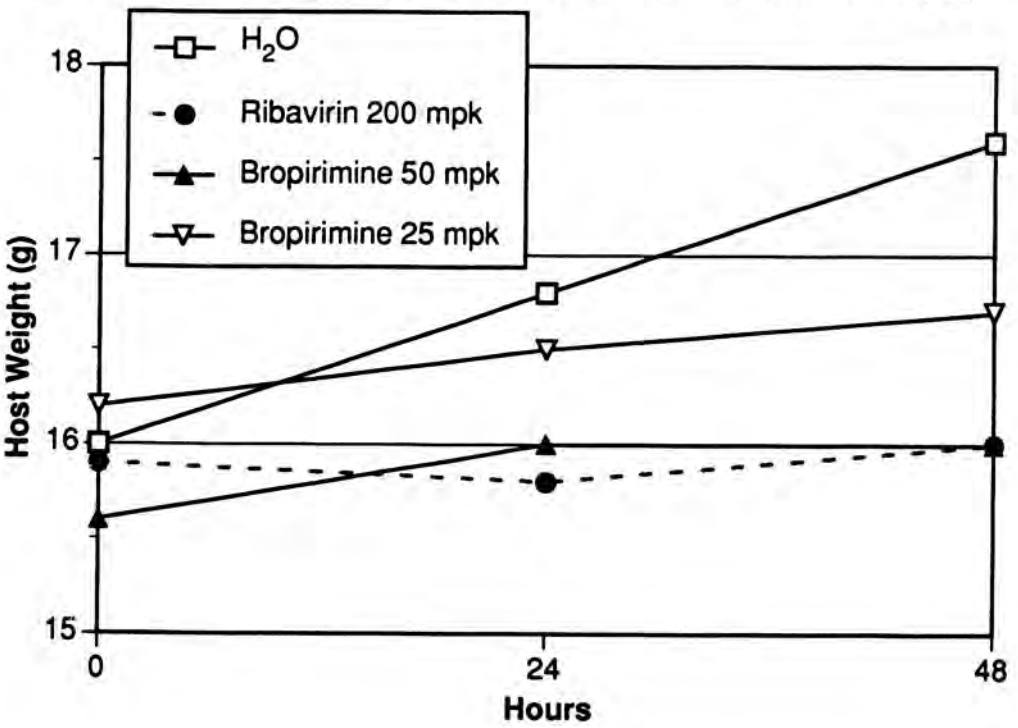


Table VIII-1. PT 331. Serum Interferon Titers in Bropirimine-Treated Mice

Treatment Group	Mean IFN Titer (log_{10} units/0.1 ml) 3 hr post	24 hr post
Ribavirin + 50 mpk Bropirimine	2.5	<1.0
H_2O Controls + 50 mpk Bropirimine	2.7	<1.0
Ribavirin + 25 mpk Bropirimine	<1.0	<1.0
H_2O Controls + 25 mpk Bropirimine	1.1	<1.0
H_2O Controls	<1.0	
Ribavirin	<1.0	

IX. INTERFERON AND INTERLEUKIN-2 INDUCTION IN MICE BY A SERIES OF AVS COMPOUNDS

Introduction

As a means of conserving drug, a series of AVS compounds were evaluated in mice for their ability to induce interferon (IFN) or interleukin-2 (IL-2) after a single injection in mice. The plan was to have additional quantities of any active compounds made up for a complete evaluation in our PTV model.

Materials and Methods

Animals: Three-week-old female C57BL/6 mice were obtained from Simonsen Laboratories (Gilroy, CA). They were maintained on standard mouse chow and tap water during the experiment. They were quarantined 24 hr prior to use in this study.

Compounds: The following AVS compounds were submitted for evaluation: AVS581, 702, 709, 710, 712, 1644, 1841, 1846, 3362, 3547, 3580, 3935, 4156, 4277, 4611, 4923, 5065, 5067, 5075, and 5603. All were dissolved or suspended in saline and used immediately.

IFN Assay: Serum samples to be assayed were diluted through a series of $\log_{10}$ dilutions from 10^{-1} through 10^{-5}. Aliquots of 0.1 ml of each dilution were added to each of 3 cups in 96-well flat-bottomed microplates in which a 24-hr monolayer of L929 cells had been established. The samples were incubated 24 hr at 37°C. After incubation, the cells were drained and 0.1 ml of a 10^3 $CCID_{50}$/0.1 ml concentration 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 (cells exposed to test medium, then to VSV), and cell controls which were exposed to test medium only. Test medium was minimum essential medium (MEM) with 2% fetal bovine serum (FBS), 0.18% $NaHCO_3$ and 50 µg gentamicin/ml.

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-mercaptoethanol. 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: Eight mice were injected with a single concentration (1 mg/mouse in 0.2 ml) of test compound. At 4 hr and 24 hr, 4 mice were killed, bled and the serum frozen for later IFN assay. Eight untreated mice were also used as normal controls. These animals were processed in an identical manner to the treated animals.

At 24 hr, spleens were removed from the treated mice and processed as above for murine IL-2 activity. These IL-2 tests were run in 2 sets, depending on the test compound. A group of normal controls were run with each set.

Results and Discussion

The results of this study are summarized in Tables IX-1 and IX-2.

Three AVS compounds, 581, 709, and 4611, induced detectable quantities of IFN. The IFN was detected only at 4 hr post-treatment in mice treated with AVS581 and 709, indicating a rapid IFN induction and a relatively short serum half-life of the IFN induced. Mice receiving AVS4611 had low levels of IFN at 4 hr, but high titers at 24 hr, indicating the IFN induction occurred more slowly with this compound. Normal controls showed no signs of serum IFN.

Insufficient compound was available for in vivo testing vs PTV; early termination of the contract precluded obtaining additional compound.

As seen in Table IX-2, the normal control animals in each group tested had mean IL-2 levels of 0.9 and 0.86. All AVS compounds evaluated increased the murine splenic IL-2 levels above these baseline values. Predominant among these were AVS709, 710, 712, 1644, 1846, 3362, 4277, and 4611. Following single treatment with these compounds, the IL-2 levels were more than double the normal controls and their ± standard deviations indicated significant differences from the controls.

Conclusions

Compounds AVS581, 702, 709, 710, 712, 1644, 1841, 3362, 3547, 3935, 4156, 4277, 4611, 4923, 5065, 5067, 5075, and 5603 were evaluated for their ability to induce IFN and IL-2 in 3 week-old C57BL/6 mice. Compounds AVS581, 709, and 4611 were shown to induce detectable levels of serum IFN. Compounds AVS709, 710, 712, 1644, 1846, 3362, 4277, and 4611 were considered to be most effective in stimulating IL-2, with the induced levels of this cytokine more than twice those of normal controls and significantly different than the normal controls.

Table IX-1. Interferon Inducing Ability of AVS Compounds in C57BL/6 Mice[a]

Compound (AVS No.)	IFN Titer[b] (log_{10} units/0.1 ml) 4 hr post	24 hr post
581	1.7 ± 0.2	<1.0
702	<1.0	<1.0
709	1.0 ± 0.6	<1.0
710	<1.0	<1.0
712	<1.0	<1.0
1644	<1.0	<1.0
1841	<1.0	<1.0
1846	<1.0	<1.0
3362	<1.0	<1.0
3547	<1.0	<1.0
3580	<1.0	<1.0
3935	<1.0	<1.0
4156	<1.0	<1.0
4277	<1.0	<1.0
4611	0.7 ± 0.4	2.4 ± 0.2
4923	<1.0	<1.0
5065	<1.0	<1.0
5067	<1.0	<1.0
5075	<1.0	<1.0
5603	<1.0	<1.0

[a]1.0 mg/mouse injected i.p.; 50, 100, 200, and 400 mg/kg of AVS3580 were tested.

[b]Mean ± SE of 4 mice per group.

Table IX-2. IL-2-Inducing Ability of AVS Compounds in C57BL/6 Mice[a]

Compound (AVS No.)	Test No.[b]	Mean IL-2 (units/ml ± SD)
581	1	1.17 ± 0.18
702	1	1.62 ± 0.20
709	1	2.66 ± 0.58
710	1	2.48 ± 0.31
712	1	2.58 ± 0.75
1644	1	3.79 ± 0.85
1841	1	1.55 ± 0.46
1846	1	2.47 ± 0.41
3362	1	2.22 ± 0.42
3547	1	1.53 ± 0.23
Normal controls	1	0.90 ± 0.14
3935	2	1.02 ± 0.40
4156	2	1.57 ± 0.34
4277	2	2.17 ± 0.64
4611	2	2.04 ± 0.42
4923	2	1.95 ± 0.73
5065	2	2.70 ± 1.75
5067	2	1.59 ± 0.63
5075	2	1.95 ± 0.79
5603	2	1.66 ± 0.37
Normal controls	2	0.86 ± 0.26

[a]1.0 mg/mouse injected i.p.

[b]IL-2 studies were run in 2 sets according to AVS number.

X. SUSCEPTIBILITY OF VARIOUS STRAINS OF MICE TO PUNTA TORO VIRUS INFECTION

Introduction

It was of interest to determine the relative susceptibility of DBA/2 mice and C57BL/6 mice, the latter as produced by a different supplier (SASCO Labs), to the hepatotropic PTV. With the start of a new contract, we were required to obtain new bids from mouse suppliers. SASCO had the lowest bid for C57BL/6 mice, and we had to then determine if the SASCO mice were acceptably susceptible to the virus used in our standard PTV chemotherapy experiments. The standard Simonsen C57BL/6 mice were run also as a control.

Materials and Methods

Virus: The Adames strain of PTV as has been previously described was used.

Animals: Male and female DBA/2 mice in three weight ranges: 9-12 g, 14-16 g, and 18-20 g were obtained from Simonsen Laboratories (Gilroy, CA). Male and female C57BL/6 mice weighing 8-10 g, 12.5-14.5 g, and 14-6-16.5 g provided by SASCO Laboratories (St. Louis, MO). Female 8-10 g C57BL/6 mice were obtained also from Simonsen Labs. All were quarantined 24 hr prior to use, and were maintained on Wayne Lab Blox and tap water throughout these studies.

Experiment Design: Mice in groups of 5 or 10 were infected s.c. with 0.2 ml of varying $\log_{10}$ or 0.5 $\log_{10}$ dilutions of virus. The animals were observed daily for death for 21 days.

Results and Discussion

The results of these titrations are summarized in Tables X-1 to X-4. The DBA/2 mice (Table X-1) were moderately susceptible to the virus, with a "window" of infectivity seen in the 9-12 g mice. The non-lethal effects of high concentrations of virus, presumably due to defective interfering particles, were especially apparent in these animals. Older mice were less susceptible to the virus. Pifat and Smith (1) have reported similar findings in DBA/2 mice infected with an earlier preparation of this virus.

The SASCO C57BL/6 mice were also moderately sensitive to the virus (Tables X-2, 3). In these animals, the non-lethal effects of high virus concentrations were not as apparent as seen in the DBA/2 mice. The LD50 of the virus was quite similar in both male and female mice. In no instance did all the mice die in a single virus dilution group. This titration was repeated with virtually identical results (Table X-3).

The Simonsen mice appeared much more susceptible to PTV as seen in Table X-4. In these animals, all mice died in 3 virus dilution groups, with mean survival times of 4 to 5 days.

These data suggest the SASCO mice to be less acceptably susceptible to PTV. The SASCO animals are shipped via truck from St. Louis to our laboratory, this taking approximately 24 hr. It is possible the trauma of shipping may have set off an immunologic reaction protecting the mice from PTV infection. Another possibility is a genetic difference between SASCO and Simonsen mice.

Conclusions

DBA/2 and SASCO C57BL/6 mice were found moderately susceptible to infection with the hepatotropic Adames strain of PTV. The DBA/2 mice had a more pronounced insensitivity to high doses of virus. The SASCO mice were less sensitive than Simonsen animals.

Literature Cited

1. Pifat, D.Y. and J.F. Smith. 1987. Punta Toro virus infection of C57BL/6J mice: A model for *Phlebovirus*-induced disease. Microb. Pathogen. 3:409-422.

Table X-1. Susceptibility of DBA/2 Mice to s.c. PTV Inoculation

Virus Dilution	Surv/ Total[a]	Mean Survival Time[b]
9-12 g mice		
$10^{-0.5}$	5/5	>21.0
$10^{-1.0}$	5/5	>21.0
$10^{-1.5}$	5/5	>21.0
$10^{-2.0}$	5/5	>21.0
$10^{-2.5}$	2/5	6.0
$10^{-3.0}$	1/5	4.8
$10^{-3.5}$	1/5	5.8
$10^{-4.0}$	1/5	4.5
$10^{-4.5}$	2/5	6.0
$10^{-5.0}$	4/5	4.0
	LD50 = $10^{-4.2}$	
14-16 g mice		
$10^{-0.5}$	5/5	>21.0
$10^{-1.0}$	5/5	>21.0
$10^{-1.5}$	5/5	>21.0
$10^{-2.0}$	3/5	4.5
$10^{-2.5}$	5/5	>21.0
$10^{-3.0}$	5/5	>21.0
$10^{-3.5}$	3/5	7.5
$10^{-4.0}$	4/5	8.0
$10^{-4.5}$	4/5	10.0
$10^{-5.0}$	2/5	5.3
	LD50 = $10^{-4.8}$	
18-20 g mice		
$10^{-0.5}$	4/5	9.0
$10^{-1.0}$	3/5	4.5
$10^{-1.5}$	5/5	>21.0
$10^{-2.0}$	4/5	6.0
$10^{-2.5}$	4/5	5.0
$10^{-3.0}$	3/5	5.0
$10^{-3.5}$	5/5	>21.0
$10^{-4.0}$	5/5	>21.0
$10^{-4.5}$	5/5	>21.0
$10^{-5.0}$	5/5	>21.0
	LD50 = $10^{-0.5}$	

[a]21 days.

[b]Animals dying on or before day 21.

Table X-2. Susceptibility of SASCO C57BL/6 Mice to PTV Inoculation

Virus Dilution	Surv/ Total[a]	Mean Survival Time[b]
8-10 g male mice		
$10^{-1.0}$	8/10	2.5
$10^{-2.0}$	5/10	2.4
$10^{-3.0}$	3/10	3.4
$10^{-4.0}$	4/10	3.8
$10^{-5.0}$	3/10	3.7
$10^{-6.0}$	2/10	3.9
$10^{-7.0}$	7/10	8.7
$10^{-8.0}$	10/10	>21.0
$10^{-9.0}$	10/10	>21.0
	LD50 = $10^{-6.4}$	
8-10 g female mice		
$10^{-1.0}$	7/10	2.0
$10^{-2.0}$	4/10	2.5
$10^{-3.0}$	2/10	3.1
$10^{-4.0}$	1/10	3.7
$10^{-5.0}$	4/10	3.7
$10^{-6.0}$	5/10	3.6
$10^{-7.0}$	7/10	4.0
$10^{-8.0}$	9/10	5.0
$10^{-9.0}$	10/10	>21.0
	LD50 = $10^{-5.7}$	
12.5-14.5 g female mice		
$10^{-1.0}$	4/6	4.5
$10^{-2.0}$	5/6	3.0
$10^{-3.0}$	1/6	4.4
$10^{-4.0}$	3/6	4.3
$10^{-5.0}$	3/6	6.0
$10^{-6.0}$	6/6	>21.0
	LD50 = $10^{-3.8}$	
8-10 g female mice		
$10^{-1.0}$	5/6	4.0
$10^{-2.0}$	3/6	3.3
$10^{-3.0}$	1/6	4.6
$10^{-4.0}$	4/6	5.0
$10^{-5.0}$	3/6	6.0
$10^{-6.0}$	5/6	5.0
	LD50 = $10^{-3.7}$	

[a]21 days.

[b]Animals dying on or before day 21.

Table X-3. Susceptibility of SASCO C57BL/6 Mice to PTV Inoculation (confirming expt.)

Virus Dilution	Surv/ Total[a]	Mean Survival Time[b]
8-10 g male mice		
$10^{-1.0}$	7/10	3.0
$10^{-2.0}$	6/10	4.8
$10^{-3.0}$	3/10	4.0
$10^{-4.0}$	1/10	3.4
$10^{-5.0}$	5/10	5.2
$10^{-6.0}$	3/10	4.7
	LD50 = $\sim 10^{-5.0}$	
8-10 g female mice		
$10^{-1.0}$	6/10	4.0
$10^{-2.0}$	8/10	4.5
$10^{-3.0}$	5/10	3.8
$10^{-4.0}$	6/10	4.5
$10^{-5.0}$	3/10	4.9
$10^{-6.0}$	6/10	4.8
	LD50 = $\sim 10^{-5.8}$	

Table X-4. Susceptibility of Simonsen C57BL/6 Mice to PTV Inoculation

Virus Dilution	Surv/ Total[a]	Mean Survival Time[b]
8-10 g mice		
$10^{-1.0}$	10/10	>21.0
$10^{-2.0}$	5/10	5.0
$10^{-3.0}$	0/10	5.0
$10^{-4.0}$	0/10	4.2
$10^{-5.0}$	0/10	4.9
$10^{-6.0}$	5/10	4.8
	LD50 = $10^{-6.0}$	

XI. INFLUENCE OF SHIPPING METHOD ON SENSITIVITY OF C57BL/6 MICE TO PUNTA TORO VIRUS

Introduction

We have continued to have problems in achieving a satisfactorily lethal infection in C57BL/6 mice supplied by SASCO Laboratories (see Section X). As was noted in the previous section, the SASCO animals were shipped to us via truck instead of by air, and the shipping period was approximately 1 day longer. To determine if the shipping method may influence the animals' susceptibility to PTV, two groups of mice of the same age and weight were shipped concomitantly by truck and by air, being trucked only from Salt Lake City to Logan. The latter trucking takes about 2 hr. The mice received via each method were then injected s.c. with PTV and their relative susceptibilities determined.

Materials and Methods

Animals: All 9-11 g female C57BL/6 mice were obtained from SASCO Laboratories (St. Louis, MO). One-half were shipped by air-conditioned truck, a journey requiring about 30 hrs. The remainder were shipped by plane, a journey requiring about 20 hrs total, including a 10 hr layover in Salt Lake City, a 30 minute truck ride to the St. Louis Airport and a 2 hr truck ride from the Salt Lake City Airport to Logan. All were maintained on Wayne Lab Blox and tap water *ad libitum*. They were all quarantined 24 hr before use.

Virus: The Adames strain of PTV as described earlier was used.

