# Tilorone Hydrochloride: Human Toxicity and Interferon Stimulation[1] (35576)

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Although interferon was discovered more than 20 years ago, the hope that this broad-spectrum antiviral might be of some use in man has not yet been realized. The discovery that the interferon system could be artificially stimulated, and a realization that synthetic, double-stranded RNA was a potent interferon inducer brought new hope for a practical way to mobilize interferon. Drugs such as poly I:C were extremely effective in mice in preventing and treating several types of virus disease, and were also effective in rabbits.

Most evidence indicates, however, that the effect in these lower animals is considerably greater than in primates and man (1). In herpetic keratitis in rabbits, poly I:C had a definite, but weak, therapeutic effect (2, 3). It could, however, prevent infection by herpes and recurrences of the keratitis for a period of approximately 6 weeks (3). In rabbits, an excellent correlation was seen between interferon production and the duration of protection against herpes infection of the cornea after topical poly I:C. This drug could be given topically in relatively high concentrations daily and was found to be an effective way to stimulate interferon production in the tears. Interferon was detected throughout the period of protection and then disappeared even though treatment was continued (4).

In man, after topical poly I:C, interferon was detected only during the first 48 hr of continued treatment. This result is similar to that reported by Hill[2] *et al.*, who, upon giving poly I:C intravenously, found that, despite repeated injection, interferon in the blood was seen only during the first 48 hr. He was able to show some slight protective effect against influenza infection in volunteers challenged approximately 24 hr after the interferon inducer was administered. Poly I:C causes only a brief production of interferon in man and it seems likely that the chemically related long-chain polyanions might be similarly ineffective.

Tilorone hydrochloride [2,7-bis[2-(diethylamino)ethoxy] fluoren-9-one dihydrochloride], however, is a chemically different type of interferon inducer (5, 6). This small molecular-weight compound, like poly I:C, was found to be effective in mice and to induce the production of interferon. This study was begun to determine whether it would be a safe and effective interferon inducer in man.

*Methods and Materials. Ocular toxicity.* A solution of tilorone HCl[3] (200 mg/ml) was made in phosphate-buffered saline, pH 7.2. It was administered to rabbits as indicated below. Rabbits were examined before the study and frequently thereafter with the slit lamp biomicroscope, and special attention was paid to the cornea, cloudiness of the anterior chamber, and presence or absence of lens opacities.

Eight eyes (4 rabbits) were treated with 1 drop of tilorone HCl three times a day for 4 days, and their corneas were carefully observed.

Eight additional eyes (4 rabbits) were treated with 1 drop each day and examined very frequently for 9 days. Of these, four eyes had treatment continued for 12 days. Examination revealed no definite abnormality.

The corneas were bisected and half were

[1] This study supported in part by U.S. Public Health Service Grants EY-00007 and EY-00446 from the National Eye Institute.

[2] Personal communication, February 1970.

[3] Supplied by William S. Merrell Company.

subjected to frozen sections. Frozen sections were done without fixation and without immersion in any fluid so that if drug was stored in the corneal tissue, it might not be removed by fixatives and other solutions. Some were stained with Thionine and some with hematoxylin and eosin. Paraffin-embedded sections of the eyes were then made.

*Human studies.* All patients were normal, healthy volunteers who had general physical examinations before the study and at the end of the study.

Detailed ocular examinations were done at frequent intervals (daily when possible). All patients were subjected to the following screening laboratory procedures: platelet counts, white blood counts, differential counts, red blood counts, hemoglobin, and hematocrit. From these, mean corpuscular volumes, hemoglobin, and hematocrits were calculated. Blood chemistry examinations included BUN, alkaline phosphatase, total bilirubin, and SGOT.

In all patients, the general physical examinations at the beginning and end of the study were normal, and no alteration of the blood chemistry studies was found. Peripheral blood smears were available from a number of the patients at the end of the study. These were sent to the Wm. Merrell Company and the company reported no significant abnormalities of the smears.

Oral tilorone was administered to three subjects and serum was collected for interferon assay. Ten additional subjects received eye drops and their tears were collected for assay.

*Interferon assay.* Human skin and muscle cell monolayers were used for the interferon assay. Tears from individual patients or the combined (pooled) tears from several patients were diluted to 2 ml with BME (2% fetal calf serum, 1% glutamine, and antibiotics). The sera were diluted 1:10 in the same media. The bottles were incubated overnight with 2 ml of sample (either tears or serum). The cell layer was washed with media the next day and 0.4 ml of media containing an appropriate amount of plaque-forming units of VSV (vesicular stomatitis virus) was added to each bottle and allowed to incubate at 37° for 1 hr, with frequent shaking of the cultures to spread the virus evenly. After incubation, the bottles were drained and overlaid with 1% methylcellulose in BME with 10% fetal calf serum. Each bottle received 4.5 ml of overlay. Bottles were incubated 48 hr or more. This was a system previously used for human interferon assays and interferon in tears had previously been demonstrated by this method in our laboratory after stimulation by double-stranded RNA (4).

