Intranasal Administration of Human Fibroblast Interferon in Mice, Rats, Rabbits and Dogs

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In previous studies on the nasal administration of human fibroblast interferon (HuIFN-β), only rabbits have been used. Therefore, this route was investigated in mice, rats, rabbits and dogs. HuIFN-β could be delivered across the nasal mucosa in mice, rats, rabbits and dogs, when it was mixed with sodium glycocholate as an absorption promoter. However, the pattern of the plasma HuIFN-β concentration–time curve was different from that in rabbits. Rabbits gave the highest value of the maximum plasma HuIFN-β concentration (C_{max}), but plasma HuIFN-β declined rapidly thereafter, upon nasal administration of the powder dosage form (8.45×10^2 IU/g). In rats and dogs, C_{max} was lower than in rabbits, but plasma HuIFN-β declined slowly after nasal administration of the powder or liquid dosage form (4—6×10^2 IU/g). These differences might be attributed to differences of absorption rate constant.

Keywords interferon; nasal administration; plasma distribution; mouse; rat; rabbit; dog

Human fibroblast interferon (HuIFN-β) could be delivered across the nasal mucosa in rabbits when it was mixed with several absorption promoters and excipients.[1-4] Rabbits have many advantages for intranasal administration, *i.e.*, their nostrils are large enough to administer the powder dosage form and they do not sneeze by nasal administration. However, their sensitivity to the antiviral action of HuIFN-β is different from that of animal cells.[5] HuIFN-β may be more active on rabbit cells than on cells of other animals.[6-8]

The purpose of this study was to elucidate whether nasal absorption of HuIFN-β occurs in other animals or not. Accordingly, the intranasal administration of HuIFN-β was investigated in mice, rats, rabbits and dogs with liquid and powder dosage forms.

Experimental

Materials The HuIFN-β used was a preparation from Toray Industries, Inc., with a specific activity of 2×10^6, 3×10^6, or 1×10^7 (specially ordered) international unit (IU) per vial. Human serum albumin (HSA) and lactose were included for stabilization, but the last one contained lower concentrations of HSA and lactose than the others. Sodium glycocholate (GC-Na, Tokyo Chemical Industry Co., Ltd.) was used as an absorption promoter.

Preparation of the Liquid Dosage Form for Mice, Rats, and Dog For mice, 5 ml of 3 w/v% GC-Na aqueous solution was added to the HuIFN-β (1×10^7 IU) vial, and 10 μl of this solution was administered nasally. The dose was 2×10^4 IU/mouse.

For rats, 1 ml of 3 w/v% GC-Na aqueous solution was added to the HuIFN-β (1×10^7 IU) vial, and 20 μl of this solution was administered nasally. The dose was 2×10^5 IU/rat.

For dog, 100 μl of 3 w/v% GC-Na aqueous solution was added to the HuIFN-β (3×10^6 IU) vial, and 200 μl of this solution in two vials was administered nasally. The dose was 6×10^6 IU/dog.

The concentration of GC-Na solution was set at 3 w/v%,[1] because Hirai reported that GC-Na solution at under 4 w/v% caused little damage to the nasal mucosa in rats.[9] The pH of this solution was discussed in our previous paper.[2]

Preparation of the Powder Dosage Form for Rabbits and Dogs The 3×10^6 IU vial of HuIFN-β was used for the powder dosage form. The weight of one vial was about 13 mg including HSA, and 3 mg of GC-Na was added to one vial. They were mixed and then passed through a 100 mesh sieve. There was no residue on the mesh after sieving. The amount of GC-Na was discussed in our previous paper.[2]

For rabbits, one 3×10^6 IU vial of HuIFN-β and 3 mg of GC-Na was used, and the dose was 3×10^6 IU/rabbit.

For dogs, the same dose per body weight as with rabbits (four 3×10^6 IU vials) could not be used because the powder volume was too large to administer. Therefore, two 3×10^6 IU vials of HuIFN-β and 6 mg of GC-Na were used per dog, and the dose was 6×10^6 IU/dog.

