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# Enzyme Immunoassay of Human Fibroblast Interferon after Intranasal Administration with Several Excipients in Rabbits[1)]

TAKESHI IGAWA, YOSHIE MAITANI,* YOSHIHARU MACHIDA and TSUNEJI NAGAI

*Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2–4–41, Shinagawa-ku, Tokyo 142, Japan*

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Human interferon-$\beta$ (HuIFN-$\beta$) titer after intranasal administration was determined by enzyme immunoassay (EIA), substituting for bioassay. The intranasal absorption was linearly related to HuIFN-$\beta$ dose. Compared with intravenous administration, the mean bioavailability of intranasal HuIFN-$\beta$ was 3%. The effects of several excipients, human serum albumin (HSA), polyethylene glycol, diethylaminoethyl-dextran, carboxy polymethylene, $\alpha$-cyclodextrin and cholesteryl stearate, on the absorption of HuIFN-$\beta$ were examined with the powder dosage form. Among these excipients, HSA and $\alpha$-cyclodextrin gave the highest HuIFN-$\beta$ concentrations in plasma. The area under the plasma concentration-time curve ($AUC$) decreased as the molecular weight of excipients was increased.

**Keywords**——interferon-$\beta$; nasal administration; enzyme immunoassay; excipient; powder dosage form; bioavailability

Human fibroblast interferon-$\beta$ (HuIFN-$\beta$) is an antiviral and antineoplastic substance. Intravenous and intraspinal administration of HuIFN-$\beta$ have been used clinically because it is a peptide.[2,3)] Many clinical studies on intranasal administration of leukocyte interferon have been carried out.[4,5)] However, HuIFN-$\beta$ has been studied only by intravenous and intramuscular administration. Recently we reported the nasal administration of HuIFN-$\beta$ as liquid and powder dosage forms in rabbits.[6,7)] In the previous studies, HuIFN-$\beta$ titer in plasma was determined by bioassay (BA). As no report of the determination of HuIFN-$\beta$ by enzyme immunoassay (EIA) after nasal administration has yet appeared, we employed the EIA which was developed by Toray Industries Inc.[8)] in the present study.

It was found that powder and liquid dosage forms showed the same bioavailability in nasal administration of HuIFN-$\beta$.[7)] However, in general a powder dosage form is more stable than a liquid one. Moreover, much higher bioavailability is expected if mucosal adhesive excipients are used.[9)] Therefore, the effects of several excipients on the absorption of HuIFN-$\beta$ were examined, using sodium glycocholate as an absorption promoter.

## Experimental

**Materials**——The HuIFN-$\beta$ used was prepared by Toray Industries, Inc.; $3 \times 10^6$ international unit (IU) per vial, and $1 \times 10^7$ IU per vial (specially ordered). The preparations included human serum albumin (HSA) and lactose for stabilization; the latter one contained lower concentrations of HSA and lactose.

The following materials were used as the excipients. HSA (Nihon Seiyaku Co., Ltd.), $\alpha$-cyclodextrin (Nihon Shokuhin Kako Co., Ltd.), polyethylene glycol 4000 (PEG, Wako Pure Chemical Industries, Ltd.), diethylaminoethyl (DEAE)-dextran 9000 synthesized from dextran 9000,[10)] cholesteryl stearate (CS, Nikko Chemical, Ltd., Tokyo, Japan), hydroxypropyl cellulose-H (HPC, Nippon Soda Co., Ltd.), and carboxypolymethylene (HIVISWAKO-204, HW, Wako Pure Chemical Industries, Ltd.). As an absorption promoter, sodium glycocholate (GC-Na, Tokyo Chemical Industry Co., Ltd.) was used.

**Preparation of the Powder and Liquid Dosage Forms**——In the studies using rabbits, the $3 \times 10^6$ IU vial of HuIFN-$\beta$ was used for preparation of the powder dosage form, and 3 mg of GC-Na was added to HuIFN-$\beta$ as an absorption promoter. They were mixed and passed through a 100 mesh sieve. In the study on the effect of excipients on the absorption, the $1 \times 10^7$ IU vials of HuIFN-$\beta$ were used.

