

DURATION OF RESISTANCE TO VIRAL INFECTION FOLLOWING ADMINISTRATION OF INTERFERON INDUCERS*

JEROME P. SCHMIDT, FRANK F. PINDAK, DAVID J.
GIRON, AND ROBERT R. IBARRA

*Infectious Diseases Branch, Biosciences Division,
USAF School of Aerospace Medicine, Aerospace Medical Division (AFSC),
Brooks Air Force Base, Texas 78235*

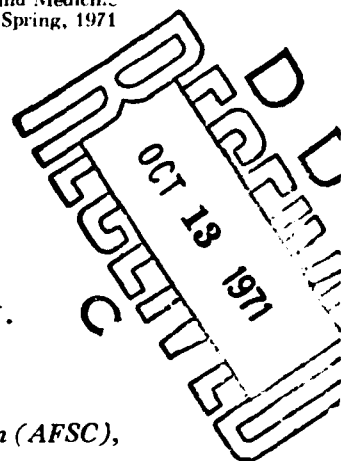
*Interferon; interferon inducers; statolon; pyran; poly I/C;
endotoxin; Newcastle disease virus; antiviral resistance*

ABSTRACT

The duration of protection afforded by 5 known interferon inducers was determined. Groups of mice were given a single dose of statolon, maleic acid-divinyl ether copolymer (pyran), polycytidylic/polyinosinic acid (I/C), Newcastle disease virus (NDV), or bacterial endotoxin. These groups and an untreated control group were uniformly challenged with MM virus. Survival data obtained during 20 days after infection revealed that the duration of protection varied with the inducer. The longest periods of significant protection elicited by the inducers were: endotoxin, 5 days; NDV, 8 days; I/C, 30 days; statolon or pyran, 55 days. These findings show that with the dosage used, the longest lasting protection was afforded by statolon and pyran.

Various investigators have suggested that some viral infections could be prevented or suppressed by appropriate use of interferon. Many types of interferon inducers are available presently, but it is difficult to ascertain which inducers have the longest lasting effect, since they have been tested under dissimilar conditions. In this paper, we compared the duration and degree of protection afforded by administration of 5 well-known interferon inducers. All inducers were studied in a homogenous group of mice from the same source and of the same age and sex. All animals received a uniform viral challenge on the same day. Thus, with experimental conditions as uniform as possible, more meaningful comparisons of the materials tested could be made.

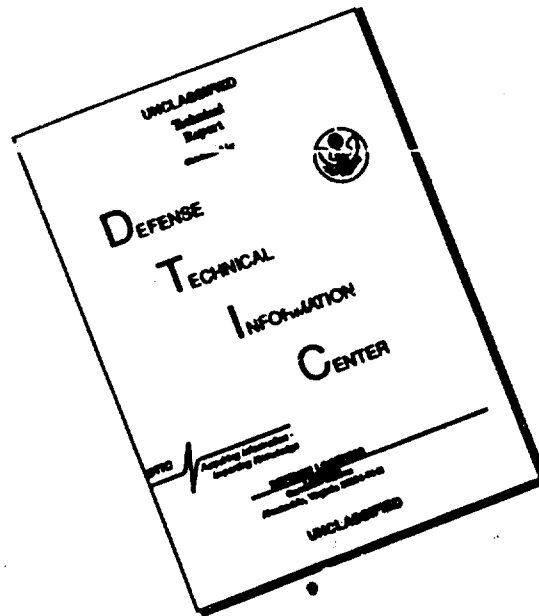
* Animals involved in this study were maintained in accordance with the *Guide for Laboratory Animal Facilities and Care*, as published by the National Academy of Sciences—National Research Council. Rec'd for pub. Aug. 11, 1970.



