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ANTIVIRAL ACTIVITY OF HUMAN LEUCOCYTE INTERFERON IN RHESUS MONKEYS AND MARMOSETS

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When applied before infection human leucocyte interferon (HLI) had a pronounced antiviral activity in vaccinia virus-infected rhesus monkeys. Even one single injection of 500,000 units/kg given before infection yielded significant protection. However, when HLI was applied after infection no significant protection was obtained. In marmosets HLI showed relatively poor antiviral activity.

rhesus monkey interferon marmoset vaccinia virus

INTRODUCTION

Interferons are now being evaluated as antiviral and antitumor drugs by various research groups [1, 13]. The treatment schedules vary considerably and they are not based on experimental data. Some such data, mainly obtained from experiments on mice, are available, but it may be unjustified to directly extrapolate these results to humans. Less interferon per body weight seems to be needed to induce a response in humans than in mice [3, 4], and a reduction in tumor size by systemic interferon treatment, as reported in man [8, 10], has not been achieved in experimental tumors in animals. Accordingly, there is an urgent need to broaden the experimental basis for designing treatment schedules. In particular a primate model in which human interferon could be tested would be useful. There have been studies on the effects of human interferon in non-human primates, but these studies have all been done in a small number of animals [15].

We recently reported the use of vaccinia virus-infected rhesus monkeys to test the efficacy of human interferon preparations [12]. We reported the dose–response effect of human leucocyte interferon in this model, the relative efficacy of human leucocyte and fibroblast interferon and the effect of the administration route [16]. We have now extended these observations by studying the effect of shorter treatment schedules and by comparing the prophylactic and curative effect of human leucocyte interferon.

There is hardly any experience with experimental tumor research in rhesus monkeys.

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In contrast, marmosets are well characterized as a model for various tumors. For this reason it seemed interesting to study the antiviral effect in vaccinia virus-infected marmosets.

EXPERIMENTAL

Source, propagation and titration of the vaccinia virus (RIV-strain) were as described previously [5].

Rhesus monkeys (*Macaca mulatta*), bred at the TNO Primate Center (Rijswijk, The Netherlands) and weighing 1.5–3 kg were used. The marmosets (*Callithrix jacchus*, 300–400 g) employed in this study were bred at the same center. Human leucocyte interferon was prepared and titrated as described previously [2] and had a specific activity of 10^6 units/mg protein. Units refer to the standard of human leucocyte interferon (Medical Research Council 69/19). Animals were kept in quarantine from 2 weeks before the start until 2 weeks after the experiments. Live vaccinia virus (0.05 ml aliquots) at different concentrations, 10^7, 10^6 and 10^5 $TCID_{50}$/ml, were inoculated on the chest by intradermal injection. Control injections consisted of heat- and UV-inactivated vaccinia virus (10^7 $TCID_{50}$/ml) before inactivation and saline. Each virus dilution and all control solutions were injected at three sites. 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 the appearance and diameter of papules and pustules. The significance of the antiviral effect of interferon was established by comparing the lesion score in the interferon-treated group with the untreated controls in the Mann–Whitney U test [14].

In our earlier experiments on rhesus monkeys the animals received 5×10^5 units/kg daily for 9 days, starting on the day before infection. Here, we have studied the effect of shorter treatment periods. The results are shown in Table 1. Two injections, one on the day before and the other on the day of infection clearly protected the animals. Only interferon given before infection had antiviral activity. Treatment with 5×10^5 units/kg for 3 days started on the first or third day after infection had no effect (Table 1). Even when the interferon dose was increased to 2×10^6 units/kg treatment started after infection had no effect (Table 2). On the other hand, single injections of 5×10^5 units/kg given as early as 5 days before infection were still protective (Table 1); no activity was found when the injections were given 10 and 9 days before infection (Table 1).

As a pilot experiment to planned studies of the antitumor effect of human interferons in marmosets, we tested the antiviral activity in this species. In the initial experiment (data not shown) we studied the effect of the standard treatment of 5×10^5 units/kg daily from the day before until 7 days after infection in vaccinia virus-infected marmosets. This treatment proved ineffective. In the subsequent experiment (Table 3) we tested the effect of higher doses of interferon. A significant protective effect could only be obtained if doses as high as 2×10^6 units/kg were used.

