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Combined use of 2-hydroxypropyl-β-cyclodextrin and a lipophilic absorption enhancer in nasal delivery of the LHRH agonist, buserelin acetate, in rats

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Abstract

The potential use of 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) as a biocompatible solubilizer for the lipophilic absorption enhancer, 1-[2-(decylthio)ethyl]azacyclopentane-2-one (HPE-101), was assessed in the nasal absorption of an LHRH agonist, buserelin acetate (BLA), in rats. HP-β-CyD increased the solubility of HPE-101 in water through the formation of an inclusion complex, and hence facilitated the transfer of HPE-101 into the nasal mucosa. The nasal administration of the combined system of HPE-101 and HP-β-CyD led to a significant increase in plasma BLA levels in the rats. This enhancement effect was ascribable to the lowering of both enzymatic and permeation barriers to the peptide in the nasal mucosa. The release of membrane proteins and scanning electron microscopic observations indicated that local mucosal damage due to the coadministration of HPE-101 and HP-β-CyD may not be a serious obstacle to their safe use. The results obtained here suggest that HP-β-CyD potentiates the action of the lipophilic absorption enhancers at the appropriate combination ratio without causing severe local irritation, and that this combination may be useful for designing an aqueous nasal formulation of BLA.

Keywords: Buserelin acetate; Nasal absorption; Peptide ; Hydroxypropyl-β-cyclodextrin; Lipophilic absorption enhancer; Biocompatible solubilizer; Local mucosal damage

1. Introduction

Since the nasal delivery of peptide and protein drugs is known to be limited by pre-systemic elimination due to enzymatic degradation or mucociliary clearance and by poor mucosal membrane permeability, many attempts have been made to overcome these problems, including coadministration of peptidase inhibitors or absorption enhancers, or modification of physicochemical properties of the drug molecules (Lee and Yamamoto, 1990). Cyclodextrins (CyDs) are attractive candidates for such an approach, since they can extract a number of specific lipids from biological membranes through the rapid and reversible formation of inclusion complexes, leading to an increase in membrane permeability to poorly absorbable drugs (Uekama and Otagiri, 1987). Recent studies have demonstrated that heptakis(2,6-di-*O*-methyl)-β-CyD (DM-β-CyD)

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markedly enhanced the nasal absorption of insulin (Merkus et al., 1991; Irie et al., 1992a; Shao et al., 1992; Watanabe et al., 1992), mimicking the effects of bile salts with regard to increased membrane permeability accompanied by the inhibition of proteolysis, although they are somewhat different from each other in their manner of action on membranes (Ohtani et al., 1989). However, the highly surface active nature of DM-β-CyD may cause local irritation (Uekama and Irie, 1990), which consequently limits the long-term use of nasal administration. In sharp contrast, 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) is ineffective in enhancing nasal membrane permeability, but can be used as a biocompatible solubilizer for hydrophobic additives in preparing aqueous nasal formulations, as it is well tolerated in long-term nasal administration to humans (Al-Nakib et al., 1989), shows minimal systemic toxicity even when given parenterally in small animals and humans, and retains excellent solubilizing ability (Pitha et al., 1988; Brewster et al., 1990; Mesens et al., 1991). In this study, we evaluated the potential use of HP-β-CyD as a solubilizer for the lipophilic absorption enhancer, 1-[2-(decylthio)ethyl]azacyclopentane-2-one (HPE-101), which has been successfully applied to dermal formulations (Adachi et al., 1993; Yano et al., 1993), but which is of limited use in nasal formulations due to the lack of suitable solubilizers. The present paper deals with the combination effects of HP-β-CyD with HPE-101 on the nasal absorption of buserelin acetate (BLA), a luteinizing hormone-releasing hormone (LHRH) agonist as a model peptide (Brogden et al., 1990). The present approach may also allow the extended use of HP-β-CyD as an absorption coenhancer to assist the transmucosal delivery of poorly absorbable peptide and protein drugs without causing local irritation.

2. Materials and methods

2.1. Materials

HPE-101 and [^{14}C]HPE-101 (0.243 mCi/ml) were donated by Hisamitsu Pharmaceutical Laboratory (Saga, Japan). BLA was a generous gift from Pharma Research Laboratories, Hoechst Japan Ltd (Saitama, Japan). HP-α-CyD, HP-β-CyD, and HP-γ-CyD were supplied by Nihon Shokuhin Kako Co. (Tokyo, Japan). The average degrees of substitution of HP-CyDs were confirmed to be 5.2 for HP-α-CyD, 5.8 for HP-β-CyD, and 4.9 for HP-γ-CyD by fast atom bombardment mass and NMR spectrometry (Pitha et al., 1986). DM-β-CyD was supplied by Toshin Chemical Co. (Tokyo, Japan). [^{3}H(G)]Inulin (312.0 mCi/g; average molecular weight 5500) was obtained from New England Nuclear (MA, USA). Aprotinin, a typical protease inhibitor, (14 TIU/mg protein, Sigma Chemical Co., MO, USA), laureth-9, a non-ionic surfactant (Sigma Chemical Co., MO, USA), and sodium deoxycholate (SDC, Nacalai Tesque Co., Kyoto, Japan) were used as supplied. Other materials and solvents were of analytical reagent grade and deionized double-distilled water was used.