AD 30836

9

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

MATERIALS AND METHODS

Interferon inducers

Statolon, lot No. 354-869B-139, was kindly supplied by W. J. Kleinschmidt of the Lilly Research Laboratories. Maleic acid-divinyl ether copolymer (pyran) was obtained from Hercules, Inc., Wilmington, Del., and synthetic double-stranded ribonucleic acid prepared from polycytidylic and polyinosinic acid (I/C) was received as the double-stranded complex from Mann Laboratories, N.Y. Lipopolysaccharide B. *E. coli* 0:128:B12 (endotoxin), was purchased from Difco Laboratories, Detroit, Mich. Each inducer was administered intraperitoneally in 0.2-ml amounts. This volume contained 8 mg of statolon (760 μ g of nondialyzable solid), 200 μ g of pyran, 400 μ g of I/C, or 100 μ g of endotoxin, respectively. Statolon, pyran, I/C, and endotoxin were suspended in Hanks' balanced salt solution. The Newcastle disease virus (NDV) was propagated in 10-day chick embryos. Two tenths ml of undiluted allantoic fluid contained 2×10^8 plaque-forming units.

Groups of 30 mice received a single dose of a given inducer. Statolon, pyran, and I/C were administered to a total of 7 groups of mice at 1, 2, 5, 8, 19, 30, or 55 days. NDV was given at 1, 2, 5, 8, or 19 days, and endotoxin at 1, 2, 5, or 8 days before viral challenge. These treatments were scheduled in such a way that all groups could be challenged with MM virus on the same day. The interferon-inducing potency of each inducer was verified in tests conducted simultaneously, with parallel groups of mice from the same population as the test animals.

Animals

The young adult male Swiss albino mice used weighed approximately 20 g were of uniform age, and were received as a single shipment from the BioScience Animal Laboratories, Oakland, Calif. They were maintained in groups of 10 per cage and had unlimited access to food and water. No signs of illness were found among them during a 14-day quarantine period prior to inoculations with the inducers.

Viral challenge

All treated animals, as well as an untreated control group, were inoculated intraperitoneally with MM virus propagated in tissue culture as previously described (21). The stock virus was diluted in Hank's balanced salt solution to contain approximately 100 plaque-forming units in 0.2 ml. All animals were inoculated from the same virus suspension, and were under observation for 20 days following the viral challenge. The deaths were recorded daily.

RESULTS

The interferon inducers alone, in dosages used, appeared to be well tolerated by the animals, since no adverse reactions following their administration were seen. Their protective effect against the challenge with MM virus was determined from the comparisons of survival in the individual treatment groups to that in the untreated control group. The percentage of mice which survived for 20 days after infection is plotted in Fig. 1.

