Effects of an Interferon Inducer, Poly I:C, on Acute Ocular Infection Produced by Intracameral Inoculation of Toxoplasma gondii

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A potent interferon inducer, synthetic polyninosinic acid-polycytidylic acid complex (poly I:C), was used prophylactically and therapeutically in experimental ocular Toxoplasma infections in rabbits. Daily intravenous injections of poly I:C alone or when combined with daily subconjunctival injections of the inducer delayed the appearance of conjunctivitis, corneal opacity, and iritis in Toxoplasma-infected eyes provided that the treatment was started 1 day before the infection. When the treatment was begun at the same time or 1 day after the infection, no delay in the production of the ocular lesions was noted. In no case was the treatment curative or completely suppressive.

In 1968, Remington and Merigan (9) reported that chick and mouse cell monolayers treated with virus-induced interferon (IF) were significantly protected from destruction by the RH strain of Toxoplasma gondii. Recently, the same authors noted that treatment of mice with an IF-inducer, pyran, also decreased mortality and prolonged life of mice infected with the C56 strain of Toxoplasma organisms (10).

A synthetic polyninosinic acid-polycytidylic acid complex (poly I:C) has been shown to be a potent IF inducer and to mediate resistance to viral and nonviral infections in vitro as well as in vivo. Therefore, an attempt was made to evaluate the therapeutic effects of poly I:C on ocular Toxoplasma infections. This was tested in rabbit eyes, and the results are reported as follows.

MATERIALS AND METHODS

Rabbits. The experimental animals were random-bred, healthy, male New Zealand white rabbits. They weighed approximately 2 kg each, and their sera were negative for the Sabin-Feldman dye test before the experiment.

T. gondii. The RH strain of Toxoplasma organisms was used in this study. Peritoneal fluid containing the parasites was obtained from mice of the Swiss Webster strain (15 to 20 g) which had been inoculated with parasites 4 days earlier. The suspension of parasites was partially purified by the method of Fulton and Sutton (2), as modified by Nozik and O'Connor (7). The number of the parasites in the suspension was determined by counting them in a hemocytometer. The 50% mouse lethal dose (MLD50) was established by injecting a series of 10-fold dilutions of the suspension into the peritoneal cavities of healthy mice. In most cases, 1 ml of the partially purified Toxoplasma suspensions contained 106 parasites, and this represented 106–1 MLD50.

IF inducer. A lyophilized form of poly I:C (Miles Laboratories, Elkhart, Ind.) was reconstituted and diluted to the desired concentrations by the addition of sterile phosphate-buffered saline (PBS) at pH 7.2. The solution contained 100 units of penicillin and 100 μg of streptomycin per ml. It was tested for bacterial sterility before use.

Intraocular inoculation of Toxoplasma organism. Rabbits were anesthetized with intravenously administered sodium pentobarbital, and tetracaine hydrochloride drops were instilled onto the cornea. By means of a previously described technique (5), 0.1 ml of the aqueous humor was removed from the anterior chamber of the eye with a 26-gauge hypodermic needle, and this volume was then replaced with an equal amount of an inoculum containing the Toxoplasma organisms. In all experiments, both eyes of all animals were inoculated.

Evaluation of ocular lesions. Each day during the first week and every other day thereafter, the eyes were examined for signs of conjunctivitis, corneal opacity, and iritis. No attempts were made to grade the severity of these lesions since the lesions progressed rapidly to maximum severity once they appeared, regardless of the treatment.

Detection of IF in serum and aqueous humor. Serum was separated from clotted blood that had been collected aseptically from an ear vein. Aqueous humor was collected from both eyes of each rabbit and pooled. Aqueous humor specimens showing gross contamination with blood cells were discarded. The titration of IF was carried out with the Indiana strain of vesicular stomatitis virus (VSV) in tubes of primary cultures.
of rabbit kidney cells by a previously described technique (3). The titers of IF (units per milliliter) were expressed as the reciprocal of the highest dilution of each specimen that completely inhibited the cytopathic effects of VSV.

RESULTS

Production of ocular lesions by various amounts of *Toxoplasma* organisms. To determine the optimum inoculation of *Toxoplasma* into the eye for the subsequent study of the therapeutic effect of poly I:C, three groups of five rabbits each were inoculated intracamerally with approximately $10^5$, $10^3$, and $10$ MLD$_{50}$ of *T. gondii*, respectively. The lesions were evaluated daily for a period of 10 days.

As shown in Fig. 1, an inoculum of $10^5$ MLD$_{50}$ of the organisms produced conjunctivitis, corneal opacity, and iritis in all eyes as early as 1 day after infection, and the lesions became progressively worse thereafter. In the eyes inoculated with $10^3$ MLD$_{50}$ of the organisms, signs of conjunctivitis and iritis began to appear in 20% of the eyes on day 2 and in all eyes on day 3. The corneal opacity, however, did not appear until day 5. With $10$ MLD$_{50}$, conjunctivitis and iritis were first noted in few eyes on day 5 and in all eyes on day 6. Corneal opacity was apparent in 50% of the eyes on day 7 and in all eyes on day 10. Heat-inactivated *Toxoplasma* organisms did not produce the ocular lesions. On this basis, it was decided that $10^3$ MLD$_{50}$ of the organisms would be used consistently in the subsequent experiments.