**Animal Experiment**——Male Japanese white rabbits (Saitama Experimental Animal Supply Co., 3.0—3.6 kg) were used. Rabbits were fasted for 20 h before intranasal or intravenous administration. The tool for nasal administration of the powder dosage form consisted of a special sprayer, Eppendorf pipette tip and polyethylene tubing. The sample powder was put in an Eppendorf pipette tip which was connected to the polyethylene tubing (1.57 mm i.d. and 2.08 mm o.d.). The tube was inserted into the nasal cavity at a position about 2.8 cm from the nostril and sprayed through the 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-$\beta$. Plasma was separated by centrifugation at 3000 rpm for 15 min. Plasma samples were stored at $-20$ °C until analysis.

**Determination of Plasma HuIFN-$\beta$ Concentration**——Plasma HuIFN-$\beta$ was assayed by EIA.[8,11] Microplate wells were coated with anti rabbit HuIFN-$\beta$ antibody as the first antibody. The enzyme reaction was determined by recording the change of optical density. The amount of HuIFN-$\beta$ in samples was calculated from the standard curves obtained with reference HuIFN-$\beta$ which had been standardized against the international reference of HuIFN-$\beta$ (G-023-902-527, NIH, Bethesda, MD) by bioassay.

## Results and Discussion

### Dose Dependence Studies

Doses of $1 \times 10^6$, $3 \times 10^6$, $6 \times 10^6$, $1 \times 10^7$ and $2 \times 10^7$ IU of HuIFN-$\beta$ were administered as powder dosage forms with 3 mg of GC-Na. Plasma HuIFN-$\beta$ concentrations determined

TABLE I. Pharmacokinetic Parameters after Nasal and Intravenous Administration of HuIFN-$\beta$ in Rabbits

| Dose (IU) | $n$ | $C_{max}$ (IU/ml) | $T_{max}$ (h) | $AUC_0^\infty$ (IU·h/ml) | $MRT$ (h) | $VRT$ ($h^2$) | $F$ (%) |
|---|---|---|---|---|---|---|---|
| $1 \times 10^6$ | 1 | 37.3 | 0.5 | 31.7 | 1.02 | 0.61 | — |
| $3 \times 10^6$ | 4 | 96.1±14.0 | 0.3±0 | 167.5± 32.6 | 1.98±0.66 | 5.11±3.37 | 3.01 |
| $6 \times 10^6$ | 4 | 107.2±48.9 | 0.4±0.1 | 387.9±234.0 | 3.72±0.88 | 16.0 ±7.31 | — |
| $1 \times 10^7$ | 1 | 135.0 | 0.3 | 362.1 | 2.75 | 8.01 | — |
| $2 \times 10^7$ | 1 | 212.0 | 1.0 | 893.9 | 3.85 | 14.0 | — |
| $3 \times 10^6$ (i.v.) | 3 | — | — | 5558.2±893.9 | 0.70±0.11 | 1.32±0.32 | — |

MRT, mean residence time; VRT, variance residence time.

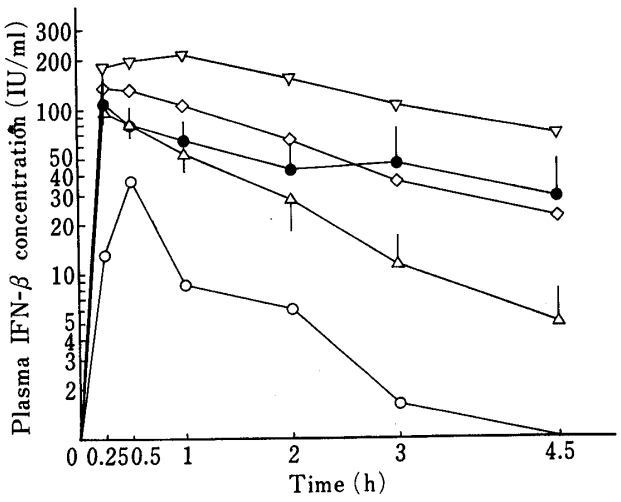


Fig. 1. Plasma HuIFN-$\beta$ Concentration after Nasal Administration in Rabbits

All doses were in powder dosage form with 3 mg of sodium glycocholate. Dose: ○, $1 \times 10^6$ IU ($n=1$); △, $3 \times 10^6$ IU ($n=4$); ●, $6 \times 10^6$ IU ($n=4$); ◇, $1 \times 10^7$ IU ($n=1$); ▽, $2 \times 10^7$ IU ($n=1$).