Fifteen of 43 untreated mice survived (34.9%). Since these animals

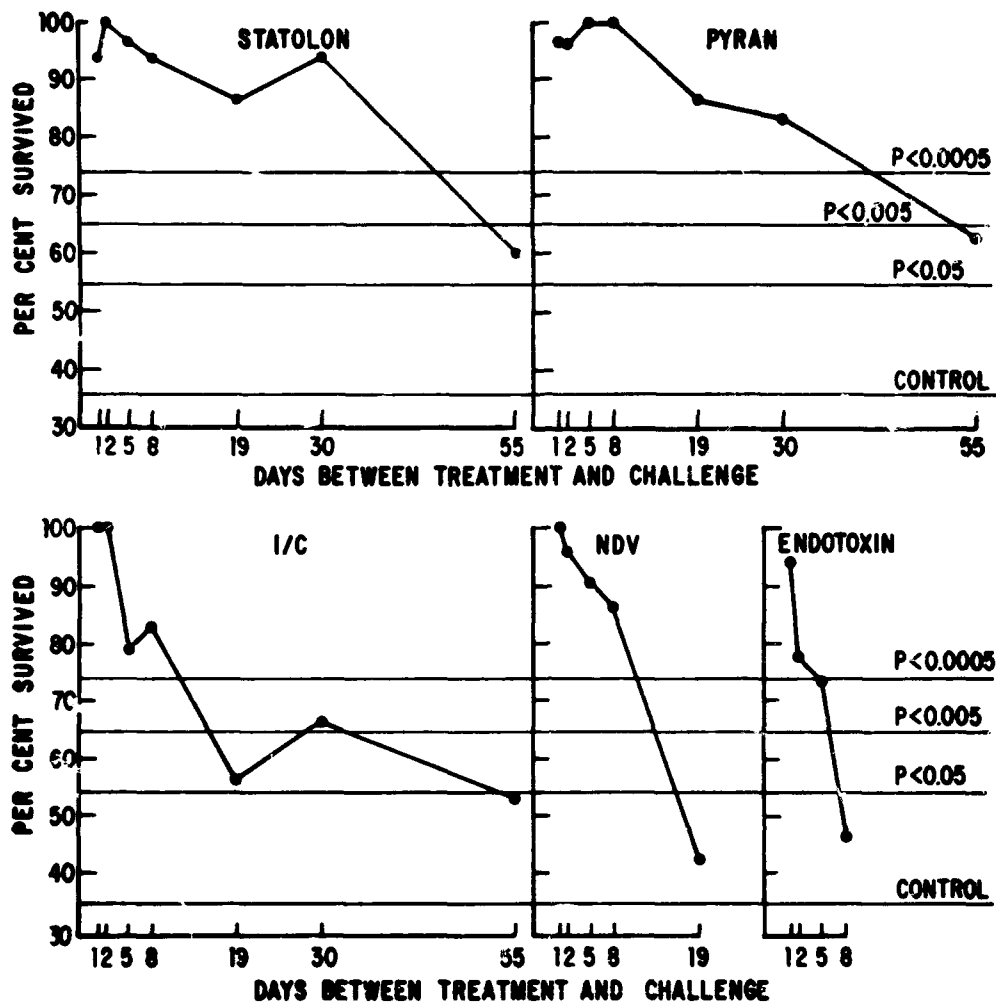


FIG. 1. Persistence and statistical significance of protection elicited by interferon inducers.

served as a control group for all other groups, their survival percentage is given as a straight line. Among the treated groups, survival was dependent on the type of interferon inducer used and on the time of its administration before infection. The three probability levels of significance of the differences between the survival in the treated groups and the control, as indicated in Fig. 1, were derived from χ^2 determinations.

There was a marked similarity in the protective effect afforded by statolon and pyran. The increased survival following administration of either one on 1, 2, 5, 8, 19, or 30 days before viral challenge was significant at the $P < 0.005$ level. Noticeably lesser effect, but still significant at the $P < 0.05$ level, was associated with treatments given at 55 days before challenge. Longer periods between treatment and

challenge were not tested. I/C was most effective when given 1 or 2 days before challenge, as the complete protection of the groups treated at these two intervals has shown. The protection afforded by this compound was of a lesser duration than that derived from statolon or pyran. Although still moderately effective at 30 days after treatment ($P < 0.005$), the increased survival at 55 days was not significant ($P > 0.05$).

Both NDV and endotoxin are also highly effective initially, but the protection afforded by them diminished to insignificant levels in a considerably shorter time. No essential increase in survival was seen in groups infected at 19 days after NDV and at 8 days after endotoxin inoculations.

A detailed analysis of the effect of the interferon inducers on the prolongation of the mean survival time reported previously for statolon (21) was not possible, since, in many groups, the scarcity of deaths would not allow meaningful evaluation. Nevertheless, certain conclusions could be drawn from the available data, as Table 1 shows. Disregarding the time interval between treatment and challenge, all deaths occurring in the groups treated with the individual inducers were summed. From these numbers, the percentages of deaths which occurred later than 11 days after challenge were determined. This demarcation point was chosen since it coincided with the latest time of death in the untreated control group. It can be seen that the postponement of deaths was considerably greater among animals treated with statolon or I/C than in the other groups.

