

Effect of Absorption Promoters in Intranasal Administration of Human Fibroblast Interferon as a Powder Dosage Form in Rabbits¹⁾

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The utility of the absorption promoters, sodium glycocholate (GC-Na), ethylenediamine dihydrochloride (EDTA-2Na), sodium caprylate (Cap-Na) and sodium salicylate (Sal-Na), in the intranasal administration of human fibroblast interferon- β (HuIFN- β) in rabbits was investigated. The optimal amount of added EDTA-2Na, Cap-Na and Sal-Na with respect to HuIFN- β was examined for nasal absorption in the powder dosage form. Formulations of HuIFN- β with GC-Na showed greatly enhanced intranasal HuIFN- β absorption, as compared to the other absorption promoters. The results of a stability study on HuIFN- β in homogenates of nasal mucosa suggested that GC-Na behaved as a hydrolysis inhibitor in the nasal mucosa and maintained the activity of HuIFN- β .

Keywords interferon beta; nasal administration; absorption promoter; bioavailability; nasal mucosa

Human fibroblast interferon- β (HuIFN- β) is an antiviral and antineoplastic substance. HuIFN- β has been administered only by the intravenous and intraspinal routes because of degradation by gastrointestinal proteases in the oral route. Recently, however, many researchers have reported alternative routes such as the nasal or intradermal route instead of injection.^{2,3)} In these studies, some absorption promoters found to be useful for water-soluble drugs and peptides were investigated. We reported previously that the plasma HuIFN- β levels in rabbits were significantly increased after nasal administration with sodium glycocholate (GC-Na).^{4–6)} However, the mechanism of HuIFN- β absorption enhancement by bile salts is not fully understood. In the present study, we investigated the effect of several absorption promoters on the nasal absorption of HuIFN- β . The substances used were GC-Na, ethylenediamine dihydrochloride (EDTA-2Na), sodium caprylate (Cap-Na) and sodium salicylate (Sal-Na), which have been employed as absorption promoters for rectal mucosal absorption.⁷⁾ The interaction of the absorption promoter and susceptibility of HuIFN- β to degradation by peptidases present in the nasal mucosa were also examined.

Experimental

Materials The HuIFN- β used was a preparation from Toray Industries, Inc. (3×10^6 international units (IU) per vial). The sources of the materials used as absorption promoters were as follows: sodium glycocholate (GC-Na, Tokyo Chemical Industry Co., Ltd.), EDTA-2Na (Wako Pure Chemical Industries, Ltd.), sodium caprylate (Cap-Na, Wako Pure Chemical Industries, Ltd.) and sodium salicylate (Sal-Na, Nakarai Chemicals, Ltd.).

Preparation of the Powder Dosage Form One vial of HuIFN- β was employed for the preparation of the powder dosage form. It included 3×10^6 IU of HuIFN- β , human serum albumin and lactose for stabilization in a total weight of 13 mg. Briefly, 1–5 mg of each absorption promoter was added to one vial of HuIFN- β for one dose, then mixed and passed through a 100 mesh sieve.

Nasal Administration of the Powder Dosage Form Male Japanese white rabbits (Saitama Experimental Animal Supply Co.; 3.0–3.6 kg) were used. The rabbits were fasted for 20 h before intranasal administration. The tool for effecting the nasal administration of the powder dosage form consisted of a special sprayer, 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 and the powder was sprayed through the special sprayer.

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, 4.5 and 6 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 analysis.

Stability Study on Homogenates of Nasal Mucosa Three male Japanese rabbits were used. They were fasted overnight and then killed by injecting sodium pentobarbital (Nembutal®, Abbot Laboratories) into a marginal ear vein. The nasal mucosa was removed immediately by scraping. Approximately 30 min were required for the excision of nasal mucosa from a single rabbit. The mucosa was rinsed in saline, and then homogenized with 5 ml of the rinse solution using a homogenizer (Ultra-turrax®, Kubota Trading Co., Ltd.). The homogenates were centrifuged at $9000 \times g$ at 5°C for 10 min to remove cellular and nuclear debris. Next, 1 ml of the resultant supernatant was preincubated at 37°C . HuIFN- β (3×10^6 IU) was dissolved in 109.1 ml of 0.04 M phosphate buffer at pH 7.4, and 0.12 mg of each promoter was added to 4 ml of this HuIFN- β solution. The pH of 7.4 was chosen because it is the same pH as that in the nasal mucosa and is also close to the optimum pH of most peptidases. One milliliter of the supernatants of the nasal mucosa homogenates was added to 4 ml of the HuIFN- β solution. Then, the 5 ml of mixed solution was incubated in 37°C for up to 180 min. The solution contained 3% (w/v) of absorption promoters. All incubations were performed at least in triplicate. A 0.05 ml aliquot of sample solution was collected before the addition of supernatants, and further aliquots after 10, 20, 30, 45, 60, 120 and 180 min; 0.05 ml of 0.01 N HCl was added to each sample, and the mixture was stored at -20°C until analysis.

