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Effect of Tilorone Hydrochloride on the Lymphoid and Interferon Responses of Athymic Mice (39188)

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Tilorone hydrochloride (2,7-bis[(2-diethylamino)ethoxy] fluoren-9-one dihydrochloride) is an orally active antiviral agent and interferon inducer (1-4). It has also been reported to enhance the antibody (IgG, IgM, and IgE) response to several types of antigens (5-7) and to suppress cell-mediated immune responses associated with T-lymphocyte function as in experimental allergic encephalomyelitis, tuberculin skin response, adjuvant arthritis, and transplant rejection (6, 8). Tilorone also induces a marked but transient lymphopenia with depletion of lymphocytes in the T-cell areas of spleen, lymph node, and Peyer's patches (9, 10). These results suggest that the action of tilorone is selectively on the T-lymphocyte. Since the nude mouse (homozygous nu/nu) essentially lacks functional thymus tissue and is therefore considered to be devoid of T-lymphocytes (11, 12), it was felt that a comparison of the effects of tilorone on the lymphoid and interferon responses of normal and athymic mice might help in elucidating the mechanism of action of this compound.

Methods. Athymic nude mice (homozygous nu/nu)[1] and phenotypically normal litter mates were housed in groups in plastic cages on pine shavings and provided food and water *ad libitum*. The mice were separated into four groups, each containing five animals of mixed sex. One group of normal mice and one group of athymic mice were each administered 100 mg/kg of tilorone hydrochloride by gavage as a solution in distilled water. Similar groups of mice were given an equal volume of distilled water by gavage and observed for control purposes. Blood samples for hematology studies were collected from each mouse before dosing and at 8, 24, 48, and 72 hr after dosing. Collections were made in capillary tubes following tail nicking with a razor blade. Following appropriate dilution, total leukocyte counts were made with a Coulter Model F electronic particle counter. Blood smears were made with fresh blood and stained with Wright-Giemsa stain for differential leukocyte counting.

In order to determine the antiviral response to tilorone treatment, several additional mice, both normal and athymic were used. Treated mice were given 150 mg/kg tilorone hydrochloride by gavage as a single dose and were sacrificed 18 hr later and pooled serum samples evaluated for the presence of *in vitro* antiviral activity against vesicular stomatitis virus by plaque reduction assay on L929 cells as has been previously described (3, 4). Eighteen hours is the approximate time when the maximum serum antiviral activity can be seen following the oral administration of tilorone to mice.

Results. The mean lymphocyte counts determined for each of the groups during the course of the study are graphically presented in Fig. 1. As expected, lymphocyte counts prior to treatment were lower in athymic mice than in their normal controls (11). A mild reduction in the lymphocyte counts in all groups, including the controls, occurred 8 hr after dosing. This effect has been noted frequently in a number of studies of this type in both mice and rats and has been attributed to the stress of handling and dosing. Otherwise control values remained relatively stable throughout the test. Following tilorone treatment, the lymphocyte counts of athymic mice did not differ from the untreated controls. In comparison nor-

[1] Microbiology Department, University of Cincinnati, College of Medicine. Derived from G1. Bomholtgaard, Lab An. Breeding and Research Centre, Ry, Denmark.

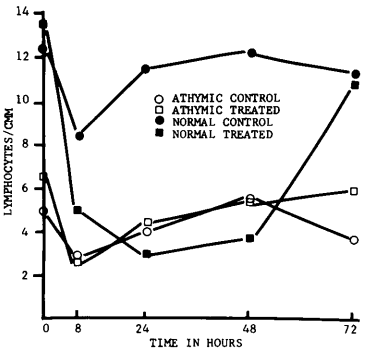

FIG. 1. Effect of oral tilorone hydrochloride administration (100 mg/kg) on the peripheral lymphocyte counts of mice (n = 5).

mal mice exhibited a definite and striking lymphopenia, which reached its nadir 24 to 48 hr after a single oral 100 mg/kg dose of tilorone. The lymphocyte counts in these mice had returned to normal levels by 72 hr after dosing.