2.2. Solubility measurements

A constant and excess amount of HPE-101 was added to an isotonic phosphate buffer solution (pH 7.4) containing a given concentration of CyDs. These were mixed by a reciprocal shaker at 25° C, and after equilibrium was attained (about 7 days) the mixture was filtered through a cellulose acetate membrane, Advantec Dismic 25CS045AN (Toyo-Roshi Co., Tokyo, Japan). HPE-101 in the filtrate was assayed by high-performance liquid chromatography (HPLC), under the following conditions: UV monitor, Hitachi L-4000 (Tokyo, Japan) at 210 nm; pump, Hitachi L-6000 (Tokyo, Japan); column, Nucleosil 100-5C18 (4.6 i.d. × 150 mm; GL-Science Co., Tokyo, Japan); mobile phase, water/methanol = 15:85 (% v/v); flow rate, 1.0 ml/min. The apparent 1:1 stability constants for the complexes of HPE-101 with HP-CyDs were calculated from the initial linear portion of the solubility diagrams according to the method of Higuchi and Connors (1965).

2.3. In vivo nasal absorption experiments

Nasal absorption studies of BLA, [^{14}C]HPE-101, and [^{3}H]inulin were performed according to

the method of Hirai et al. (1981), the experimental procedures being essentially the same as those described previously (Irie et al., 1992a). Male Wistar rats weighing 220–280 g were fasted for 16 h and anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg). BLA was dissolved in isotonic phosphate buffer (pH 7.4, 1.0 mg/ml) with or without HPE-101 and HP-β-CyD. Each solution was given via the nostril in a volume of 25 μl. Blood samples (0.4 ml) were corrected periodically from the jugular vein and the plasma BLA levels were determined by radioimmunoassay (Saito et al., 1985), where CyDs did not interfere with this assay. In the case of HPE-101, labeled [^{14}C]HPE-101 was diluted with unlabeled HPE-101 and finally made up to 2.0 μCi/25 μl with or without HP-β-CyD in isotonic phosphate buffer (pH 7.4). These samples were administered to rats, and the radioactivities in plasma were determined using a liquid scintillation counter (Aloka LSC-3500, Tokyo, Japan). Similarly, [^{3}H]inulin (2.5 μCi/body) with or without HPE-101 and HP-β-CyD was administered to rats, and the plasma levels of [^{3}H]inulin were also determined with the liquid scintillation counter.

2.4. Stability of BLA in nasal membrane homogenates

Male Wistar rats weighing 220–280 g were anesthetized with diethyl ether and decapitated. The nasal mucosa on the septal cartilage was isolated from the frontal bone and homogenized in a 10-fold volume of cold isotonic phosphate buffer (pH 7.4) using a blade homogenizer (Physcotron® NS-50, Niti-On Co. Ltd, Chiba, Japan). The homogenates were centrifuged at 9000 × g for 10 min at 4° C and the resulting supernatant (0.2 ml) was added to the buffer solution (0.8 ml, pH 7.4) containing BLA (0.1 mg/ml) and HPE-101 (1% w/v) and/or HP-β-CyD (10% w/v). After the mixture had been incubated for 3 h at 37° C, the reaction was terminated by the addition of a 0.1 ml aliquot of the mixture to 0.1 N hydrochloric acid solution (1 ml) at 0° C. The residual BLA in the mixture was determined by HPLC under the following conditions: monitor, Shimadzu RF-550A fluorescence spectrophotometer (Kyoto, Japan) at excitation wavelength 280 nm and emission wavelength 350 nm; pump, Hitachi L-6000 (Tokyo, Japan); column, GL-Science Inertsil ODS-2, 6.0 i.d. × 150 mm (Tokyo, Japan); mobile phase, 0.8% KH_2PO_4 (pH 6.2)/acetonitrile = 2:1 (v/v%); flow rate, 1.0 ml/min (Uekama et al., 1989).

