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ADMINISTRATION ROUTE DEPENDENT BIOAVAILABILITY OF INTERFERON-α AND EFFECT OF BILE SALTS ON THE NASAL ABSORPTION

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ABSTRACT

Administration route dependent bioavailability of recombinant human interferon alpha (IFN-α) and effect of seven bile salts and polyoxyethylene-9-lauryl ether (BL-9) on nasal absorption of IFN-α were studied in rats. IFN-α (1.5×10^7 IU/kg) was administered through *iv, pv, po* and *ip* routes and *AUC* of the routes were compared. As a result, it was found that IFN-α is extracted almost completely during its passage through the GI lumen, and is not absorbed from the GI lumen. Moreover, IFN-α sparingly transported through the GI lumen suffers additional extraction by the GI mucosa (57 %) and the liver (8 %) consecutively and only about 40 % of it can reach the systemic circulation. Therefore, a high bioavailability of IFN-α cannot be expected through the oral route even with the

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aid of absorption enhancers. On the other hand, significant absorption of IFN-α could be attained through the nasal route with some absorption enhancers (1 % w/v). Among the enhancers examined, sodium cholate (CH), sodium glycocholate (GC), sodium taurocholate (TC), sodium glycodeoxycholate (GDC), sodium taurodeoxycholate (TDC) and BL-9 increased the nasal bioavailability of IFN-α. However, sodium dehydrocholate (DHC) and sodium deoxycholate (DOC) did not show such effect. Nasal bioavailability of IFN-α was increased up to 32.3 (± 15.5) % by 1 % TC. The enhancing effect of TC was significantly ($p<0.05$) greater than those of CH, DOC, DHC and BL-9. TC and GC seemed to be potential candidates for the nasal absorption enhancers of IFN-α, considering that they are reportedly less toxic than GDC and TDC.

INTRODUCTION

Interferon (IFN) has been administered to patients usually intravenously or intramuscularly. Much effort has been dedicated to formulate alternative effective delivery systems other than parenteral administration. Development of oral formulation of IFN would greatly expand its clinical application. However, bioavailability of oral IFN has been known to be low [1-4]. This is likely to be due to its proteolysis in GI tract [2], poor GI absorption and/or presystemic first-pass extraction. In this respect, reasons for the low bioavailability of oral IFN should be explained before the attempts to increase its bioavailability with the aid of absorption enhancers.

To assess the mechanism of the low oral bioavailability of recombinant human interferon-α (IFN-α), IFN-α was administered intravenously (*iv*), perorally (*po*), intraperitoneally (*ip*), and portal venously (*pv*) to rats, and bioavailabilities of each administration routes were compared in this study.

As a potential alternative way for parenteral and oral administrations, the nasal route has been actively investigated in the last few years for IFN [5-14]. However, even with the nasal route, the use of certain types of absorption enhancers seems necessary for significant absorption of peptide drugs. Various groups have explored the possibility of enhancing the nasal absorption of peptide drugs by means of absorption enhancers, including nonionic surfactants [15, 16], chelators [6, 16], fatty acids [6, 16], bile salts [6, 8-10, 12, 16-22] and many others [6, 23, 24].

Bile salts are the most studied absorption enhancers in both animals and human. Based on scanning electron microscopic observations, the absorption enhancement mechanism of bile salts is believed to be due to the disturbance of the nasal membrane integrity [22, 25]. Nevertheless, they are less irritating to the nasal mucosa than synthetic surfactants such as polyoxyethylene-9-lauryl ether (BL-9) and are reported to be well-tolerable for animals and human subjects, at least for a short-term treatment [17, 21].

Amongst the bile salts tested as absorption enhancers, dihydroxy bile salts appear to be more toxic than trihydroxy bile salts [26]. Therefore, their use has been restricted mainly to the trihydroxy bile salts such as sodium taurocholate (TC) for nicardipine [27], and sodium glycocholate (GC) for IFN-β [6-8], insulin [12, 24] and growth hormone [15].

