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Effect of Administered Interferon on Rabies in Rabbits

BOSKO POSTIC AND PAUL FENJE

The Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15213, and The Connaught Medical Research Laboratories, University of Toronto, Willowdale, Ontario

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This study describes the effect of interferon on the survival of rabbits infected with a street strain of rabies virus. Interferon was prepared by collecting serum from rabbits injected with Newcastle disease virus and was characterized by biological and physicochemical methods. Rabbit serum interferon mixed and incubated with a suspension of rabies virus did not neutralize its infectivity. Rabbits were inoculated into the hind leg muscle with approximately 80 LD_{50} of virus. Interferon was administered intravenously or intramuscularly, or by both methods, in the same or opposite leg as virus. Mortality due to rabies was significantly reduced by the concurrent administration of 8×10^5 units of interferon divided between the site of virus inoculation and intravenously. There was less protection if 3 hr elapsed between the inoculation of virus and interferon. Treatment given 24 hr after infection did not prevent death but prolonged the incubation period.

Several recent studies in rabbits suggest the usefulness of interferon in the prophylaxis of rabies. Partial protection resulted from injections with virus-free filtrates of skin and brain homogenates from rabbits infected with vaccinia virus, presumably due to interferon (22, 23). Rabbits treated with an inducer of interferon, the polyribonucleotide complex of inosinic and cytidylic acids, poly I·poly C (10), resisted challenge with rabies virus (8, 9; B. Janis and K. Habel, Fed. Proc. **29:** 636, 1970). Injections with infectious or inactivated myxoviruses, which may have acted by inducing interferon, were also effective (1, 6).

The purpose of this investigation was to examine the efficacy of administered interferon in preventing rabies. The administration of rabbit serum interferon of high potency protected from encephalitis and death due to rabies virus.

MATERIALS AND METHODS

The rabies street virus originated from a naturally infected fox (7). The virus suspension was stored as a 10% homogenate of the salivary gland and contained 8,000 rabbit intramuscular (im) LD_{50} per 0.4 ml. For the titration of virus, as well as for challenge of interferon-treated and untreated (control) rabbits, the suspension was diluted as described below, and 0.4 ml was injected into the quadriceps femoris muscle. The dose of the injected virus, as rabbit LD_{50}, was calculated by the Reed-Muench method. New Zealand white rabbits of both sexes, purchased from a Toronto breeder, weighed approximately 1 kg each when injected with rabies virus. After injection, they were observed for 6 weeks as in previous studies (8, 9). With the given strain of street virus, the encephalitic signs of disease were invariably followed by the death of rabbits in 2 to 3 days.

For the preparation of exogenous interferon, 2- to 3-kg albino rabbits from Animal Resources, Inc., Baltimore, Md., were injected intravenously (iv) with 1.5×10^9 plaque-forming units (PFU) of Newcastle disease virus (NDV) CG strain (13). Four hours later the animals were anesthetized with sodium pentocarbital (Diamond Laboratories) and exsanguinated, and the serum was obtained. To assure inactivation of residual infectivity, it was heated at 56 C for 1 hr (18) and then stored at −70 C. Sera showing the highest interferon titers were pooled. Two such pools (A and B), containing 1.6×10^6 and 4×10^5 units per 2 ml, respectively, were used for iv or im injections of rabbits. The ear vein was used for iv and the quadriceps femoris muscle was used for im administration of interferon.

The interferon activity was assayed by reduction of vesicular stomatitis virus (VSV) plaque formation in primary rabbit kidney monolayers. Titers were expressed in international units per 2 ml by comparison to the standard rabbit interferon (NIH Research Reagent G-019-901-028) and represent reciprocals of serum dilutions inhibiting 50% of VSV plaques (14, 19).

For the identification of interferon, samples of serum, diluted at 1: 10 in Hanks balanced salt solution (*p*H 6.8), were incubated with pancreatic ribonuclease, crystalline trypsin (both from Worthington Corp.), or antiserum to NDV, at concentrations as described below. Antiserum was prepared by injecting two rabbits intramuscularly with weekly doses of 5×10^{10} PFU of NDV suspended in phosphate-buffered saline

(*p*H 7.2). The animals were bled 6 weeks after the beginning of immunization, and their sera were pooled and then heated for 1 hr at 56 C. This antiserum possessed the neutralizing titer of 1:6,000 for the homologous virus as determined by the standard serum dilution end-point method. Virus neutralization and the titrations of the infectivity of NDV and VSV were done by plaque formation on chick embryo monolayers as previously described for Sindbis virus (19, 20).