Experiment Design: Each group of mice (shipped by truck or shipped by air) were inoculated i.p. with varying 10-fold dilutions of PTV. Five animals were used per dilution. All were held through 21 days and deaths recorded daily.

Results and Discussion

The results of this study are summarized in Table XI-1. Of the mice shipped by truck, only those receiving a 10^{-3} virus dilution all died of the infection. Of those shipped by air, virus dilutions of 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-6} were 100% fatal to the animals.

These data indicate the method of shipping is an important factor affecting the sensitivity of this mouse to PTV, with those shipped by air being much more susceptible to lethal effects of the virus.

It may be speculated that the long period involved in truck shipping, with the continual stops and starts, vibration, excessive noise, and continuous holding in a dark atmosphere may stress the animals sufficiently that they release some immunologic substance such as interferon which may prevent the virus infection.

Conclusions

C57BL/6 mice shipped by air were much more susceptible to lethal effects of i.p.-inoculated Adames strain PTV than were similar mice shipped via truck.

Table XI-1. Comparison of the Infectivity of Punta Toro Virus[a] in SASCO C57BL/6 Mice Shipped by Truck and by Air

Mice shipped by truck

Virus Dilution	Survivors/ Total[b]	Mean Survival Time[c] (days)
10^{-1}	4/5	3.0
10^{-2}	4/5	3.0
10^{-3}	0/5	4.8
10^{-4}	4/5	4.0
10^{-5}	4/5	4.0
10^{-6}	3/5	5.5
10^{-7}	4/5	6.0

Mice shipped by air

Virus Dilution	Survivors/ Total[b]	Mean Survival Time[c] (days)
10^{-1}	3/5	4.0
10^{-2}	0/5	4.4
10^{-3}	0/5	3.8
10^{-4}	0/5	4.2
10^{-5}	2/5	4.6
10^{-6}	0/5	5.4
10^{-7}	5/5	>21.0

[a]Adames strain inoculated subcutaneously.

[b]Mice held for 21 days.

[c]Mice dying on or before day 21.

XII. DETERMINATION OF POTENTIAL HEPATIC TOXICITY OF HUMAN INTERLEUKIN-2 (AVS5079) IN C57BL/6 MICE

Introduction

A reviewer of a recently submitted manuscript describing the anti-PTV effects of AVS5079 questioned whether this material was hepatotoxic to mice at the doses used in the antiviral experiments, this toxicity as manifested by increases in serum glutamic oxalocetate and pyruvate transminases (SGOT, SGPT). An experiment was subsequently run to determine if increases in these transaminase values were seen in the IL-2-treated animals.

Materials and Methods

Compound: Human recombinant IL-2 (AVS5079) was provided by Biological Research Faculty and Facility, Inc. (Rockville, MD). The material was maintained at 4°C until used. It was diluted in sterile water containing 5% dextrose to the concentration desired.

Animals: Three-week-old C57BL/6 mice weighing 10-13 g were obtained from Simonsen. Quarantine, caging and feeding of these mice was as described earlier.

Experiment Design: Groups of eight mice were treated i.p. with 12,500 or 25,000 cetus units of IL-2/mouse/day once daily for 5 days. Four hours after the final treatment, the mice, and 8 normal control animals, were exsanguinated and their serum assayed for SGOT and SGPT using colorimetric kits purchased from Sigma Chemical Co. (St. Louis, MO).

Results and Discussion

The results of this experiment are summarized in Table XII-1. No significant increases in SGOT or SGPT values were seen following treatment with either dose of AVS5079. These data suggest the material is not hepatotoxic at the dosages used.

Summary

AVS5079, administered i.p. to mice qd x 5, did not cause significant increases in SGOT or SGPT when the serum was assayed 4 hr after the final treatment.

Table XII-1. PT329. SGOT and SGPT Values in C57BL/6 Mice Treated i.p. with AVS5079[a].

Treatment	Mean SGOT[b] ± SE	Mean SGPT[b] ± SE
AVS5079, 25,000 units/ mouse/day	173 ± 28.9	34.5 ± 3.9
AVS5079, 12,500 units/ mouse/day	96 ± 11.1	22.6 ± 1.7
Normal Controls	105 ± 28.0	24.8 ± 3.4

[a]i.p., qd x 5.

[b]Expressed in Sigma-Fraenkel units/ml.

XIII. FAILURE OF PICHINDE VIRUS TO CAUSE LETHAL INFECTION IN GENETICALLY IMMUNOSUPPRESSED MICE

Introduction

With the beginning of this new contract, which included the use of Pichinde virus (PCV) in antiviral studies, it was of interest to determine if the virus would induce a lethal infection in genetically immunosuppressed mice. The animals used were: 1) severe combined immunodeficiency (SCID) mice, which are C.B-17 scid/scid mice, a congenic partner strain of BALB/c Anlcr which lack functional T or B cells (1). The animals are hypogamma-globulinemic, poor mitogen responders, and fail to reject allogenic skin grafts. Other hematopoietic cell types (monocytes, granulocytes, erythrocytes, natural killer cells) are present and function normally. 2) NIH-III nude mice, which have the unique genotype: bg/nu/xid. These mice combine beige (bg/bg, reduced NK cell activity), nude (nu/nu, athymic) and x-linked immunodeficiency (xid/xid, reduced T-independent B-cell response and reduced and reduced lymphokine activated killer cell activity) traits (2, 3).

Materials and Methods

Virus: The AN4763 strain of PCV was obtained from Dr. Joseph D. Gangemi, University of South Carolina School of Medicine, Columbia, SC. A virus pool was produced in Vero cells. The pool had a titer of 1.6×10^6 LD50/ml when titrated in MHA hamsters.

Animals: Female NIH-III and SCID mice were used in these studies. The SCID mice were initially provided by Dr. Norman Klinman of Scripps Institute. The NIH-III mice were originally obtained from Charles River Laboratory (Wilmington, DE). Both mouse strains were then used to establish colonies in our laboratory. All were housed in microisolator cages containing sterilized bedding, food and water. Cages were maintained in HEPA-filtered horizontal laminar flow hoods (Lab Products, Maywood, NJ). Cages were changed under a laminar flow hood. All personnel working with these animals wore sterile gloves, gowns, and masks.

Experiment Design: Each strain of mice was injected s.c. with undilute, 10^{-1} or 10^{-2} dilutions of PCV. Five mice were used in each group. The animals were observed daily for death over a 21-day period.

Results and Discussion

No animals showed any signs of disease or died during the observation period of this experiment. We conclude that PCV is not acceptably virulent for these mouse strains, despite their inherent immunosuppressive properties.

Conclusions

The genetically immunodeficient NIH-III and SCID mice were not visibly susceptible to infection by PCV when the virus was inoculated s.c.

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XIV. STUDIES ON THE MECHANISMS OF AVS01 MURINE TOXICITY: IMMUNOLOGIC EFFECTS

Introduction

We have previously described combination studies in which ribavirin (AVS01) was used with various immunomodulators. An interesting and potentially significant observation was the reduction of high-dose ribavirin lethal toxicity using certain of the immunomodulators. In order to understand more fully the mechanism(s) by which this toxicity was reversed, studies have been initiated to determine how the mice die when treated with high doses of ribavirin. This report describes initial studies in which immunologic effects and gross pathology are determined.

Materials and Methods

Animals: Ten-twelve gram male C57BL/6 mice were obtained from Simonsen Laboratories (Gilroy, CA).

Compounds: Ribavirin (AVS01) was provided by USAMRIID.

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.

T Cell Function Assay: T cell function was expressed in a phytohemagglutinin (PHA)-induced blastogenesis assay. This was performed by pipetting 1×10^5 spleen cells into triplicate wells of flat-bottom 96-well microplates in a volume of 0.1 ml. PHA of various concentration was added to each well in 0.1 ml aliquots and was used to monitor T cell functions. During the last 24 hr of a 48 hr incubation at 37°C, the cells were pulsed with 0.4 µCi of [^{3}H]thymidine. The cells were then harvested on glass fiber filter paper disks using a Skatron cell harvester (Flow Labs, Irvine, CA) and the uptake of radioactivity determined using a Packard Matrix 96 Direct Beta Counter. The proliferative responses were expressed as counts per minute (CPM) of [^{3}H]thymidine incorporation into splenocytes.

B Cell Function Assay: B cell function was expressed in a lipopolysaccharide (LPS)-induced blastogenesis assay. This was performed as above, with LPS substituted for PHA.

Experiment Design: Mice were treated i.p. with 2000, 1500, or 1000 mg/kg/day of ribavirin twice daily for 3 days. On days 1, 2, and 3, 3 animals in each group were killed, gross pathology determined and hematocrits determined on their blood. On day 4, the spleens were assayed for NK activity, T and B cell function and T, T helper, T suppressor, and B cell enumeration. Mice in each group were also weighted daily through 6 days.

Results and Discussion

The mice receiving all 3 ribavirin doses lost weight beginning immediately after initiation of treatment (Figure XIV-1). The animals receiving 2000 and 1500 mg/kg/day all died by day 6. Hematocrit values decreased initially on day 1, then increased during the last 2 days of treatment (Table XIV-1), a rather surprising observation, since prolonged ribavirin treatment causes anemia (1). It is possible that if therapy had been continued, the hematocrit values would have fallen, however.

The increased hematocrit may have been due to the animals' attempt to make up with immature red blood cells the depleted cells seen after the first day of treatment. No attempt was made in this experiment to determine the maturity of the red blood cells.

The immunologic data are summarized in Figure XIV-2-4. Both T and B cell function were significantly depressed 24 hr after termination of ribavirin therapy at all dosage levels (Figure XIV-

2). NK cell activity, however, at this same time period appeared to be enhanced (Figure XIV-3). The percentage of T, T helper and T suppressor cells were much higher in the ribavirin-treated groups; the total B cells were significantly depressed, however.

The cell enumeration data indicate high dosages of ribavirin have a marked effect on depleting B cells in the mouse. This cell depletion would result in a considerable imbalance of T cells, reflected in the apparent increase in the treated mice. This does not mean the drug enhanced T cell numbers, but the percentage would have to increase if the percentage of B cells decrease.

This decreased number of B cells is also expressed in the he significantly decreased B cell function, which may be only a reflection of less numbers of B cells available to be assayed. The depressed T cell function, however, in light of the significantly increased number of T cells, strongly indicates ribavirin to have a markedly suppressive effect on T cell function.

The increased NK activity seen in the ribavirin-treated groups may be a reflection of increased NK cells in the spleen in response to the decreased B cells.

Gross pathologic examination revealed the mice had marked bleeding into the intestinal tract, as especially indicated by a black appearance particularly of that upper intestinal area. We have, in a previous report, found that the arterial oxygen saturation falls precipitously at the time the intestinal bleeding appears, which would coincide with less red blood cells available to carry oxygen in the blood.

Summary

C57BL/6 mice treated i.p. twice daily for 3 days with 2000 or 1500 mg/kg/day of ribavirin exhibited weight loss and death within 2-3 days after treatment termination. The major gross pathologic finding was excessive intestinal hemorrhage. Hematocrit declined initially, but increased by day 3, perhaps due to a release of immature red blood cells. The ribavirin therapy caused significant T and B cell function and a depletion of splenic B cells. NK cell activity appeared to increase, but this may have been a reflection of more NK cells in the spleen in place of the decreased B cells.

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Table XIV-1. Mean Hematocrit Values of C57BL/6 Mice Treated i.p. with Ribavirin[a]

Dose (mg/kg/day)	Day of Assay		
	1[b]	2[c]	3[d]
2000	30.7	40.0	all died
1500	30.6	41.3	46.0
1000	37.0	37.0	39.3
0	36.5	—	—

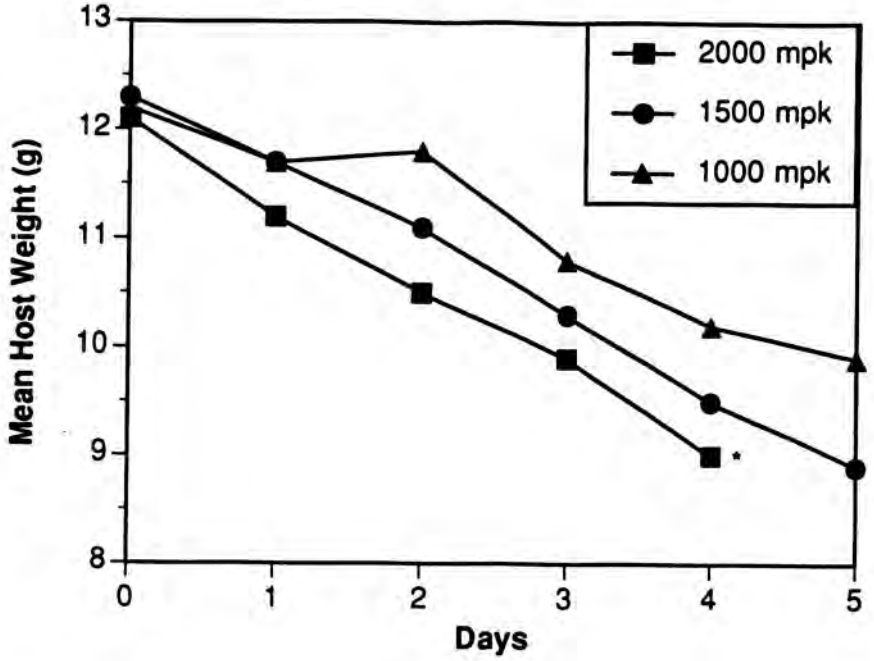
[a]bid x 3.

[b]Treated 2 times.

[c]Treated 4 times.

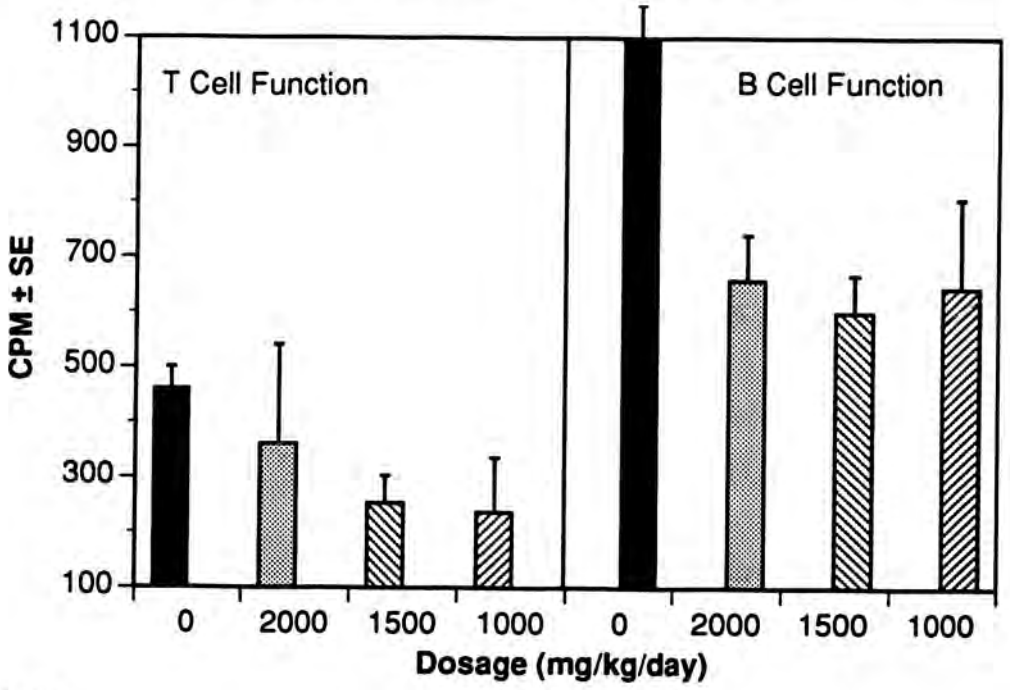
[d]Treated 6 times.

Figure XIV-1. Host Weight Change in C57BL/6 Mice Treated i.p. with Ribavirin[a].



[a]bid x 3

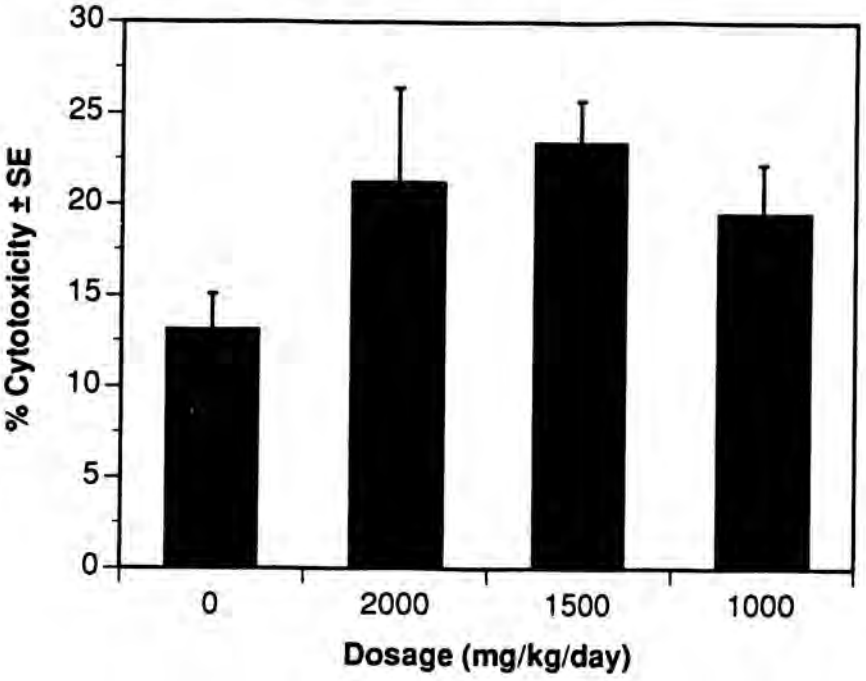
Figure XIV-2. Effect of High Dose i.p. Ribavirin Therapy[a] on T and B Cell Function in C57BL/6 Mice[b].



[a]bid x 3

[b]Determined on day 4.