Thus, the objective of this study is two-fold. The first is to elucidate the mechanism for the low bioavailability of oral IFN-α to examine the possibility of increasing the bioavailability by absorption enhancers. The second aim is to compare systematically the efficiency of several bile salt enhancers on the nasal absorption of IFN-α. This would allow rational selection of the appropriate bile salt enhancer for nasal absorption.

Trihydroxy bile salts such as sodium cholate (CH), GC and TC, and dihydroxy bile salts such as sodium dehydrocholate (DHC),

sodium deoxycholate (DOC), sodium glycodeoxycholate (GDC) and sodium taurodeoxycholate (TDC) were selected as bile salt enhancers. BL-9, a reported nasal absorption enhancer for insulin [21], recombinant methionyl-human growth hormone [15] and gentamicin [26], was also tested and compared with the bile salt enhancers for their enhancing effect.

MATERIALS

Recombinant human lymphoblastoid interferon alpha (IFN-α) was obtained from Cheil Sugar Co., Seoul, Korea. The specific activity of IFN-α was 1×10^8 reference units/mg protein and its purity was over 99.0 %. Molecular weight of IFN-α was approximately 18,000 - 22,000 daltons. CH, GC, TC, DOC, DHC, GDC, TDC and BL-9 were obtained from Sigma Chemical Company, Poole, England, and their effectiveness as a nasal absorption enhancer was examined. All other chemicals were of reagent grade.

METHODS

Administration of IFN-α via Various Routes-Male Wistar rats (Experimental Animal Center, Seoul National University) weighing 230 - 300 g were used in this experiment. IFN-α was administered to the rats through *iv*, *po*, *pv* and *ip* routes. IFN-α was dissolved in sterilized physiological saline for the appropriate concentrations. Under urethane anesthesia, femoral arteries of the rats were cannulated with polyethylene tubings (PE-50, Intramedic, Clay Adams, USA) for blood sampling. Femoral veins were also cannulated with PE-50 for *iv* administration. For *pv* administration, the pyloric vein was cannulated as follows: the abdomen was opened through a midline incision and the tip of an injection

needle (25 gauge) attached to a PE-50 was inserted into the hepatic portal vein and fixed with adhesive agent (Aron Alpha, Sankyo Co., Tokyo, Japan). The needle was bent 120° for the convenience of insertion. This catheter was connected to a 1 ml syringe and the dose of IFN-α was given through the syringe into the portal vein. For *po* administration, a round-tip needle connected to a 1 ml syringe was inserted into the stomach.

After complete recovery from the anesthesia, IFN-α was administered at the dose of 1.5 x 10^7 IU/kg (2 ml/kg) for *iv* , *po, pv* and *ip* study. Blood samples (0.15 ml) were withdrawn from the femoral artery via PE-50 catheter prior to administration and at 10, 20, 40, 60, 120, 180 and 240 min post-administration. Plasma samples were obtained by immediate centrifuging the blood samples at 4,000 x g for 10 min and stored at -20 °C until analysis.

Nasal Administration of IFN-α – The *in vivo* experimental model described by Hirai et al [22] and slightly modified by us [28] was used to study the effect of the absorption enhancers on the nasal absorption of IFN-α. Male Wistar rats of 230 - 300 g were anesthetized by *ip* injection of 1125 mg/kg urethane (250 mg/ml). After 20 min of the urethane injection, the neck of the rat was incised and the trachea was cannulated with polyethylene catheter (Tubing No. 7, Hidiko, Japan). The esophagus was cannulated with the same catheter of which one end was sealed to prevent leaking of the administered drug from the nasal cavity. After suturing the incision, the nasopalatine was closed with Aron Alpha to prevent drug draining from the nasal cavity to the mouth. The carotid artery was cannulated with polyethylene catheter (PE-50, Intramedic) for blood sampling. After 30 min, the saline solution of IFN-α with and without the absorption enhancers (1 %, w/v) was prepared freshly and instilled into the nasal cavity with a Hamilton syringe at the

dose of 0.1 ml/kg rat (1.5×10^7 IU/kg for IFN-α and 1 mg/kg for the enhancers). Then the nostrils were sealed with Aron alpha.

