NOTES

Tilorone Hydrochloride: Lack of Correlation Between Interferon Induction and Viral Protection

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

Environmental Microbiology Branch, Epidemiology Division, USAF School of Aerospace Medicine (AMD-AFSC), Brooks Air Force Base, Texas 78235

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The protection of mice against MM virus infection and the induction of circulating interferon by tilorone hydrochloride were determined. Whereas protection was evident with doses of 0.15 and 1.5 mg/kg, interferon was not detected with doses lower than 150 mg/kg. Protection was apparently not dependent on interferon induction.

Tilorone hydrochloride, the orange, water-soluble dihydrochloride salt of 2,7-bis[2-(diethylamino) ethoxy] fluoren-9-one, is a broad-spectrum antiviral agent (5). In mice, it is effective against Semliki Forest virus by the oral, subcutaneous, and intraperitoneal treatment routes. It has also been shown to be effective against intranasal infection by vesicular stomatitis virus (VSV; 2). Its mode of action is presumably interferon stimulation (2, 6). We have investigated the protection elicited by different doses of tilorone given intraperitoneally against MM virus infection in mice. At the same time, the amount of circulating interferon induced by each dose of tilorone was determined.

Female Swiss Webster mice weighing about 14 g were treated intraperitoneally with different doses of tilorone diluted in Hanks balanced salt solution. Ten mice from each group were bled at 6, 12, and 24 hr after the administration of tilorone. The blood was pooled, and the plasma was collected and assayed for interferon activity. At 24 hr after the injection of tilorone, the mice remaining in each group were challenged intraperitoneally with about 100 plaque-forming units of MM virus (2 LD₅₀). Deaths were recorded daily for 20 days. The results (Table 1) show that, in terms of mortality and mean survival time, a tilorone dose of 7.5 mg/kg was sufficient to protect mice. They also suggest that doses as low as 0.15 and 1.5 mg/kg can have a protective effect, although at these doses the results were variable. Circulating interferon, however, was not detected with tilorone doses

of less than 150 mg/kg. As these data show, in our system there was no correlation between the protection of mice against MM virus infection and the amount of circulating interferon induced by tilorone.

These results are not in agreement with those of De Clercq and Merigan (2), who reported that the degree of protection of mice against intranasal infection with VSV was directly related to the titers of interferon induced by different doses of tilorone. Their interferon titers in response to tilorone were much higher than those in the present study. Since the 50% reduction in plaque count as a method for assay of interferon is more sensitive when MM virus is the challenge agent than when VSV is used (1), we must conclude that the differences in interferon titers in the two studies are real. This discrepancy could be due to a genotypic difference in the animals. Variations in titer of interferon have been reported to result not only from differences in age and genotype of the mice but to occur also among animals of the same age and genotype in different experiments (3, 4). We, too, have observed great differences, with either tilorone or other inducers, in the amounts of circulating interferon between experiments, but we have not seen such marked variations in the protective effects of the drugs (*unpublished data*). It is also apparent that MM virus is much more sensitive to the protective action of tilorone than is VSV (2). Thus, lower doses of tilorone, which do not induce detectable amounts of circulating interferon, were effective.

TABLE 1. *Interferon induction and protection of mice against MM virus infection by tilorone hydrochloride*

Tilorone ^a (mg/kg)	Mortality (dead/total)	Significance (P) ^b	MST ^c (days)	Significance (P) ^d	Circulating interferon (PR ₅₀ units) ^e		
					6 hr	12 hr	24 hr
0	35/40	— ^f	4.60	— ^f	<4	<4	<4
0.15	25/40	<.05	5.35	NS	<4	<4	<4
1.5	29/40	NS	5.96	<.005	<4	<4	<4
7.5	24/40	<.01	6.08	<.001	<4	<4	<4
37.5	10/39	<.001	7.22	<.001	<4	<4	<4
75.0	1/40	<.001	9.00	— ^f	<4	<4	<4
150.0	2/35	<.001	7.50	— ^f	<4	500	375

^a Groups of mice were treated intraperitoneally with the indicated doses; 24 hr later they were challenged with 2 LD₅₀ of MM virus.

^b Determined by chi-square. NS = not significant.

^c Mean survival time.

^d Determined by *t* test (two-tailed).

^e Interferon expressed as the reciprocal of the highest dilution inhibiting the number of MM virus plaques by 50%. Plasma samples were taken at the indicated times after tilorone administration.

^f Insufficient number of deaths to do statistical analysis.

Pindak et al. (*in press*) found that in mice there was no direct relationship between the amounts of demonstrable interferon induced by bacterial endotoxin, statolon, pyran, or polyinosinic-polycytidylic acid and the protection against MM virus infection. Merigan et al. (7) presented data which show a lack of correlation between the amounts of circulating interferon induced by pyran and polyinosinic-polycytidylic acid and the degree of protection by these agents against mengovirus infection in mice. These reports, together with the present study, imply that determination of circulating interferon titers is of limited value in assessing the potential of a substance as an antiviral agent.

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