International Journal of Pharmaceutics, 93 (1993) 91–99

IJP 03027

Effect of absorption promoters on the nasal absorption of drugs with various molecular weights

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(Received 3 May 1992)
(Modified version received 30 July 1992)
(Accepted 1 September 1992)

Key words: Absorption promoter; Nasal absorption; Drug delivery; Molecular weight dependence; Phenol red; Fluorescein isothiocyanate-dextran

Summary

The effect of absorption promoters on the nasal absorption of drugs with different average molecular weights was examined in rats. Phenol red and fluorescein isothiocyanate-dextrans (FITC-dextrans) with various molecular weights were chosen as model drugs while the absorption promoters used in this study were Na glycocholate, Na taurocholate, Na deoxycholate, Na salicylate, EDTA, Na caprylate and Na caprate, all at a concentration of 1%. Of the absorption promoters, bile salts such as Na glycocholate, Na taurocholate, Na deoxycholate, Na caprate and EDTA appeared to be more effective at enhancing the nasal absorption of drugs as compared to Na salicylate and Na caprylate. Na glycocholate, Na caprate and EDTA showed the strongest promoting effect of drugs with an approximate molecular weight 4000, while the maximal effect of Na taurocholate and Na deoxycholate was observed in the nasal absorption of drugs with an approximate molecular weight of 10000. In contrast, Na salicylate and Na caprylate did not affect the absorption of drugs having various molecular weights. These findings indicated that the efficacy of absorption promoters on the nasal absorption of drugs was dependent on their molecular weights.

Introduction

Accompanying recent progress in the field of biotechnology utilizing genetic recombination and cell fusion technology, increased interest has been focussed on the use of physiologically active peptides as pharmaceutical drugs. However, oral administration of peptide drugs is often limited by their instability in the gastrointestinal environment and poor absorption from the gut (Lee and Yamamoto, 1990). Consequently, although the clinical administration of peptides is presently limited to administration by injection, such frequent administration subjects the patients to considerable pain, and there is also the possibility of the manifestation of serious side effects. Therefore, we have a strong desire to develop an administration method for peptides that can serve as an alternative to oral and injection administration. Various mucosal absorption routes have been utilized thus far, including nasal (Hirai et al., 1981), buccal (Ishida et al., 1981), ocular

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(Yamamoto et al., 1989), vaginal (Okada et al., 1982) and rectal (Nishihata et al., 1984) absorption. Of all these routes, the nasal pathway has been pursued as an alternative to the parenteral route for the administration of a variety of peptides and proteins. Indeed, some small peptides such as enkephalins appear to be absorbed effectively via the nasal route with bioavailabilities comparable to those for the intravenous route of administration (Su et al., 1985). However, many higher molecular weight peptides and proteins such as horseradish peroxidase (HRP) and secretin show much lower absorption efficiencies when administered intranasally (Ohwaki et al., 1985; McMartin et al., 1987). Consequently, absorption promoters are required to enhance the nasal absorption of peptides and proteins. It has been reported that a large number of absorption promoters including surfactants, bile salts, chelators and fatty acids can be used to enhance the nasal absorption of macromolecules with varying degrees of success (Chien and Chang, 1987). However, few studies have been performed to determine the relationship between the molecular size and absorption promoting efficacy of various absorption promoters. In our previous investigation, it was indicated that the effectiveness of absorption enhancers in the lung depended on the drug's molecular size (Ohtani et al., 1991).

In the present study, phenol red and fluorescein isothiocyanate dextrans (FDs) with different average molecular weights were chosen as model drugs. The nasal absorption of these drugs was then examined. We also describe the effect of various absorption promoters on the nasal absorption of these drugs and examine the relationship between the molecular weights of drugs and their absorption promoting efficacy.

Materials and Methods

Materials

Fluorescein isothiocyanate-labelled dextrans (FITC-dextrans), sodium glycocholate, sodium taurocholate and sodium deoxycholate were purchased from Sigma Chemical Co., St. Louis, MO. The mean molecular weights of the FITC-dextrans employed were 4400, 9400, and 71 200 (referred to as FD-4, FD-10 and FD-70, respectively). Sodium caprylate and sodium caprate were supplied by Tokyo Kasei Kougyou, Co. Ltd, Japan. Phenol red, EDTA, sodium salicylate and all other reagents used in these experiments were of reagent grade and obtained from Nacalai Tesque, Inc., Japan.

Preparation of drug solution

The isotonic buffer solution of pH 7.4 was prepared from 0.123 M Na_2HPO_4 and 0.163 M NaH_2PO_4. Each drug was dissolved in this buffer solution at a concentration of 2 mg/25 μl for absorption studies. In certain experiments, the dosing solutions were added with absorption promoters such as sodium glycocholate, sodium taurocholate, sodium deoxycholate, sodium caprylate, sodium caprate, EDTA and sodium salicylate to yield a final concentration of 1 w/v%.