Effect of daily intravenous injections of poly I:C on *Toxoplasma* lesions. Three groups of six rabbits each received daily intravenous (iv) injections of 0.5 mg of poly I:C for 8 days. The injections of poly I:C were started 1 day before infection, on the day of infection, or 1 day after intraocular inoculation of $10^3$ MLD$_{50}$ of *T. gondii*, respectively. Another group of six rabbits served as a control series and received daily iv injections of PBS starting 1 day before the *Toxoplasma* inoculation.

As shown in Fig. 2, conjunctivitis and iritis began to appear as early as day 1 in a few eyes, and almost all eyes were affected by day 4 in the control group. In contradistinction to these findings, the group which received daily poly I:C injections beginning 1 day before infection showed no sign of conjunctivitis or iritis up to day 4. However, all eyes of the animals in this group showed the classical signs of infection by day 10. When daily iv injections of poly I:C were initiated at the same time or 1 day after the *Toxoplasma* injection, no significant delaying effect was noted in the production of corneal opacity or iritis, although a slight delay in the appearance of conjunctivitis was noted. Poly I:C treatment had no deterrent effect on the death rate of *Toxoplasma*-infected rabbits. All rabbits of the treated as well as the control groups died of widely disseminated toxoplasmosis 2 to 4 weeks after infection.

Induction of IF in blood and aqueous humor by daily iv injections of poly I:C. The kinetics of IF induction in blood and aqueous humor after daily iv injections of poly I:C was studied. Blood was collected from five rabbits at 3, 6, and 24 hr after each iv injection of 0.5 mg of poly I:C. Aqueous humor was also collected from the same 3 hr after poly I:C injection on days 0, 2, and 4.

As shown in Fig. 3, the levels of IF in both serum and aqueous humor were highest 3 hr after the first iv injection of poly I:C, but the levels declined rapidly thereafter, and no IF was detected at 24 hr. Each subsequent daily iv injection of the same amount of poly I:C induced a high titer of IF in both serum and aqueous humor, but on each succeeding day a lesser amount was produced than on the preceding day, and very
low titers (essentially no IF) were detected in either the serum or the aqueous humor from day 4 onward.

**Effect of daily iv and subconjunctival injections of poly I:C on ocular toxoplasmosis.** In the foregoing experiments, daily iv injections of poly I:C delayed the appearance of the ocular lesions but did not prevent the infection. The treatment was ineffective if it was started either on the same day or 1 day after the infection. This limited effectiveness of poly I:C could be due to the brevity of the presence of IF in the ocular sites (Fig. 3). Therefore, it was thought that daily iv injections of poly I:C in combination with daily subconjunctival injections of poly I:C might maintain higher levels of IF in the ocular tissues for longer periods. This combination, it was thought, might provide more effective protection.
This possibility was tested, and the results are shown in Fig. 4. When daily iv (0.5 mg) and subconjunctival (0.1 mg) injections of poly I:C were initiated 1 day before Toxoplasma inoculation, the production of the ocular lesions was delayed 2 to 3 days, as compared to the control rabbits, but all eyes showed signs of infection between days 6 and 8. If the treatment was started 1 day after infection, it was definitely less effective. Daily subconjunctival injections of 0.1 mg of poly I:C alone resulted in a mild transient conjunctivitis.

**DISCUSSION**

The present study showed that daily iv injections of poly I:C delayed the production of ocular lesions by the RH strain of T. gondii provided the treatment was started 1 day before the injection. No such effect was noted if the treatment was begun at the same time or 1 day after the infection. The RH strain of Toxoplasma organism is a virulent strain. The response of the infection to poly I:C treatment might have been more favorable if less virulent strains, such as the Beverley strain, had been used for the production of the ocular infection. Such a possibility was substantiated in Herpesvirus hominis infections in which various strains revealed differences in their sensitivity to the antiviral effects of poly I:C.

The mechanisms by which daily iv injections of poly I:C delay the appearance of the toxoplasmic ocular lesions in rabbits are not clear at present. Although IF has been shown to have a protective effect against Toxoplasma infections in vitro, the observed delaying effect of poly I:C treatment on toxoplasmic ocular lesions could have been mediated by a factor(s) other than IF. Such was also the case with experimental trachoma infections of rabbit eyes treated with iv injections of poly I:C and with Listeria monocytogenes infections of mice treated with pyran and poly I:C. In addition to their ability to induce IF production, polynucleotides have also been known to enhance in vivo immunological responsiveness, to stimulate phagocytic activities, and to inhibit serum complement (W. Regelson and A. Munson, personal communication). With regard to the last of these, Strannegard (11) has shown that "accessory factor," a complement-like substance believed to be identical to properdin, is essential for the immunoinactivation of Toxoplasma. It remains to be seen whether any of these mechanisms is responsible for the observed effect of poly I:C on experimental Toxoplasma infections in rabbit eyes.

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**LITERATURE CITED**