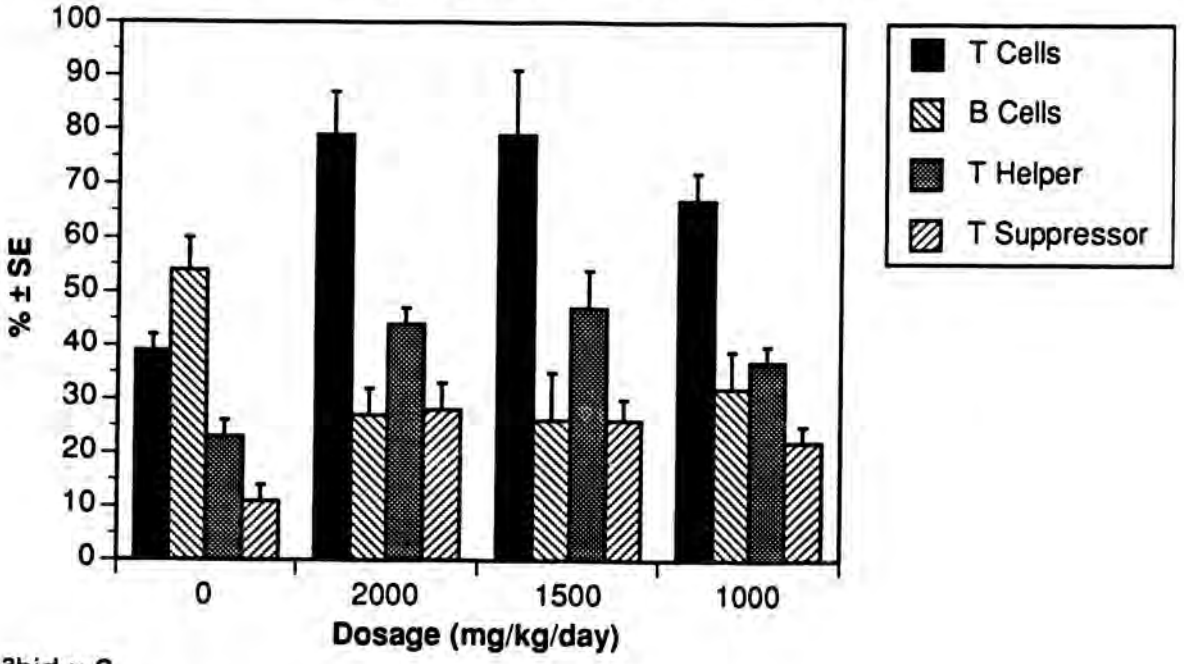
Figure XIV-3. Effect of i.p. Ribavirin Treatment[a] on NK Cell Activity[b] in C57BL/6 Mice.



[a]bid x 3

[b]Determined on day 4.

Figure XIV-4. Effect of i.p. Ribavirin Treatment[a] on Splenic Cell Enumeration[b] in C57BL/6 Mice.



[a]bid x 3

[b]Determined on day 4.

XV. EFFECTS OF BCH-523, 524, 525, 526, AND 527 ON PUNTA TORO VIRUS-INFECTED MICE

Introduction

A series of 5 lipophilic desmuryl MDP analogues were submitted to us from Dr. Christopher L. Penney of IAF BioChem International, Inc. for testing against in vivo Punta Toro virus (PTV) infections experimentally induced in mice. This was done in response to a request from Dr. Meir Kende, Head of the Immunomodulator Program at the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID), Fort Detrick (Frederick, MD. In a telephone conversation with Dr. Penney, a treatment regimen was planned, and, in addition, experiments were decided upon to confirm the immunologic activity of each MDP analogue.

The results of this study are the subject of this report.

Materials and Methods

Virus: The Adames strain of PTV was used. The virus was as we have described (1). A twice plaque-isolated virus prepared in LLC-MK_2 cells was used, after being titrated in the appropriate mice.

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: The 5 MDP analogues submitted from Dr. Penney were: BCH-523, BCH-524, BCH-525, BCH-526, and BCH-527. Information provided with the compounds suggested each were poorly soluble in water, so 0.4% carboxymethylcellulose (CMC) was used as vehicle. All were prepared at the same time and held at 4°C until used. Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) was included in each experiment as a known positive control. The PTV-inhibitory activity of this compound has been previously reported (1).

Natural Killer (NK) Cell Activity: Splenic cells were tested for their ability to lyse YAC-1 tumor cells in a conventional 4 hr chromium release assay as an indicator of NK cell function (2). Ratios of 50 and 25 splenic cells to 1 tumor cell were used. Cytotoxicity was expressed as: % chromium release = (experimental counts per minute (cpm) - background cpm)/(maximum cpm - background cpm).

Splenic Cell Enumeration Assay: Dispersed splenocytes were reacted with fluorescein isothiocyanate-labeled murine monoclonal antibody anti-Ly5 for B cell enumeration and phycorythrin-labeled monoclonal antibody anti-Thy 1.2 for T cell counts. The labeled cells were then enumerated with a fluorescence-activated cell sorter (FACS) (EPICS-C, Coulter Corp., Hialeah, FL).

Macrophage Function Determinations: Macrophage function was assessed by an interleukin-1 (IL-1) assay that utilizes responsiveness of mouse thymocytes to phytohemagglutin (PHA) which is dependent on IL-1 for its reactivity. Splenocytes from treated animals were incubated for 24 hr at 37°C and 5% CO_2 in the presence of 20 µg/ml lipopolysaccharide. The cells were removed by low speed centrifugation and the supernate frozen at -20°C until later assay for IL-1. Murine thymocytes in a concentration of 10^7 cells/ml were suspended in RPMI 1640 medium containing 2% PHA, 5% fetal bovine serum, and 0.05 mM 2-mercaptoethanol/penicillin-streptomycin. A total of 100 µl of this suspension was added to each well of a 96-well flat-bottomed microplate containing serial dilutions of supernate to be assayed for IL-1. The cells were incubated 72 hr at 37°C; the last 4 hr of the incubation the cells were pulsed with [^{3}H]thymidine (1 µCi/well). The cells were then harvested and [^{3}H]thymidine incorporation determined using a direct counter (Packard, Downers Grove, IL).

Experiment Design: A total of 15 mice infected subcutaneously (s.c.) with PTV were treated intraperitoneally (i.p.) with each dose of compound, the dosages being 1.6, 5, 16, and 50 mg/kg/day. Treatments began 18 hr pre-virus inoculation and continued every other day for a total of 4 treatments. Ribavirin at a dosage of 75 mg/kg/day was administered i.p. twice daily for 3 days beginning 4 hr post-virus inoculation. A total of 30 infected mice were treated with CMC as virus controls. Five infected, drug-treated mice were sacrificed on infection day 4. Their livers

were removed and assigned a icterus score of 0 (normal) to 4 (maximal discoloration). The livers and serum were frozen at -70°C until assayed for infectious virus titers. This was done by assay of 10-fold dilutions of liver homogenates or serum in triplicate 96 well microplate cups containing LLC-MK_2 cell monolayers. Viral cytopathic effect determined after 5 days incubation at 37°C was used as endpoint. The serum was also assayed for glutamic oxaloacetic and pyruvic acid transaminases (SGOT, SGPT) determined by colorimetric kits from Sigma Chemical Co. (St. Louis, MO). Spectrophotometric readings of the colormetric assays were performed in duplicate using a microplate autoreader (EL309, Bio-Tek Instruments, Inc., Winooski, VT). Infected animals not killed on day 4 were observed for 21 days, with deaths recorded daily.

Toxicity and normal controls were weighed immediately prior to treatment and again 18 hr after final treatment to determine weight loss or failure to gain weight.

Five toxicity control animals treated with the highest (50 mg/kg/day) dosage of each BCH compound were killed 24 hr after the final treatment and their spleens removed. Each spleen was suspended in RPMI-1640 medium and homogenized using a stomacher (Tekmar, Cincinnati, OH). Red blood cells were removed by hemolytic lysis. Remaining splenocytes were washed three times in RPMI-1640 and resuspended in medium containing 20% fetal calf serum and counted using a Coulter counter (Hialeah, FL) before use in NK and macrophage function assays and T and B cell enumeration studies.

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. The immunological data were expressed as means ± computer-derived standard deviations.

Results and Discussion

Tables XV-1–5 summarize the effects of these compounds on the PTV infection. Only BCH-523 exerted any effect which may be construed as inhibitory to the infection; this effect was evidenced as moderate decreases in SGOT, SGPT, and liver virus in mice receiving the highest dosage. It should be noted that this dosage was well tolerated in the toxicity control mice, suggesting a higher dosage may exert a more positive effect.

Ribavirin exerted the positive activity expected. We have previously described the PTV-inhibitory effects of this drug (1).

All the BCH compounds appeared well tolerated by the concomitantly run toxicity control animals. In view of the lack of aqueous solubility of these materials, one would wonder if the suspension injected was being adsorbed by the mouse. In our experience with other, similarly insoluble materials, however, an adsorption does occur and positive effects can be seen (3). In addition, as will be discussed subsequently, a significant immunological effect was observed, which indicates biologically active levels were being achieved in the animals.

Table XV-6 summarizes the effect of these compounds on macrophage function as expressed by IL-1 activity in splenocytes from the treated mice. A considerable variation occurred in most groups, which may have been lessened by using a larger number of animals. Thus, while no statistical significance was seen, compounds BCH-523, 524, and 527 appeared to be stimulatory, whereas BCH-525 and 526 were inhibitory.

The NK cell activity of splenocytes taken from mice treated with the BCH compounds is summarized in Table XV-7. Only BCH-527 appeared to significantly stimulate this activity; a similar effect was seen at both effector:target cell ratios. BCH-524 and 525 were marginally suppressive to the NK cell activity in this study. The stimulation seen with BCH-527 compares well with the stimulation we have seen with two other immunomodulators, 7-thia-8-oxoguanosine (4) and Aviron (ImuVert) (5).

The splenic T and B cell enumeration data obtained using these BCH compounds are shown in Table XV-8. BCH-526 and 527 appeared to increase % B cells while suppressing T cells. BCH-524 appeared suppressive both to T and B cells.

It should be pointed out that the 24 hr post-treated time for determination of the immunologic parameters is quite arbitrary; it is very possible that we are seeing the end of a

response, with maximal differences occurring earlier. In addition, the every other day treatment regimen was selected rather arbitrarily, with the supposition that daily treatments may exhaust the immune system and possibly cause a hyporesponsive state in the animal. It was presumed that 48 hr would allow normalcy to return to the immune system, although we have found some compounds to require a 12–24 hr longer period before the host's immune system returned to a normal state. The dosage selected for evaluation was also arbitrary; it was assumed the highest dose would have the greatest effect. In view of the marked lack of signs of toxicity using these compounds, higher dosages may render a greater effect, although with immunopotentiating agents, biphasic immune stimulation is common.

The PTV infection is highly sensitive to interferon (3, 6), but other immunological alterations, with the possible exception of IL-2 stimulation (7), have not significantly affected the outcome of the disease. We understand the BCH compounds are not interferon inducers, so it is not surprising that a lack of PTV-inhibitory effect was seen.

Summary

The lipophilic desmuryl MDP analogs BCH-523, 524, 525, 526, and 527 were evaluated for efficacy against the hepatotropic PTV infection in C57BL/6 mice. Treatments were i.p. every other day for a total of 4 injections beginning 18 hr pre-virus inoculation. Only BCH-523 exerted an inhibitory effect; this was seen as decreased SGOT, SGPT, and liver virus titers in mice receiving the maximal dose. All the BCH compounds were well tolerated in the mice. Immunologic assays of splenocytes taken 24 hr after final treatment with the 50 mg/kg/day dose of each compound indicated the following: *Macrophage function:* Stimulation with BCH-523, 524, and 527; moderate suppression with BCH-525 and 526. *NK cell activity:* Stimulation with BCH-527, marginal suppression with BCH-524 and 525. *T and B cell enumeration:* B cell increase by BCH-526 and 527 with concomitant T cell suppression. T and B cell suppression by BCH 524. The time of assay as well as dosage of each compound used may markedly influence the outcome of these immunologic tests.

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Table XV-1. Expt. PtA948. Effect of I.p. Treatment with BCH-523 on Punta Toro Virus Infections in Mice.

Animals: 8.0-10.5 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC (Ribavirin: Sterile physiological saline)

Treatment Schedule: eod x 3, beginning 18 hr pre-virus inoculation. (Ribavirin: bid x 3 beginning 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})
BCH-523	50	5/5	1.1	0/10	3.8	3.6	0/5(10,431*)	0/5(6110*)	6.2	6.2
	16	5/5	1.9	0/10	3.3	4.0	0/5(15,850)	0/5(8900)	7.2	6.5
	5	5/5	2.0	0/10	3.2	4.0	0/5(15,850)	0/5(8900)	7.2	6.5
	1.6	5/5	3.1	0/10	4.0	4.0	0/5(15,850)	0/5(8900)	7.2	6.5
Ribavirin	75	5/5	1.3	9/10**	8.0	0.2**	4/5**(186**)	2/5*(88**)	4.2**	4.7**
CMC	-	-	-	1/20	4.0	3.8	0/10(15,850)	0/10(8900)	7.2	6.5
Normals	-	5/5	1.8	-	-	0.5	3/5(300)	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: BCH-523 was active only in significantly decreasing the SGOT and SGPT values at the highest dosage used. It is noted some decrease (1 log_{10}) in liver virus titer was also seen at this dosage. The compound was well tolerated in the mice, suggesting higher dosages may be more useful.

Table XV-2. Expt. PtA949. Effect of i.p. Treatment with BCH-524 on Punta Toro Virus Infections in Mice.

Animals: 8.0-10.5 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC (Ribavirin: Sterile physiological saline)

Treatment Schedule: eod x 3, beginning 18 hr pre-virus inoculation. (Ribavirin: bid x 3 beginning 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})
BCH-524	50	5/5	1.8	0/10	3.9	4.0	0/5(15,850)	0/5(8900)	7.2	6.5
	16	5/5	2.6	0/10	4.0	4.0	0/5(15,850)	0/5(8900)	7.2	6.5
	5	5/5	3.1	0/10	4.0	4.0	0/5(15,850)	0/5(8900)	7.2	6.5
	1.6	5/5	2.3	0/10	4.0	4.0	0/5(15,850)	0/5(8900)	7.2	6.5
Ribavirin	75	5/5	1.3	9/10**	8.0	0.2**	4/5**(186**)	2/5*(88**)	4.2**	4.7**
CMC	-	-	-	1/20	4.0	3.8	0/10(15,850)	0/10(8900)	7.2	6.5
Normals	-	5/5	1.8	-	-	0.5	3/5(300)	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: BCH-524 was not considered active vs PTV infections in this study. The material was well tolerated at all dosages used.

Table XV-3. Expt. PtA950. Effect of i.p. Treatment with BCH-525 on Punta Toro Virus Infections in Mice.

Animals: 8.0-10.5 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC (Ribavirin: Sterile physiological saline)

Treatment Schedule: eod x 3, beginning 18 hr pre-virus inoculation. (Ribavirin: bid x 3 beginning 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})
BCH-525	50	5/5	2.7	1/10	4.2	3.7	0/5(13,890)	0/5(8010)	6.9	6.3
	16	5/5	2.1	0/10	3.9	4.0	0/5(15,850)	0/5(8900)	7.2	6.5
	5	5/5	1.7	0/10	4.5	4.0	0/5(15,850)	0/5(8900)	7.2	6.5
	1.6	5/5	2.7	0/10	4.2	4.0	0/5(15,850)	0/5(8900)	7.2	6.5
Ribavirin	75	5/5	1.3	9/10**	8.0	0.2**	4/5**(186**)	2/5*(88**)	4.2**	4.7**
CMC	-	-	-	1/20	4.0	3.8	0/10(15,850)	0/10(8900)	7.2	6.5
Normals	-	5/5	1.8	-	-	0.5	3/5(300)	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: BCH-525 was not considered active vs PTV infections in this study. The material was well tolerated at all dosages used.

Table XV-4. Expt. PtA951. Effect of I.p. Treatment with BCH-526 on Punta Toro Virus Infections in Mice.

Animals: 8.0-10.5 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC (Ribavirin: Sterile physiological saline)

Treatment Schedule: eod x 3, beginning 18 hr pre-virus inoculation. (Ribavirin: bid x 3 beginning 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})
BCH-526	50	5/5	2.4	2/10	6.4	3.2	0/5(3906)	0/5(3060)	5.5	6.0
	16	5/5	2.4	1/10	6.0	3.5	0/5(4052)	0/5(2769)	6.7	6.1
	5	5/5	2.4	0/10	5.3	4.0	0/5(7350)	0/5(5350)	7.2	6.5
	1.6	5/5	2.8	0/10	6.0	3.1	1/5(4823)	1/5(3464)	5.3	5.1
Ribavirin	75	5/5	1.6	8/10**	8.0*	0.4**	5/5**(97**)	5/5**(31**)	4.1*	4.8
CMC	-	-	-	5/20	5.7	3.5	0/9(5190)	0/9(3456)	6.3	6.3
Normals	-	5/5	1.7	-	-	0.1	3/5(182)	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.

*P<0.05 **P<0.01

Conclusions: BCH-526 was not considered active vs PTV infections in this study. The material was well tolerated at all dosages used.

Table XV-5. Expt. PtA952. Effect of i.p. Treatment with BCH-527 on Punta Toro Virus Infections in Mice.

Animals: 8.0-10.5 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC (Ribavirin: Sterile physiological saline)

Treatment Schedule: eod x 3, beginning 18 hr pre-virus inoculation. (Ribavirin: bid x 3 beginning 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})
BCH-527	50	5/5	2.8	0/10	6.3	3.9	0.5(4748)	0.5(3040)	5.8	6.3
	16	5/5	3.1	0/10	6.2	4.0	0/5(7300)	0/5(4550)	7.5	6.5
	5	5/5	3.3	0/10	5.7	3.2	1/5(5855)	1/5(3645)	6.5	5.2
	1.6	5/5	2.8	0/10	6.4	2.6*	0/5(4125)	0/5(2649)	5.9	6.2
Ribavirin	75	5/5	1.6	8/10**	8.0*	0.4**	5/5**(97**)	5/5**(31**)	4.1*	4.8
CMC	-	-	-	5/20	5.7	3.5	0/9(5190)	0/9(3456)	6.3	6.3
Normals	-	5/5	1.7	-	-	0.1	3/5(182)	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.

*P<0.05 **P<0.01

Conclusions: BCH-527 was not considered active vs PTV infections in this study. The material was well tolerated at all dosages used.

Table XV-6. Expts. PtA 948-952. Effect of BCH Compounds[a] on Macrophage Function[b] in C57BL/6 Mice.

Compound	Dosage (mg/kg/day)	Mean CPM of Treated Thymocytes±SD[c]
BCH-523	50	5236±820
BCH-524	50	5491±1269
BCH-525	50	3392±201
BCH-526	50	3413±206
BCH-527	50	5660±1011
Normal Controls	0	4791±426

[a]All compounds administered i.p. every other day for 4 injections; splenocytes taken 24 hr after final treatment for assay.