Absorption studies

Male Wistar albino rats weighing 180–230 g were anesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 40 mg/kg. A surgical operation for the in vivo nasal absorption study was carried out according to the modified method of Hirai et al. (1981). After the trachea had been exposed, a polyethylene tube (o.d. 2 mm, i.d. 1.2 mm, Hibiki, Co. Ltd, Japan) was inserted toward the lungs of the animal to maintain respiration. The same tube was also inserted through the esophagus to the posterior part of the nasal cavity. This cannula served to introduce the drug solution into the nasal cavity. The nasopalatine was then sealed with an adhesive agent to block any drainage of the drug solution from the nasal cavity into the mouth. In order to collect blood samples during the experiment, 0.2 ml of heparin solution (1000 U/ml solution, Nacalai Tesque, Inc., Japan) was injected intravenously. A cannulation was also made in the jugular artery with a polyethylene tube (i.d. 0.5 mm, o.d. 0.8 mm; Dural Plastics, Dural, Australia). After the surgical operation, drug solution (2 mg/25 μl) was administered into the nasal cavity by means of a micropipette through the nostril. After administration, 0.5 ml of blood was

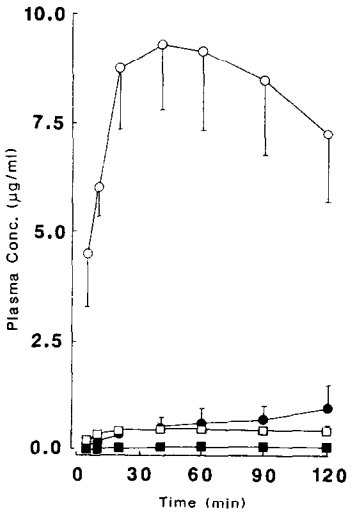

Fig. 1. Plasma concentration of drugs with various molecular weight after nasal administration to rats at a dose of 2 mg. (○) Phenol red, (●) FD-4, (□) FD-10, (■) FD-70. Results are expressed as the mean ± S.E. of 3–4 rats. The absence of bars indicates that the S.E. is within the size of the symbol.

taken periodically from the jugular artery. The plasma was separated by centrifugation at 3000 rpm and the drug concentration in the plasma was determined. At the end of the experiments, the drug solution in the nasal cavity was washed with pH 7.4 buffer solution. The washings were combined with the drug solution and made up to 10 ml with pH 7.4 buffer solution. The percent absorption of these drugs from the nasal cavity was estimated as the difference between the dosing amount of the drug in the initial solution and the percentage of drug remaining in the nose at the end of the experiments.

Analytical methods

Fluorescein isothiocyanate dextrans: 200 μl of a plasma sample solution was diluted with 4 ml of pH 7.4 phosphate buffer. The fluorescence intensity was measured on a fluorescence spectrophotometer (Shimadzu model, RFA-540) at an excitation wavelength of 490 nm and emission wavelength of 520 nm. Phenol red: 200 μl of a plasma sample solution was alkalinized with 3 ml of 1 N NaOH and determined spectrophotometrically at 560 nm.

Pharmacokinetic analyses

The pharmacokinetic parameters after intravenous administration were calculated by MULTI (Yamaoka et al., 1981). In Fig. 7, the absorption percentages of these drugs in 120 min were estimated by a deconvolution method (Yamaoka et al., 1981) using the plasma concentration-time course data after intravenous injection.

Statistical analyses

Results were expressed as the mean ± standard error of the mean (S.E.). Statistical analyses were performed using Student's *t*-test.

Results

Nasal absorption of drugs with various molecular weights

Fig. 1 shows the plasma concentration profiles of phenol red and FDs following intranasal administration in rats. As shown in Fig. 1, the plasma concentrations of the drugs gradually decreased with increasing molecular weights of the compounds.

Fig. 2 indicates the relationship between the molecular weight of drugs and drug absorption % in 2 h. A linear correlation exists between log absorption % of the drugs and log molecular weights for these drugs over the range of 350–70000.

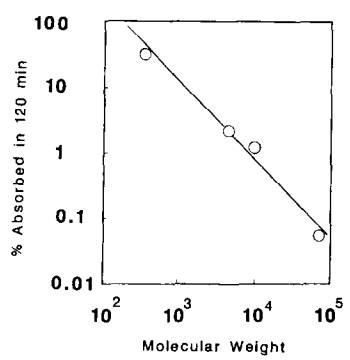

Fig. 2. Relationship between absorption % and molecular weight of drugs on nasal absorption.