Blood samples (0.3 ml) were collected into the tubes containing 10 μl of heparinized saline (150 IU/ml) prior to administration and at 10, 20, 40, 60, 120, 180 and 240 min post-administration. The blood samples were immediately centrifuged at 4,000 x g for 10 min and the separated plasma samples were stored frozen at -20 °C until assayed.

Assay of IFN-α in Plasma-IFN-α in plasma was assayed according to Jung et al [29]. The freeze-thawing was found not to affect the activity of IFN-α [29]. After thawing the frozen plasma samples, IFN-α was titrated by 50 % cytopathic effect reduction method using vesicular stomatitis virus (Indiana strain) and human amnionic cells (WISH). The effect was determined by measuring the cellular uptake of neutral red dye using auto-reader (Microelisa MR 580) at 570 nm [30]. All the assays were made employing the international reference preparations for IFN-α obtained from the National Institute for Biological Standards and Control, London. All titres were reported in IU/ml.

Pharmacokinetic Analysis-Total body plasma clearance (CL_t), distribution volume at steady-state (Vd_{ss}) and pharmacokinetic half life ($t_{1/2\beta}$) were calculated using *iv* data as follows,

$$CL_t = Dose/AUC \quad (1)$$

$$Vd_{ss} = Dose \ \ AUMC/AUC^2 \quad (2)$$

$$t_{1/2\beta} = CL_t/\beta \quad (3)$$

where *AUC*, *AUMC* and *β* are the area under the plasma activity-time curve, area under the moment of the plasma activity-time curve and the slope of the natural log concentration-time curve at postdistributive phase (*β*-phase), respectively. *AUC* and *AUMC*

were calculated by trapezoidal rule and β was obtained by fitting the plasma activity data to the conventional 2-compartment model by MULTI program [31].

Using mean *AUC* data after various routes of administration, fraction of IFN-α transported (*F*; Availability = 1 - Extraction Ratio) across the liver (*F3*), GI mucosa (*F4*) and GI lumen (*F5*) were calculated from the following relationships based on the drug disposition model [32, 33], where IFN-α was presumed neither to be cleared in the lung [34] nor subjected to enterohepatic recirculation.

$$F3 = AUCpv/AUCiv \quad (4)$$

$$F4 = AUCip/AUCpv \quad (5)$$

$$F5 = AUCpo/AUCip \quad (6)$$

Analyses of Data - One-way analysis of variance (ANOVA) with Turkey's multiple range comparison procedure was used to compare each data, and a *p* value of 0.05 or less was considered to be significant.

RESULTS

Pharmacokinetics and Bioavailability of IFN-α–Plasma levels of IFN-α after *iv, pv, ip, po* and *in* administration at the dose of 1.5×10^7 IU/kg were plotted as a function of time and are shown in Fig.1. Plasma profile after *iv* administration showed biexponential decay. CL_t, Vd_{ss} and $t_{1/2\beta}$ calculated from the *iv* data using Eq. 1 - 3 were 4.6 (± 0.9) ml/min/kg, 134.2 (±36.0) ml/kg and 53.2 (± 12.6) min, respectively. These values were consistent with our previous work [35]. Plasma profile after *pv* administration was almost parallel to that of *iv* administration. Plasma profile after *ip* administration showed rapid absorption, but those after *po* and *in*