DISCUSSION

Induction of interferon following treatment with statolon has been demonstrated in tissue culture (11-13), in the mouse (12, 25, 27),

TABLE 1

Effect of the various interferon inducers on the time of death of challenged animals

Groups treated with	Deaths* total	Percent of deaths occurring later than 11 days after challenge
Statolon	23	39.1
Pyran	22	4.5
I/C	48	12.5
NDV	24	4.2
Endotoxin	33	3.0
Control	28	0.0

* Deaths in all treated groups pooled, regardless of the time interval between treatment and challenge.

and in the monkey (12). Mammalian cell cultures treated with pyran, I/C or NDV (7) have been shown to release varying amounts of interferon. Similarly, interferon has been induced in the mouse by pyran (16), I/C (4, 8), NDV (2, 8, 9, 15), or endotoxin (9, 24, 27, 28). We verified the interferon-inducing potency of the materials studied but did not determine the levels of interferon in our animals at the time of challenge because the kinetics of interferon induction and decay of these agents have been amply documented by other investigators. As a more meaningful gauge of effectiveness, we compared the protection elicited by them against a uniform challenge.

Statolon has been shown to protect mice against Columbia SK virus (1), MM virus (12), mengovirus (20, 23), Friend leukemia virus (25, 26), and several other viral agents. The protective effect of pyran against mengovirus and vaccinia virus has been also described (17, 18). Significant levels of protection by I/C have been achieved in mice against several human viruses (14, 19), Columbia SK (4, 6), mengovirus (17), leukemia (29), and pneumonia virus in mice (6). Protection against the latter agent by bacterial endotoxin had been described long before the existence of interferon became known (10).

Our experience has shown that at the dose levels used highly significant protection of challenged animals was obtained with all inducers tested. The increased survival in the groups which were treated one day before infection differed from the untreated control at the $P < 0.0005$ level (Fig. 1), and the differences among the individual treated groups were not significant ($P > 0.05$). However, when longer time intervals were allowed between treatment and challenge, individual patterns in the persistence of the refractoriness developed. The protective effect waned gradually, at rates which appeared characteristic of the particular inducers. The protection resulting from the endotoxin treatment was of the shortest duration. Mice treated with NDV remained protected longer than those which received endotoxin, but not as long as those treated with I/C. The longest lasting protection was observed in groups treated with statolon or pyran. The effectiveness of these two substances appeared to be markedly similar.

Neither the degree nor the duration of the increased resistance could be correlated with either the amount or the persistence of circulating interferon as cited in the literature. Endotoxin-induced interferon was reported to reach the peak level in about 2 hours, and little or none was demonstrable at 6 hours after this inducer was administered (5, 9, 24, 27). Interferon induced by NDV reached maximal serum concentration in 4 to 12 hours, falling to subdetectable levels in approximately 72 hours (2, 9, 24, 26). The peak for I/C-induced interferon

was 3 hours, followed by gradual decline after 8 to 12 hours (4, 15). Interferon was no longer demonstrable at 48 hours after administration of pyran (16) and statolon (27). These reports indicate that, at the time our animals were challenged, residual levels of interferon could have been demonstrable only in the groups treated 1 or 2 days earlier with NDV (26), pyran (16), or statolon (27). In all other groups, regardless of the inducer used, or the time of its administration before the challenge, interferon could no longer be demonstrated by the presently available assay techniques. Thus, the differences in the duration of protection seen could not be adequately explained solely on the basis of the persistence of demonstrable interferon.

The observations here described suggest that either the qualities of interferons induced by the various substances are markedly dissimilar or that other mechanism(s) than interferon take part in the protection of the intact animal challenged with the virus. This possibility was suggested by other investigators (3). Whatever the true nature of the protection, statolon and pyran provided the longest lasting effect in these studies and should be further investigated using additional viral agents and animal species.