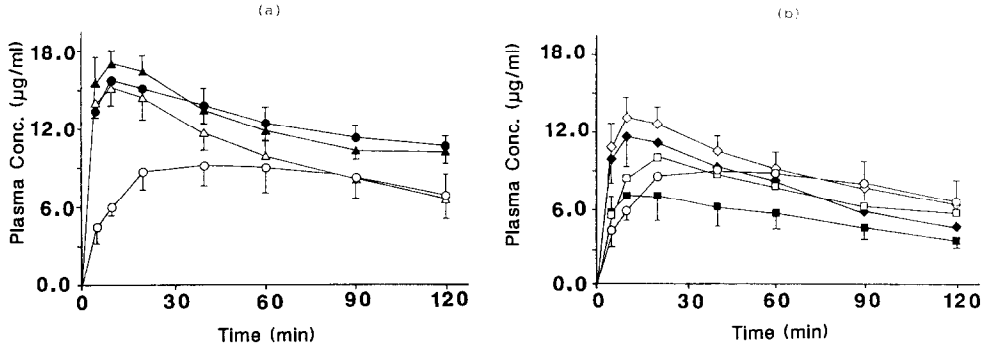

Fig. 3. Plasma concentration-time profiles of phenol red after nasal administration in the presence of various absorption promoters in rats. (a) (○) Control, (●) 1% Na glycocholate, (△) 1% Na taurocholate, (▲) 1% Na deoxycholate; (b) (○) control, (□) 1% EDTA, (■) 1% Na salicylate, (◇) 1% Na caprylate, (◆) 1% Na caprate. Each point represents the mean ± S.E. of 3–4 rats.

TABLE 1

Peak plasma concentration (C_{max}), time to peak (T_{max}), area under the curve (AUC), absorption % in 2 h after intranasal administration of phenol red in rats (n = 3 or 4)

	C_{max} (μg/ml)	T_{max} (min)	AUC (0–120 min) (μg min ml^{-1})	Absorption (%)
Control	9.6 ± 1.6	40.0 ± 8.2	977.8 ± 174.4	32.9 ± 5.6
Glycocholate	16.1 ± 1.0	15.0 ± 2.9	1523.2 ± 124.3 [a]	55.7 ± 4.0 [b]
Taurocholate	15.3 ± 1.4	12.5 ± 2.5	1246.1 ± 159.7 [d]	46.2 ± 6.1 [d]
Deoxycholate	18.1 ± 0.4	11.3 ± 3.1	1501.7 ± 67.6 [a]	63.4 ± 7.1 [c]
EDTA	10.6 + 2.6	20.0 ± 7.1	927.4 ± 235.9 [d]	32.6 ± 8.2 [d]
Salicylate	7.6 ± 1.7	32.5 ± 19.3	687.0 ± 133.5 [d]	24.7 ± 4.7 [d]
Caprylate	13.4 ± 1.4	12.5 ± 2.5	1293.3 ± 61.8 [d]	42.2 ± 5.3 [d]
Caprate	10.7 ± 0.6	15.0 ± 2.9	937.1 ± 42.3 [d]	34.0 ± 1.7 [d]

[a] $P < 0.05$, [b] $P < 0.02$, [c] $P < 0.01$, [d] not significantly different, compared with the control.

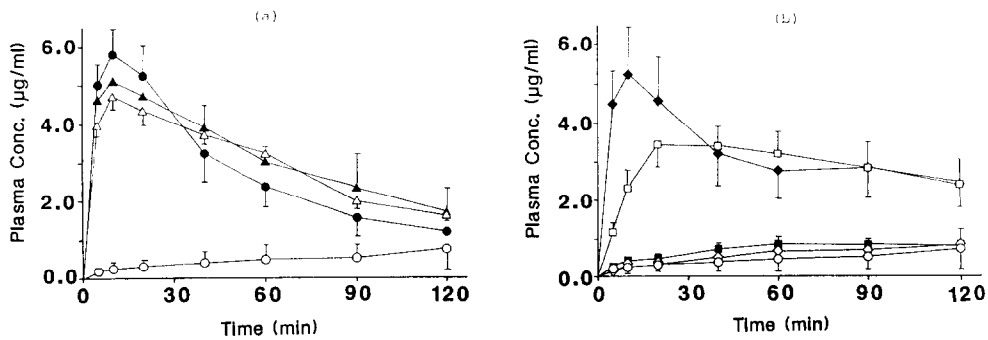

Fig. 4. Plasma concentration-time profiles of FD-4 after nasal administration in the presence of various absorption promoters in rats. (a) (○) Control, (●) 1% Na glycocholate, (△) 1% Na taurocholate, (▲) 1% Na deoxycholate; (b) (○) control, (□) 1% EDTA, (■) 1% Na salicylate, (◇) 1% Na caprylate, (◆) 1% Na caprate. Each point represents the mean ± S.E. of 3–4 rats.