[b]Macrophage funtion expressed as IL-1 activity in splenocytes, measured by [^{3}H]thymidine uptake in IL-1-dependent PHA-stimulated thymocytes.

[c]Standard deviation (n=5).

Conclusions: BCH-523, 524, and 527 all appeared to stimulate macrophage function in this test. BCH-525 and 526 appeared to be slightly suppressive in the same assay. However, the wide standard deviations, presumably due to the relatively small sample size, prevented these effects from being statistically signficant.

Table XV-7. Expts. PtA 948-952. Effect of BCH Compounds[a] on Natural Killer Cell Activity[b] in C57BL/6 Mice.

Compound	Dosage (mg/kg/day)	% Chromium Release±SD Effector:Target Ratio 50:1	Effector:Target Ratio 25:1
BCH-523	50	15.2±2.3	10.6±1.5
BCH-524	50	14.3±1.9	9.8±3.6
BCH-525	50	13.9±1.2	7.2±2.3
BCH-526	50	17.2±3.3	12.8±2.1
BCH-527	50	24.0±1.7	18.9±2.0
Normal Controls	0	19.5±3.9	11.6±1.8

[a]All compounds administered i.p. every other day for 4 injections; splenocytes taken 24 hr after final treatment for assay.

[b]NK cell activity expressed as % chromium release in YAC-1 tumor cells lysed by splenocytes (2).

[c]Standard deviation (n=5).

Conclusions: BCH-527 significantly stimulated NK cell activity at both effector:target cell ratios. BCH-524 and BCH-525 appeared to be marginally suppressive.

Table XV-8. Expts. PtA 948-952. Effect of BCH Compounds[a] on Total T and B Cells in Splenocytes[b] from C57BL/6 Mice.

Compound	Dosage (mg/kg/day)	% Cells/Spleen±SD	
		T Cells	B Cells
BCH-523	50	49±7.7	39±9.3
BCH-524	50	35±9.6	33±10.2
BCH-525	50	53±9.5	34±10.6
BCH-526	50	40±2.4	43±1.4
BCH-527	50	39±2.3	43±1.5
Normal Controls	0	49±3.9	37±2.7

[a]All compounds administered i.p. every other day for 4 injections; splenocytes taken 24 hr after final treatment for assay.

[b]Cell enumeration performed by FACS analysis using monoclonal antibodies anti-Thy 1.2 for T cells, anti-Ly5 for B cells.

[c]Standard deviation (n=5).

Conclusions: BCH-526 and BCH-527 appeared to increase % B cells while suppressing T cells in this study. BCH-524 appeared suppressive to both T and B cells.

XVI. TREATMENT OF LETHAL PICHINDE VIRUS INFECTIONS IN WEANLING LVG/LAK HAMSTERS WITH RIBAVIRIN, RIBAMIDINE, SELENAZOFURIN, AND AMPLIGEN

Introduction

Arenaviruses are a group of rodent-transmitted infectious agents that cause serious life-threatening hemorrhagic fevers in man (1). Some of the more dangerous viruses in the group include Junin, Machupo, and Lassa fever, which are endemic to South America or Africa (2). Pichinde virus is an arenavirus that is much less pathogenic to humans, and thus has been used in infection studies in guinea pigs (3) and hamsters (4, 5). Formerly, only the MHA strain of hamster was thought to develop lethal Pichinde virus infections in adult animals (6–8). These animals, when inoculated subcutaneously with virus, die in 10-13 days, whereas random-bred adult LVG/Lak hamsters survive the infection if similarly infected. We have determined that intraperitoneal Pichinde virus challenges are lethal to random-bred 3 week-old LVG/Lak hamsters using the An 4763 strain of virus, and that this animal species is suitable for conducting antiviral chemotherapy experiments. LVG/Lak hamsters are much more readily available than MHA hamsters, have a milder temperament, and are considerably less expensive.

Because of the serious and often life-threatening nature of arenavirus infections, development of new treatments for these diseases is warranted. Ribavirin, first reported to be active against Pichinde and Lassa fever arenaviruses in animals (9, 4), was later shown to be effective against Lassa fever virus in humans (10). In our laboratory we first evaluated ribavirin in the LVG/Lak hamster model of Pichinde virus infection to establish the drug as a positive control for future studies. Three previously untested compounds were also evaluated in the same model. These included ribamidine, a ribavirin derivative with activity similar to that of ribavirin against other viruses (11, 12); selenazofurin, a nucleoside analog with demonstrated anti-arenavirus activity *in vitro* (13); and ampligen, an interferon-inducing mismatched double-stranded RNA molecule that has virus-inhibitory properties (14, 15). In the present studies, we found ribavirin and ribamidine to be active against Pichinde virus in hamsters, whereas the other two compounds appeared ineffective against this infection.

Materials and Methods

Compounds: Ribavirin, ribamidine, selenazofurin, and ampligen were provided in dry powder form by the U.S. Army Medical Research Institute for Infectious Disease (USAMRIID) via Technassociates, Inc. (Rockville, MD). They were dissolved in sterile saline for injection into hamsters. Ampligen required heating at 67°C for 16 h then at 37°C for 1 h in order to anneal the strands of the polymer prior to animal treatments.

Virus and Cells: Pichinde virus (PCV) strain An 4763 was provided by Joseph D. Gangemi, University of South Carolina School of Medicine, Columbia, SC. Virus stocks were prepared in Vero 76 cells (obtained from the American Type Culture Collection, Rockville, MD) from twice plaque-purified PCV, then were stored frozen at -80°C. The cells were grown in Eagle's medium containing 10% fetal bovine serum (FBS), 0.1% sodium bicarbonate and 50 µg gentamicin/ml in 5% $C0_2$ at 37°C.

Animals and Virus Infection Model: Three week-old specific pathogen-free female random-bred Golden Syrian (LVG/Lak strain) hamsters, weighing approximately 50 grams each, were obtained from SASCO, Inc. (a division of Charles River Labs), Omaha, NE. PCV, in a volume of 0.2 ml per injection, was inoculated into the animals intraperitoneally (i.p.) both on the right and left sides of the abdomen to insure that an i.p. injection was achieved, since subcutaneous (s.c.) inoculations of the virus are not lethal to weanling animals (6). Other hamsters were not infected and served as drug toxicity controls. The animals were quarantined 24-48 h prior to use, housed 5 to a cage, and fed hamster chow and tap water ad libitum.

In Vivo Chemotherapy Studies: Except where indicated, PCV was inoculated i.p. into hamsters at a dose of 1000 plaque forming units (PFU) per animal. Starting 24 h after virus challenge, the nucleoside analogs (ribavirin, ribamidine, and selenazofurin) were administered i.p. twice daily for 10 days. Ampligen was administered i.p. every other day for 5 injections in order to avoid the hyporesponsive phenomenon that accompanies treatment with interferon inducers (16). The animals were weighed daily to insure constant mg/kg dosages. Doses of each compound (see tables and text) were selected based upon our experience with each

substance in mice infected with Punta Toro virus (12, 15, 17, 18) or as was reported by others using ribavirin against PCV in MHA hamsters (4). Death was monitored daily for 21 days using 10 animals in each drug-treated group and 20 hamsters in the placebo control. An additional 5 hamsters/group were held for tissue virus titer and serum alanine aminotransferase determinations. Five uninfected animals/group, maintained in an area remote from the infected hamsters, were used to monitor drug toxicity. Their numbers were recorded daily, and weights were noted before the first and 24 h after the last treatment.

For virus titer determinations, serum was obtained and tissues removed and stored frozen at -80°C until assayed. Ten percent homogenates of tissues were made using a Stomacher™ (Techmar Co., Cincinnati, OH) in cell culture medium. Tissues and serum from infected hamsters were each titrated separately. Samples were titrated at 10^{-1} to 10^{-8} dilutions in Vero 76 cells in 96-well microplates by end point dilution method (19), and the virus titers expressed as $\log_{10}$ cell culture infectious units ($CCID_{50}$) per gram of tissue or serum. Because PCV does not readily exhibit a discernible cytopathic effect, an immunofluorescence assay was used to detect the presence or absence of virus in each well. Briefly, cells inoculated with dilutions of virus-containing tissue homogenates were incubated in medium with 2% FBS for 6 days. Plates were inverted and blotted to remove the medium, then were dried 1 week or longer. A fluorescein-labeled monoclonal antibody against PCV described previously (20) was used to stain the infected monolayers for 2 h at 37°C. Plates were inverted and blotted to remove the immunoconjugate. When wells were dry, they were checked for virus using an inverted fluorescence microscope.

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) determinations were made using colorimetric kits (Sigma Chemical Co., St. Louis, MO) following the manufacturer's instructions. Animals were bled by cardiac puncture to obtain serum samples.

Survivor increases in infected and uninfected groups were evaluated using chi-square analysis with Yates' correction. The Mann-Whitney U test was used to analyze increases in mean survival times of animals that died before day 21 and reductions in tissue and serum virus titers. Since virus titers in placebo control groups exceeded the dilution endpoint, we assumed an arbitrary standard deviation of 2.0 for statistical analyses. This we considered to be reasonable, since most other standard deviations on the tables were less than this. Significant decreases in ALT levels were determined using the Student's *t* test. In all cases, values of statistical significance were made comparing drug-treated groups to respective placebo controls. The thresholds of statistical significance were $P<0.05$ and $P<0.01$, using two-tailed analyses.

Results

Infection parameters in PCV-infected LVG/Lak hamsters: As part of developing the LVG/Lak hamster model of PCV infection, various disease parameters were determined daily though 8 days of infection in animals inoculated with 1000 PFU of virus (Figure 1). Virus in kidney, liver, lung, spleen, brain, heart, serum, and salivary gland tissues rose steadily through the acute infection, and mean virus titers exceeded 10^7 $\log_{10}$ $CCID_{50}$/gram in all tissues analyzed. ALT and AST values in serum increased to high levels by day 8, indicating severe liver damage. In addition, spleens of PCV-infected animals were markedly necrotic relative to uninfected animals. The virus, when inoculated i.p. into these animals was uniformly fatal, causing death between 6 and 9 days post-virus inoculation.

Effect of virus dose on ribavirin activity: Since antiviral activity is dependent upon virus dose, an experiment was conducted to determine an appropriate PCV challenge dose for subsequent studies. This was accomplished by evaluating the efficacy of ribavirin in hamsters inoculated with different PFU of virus (Table XVI-1). Ribavirin-treated (40 mg/kg) animals inoculated with 10^4 PFU survived the infection, but only half of the animals survived in the 20 mg/kg group. With two exceptions, virus titers in tissues and serum were only moderately reduced in the ribavirin-treated groups at this virus challenge dose. Serum and spleen virus titers were markedly decreased in the 40 mg/kg group, with inhibition of spleen virus titers being statistically significant.

As the infecting virus dose decreased to 10^3 and 10^2 PFU/animal, the degree of antiviral activity of ribavirin increased (Table XVI-1). All ribavirin-treated animals survived these virus challenge doses. The amounts of virus recovered from tissues and sera of ribavirin-treated

hamsters were much less than those seen in the placebo controls. Virus titers were suppressed to a greater extent in ribavirin-treated groups infected with 10^2 PFU than in the groups receiving higher virus challenge inocula. In this experiment, it appeared that low spleen and serum virus titers correlated well with a favorable prognosis for recovery from the lethal infection.

Comparative antiviral activities of ribavirin and ribamidine: Following the conclusion of the above study, a virus infecting dose of 10^3 PFU/animal was selected for dose-response evaluations of ribavirin and ribamidine (Table XVI-2). Ribavirin completely protected hamsters from mortality at 32 mg/kg, was weakly active at 10 mg/kg and ineffective at 3.2 and 1 mg/kg. The 10 mg/kg dose prolonged life in those animals that died from the infection. From previous studies (12) we predicted that ribamidine would be active but less potent than ribavirin against PCV in vivo, thus higher doses of this agent were chosen for evaluation. Only the 320 mg/kg dose of ribamidine prevented death in all hamsters, and the 100 and 32 mg/kg doses protected a significant number of animals. In comparing virus titer and ALT results, the doses of ribavirin and ribamidine that were most protective from mortality caused statistically significant reductions in all of the virological and enzymatic parameters. Ribavirin at 32 mg/kg had a more pronounced inhibitory effect on brain virus titers than did ribamidine, suggesting a better entry of the former compound into the central nervous system. As was observed in the results of Table XVI-1, significant reductions in spleen and serum virus titers correlated with increased survival in drug-treated groups. Overall, ribavirin activity at 32 mg/kg was similar to ribamidine activity at 100 and 320 mg/kg, indicating that the two compounds were approximately equally inhibitory to the infection but that ribavirin was at least 3 times more potent.

Toxicity evaluations of ribavirin and ribamidine were performed in uninfected hamsters in parallel with the above experiments. Ten-day treatments with these compounds were not acutely toxic, since no animals died or lost weight. There were moderate degrees of suppression of weight gain at certain doses, however. The placebo controls gained a mean of 21.6 g over 10 days compared to 14.7 g, 11.2 g, and 15.5 g for ribamidine groups treated with 320, 100, and 32 mg/kg/day, respectively. By comparison, the 32 mg ribavirin/kg/day group gained 16.7 g, which is also less than the placebo control. Lower doses of either compound did not suppress weight gain.

Antiviral activities of selenazofurin and ampligen: In experiments performed similar to those described above, selenazofurin and ampligen were administered 24 h after a lethal PCV challenge (1000 PFU/hamster). Doses of selenazofurin, ranging by half-$\log_{10}$ increments from 1 to 100 mg/kg/day, protected no animals from death nor extended mean survival times relative to placebo controls. Similarly, ampligen at 0.5 or 5 mg/kg given every other day for 5 treatments provided no protection to the animals. In this experiment, the drug-treated animals died between 6.3 and 8.4 days. All placebo-treated animals died, with a mean day to death of 7.6 days. Ribavirin (32 mg/kg/day), included as a positive control, protected all of the hamsters from the lethal PCV infection.

In addition to the lack of antiviral activity of selenazofurin, the compound was overtly toxic to uninfected animals at two doses. The 100 mg/kg/day dose killed all hamsters, with a mean day to death of 6.5 days. At 32 mg/kg/day the animals all died, with a mean day to death of 13.3 days. Doses <10 mg/kg were not overtly toxic, nor did they suppress weight gain relative to the placebo control. Ampligen was not lethally toxic nor suppressed weight gain at the two doses tested.

Discussion

These studies demonstrated that the LVG/Lak strain of Golden Syrian hamster could be used as a viable model for evaluating antiviral agents against PCV. In each antiviral experiment we performed, the mortality rate in the placebo group was 100%. This was achieved by i.p. injection of a suitable virus challenge dose, as opposed to s.c inoculation which causes non-fatal infections in these animals (6, and as confirmed by us in unpublished experiments). Formerly, antiviral chemotherapy studies of PCV infections in hamsters utilized the s.c.-infected MHA animal strain (4). It may have been assumed that lethal infections could not be achieved in weanling LVG/Lak hamsters, based upon the published literature (6–8). After reading these reports, it is unclear to us whether these investigators ever attempted i.p. inoculation of weanling (3 week-old) LVG/Lak hamsters using the An 4763 strain of PCV. For example, the studies of

Buchmeier and Rawls (6) describes only s.c. inoculation using the An 3739 virus strain. By this method, animals less than 8 days old or older animals immunosuppressed using cyclophosphamide died from the infection. Infection of animals by i.p. route was not mentioned in the article. Gee et al. (8) inoculated PCV (An 3739 strain) i.p. into two inbred hamster strains (MHA and LSH), and found only the MHA strain to be lethally infected. In the same article they reported non-lethal infection experiments in random-bred LVG/Lak hamsters, but did not mention using the i.p. infection route for this particular animal strain. Jahrling et al. (3) indicated that PCV (An 4763 strain) adapted to kill guinea pigs (a variant of the virus we used) was not lethal to Syrian hamsters. The strain of hamster and route of virus challenge were not described in that report, however.

Whether the strain of virus or particular source of LVG/Lak hamster we used was critical to establishing this new animal model remains to be determined. The An 4763 strain of PCV was the only virus we had in our collection, thus the reason for its use in the present studies. The SASCO brand of LVG/Lak hamster is specific pathogen-free, whereas the same type of hamster obtained from other sources may not be. Whether the use of these animals contributed to the present results will require analyses in hamsters obtained from other venders. One titration of PCV was conducted using 3 week-old animals obtained from Simonsen Labs (Gilroy, CA) , and most of those animals died from the infection (unpublished results), suggesting that the source of the animal may not be critical. Another unanswered question is how old of an animal can be lethally infected with this virus. For our purposes, 3 week-old hamsters were quite suitable for antiviral drug evaluations. What appears to be essential to achieve lethal infections in weanling animals is correctly-delivered i.p. virus inoculations. For this reason we delivered the virus in two injections (one on each side of the abdomen), using the full length of a 1 inch needle.

The mean day to death in i.p.-infected LVG/Lak hamsters is shorter (7-9 days) than for s.c.-infected MHA hamsters (10-13 days). Development of high virus titers in both strains of hamster appears to be similar (4, and this report). The main advantages to using LVG/Lak hamsters over MHA hamsters are reduced cost and greater availability. Although the guinea pig model of PCV infection has also been employed for antiviral studies (3, 22), guinea pigs are very costly and require substantially more drug for treatments than do hamsters.

The effects of ribavirin to inhibit PCV disease in LVG/Lak hamsters was similar to those reported using MHA hamsters (4). Ribavirin appears to be less effective in guinea pigs than in hamsters infected with PCV, however (22). Although personnel affiliated with USAMRIID have evaluated other nucleoside analogs against PCV in animal models, the results have not been published, probably because the compounds have failed to exhibit antiviral activity. Here we report that the ribavirin derivative, ribamidine, exhibited anti-PCV activity *in vivo*. The potency of ribamidine against this virus infection was about one-third that of ribavirin, as was observed in studies against Punta Toro virus (12, 17). Since ribamidine was also well tolerated, the results suggest that the therapeutic indices (maximum tolerated dose divided by minimum effective dose) of both compounds are similar.

These studies illustrate the importance of evaluating compounds in animal models to confirm antiviral activity initially established *in vitro*, as evidenced by the behavior of selenazofurin in both types of assays. Although selenazofurin showed potent anti-PCV activity in cell culture (13), the agent proved to be inactive (and toxic) in infected hamsters. These results would not be predicted, especially knowing that selenazofurin inhibits other RNA viruses in mice (23, 18). It may be that the pharmacology or toxicology of selenazofurin in hamsters is unfavorable relative to mice for providing antiviral protection to the animals.