A zero time or premedication sample was obtained from all of the subjects. Blood was drawn for interferon assay at intervals as indicated. Similarly, tears were collected and assayed for interferon.

*Results. Ocular toxicity.* Gross examination of the rabbit eyes revealed no ocular abnormalities at any time during or at the end of treatment. Histological examination of both frozen and paraffin-embedded eyes was normal. No crystals of drug were seen when unfixed specimens were examined with polarized light.

*Human studies.* The volunteers received oral tilorone in the regimen listed in Table I. They had some gastrointestinal upset with a little diarrhea and, in one case, vomiting. This stopped when the drug was stopped, and there were no general or ocular signs of toxicity from systemic administration.

The patients receiving the eye drops developed a corneal epithelial change which at first looked like a small amount of corneal edema, first visible about 10 days after treatment began. Later, it appeared through the biomicroscope that this consisted of a fine amount of drug stored in the epithelium of the cornea. There was no deposit in the stroma or endothelium, and no lens or fundus changes were seen at any time during the examinations or at the conclusion of the study. The epithelial deposit was accompanied by blurred vision and halos around lights without significant reduction in visual acuity. Approximately 2 months were required for the drug to disappear from the epithelium, but no reduction of visual acuity or other abnormalities of the eyes were noted.

*Interferon assay.* Serum was assayed from persons receiving the oral tilorone at 0 time,

TABLE I. Schedule for Tilorone Hydrochloride Treatment.

| Subject no. | Day 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Oral medication (mg) | | | | | |
| 1 | 1000 | 1000 | 1000 | 0 | 0 | 1000 | 750 | 750 | 750 |
| 2 | 1000 | 1000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 1000 | 1000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | Eye drops, 200 mg/ml[a] | | | | | |
| 4 | x | x | x | x | 0 | x | 0 | 0 | 0 |
| 5 | x | x | x | 0 | 0 | x | 0 | x | 0 |
| 6 | x | x | x | x | 0 | 0 | 0 | x | 0 |
| 7 | x | x | x | x | x | x | 0 | x | 0 |
| 8 | x | x | x | x | x | x | 0 | x | 0 |
| 9 | x | x | x | x | x | x | 0 | x | 0 |
| 10 | x | x | x | x | x | x | 0 | x | 0 |
| 11 | x | x | x | x | x | x | 0 | 0 | 0 |
| 12 | x | x | x | x | x | x | 0 | x | 0 |
| 13 | x | x | x | x | 0 | 0 | 0 | x | 0 |

[a] x = 1 drop given/day in both eyes; 0 = no medication.

5, 12, 24, and 48 hr after starting treatment.

Control serum was assayed from two subjects not receiving any medications. There was no difference found in the number of plaques between zero time and any other time in the experimental subjects, and all counts were similar to that of the controls.

Tears were assayed from persons receiving eye drops at 0 time, 5, 12, 24, 48, and 72 hr after starting medication. Again, no difference was found between controls, zero time, and any other times.

*Discussion.* A human skin and muscle tissue culture system with vesicular stomatitis virus as the indicator virus has been effective as a method for detecting interferon in man (4), and, although it is impossible to be certain that its sensitivity is comparable to the assays used in lower animals, the complete failure to detect any interferon in the blood, or in the tears of man, despite very large doses of tilorone, suggests that tilorone, like poly I:C, is relatively ineffective in inducing interferon in man. The topical administration permits us to use high drug concentrations and the study of tears indicates that even in high concentrations, the drug is ineffective. Furthermore, whether using poly I:C or tilorone, the resultant differences seen in mouse and man suggests that the interferon system in man is different from that of lower animals. It appears more difficult to stimulate and impossible to maintain in a stimulated state by artificial means for a significant period of time. If this is correct, the prophylactic or therapeutic potential of exogenous human interferon stimulation is very limited indeed.

The apparent storage of tilorone and its toxicity for epithelial cells, as well as the gastrointestinal toxicity observed, suggests that, even if the drug were effective as an interferon inducer, this particular compound would not be sufficiently safe for use in man. The accumulation and storage in the corneal epithelium suggest the likelihood of similar accumulation in other epithelial tissues. In the eye, the retinal pigment epithelium and the lens are possible sites of toxicity, and we must conclude that the drug appears neither safe nor effective.

*Summary.* Tilorone hydrochloride was studied in humans to determine whether it would be a safe and effective interferon inducer in man. Both systemic and topical application failed to produce detectible interferon, and toxicity was noted using both routes of administration. This study indicates that even in the high topical doses, tilorone HCl is neither safe nor effective.

Poly I:C has been shown to have a very brief, reduced effect in man as apposed to lower animals. The fact that tilorone, a totally unrelated compound, is very effective in the mouse but not in man suggests that the interferon system in man may not be capable of intense or prolonged exogenous stimulation and that findings in lower animals may not be applicable to man.

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