RESULTS

With pool A interferon, four groups of six rabbits each received different doses by different routes of administration. They were concurrently injected into the quadriceps femoris muscle with 0.4 ml of the 1:100 dilution of the suspension of rabies street virus. This dose corresponded to 80 rabbit im LD_{50}, as determined by a separate titration. The results of treatment with interferon are shown in Table 1. The most effective treatment resulting in net protection was that given to group 4, which received the maximal dose of interferon by two im and one iv injection. The im route appeared to be superior to iv administration, provided that interferon was injected into the same leg muscle as virus (compare group 1 with groups 2 and 3). The incubation period was extended in all treated groups, even when the rabbits succumbed, suggesting some antiviral effect. Untreated animals (groups 5 to 7) all died after a shorter incubation period.

Parallel to the above-described experiment, pool B interferon was injected into another set of 18 rabbits either concurrently or after rabies virus (Table 2). All animals were inoculated im into the hind leg with a 1:100 dilution of the virus suspension. Thereupon, each rabbit received two injections of interferon, one into the site of virus inoculation and another one iv. Protection resulted when rabbits were treated at the time of infection, but it was reduced when interferon was administered 3 hr later. Interferon only prolonged the incubation period when used 24 hr after the infection.

To test whether the rabbit serum interferon had a direct inactivating effect on the infectivity of rabies virus, the following two experiments were performed. (i) The CVS strain of fixed rabies virus, diluted to contain 100 to 200 mouse LD_{50} per 0.03 ml, was mixed with equal amounts of either interferon pool B or with serum from uninoculated rabbits (control). After incubation of these sets at 37 C for 1.5 hr, the standard mouse test for the determination of rabies virus-neutralizing antibodies was performed as in a previous study (7). No neutralizing activity to CVS was detected in either rabbit serum. (ii) A 0.5-ml amount of street virus suspension, appropriately diluted to contain 80 rabbit LD_{50}, was mixed and incubated as above with an equal volume of undiluted interferon (pool B) or control serum. The residual infectivity of these two sets was determined by inoculating im groups of four rabbits each with fivefold dilutions of the virus-serum mixtures. The titration of the mixtures showed that both the interferon and the control set contained 20 rabbit LD_{50} of virus per 0.4 ml. The largest dose of interferon contained in the inoculum (virus-serum mixture diluted at 1:5) was 8,000 units. This was insufficient to affect either

TABLE 1. *Effect of exogenous interferon on rabies in rabbits*

Group	Injections (concurrently)[a]				Outcome[b]	
	Interferon			Rabies virus (dilution)	Dead/inoculated	Incubation (days)
	Amt (ml)	Route	Total[c]			
1	2	im(s)	1.60	1:100	3/6	26
2	2	im(o)	1.60	1:100	6/6	17.5
3	4	iv	3.20	1:100	5/6	18
4	2	im(s)	6.40	1:100	0/6	
	2	im(o)				
	2	iv				
5	None			1:100	4/4	14.5
6	None			1:500	4/4	13.5
7	None			1:2,500	4/4	15

[a] Interferon intravenously (iv) or intramuscularly (im), or by both methods, either into the same (s) or opposite (o) leg in respect to the virus.

[b] Incubation is the median period from the inoculation to the onset of encephalitic symptoms in rabbits succumbing to rabies.

[c] Units per rabbit. Values expressed $\times 10^6$.

the incubation period or the lethal outcome of the infection. Taken together, the results of these two experiments indicate that interferon administered into the site of virus entry (Table 1) probably did not act by inactivating the inoculum directly.

To identify interferon, samples from serum pool B were subjected to treatments listed in Table 3. The serum interferon was diluted at 1:10 in Hanks solution and incubated for 1 hr at 37 C with one of three substances: trypsin, ribonuclease, or antiserum to NDV. Interferon activity was also tested after high-speed centrifugation, heating (56 C), and exposure to acid *p*H. The only procedure which significantly reduced the antiviral activity on rabbit kidney cells was the digestion with trypsin. Treatment with potent antiserum to the inducing virus or high-speed centrifugation maintained the virus-inhibitory activity. After exposure to *p*H 2.5 (4 C), the titer was slightly increased. Variations of this magnitude were observed within a single experiment previously (14). In addition, pools A or B (both at 1:50 and 1:200) showed no inhibitory activity against VSV on chicken embryo cells.