Total white-cell counts, although not reported graphically in this report, revealed a definite leukopenia only in tilorone-treated normal mice. This leukopenia had a similar time sequence to that just described. No other group had this response. Total neutrophil counts exhibited a slight neutrophilia in both treated groups 8 hr after dosing and were back to normal levels by 24 hr. Such responses have been reported previously in normal mice and rats (9, 10).

The results of the serum antiviral assays are presented in Table 1. From these data it can be seen that the 150 mg/kg oral dose of tilorone did induce serum antiviral activity in both normal and athymic mice, when compared to their respective controls not receiving compound. The response of the athymic mice was very distinct, in spite of their lack of T-lymphocytes. The serum antiviral activity produced in normal mice was consistently higher than that produced in athymic mice, although the differences were not great.

Discussion. Since T-lymphocytes normally comprise a large percentage of the circulating lymphocytes of normal mice (13), a reduction in these cells will produce an obvious effect on peripheral lymphocyte counts in this species. Such was the case following tilorone treatment in normal mice, when the transient lymphopenia so characteristically seen following tilorone treatment was noted. Nude athymic mice, which lack T-lymphocytes (13), failed to develop this effect, however. These findings, in conjunction with the transient lymphoid depletion of T-cell areas previously reported in the spleen, lymph nodes, and Peyer's patches of normal mice (10), tend to confirm the specificity of tilorone for T-lymphocytes and explain the reason for the suppressed cell mediated immunity induced by this compound. The mechanism for the stimulatory effect of tilorone on antibody response is not clear at this time. Its effect on T-lymphocytes may also be responsible, however, since it has been shown that certain T-cells may exert an inhibitory effect on B-lymphocyte response to antigens (14).

The serum antiviral activity induced in mice by tilorone has been shown to be, at least to a considerable degree, due to interferon production (2–4). T-lymphocyte depletion following tilorone treatment occurs more or less simultaneously with the appearance of antiviral activity (both reaching maximum effect within 24 hr), and in addition both exhibit hyporesponsiveness to a second dose of tilorone when given within a few days of the first dose (2, 3, 10). For these it appeared that T-lymphocytes were probably responsible for both the interferon induction and suppressed cell mediated immunity produced by this compound. The fact that the absence of T-lymphocytes in athymic mice did not prevent a distinct antiviral response to tilorone suggested that cells other than T-lymphocytes account for a major portion of this activity. T-lympho-

TABLE I. SERUM ANTIVIRAL ACTIVITY INDUCED BY A 150 mg/kg ORAL DOSE OF TILORONE HYDROCHLORIDE IN NORMAL AND ATHYMIC MICE.

Dose group	Number of samples tested[a]	Geometric mean titer[b]	(95% Conf. interval)
Normal control	3	<25	–
Normal treated	5	1600	(1032–2477)
Athymic control	2	<25	–
Athymic treated	3	1007	(634–1600)

[a] Pooled samples containing the serum of two to six mice each.

[b] Dilution producing 50% reduction in plaque-forming units.

cytes apparently contribute to this activity, but, as we have shown, are not absolutely necessary for mediation of this response. These findings tend to corroborate those of Stringfellow and Glasgow (3), who reported that antilymphocyte serum treatment had no effect on the interferon response produced by tilorone in normal mice.

Summary. Nude athymic mice, which lack T-lymphocytes and are unable to mount a cellular immune response, failed to develop the lymphopenia so characteristically produced by a 100 mg/kg oral dose of tilorone hydrochloride in normal mice. The antiviral activity detected in athymic mice 18 hr after a 150 mg/kg oral dose of tilorone was significant, although somewhat less than that found in treated normal mice. These findings suggest that tilorone hydrochloride has a specific effect on T-lymphocytes that accounts for its effect on cellular immunity, but that its antiviral activity is not primarily mediated by T-lymphocytes.

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