Regarding the lack of efficacy of ampligen in the hamster model, PCV infections were previously found to not be inhibited by interferon or an inducer of interferon (22), suggesting that a similar-acting agent such as ampligen would also be inactive. Arenaviruses are known to be relatively insensitive to the action of interferons (1). Appar-ently, the immunological events accompanying interferon induction in the host also do not play a major role in combating PCV infections.

Up to the present time, the most potent and useful anti-arenavirus agent known continues to be ribavirin. Ribamidine represents a second compound that may hold clinical promise. A number of other potent anti-arenavirus agents (24) and ribavirin-like agents (25) have been

identified as active in cell culture screens. These await experimentation in animals to establish their potential utility in the treatment of human arenavirus infections.

Summary

A lethal Pichinde (An 4763 strain) virus infection was produced in 3 week-old random-bred Golden Syrian (LVG/Lak strain) hamsters inoculated intraperitoneally with virus, causing mortality in 6-9 days. High virus titers ($\geq 10^{7.5}$ cell culture infectious doses/gram) were present in visceral organs, serum, brain and salivary glands near the time of death. Intraperitoneal treatments with ribavirin (10 and 32 mg/kg) and ribamidine (32, 100, and 320 mg/kg) for 10 days starting 24 h after virus challenge significantly decreased mortality and reduced virus titers by 100- to >10,000-fold in liver, spleen, brain, and serum. Serum alanine aminotransferase (an indicator of liver damage) was also reduced in animals treated with the two compounds (ribavirin at 32 mg/kg; ribamidine at 100 and 320 mg/kg). Intraperitoneal selenazofurin (1-100 mg/kg/day for 10 days) and ampligen (0.5 and 5 mg/kg every other day for 5 injections) treatments provided no protection from the lethal infection nor increased mean survival times. In fact, selenazofurin was overtly toxic causing death of uninfected hamsters at 32 and 100 mg/kg. The random-bred LVG/Lak hamster appears to be a viable and cost-effective model for evaluating new therapies for arenavirus infections.

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Table XVI-1. Effect of virus challenge dose on the PCV disease-inhibitory activity of ribavirin in LVG/Lak hamsters.

Virus Challenge (Log_{10} PFU/animal)	Ribavirin[a] (mg/kg/day)	Survivors/ Total	Mean Day to Death	Virus Titer[b] (Log_{10} $CCID_{50}$/gram) in Brain	Liver	Spleen	Serum
10^2	0	0/20	7.3 ± 0.7[c]	>10.5 ± 0.0	>10.5 ± 0.0	>10.5 ±0.0	>9.5 ±0.0
10^2	20	10/10**	>21	8.6 ± 1.8	6.5 ± 2.3**	5.9 ± 1.0**	5.4 ±1.3**
10^2	40	10/10**	>21	7.3 ± 1.6*	6.4 ± 2.1**	5.4 ± 0.6**	4.1 ±2.7**
10^3	0	0/20	6.9 ± 0.5	≥10.5 ± 0.0	≥10.5 ± 0.0	≥10.5 ±0.0	≥9.5 ±0.0
10^3	20	10/10**	>21	8.3 ± 2.0	7.8 ± 1.8	6.8 ±2.1**	6.0 ±2.1**
10^3	40	10/10**	>21	8.8 ± 1.8	8.6 ± 1.7	6.0 ± 0.8**	5.6 ±1.5**
10^4	0	0/20	6.8 ± 0.4	≥10.5 ± 0.0	≥10.5 ± 0.0	≥10.5 ±0.0	≥9.5 ±0.0
10^4	20	5/10**	10.4 ± 3.5*	9.7 ± 1.4	9.9 ± 1.7	9.2 ± 1.8	9.2 ±0.9
10^4	40	10/10**	>21	9.0 ± 2.0	9.6 ± 1.4	6.9 ± 2.4*	6.9 ±2.4

[a]Treatments were twice daily for 10 days starting 24 h after virus inoculation.

[b]Determined 7 days after virus challenge.

[c]Standard deviation.

* $P<0.05$, ** $P<0.01$.

Table 2. Effects of ribavirin and ribamidine on PCV infections in LVG/Lak hamsters.

Compound	Dose[a] (mg/kg/day)	Survivors/ Total	Mean Day to Death	Virus Titer[b] (Log_{10} CCID50/gram) in Brain	Liver	Spleen	Serum	ALT[c]
Placebo	-	0/20	7.3 ± 1.1[d]	≥10.5 ± 0.0	≥10.5 ± 0.0	≥10.5 ± 0.0	>9.5 ± 0.0	2973 ± 1714
Ribavirin	1	0/10	7.9 ± 0.6	10.1 ± 0.5	10.1 ± 0.9	10.4 ± 0.3	9.4 ± 0.3	5000 ± 608
Ribavirin	3.2	0/10	8.0 ± 1.2	9.5 ± 0.8	6.4 ± 0.7**	9.3 ± 0.5	6.9 ± 1.1*	5150 ± 233
Ribavirin	10	3/10*	9.6 ± 1.5*	8.5 ± 1.4	7.0 ± 1.5*	7.3 ± 1.1*	5.3 ± 1.4**	3400 ± 200
Ribavirin	32	10/10**	>21	5.7 ± 0.2**	5.4 ± 1.0**	4.9 ± 1.0**	4.5 ± 0.5**	264 ± 143*
Ribamidine	10	2/10	7.5 ± 0.5	>10.5 ± 0.0	10.0 ± 0.5	10.4 ± 0.4	9.3 ± 0.6	4700 ± 246
Ribamidine	32	5/10**	11.2 ± 4.5*	9.1 ± 1.3	8.9 ± 1.9	8.3 ± 1.9	6.4 ± 0.7*	1987 ± 207
Ribamidine	100	8/10**	9.0 ± 0.0*	7.9 ± 1.3*	8.2 ± 1.7	6.8 ± 0.9**	5.3 ± 0.4**	80 ± 94**
Ribamidine	320	10/10**	>21	8.6 ± 0.7*	6.6 ± 0.9**	5.4 ± 0.7**	3.4 ± 0.3**	23 ± 6**

[a] Treatments were twice daily for 10 days starting 24 h after virus inoculation.

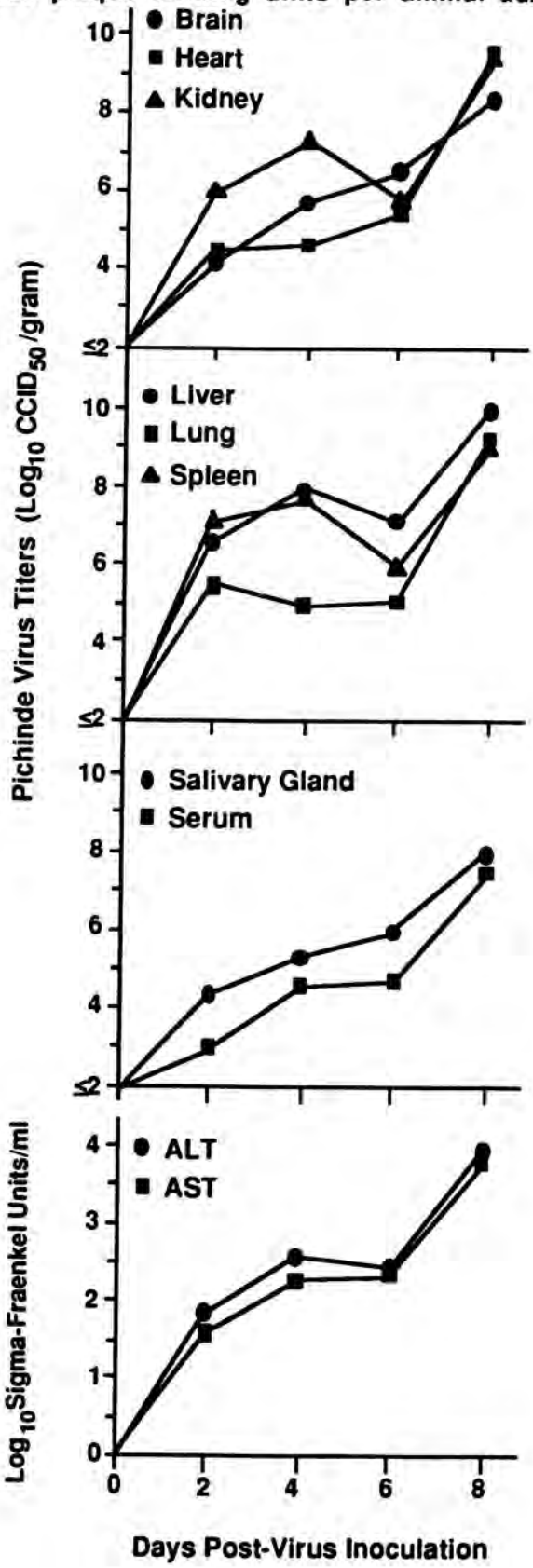
[b] Determined 7 days after virus challenge.

[c] Serum alanine aminotransferase activity expressed in Sigma-Fraenkel units/ml.

[d] Standard deviation.

* $P<0.05$, ** $P<0.01$.

Figure XVI-1. Development of PCV titers, and effects of infection on serum alanine (AST) and aspartate (AST) aminotransferase activities in LVG/Lak hamsters. The virus challenge dose was 1000 plaque forming units per animal administered by I.p. route.



XVII. OVERVIEW OF *IN VIVO* ANTI-PUNTA TORO VIRUS ACTIVITY OF AVS COMPOUNDS: SUMMARY OF SIX 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 XVII-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 XVII-1. Overview of In Vivo Anti-Punta Toro Virus Activity of AVS Compounds: Summary of Six Year's Testing

PtA In Vivo Evaluations Dec. 1985–Dec. 1991

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
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	1/5/89	bid x 5, beg 24 hr post	po	1-300	>300	+	1	COMBINATION
1	Ribavirin	584	1/11/89	single, beg 4 hr pre	iv	62.5-500	>4500	±	500	BALLIET
1	Ribavirin	647	3/16/89	bid x 3, beg 24 hr post	po	3.13-1200	>1200	+	12.5	COMBINATION
1	Ribavirin	669	4/19/89	bid x 5, beg 24 hr post	sc	3.2-1000	1000	+	3.2	EXPANDED ALI
1	Ribavirin	687	5/17/89	bid x 5, beg 24 hr post	po	6.4-2000	2000	+	6.4	EXPANDED ALI
1	Ribavirin	690	5/25/89	qd x5, varying times	sc	140	>140	+	140	
1	Ribavirin	693	6/2/89	bid x 5, varying times	sc	140	>140	+	140	
1	Ribavirin	696	6/8/89	bid x 5, varying times	po	325	>325	+	48 post	
1	Ribavirin	701	7/14/89	qd x 5, varying times	po	325	>325	+	72 post	
1	Ribavirin	704	7/14/89	bid x 1-5, beg 24 hr post	po	325	~325	+	325	
1	Ribavirin	705	7/14/89	single, beg 24 hr post	po	325	~325	+	325	
1	Ribavirin	711	7/14/89	bid x 5, beg 4 hr post	sc	16	>16	?	?	BALLIET
1	Ribavirin	712	7/20/89	bid x 1-5, beg 24 hr post	sc	140	>140	+	140	
1	Ribavirin	713	7/20/89	single, beg 24 hr post	sc	140	>140	+	140	
1	Ribavirin	719	7/28/89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	720	7/28/89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF

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1	Ribavirin	721	7/28/89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	722	7/28/89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	723	7/28/89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	736	8/10/89	bid x 1-5, beg 24 hr post	po	81	>81	+	81	
1	Ribavirin	737	8/10/89	single, beg 24 hr post	po	81	>81	+	81	
1	Ribavirin	761	9/15/89	bid x 5, beg 4 hr pre	ip	75-600	600	±	300	BALLIET
1	Ribavirin	765	9/21/89	bid x 1-5, beg 24 hr post	po	20	>20	+	20	
1	Ribavirin	766	9/21/89	single, beg 24 hr post	po	20	>20	+	20	
1	Ribavirin	771	9/27/89	single, beg 24 hr post	po	41	>41	+	41	EXPANDED ALI
1	Ribavirin	774	10/6/89	bid x 3, beg 24 hr post	po	6.25-1250	1250	+	25	COMBINATION
1	Ribavirin	788	11/3/89	bid x 5, beg 4 hr post	sc	16	>16	+	16	EXPANDED ALI
1	Ribavirin	813	2/22/90	bid x 3, beg 24 hr post	po	1.60-2000	2000	+	1.6	COMBINATION
1	Ribavirin	844	6/21/90	bid x 3, beg 24 hr post	po	2.5 - 1500	1200	±	10	COMBINATION
1	Ribavirin	900	12/13/90	bid x 3, beg 24 hr post	po	12.5 - 1500	1500	+	12.5	COMBINATION
1	Ribavirin	908	2/21/91	bid x 3, beg 24 hr post	po	2.5 - 1500	1500	+	5	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	125-1000	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	4/19/89	bid x 5, beg 24 hr post	sc	9.6-3000	3000	+	9.6	EXPANDED ALI
2	Ribavirin triacetate	689	5/17/89	bid x 5, beg 24 hr post	po	12.8-4000	4000	+	12.8	EXPANDED ALI
2	Ribavirin triacetate	692	5/25/89	qd x 5, varying times	sc	425	>425	+	425	
2	Ribavirin triacetate	695	6/2/89	bid x 5, varying times	sc	425	>425	+	425	
2	Ribavirin triacetate	698	6/8/89	bid x 5, varying times	po	650	>563	+	96 post	
2	Ribavirin triacetate	702	7/14/89	qd x 5, varying times	po	563	>563	+	48 post	
2	Ribavirin triacetate	706	7/14/89	bid x 1-5, beg 24 hr post	po	563	>563	+	563	
2	Ribavirin triacetate	707	7/14/89	single, beg 24 hr post	po	563	>563	+	563	
2	Ribavirin triacetate	714	7/20/89	bid x 1-5, beg 24 hr post	sc	425	>425	+	425	
2	Ribavirin triacetate	715	7/20/89	single, beg 24 hr post	sc	425	>425	+	425	
2	Ribavirin triacetate	724	7/28/89	bid x 5, beg 24 hr post	po	11.3-1126	>1126	+	112.6	MMF

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2	Ribavirin triacetate	725	7/28/89	bid x 5, beg 24 hr post	po	11.3-1126	>1126	+	112.6	MMF
2	Ribavirin triacetate	726	7/28/89	bid x 5, beg 24 hr post	po	11.3-1126	>1126	+	112.6	MMF
2	Ribavirin triacetate	727	7/28/89	bid x 5, beg 24 hr post	po	11.3-1126	>1126	+	112.6	MMF
2	Ribavirin triacetate	728	7/28/89	bid x 5, beg 24 hr post	po	11.3-1126	>1126	+	11.3	MMF
2	Ribavirin triacetate	738	8/10/89	bid x 1-5, beg 24 hr post	po	141	>141	+	141	
2	Ribavirin triacetate	739	8/10/89	single, beg 24 hr post	po	141	>141	+	141	
2	Ribavirin triacetate	762	9/15/89	bid x 5, beg 4 hr pre	ip	225-1800	900	-	>1800	BALLIET
2	Ribavirin triacetate	767	9/21/89	bid x 1-5, beg 24 hr post	po	35	>35	+	35	
2	Ribavirin triacetate	768	9/21/89	single, beg 24 hr post	po	35	>35	+	35	
2	Ribavirin triacetate	772	9/27/89	single, beg 24 hr post	po	71	>71	+	71	EXPANDED ALL
52	Thioformycin B	2	10/10/86	bid x 5, beg 4 hr pre	sc	62.5-250	>250	-	>250	
52	Thioformycin B	22	1/22/87	single, beg 4 hr post	sc	300-1200	>1200	-	>1200	
52	Thioformycin B	23	1/22/87	single, beg 8 hr post	sc	300-1200	>1200	-	>1200	
52	Thioformycin B	24	1/22/87	single, beg 24 hr post	sc	300-1200	>1200	-	>1200	
52	Thioformycin B	153	10/9/87	tid x 5, beg 4 hr pre	sc	62.5-500	>500	+	250	
52	Thioformycin B	231A	12/18/87	qid x 5, beg 4 hr pre	sc	25-400	>400	±	50	
52	Thioformycin B	342	4/22/88	tid x 5, beg 4 hr pre	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	12/1/88	tid x 5, beg 4 hr pre	sc	31.3-500	>500	+	125	
65	Formycin B	560	12/8/88	single, beg 4 hr pre	sc	31.3-500	>500	-	>500	
65	Formycin B	561	12/8/88	single, beg 24 hr post	sc	31.3-500	>500	+	62.5	
65	Formycin B	596	1/19/89	single, beg 24 hr post	sc	100-800	>800	-	>800	
65	Formycin B	597	1/19/89	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	2/8/90	tid x 5, beg 4 hr pre	ip	62.5-500	>500	±	125	EXPANDED
65	Formycin B	818	3/1/90	tid x 5, beg 4 hr pre	ip	125-1000	1000	-	>1000	EXPANDED
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	3	10/10/86	bid x 5, beg 4 hr pre	sc	25-100	100	+	25	
79	9-ß-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-ß-D-ribofuranosylpurine-6-thiocarboxamide	18	12/3/86	bid x 5, beg 24,4 hr pre	sc	9.4-75	>75	-	>75	BALLIET
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	25	1/22/87	single, beg 4 hr post	sc	175-700	700	?		
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	26	1/22/87	single, beg 8 hr post	sc	175-700	700	?		
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	27	1/22/87	single, beg 24 hr post	sc	175-700	700	?		
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	95	7/30/87	qd x 5, beg 4 hr pre	sc	25-200	200	?		
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	102	8/7/87	bid x 5, beg 4 hr pre	po	25-200	>200	±	>200	EXPANDED
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	107	8/14/87	bid x 5, beg 24 hr post	sc	18.8-150	>150	-	18.8	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	108	8/14/87	bid x 5, beg 36 hr post	sc	18.8-150	>150	-	37.5	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	109	8/14/87	bid x 5, beg 48 hr post	sc	18.8-150	>150	-	>150	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	133	9/11/87	qd x 5, beg 4 hr pre	sc	25-200	200	+	50	REPEAT #95
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	154	10/9/87	single, beg 4 hr post	sc	87.5-700	>700	±	350	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	155	10/9/87	single, beg 24 hr post	sc	87.5-700	>700	±	175	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	156	10/9/87	single, beg 48 hr post	sc	87.5-700	>700	+	87.5	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	157	10/9/87	single, beg 72 hr post	sc	87.5-700	>700	-	>700	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	158	10/9/87	single, beg 96 hr post	sc	87.5-700	>700	-	>700	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	187	11/6/87	bid x 5, beg 4 hr pre	ip	6.25-200	200	-	200	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	188	11/6/87	tid x 5, beg 4 hr pre	sc	6.25-200	200	+	6.25	

