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ANTIVIRAL AND SIDE EFFECTS OF INTERFERONS PRODUCED BY RECOMBINANT DNA TECHNIQUES AS TESTED IN RHESUS MONKEYS

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Human interferon type α_2 (HuIFN-α_2) produced by *Escherichia coli* was found to be as active as natural leukocyte interferon in protecting rhesus monkeys against intradermal vaccinia virus infection. HuIFN-β_1 produced in *E. coli* had similar but less pronounced activity. HuIFN-α_2 induced fever but not leukopenia, while HuIFN-β_1 had opposite effects. Concurrent treatment with acetosalicylic acid and prednisolone/azothioprine combinations did not interfere with the efficacy of the human interferons.

interferon vaccinia virus rhesus monkey

INTRODUCTION

Clinical evaluation of interferons as antitumor and antiviral agent in man is hampered by the scarce availability of accurate data obtained from studies in animals. In order to provide such experimental data, we employ rhesus monkeys and study the effects of human interferons on the development of skin lesions after intradermal infection with vaccinia virus. The model allows to define dose–response relationships, relative effects of different types and subtypes of interferon, relative effectiveness of different routes of administration [11] and the influence of the time application of interferon (IFN) [6]. We have also used the model to study the mode of action of IFN in vivo [7,8].

Until recently, all IFN for clinical use had to be produced in cell cultures, which made it scarce and expensive. Recombinant DNA technqiues have been successfully used to clone and express human interferon (HuIFN) genes in microorganisms. The production of interferon by bacteria is less expensive and makes application on a wider scale possible. The question is whether interferons produced by recombinant DNA techniques are as active as in vivo as the natural products made by human cells. In our rhesus monkey/vaccinia virus model, we have already established that HuIFN-α_2 produced in *Escherichia coli* is as active as natural, buffy coat-derived leukocyte interferon [6]. We report here on the efficacy of HuIFN-α_2 from another source as well as on the efficacy of HuIFN-β_1 produced in *E. coli*. It is likely that, when interferon is applied in man, it will be com-

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bined with other forms of therapy. It is important therefore to test the influence if certain drugs on the efficacy of interferon in vivo. A potential use of interferon is the prevention of viral infections in immunosuppressed transplant recipients. We have therefore tested the influence of the combination azothioprine/prednisolone on the activity of human interferon. One of the side effects of interferon in man is fever and this can be effectively treated with standard antipyretics. Therefore, we considered it of interest to see whether acetosalicylic acid interferes with the in vivo antiviral activity of interferon.

EXPERIMENTAL

The source, propagation and titration of the vaccinia virus strain (RIV) were as described previously [2]. Rhesus monkeys (*Macaca mulatta*) bred at the TNO Primate Center (Rijswijk, The Netherlands) and weighing 1,5–2 kg were used. Natural human leukocyte interferon was prepared and titrated as previously described [1] and had a specific activity of 10^6 units/mg protein. The bacteria-derived human interferons used in this study were highly purified preparations of HuIFN-α_2 (LeIF-A) or HuIFN-β_1. These interferons have been purified to homogeneity as determined by polyacrylamide gel electrophoresis and have specific activities of 1.0×10^8 international units (I.U.)/mg and 2.0×10^8 I.U./mg protein, respectively, as determined on human WISH cells challenged with vesicular stomatitis virus (VSV) in a microtiter assay, standardized to the NIH leukocyte (GO23-901-527) and fibroblast interferon (GO23-902-527) standards. The in vitro antiviral activity of HuIFN-α_2 (LeIF-A) has been described previously [10].

Acetosalicylic acid (Aspegic®, 0.5 g/ampoule) was obtained from Egic (France). Prednisolone (Di-adreson F aquosum®; 25 mg/ampoule) was purchased from Organon (Oss, The Netherlands). Azothioprine (Imuran®; 50 mg/ampoule) was a kind gift of Burroughs-Wellcome (Beckenham, U.K.).

The monkeys were infected with vaccinia virus containing 10^8 plaque-forming units (p.f.u.)/ml using a Sterneedle® device at three different sites on the chest. Skin lesions were scored 7 days after infection by two independent investigators using an arbitrary scale of 0–4 based on the appearance and diameter of papules and pustules. The significance of the antiviral effect was established by comparing the lesion score with those of the control monkeys, not treated with interferon, using a Mann–Whitney U test.

The animals were treated daily with 500,000 units/kg intramuscularly from the day before infection until 6 days after. This dose has been shown before to be virtually completely protective [7,11]. On the day of infection, the animals were sedated with ketamine hydrochloride 3 h after interferon injection to determine the body temperature rectally and to collect blood for interferon titration and haematology.