REFERENCES

1. Akers, T. G. and B. D. Stirling: Statolon-induced resistance of mice to lethal aerosols of Columbia-SK virus. *Proc. Soc. Exp. Biol. Med.*, 128: 931-934, 1968.
2. Baron, S. and C. E. Buckler: Circulating interferon in mice after intravenous injection of virus. *Science*, 141: 1061-1063, 1963.
3. DeClerq, E. and P. DeSommer: Prolonged antiviral protection by interferon inducers. *Proc. Soc. Exp. Biol. Med.*, 132: 699-703, 1969.
4. Dianzini, F., G. Rita, P. Cantagalli and S. Gagnoni: Effect of DEAE-dextran on interferon production and protective effect in mice treated with the double-stranded polynucleotide complex polyinosinic-polycytidilic acid. *J. Immun.*, 102: 24-27, 1969.
5. DeSommer, P. and A. Billiau: Interferon production by the spleen of rats after intravenous injection of sindbis virus or heat-killed *Escherichia coli*. *Arch. Ges. Virusforsch.*, 19: 143-154, 1966.
6. Field, A. K., A. A. Tytell, G. P. Lampson and M. R. Hilleman: Inducers of interferon and host resistance. II, Multistranded synthetic polynucleotide complexes. *Proc. Nat. Acad. Sci. U.S.A.*, 58: 1004-1010, 1967.
7. Finkelstein, M. S., G. H. Bausek and T. C. Merigan: Interferon inducers in vitro: difference in sensitivity to inhibitors of RNA and protein synthesis. *Science*, 161: 465-468, 1968.
8. Gresser, I., C. Fontaine-Brouty-Boyé, C. Bourali and M. T. Thomas: A comparison of the efficacy of endogenous, exogenous, and combined endogenous-

- exogenous interferon in the treatment of mice infected with encephalomyocarditis virus. *Proc. Soc. Exp. Med.*, 130: 236-242, 1969.
9. Hallum, J. V., J. S. Younger, and W. R. Stinebring: Interferon activity associated with high molecular weight proteins in the circulation of mice injected with endotoxin or bacteria. *Virology*, 27: 429-431, 1965.
 10. Horsfall, F. L., Jr. and M. McCarty: The modifying effects of certain substances of bacterial origin on the course of infection with pneumonia virus of mice (PVM). *J. Exp. Med.*, 85: 623-646, 1947.
 11. Kleinschmidt, W. J. and E. B. Murphy: Investigations on interferon induced by statolon. *Virology*, 27: 484-489, 1965.
 12. ———: Interferon induction with statolon in the intact animal. *Bact. Rev.*, 31: 132-137, 1967.
 13. Kleinschmidt, W. J. and G. W. Probst: The nature of statolon, an antiviral agent. *Antibiot. Chemother.*, 12: 298-309, 1962.
 14. Lindh, H. F., H. L. Lindsay, B. R. Mayberry and M. Forbes: Polyinosinic-cytidylic acid complex (poly I:C) and viral infections in mice. *Proc. Soc. Exp. Biol. Med.*, 132: 83-87, 1969.
 15. Merigan, T. C.: Purified interferons: physical properties and species specificity. *Science*, 145: 811-813, 1964.
 16. ———: Induction of circulating interferon by synthetic anionic polymers of known composition. *Nature (London)*, 214: 416-417, 1967.
 17. Merigan, T. C. and M. S. Finkelstein: Interferon-stimulating and *in vivo* effects of various synthetic anionic polymers. *Virology*, 35: 363-374, 1968.
 18. Merigan, T. C. and W. Regelson: Interferon induction in man by a synthetic polyanion of defined composition. *New Eng. J. Med.*, 277: 1283-1287, 1967.
 19. Nemes, M. M., A. A. Tytell, G. P. Lampson, A. K. Field and M. R. Hilleman: Inducers of interferon and host resistance. VI. Antiviral efficacy of poly I:C in animal models. *Proc. Soc. Exp. Biol. Med.*, 132: 776-783, 1969.
 20. Pindak, F. F. and J. P. Schmidt: Treatment of mengovirus infection in mice with statolon. *Appl. Microbiol.*, 15: 1240-1243, 1967.
 21. ———: Delayed death associated with statolon treatment of mice infected with mengovirus. *Texas Rep. Biol. Med.*, 26: 573-580, 1968.
 22. ———: Propagation of MM virus in continuous cell lines. *Appl. Microbiol.*, 17: 815-818, 1969.
 23. ———: Duration of prophylactic effect of statolon against mengovirus in mice. *Appl. Microbiol.*, 16: 468-469, 1968.
 24. Stinebring, W. R. and J. S. Younger: Patterns of interferon appearance in mice injected with bacteria or bacterial endotoxin. *Nature (London)*, 204: 712, 1964.
 25. Wheelock, E. F.: Effect of statolon on Friend virus leukemia in mice. *Proc. Soc. Exp. Biol. Med.*, 124: 855-858, 1967.
 26. Wheelock, E. F., N. L. Caroline, and R. D. Moore: Suppression of established Friend virus leukemia by statolon. I. Demonstration of latent infection in clinically normal mice. *J. Virol.*, 4: 1-6, 1969.