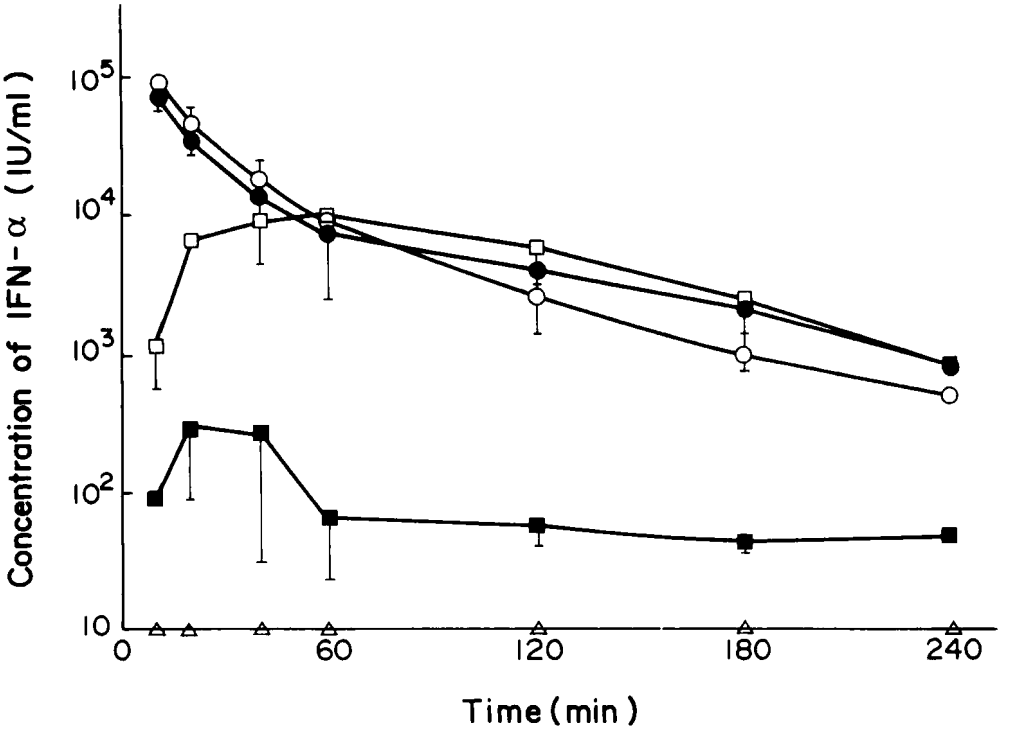

Fig. 1-Plasma profiles of IFN-α after *iv* (○), *pv* (●), *ip* (❑), *po* (■) and *in* (△) administration of 1.5 x 10 ^{7} IU/kg doses to rats without any absorption enhancers. Each point represents mean ± SE of four experiments.

administration were much lower than that of *iv* administration. Especially, plasma level after *in* administration was so low that it was neglected in Fig. 1.

Bioavailabilities of IFN-α calculated by *AUC* ratio after *pv, ip, po* and *in* administration were 92.0, 39.3, 0.6 and 0.0 %, respectively (Table 1). From the relationships shown by Eq. 4 - 6, the availability of IFN-α across the liver (*F3*), GI mucosa (*F4*) and GI lumen (*F5*) were calculated using mean *AUC* values from Table 1 and are shown in Table 2. It indicates that the first-pass extraction by the GI

TABLE 1. *Bioavailability parameters of IFN-α after iv, po, ip, pv and in administration at a Dose of 1.5 x 10 7 IU/kg to rats*[a]

Administration Route	C_{max} (IU/ml)	T_{max} (min)	*AUC* (IU min/ml)	*Bioavailability* %
iv	-	-	3111113.7 (± 324343.4)	100.0
po	343.0 (± 218.8)	16.7 (± 3.3)	19915.1 (± 10008.1)	0.6 (± 0.3)
ip	9916.0[b] (±1230.0)	60.0[b] (± 0.0)	1223612.8 (± 170197.0)	39.3 (± 5.5)
pv	-	-	2862224.6[b] (± 733485.5)	92.0[b] (± 23.6)
in	ND	ND	ND	0.0

[a] Each value represents mean ± SE of four experiments. C_{max} ; Maximum plasma activity of IFN-α after *po*, *ip* and *in* administration. T_{max} ; Time to reach maximum plasma activity. ND; not detectable. Bioavailabilities of each administration routes were calculated by comparing each *AUC* value with mean *AUC* value of *iv* administration. [b] Significantly ($p<0.05$) different from *po* data.