TABLE 2. *Influence of the time of interferon administration on rabies in rabbits*

Time of treatment[a] (hr after virus)	Outcome[b]	
	Mortality	Incubation (days)
0	1/6	23
3	4/6	16.5
24	6/6	18.5

[a] Each rabbit was injected with 0.4 ml of the 1:100 dilution of virus (0 hr) into the hind leg muscle and received a total of 8×10^5 units of interferon. One half of the interferon dose (2 ml) was injected into the same site as virus and the rest was injected intravenously.

[b] Observations as under Table 1. For outcome in untreated rabbits, refer to Table 1 (group 5).

TABLE 3. *Identification of rabbit serum interferon*

Treatment	Result[a]	
	Units/2 ml	Per cent original activity
None	400,000	100
Crystalline trypsin (0.1%)	<100	<1
Pancreatic ribonuclease (50 μg/ml)	280,000	67
Antiserum to NDV (10%)	400,000	100
100,000 × *g* for 1 hr[b]	400,000	100
Heating at 56 C for 1 hr	300,000	75
*p*H 2.5 for 5 days at 4 C[c]	500,000	125

[a] Interferon was assayed on primary rabbit kidney cells (14, 19).

[b] Spinco L centrifuge and rotor 40 were used; the supernatant fluid was tested for interferon.

[c] For acidification, 1 *N* HCl was used. The *p*H was readjusted to 7.0 with 1 *N* NaOH prior to assay.

DISCUSSION

The experiments described herein showed that an antiviral substance identified as interferon suppressed rabies in rabbits. The present study confirms the impression gained by Vieuchange (22, 23) and Bahmanyar and co-workers (2). However, their interferon preparations were not characterized and some antirabies effect was noted with normal tissue constituents (2).

The site of interferon injection in respect to that of rabies virus was important for eliciting the antiviral effect. Best results were obtained when interferon was given into the same muscle in addition to iv administration. This could not be attributed to the direct virus-inactivating effect of rabbit serum interferon. Namely, the infectivity of rabies virus (either for the heterologous or the homologous host) was not affected by prior treatment with rabbit interferon in vitro. Normal rabbit serum, given iv and im into the same site and concurrently with virus, had no antirabies effect (P. Jenje, *unpublished data*). Vieuchange (22) also noted the effectiveness of local administration of interferon, and, subsequently, Janis and Habel (Fed. Proc. **29:** 636, 1970) obtained somewhat better protection when poly I:C was inoculated into the same leg as virus. The reason for the apparent local effect of interferon on rabies is not clear. Studies bearing on the fate of rabies virus at inoculation site showed persistence of infectivity for 4 to 6 days, but replication was not demonstrated (12, 17). It is conceivable that serum interferon may suppress rabies virus replication in the dorsal ganglia (R. T. Johnson, *In* F. Davenport and Y. Nagano (ed.), Symposium on Rabies, Univ. of Tokyo Press, *in press*), rather than in the central nervous system into which it diffuses poorly (5).

Contrary to the experience in rabbits, interferon or interferon inducers failed to protect mice challenged with fixed rabies virus (1, 3, 6, 11, 21). Poly I·poly C offered limited protection in this model (16). However, inactivated or live NDV was clearly effective in the hamster (1). The basis for the above differences between animal species is not understood, since the replication of fixed rabies virus was inhibited in cell culture by mouse interferon (4).

The present study may be relevant to the prevention of rabies in man. In the absence of a non-

toxic inducer, the application of exogenous interferon is justly entertained. Although the potency of interferons from different species cannot be equated, encouragingly a semipurified human interferon preparation titering above 10^6 units per ml was produced and shown to be active in primates against systemic infection with yellow fever virus (24). The possible addition of interferon to the prophylaxis of rabies in man would not be expected to decrease the effect of either vaccine or antiserum. Rather, it may contribute a needed antiviral measure (15) and reduce the number of therapeutic failures.

