Differences in Natural and Recombinant Interferon for Herpes Keratitis in Two Animal Models

James J. Saniroro, Emily D. Varnell, Herbert E. Kaufman, and V. K. Raju

Natural human leukocyte interferon (natural HuIFN-α) and recombinant leukocyte A interferon (recombinant A HuIFN-α) were tested for prophylactic and/or therapeutic effects in reducing the severity of herpetic keratitis in rabbit and monkey eyes infected with McKrae strain herpesvirus. The results showed that the two interferons acted differently in the rabbit eye; combined prophylactic and therapeutic administration of natural interferon mitigated the disease, while recombinant interferon had no effect. In monkeys, the two interferons acted similarly. Combined prophylactic and therapeutic administration reduced disease findings, while therapeutic administration alone had no effect. Thus, studies in rabbits are not accurate predictors of primate study results; whether or not nonhuman primate results can be extrapolated to humans remains to be seen. Invest Ophthalmol Vis Sci 25:874–876, 1984

Human leukocyte interferon was first described by Isaacs and Lindenmann in 1957,1 and its potential as a useful ocular antiviral agent was proposed as early as 1962.2 Since that time, topically applied, natural interferon has been tested successfully for the treatment of experimental herpes keratitis.3 Other studies have shown that this type of interferon exerts a prophylactic effect against both primary ocular herpetic infection and recurrent ocular disease in owl monkeys4 and prophylactic and therapeutic effects in rabbits.5,6 However, because of the expense and complexity of preparation, interferon has not, until recently, been a practical agent for the treatment or prevention of herpetic infection.

Recent advances in recombinant DNA technology7 have resulted in the commercial synthesis of recombinant interferon, which is now available relatively inexpensively and in quantities large enough to support widespread experimental usage. Two questions relating to this research effort are of immediate importance. First, does recombinant interferon have the same antiviral effect as the natural agent? Second, is the efficacy of recombinant interferon the same in different species? The latter answer also would determine if studies involving a nonprimate animal model are an adequate predictor of the primate response.

For answers to these questions, we compared natural HuIFN-α and recombinant A HuIFN-α given prophylactically and/or therapeutically in owl monkeys and New Zealand white rabbits infected with McKrae strain herpesvirus.

Materials and Methods. Natural HuIFN-α (Revlon Health Care Group, Meloy Laboratories, Springfield, VA) was supplied frozen in a concentration of $4 \times 10^6$ units/ml. When thawed, it was stored at $4^\circ C$ and used within 24 h. Recombinant A HuIFN-α (Hoffmann-La Roche Inc.; Nutley, NJ) was supplied as a 95% pure, lyophilized powder. In our laboratory, the powder was stored at $-90^\circ C$, thawed and reconstituted for use with sterile water to a concentration of $50 \times 10^6$ units/ml, predetermined by Hoffmann-La Roche Inc., as titered against VSV using bovine kidney cells. When reconstituted, the interferon was stored at $4^\circ C$ and used within 24 hr.

New Zealand white rabbits (2–3 kg, both sexes) and adult owl monkeys (Aotus trivirgatus) (1–2 kg; both sexes) were used as the nonprimate and primate models, respectively. For inoculation of the herpesvirus, the animal corneas were anesthetized with proparacaine hydrochloride (Ophthaine) eye drops and abraded gently with a 30-gauge needle. Two drops of McKrae strain virus suspension ($10^5$ PFU) were instilled into each eye. For both prophylactic and therapeutic interferon applications, two drops of the interferon solution were administered topically to each eye three times a day, at 7 AM, 12 PM, and 3 PM, unless otherwise stated below. Virus inoculation was performed at 10 AM, that is, between the first and second interferon application times.

The animal eyes were examined by means of the slit-lamp biomicroscope prior to inoculation with the herpesvirus and daily thereafter for 7 days. Fluorescein staining was used to grade the severity of the herpetic keratitis on a scale of 0–4, with 4 being the most severe disease, as described previously.8 Examinations and treatment regimens were carried out in a double-blind, randomized fashion.

Results were evaluated by means of analysis of variance and nonparametric analysis of variance testing (experiments 1 and 3), as well as pairwise t-testing (experiment 2). Statistical analyses were carried out by Grace E. Kissling, PhD, Department of Biometry, LSU Medical Center School of Medicine (New Orleans, LA).

These investigations conformed to the ARVO Resolution on the Use of Animals in Research.

