The influence of polyinosinic-polycytidylic acid complex on vaccinia keratitis in rabbits

Endliam Chowchuvech, Mercedes Weissenbacher, Miles A. Galin, and Samuel Baron

Topical polyinosinic-polycytidylic acid complex was used prophylactically and therapeutically in experimental vaccinia keratitis of rabbits. It was found that this interferon inducer protects against vaccinia infection when administered from three days up to eight hours before infection. Its therapeutic efficacy diminished with time and was absent 24 hours after infection.

Key words: Polyinosinic-polycytidylic acid complex (In-Cn), vaccinia keratitis, blindness, interferon inducers, rabbits.

Ocular complications after smallpox vaccination are rare occurrences, but still may cause considerable morbidity. In some of the developing countries, vaccinia keratitis is still an important cause of blindness. Keratitis is the most serious ocular problem and occurs in about 20 to 30 per cent of ocular vaccinia.

A diverse number of therapeutic regimes have been used in the treatment of ocular vaccinia, including vaccinia antiserum, x-ray therapy, gamma globulin, antibiotics, and cytosine arabinoside, with essentially mixed to poor results. The interferons, however, have demonstrated some effectiveness against vaccinia virus in vitro and in experimental animals. It has been shown that exogenous rabbit interferon can delay the onset of vaccinia keratitis in rabbits when applied topically immediately after corneal virus inoculation. The prophylactic and therapeutic effectiveness of interferon against vaccinia infection in man also has been reported.

It has been demonstrated recently that a double-stranded, synthetic polynucleotide, polyinosinic-polycytidylic acid complex (In-Cn), is a potent interferon inducer. The significant prophylactic and minor therapeutic effectiveness of this compound was shown in experiments with herpes simplex keratitis and conjunctivitis. Therefore, a study was designed to test the efficacy of topical In-Cn in the prevention and treatment of vaccinia keratitis in rabbits.

Materials and methods

Vaccinia virus of the Mill-Hill strain, obtained from Dr. Samuel Baron, was used throughout
this experiment to produce primary acute keratitis. Its titer was $10^{6.0}$ when titrated in primary rabbit kidney cells for TCID$_{50}$ (tissue culture infective dose).

Albino New Zealand rabbits, weighing approximately 1.5 to 2.0 kilograms, were used for this experiment. The animals were examined prior to the experiment; those with corneal changes or conjunctival hyperemia were excluded.

In-Cn was obtained in solution in a concentration of 1 mg. per milliliter. It was stored at $4^\circ$ C. in vials containing enough solution for one day's treatment.

**In vivo studies.**

**Infection.** Two superficial 5 mm. crisscrossing corneal scratches were made on each nonanesthetized, proptosed eye with a 25 gauge needle. One drop of undiluted vaccinia virus (1/20 ml.) was dropped into the lower conjunctival cul-de-sac immediately after scarification. The lids were then held closed and gently rubbed against the cornea for ten seconds.

**Treatment.** Rabbits were divided into groups of eight (16 eyes); each group contained four control and four experimental animals. The day of inception of therapy varied but treatment was identical for all animals. Each animal received one drop (1/20 ml.) of In-Cn in each eye every hour for eight hours on the initial day of therapy, followed thereafter by drops, four times daily for 14 days. In Groups 1, 2, and 3, treatment was initiated 48, 24, and 8 hours, respectively, prior to inoculation. Treatment was given 30 minutes, 18 hours, 24 hours, and 48 hours after infection in Groups 4, 5, 6, and 7, respectively. Control eyes were treated with normal saline in an identical manner to infected eyes.

Animals were examined daily utilizing a slit lamp, with and without fluorescein staining. The severity, extent, staining characteristics, and topography of lesions were recorded in comparative drawings and graded from I to IV depending on the extent of stromal involvement.

**Clinical course.** Three days after viral inoculation, minute central epithelial lesions appear. These lesions become larger and reach their maximum size between days 7 and 12. Some stromal involvement may become apparent on day 3, but usually first appears on day 5, to reach a maximum between days 7 and 12. Most of the

---

**Fig. 1.** Therapy was initiated two days prior to infection and continued for 14 days.

**Fig. 2.** Therapy was initiated one day prior to infection and continued for 14 days.
Fig. 3. Therapy was initiated eight hours prior to infection and continued for 14 days.

Fig. 4. Therapy was initiated 1/2 hour after infection and continued for 14 days.

Fig. 5. Therapy was initiated 16 hours after infection and continued for 14 days.

Fig. 6. Therapy was initiated 24 hours after infection and continued for 14 days.
epithelial lesions start to heal on days 12 to 14 almost at the same time that stromal involvement starts to resolve.

By day 28, all stromal involvement has completely resolved, leaving a variable degree of scarring; some epithelial lesions still persist. Vasculization commences on about day 9, becomes most active between days 14 and 17, and starts to resolve thereafter, so that some of the eyes are completely free of vascularization by day 28.

**Results.** As is shown in Figs. 1 to 3, In-Cn can prevent the appearance of vaccinia keratitis when administered topically prior to infection. Though some eyes still develop keratitis, the severity and extent of the lesions are much less than in control eyes; these lesions heal more rapidly.