Nasal Administration of HuIFN-β to Animals Male ddY mice (27—33 g), male Wistar rats (270—330 g), male Japanese white rabbits (3.0—3.6 kg) and male beagle dogs (12—15 kg) were used. They were fasted for 20 h before nasal administration. Mice and rats were anesthetized with diethyl ether and cannulation was performed to prevent suffocation before the nasal administration. Dogs were anesthetized by intravenous injection of sodium pentobarbital (Nembutal®, Abbot Laboratories, 25 mg/kg) at 30 min before the nasal administration to avoid reflex action such as sneeze.

For administration of liquid dosage forms, sample solution was applied into a nostril with polyethylene tube connected to an Eppendorf pipette or disposable syringe.

The tool for the administration of powder dosage form in rabbits and dogs consisted of a special sprayer (rubber bulb with reservoir), an Eppendorf pipette tip and polyethylene tubing. The sample powder was placed in the Eppendorf pipette tip which was connected to the polyethylene tubing (1.57 mm i.d. and 2.08 mm o.d.). The tubing was inserted into the nasal cavity at a position about 2.8 cm from the nostril.

Intravenous Administration of HuIFN-β in Dog A male beagle dog (13.5 kg) was used. The dog was fasted for 20 h before intravenous injection. HuIFN-β (2×10^6 IU) in a vial was dissolved in 1 ml of saline, and the resulting solution was administered intravenously.

Collection of Blood Samples Blood (1.5 ml) was collected in a heparinized syringe from the vena auricularis just before administration and at 0.25, 0.5, 1, 2, 3 and 4.5 h after nasal administration of HuIFN-β. Plasma was separated by centrifugation at 3000 rpm for 15 min. The plasma samples were stored at −20 °C until assay.

Determination of Plasma HuIFN-β Concentration Plasma HuIFN-β was assayed by enzyme immunoassay[10] based on the antibody sandwich method using 96-well microplate. Each microplate well was coated with antirabbit HuIFN-β antibody (first antibody). The enzyme reaction was determined by recording the optical density difference. The amount of HuIFN-β in samples was calculated from the standard curves prepared by using a reference HuIFN-β which had been standardized against the international reference for HuIFN-β (G-023-902-527, NIH, Bethesda, MD) by bioassay.

Statistical evaluation was performed using Student's *t*-test with $p < 0.01$ or $p < 0.05$, as the criterion of significance.

Results and Discussion

The intransasal administration of HuIFN-β was investigated in mice, rats, rabbits and dogs. In mice and rats, the nostrils are too small for administration of the powder dosage form, so only liquid form was administered. In a previous study using rabbits, the powder and liquid dosage forms showed the same bioavailability,[2] and therefore, only the powder dosage form was used in rabbits.

Plasma HuIFN-β concentrations after nasal administration in mice, rats, rabbits and dogs are shown in Fig. 1. The absorption of HuIFN-β from the nasal mucosa is observed not only in rabbits but also in mice, rats and dogs, using GC-Na as an absorption promoter. However, the

TABLE I. Pharmacokinetic Parameters and Related Data after Intranasal Administration of HuIFN-β in Mice, Rats, Rabbits and Dogs

Animal	Dosage[a] form	n	Body weight (g)	Dose (IU)	D/b[b] (IU/g)	C_{max} (IU/ml)	P.D.[c] (%)	$AUC_0^{4.5}$ (IU·h/ml)
Mouse	l	3	30.0± 1.7	2×10^4	6.67×10^2	95.23±13.98[d]	0.643±0.068	
Rat	l	3	300.0± 17.0	2×10^5	6.67×10^2	75.33± 6.67	0.540±0.066	240.25±45.97
Rabbit	p	4	3550.0± 250.0	3×10^6	8.45×10^2	96.05±13.98	0.526±0.100	147.99±35.51
Dog	p	3	14500.0±1000.0	6×10^6	4.14×10^2	49.33± 9.82	0.683±0.194	161.96±29.77
Dog	l	1	15000.0	6×10^6	4.00×10^2	11.50	0.129	33.36

a) Dosage forms: p, powder; l, liquid. *b*) (dose)/(body weight). *c*) HuIFN-β distribution percent in plasma at T_{max} after nasal administration, in mice at 0.5 h after nasal administration. P.D.(%)=(plasma HuIFN-β concentration)×(body weight)×0.045×100/(dose). *d*) Plasma HuIFN-β concentration at 0.5 h after nasal administration. The data are expressed as the mean values±S.E.