by EIA are shown in Fig. 1. Table I shows pharmacokinetic parameters obtained from the results in Fig. 1. These parameters were calculated by using the computer program MULTI.[12] The area under the plasma HuIFN-$\beta$ concentration curve ($AUC_0^\infty$) after nasal administration was calculated by applying a trapezoidal formula from 0 to 4.5 h, and by extrapolation using an exponential function after 4.5 h. The maximum plasma HuIFN-$\beta$ concentration ($C_{max}$) was reached within 1 h ($T_{max}$) and $C_{max}$ increased with increase of the dose. The fraction absorbed ($F$) was calculated by means of Eq. 1.

$$F=\frac{(AUC_{nasal})\times(dose_{i.v.})}{(AUC_{i.v.})\times(dose_{nasal})}\times 100 \quad (1)$$

The absolute bioavailability at the HuIFN-$\beta$ dose of $3\times10^6$ IU was 3%. This value is almost the same as that obtained by BA.[7]

The relationship between $AUC_0^\infty$ and dose after nasal administration to rabbits is shown in Fig. 2. A linear relationship was observed and the coefficient of correlation was 0.975.

**Effect of Excipients on the Absorption of HuIFN-$\beta$**

Excipients in the powder dosage form have an important role in the absorption of insulin from the nasal mucosa.[9] Therefore, we studied the effects of several excipients on HuIFN-$\beta$ absorption from the nasal mucosa, as shown in Fig. 3. One dose of HuIFN-$\beta$ ($3\times10^6$ IU) contained 3 mg of GC-Na and 13 mg of excipient. HSA gave the highest plasma concentration of HuIFN-$\beta$; $T_{max}$ was 0.25 h. The plasma concentration of HuIFN-$\beta$ decreased rapidly after the nasal administration. The elimination rate constant ($k_e$) was 0.823 $h^{-1}$ at the dose of HuIFN-$\beta$ of $3\times10^6$ IU. The plasma concentration of HuIFN-$\beta$ decreased rapidly with all excipients used except for HPC and HW. Namely, $T_{max}$ was less than 1 h. The results obtained by the addition of HPC and HW, gel-forming polymers, were different from those with other excipients. The $C_{max}$ values were one-fifteenth of $C_{max}$ with HSA. The low $C_{max}$ with these polymers might be due to the entrapment of HuIFN-$\beta$ by gelled HPC and HW. Alternatively, these excipients may interact with HSA which was present with HuIFN-$\beta$ as a stabilizer. The

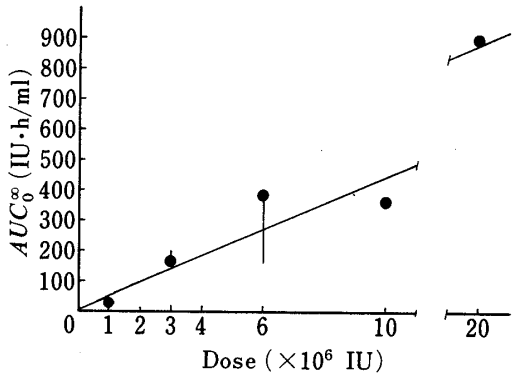


Fig. 2. Relationship between Dose and $AUC_0^\infty$ of HuIFN-$\beta$ after Nasal Administration in Rabbits

Doses used were $1\times10^6$ IU ($n=1$), $3\times10^6$ IU ($n=4$), $6\times10^6$ IU ($n=4$), $1\times10^7$ IU ($n=1$) and $2\times10^7$ IU ($n=1$). ↓ means ± S.E. The coefficient of correlation is 0.975.

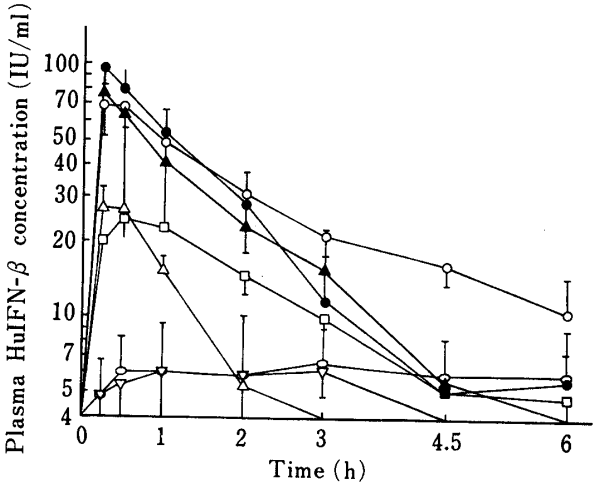


Fig. 3. HuIFN-$\beta$ Concentration in Plasma after Nasal Administration with Different Excipients in Rabbits

One dose ($3\times10^6$ IU) contained 3 mg of sodium glycocholate and 13 mg of excipient. ▲, cholesteryl stearate ($n=3$); ●, HSA ($n=4$); ○, α-cyclodextrin ($n=3$); □, PEG ($n=3$); △, DEAE-dextran 9000 ($n=3$); ▽, carboxypolymethylene ($n=3$); ○, the mixture of HPC and carboxypolymethylene (3 : 1), ($n=3$). All data are expressed as mean ± S.E.