TABLE 2

Peak plasma concentration (C_{max}), time to peak (T_{max}), area under the curve (AUC), absorption % in 2 h after intranasal administration of FD4 in rats (n = 3 or 4)

	C_{max} (μg/ml)	T_{max} (min)	AUC (0–120 min) (μg min ml^{-1})	Absorption (%)
Control	–	–	69.8 ± 38.8	2.2 ± 1.3
Glycocholate	5.8 ± 0.7	10.0 ± 0.0	350.2 ± 68.1 [a]	11.7 ± 2.0 [b]
Taurocholate	4.7 ± 0.3	10.0 ± 0.0	374.0 ± 22.5 [c]	11.5 ± 0.6 [c]
Deoxycholate	5.4 ± 0.7	20.0 ± 10.0	396.7 ± 61.4 [b]	12.4 ± 1.6 [c]
EDTA	3.6 ± 0.6	30.0 ± 5.8	359.6 ± 66.0 [b]	10.1 ± 1.9 [a]
Salicylate	–	–	100.3 ± 19.4 [d]	2.9 ± 0.6 [d]
Caprylate	–	–	84.1 ± 16.7 [d]	2.6 ± 0.6 [d]
Caprate	5.3 ± 1.2	10.0 ± 0.0	399.7 ± 93.7 [a]	13.0 ± 3.0 [a]

[a] $P < 0.05$, [b] $P < 0.02$, [c] $P < 0.01$, [d] not significantly different, compared with the control.

Effect of absorption promoters on nasal absorption of drugs with various molecular weights

Fig. 3 depicts the concentration-time profiles of phenol red in plasma after intranasal administration in the presence of various absorption promoters in rats. A marked increase in plasma concentration of phenol red was observed in the presence of three bile salts, while Na caprylate and Na caprate induced only a slight increase in the plasma concentration of phenol red. We found no significant increase in the plasma concentration of phenol red with EDTA and salicylate. Table 1 lists various pharmacokinetic parameters after intranasal administration of phenol red. A significant increase in AUC and absorption % of phenol red was noted in the presence of Na glycocholate and Na deoxycholate, whereas there was no significant difference in the presence of the other absorption promoters.

Fig. 4 shows plasma concentration-time profiles of FD-4 after intranasal administration in the presence of various absorption promoters. A significant increase in plasma concentration of FD-4 occurred in the presence of all three bile salts. Similar results were also noted concerning the effect of Na caprate and EDTA on the plasma concentration of FD-4. In contrast, Na caprylate and salicylate exerted no significant influence on the plasma concentration of FD-4. Table 2 summarizes the pharmacokinetic parameters after intranasal administration of FD-4 in rats. We found a significant increase in AUC and absorption % of FD-4 in all cases except for Na salicylate and Na caprylate.

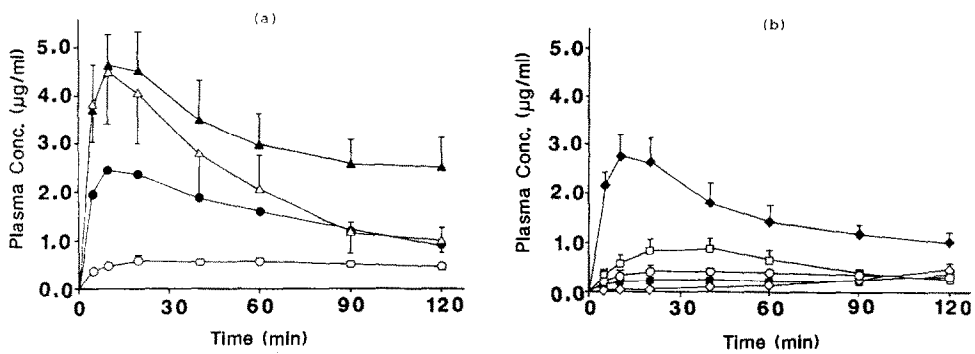

Fig. 5. Plasma concentration-time profiles of FD-10 after nasal administration in the presence of various absorption promoters in rats. (a) (○) Control, (●) 1% Na glycocholate, (△) 1% Na taurocholate, (▲) 1% Na deoxycholate; (b) (○) control, (□) 1% EDTA, (■) 1% Na salicylate, (◇) 1% Na caprylate, (◆) 1% Na caprate. Each point represents the mean ± S.E. of 3–4 rats.