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LITERATURE CITED

1. Atanasiu, P., M. Barroeta, H. Tsiang, and S. Favre. 1970. Inhibition in vivo de la multiplication du virus rabique par un interféron endogène. Ann. Inst. Pasteur **119**:767–777.
2. Bahmanyar, M., A. Fayaz, and J. Vieuchang, 1967. Présence d'une substance du type interféron active a l'égard de la rage des rues, dans les extraits de tissus infectés de vaccine. C. R. Seances Soc. Biol. **161**:225–228.
3. Baron, S. 1967. Discussion, p. 215–216. *In* G. E. W. Wolstenholme and J. O'Connor (ed.), Ciba Found. Symp. Interferon. J & A Churchill, Ltd., London.
4. Barroeta, M., and P. Atanasiu. 1969. Action inhibitrice de l'interféron sur le développement du virion rabique en culture cellulaire. C. R. Acad. Sci. **269**:1353–1355.
5. Cathala, F., and S. Baron. 1970. Interferon in rabbit brain, cerebrospinal fluid and serum following administration of polyinosinic-polycytidylic acid. J. Immunol. **104**:1355–1358.
6. Fayaz, A., A. Afshaz, and M. Bahmanyar. 1970. Interference between bovine parainfluenza 3 virus and a street strain of rabies virus in rabbits. Arch. Gesamte Virusforsch. **29**: 159–162.
7. Fenje, P., and L. Pinteric. 1966. Potentiation of tissue culture rabies vaccine by adjuvants. Amer. J. Pub. Health **56**: 2106–2113.
8. Fenje, P., and B. Postic. 1970. Protection of rabbits against experimental rabies by poly I. poly C. Nature (London) **226**:171–172.
9. Fenje, P., and B. Postic. 1971. Prophylaxis of experimental rabies with the polyriboinosinic-polyribocytidylic acid complex (poly I:C). J Infec. Dis. **123**:426–428.
10. Field, A. K., A. A. Tytell, G. P. Lampson, and M. R. Hilleman. 1967. Inducers of interferon and host resistance. II. multistranded synthetic polynucleotide complexes. Proc. Nat. Acad. Sci. U.S.A. **58**:1004–1010.
11. Finter, N. B. 1967. Of mice and men: studies with interferon p. 204–215. *In*, G. E. W. Wolstenholme and M. O'Connor (ed.), Ciba Found. Symp. Interferon. J & A Churchill, Ltd., London.
12. Habel, K. 1941. Tissue factors in antirabies immunity of experimental animals. Pub. Health Rep. **56**:692–702.
13. Ho, M., M. K. Breinig, B. Postic, and J. A. Armstrong. 1970. The effect of preinjections on the stimulation of interferon by a complexed polynucleotide, endotoxin and virus. Ann. N.Y. Acad. Sci. **173**:680–963.
14. Ke, Y. H., J. A. Armstrong, M. K. Breinig, L. Ople, B. Postic, and M. Ho. 1970. The assay and standardization of rabbit interferons. Symp. Ser. Immunobiol. Stand. **14**:131–144.
15. Koprowski, H. 1967. Vaccines against rabies: present and future. *In* First Int. Conf. Vaccines Against Viral and Rickettsial Diseases of Man, p. 488–493. Sci. Publ. 147, PAHO/WHO.
16. Nemes, M. M., A. A. Tytell, G. P. Lampson, A. K. Field, and M. R. Hilleman. 1969. Inducers of interferon and host resistance. VI. Antiviral efficacy of poly I:C in animal models. Proc. Soc. Exp. Biol. Med. **132**:776–783.
17. Petrovic, M., and H. Timm. 1969. Experimenteller Beitrag zur Pathogenese der Tollwut. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. I Orig. **211**:149–161.
18. Postic, B., C. DeAngelis, M. K. Breinig, and M. Ho. 1966. Effect of temperature on the induction of interferons by endotoxin and virus. J. Bacteriol. **91**:1277–1281.
19. Postic, B., C. J. Schleupner, J. A. Armstrong, and M. Ho 1969. Two variants of sindbis virus which differ in interferon induction and serum clearance. I. The phenomenon. J. Infec. Dis. **120**:339–347.
20. Schleupner, C. J., B. Postic, J. A. Armstrong, R. W. Atchison, and M. Ho. 1969. Two variants of sindbis virus which differ in interferon induction and serum clearance. II. Virological characterizations. J. Infec. Dis. **120**:348–355.
21. Soave, O. A. 1968. Influence of statolon-induced interferon on rabies virus infection in mice. Amer. J. Vet. Res. **29**:1507–1508.
22. Vieuchange, J. 1967. Interférence entre le virus vaccinal et le virus rabique; rôle éventuel d'un interféron. Arch. Gesamte Virusforsch. **22**:87–96.
23. Vieuchange, J. 1969. Recherches sur l'action d'un interféron a l'égard de la rage experimentale du lapin. Bull. Acad. Nat. Med. (Paris) **153**:374–378.
24. Working Party on Human Interferon of the U.K. Scientific Committee on Interferon. 1970. Progress toward trials of human interferon in man. Ann. N.Y. Acad. Sci. **173**:770–781.