The antiviral activity of natural and bacteria-derived interferons in vaccinia-infected rhesus monkeys and the effect of azothioprine, prednisolone and acetosalicylic acid are shown in Table 1. HuIFN-α_2 obtained by recombinant DNA technology appeared to be as active as natural HuIFN-α. HuIFN-β from *E. coli* also has a significant antiviral activity in the rhesus monkeys but was clearly less active than natural or bacteria-derived HuIFN-

TABLE 1

Protective effects of natural and bacteria-derived interferons in rhesus monkeys infected with vaccinia virus, and effects of antipyretic and immunosuppressive drugs

Treatment	Lesion score[a] on day 7 post-infection	*P* value[b] for comparison with untreated control	*P* value[b] for comparison with treated control
None	4.0 (0)		
Prednisolone/azothioprine[d]	4.0 (0)		
Natural leukocyte interferon	0.3 (0.6)	<0.01	
Natural leukocyte interferon + acetosalicylic acid[c]	0.5 (0.9)	<0.01	
Bacteria-derived HuIFN-α_2	0.8 (0.8)	<0.01	
Bacteria-derived HuIFN-β_1	2.5 (0.9)	<0.01	
Bacteria-derived HuIFN-α_2 + prednisolone/azothioprine[d]	0.3 (0.6)	<0.01	<0.05
Bacteria-derived HuIFN-β_1 + prednisolone/azothioprine[d]	2.8 (1.0)	<0.05	0.06

a Mean (standard deviation) $n = 3$; except for untreated controls; $n = 4$.
b Mann–Whitney U test.
c 1 g daily intramuscularly from day −5 until day 7.
d Prednisolone 1 mg/kg, azothioprine 2 mg/kg daily intramuscularly from day −5 until day 7.

α_2. Combined azothioprine/prednisolone treatment had no effect on the antiviral activity of the bacteria-derived interferons and acetosalicylic acid did not interfere with the antiviral activity of natural leukocyte IFN in vitro. Table 2 shows the interferon levels in the serum of rhesus monkeys 3 h after intramuscular injection of 500,000 units/kg. The titers for natural and bacteria-derived HuIFN-α were comparable. After administration of *E. coli*-derived HuIFN-β, no antiviral activity became detectable in the serum. The side effects of the different interferons are shown in Table 3. Natural leukocyte interferon in-

TABLE 2

Levels of circulating interferon, 3 h after intramuscular injection of interferons in rhesus monkeys

Interferon preparation injected[a]	No. of monkeys	Serum interferon level ($\log_{10}$ units/ml)[b]
None	4	<1.3
Natural leukocyte interferon	3	2.50 (108)
Bacteria-derived HuIFN-α_2	3	2.18 (18)
Bacteria-derived HuIFN-β_1	3	<1.3

a 500,000 units/kg intramuscularly.
b Mean (standard deviation).

TABLE 3

Side effects of human interferon treatment in rhesus monkeys

Treatment[a]	No. of monkeys	Body temperature (°C)[b]	*P* value for comparison with untreated controls[c]	Peripheral blood leukocyte count ($\times 10^{-9}$/l)[d]		*P* value
				Base level (day before 1st interferon injection)	Level after (24 h after 1st interferon injection)	
None	4	36.9 (0.6)		9.9 (4.0)	7.5 (2.7)	> 0.05
Natural leukocyte interferon	3	38.7 (0.2)	< 0.05	10.5 (2.3)	8.6 (2.4)	> 0.05
Bacteria-derived HuIFN-α_2	3	37.0 (0.8)	> 0.05	17.3 (6.5)	5.8 (1.0)	< 0.05
Bacteria-derived HuIFN-β_1	3	38.0 (0.3)	< 0.05	11.8 (3.1)	9.2 (2.1)	> 0.05

[a] Interferon given at 500,000 units/kg intramuscularly.
[b] Taken 3 h after injection. Mean (standard deviation).
[c] Mann–Whitney U test.
[d] Mean with standard deviation.

duced fever but not leukopenia. Bacteria-derived HuIFN-α had the opposite effect. *E. coli*-derived HuIFN-β was pyrogenic but did not affect the leukocyte count. The liver and kidney functions were not influenced by either of the three types of interferon preparation (data not shown). Concurrent treatment with azothioprine/prednisolone had no influence on interferon titers or on the occurrence of side effects (data not shown).

The efficacy of the bacteria-derived HuIFN-α_2 in the rhesus monkey–vaccinia model was similar to that seen with HuIFN-α_2 produced in *E. coli* in the laboratory of Charles Weissmann [5]. However, the latter did not induce side effects, while the bacteria-derived HuIFN-α_2 used in the present study did induce fever. However, the number of animals studied was small and, during the measurement of temperature, the monkeys were sedated with ketamine hydrochloride which can interfere with pyrexial reactions.

Bacteria-derived HuIFN-β exerted a significant antiviral effect but it was clearly less effective than natural leukocyte interferon and bacteria-derived HuIFN-α_2. In previous studies from our laboratoriy [11] natural HuIFN-β was also found to be less active in the vaccinia virus model. One explanation for this is the fact that after intramuscular injection natural HuIFN-β hardly ever appears in the blood. It is either poorly resorbed from or inactivated at the injection site or removed rapidly from the circulation. We could not detect activity in the serum in this study after intramuscular injection of as much as 500,000 units/kg of HuIFN-β. The relative efficacy of natural and bacterially derived HuIFN-β should, however, be established in direct comparison.

A possible clinical application of interferon is the prevention of viral infection and reactivation in immunosuppressed kidney transplant recipients [3]. Immunosuppression, however, could interfere with the host defense mechanisms which can mediate the antiviral effect of interferon in vivo [7–9]. This study shows that, at least in vaccinia virus-infected rhesus monkeys, the standard immunosuppressive regimen of prednisolone and azothioprine did not influence the in vivo activity of human interferons.

A common side effect of interferon administration in man is fever. This can be effectively treated with common antipyretics such as acetosalicylic acid. Acetosalicylic acid has been reported to interfere with the antiviral activity of interferon in vitro [4]. In vaccinia virus-infected rhesus monkeys, however, comparatively high doses of acetosalicylic acid has no deleterious effect on the antiviral effects of human interferons.

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