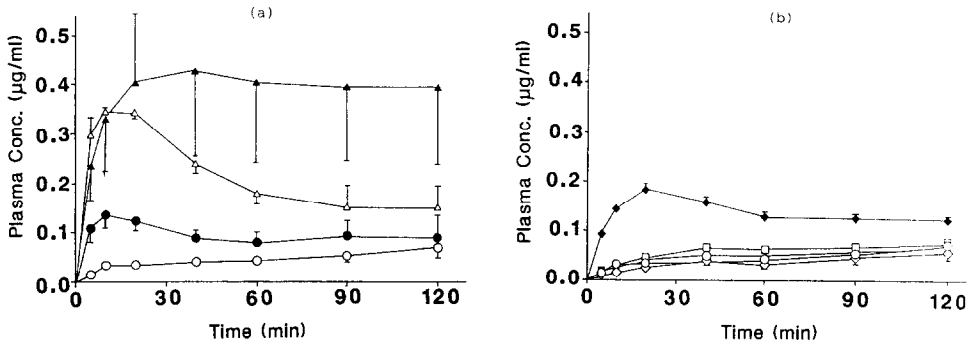

Fig. 6. Plasma concentration-time profiles of FD-70 after nasal administration in the presence of various absorption promoters in rats. (a) (○) Control, (●) 1% Na glycocholate, (△) 1% Na taurocholate, (▲) 1% Na deoxycholate; (b) (○) control, (□) 1% EDTA, (■) 1% Na salicylate, (◇) 1% Na caprylate, (◆) 1% Na caprate. Each point represents the mean ± S.E. of 3–4 rats.

TABLE 3

Peak plasma concentration (C_{max}), time to peak (T_{max}), area under the curve (AUC), absorption % in 2 h after intranasal administration of FD-10 in rats (n = 3 or 4)

	C_{max} (μg/ml)	T_{max} (min)	AUC (0–120 min) (μg min ml^{-1})	Absorption (%)
Control	–	–	50.8 ± 9.3	1.2 ± 0.2
Glycocholate	2.3 ± 0.1	10.0 ± 0.0	180.9 ± 10.7 [d]	4.9 ± 0.3 [d]
Taurocholate	4.3 ± 1.1	10.0 ± 0.0	260.0 ± 79.7 [a]	7.6 ± 2.1 [b]
Deoxycholate	4.6 ± 0.7	13.3 ± 3.3	440.3 ± 53.2 [c]	10.1 ± 2.0 [c]
EDTA	0.9 ± 0.2	33.3 ± 6.7	75.3 ± 16.6 [e]	1.8 ± 0.4 [e]
Salicylate	–	–	35.9 ± 7.9 [e]	0.9 ± 0.2 [e]
Caprylate	–	–	29.1 ± 4.3 [e]	0.7 ± 0.1 [e]
Caprate	2.9 ± 0.5	13.3 ± 3.3	203.2 ± 32.9 [c]	5.7 ± 0.9 [d]

[a] $P < 0.05$, [b] $P < 0.02$, [c] $P < 0.01$, [d] $P < 0.001$, [e] not significantly different, compared with the control.

TABLE 4

Peak plasma concentration (C_{max}), time to peak (T_{max}), area under the curve (AUC), absorption % in 2 h after intranasal administration of FD-70 in rats (n = 3 or 4)

	C_{max} (μg/ml)	T_{max} (min)	AUC (0–120 min) (μg min ml^{-1})	Absorption (%)
Control	–	–	6.1 ± 0.8	0.05 ± 0.009
Glycocholate	0.14 ± 0.03	10.0 ± 0.0	13.4 ± 2.3 [a]	0.13 ± 0.04 [e]
Taurocholate	0.35 ± 0.01	15.0 ± 5.0	26.1 ± 3.4 [c]	0.21 ± 0.02 [d]
Deoxycholate	0.44 ± 0.17	26.7 ± 6.7	38.8 ± 14.0 [a]	0.18 ± 0.03 [c]
EDTA	–	–	7.2 ± 0.5 [e]	0.06 ± 0.006 [e]
Salicylate	–	–	6.7 ± 1.1 [e]	0.05 ± 0.007 [c]
Caprylate	–	–	5.1 ± 1.3 [e]	0.04 ± 0.009 [e]
Caprate	0.18 ± 0.01	20.0 ± 0.0	16.7 ± 1.0 [d]	0.12 ± 0.006 [b]

[a] $P < 0.05$, [b] $P < 0.02$, [c] $P < 0.01$, [d] $P < 0.001$, [e] not significantly different, compared with the control.

