Prevention of Vaccinia Lesions in Rhesus Monkeys by Human Leucocyte and Fibroblast Interferon

By W. WEIMAR, 1 L. STITZ, 2 A. BILLIAU, 3 K. CANTELL 4 and H. SCHELLEKENS 5

1 Department of Internal Medicine and 5 Department of Virology, Erasmus University Rotterdam, P.O. Box 1738, Rotterdam, The Netherlands, 2 Primate Centre TNO, Virology Section, 151 Lange Kleiweg, Rijswijk, The Netherlands, 3 Rega Institute, University of Leuven, Minderbroedersstraat 10, Leuven, Belgium and 4 State Serum Institute, Mannerheimintie 166, Helsinki, Finland

(Accepted 26 November 1979)

SUMMARY

The prophylactic antiviral activity of systemically administered human interferon preparations was tested in 36 rhesus monkeys against vaccinia virus injected into the skin. All nine control monkeys developed typical vaccinia skin lesions. Eight of nine monkeys treated with daily intramuscular injections of leucocyte interferon (5 x 10^5 units/kg) from day -1 to day +7 after vaccination were completely protected. No lesions developed after discontinuation of therapy. Administration of the same amounts of leucocyte interferon intravenously (i.v.) was equally effective. Daily intramuscular (i.m.) injections of lower doses of leucocyte interferon (1.25 x 10^5 units/kg; 0.5 x 10^5 units/kg) decreased the severity of the skin lesions. Lesion scores correlated inversely with the dose of interferon. Four of six animals receiving daily i.m. injections of fibroblast interferon (5 x 10^5 units/kg) and one of three animals treated i.v. with the same dose were protected against vaccinia virus, and the lesions in the other monkeys were smaller. Intramuscular injections of 5 x 10^5 units/kg of fibroblast interferon or 1.25 x 10^5 units/kg of leucocyte interferon resulted in comparable serum levels and had comparable efficacy in reducing lesion scores.

INTRODUCTION

In recent years evidence has been accumulating for the effectiveness of systemic interferon administration in the treatment of both virus and neoplastic diseases in man (Dunnick & Galasso, 1979). Treatment schedules in different studies vary considerably: clearly the optimal dose, frequency and route of administration are not known. High doses of interferon are found to be necessary to influence the course of an established virus infection (Merigan et al. 1978). Less interferon may be needed for prevention of virus disease. However, results of studies on the prophylactic efficacy are conflicting (Strander et al. 1976; Weimar et al. 1978; Cheeseman et al. 1979).

The interferon preparations used so far for clinical studies have been derived from two sources: human leucocytes induced with Sendai virus (human leucocyte interferon, HLI) and human diploid fibroblasts induced with double-stranded RNA (human fibroblast interferon, HFI). Little is known of the comparative efficacy of these preparations. When applied topically, no difference was found in the effect on herpes keratitis (Sundmacher et al. 1978).
Used systemically in HBsAg-positive chronic hepatitis, differential effects of these interferons were reported (Weimar et al. 1979).

In an attempt to determine an optimal treatment regimen and to clarify possible differences in the efficacy of these interferon preparations, we have performed dose-response studies with HLI and compared the activity of HLI and HFI in Rhesus monkeys inoculated intradermally (i.d.) with vaccinia virus. It has been reported that relatively crude interferon preparations can suppress vaccinia lesions in monkeys, whether injected i.d. or i.v. (Andrews, 1961; Pinto et al. 1969). Recently, we have described the effectiveness of purified HLI in this animal model (Schellekens et al. 1979).

**METHODS**

**Virus.** The source, propagation and titration of vaccinia virus (RIV strain) have been described by Hekker et al. (1973).

**Animals.** Rhesus monkeys (Macaca mulatta) bred at the Primate Centre TNO (Rijswijk, The Netherlands) and weighing 1.5 to 3 kg were used. Only animals lacking antibodies to vaccinia virus as tested by a serum neutralization test, were used.

**Interferons.** HLI was prepared as described previously (Cantell et al. 1974) and had a specific activity of $10^6$ units/mg protein. HFI, prepared and partially purified as described elsewhere (Billiau et al. 1979), had a specific activity of $10^6$ units/mg protein.

**Interferon titration.** Interferon activity was measured by a dye-uptake method using diploid skin fibroblasts and vesicular stomatitis virus (VSV) as a challenge virus (Finter, 1969); units refer to the standard of HFI (G-023-902-527) provided by the National Institutes of Health (Bethesda, Md., U.S.A.). Interferon activity was also determined by a cytopathic effect inhibitory assay, employing Rous sarcoma virus-transformed human fibroblasts (RSb cells) and VSV as a challenge virus; units refer to the standard of HLI (Medical Research Council 69/19).