TABLE 2. *Fraction of IFN-α transported across the liver, GI mucosa and GI lumen calculated from Eqs. 4 -6 using AUC data in Table 1.*

Organ	Fraction Transported *(F)*
Liver *(F_3)*	0.92
GI Mucosa *(F_4)*	0.43
GI Lumen *(F_5)*	0.02

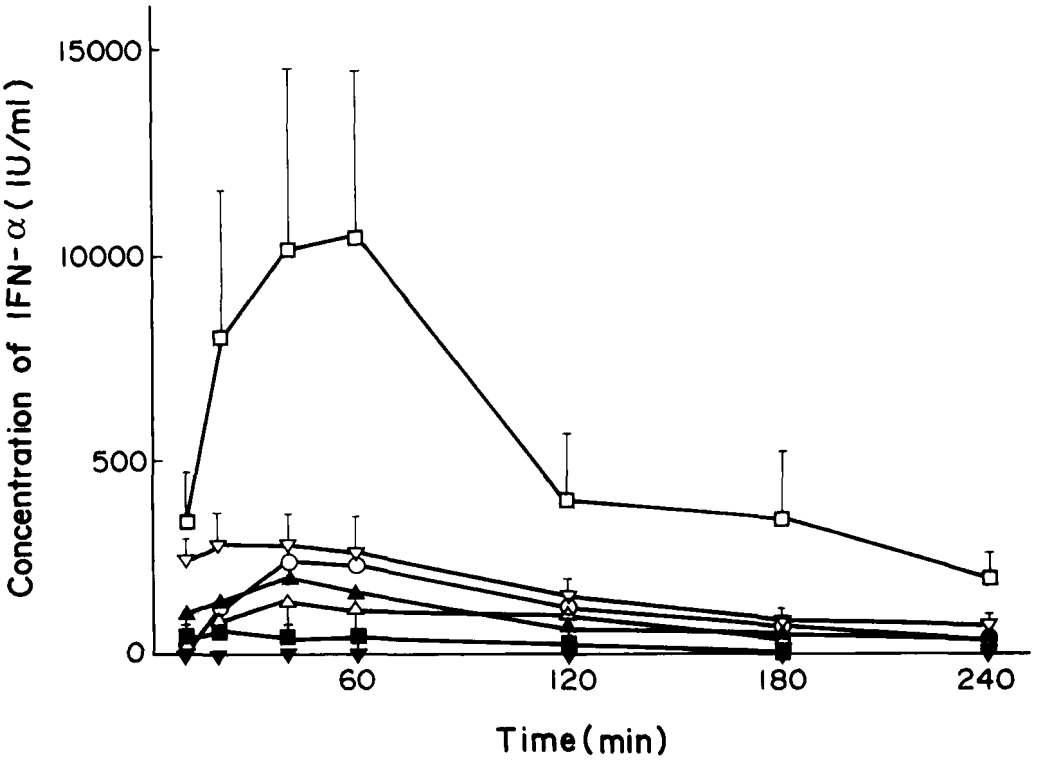

Fig. 2-Plasma profiles of IFN-α after *in* administration with each of eight absorption enhancers (1 % w/v); TC (❑), GDC ( ), TDC (○), GC (▲), BL-9 (△), CH (■), DOC and DHC ( ).

mucosa is rather higher than that by the liver, and absorption from the GI lumen to the GI mucosa (*F5*) is negligible.