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79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	336	4/15/88	single, beg 4 hr post	po	87.5-700	>700	±	175	EXPANDED
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	337	4/15/88	single, beg 24 hr post	po	87.5-700	>700	+	87.5	EXPANDED
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	338	4/15/88	single, beg 48 hr post	po	87.5-700	>700	+	87.5	EXPANDED
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	374	5/20/88	single, beg 4 hr post	po	87.5-700	>700	±	350	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	375	5/20/88	single, beg 24 hr post	po	87.5-700	>700	±	700	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	376	5/20/88	single, beg 48 hr post	po	87.5-700	>700	±	175	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	403	6/17/88	single, beg 60 hr post	po	43.8-700	>700	-	>700	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	534	11/22/88	single, beg 24 hr post	ip	62.5-500	>500	-	>500	BALLIET
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	799	1/4/90	tid x 4, beg 4 hr pre	ip	125-1000	250			
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	819	3/1/90	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
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	4/19/90	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	11/2/88	single, beg 24 hr pre	po	150-1200	1200	-	>1200	EXPANDED
147	Enviroxime	523	11/3/88	single, beg 4 hr post	po	150-1200	1200	±	300	EXPANDED
147	Enviroxime	524	11/3/88	single, beg 24 hr post	po	150-1200	1200	-	>1200	EXPANDED
147	Enviroxime	817	3/1/90	tid x 5, beg 4 hr pre	sc	75-500	>500	±	75	EXPANDED
147	Enviroxime	820	3/8/90	bid x 5, beg 4 hr pre	sc	75-500	>500	±	125	EXPANDED
148	Pyrazofurin	914	4/11/91	bid x 5, beg 4 hr pre	ip	1.25 - 20	5	+	1.25	EXPANDED
148	Pyrazofurin	929	6/20/91	bid x 5, beg 4 hr pre	ip	0.3125 - 2.5	>2.5	+	0.3125	EXPANDED
148	Pyrazofurin	930	6/27/91	bid x 5, beg 4 hr pre	po	1.25 - 10	5	+	1.25	EXPANDED
148	Pyrazofurin	931	7/11/91	bid x 5, beg 4 hr post	ip	0.3125 - 2.5	>2.5	+	0.3125	EXPANDED
148	Pyrazofurin	932	7/11/91	bid x 5, beg 24 hr post	ip	0.3125 - 2.5	>2.5	+	0.3125	EXPANDED
148	Pyrazofurin	934	7/26/91	bid x 5, beg 48 hr post	ip	0.3125 - 2.5	>2.5	±	0.3125	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	tid 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	

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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	12/18/87	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	4/19/89	bid x 5, beg 24 hr post	sc	9.6-3000	3000	+	30	EXPANDED ALL
206	Ribamidine	688	5/17/89	bid x 5, beg 24 hr post	po	12.8-4000	4000	+	12.8	EXPANDED ALL
206	Ribamidine	691	5/25/89	qd x 5, varying times	sc	425	>425	+	425	
206	Ribamidine	694	6/2/89	bid x 5, varying times	sc	425	>425	+	425	
206	Ribamidine	697	6/8/89	bid x 5, varying times	po	650	>650	+	48 post	
206	Ribamidine	703	7/14/89	qd x 5, varying times	po	650	>650	+	48 post	
206	Ribamidine	708	7/14/89	bid x 1-5, beg 24 hr post	po	650	>650	+	650	
206	Ribamidine	709	7/14/89	single, beg 24 hr post	po	650	>650	+	650	
206	Ribamidine	716	7/20/89	bid x 1-5, beg 24 hr post	sc	425	>425	+	425	
206	Ribamidine	717	7/20/89	single, beg 24 hr post	sc	425	>425	+	425	
206	Ribamidine	729	7/28/89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	13	MMF
206	Ribamidine	730	7/28/89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	130	MMF
206	Ribamidine	731	7/28/89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	130	MMF
206	Ribamidine	732	7/28/89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	130	MMF
206	Ribamidine	733	7/28/89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	41.1	MMF
206	Ribamidine	740	8/10/89	bid x 1-5, beg 24 hr post	po	163	>163	+	163	
206	Ribamidine	741	8/10/89	single, beg 24 hr post	po	163	>163	+	163	
206	Ribamidine	763	9/15/89	bid x 5, beg 4 hr pre	ip	225-1800	1800	-	>1800	BALLIET
206	Ribamidine	769	9/21/89	bid x 1-5, beg 24 hr post	po	41	>41	+	41	
206	Ribamidine	770	9/21/89	single, beg 24 hr post	po	41	>41	+	41	
206	Ribamidine	773	9/27/89	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	

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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	12/8/88	tid x 5, beg 4 hr pre	sc	12.5-100	>100	-	>100	
215	3-Deazaguanosine	558	12/8/88	bid x 5, beg 4 hr pre	sc	12.5-100	>100	+	50	
215	3-Deazaguanosine	559	12/8/88	bid x 5, beg 4 hr pre	ip	12.5-100	>100	+	25	
215	3-Deazaguanosine	591	1/19/89	tid x 5, beg 4 hr pre	ip	12.5-100	>100	+	12.5	EXPANDED
215	3-Deazaguanosine	592	1/19/89	bid x 5, beg 4 hr pre	ip	12.5-100	>100	+	25	EXPANDED
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	55	3/12/87	bid x 5, beg 24 hr pre	ip	3.13-50	>50	-	>50	BALLIET
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	-	>250	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	302	3/3/88	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	366	5/6/88	qd x 5, beg 24 hr pre	sc	62.5-500	>500	-	>500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	437	7/20/88	bid x 5, beg 4 hr pre	sc	2000	2000	-	>2000	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	438	7/21/88	single, 24 hr pre	ip	187.5-1500	>1500	±	>1500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	439	7/21/88	single, 4 hr pre	ip	187.5-1500	>1500	-	>1500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	440	7/21/88	single, 24 hr post	ip	187.5-1500	>1500	-	>1500	
233	Formycin	17	11/19/86	tid x 5, beg 4 hr pre	sc	62.5-500	>500	-	>500	
233	Formycin	40	1/29/87	bid x 5, beg 4 hr pre	sc	100-400	>400	-	>400	
233	Formycin	41	1/29/87	single, beg 12 hr post	sc	450-1800	900	±	450	
253	Selenazofurin	5	10/10/86	single, beg 12 hr post	sc	450-1800	900	±	450	
253	Selenazofurin	14	11/14/86	bid x 5, beg 4 hr pre	sc	80-320	160	+	80	
253	Selenazofurin	19	12/3/86	bid x 5, beg 4 hr pre	sc	20-160	>160	+	80	
253	Selenazofurin	97	7/30/87	bid x 5, beg 24,4 hr pre	sc	18.8-150	>150	+	>150	BALLIET
253	Selenazofurin	104	8/7/87	qd x 5, beg 4 hr pre	sc	40-320	320	±	40	REPEAT
253	Selenazofurin	538	11/22/88	bid x 5, beg 4 hr pre	po	40-320	320	+	40	EXPANDED
253	Selenazofurin	800	1/4/90	single, beg 4 hr post	ip	93.75-750	>750	±	93.8	BALLIET
253	Selenazofurin	801	1/4/90	qd x 4, 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	125-1000	1000	+		EXPANDED
257	Tiazofurin 5'-MP	449	9/2/88	bid x 5, beg 4 hr pre	ip	25-400	>400	+	400	
272	3-Deazaguanine	186	11/6/87	bid x 5, beg 4 hr pre	ip	50-400	>400	+	200	EXPANDED
272	3-Deazaguanine	232	12/18/87	bid x 5, beg 4 hr pre	sc	25-200	100	-	>200	
272	3-Deazaguanine	280	2/11/88	qd x 5, beg 4 hr pre	sc	25-200	>200	+	25	
272	3-Deazaguanine	317	3/18/88	bid x 5, beg 24 hr pre	ip	12.5-100	>100	?		
272	3-Deazaguanine	343	4/22/88	bid x 5, beg 24 hr pre	ip	12.5-100	100	-	>12.5	
272	3-Deazaguanine	370	5/13/88	qd x 5, beg	ip	25-200	200	-	>200	BALLIET
272	3-Deazaguanine	498	10/13/88	qd x 5, beg 4 hr pre	po	18.8-300	>300	+	18.8	EXPANDED
272	3-Deazaguanine	539	11/22/88	qd x 5, beg 4 hr pre	sc	18.8-300	>300	-	>300	EXPANDED
272	3-Deazaguanine	802	1/12/90	single, beg 4 hr post	ip	93.75-750	>750	-	>750	BALLIET
347	Phyllanthoside	829	4/12/90	bid x 5, beg 4 hr pre	sc	62.5-500	500	+	62.5	EXPANDED
360	7-Deoxynarciclasin	42	1/29/87	bid x 4, beg 4 hr pre	sc	15-120	120	±	30	EXPANDED
361	Pancratistatin	417	6/24/88	bid x 5, beg 4 hr pre	sc	62.5-500	>500	±	250	
1018	Phenyleneamine	791	11/16/89	qd x 7, beg 24 hr pre	sc	.5-4	>4	-	>4	
1018	Phenyleneamine	792	11/16/89	single, beg 4 hr post	po	1.56-12.5	>12.5	±	12.5	EXPANDED
1018	Phenyleneamine	830	4/12/90	3 shots in 9 days, beg 24 hr post	po	1.56-12.5	>12.5	+	1.56	EXPANDED
				single, beg 24 hr post	po	3.13-25	>25	+	6.25	EXPANDED

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1018	Phenyleneamine	831	4/12/90	single, beg 36 hr post	po	3.13-25	>25	+	6.25	EXPANDED
1018	Phenyleneamine	838	5/31/90	4 hr pre, day 4	po	3.13-25	>25	±	3.13	BALLIET
1018	Phenyleneamine	904	2/14/91	single, beg 24 hr post	ip	3.13-25	>25	+	6.25	
1018	Phenyleneamine	905	2/14/91	single, beg 48 hr post	ip	3.13-25	>25	-	>25	
1018	Phenyleneamine	906	2/14/91	single, beg 24 hr post	sc	3.13-25	>25	+	3.13	
1018	Phenyleneamine	907	2/14/91	single, beg 48 hr post	sc	3.13-25	>25	±	12.5	
1018	Phenyleneamine	936	8/8/91	single, beg 24 hr post	ip	6.25 - 50	>50	±	6.25	BALLIET
1018	Phenyleneamine	937	8/8/91	single, beg 72 hr post	ip	6.25 - 50	>50	±	25	BALLIET
1212	Uridine 2',3'-dialdehyde	550	12/1/88	bid x 5, beg 4 hr pre	sc	25-400	>400	-	>400	INITIAL
1212	Uridine 2',3'-dialdehyde	562	12/8/88	single, beg 4 hr pre	sc	25-400	>400	±	25	
1212	Uridine 2',3'-dialdehyde	563	12/8/88	single, beg 24 hr post	sc	25-400	>400	+	25	
1212	Uridine 2',3'-dialdehyde	598	1/19/89	single, beg 24 hr post	sc	12.5-100	>100	-	>100	
1212	Uridine 2',3'-dialdehyde	599	1/19/89	single, beg 24 hr post	ip	12.5-100	>100	+	100	
1212	Uridine 2',3'-dialdehyde	661	4/6/89	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	1/8/88	qd x 3, beg 4 hr pre	ip	3.13-50	50	+	6.25	
1754	MVE-2	240	1/8/88	single, beg 72 hr post	ip	6.25-100	>100	-	>100	
1754	MVE-2	241	1/8/88	single, beg 96 hr post	ip	6.25-100	>100	-	>100	
1754	MVE-2	249	1/15/88	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	FN, EXPANDED
1754	MVE-2	603	1/26/89	bid x 5, beg 24 hr post	ip	1.56-50	>50	+	12.5	EXPANDED
1754	MVE-2	624	2/24/89	single, beg 24 hr post	ip	0.75-25	>25	±	12.5	MMF
1754	MVE-2	625	2/24/89	single, beg 24 hr post	ip	0.75-25	>25	+	12.5	MMF
1754	MVE-2	626	2/24/89	single, beg 24 hr post	ip	0.75-25	>25	+	12.5	MMF
1754	MVE-2	627	2/24/89	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

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1761	Poly IC•LC	672	4/27/89	3 in 7 days, beg 4 hr post	ip	0.125-1	>1	+	0.125	EXPANDED
1761	Poly IC•LC	734	8/4/89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.0032	EXPANDED
1761	Poly IC•LC	742	8/10/89	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	8/24/89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.01	EXPANDED
1761	Poly IC•LC	814	2/22/90	eod x 3, beg 24 hr post	ip	0.32	>0.32	+	0.32	COMBINATION
1761	Poly IC•LC	821	3/8/90	eod x 3, beg 24 hr post	ip	0.001-0.01	>0.01	+	0.001	COMBINATION
1761	Poly IC•LC	909	2/21/91	single, 23 hr post	ip	0.005 - 5	>5	+	0.005	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	1/15/88	single, beg 4 hr pre	sc	25-400	>400	+	50	
1767	AM-3	244	1/15/88	single, beg 4 hr post	sc	25-400	>400	+	25	
1767	AM-3	245	1/15/88	single, beg 24 hr post	sc	25-400	>400	+	25	
1767	AM-3	246	1/15/88	single, beg 48 hr post	sc	25-400	>400	+	25	
1767	AM-3	247	1/15/88	single, beg 72 hr post	sc	25-400	>400	-	>400	
1767	AM-3	248	1/15/88	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	tid 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	
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	

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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	1/8/88	qd x 5, beg 4 hr pre	sc	9.4-150	>150	?		
1778	Mannozym	250	1/15/88	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	CL246,738	797	12/14/89	single, 4 hr pre	po	12.5-100	>100	+	12.5	EXPANDED
1968	CL246,738	798	12/14/89	3 shots, beg 24 hr post	po	12.5-100	>100	+	12.5	EXPANDED
1968	CL246,738	839	5/31/90	single, 4 hr pre	po	12.5-100	>100	-	>100	BALLIET
1969	CL259763	356	4/29/88	single, beg 24 hr pre	po	2, 2	>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	May-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	
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

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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	1/22/88	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	3/23/89	qd x 5, beg 4 hr pre	ip	0.05-5	>5	+	0.05	MMF
2149	Ampligen	654	3/23/89	qd x 5, beg 4 hr pre	ip	0.05-5	>5	+	0.05	MMF
2149	Ampligen	655	3/23/89	qd x 5, beg 4 hr pre	ip	0.05-5	>5	+	0.05	MMF
2149	Ampligen	656	3/23/89	qd x 5, beg 4 hr pre	ip	0.05-5	>5	+	0.05	MMF
2149	Ampligen	668	4/12/89	single, beg 48 hr post	ip	2.5	>2.5	ON TEST	ON TEST	IMMUNOLOGY
2149	Ampligen	673	4/27/89	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	6/21/90	single, beg 23 hr post	ip	0.005-5	>5	+	0.005	COMBINATION
2276	Theracel No. BL-002	867	9/13/90	qd x 5, beg 4 hr post	po	10-500	>500	±	32	EXPANDED
2276	Theracel No. BL-002	879	10/17/90	qd x 5, beg 24 hr pre	po	125-2000	>2000	+	250	EXPANDED
2276	Theracel No. BL-002	881	10/22/90	qd x 1	po	125-2000	>2000	ON TEST	ON TEST	IFN
2285	Theracel No. BL-012	866	9/13/90	qd x 5, beg 4 hr post	po	10-500	>500	±	32	EXPANDED
2285	Theracel No. BL-012	880	10/17/90	qd x 5, beg 24 hr pre	po	125-2000	>2000	+	125	EXPANDED
2285	Theracel No. BL-012	882	10/22/90	qd x 1	po	125-2000	>2000	ON TEST	ON TEST	IFN
2318	UNIDENTIFIED	942	9/5/91	bid x 5, beg 4 hr pre	ip	275 - 1100	550	+	275	EXPANDED
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	1/19/89	bid x 5, beg 4 hr pre	ip	12.5-100	>100	+	12.5	EXPANDED
2700	6-Ethyl thiopurine riboside	594	1/19/89	single, beg 4 hr pre	ip	31.3-500	>500	-	>500	

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2700	6-Ethyl thiopurine riboside	595	1/19/89	single, beg 24 hr post	ip	31.3-500	>500	+	62.5	
2700	6-Ethyl thiopurine riboside	600	1/26/89	single, beg 4 hr pre	ip	31.3-500	250	-	>500	
2700	6-Ethyl thiopurine riboside	601	1/26/89	single, beg 24 hr post	ip	31.3-500	250	±	31.3	
2700	6-Ethyl thiopurine riboside	602	1/26/89	bid x 5, beg 4 hr pre	ip	12.5-100	>100	+	50	EXPANDED
2700	6-Ethyl thiopurine riboside	645	3/9/89	bid x 5, beg 24 hr pre	ip	9.4-150	>150	-	>150	BALLIET
2700	6-Ethyl thiopurine riboside	657	3/30/89	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	5/20/88	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	12/8/88	tid 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	5/20/88	bid x 5, beg 4 hr pre	ip	May-40	>40	-	>40	
2716	UNIDENTIFIED	666	4/13/89	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	2/9/89	single, beg 4 hr post	sc	31.3-500	>500	±	250	
2742	Ribavirin 5-OH tetrahydropyrimidine	608	2/9/89	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	1/21/88	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