The effect of various absorption promoters on nasal absorption of FD-10 and FD-70 was also examined. As shown in Fig. 5, the plasma concentration of FD-10 increased on coadministration with three bile salts and caprate, while we did not observe a significant effect on the addition of salicylate and caprylate. Similarly, we found a significant enhancement in plasma concentration of FD-70 by the addition of these bile salts and caprate (Fig. 6). The pharmacokinetic parameters of FD-10 and FD-70 are listed in Tables 3 and 4. As indicated in Tables 3 and 4, bile salts and Na caprate significantly increased the AUC and absorption % of both FD-10 and FD-70. However, EDTA, Na salicylate and Na caprylate did not produce any significant absorption of these drugs. Overall, bile salts such as Na glycocholate, Na taurocholate, Na deoxycholate, Na caprate and EDTA appeared to be more effective in enhancing the nasal absorption of drugs than Na salicylate and Na caprylate.

The absorption enhancement by these absorption promoters was evaluated from the ratio of absorption percentages in 120 min between control and enhancers by a deconvolution method. As shown in Fig. 7a and b, Na glycocholate, Na caprate and EDTA showed the highest promoting effect to drugs with an approximate molecular weight of 4000, while the maximal effect of Na taurocholate and Na deoxycholate was observed in the nasal absorption of drug with an approximate molecular weight of 10000.

Discussion

The nasal route has been actively pursued as an alternative to the parenteral route for the administration of a variety of peptides and proteins (Chien and Chang, 1987). Recently, Fisher et al. (1987) studied the relationship between the molecular weight of hydrophilic compounds and their systemic absorption via the nasal route and found that the uptake of drugs decreased with increasing molecular weight. Similarly, McMartin et al. (1987) studied bioavailability for many compounds using the literature value and found that the transport of drugs across the nasal membrane fell off sharply at molecular weights greater than 1000. Our present result that a close correlation exists between the bioavailability of the nasal absorption of drugs and their molecular weights drugs is fairly consistent with these previous reports. Similar findings have also been reported for the nasal absorption of drugs with various molecular weights by Maitani et al. (1989) and Donovan et al. (1990).

In the present study, we found that phenol red was relatively absorbed from the nasal mucosa even in the absence of absorption promoters.

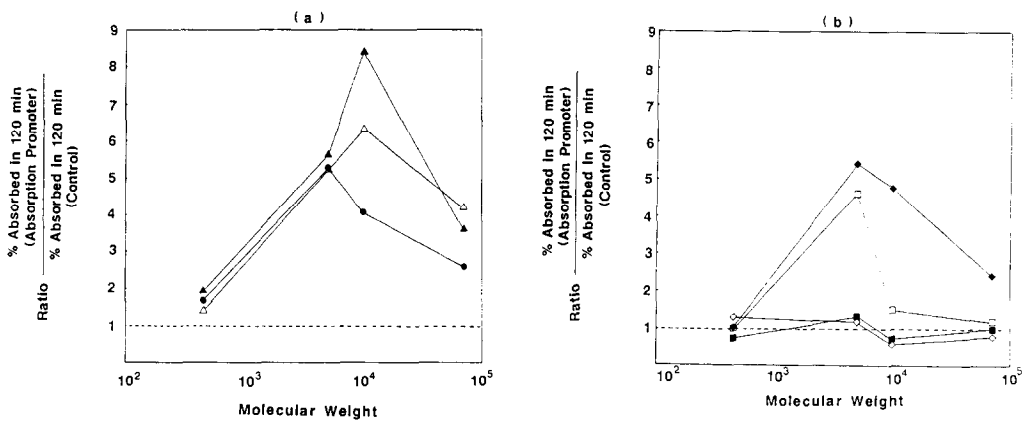

Fig. 7. Relationship between enhancement effect of various absorption promoters and molecular weight of drugs on nasal absorption. (a) (●) 1% Na glycocholate, (△) 1% Na taurocholate, (▲) 1% Na deoxycholate; (b) (□) 1% EDTA, (■) 1% Na salicylate, (◇) 1% Na caprylate, (◆) 1% Na caprate.

Similarly, Duchateau et al. (1987) reported that the nasal bioavailability of phenol red in the absence of absorption enhancers was 22.1 ± 11.0%. In contrast, Kakemi et al. (1970) reported that phenol red was poorly absorbed from the rat small intestine unless absorption promoters were used. The difference between nasal and intestinal absorption characteristics of phenol red may be partly explained by the morphological difference of these regions. The nose has a large epithelial surface area available due to numerous microvilli and the subepithelial layer is highly vascularized compared with the gastrointestinal tract (Chien and Chang, 1987).

As already mentioned, absorption promoters are reqiured to promote the nasal absorption of peptides and proteins (Chien and Chang et al., 1987). In this study, bile salts, EDTA, fatty acids and salicylate were used as absorption promoters for the enhancement of drugs with various molecular weights.

Bile salts have been shown to facilitate peptide and protein absorption from various absorption sites (Gordon et al., 1985; Aungst et al., 1988). Some investigators have proposed that bile salts enhance nasal permeability by removing the epithelial cells, which constitute a major permeability barrier, rather than causing chemical modification of the mucosal cells (Hersey et al., 1987). Murakami et al. (1984) reported that the effect of deoxycholate on the rectal absorption of sodium ampicillin was more marked than that of sodium glycocholate and sodium taurocholate, which was in good agreement with our data.