When In-Cn was administered ½ hour after viral inoculation (Fig. 4), its effectiveness was diminished in comparison to the effectiveness of prophylactic employment. However, the severity and extent of lesions were still less than in controls and this beneficial effect, albeit at a decreasing rate, still persisted when In-Cn was administered 16 hours after infection (Figs. 5 and 8).

Administration of In-Cn 24 or 48 hours after infection resulted in epithelial lesions that were still smaller than those in the control animals, however, there were no differences in the degree of stromal involvement or healing time between treated and control eyes (Figs. 6 to 8).

**In vitro studies.** Ten-day-old primary rabbit corneal cell cultures in screw-top tubes were used in these experiments. Eight tubes were incubated at 37°C with 1 ml. of medium containing 0.1 mg per milliliter of InCn in minimal essential medium (MEM) with 2 per cent fetal bovine serum (FBS). Ten tubes served as controls. These were incubated in the same manner with 1 ml. of MEM plus 2 per cent FBS, but without InCn. After 18 hours of incubation, the medium was decanted and corneal cell cultures were challenged with tenfold, progressively decreasing titers of vaccinia virus starting at 100,000 infective dose (ID₅₀). Two tubes were used per each dilution. After an adsorption period of one hour at 37°C, corneal cell cultures were washed two times with Hank's balanced salt solution (BSS) to remove excess virus and then refed with 1 ml. of mainte-
Fig. 9. Influence of viral titer on ability to prevent vaccinial keratitis with polyinosinic-poly-cytidylic acid complex.

The cultures were then incubated at 37° C. After 72 hours of incubation, the medium from each dilution was harvested, pooled, and virus titers assayed in primary rabbit kidney cell cultures. Two tubes of primary rabbit kidney cells were seeded with 1 ml of serial tenfold dilutions of pooled medium and the ID₅₀ endpoints were determined on day 7 of incubation. This experiment was repeated twice with similar results, which are shown in Fig. 9.

An additional experiment was carried out to determine the presence of interferon. Three sets of primary rabbit corneal cells of five tubes each were incubated with 1 ml. of In-Cn in concentrations of 0.1, 0.05, and 0.01 mg. per milliliter, respectively. A fourth set received no In-Cn and served as a control. After an incubation period of 24 hours, the medium was harvested and interferon titers were assayed by the cytopathic protection method in primary rabbit kidney cells. It was found that 10 units of interferon were present in the media harvested from cells treated with 0.1 mg. per milliliter of In-Cn. No interferon was detected in media harvested from cells treated with lesser amounts of In-Cn.

Immediately after the removal of fluid for interferon titration, the cells were washed and challenged with 1,000 ID₅₀ of vesicular stomatitis virus (VSV). Observation for any cytopathic effect of this virus was continued for 3 days. Protection was found even in corneal cells where the presence of interferon was not detected. These results tend to imply that rabbit corneal cells may produce interferon to protect against viral infection after treatment with In-Cn, but that the concentration of interferon in the media may not be sufficient for detection by the employed technique.

Discussion

This experiment clearly shows that In-Cn has prophylactic efficacy against vaccinia keratitis in rabbits. The topical effectiveness is of the same degree whether or not therapy is initiated three days or eight hours before infection. The explanation for this might be that corneal cells which are engaged in the production of interferon may be unable to produce more interferon after reaching a maximum rate of production. Baron and his colleagues have shown that interferon produced in the early phases of a viral infection tends to inhibit subsequent interferon production.

As with other viral infections, the effectiveness of In-Cn decreased when administered to 18 hours after infection. Despite this, as has been shown with herpes simplex virus infection of rabbit cornea, the extensiveness of stromal involvement and epithelial lesions were still less than in controls. When In-Cn was administered 24 to 48 hours after the infection, there was no difference between control and treated eyes.

It has been shown that the most efficient inhibition of vaccinia virus reproduction occurs when interferon is applied directly within 15 to 30 minutes after virus infection. Since In-Cn is an interferon inducer, it probably requires some time to create an antiviral effect after being adsorbed into interferon-producing cells. It has been well demonstrated in rabbits that a period of at least 2 to 2½ hours is required before circulating interferon reaches its maximal level after intravenous injection of In-Cn. Therefore, it is possible that In-Cn does not prevent the appearance of keratitis when it is administered early after an induced corneal infection; no "interference" can be created during the period the virus is penetrating corneal cells, uncoating, and initiating synthesis of its components. Additional cells will become infected until sufficient interferon is produced to permit
recovery or prevent uninvolved cells from becoming infected. When the administration of InCn was delayed for 24 to 48 hours after infection, no difference between control and treated eyes was observed. Presumably, during this 24 to 48 hours, the titer of tissue virus significantly increased and the larger number of attacking virus particles per new cell was able to overcome the inhibiting action of interferon. This concept is borne out by the demonstration that InCn-treated cells were resistant to vaccinia virus in doses of 100 ID₅₀ and 1,000 ID₅₀, and the virus titers in such cells were markedly reduced. This response was significantly less when cells were infected with 10,000 and 100,000 ID₅₀ (Fig. 9).

The observation that interferon has a significant effect on vaccinia infection has not been applied clinically because purified interferon is difficult to obtain in high concentration. InCn, a potent interferon inducer, may prove to be a prophylactic measure with some therapeutic potential.

The authors wish to acknowledge the excellent technical assistance of Miss Anita Panic-Pesika and Mr. Thomas Kelly.

REFERENCES