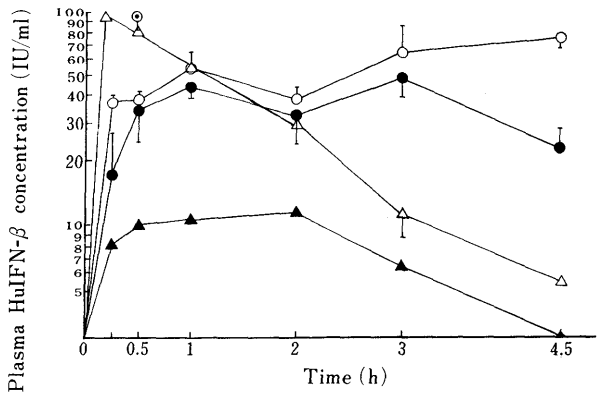

Fig. 1. Plasma HuIFN-β Concentration after Nasal Administration in Mice, Rats, Rabbits and Dogs

⊙, mice (2×10^4 IU/mouse, $n=3$); ○, rats (2×10^5 IU/rat, $n=3$); △, rabbits (3×10^6 IU/rabbit, $n=4$); ●, dogs, powder dosage form (6×10^6 IU/dog, $n=3$); ▲, dog, liquid dosage form (6×10^6 IU/dog, $n=1$). The data ($n=3$ and $n=4$) are expressed as the mean values±S.E.

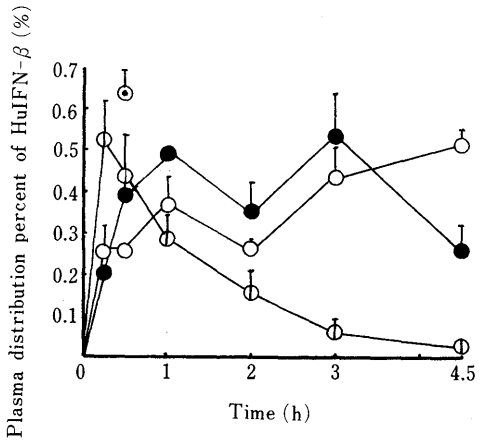

Fig. 2. Plasma Distribution Percent of HuIFN-β after Nasal Administration in Mice, Rats, Rabbits and Dogs

⊙, mice (2×10^4 IU/mouse, $n=3$); ○, rats (2×10^5 IU/rat, $n=3$); ⊕, rabbits (3×10^6 IU/rabbit, $n=4$); ●, dogs, powder dosage form (6×10^6 IU/dog, $n=3$). The data ($n=3$ and $n=4$) are expressed as the mean values±S.E.

patterns of the plasma HuIFN-β concentration–time curves in rats and dogs were different from that in the case of rabbits. In mice, only the plasma HuIFN-β concentration at 0.5 h after nasal administration of 2×10^4 IU is shown. In rats, the data are not the time course, since plasma HuIFN-β concentration was determined in different animals at each sampling point. Pharmacokinetic parameters and related data after intranasal administration of HuIFN-β are summarized in Table I.

In rabbits, the maximum plasma HuIFN-β concentration (C_{max}) was 96.05 IU/ml, and it was observed at 0.25 h (T_{max}). After T_{max}, the concentration decreased linearly to less than 10 IU/ml at 4.5 h. Therefore, the area under the plasma HuIFN-β concentration–time curve from 0 to 4.5 h ($AUC_0^{4.5}$) was the smallest, in spite of the larger C_{max} than those in other animals.

In rats, C_{max} was 75.33 IU/ml, and this was the same value as in the rabbits. However, the pattern of the plasma HuIFN-β concentration–time curve was different from that in rabbit, and there was no marked decline at 4.5 h after administration. The elimination phase could not be confirmed. The highest value of $AUC_0^{4.5}$ was observed in this animal.