It is believed that EDTA increases intestinal mucosal permeability by depleting Ca^{2+} from the tight junctional areas, thereby opening these normally tight junctions. Cassidy and Tidball (1967) reported that phenol red absorption was greatly increased, while tissue levels of Ca^{2+} or Mg^{2+} were depleted, by sodium EDTA. They also found that replacement of Ca^{2+} or Mg^{2+} in the perfusion solutions restored the normally low permeability of phenol red and normalized the tissue levels of these cations. Recently, Yamashita et al. (1987) examined the effect of EDTA on the electrical parameters of sulfanilic acid permeation through rat jejunum in vitro and found that the permeation-enhancing effect of EDTA occurred primarily through paracellular pathways. Our result that EDTA showed the strongest promoting effect on drug with an approximate molecular weight of 4000, as compared with the effectiveness of the other compounds such as phenol red, FD-10 and FD-70, may be partly explained by the assumption that enlargement of the pore radius existing in the paracellular pathway was restricted even in the presence of EDTA.

Kajii et al. (1988) reported that sodium caprylate can increase the permeability of rat intestinal brush border membrane (BBM) through perturbation of the membrane, promoting the absorption of water-soluble and poorly absorbed drugs. Recently, Mishima et al. (1987) showed that fatty acids can reduce the activity of leucine aminopeptidase and inhibit the degradation of insulin in the nasal mucosa, which is also an important factor for improving the absorption of peptides and proteins. They also demonstrated that Na caprate exhibited the strongest promoting effect on insulin among these fatty acid salts. Our result that Na caprate is more effective for enhancing the nasal absorption of drugs than Na caplylate is quite consistent with their data.

Na salicylate has been mostly used to promote rectal and intestinal membrane permeability (Nishihata et al., 1984). It was suggested that Na salicylate interacts with membrane proteins and reduces the levels of membrane protein thiols to increase transcellular absorption and may also increase paracellular transport by calcium chelation (Nishihata et al., 1986a,b) Our present data indicate that Na salicylate had no significant promoting effect on the nasal absorption of drugs with various molecular weights. Similarly, Aungst et al. (1988) reported that Na salicylate had no significant promoting effect on nasal or buccal insulin absorption but significantly improved rectal insulin efficacy, although the mechanisms of these regional differences are unknown. From these results, it was suggested that the efficacy of absorption promotion by Na salicylate depends on the route of administration.

In conclusion, the present results suggested that bile salts such as Na glycocholate, Na deoxycholate and Na taurocholate, Na caprate and

EDTA appeared to be more effective for enhancing the nasal absorption of drugs with various molecular weights than Na salicylate and Na caprylate. The efficacy of absorption promoters on nasal absorption of drugs was dependent on their molecular weights.

References

Aungst, B.J. and Rogers, N.J., Site dependence of absorption-promoting actions of laureth-9, Na-salicylate, Na_2EDTA, and aprotinin on rectal, nasal, and buccal insulin delivery. *Pharm. Res.*, 5 (1988) 305–308.

Cassidy, M.M. and Tidball, C.S., Cellular mechanism of intestinal permeability alterations produced by chelation depletion. *J. Cell Biol.*, 32 (1967) 685–698.

Chien, Y.W. and Chang, S.-F., Intranasal drug delivery for systemic medications. *CRC Crit. Rev. Ther. Drug Carrier Syst.*, 4 (1987) 67–194.

Donovan, M.D., Flynn, G.L. and Amidon, G.L., Absorption of polyethylene glycols 600 through 2000: The molecular weight dependence of gastrointestinal and nasal absorption. *Pharm. Res.*, 7 (1990) 863–868.

Duchateau, G.S.M.J.E., Zuidema, J. and Basseleur, S.W.J., Influence of some surface-active agents on nasal absorption in rabbits. *Int. J. Pharm.*, 39 (1987) 87–92.

Fisher, A.N., Brown, K., Davis, S.S., Park, G.D. and Smith, D.A., The effect of molecular size on the nasal absorption of water-soluble compounds in the albino rat. *J. Pharm. Pharmacol.*, 39 (1987) 357–362.

Gordon, G.S., Moses, A.C., Silver, R.D., Flier, J.S. and Carey, M.C., Nasal absorption of insulin: Enhancement by hydrophobic bile salts. *Proc. Natl. Acad. Sci. USA*, 82 (1985) 7419–7423.

Hersey, S.J. and Jackson, R.T., Effect of bile salts on nasal permeability in vitro. *J. Pharm. Sci.*, 76 (1987) 876–879.