Determination of Plasma HuIFN- β Concentration HuIFN- β was determined by enzymeimmunoassay (EIA).⁸⁾ Microplate wells were coated with anti rabbit HuIFN- β antibody as the first antibody. The enzyme reaction was determined by recording the optical density difference. The amount of HuIFN- β in the samples was calculated from the standard curves obtained with reference HuIFN- β which had been standardized against the international reference for HuIFN- β (G-023-902-527, NIH, Bethesda, MD) by bioassay.

Results and Discussion

Effect of Absorption Promoters The optimal amount of added GC-Na has already been confirmed.⁶⁾ Addition of 3 mg of GC-Na greatly enhanced the intranasal absorption of HuIFN- β in the range between 1 and 5 mg. As shown in Fig. 1, when 3×10^6 IU of HuIFN- β was administered in the powder dosage form with 3 mg of various absorption promoters, the HuIFN- β concentration–time pattern in the plasma was very different between GC-Na and the other promoters.

Table I lists the pharmacokinetic parameters obtained from the results in Fig. 1. These parameters were calculated by employing the computer program MULTI.⁹⁾ The area under the plasma HuIFN- β concentration curve (AUC_0^∞) after nasal administration was calculated by applying a

TABLE I. Bioavailability Parameters after Nasal Administration of HuIFN-β in Rabbits

Absorption promoter	C_{max} (IU/ml)	T_{max} (h)	AUC_0^∞ (IU·h/ml)	k_e (h^{-1})	MRT (h)	VRT (h^2)
Sodium glycocholate ^{a)}	96.1 ± 14.0	0.25 ± 0	167.5 ± 32.6	0.82 ± 0.18	1.98 ± 0.66	5.11 ± 3.37
Sodium salicylate ^{b)}	22.4 ± 3.5	0.83 ± 0.17	167.5 ± 73.6	0.21 ± 0.09	6.3 ± 2.5	48.7 ± 35.7

MRT, mean residence time; VRT, variance residence time. Dose: HuIFN-β, 3 × 10⁶ IU; absorption promoter, 3 mg. The data are expressed as the mean ± S.E. a) n=4. b) n=3.

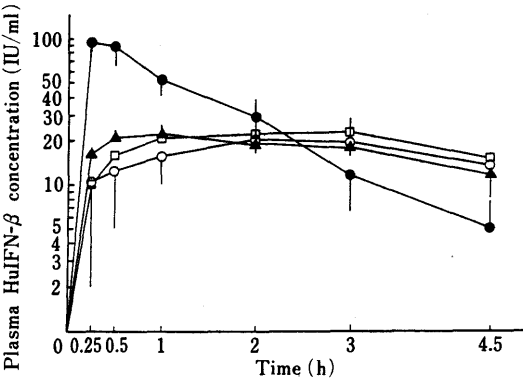


Fig. 1. Plasma HuIFN-β Concentration after Nasal Administration

All doses (3 × 10⁶ IU) were given in the powder dosage form with 3 mg of absorption promoters. ●, sodium glycocholate (n=4); ▲, sodium salicylate (n=3); □, sodium caprylate (n=1); ○, EDTA-2Na (n=3).

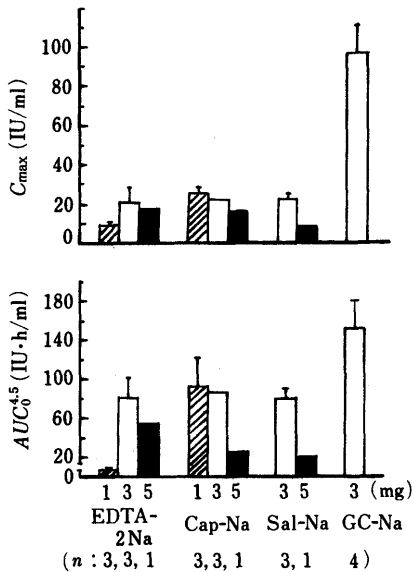


Fig. 2. C_{max} and $AUC_0^{4.5}$ of HuIFN-β (3 × 10⁶ IU) after Nasal Administration with Various Amounts of Different Absorption Promoters

The data (n=3 and n=4) are expressed as the mean ± S.E. Hatched, open and closed columns indicate 1, 3 and 5 mg of absorption promoters, respectively.