In dogs, the C_{max} value was 49.33 IU/ml, being smaller than that in rabbits. On the other hand, the T_{max} value, 1.5 h, was later than that in rabbits. The plasma HuIFN-β concentration–time curve for the powder dosage form showed almost the same pattern as in rats, and the elimination phase could not be confirmed up to 4.5 h. The pattern of plasma HuIFN-β concentration following intranasal administration in rats and dogs was different from that in rabbit, and resembled the pattern after intramuscular injection.[11,12]

To compare the elimination rate constants (k_e) in rabbits, rats and dogs, intravenous injection of HuIFN-β was carried out in a dog. In rats, plasma HuIFN-β concentrations after intravenous injection were taken from the report by Satoh.[13] Their plasma HuIFN-β concentration data fitted a two-compartment model. The k_e values obtained in intravenous injection of 2×10^6 IU/13.5 kg for dog, 2×10^6 IU/2.8—3.2 kg for rabbits,[1] 6×10^6 IU/kg for rats[13] were 0.78, 0.52 and 1.82 h^{-1}, respectively. It seems that the k_e values are rather similar. From these results, the differences in the patterns of plasma HuIFN-β concentration–time curves might be attributed to the differences of absorption rate constant (k_a) after nasal HuIFN-β administration. The k_a values in rats and dogs might be smaller than k_e, as in the case of intramuscular injection.[11,12]

The values of plasma distribution percent of HuIFN-β in mice, rats, rabbits and dogs were calculated using Eq. 1.

$$\text{plasma distribution percent (P.D.\%)} = \text{(plasma HuIFN-}\beta\text{ concentration)} \times \text{(body weight)} \times 0.045 \times 100/\text{dose} \quad (1)^{14}$$

The term of "(body weight)×0.045" means plasma volume of an animal. When P.D.% is 100%, it means all the HuIFN-β administered exists in the plasma. The plasma

distribution percent at each sampling time is shown in Fig. 2. Until 2 h after nasal administration, values of P.D.% of HuIFN-β were almost the same among the various animals ($p > 0.05$). But, at 3 and 4.5 h after nasal administration, it was significantly different in rats and dogs compared with rabbits ($p < 0.05$). In Table I, the P.D.% values of HuIFN-β in rats, rabbits and dogs at T_{max} are also shown. In mice, only the value at 0.5 h after nasal administration is shown. The P.D.% values in mice, rats, rabbits and dogs were 0.643, 0.540, 0.526 and 0.683%, respectively. There is no significant difference among these values ($p > 0.01$). It was suggested that the extent bioavailability were almost the same. Therefore, it appeared that the difference of HuIFN-β concentration–time curve pattern was responsible for the difference of k_a.

The above experimental results suggest that the plasma HuIFN-β concentration following intranasal administration in rats and dogs might be different from that in rabbits, even considering the differences in nostrils, and HuIFN-β might have a species-specificity.[8)]

Conclusion

The absorption of HuIFN-β from the nasal mucosa was observed not only in rabbits but also in mice, rats and dogs. But the patterns of the plasma HuIFN-β concentration–time curves were different among rabbits, rats and dogs. The plasma HuIFN-β concentration in rabbits declined rapidly and showed very low values at 4.5 h after the intranasal administration of 3×10^6 IU. But in rats and dogs, plasma HuIFN-β concentration could be detected up to 4.5 h after the intranasal administration of powder (dogs) and liquid (rats) dosage forms at $4—8 \times 10^2$ IU/g. These patterns resembled the one after intramuscular injection. The difference seemed to be attributable to the different k_a values, because k_e is not significantly different among these species. Values of plasma distribution percent of HuIFN-β were almost the same in mice, rats, rabbits and dogs at 2 h after intranasal administration. HuIFN-β may show different pharmacokinetic behavior following intranasal administration in rats and dogs as compared with rabbits, even considering the differences in their nostrils. The results suggest that animal experiments with HuIFN-β are likely to provide useful information about the pharmacokinetics of HuIFN-β in human, but it is necessary to consider the species-specificity of HuIFN-β.

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