Effect of Absorption Enhancers on *In* Absorption of IFN-α–Effect of some absorption enhancers on the *in* absorption of IFN-α was plotted in Fig. 2. The concentrations of the enhancers in the IFN-α solution were 1 % (w/v). IFN-α was not absorbed intranasally from the solution without enhancer. However, significant *in* absorption of IFN-α was obtained with the aid of some enhancers. Among the enhancers examined, CH, GC, TC, GDC, TDC and BL-

TABLE 3. *Relative potencies of bile salts and BL-9 for the in absorption of IFN-α in rats*[a]

Enhancer (1 % w/v)	*AUC* (IU min/ml)	C_{max} (IU/ml)	T_{max} (min)	*Bioavailability* (%)
CH	67630.0 (± 12712.8)	567.7 (± 249.8)	27.5 (± 7.5)	2.2 (± 0.4)
GC	288842.2 (± 149262.5)	3210.0 (± 1853.3)	23.3 (± 8.8)	9.3 (± 4.8)
TC	1004431.5[b] (± 482503.0)	17192.7[c] (± 6995.0)	40.0 (± 11.5)	32.3[b] (± 15.5)
DOC	ND	ND	ND	ND
GDC	371594.2 (± 132310.8)	2978.0 (± 944.8)	30.0 (± 15.3)	11.9 (± 4.2)
TDC	344325.1 (± 97066.6)	1722.6 (± 779.4)	50.0 (± 5.8)	9.2 (± 2.9)
DHC	ND	ND	ND	ND
BL-9	235216.6 (± 93608.3)	1758.8 (± 688.7)	45.0 (± 9.6)	7.6 (± 3.0)

[a] Each value represents mean ± SE of 3-4 experiments. Bioavailability was calculated relative to mean *AUC* achieved following *iv* injection of equal dose (1.5×10^7 IU/kg). ND; not detectable. [b] Significantly ($p<0.05$) different from CH, DOC, DHC and BL-9. [c] Significantly ($p<0.05$) different from CH, DOC, TDC, DHC and BL-9. For abbreviations, see text.

9 increased the *in* absorption of IFN-α, but DOC and DHC did not exert any effect. The effect of TC was significantly ($p<0.05$) greater than those of CH, DOC, DHC and BL-9.

Bioavailability parameters of *in* IFN-α administered together with 1 % (w/v) enhancers were summarized in Table 3. Absolute bioavailability of *in* IFN-α could be increased up to 32.3 % with the aid of 1 % (w/v) TC.

DISCUSSION

Pharmacokinetic parameters of *iv* IFN-α such as CL_t, Vd_{ss} and $t_{1/2\beta}$ agreed with our previous report [35]. Vd_{ss} of IFN-α (134.2 ± 36.0 ml/kg) was far below the body weight which indicates poor distribution within the body tissues. Hepatic clearance of IFN-α (CL_h) was calculated to be 2.6 ml/min/kg from the relationship

$$CL_h = ER_h \times HPF \qquad (7)$$

where ER_h and HPF represent the hepatic extraction ratio of IFN-α and the hepatic plasma flow respectively. ER_h was calculated to be 0.08 from the relationship $ER_h = 1 - F_3$, and HPF (32.5 ml/min/kg) was cited from the literature [36]. The calculated CL_h (2.6 ml/min/kg) occupied 56.5 % of the CL_t. The rest of CL_t might be explained primarily by the additional elimination in the GI mucus considering the high extraction ratio of GI mucosa ($1 - F_4 = 0.57$) from Table 2. Besides the elimination in the GI mucus, renal elimination might be at least partially responsible for the nonhepatic clearance since profound renal catabolism of IFN-α has been reported [37] in spite of its negligibly small urinary excretion [35]. Pulmonary catabolism of interferons was reported to be negligible [34].

Table 2 indicates that IFN-α is in fact not absorbed from the GI lumen and is additionally extracted by the GI mucosa (57 %) and liver (8%). Therefore, only 39.6 % of IFN-α absorbed from the GI lumen would reach the systemic circulation. It is interesting that IFN-α is extracted much more by the GI mucosa than by the liver. The extremely high extraction of IFN-α by the GI lumen (98 %) is thought to be primarily due to its gastrointestinal proteolysis by pancreatic enzymes such as trypsin and chymotrypsin. Aminopeptidase might contribute to the proteolysis in part [2]. Besides proteolysis, the large molecular size of IFN-α might also be responsible for its poor GI absorption [3]. Because IFN-α will suffer additional extraction during its passage through GI mucosa (57 %) and liver (8 %), a high bioavailability through the oral route can hardly be expected even with the aid of absorption enhancers or proteinase inhibitors [1, 2].