trapezoidal formula from 0 to 4.5 h, and by extrapolation using an exponential function after 4.5 h. It was shown that GC-Na gives the maximum plasma concentration of HuIFN-β (C_{max} , 96.1 IU/ml), and the peak plasma HuIFN-β levels occurred at 15 min (T_{max}) after the administration. The HuIFN-β levels in the plasma with the other absorption promoters were comparatively low but decreased slowly. The C_{max} values of HuIFN-β with EDTA-2Na, Cap-Na and Sal-Na were significantly lower than those with GC-Na ($p < 0.05$) and their T_{max} values were much slower than with GC-Na (Fig. 1). In the case of Sal-Na, T_{max}

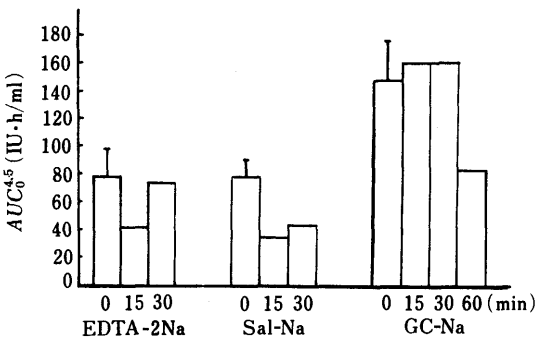


Fig. 3. Duration of Effect of Absorption Promoters on the Nasal Mucosa

EDTA-2Na, Sal-Na or GC-Na (3 mg) was administered at 15, 30 or 60 min prior to the administration of HuIFN-β (13 mg). The data are expressed as the mean ± S.E. (simultaneous administrations, n=3; other times, n=1).

was very late (0.83 ± 0.17 h) and C_{max} was low (22.4 IU/ml), and HuIFN-β was detected at 6 h following administration. The elimination rate constants (k_e) of GC-Na and Sal-Na were 0.82 ± 0.18 and 0.21 ± 0.09 h^{-1} , respectively. These findings might reflect the different promoting mechanisms in the absorption of HuIFN-β. Since the C_{max} values were low but the k_e values were also low, the AUC_0^∞ values were not significantly different among the absorption promoters examined.

Figure 2 depicts C_{max} and $AUC_0^{4.5}$ for the administration of HuIFN-β with GC-Na, EDTA-2Na, Cap-Na and Sal-Na. The data were generated by administering the dosage form containing 1 to 5 mg of each absorption promoter and 13 mg of HuIFN-β. Addition of 3 mg of GC-Na was clearly much more effective for nasal HuIFN-β absorption than addition of other absorption promoters.

GC-Na appeared to affect the nasal mucosa very quickly after the administration, because T_{max} was 15 min. We carried out pretreatment with these promoters, in which 3 mg of EDTA-2Na, Sal-Na or GC-Na was administered prior to the administration of HuIFN-β. Then, after 15, 30 or 60 min, 13 mg of HuIFN-β (3 × 10⁶ IU) was administered. As shown in Fig. 3, the values of $AUC_0^{4.5}$ on separate administration of the absorption promoters and HuIFN-β, did not differ significantly from the $AUC_0^{4.5}$ on simultaneous administration of the absorption promoters and HuIFN-β. However, the effect of GC-Na on the nasal mucosa appeared to disappear at 60 min after the administration, since the $AUC_0^{4.5}$ value in this case was much lower than those at the earlier times.

Stability of HuIFN-β in Phosphate Buffer and Homogenates of Nasal Mucosa The stability of HuIFN-β in homogenates of nasal mucosa was studied in an attempt to clarify the mechanism of the enhanced HuIFN-β absorption induced by absorption promoters given *via* the nasal

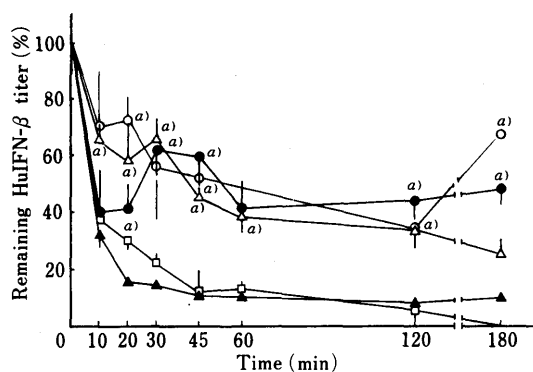


Fig. 4. Disappearance of HuIFN- β with Absorption Promoters on Incubation in 0.04 M Phosphate Buffer (pH 7.4) at 37 °C ($n=3$)

▲, control; ●, sodium glycocholate; △, EDTA-2Na; ○, sodium caprylate; □, sodium salicylate. *a*) Indicates $p < 0.05$ (compared to the control).