Results. Experiment 1. Nonprimate model: Twenty-eight New Zealand white rabbits were divided randomly into three groups: natural interferon-treated (10 rabbits); recombinant interferon-treated (10 rabbits); and untreated controls (eight rabbits). The experimental animals were given interferon prophylactically (two drops, three times a day for two days), then inoculated with herpesvirus, followed by interferon treatment on the same schedule for 5 days after ocular infection.
Table 1. Prophylactic and therapeutic effects of natural HuIFN-α and recombinant A HuIFN-α on the severity of herpetic keratitis in herpesvirus-infected eyes of rabbits and owl monkeys

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Interferon administration</th>
<th>Disease severity reduced</th>
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<tbody>
<tr>
<td></td>
<td>Prophylactic</td>
<td>Therapeutic</td>
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<tr>
<td>Exp. I: Rabbits</td>
<td>Natural HuIFN-α</td>
<td>X</td>
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<tr>
<td></td>
<td>Recombinant A HuIFN-α</td>
<td>X</td>
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<tr>
<td>Exp. II: Monkeys</td>
<td>Natural HuIFN-α</td>
<td>X (4 doses)</td>
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<tr>
<td></td>
<td>Recombinant A HuIFN-α</td>
<td>X (4 doses)</td>
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<tr>
<td></td>
<td>Recombinant A HuIFN-α</td>
<td>1 (dose)</td>
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<tr>
<td>Exp. III: Monkeys</td>
<td>Natural HuIFN-α</td>
<td>X</td>
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<tr>
<td></td>
<td>Recombinant A HuIFN-α</td>
<td>X</td>
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The severity of the dendritic keratitis in the natural interferon-treated rabbit eyes was significantly less than in the untreated control eyes (P = 0.001), but there was no significant reduction in severity in the recombinant interferon-treated eyes (Table 1, Fig. 1). Thus, recombinant A HuIFN-α was ineffective in the rabbit.

Experiment 2. Primate model: Twenty-seven owl monkeys were randomly divided into four groups: natural interferon-treated (seven); two groups of recombinant interferon-treated (seven and seven); and untreated controls (six). The natural interferon-treated group received prophylactic interferon three times on the day before virus inoculation, as well as once in the morning on the day of infection, followed by two additional doses on that day after inoculation and three treatments a day for 7 days thereafter.

Of the two recombinant interferon-treated groups, one group (seven monkeys) received four prophylactic doses of interferon, three on the days before infection and one in the morning on the day of infection. The other recombinant interferon-treated group (seven monkeys) received only one dose of interferon in the morning on the same day as virus inoculation. Both groups received the noon and 3 PM doses of interferon on the day of infection, and three treatments a day for the next 7 days.

Eyes receiving four prophylactic doses of interferon showed a statistically significant reduction in ocular disease severity (P = 0.04 on day 2; 0.012 on day 4; and 0.0004 on day 5) compared with untreated controls; neither natural nor recombinant interferon was more effective than the other in decreasing clinical keratitis findings. Eyes receiving only one prophylactic dose the morning of infection showed no decrease in severity of disease (Table 1, Fig. 2). In contrast to the results in rabbits, recombinant A HuIFN-α was effective in monkeys.

Experiment 3. Primate model: Twenty-seven owl monkeys were divided randomly into three groups: natural interferon-treated (nine); recombinant interferon-treated (nine); and untreated controls (nine). No prophylactic interferon was administered; infected eyes were treated with two drops of interferon twice a day for 7 days beginning the day after inoculation with the herpesvirus.

No statistically significant reduction in severity of ocular disease was seen in either treatment group, compared with the untreated controls (Table 1, Fig. 3), i.e., neither natural HuIFN-α nor recombinant A HuIFN-α was significantly effective therapeutically.

Discussion. The results of this study show that in rabbits, combined prophylactic and therapeutic natural...
HuIFN-α treatment mitigated the severity of dendritic herpes keratitis, while recombinant A IFN-α, similarly administered, had no apparent effect on the ocular disease. In the primate model, four doses of prophylactic interferon combined with therapeutic application were effective in reducing clinical herpes keratitis findings, with no difference between the two types of interferon in terms of efficacy. However, a single prophylactic application had no effect. Also in the primate model, postinfection therapeutic use of interferon alone, with no prophylactic administration, had no effect on the severity of the disease, in contrast to the results of some previous studies.

This work supports previous demonstrations of interferon as a useful prophylactic agent against herpesvirus. However, little effect was obtained when interferon was applied only after the infection had become established. These results imply that interferon would be of value by itself only if given prior to primary infection with McKrae strain herpesvirus.

In answer to our original questions, it appears that natural HuIFN-α and recombinant A IFN-α have different antiviral effects in the rabbit and similar antiviral effects in the monkey. Therefore, results in nonprimate models are not accurate predictors of interferon effects in primates. Any theoretical, qualitative and quantitative assessments of interferon activity in the primate based on rabbit studies could well be inaccurate. Whether or not primate findings can be extrapolated successfully to humans remains to be seen.

Key words: human leukocyte interferon, recombinant leukocyte A interferon, HuIFN-α, herpes keratitis, owl monkey, rabbit.

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