Not only the oral absorption but also the colorectal absorption of IFN-α has been reported to be poor even with the aid of absorption enhancers [38-41]. This implies that the colorectal mucosa is comparable to the GI mucosa in terms of IFN-α extraction.

The *in* absorption of IFN-α could not be obtained without absorption enhancers, as reported earlier [5-11, 13]. Reasons for this poor *in* absorption have been attributed to the loss of administered drug into the throat [6], instability on the nasal mucosa due to pH and temperature [6], loss of activity on the nasal mucosa due to peptidase action [11], and binding of IFN to nasal mucosal cells [5].

Absorption enhancers have been employed in the attempt to increase the extent of *in* IFN absorption [6-10, 13]. GC has been reported to enhance the *in* absorption of IFN-β greatly from the powder dosage form [6-8] in rabbits at the dose of 1 mg/kg. It stimulated us to compare the *in* enhancing effects of some bile

salts systematically. All the enhancers in Table 2 except DHC and DOC enhanced *in* absorption of IFN-α significantly with 1 mg/kg dose. Among the six effective enhancers, TC enhanced the *in* absorption of IFN-α to achieve a bioavailability of 32.3 %. This result is consistent with the effect of TC on *in* absorption of nicardipine [27]. The enhancing effect of TC was significantly ($p<0.05$) greater than those of CH, DOC, DHC and BL-9, but not much different from those of GC, GDC and TDC.

Bile salts are generally believed to enhance the *in* absorption of drugs by disturbing the nasal membrane integrity [22, 25]. GC was found to enhance the *in* absorption of IFN-β probably by increasing the permeability of the nasal mucosa and inhibiting the aminopeptidase activity [6]. In this study, the enhancing mechanism of TC for the *in* absorption of IFN-α was not investigated, though it is likely to be similar to that of GC.

Gordon et al [12] found a positive correlation between hydrophobicity of bile salts and their adjuvant potency in enhancing insulin *in* absorption. They demonstrated that DOC, a more hydrophobic bile salt than GC, was much more effective than GC in enhancing *in* insulin absorption. In the present study, however, GC enhanced the *in* absorption of IFN-α significantly, while DOC did not.

Duchateau et al [26] also reported that the enhancing efficiency of bile salts on *in* absorption of gentamicin is related to the physicochemical properties of the bile salts such as cholesterol-solubilizing capacity and hydrophobicity. In this sense, they concluded that CH rather than TC will be the most active *in* absorption enhancer. Their conclusion, however, does not hold for *in* absorption of IFN-α of this study.

Therefore, the enhancement mechanisms of bile salts for the *in* absorption of drugs seem to differ greatly between IFN-α and insulin or gentamicin. As for IFN-α, hydrophobicity of the bile salts

seems not likely to be the major factor enhancing its *in* absorption. More studies on the *in* absorption mechanism of IFN-α including absorption pathway (transcellular or paracellular [24]) together with the physicochemical properties of bile salts should be performed before the conclusion on the enhancement mechanism of bile salts can be drawn.

Considering the fact that trihydroxy bile salts are generally less toxic than dihydroxy bile salts [24], TC as well as GC is considered as a potential candidate for *in* absorption enhancer for IFN-α.

In conclusion, the results obtained from this study show that not more than 40 % of oral dose cannot be expected to be absorbed even with the aid of absorption enhancers since there is a severe additional extraction of IFN-α by the GI mucosa. Among possible alternative routes, intranasal administration together with 1 % TC or GC seems to be promising. However, evaluation of the practical usefulness of TC as an absorption enhancer needs further careful examination especially in terms of its toxic effects on nasal mucosa.

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