**Experimental design.** Animals were kept in quarantine from 2 weeks before the start until 2 weeks after the experiments. Interferon was injected daily, starting on the day before vaccination until 7 days after vaccination. Each monkey was inoculated on the chest by i.d. injection of 0.05 ml aliquots of live vaccinia virus at different concentrations ($10^7$, $10^8$ and $10^6$ TCID$_{50}$/ml), u.v.- and heat-inactivated vaccinia virus ($10^7$ TCID$_{50}$/ml before inactivation) and saline. Each virus dilution and all controls were injected at three sites. Animals were kept under general anaesthesia during vaccination. The monkeys were examined daily and the skin lesions were scored by two independent observers on an arbitrary scale from 0 to 4 based on appearance and diameter of papules and pustules. Each day one third of the animals in turn were anaesthetized to allow photographic recording of the lesions as well as blood sampling for various tests. Blood samples were taken 3 to 4 h after the first interferon injection.

**RESULTS**

In the first experiment, groups of three monkeys received i.m. injections of either saline, or $5 	imes 10^5$ units/kg of HFI or HLI. Typical vaccinia skin lesions developed in all untreated monkeys: pustules appeared between day 4 and day 7 p.i. All virus dilutions induced these lesions, while no lesions were produced by the inactivated virus or the saline. All three monkeys treated with HLI and one monkey treated with HFI were completely protected against vaccinia virus. In the two other animals injected with HFI, the pustules were smaller than in the control animals. In the protected monkeys, no vaccinia lesions developed after discontinuation of interferon treatment during the observation period of 42 days.

In the second experiment, three groups of three animals were given different dose schedules of HLI i.m. ($5 	imes 10^6$, $1.25 	imes 10^6$ and $0.5 	imes 10^6$ units/kg, respectively); a control
Prevention of vaccinia lesions by interferon

Fig. 1. Skin lesion scores in rhesus monkeys treated i.m. with human leucocyte interferon from day -1 to day +7: ■ -■, controls (n = 6); □ -■, daily dose 0.5 x 10⁵ units/kg (n = 3); ○-○, daily dose 1.25 x 10⁵ units/kg (n = 3); •-•, daily dose 5 x 10⁵ units/kg (n = 6).

Fig. 2. Skin lesion scores in rhesus monkeys treated with 5 x 10⁵ units/kg human fibroblast interferon from day -1 to day +7: ■-■, controls (n = 6); △-△, i.v. route (n = 3); ○-○, i.m. route (n = 6).

All control animals developed vaccinia lesions. The highest dose of HLI protected two monkeys completely; the two other dosage regimens inhibited formation of pustules in one monkey of each group. Lesion size appeared to be influenced by the dosage of interferon. Fig. 1 shows the inhibition of lesion scores in monkeys treated with different doses of HLI.

In the third experiment, two groups of three monkeys received HLI (5 x 10⁵ units/kg) i.m. or i.v.; two other groups received HFI (5 x 10⁶ units/kg) i.m. or i.v.; again, a control group received i.m. injections of saline. The three control monkeys developed pustules. All animals treated with HLI, whether by the i.m. or the i.v. route, were protected. All monkeys treated with HFI intramuscularly were equally well protected. HFI given i.v. depressed lesion size in two monkeys, while one monkey was completely protected. Fig. 2 shows the inhibition of skin lesion scores in monkeys treated with HFI given by different routes.
Table I. Levels of serum interferon and vaccinia lesion score in rhesus monkeys treated with intramuscular injections of human interferon

<table>
<thead>
<tr>
<th>Type</th>
<th>Dose (units/kg)</th>
<th>Serum interferon* (units/ml)</th>
<th>Mean lesion score on day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLI</td>
<td>$5 \times 10^5$</td>
<td>250</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>$1.25 \times 10^5$</td>
<td>120</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>$0.5 \times 10^6$</td>
<td>80</td>
<td>3.0</td>
</tr>
<tr>
<td>HFI</td>
<td>$5 \times 10^5$</td>
<td>140</td>
<td>1.3</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>&lt;25</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* Blood samples taken 3 h after injection; average of three animals; interferon activity assayed on diploid fibroblasts against HFI standard.

Serum interferon levels 3 h after i.m. injections are shown in Table I. Similar levels were found after injections of HLI, whether the sera were assayed on diploid skin fibroblasts against the HFI standard, or on a continuous cell line (RSt cells) against the HFI standard. After i.m. injections of HFI, interferon activity in the sera was detected only when the assay on diploid fibroblasts was used.

Table I shows that injections of $5 \times 10^5$ units/kg of HFI resulted in serum levels comparable with those after HLI given in a dose of $1.25 \times 10^5$ units/kg. Furthermore, these doses were about equally effective in reducing lesion scores.