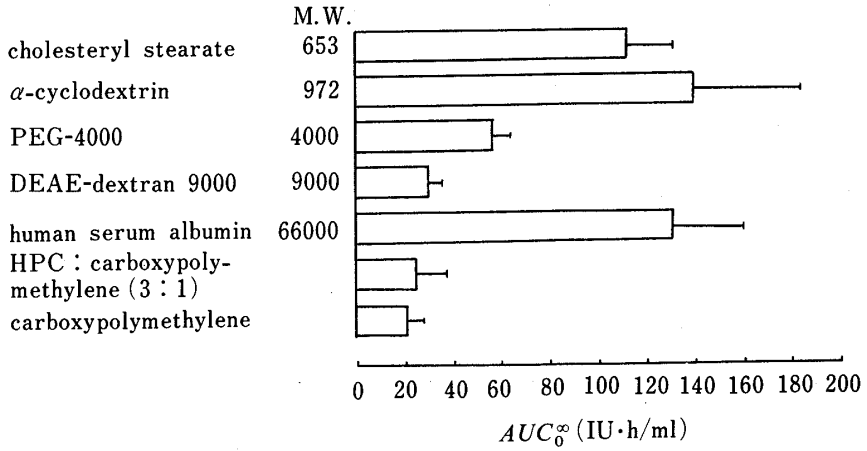


Fig. 4. $AUC_0^\infty$ of Plasma HuIFN-$\beta$ Concentration after Nasal Administration of HuIFN-$\beta$ as a Powder Dosage Form ($3\times10^6$ IU) with 3 mg of Sodium Glycocholate and 13 mg of Various Excipients in Rabbits

All data are expressed as mean ± S.E. ($n=3$).

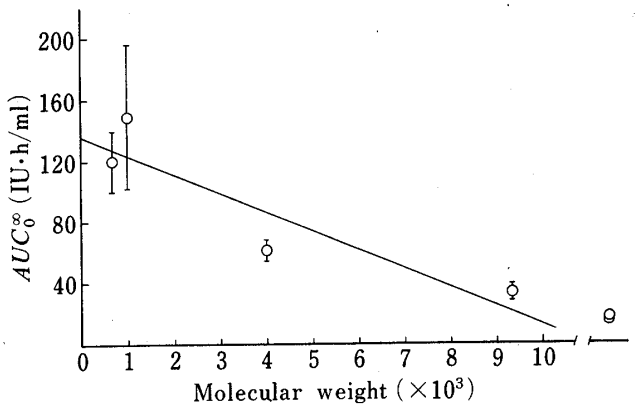


Fig. 5. Relationship between Molecular Weight of Excipients and $AUC_0^\infty$ of HuIFN-$\beta$ Concentration Curve after Nasal Administration of a Powder Dosage Form to Rabbits

The coefficient of correlation is 0.913. All data are expressed as mean ± S.E. ($n=3$).

$AUC_0^\infty$ with each excipient is shown in Fig. 4. It was observed that $AUC_0^\infty$ decreased as the molecular weight of excipients was increased except for HSA. There was a significant linear relationship between the molecular weight of excipients and $AUC_0^\infty$, as shown in Fig. 5; the coefficient of correlation was 0.913. HSA (molecular weight, 66000) was an exception to this relationship, because of its stabilizing effect on HuIFN-$\beta$.

## Conclusion

The assay of HuIFN-$\beta$ by EIA was effective, and EIA could be substituted for BA. Intranasal absorption was linearly related to HuIFN-$\beta$ dose, as determined by EIA. In the case of a powder dosage form, the absorption of HuIFN-$\beta$ seemed to be influenced by the molecular weight of excipients. $AUC_0^\infty$ after nasal administration of HuIFN-$\beta$ decreased with increase of the molecular weight of excipients, except for HSA, which is a stabilizer.

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