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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
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	3/1/89	qd x 3, beg 24 hr pre	po	25-400	>400	+	25	EXPANDED
2776	Bropirimine	632	3/2/89	qd x 3, beg 4 hr post	po	25-400	>400	+	50	EXPANDED
2776	Bropirimine	633	3/1/89	qd x 3, beg 24 hr pre	ip	25-400	>400	+	25	
2776	Bropirimine	634	3/2/89	qd x 3, beg 4 hr post	ip	25-400	>400	+	25	
2776	Bropirimine	635	3/2/89	qd x 3, beg 24 hr pos.	ip	25-400	>400	+	50	
2776	Bropirimine	636	3/2/89	qd x 3, beg 24 hr pre	ip	62.5-1000	1000	+	62.5	BALLIET
2776	Bropirimine	637	3/8/89	single, beg 24 hr pre	po	25-800	>800	+	50	
2776	Bropirimine	638	3/9/89	single, beg 24 hr post	po	25-800	>800	+	100	
2776	Bropirimine	639	3/9/89	single, beg 48 hr post	po	25-800	>800	±	400	
2776	Bropirimine	640	3/9/89	single, beg 72 hr post	po	25-800	>800	-	>800	
2776	Bropirimine	641	3/8/89	eod x 3, beg 24 hr pre	po	25-400	>400	+	100	
2776	Bropirimine	642	3/8/89	e2d x 3, beg 24 hr pre	po	25-400	>400	+	50	
2776	Bropirimine	643	3/8/89	single, beg 24 hr pre	sc	25-400	>400	+	50	
2776	Bropirimine	644	3/8/89	bid x 3, beg 24 hr pre	ip	25-400	>400	+	200	
2776	Bropirimine	648	3/16/89	qd x 3, beg 24 hr post	po	25-100	>100	+	25	COMBINATION
2776	Bropirimine	658	3/29/89	single, beg 24 hr post	ip	200	ON TEST	ON TEST	ON TEST	IMMUNOLOGY
2776	Bropirimine	662	4/6/89	single, beg 4 hr pre	sc	25-400	>400	+	25	
2776	Bropirimine	663	4/5/89	eod x 3, beg 24 hr pre	po	50-400	>400	+	50	
2776	Bropirimine	664	4/5/89	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	3/11/88	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	4/13/89	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	12/1/88	single, beg 4 hr pre	ip	0.78-100	>100	+	3.13	EXPANDED
2779	MVE-1	581	1/5/89	single, beg 24 hr post	ip	3.13-12.5	>12.5	±	6.25	COMBINATION
2779	MVE-1	585	1/13/89	qd x 3, beg 24 hr post	ip	1.56-50	>50	+	1.56	

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2779	MVE-1	586	1/13/89	bid x 3, beg 24 hr post	ip	1.56-50	>50	-	>50	
2779	MVE-1	587	1/13/89	single, beg 24 hr post	sc	6.25-100	>100	+	6.25	
2779	MVE-1	588	1/13/89	single , beg 36 hr post	ip	6.25-100	>100	-	>100	
2779	MVE-1	589	1/13/89	single , beg 48 hr post	ip	6.25-100	>100	+	12.5	
2779	MVE-1	590	1/13/89	single, beg 4 hr pre	po	6.25-200	>200	-	>200	EXPANDED
2779	MVE-1	604	1/26/89	eod x 5, beg 4 hr pre	ip	3.13-100	>100	+	3.13	
2779	MVE-1	652	3/23/89	single, beg 4 hr pre	po	6.25-200	>200	±	25	EXPANDED
2779	MVE-1	660	4/6/89	single, beg 4 hr pre	po	9.4-150	>150	±	9.4	EXPANDED
2786	UNIDENTIFIED	667	4/13/89	bid x 5, beg 4 hr pre	sc	18.8-300	>300	-	>300	
2811	7-Deoxynarciclasine	236	1/8/88	qd x 5, beg 4 hr pre	ip	3.13-25	>25	-	>25	
2811	7-Deoxynarciclasine	369	5/13/88	bid x 5, beg 4 hr pre	ip	8-Jan	>8	±	4	
2812	Narciclasine	237	1/8/88	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	1/25/90	bid x 5, beg 4 hr pre	sc	0.75-25	6.25	±	3.13	EXPANDED
2812	Narciclasine	808	1/25/90	qd x 5, beg 4 hr pre	sc	0.75-25	6.25	+	0.75	EXPANDED
2812	Narciclasine	809	2/1/90	bid x 5, beg 4 hr pre	ip	0.195-3.13	3.13	-	>3.13	EXPANDED
2812	Narciclasine	810	2/1/90	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	1/21/88	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	0.76-50	>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	4/19/90	bid x 5, beg 4 hr pre	sc	25-400	>400	±	100	INITIAL
2885	3-T-butyl-1-adamantylthiourea	841	6/7/90	bid x 5, beg 4 hr pre	sc	25-100	>100	±	25	EXPANDED
2885	3-T-butyl-1-adamantylthiourea	850	6/28/90	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

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2933	CGP 19835 A Lipid	609	2/8/89	qd x 3, beg 24 hr pre	ip	1-1000µ	>1000 µg	+	100	
2933	CGP 19835 A Lipid	610	2/9/89	single, beg 24 hr post	po	1,10,100,1000µ	>1000 µg	-	>1000	EXPANDED
2933	CGP 19835 A Lipid	859	8/9/90	single, beg 4 hr post	ip	1250-10000 µ	>10000µg	-	>10000	BALLIET
2933	CGP 19835 A Lipid	860	8/9/90	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	
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	11/2/88	bid x 5, beg 24 hr pre	po	15.6-250	>250	-	>250	
3425	8-Bromoguanosine	526	11/9/88	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	12/8/88	qd x 5, beg 4 hr pre	sc	15.7-250	>250	-	>250	
3580	Sodium diethyldithiocarbamate	404	6/17/88	bid x 5, beg 4 hr pre	ip	6.25-100	>100	±	25	
3580	Sodium diethyldithiocarbamate	532	11/9/88	single, beg 24 hr pre	sc	37.5-300	>300	±	37.5	
3580	Sodium diethyldithiocarbamate	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	24-Mar	>24	-	>400	
3585	Neurotropin	127	9/3/87	single, beg 24 hr pre	ip	24-Mar	>24	-	>400	
3585	Neurotropin	140	9/24/87	qd x 3, beg 24 hr pre	ip	24-Mar	>24	-	>24	
3585	Neurotropin	141	9/24/87	eod x 3, beg 24 hr pre	ip	24-Mar	>24	-	>24	
3585	Neurotropin	278	2/11/88	single, beg 24 hr pre	po	24-Mar		?		
3585	Neurotropin	316	3/17/88	single, beg 24 hr pre	po	24-Mar	>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	?		

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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	
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	9/8/88	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	1-(4-methoxybenzyloxy)adenosine perchloric acid salt	836	5/10/90	bid x 5, beg 4 hr pre	sc	25-400	>400	-	>400	INITIAL
3679	1-(4-methoxybenzyloxy)adenosine perchloric acid salt	926	5/31/91	bid x 5, beg 4 hr pre	ip	62.5-500	500	-	>500	
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	11/9/88	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	

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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	2/16/89	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	
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	10/5/88	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	12/6/88	tid x 7, beg 48 hr pre	po	4.7-600	>600	±	37.5	EXPANDED
3934	Ge 132, Germanium	611	2/8/89	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	Acetamido-7-amino-6-methyl-7H-S-triazolo[5,1-C]-S-triaz	833	4/19/90	bid x 5, beg 4 hr pre	sc	25-400	>400	±	100	INITIAL
4206	Acetamido-7-amino-6-methyl-7H-S-triazolo[5,1-C]-S-triaz	842	6/7/90	bid x 5, beg 4 hr pre	sc	25-100	>100	±	50	EXPANDED
4206	Acetamido-7-amino-6-methyl-7H-S-triazolo[5,1-C]-S-triaz	851	6/28/90	bid x 5, beg 4 hr pre	ip	75-600	>600	-	>600	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	862	9/6/90	bid x 5, beg 4 hr pre	sc	3.13 - 50	>50	-	>50	INITIAL
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	863	9/6/90	bid x 5, beg 4 hr pre	ip	3.13 - 50	25	+	3.13	INITIAL

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4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	868	9/20/90	single, beg 4 hr post	ip	12.5 - 200	25	±	12.5	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	870	9/20/90	single, beg 24 hr post	ip	12.5 - 200	25	-	>200	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	873	10/5/90	bid x 5, beg 4 hr pre	sc	3/13/50	50	+	3.13	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	874	10/5/90	bid x 5, beg 4 hr pre	ip	3.13 - 50	25	-	12.5	EXPANDED
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	875	10/5/90	bid x 5, beg 4 hr pre	po	3.13 - 50	>50	-	>50	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	888	11/1/90	single, 4 hr post	ip	1.56-12.5	>12.5	±	3.13	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	889	11/1/90	bid x 5, beg 4 hr pre	ip	0.8-6.25	>6.25	±	0.8	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	890	11/8/90	bid x 5, beg 4 hr pre	sc	6/25/50	>50	-	25	
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	864	9/6/90	bid x 5, beg 4 hr pre	sc	6.25 - 100	>100	-	>100	INITIAL
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	865	9/6/90	bid x 5, beg 4 hr pre	ip	6.25 - 100	>100	+	6.25	INITIAL
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	869	9/20/90	single, beg 4 hr post	ip	12.5 - 200	>200	-	>200	
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	871	9/20/90	single, beg 24 hr post	ip	12.5 - 200	>200	-	>200	
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	876	10/11/90	bid x 5, beg 4 hr pre	sc	6.25 - 100	100	-	>100	EXPANDED
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	877	10/11/90	bid x 5, beg 4 hr pre	ip	6.25 - 100	100	-	>100	EXPANDED
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	878	10/11/90	bid x 5, beg 4 hr pre	po	6.25 - 100	>100	-	>100	
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	891	11/8/90	bid x 5, beg 4 hr pre	ip	1.56 - 12.5	>12.5	-	>12.5	
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	892	11/8/90	bid x 5, beg 4 hr pre	sc	1.56 - 12.5	>12.5	-	>12.5	
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	928	6/6/91	single, 24 hr pre	ip	12.5 - 200	>200	-	>200	
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	
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	12/1/88	single, beg 24 hr post	ip	0.025-0.8	0.4	+	0.025	EXPANDED
4282	AM-5	553	12/1/88	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	0.38	-	>1.5	
4282	AM-5	605	2/2/89	single, beg 4 hr pre	po	0.05-0.8	>0.8	+	0.05	EXPANDED
4282	AM-5	606	2/2/89	single, beg 24 hr post	po	0.05-0.8	>0.8	+	0.05	EXPANDED
4282	AM-5	616	2/17/89	single, beg 4 hr pre	ip	0.025-0.2	>0.2	-	>0.2	BALLIET
4282	AM-5	617	2/17/89	single, beg 4 hr post	ip	0.025-0.2	>0.2	-	>0.2	BALLIET
4282	AM-5	618	2/17/89	single, beg 24 hr post	ip	0.025-0.2	>0.2	-	>0.2	BALLIET
4282	AM-5	630	2/23/89	eod x 3, beg 24 hr pre	ip	0.025-0.8	0.8	+	0.025	EXPANDED
4282	AM-5	659	4/6/89	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	2/16/89	single, beg 24 hr post	ip	6.3-50	>50	Terminate	Terminate	TERMINATED
4285	AM-8	628	2/24/89	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	

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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	4/19/90	bid x 5, beg 4 hr pre	sc	25-400	>400	±	100	INITIAL
4588	1-aminoadenosinium mesitylenesulfonate	843	6/7/90	bid x 5, beg 4 hr pre	sc	25-100	>100	-	>100	EXPANDED
4588	1-aminoadenosinium mesitylenesulfonate	852	6/28/90	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	2/16/89	qd x 5, beg 4 hr pre	sc	25-200	>200	-	>200	
4616	Noxymethyl pennicillinic acid	622	2/16/89	single, beg 4 hr pre	sc	62.5-500	>500	Terminate	Terminate	TERMINATED
4616	Noxymethyl pennicillinic acid	623	2/16/89	single, beg 24 hr post	sc	62.5-500	>500	Terminate	Terminate	TERMINATED
4616	Noxymethyl pennicillinic acid	629	2/24/89	single, beg 24 hr post	sc	62.5-500	>500	-	>500	
4617	206-glycine	718	7/20/89	bid x 5, beg 4 hr pre	sc	50-800	>800	+	200	
4617	206-glycine	938	8/15/91	bid x 3, beg 4 hr pre	sc	300 - 1200	>1200	+	600	EXPANDED
4618	5'-N,N-diethylthiocarbamate-5'-deoxy-5'-thioadenosine	837	5/10/90	bid x 5, beg 4 hr pre	sc	25-400	>400	±	50	INITIAL
4618	5'-N,N-diethylthiocarbamate-5'-deoxy-5'-thioadenosine	853	6/28/90	bid x 5, beg 4 hr pre	sc	6.25 - 50	>50	-	>50	EXPANDED
4618	5'-N,N-diethylthiocarbamate-5'-deoxy-5'-thioadenosine	925	5/31/91	qd x 5, beg 4 hr pre	ip	12.5 - 100	>100	±	12.5	INITIAL
4726	CPG 19835 A Lipid - Placebo	462	9/8/88	single, beg 24 hr post	ip	undilute	no	-	>undilute	EXPANDED
4785	Actidione	924	5/31/91	bid x 5, beg 4 hr pre	sc	25 - 200	25	±	25	INITIAL
4785	Actidione	935	8/8/91	bid x 5, beg 4 hr pre	sc	0.625 - 10	10	+	1.25	EXPANDED
4785	Actidione	944	10/24/91	bid x 5, beg 4 hr pre	po	0.625-10	ON TEST	ON TEST	ON TEST	EXPANDED
4785	Actidione	945	10/24/91	bid x 5, beg 24 hr post	sc	1.25 - 5	ON TEST	ON TEST	ON TEST	EXPANDED
4785	Actidione	946	10/24/91	bid x 5, beg 48 hr post	sc	1.25 - 5	ON TEST	ON TEST	ON TEST	EXPANDED
5027	Imexon	699	7/7/89	qd x 5, beg 4 hr pre	ip	18.8-150	>150	-	>150	
5027	Imexon	700	7/7/89	qd x 5, beg 24 hr post	ip	18.8-150	>150	-	>150	
5054	1-[5'-(N-methyl-3-carbonyl-1,4-dihydropyridine)2',3'-bis-	612	2/15/89	single, beg 4 hr post	iv	4.3-34	34	-	>34	BALLIET
5055	UNIDENTIFIED	613	2/15/89	single, beg 4 hr post	iv	21.9-175	>175	-	>175	BALLIET
5056	UNIDENTIFIED	614	2/15/89	single, beg 4 hr post	iv	1.9-15	>15	-	>15	BALLIET
5057	UNIDENTIFIED	615	2/15/89	single, beg 4 hr post	iv	3.13-25	>25	-	>25	BALLIET
5058	N-methyl-206	915	4/11/91	bid x 4, beg 4 hr pre	ip	125-1000	>1000	+	1000	EXPANDED
5058	N-methyl-206	941	8/30/91	bid x 4, beg 4 hr pre	sc	150 - 1200	>1200	±	1200	EXPANDED
5079	Human Recombinant Interleukin II	758	9/14/89	qd x 5, beg 4 hr post	ip	1,563-25,000 cum	>25,000	+	1563	EXPND; IMMUN
5079	Human Recombinant Interleukin II	812	2/8/90	qd x 5, beg 4 hr post	ip	1,563-12,500 cum	>12,500	+	1563	EXPND; IMMUN
5079	Human Recombinant Interleukin II	899	12/13/90	qd x 5, beg 4 hr post	ip	3000-12,000 cum	>12,000	+	12000	COMBINATION
5221	Ribavirin 2'-3'-acetonide	582	1/11/89	single, beg 4 hr pre	iv	62.5-500	>500	-	>500	BALLIET
5222	2',3',N'-triisobutyrate-5',1,4-dihydrotri. of AVS01	583	1/11/89	single, beg 4 hr pre	iv	1.95-15.6	>15.6	-	>15.6	BALLIET
5311	rIFN	789	11/9/89	single, beg 4 hr post	ip	10^3.5-10^5 upm	>10^5	+	4	EXPANDED
5311	rIFN	790	11/9/89	qd x 9, beg 4 hr post	ip	10^3.5-10^5 upm	>10^5	+	3.5	EXPANDED
5311	rIFN	826	4/5/90	qd x 5, beg 24 hr post	ip	10^3.5-10^5 upm	>10^5	+	5	EXPANDED
5311	rIFN	827	4/5/90	qd x 5, beg 36 hr post	ip	10^3.5-10^5 upm	>10^5	-	>10^5	EXPANDED