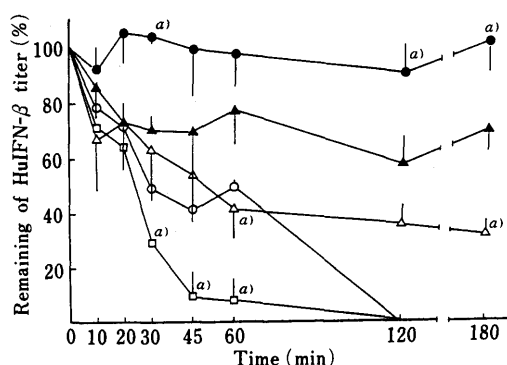


Fig. 5. Disappearance of HuIFN- β with Absorption Promoters on Incubation in Homogenates of Nasal Mucosa in 0.04 M Phosphate Buffer (pH 7.4) at 37 °C ($n=3$)

▲, control; ●, sodium glycocholate; △, EDTA-2Na; ○, sodium caprylate; □, sodium salicylate. *a*) Indicates $p < 0.05$ (compared to the control).

route. First, the effects of the various absorption promoters on HuIFN- β were examined in phosphate buffer (Fig. 4). Then, the effects in homogenates of nasal mucosa (Fig. 5) were investigated.

Figure 4 compares the disappearance profiles of HuIFN- β in phosphate buffer at pH 7.4, in the presence of various absorption promoters. In the control, the HuIFN- β activity decreased by more than 80% within 1 h of incubation. The results indicated that HuIFN- β was not chemically stable over the time of incubation. Sal-Na revealed a similar, very low remaining HuIFN- β activity upon incubation in the phosphate buffer. Cap-Na and GC-Na showed similar patterns of HuIFN- β activity, but a higher percent of HuIFN- β than in the control remained. In particular, Cap-Na maintained a high activity of HuIFN- β after 3 h. Although a clear explanation cannot be given, these results suggest that Cap-Na and GC-Na may make the conformation of HuIFN- β more stable by associating with the HuIFN- β molecule. These promoters might form micelles containing HuIFN- β as reported in the case of insulin.¹⁰⁾

Figure 5 compares the disappearance profiles of HuIFN- β in the homogenates of nasal mucosa. The control contained no absorption promoter and the HuIFN- β titer decreased to 30% after 30 min of incubation. The results indicated that the supernatants retained their peptidase activity over the time course of incubation¹¹⁾; the stability

of HuIFN- β in the homogenates of nasal mucosa was increased compared to that in the control with phosphate buffer. The proteins and peptides in the nasal mucosa should be partly destroyed upon homogenization, and degradation products might interact with HuIFN- β , as in the case of the human serum albumin which is added as a stabilizer. With addition of Sal-Na and Cap-Na, the titer of HuIFN- β decreased very quickly in the homogenates of nasal mucosa. With EDTA-2Na, the values of the remaining HuIFN- β (percent) in the homogenates of nasal mucosa were the same as the remaining HuIFN- β titers in phosphate buffer. GC-Na significantly increased the stability of HuIFN- β in the homogenates of nasal mucosa. The hydrolytic rate of HuIFN- β was reduced by at least 30% in the presence of GC-Na. GC-Na appeared to behave as an effective hydrolysis inhibitor and to decrease the susceptibility of HuIFN- β to hydrolysis. GC-Na appears to act quite quickly on the nasal mucosa (Fig. 3).

The bioavailability of HuIFN- β from the nasal route was estimated to correspond to only 3% of that of intravenously administered HuIFN- β by EIA.⁵⁾ This decrease in HuIFN- β titer might be attributable to three main factors, as follows: (1) loss into the throat; (2) instability on the nasal mucosa due to the pH and temperature; and (3) loss of activity on the nasal mucosa due to peptidase action.¹¹⁾ The absorption promoters appeared to increase the permeability of the nasal mucosa by interacting with it¹⁰⁾ and also to reduce the loss of the HuIFN- β activity. These effects may involve interactions with HuIFN- β such as micelle formation and inhibition of the peptidase in the nasal mucosa, respectively. EDTA-2Na and Cap-Na have a Ca^{2+} -chelating ability and remove Ca^{2+} ion from the mucous membrane.¹²⁾ Cap-Na and GC-Na appeared to make the conformation of HuIFN- β more stable by associating with the HuIFN- β molecule in the phosphate buffer (Fig. 4). GC-Na and Sal-Na are known to affect the nasal mucosa.^{10,13)} However, their interactions with it may be different, since the elimination rate constants (k_e) of GC-Na and Sal-Na were very different (0.82 ± 0.18 and $0.21 \pm 0.09 \text{ h}^{-1}$, respectively). In addition to enhancing the permeability of the nasal mucosa by interacting with it, GC-Na appears to behave as a peptidase inhibitor. GC-Na was therefore the most effective promoter of HuIFN- β absorption in the nasal mucosa.

Acknowledgments The authors are grateful to Mr. Shigeru Ishikawa, Mr. Takao Yamada and Mr. Naoki Uchida for their assistance with the experimental work.

References and Notes

- 1) Part of this work was presented at the 107th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, 1987.
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