Hirai, S., Yashiki, T. and Mima, H.,.Effect of surfactants on the nasal absorption of insulin in rats. *Int. J. Pharm.*, 9 (1981) 165–172.

Ishida, M., Machida, Y., Nambu, N. and Nagai, T., New mucosal dosage form of insulin. *Chem. Pharm. Bull.*, 29 (1981) 810–816.

Kajii, H., Horie, T., Hayashi, M., and Awazu, S., Fluorescence study of the membrane-purturbing action of sodium caprylate as related to promotion of drug absorption. *J. Pharm. Sci.*, 77 (1988) 390–392.

Kakemi, K., Sezaki, H., Konishi, R., Kimura, T. and Murakami, M., Effect of bile salts on the gastrointestinal absorption of drugs. I: *Chem. Pharm. Bull.*, 18 (1970) 275–280.

Lee, V.H.L. and Yamamoto, A., Penetration and enzymatic barriers to peptide and protein absorption. *Adv. Drug Delivery Rev.*, 4 (1990) 171–207.

Maitani, Y., Machida, Y. and Nagai, T., Influence of molecular weight and charge on nasal absorption of dextran and DEAE-dextran in rabbits. *Int. J. Pharm.*, 49 (1989) 23–27.

McMartin, C., Hutchinson, L.E.F., Hyde, R. and Peters, G.E., Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. *J. Pharm. Sci.*, 76 (1987) 535–540.

Mishima, M., Wakita, Y. and Nakano, M., Studies on the promoting effects of medium chain fatty acid salts on the nasal absorption of insulin in rats. *J. Pharmacobio-Dyn.*, 10 (1987) 624–631.

Murakami, T., Sakai, Y., Yamajo, R. and Yata, N., Effect of bile salts on the rectal absorption of ampicillin in rats. *Chem. Pharm. Bull.*, 32 (1984) 1948–1955.

Nishihata, T., Miyake, M. and Kamada, A., Study on the mechanism behind adjuvant action of diethylethoxymethylene malonate enhancing the rectal absorption of cefmetazole and lysozyme. *J. Pharmacobio-Dyn.*, 7 (1984) 607–613.

Nishihata, T., Ngheim, B.T., Yoshitomi, H., Lee, C.-S., Dillsaver, M., Higuchi, T., Choh, R., Suzuka, T., Furuya, A. and Kamada, A., Changes in intestinal mucosal permeability caused by nonprotein thiol loss in rats. *Pharm. Res.*, 3 (1986a) 345–351.

Nishihata, T., Miyake, M., Takahata, H. and Kamada, A., The effect of adjuvants on the colonic absorption of cefmetazole and $(Asu^{1,7})$-eel calcitonin in rats: concentration dependent absorption pathways. *Int. J. Pharm.*, 33 (1986b) 89–97.

Ohtani, T., Murakami, M., Yamamoto, A., Takada, K. and Muranishi, S., Effect of absorption enhancers on pulmonary absorption of fluorescein isothiocyanate dextrans with various molecular weights. *Int. J. Pharm.*, 77 (1991) 141–150.

Ohwaki, T., Ando, H., Watanabe, S. and Miyake, Y., Effect of dose, pH, and osmolarity on nasal absorption of secretin in rats. *J. Pharm. Sci.*, 74 (1985) 550–552.

Okada, H., Yamazaki, I., Ogawa, Y., Hirai, S., Yashiki, T. and Mima, H., Vaginal absorption of a potent luteinizing hormone releasing hormone analog(leuprolide) in rat. I: Absorption by various routes and absorption enhancement. *J. Pharm. Sci.*, 71 (1982) 1367–1371.

Su, K.S.E., Campanale, K.M., Mendelsohn, L.G., Kerchner, G.A. and Gries, C.L., Nasal delivery of polypeptides. I: Nasal absorption of enkephalins in rats. *J. Pharm. Sci.*, 74 (1985) 394–398

Yamamoto, A., Luo, A.M., Dodda-Kashi, S. and Lee, V.H.L., The ocular route for systemic insulin delivery in the albino rabbit. *J. Pharmacol. Exp. Ther.*, 249 (1989) 249–255.

Yamaoka, K., Tanigawara, Y., Nakagawa, T. and Uno, T., A pharmacokinetic analysis program(MULTI) for microcomputer. *J. Pharmacobio-Dyn.*, 4 (1981) 879–885.

Yamashita, S., Saitoh, H., Nakanishi, K., Masada, M., Nadai, T. and Kimura, T., Effects of diclofenac sodium and disodium ethylenediaminetetraacetate on electrical parameters of the mucosal membrane and their relation to the permeability enhancing effects in the rat jejunum. 39 (1987) 621–626.