This study shows that human leucocyte interferon is an efficient prophylactic antiviral agent in vaccinia virus-infected rhesus monkeys: a single injection of interferon before

TABLE 1

The antiviral effect of human leucocyte interferon injected before or after intradermal infection of rhesus monkeys with vaccinia virus

Experiment No.	Days of doses[a]	Mean lesions score at day 7[b]	*P* value for comparison with untreated control[c]
1	-5	1.0 (0.0)	< 0.05
	-1 and 0	0.8 (0.7)	< 0.05
	1, 2 and 3	3.0 (1.2)	> 0.05
	3, 4 and 5	2.8 (0.8)	> 0.05
	None	2.6 (1.9)	–
2	-10 and -9	2.9 (0.9)	> 0.05
	-5	0.4 (0.5)	< 0.05
	-1 and 0	0.2 (0.6)	< 0.05
	None	2.6 (1.9)	

[a] 0 = day of infection; 10^5 units/kg intramuscularly.
[b] Mean (standard deviation, $n = 3$).
[c] Mann–Whitney U test.

infection can be completely protective. However, when interferon is injected after infection it has little antiviral activity. We have preliminary data that the kinetics of the effect on yellow fever virus infection is similar. The difficulties in monitoring this infection by the rather variable viremia make it a model more difficult to use than the vaccinia virus-infected rhesus monkey. The question is to what extent the results obtained in the rhesus monkey can be extrapolated to the treatment of patients. Studies on immunosuppressed patients with herpes zoster indicate that in man the minimum antiviral dose

TABLE 2

Effect of human leucocyte interferon treatment given after intradermal vaccinia virus infection in rhesus monkeys

No. of animals	Interferon dose (units/kg)	Days of injection[a]	Mean lesion score at day 7[b]	*P* value for comparison with untreated controls[c]
5	2×10^6	+1 and +2	2.2 (0.5)	> 0.05
5	5×10^5	+1 and +2	2.6 (0.5)	> 0.05
3	5×10^5	-1 and 0	0.6 (0.1)	< 0.05
3	None	–	3.0 (0.9)	–

[a] 0 = day of infection.
[b] Mean (standard deviation, $n = 3$).
[c] Mann–Witney U test.

TABLE 3

The antiviral effect of human leucocyte interferon on intradermal vaccinia infection in marmosets

Interferon dose (units/kg)	Mean lesion score at day 7[a]	*P* value for comparison with untreated controls[b]
None	3.2 (0.4)	–
2×10^6	1.8 (0.8)	< 0.05
5×10^5	2.2 (0.8)	> 0.05
1×10^5	3.3 (0.6)	> 0.05

[a] 0 = day of infection; mean (standard deviation, $n = 3$).

[b] Mann–Whitney U test.

of human leucocyte interferon is also in the order of magnitude of 500,000 units/kg [9]. It has, however, also been reported that 70,000 units/kg significantly reduced the incidence of herpes reactivation after microsurgery of the trigeminal nerve [11].

The above experiments show that the protection afforded by interferon can persist for several days. Thus the main suggestion from the present work is that daily injections of interferon may not be needed in clinical studies. Protection against viral infections may be maintained by administration of a sufficient dose every 3–5 days. Furthermore, our findings suggest that interferon will be an effective prophylactic antiviral but a rather ineffective therapeutic agent. This is also suggested by several studies in mice [6, 15].

Presently interferon is also evaluated as an anticancer drug in man [7]. The optimal way to use interferon in these conditions is as yet not known. We intended to study this in experimental tumors in marmosets. The inefficient antiviral activity of human interferon in vaccinia virus-infected marmosets reported here is in agreement with the report of Billiau et al. that human fibroblast or leucocyte interferon (10^6 units/kg) had no effect on the development of malignancy in marmosets infected with herpes virus samiri (A. Billiau, personal communication). Therefore we decided to abandon this model and give priority to the development of an experimental tumor model in rhesus monkeys.

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