DISCUSSION

The present study shows that i.m. administration of either HLI or HFI can protect rhesus monkeys against vaccinia virus-induced skin lesions. With HLI the development of lesions was suppressed with all three dose regimens used: $5 \times 10^5$, $1.25 \times 10^5$ and $0.5 \times 10^5$ units/kg. However, only the highest dose was capable of completely suppressing lesion development; it is interesting that this same dose ($5 \times 10^5$ units/kg) was needed in immunosuppressed patients with varicella-zoster to prevent the appearance of any disseminated lesions (Merigan et al. 1978).

From previous studies it would seem that the direct antiviral effect of interferon on cells does not play the main role in the in vivo protection of monkeys against vaccinia virus. In vitro, the monkey cell/vaccinia virus system is virtually insensitive to both HFI and HLI. Therefore, it has been proposed that the effect against vaccinia virus in monkeys results from the activation of other host defence mechanisms, such as NK-cells or macrophages (Schellekens et al. 1979). It may well be that lower doses would be sufficient to provide in vivo protection in those host/virus systems where the direct effect of interferon on virus replication does play an important role in limiting initiation or progression of disease. In patients with malignancies treated with $3 \times 10^6$ units of HLI three times a week the incidence of symptoms of virus infections was reported to be reduced (Strander et al. 1976). In general, higher dosages are probably needed if therapy is started after the appearance of symptoms of virus infections. The dose of HLI necessary to influence the course of varicella or herpes zoster in patients roughly corresponded to that necessary in our vaccinia/monkey model (Arvin et al. 1978; Merigan et al. 1978).

One can hypothesize that in a therapeutic, as opposed to a prophylactic situation, the direct effect of interferon on virus replication is of less importance. Therapeutic effects may result mainly from activation of other host defence mechanisms which require higher doses of interferon. The prevention of vaccinia virus-induced skin lesions was not dependent on the amount of virus inoculated. Lesions appeared at several inoculation sites with different
virus doses or not at all; lesion sizes correlated inversely with the dose of interferon. This is in line with our earlier hypothesis that interferon does not exert its action by inhibiting virus replication but rather by activating other host-defence mechanisms. This hypothesis could explain why interferon is not fully effective in immunocompromised patients (Weimar et al. 1978; Cheeseman et al. 1979).

HFI inhibited lesion development in much the same way as HLI. Quantitative comparisons between the effects of these two interferons must take into account the fact that they are two different molecules with different host ranges and different dose response curves in vitro (Edy et al. 1976). In the vaccinia/monkey model the effect of $5 \times 10^6$ units/kg of HFI was about the same as that of $1.25 \times 10^6$ units/kg of HLI. Thus, on the basis of nominal units, HFI was about four times less effective than HLI. One could explain this difference by the fact that i.m.-injected HFI results in lower blood titres than HLI (Edy et al. 1978). Recent evidence indicates that HFI is inactivated at the i.m. injection site (W. E. Stewart, personal communication). In our experiments the groups of HLI-treated and HFI-treated monkeys with comparable protection also had comparable blood titres. Thus, our experiments indicate that quite high serum levels are a requirement for interferon to be active against vaccinia virus in vivo.

In order to circumvent the problem of insufficient absorption of HFI, one group of monkeys was given HFI by the i.v. route. These monkeys were also partially protected, but less so than monkeys given the same dose by the i.m. route. It is known that i.v.-injected interferon is rapidly cleared from the circulation. Therefore, while this procedure ensures that all interferon reaches the blood stream, it may also allow too little time for fibroblast interferon to activate host-mediated mechanisms.

The Scientific Committee (1970) reported that the insensitivity of vaccinia virus to interferon in vitro discouraged their plans to modify this infection by systemic interferon administration. In our opinion this insensitivity provides an interesting model to study the antiviral mechanisms by which interferon acts in vivo. Treatment regimens inferred from this model may also give some guidelines for the treatment of patients with interferon. However, the limitations of this model must also be kept in mind and the crucial comparisons between HLI and HFI will have to be made in clinical trials.

This study was partly supported by the Netherlands Kidney Foundation grant no. C 197. We thank Dr H. Balner and Professor N. Masurel for their advice, R. Sahadat for technical assistance, Mrs R. S. Engels-Bakker for preparation of the manuscript and Mrs L. M. Hombroek-Claeys for drawing of the figures. The production of HFI was made possible by grants from the Belgian Department of the Economy (Prototype Projects) and from the Belgian ASLK (General Savings and Retirement Fund).

REFERENCES


CHEESEMAN, S. H., RUBIN, R. H., STEWART, J. A., TOLKOFF-RUBIN, N. E., COSIMI, A. B., CANTELL, K., GILBERT, J.,
W. WEIMAR AND OTHERS


(Received 20 July 1979)