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5311	rIFN	828	4/5/90	qd x 5, beg 48 hr post	ip	10^3.5-10^5 upm	>10^5	±	10^5	EXPANDED
5311	rIFN	840	5/31/90	qd x 8, beg 4 hr pre	ip	10^3.5-10^5 upm	>10^5	-	>10^5	BALLIET
5311	rIFN	858	7/26/90	qd x 5, beg 4 hr post	ip	10^3-10^5 upm	ON TEST	ON TEST	ON TEST	COMBINATION
5581	1-[5'-(1-methyl-3-carbonyl-1,4-dihydropyridine)2',3'-bis-O-	779	10/9/89	qd x 5, beg 4 hr post	iv,ip	31.3-125	>125	-	>125	BALLIET
5582	1-[5'-(1-methyl-3-carbonyl-1,4-dihydropyridine-ß-D-	780	10/9/89	qd x 5, beg 4 hr post	iv,ip	125-500	>500	-	>500	BALLIET
5587	7-Thia-8-oxoguanosine	674	5/3/89	2 times, 24 hr pre	ip	6.25-100	>100	+	50	EXPANDED
5587	7-Thia-8-oxoguanosine	675	5/4/89	2 times, 4 hr pre	ip	6.25-100	>100	+	6.25	EXPANDED
5587	7-Thia-8-oxoguanosine	676	5/4/89	2 times, 24 hr post	ip	6.25-100	>100	+	6.25	EXPANDED
5587	7-Thia-8-oxoguanosine	677	5/4/89	single, beg 24 hr post	ip	6.25-100	>100	+	25	EXPANDED
5587	7-Thia-8-oxoguanosine	757	9/8/89	2 shots, beg 36 hr post	ip	12.5-100	ON TEST	ON TEST	ON TEST	EXPANDED
5587	7-Thia-8-oxoguanosine	775	10/6/89	2 shots, 24, 31 hr post	ip	6.25-25	>25	+	12.5	COMBINATION
5587	7-Thia-8-oxoguanosine	872	9/20/90	2 shots, 24, 31 hr post	ip	25-50	>50	+	25	
5588	ICLC	679	5/11/89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	0.25	EXPANDED
5588	ICLC	750	8/24/89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.032	EXPANDED
5589	ICL-CMA	680	5/11/89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	0.25	EXPANDED
5589	ICL-CMA	735	8/4/89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	-	>0.1	EXPANDED
5590	ICL-CMD	681	5/11/89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	0.25	EXPANDED
5590	ICL-CMD	743	8/10/89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.032	EXPANDED
5591	ICL-CM-Beta-C-Dextrin	682	5/11/89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5591	ICL-CM-Beta-C-Dextrin	744	8/10/89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.0032	EXPANDED
5592	ICL-GEL	683	5/11/89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5592	ICL-GEL	751	8/24/89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.032	EXPANDED
5593	ICL-Sulfated Gel	684	5/11/89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5593	ICL-Sulfated Gel	746	8/10/89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.01	EXPANDED
5594	IC-(PLL-Dextran)	685	5/11/89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5594	IC-(PLL-Dextran)	752	8/24/89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.1	EXPANDED
5595	IC-(PLL-Dextran)	686	5/11/89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5595	IC-(PLL-Dextran)	747	8/18/89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	±	0.1	EXPANDED
5596	ICLC (heat cycled)	678	5/11/89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	1	EXPANDED
5596	ICLC (heat cycled)	748	8/18/89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	-	>0.1	EXPANDED
5601	UNIDENTIFIED	939	8/15/91	bid x 4, beg 4 hr pre	sc	300-1200	>1200	-	>1200	EXPANDED
5786	UNIDENTIFIED	759	9/11/89	qd x 5, beg 4 hr post	iv,ip	4.0-16	>16	-	>16	BALLIET
5896	UNIDENTIFIED	760	9/11/89	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	9/18/89	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	Aug-32	>32	-	>32	BALLIET
6082	UNIDENTIFIED	793	12/4/89	qd x 5, beg 4 hr post	iv,ip	18.8-75	>75	+	75	BALLIET
6083	UNIDENTIFIED	794	12/4/89	qd x 5, beg 4 hr post	iv,ip	8.0-32	>32	-	>32	BALLIET
6290	UNIDENTIFIED	805	1/22/90	qd x 5, beg 4 hr post	iv,ip	39.5-158	>158	-	>158	BALLIET
6291	UNIDENTIFIED	803	1/22/90	qd x 5, beg 4 hr post	iv,ip	12.5-50	>50	-	>50	BALLIET
6292	UNIDENTIFIED	804	1/22/90	qd x 5, beg 4 hr post	iv,ip	12.5-50	>50	-	>50	BALLIET
6297	UNIDENTIFIED	824	3/26/90	qd x 5, beg 4 hr post	iv,ip	6.25-25	>25	-	>25	BALLIET
6300	UNIDENTIFIED	825	3/26/90	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	

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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	
6724	2-Thio-6-azauridine	916	4/18/91	bid x 5, beg 4 hr pre	ip	125 - 2000	>2000	±	500	EXPANDED
8361	Carrisyn	898	11/29/90	eod x 3, beg 24 hr pre	ip	0.1 - 10	>10	+	3.2	INITIAL
8361	Carrisyn	917	5/16/91	eod x 6, beg 24 hr pre	ip	1.0-10	>10	-	>10	EXPANDED
8361	Carrisyn	918	5/16/91	eod x 3, beg 4 hr pre	ip	1.0-10	>10	±	10	EXPANDED
8361	Carrisyn	919	5/16/91	eod x 3, beg 24 hr post	ip	1.0-10	>10	±	1	EXPANDED
8361	Carrisyn	920	5/24/91	eod x 3, beg 24 hr pre	ip	0.32-10	>10	-	>10	EXPANDED
8361	Carrisyn	921	5/24/91	eod x 3, beg 4 hr pre	ip	0.32-10	>10	±	0.32	EXPANDED
8361	Carrisyn	922	5/24/91	single, 24 hr post	ip	0.32-10	>10	±	0.32	EXPANDED
8361	Carrisyn	923	5/24/91	single, 48 hr post	ip	0.32-10	>10	±	1	EXPANDED
8361	Carrisyn	927	6/6/91	single, 4 hr pre	po	0.32-10	>10	±	0.32	EXPANDED
11717	UNIDENTIFIED	940	8/15/91	bid x 4, beg 4 hr pre	sc	300-1200	>1200	-	>1200	EXPANDED
11941	Phosphoramidate prodrug of AVS2318 5'-monophosphat	943	9/5/91	bid x 5, beg 4 hr pre	ip	250 - 1100	>1100	+	275	EXPANDED
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	1/5/89	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	1/5/89	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	1/5/89	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	3/16/89	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	3/16/89	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	3/16/89	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	10/6/89	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	10/6/89	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	10/6/89	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	2/22/90	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	2/22/90	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	3/8/90	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	3/8/90	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	6/21/90	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	6/21/90	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	6/21/90	01 bid x 3, 2149 single 23 hr post	po,ip	2.5-1500+0.05	1500+0.05	+	2.5+0.05	COMBINATION

PtA In Vivo Evaluations Dec. 1985–Dec. 1991

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
01 + 2149	Ribavirin + Ampligen	848	6/21/90	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	8/30/90	2 shots, 24 hr post, 24.5 hr post	ip	25-50 + 2000	>50+2000	+	25	COMBINATION
01 + 5311	Ribavirin + rHuIFN	856	7/26/90	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	7/26/90	01 bidx3 24 post, 5311 qdx5 4 post	po,ip	6.25-1500+10^3	ON TEST	ON TEST	ON TEST	COMBINATION
01 + 5079	Ribavirin + Human IL-2	901	2/7/91	01 bidx3 24 post, 5079 qdx5 4 post	po,ip	12.5-1500 + 12000	>1500+12000	+	12.5 + 1200	COMBINATION
01 + 5079	Ribavirin + Human IL-2	902	2/7/91	01 bidx3 24 post, 5079 qdx5 4 post	po,ip	12.5-1500 + 6000	>1500+6000	+	12.5 + 6000	COMBINATION
01 + 5079	Ribavirin + Human IL-2	903	2/7/91	01 bidx3 24 post, 5079 qdx5 4 post	po,ip	12.5-1500 + 3000	1500+3000	+	12.5 + 3000	COMBINATION
01 + 1761	Ribavirin + Poly ICLC	910	2/28/91	01 bidx3 24 post, 1761 single 23 pos	po,ip	2.5-1500 + 5	10 + 5	+	2.5 + 5	COMBINATION
01 + 1761	Ribavirin + Poly ICLC	911	2/28/91	01 bidx3 24 post, 1761 single 23 pos	po,ip	2.5-1500 + 0.5	1200 + 0.5	+	2.5 + 0.5	COMBINATION
01 + 1761	Ribavirin + Poly ICLC	912	3/7/91	01 bidx3 24 post, 1761 single 23 pos	po,ip	2.5-1500 + 0.05	1200 + 0.05	+	2.5 + 0.05	COMBINATION
01 + 1761	Ribavirin + Poly ICLC	913	3/7/91	01 bidx3 24 post, 1761 single 23 pos	po,ip	2.5-1500 + 0.005	1500 + 0.005	+	2.5 + 0.005	COMBINATION
gm CSF	gm CSF	947	10/24/91	eod x 3, beg 24 hr pre	ip	0.38 - 3	ON TEST	ON TEST	ON TEST	EXPANDED
BCH-523	BCH-523	948	10/31/91	eod x 3, beg 18 hr pre	ip	1.6 - 50	ON TEST	ON TEST	ON TEST	EXPANDED
BCH-524	BCH-524	949	10/31/91	eod x 3, beg 18 hr pre	ip	1.6 - 50	ON TEST	ON TEST	ON TEST	EXPANDED
BCH-525	BCH-525	950	10/31/91	eod x 3, beg 18 hr pre	ip	1.6 - 50	ON TEST	ON TEST	ON TEST	EXPANDED

XVIII. PRESENTATIONS AND PUBLICATIONS

Presentations

1. Singh, V. K., R. W. Sidwell, and R. P. Warren. (1989) Immunologic properties of bropirimine in Punta Toro virus-infected mice. Abst. Intmtn. Br., Amer. Soc. Microbiol., p.1.
2. Barnard, D. L. and R. W. Sidwell. (1989) The effects of a *Phlebovirus* inhibitory agent, ribamidine, on cell proliferation and macromolecular synthesis in LLC-MK_2 cells. Abst. Intmtn. Br., Amer. Soc. Microbiol., p.1.
3. Sidwell, R. W. J. H. Huffman, H. Renis, M. Kende and J. Huggins. (1989) *In vivo* antiviral activity of bropirimine, an orally effective immunomodulator. Abstracts of the 89th Annual Meeting of the American Society for Microbiology, 1989, p. 29.
4. Barnard, D. L. and R. W. Sidwell. (1989) The effects of a *Phlebovirus* inhibitory agent, ribamidine, on cell proliferation and macromolecular synthesis in LLC-MK_2 cells. Abstracts of the 89th Annual Meeting of the American Society for Microbiology, 1989, p. 386.
5. Sidwell, R. W., J. H. Huffman, V. K. Singh, R. P. Warren, J. Coombs, R. Burger, M. Kende, and J. H. Huggins. (1989) Use of a murine Phlebovirus infection model for the evaluation of immunomodulating agents. Presented at a workshop of the International Conference on Comparative and Applied Virology, Banff, British Columbia, Canada, October, 1989.
6. Sidwell, R. W. (1989) A comparison of the anti-Punta Toro virus activity of ribavirin, ribavirin triacetate, and ribamidine. Seminar presented to USAMRIID, September 13, 1989.
7. Sidwell, R. W. (1989) In vivo antiviral experiences with combinations of immunomodulators and antiviral agents. Presented at the symposium, Immunomodulators as Antiviral Agents, International Congress of Chemotherapy, Jerusalem, Israel, June, 1989.
8. Sidwell, R. W., J. H. Huffman, V.K. Singh, R.P. Warren, J. Coombs, R. Burger, M. Kende, and J. Huggins. (1990) Use of the Punta Toro virus murine phlebovirus model for the evaluation of immunomodulating agents alone and in combination with antivirals. Presented at the UCLA Symposium Workshop, Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics. Keystone, Colorado.
9. Sidwell, R. W. (1990) Effect of drug combinations on in vivo Punta Toro virus infections in mice. Seminar presented to USAMRIID, May 22, 1990.
10. Sidwell, R. W., J. H. Huffman, V. K. Singh, R. P. Warren, R. Burger, L. Hall, J. Coombs, M. Kende, and J. H. Huggins. (1990) Antiviral activity of ampligen used alone and in combination with ribavirin. Am. Soc. Virology, Salt Lake City, UT, July 10, 1990.
11. Mead, J., R. Burger, Y. Yonk, J. Coombs, R. Warren, M. Kende, J. Huggins, and R. Sidwell. (1990) Effect of recombinant human interleukin-2 (rIL-2) on Punta Toro virus infections in C57BL/6 mice. Am. Soc. Virology, Salt Lake City, UT, July 10, 1990.
12. Coombs, J., R. W. Sidwell, J. Huffman, H. Renis, J. Huggins, and M. Kende. (1990) A comparison of pyrimidinone analog immunomodulators for treatment of Phlebovirus infections in mice. Intmtn. Amer. Soc. Microbiology, Pocatello, ID, April 21, 1990.
13. Smee, D. F., J. H. Huffman, J. Coombs, J. W. Huggins, and R. W. Sidwell. (1990) Effects of 7-thia-8-oxoguanosine alone and in combination with ribavirin on Punta Toro virus infections in mice. Third Internatl. Conf. on Antiviral Res. Abst. 153.
14. Huffman, J. H. , R. W. Sidwell, J. Coombs, J. Huggins, and M. Kende. (1991) Treatment of Phlebovirus infections of mice by poly ICLC. Presented at the Fourth International Conference on Antiviral Research, New Orleans, LA. 25 April, 1991.
15. Sidwell, R. W. 1990. Combinations of ribavirin with biological response modifiers. Presented at the World AIDS Day Symposium, "The Place of Ribavirin in the Therapy of HIV Infection", Dublin, Ireland, 1 December, 1990.

16. Sidwell, R. W. 1991. Utilizing BRMs in combination with antivirals against experimentally induced virus infections. Presented at the symposium, "BRMs as Antiviral Substances", at the First International Congress on Biological Response Modifiers, Quebec City, Canada, March 23, 1991.

17. Smee, D. F., J. L. B. Morris, D. L. Barnard and A. Van Aerschot (1991). Selective inhibition of alpha-, arena-, bunya-, and flaviviruses by 3'-fluoro-3'-deoxyadenosine. Presented at the Intermountain Branch Meeting of the American Society for Microbiology, Logan, Utah April 13, 1991.

18. Sidwell, R. W. 1991. Effects of combined therapies with ribavirin and ampligen on Punta Toro virus infections in mice. Presented at the National Institutes of Health meeting on Combination Therapies.

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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.

2. Sidwell, R. W., J. H. Huffman, B. B. Barnett, M. Kende, and D. Y. Pifat. 1988. Effects of a series of immunomodulators on experimental *Phlebovirus* infections. Antiviral Res. 9:125.

3. Gabrielsen, B. J., M. A. Ussery, P. G. Canonico, G. R. Pettit, E. M. Schubert, and R. W. Sidwell. 1988. Anti-RNA-viral activities of phenanthridones related to narciclasine. Antiviral Res. 9:97.

4. Sidwell, R. W., J. H. Huffman, D. L. Barnard, and D. Y. Pifat. 1988. Effects of ribamidine, a 3-carboxamidine derivative of ribavirin, on experimentally induced *phlebovirus* infections. Antiviral Res. 10:193-208.

5. Huffman, J. H., R. W. Sidwell, R. K. Robins, G. R. Revankar, and D. Y. Pifat. 1989. *In vitro* and *in vivo Phlebovirus* inhibition by nucleosides related to ribavirin. Nucleotides and Nucleosides 8:1159-1160.

6. Sidwell, R. W. 1989. *In vivo* antiviral experiences with combinations of immunomodulators and antiviral agents. *In*: Recent Adv. Chemotherapy (E. Rubinstein and D. Adam, eds.) pp. 713, 1-3. Lewin-Epstein Ltd., Jerusalem.

7. Smee, D.F., J.H. Huffman, L.L. Hall, J.W. Huggins, and R.W. Sidwell. 1990. Inhibition of *Phlebovirus* infections in vivo by tiazofurin and selenazofurin. Antiviral Chem. & Chemother. 1:211-216.

8. Sidwell, R.W., J.H. Huffman, J. Coombs, H. Renis, J. Huggins, and M. Kende. 1990. A comparison of pyrimidinone analog immunomodulators for treatment of *Phlebovirus* infections in mice. Antiviral Chem. and Chemother. 1:241-248.

9. Smee, D.F., J.H. Huffman, J. Coombs, J.W. Huggins, and R.W. Sidwell. 1991. Prophylactic and therapeutic activities of 7-thia-8-oxoguanosine against Punta Toro virus infections in mice. Antiviral Res. 15:229-239.

10. Smee, D.F., J. Coombs, J.H. Huffman, J.W. Huggins, and R.W. Sidwell. 1991. Combination chemotherapy of Punta Toro virus infections in mice using ribavirin and 7-thia-8-oxoguanosine. Antiviral Chem. and Chemother. 2:93-97.

11. Sidwell, R.W., J.H. Huffman, D.F. Smee, M. Kende, and J. Huggins. 1991. Synergistic use of ribavirin in combination with immune modulators for the treatment of *Phlebovirus* infections. In: Proc. Irish Conference on Ribavirin (Smith, R.E., ed.) Academic, NY (in press).

12. Deyrup, M., R. Sidwell, R. Little, P. Druzgala, N. Bordor, and M.E. Brewster. 1991. Improved delivery through biological membranes. 54. Synthesis, and antiviral activity of a series of ribavirin chemical delivery systems: 5' and carboxamide derivatives. Antiviral Chem. and Chemother. 2:337-356.

13. Sidwell, R. W., J. H. Huffman, D. F. Smee, J. Gilbert, M. Kende, and J. Huggins. 1991. Utilizing BRMs in combination with antivirals against experimentally induced virus infections. Canad. J. Infect. Dis. (in press).

14. Mead, J. R., R. A. Burger, L. J. Yonk, J. Coombs, R. P. Warren, M. Kende, J. Huggins and R. W. Sidwell. 1991. Effect of human, recombinant interleukin 2 on Punta Toro virus infections in C57BL/6 mice. Antiviral Res. 15:331-340.

15. Bhagrath, M., R. Sidwell, K. Czako, K. Seyda, W. Anderson, N. Bordor, and M.E. Brewster. 1991. Improved delivery through biological membranes. Synthesis, characterization and antiviral activitiy of a series of ribavirin chemical delivery systems: 5' and carboxamide derivatives. Antiviral Chem. and Chemother. 2:265-286.

16. Sidwell, R.W., J.H. Huffman, D.F. Smee, J. Gilbert, A. Gessaman, A. Pease, R.P. Warren, J. Huggins, and M. Kende. 1991. Potential role of immunomodulators for treatment of *Phlebovirus* infections of animals. N.Y. Acad. Sci. (in press).

17. Gabrielsen, B.J., T.P. Monath, J.T. Rankin, J.W. Huggins, D.F. Kefauver, C.D. Kwong, C.A. Krauth, J.A. Secrist III, J.H. Huffman, D.F. Smee, and R.W. Sidwell. 1992. Synthesis and in vivo anti-RNA viral evaluation of a phosphoramidate derivative of 6-thiouridine; orotidylic acid decarboxylase inhibitors, pyrazofurin and 6-azauridine; and 2-thio-6-azauridine and its triacetate. Antiviral Res. 17(suppl. I):149.

18. Huffman, J.H., R.W. Sidwell, J. Gilbert, J.W. Huggins, and T. Monath. 1992. Comparison of the in vivo anti-Punta Toro virus activity of ribavirin, ribavirin triacetate and ribamidine in mice. Antiviral Res. 17(suppl. I):152.

19. Smee, D.F., J. Gilbert, B.B. Barnett, J.H. Huggins, and R.W. Sidwell. 1992. Treatment of lethal Pichinde virus infections in weanling LVG/Lak hamsters with ribavirin, ribamidine, selenazofurin, and ampligen. Antiviral Res. (submitted Feb. 1992).

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[a]The majority of the technicians were students and worked part-time; some were only employed 2–3 months.

[b]Students.

STUDENTS RECEIVING DEGREES FOR THIS SUPPORT

No students received degrees due to the less-than-one-year duration of this contract. See above for students who received support, however.