27. Youngner, J. S. and W. R. Stinebring: Interferon appearance stimulated by endotoxin, bacteria, or viruses in mice pretreated with *Escherichia coli* endotoxin or injected with *Mycobacterium tuberculosis*. *Nature* (London), 208: 456-458, 1965.
28. —————: Comparison of interferon production in mice by bacterial endotoxin statolon. *Virology*, 29: 310-316, 1966.
29. Zelenick, L. D. and B. K. Bhuyan: Treatment of leukemic (L-1210) mice with double-stranded polyribonucleotides. *Proc. Soc. Exp. Biol., Med.*, 130: 126-128, 1969.

ACCESSION FOR		
CFSTI	WHITE SECTION	<input checked="" type="checkbox"/>
DDC	BUFF SECTION	<input type="checkbox"/>
UNPUBLISHED		<input type="checkbox"/>
JURISDICTION		
BY		
DISTRIBUTION/AVAILABILITY CODES		
DIST.	AVAIL.	and/or SPECIAL
A	20	

Unclassified
Security Classification

AD-730 836

DOCUMENT CONTROL DATA - R & D		
<i>(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)</i>		
1. ORIGINATING ACTIVITY (Corporate author) USAF School of Aerospace Medicine Aerospace Medical Division (AFSC) Brooks Air Force Base, Texas 78235		2a. REPORT SECURITY CLASSIFICATION Unclassified
		2b. GROUP
3. REPORT TITLE DURATION OF RESISTANCE TO VIRAL INFECTION FOLLOWING ADMINISTRATION OF INTERFERON INDUCERS		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates) Final report June 1969 - January 1970		
5. AUTHOR(S) (First name, middle initial, last name) Jerome P. Schmidt David J. Giron Frank F. Pindak Robert R. Ibarra		
6. REPORT DATE March 1970	7a. TOTAL NO. OF PAGES 8	7b. NO. OF REFS 29
8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S) SAM-TR-70-299	
b. PROJECT NO. 7753 Task No. 775308	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
d. Work Unit No. 009		
10. DISTRIBUTION STATEMENT Approved for public release; distribution unlimited.		
11. SUPPLEMENTARY NOTES Texas Rep. Biol. Med. 29:1, 1971	12. SPONSORING MILITARY ACTIVITY USAF School of Aerospace Medicine Aerospace Medical Division (AFSC) Brooks Air Force Base, Texas 78235	
13. ABSTRACT The duration of protection afforded by 5 known interferon inducers was determined. Groups of mice were given a single dose of statolon, maleic acid-divinyl ether copolymer (pyran), polycytidylic/polyinosinic acid (I/C), Newcastle disease virus (NDV), or bacterial endotoxin. These groups and an untreated control group were uniformly challenged with MM virus. Survival data obtained during 20 days after infection revealed that the duration of protection varied with the inducer. The longest periods of significant protection elicited by the inducers were: endotoxin, 5 days; NDV, 8 days; I/C, 30 days; statolon or pyran, 55 days. These findings show that with the dosage used, the longest lasting protection was afforded by statolon and pyran.		

DD FORM 1 NOV 65 1473

Unclassified
Security Classification

14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Interferon Endotoxin Statolon Pyran Poly I/C MM virus Interferon inducers Newcastle disease virus Antiviral resistance						