2.5. In situ experiments for protein release from nasal mucosa

The experimental procedures were essentially the same as those described previously (Irie et al., 1992a). Each preparation was given via the left nostril in a volume of 25 μl, which was the same condition as that employed in the in vivo nasal absorption experiment. After 10 min exposure of the membrane, isotonic phosphate buffer (5 ml, pH 7.4) was recirculated from the posterior side of the nasal cavity through the nostrils at a flow rate of 1.5 ml/min for 30 min at 37° C. The amounts of proteins released from rat nasal mucosa into the perfusate were determined using the BCA protein assay kit (Pierce Co., IL, USA).

2.6. Morphological evaluation of nasal mucosal surface

These studies were carried out according to the method of Ennis et al. (1990). Male Wistar rats weighing 250–300 g were treated in the same manner as for the in situ experiments, and the sample solutions were dosed at a volume of 1 ml from an esophageal cannula. After 10 min exposure of the membrane, saline was infused into the nasal cavity to terminate the treatment for 5 min at a flow rate of 1.5 ml/min. Consecutive perfusion was followed with 2% w/v glutaraldehyde in 100 mM sodium phosphate buffer (pH 7.4) to fix the nasal membrane for 5 min. Nasal membrane damage was assessed by scanning electron microscopy (Hitachi S-510, Tokyo, Japan), and morphological scoring was performed according to the following five-point grading for each category as reported (Ennis et al., 1990): category 1, mucosal surface integrity [from 1 (normal) to 5 (unrecognizable)]; category 2, ciliary morphology

[from 1 (normal) to 5 (gross deformation); category 3, mucus/extracellular debris [from 1 (little) to 5 (abundant)].

3. Results and discussion

3.1. Enhancement of nasal absorption of BLA

The solubilizing abilities of the three kinds of HP-CyDs on HPE-101 were surveyed in isotonic phosphate buffer (pH 7.4) at 25° C. As shown in Fig. 1, the solubility of HPE-101 increased almost linearly as a function of HP-CyD concentration, owing to the formation of a water-soluble complex. From the linear portion of the phase solubility diagrams, the apparent 1:1 stability constants for the HPE-101 complexes were determined to be 40000, 44000, and 400 M^{-1} for HP-α-CyD, HP-β-CyD, and HP-γ-CyD systems, respectively. These results suggest that the binding capacity of HP-α-CyD and HP-β-CyD is extremely large, while that of HP-γ-CyD is not sufficient to form a stable complex with HPE-101, probably due to the larger cavity size. Thus, HP-β-CyD which has the highest binding capacity was selected as the preferred solubilizer for HPE-101 and was used in the following studies.

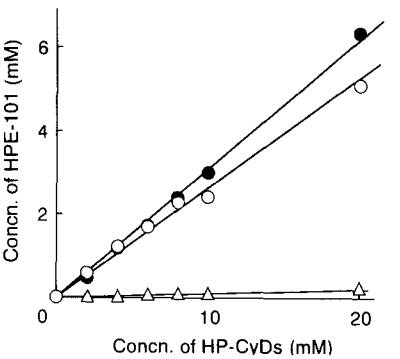

Fig. 1. Phase solubility diagrams of HPE-101/HP-CyD systems in isotonic phosphate buffer (pH 7.4) at 25° C: (○) HP-α-CyD, (●) HP-β-CyD, (△) HP-γ-CyD.

Fig. 2 shows a typical example of the effects of HPE-101 (1% w/v) and HP-β-CyD (10% w/v) on the nasal absorption of BLA in rats. The plasma levels of BLA were significantly increased by coadministration of HPE-101 and HP-β-CyD, compared with the sole use of either HPE-101 or HP-β-CyD. The pharmacokinetic parameters for BLA systems at various concentrations of HP-β-CyD are summarized in Table 1. Although the area under the plasma concentration-time curve (AUC) of the BLA-HPE-101 system was about 3-fold greater than that of BLA alone, the coad-

Table 1
Pharmacokinetic parameters[a] of BLA after nasal administration of BLA (0.1 mg/kg) with HPE-101 (1% w/v) and HP-β-CyD to rats

System	C_{max} [b] (ng/ml)	T_{max} [c] (h)	MRT [d] (h)	AUC [e] (ng ml^{-1} h)	F [f] (%)
BLA alone	24.7 ± 4.0 [h]	1.5 ± 0.2	1.2 ± 0.0 [h]	33.2 ± 5.5 [h]	11.9 ± 2.0 [h]
With HPE-101	56.9 ± 7.9 [g]	1.0 ± 0.4	1.0 ± 0.1 [g]	87.7 ± 14.8 [g]	31.5 ± 5.3 [g]
With HP-β-CyD (10% w/v)	22.8 ± 5.3 [h]	1.4 ± 0.3	1.2 ± 0.1 [h]	26.5 ± 6.7 [h]	9.5 ± 2.4 [h]
With HPE-101 and HP-β-CyD (5% w/v)	161.3 ± 7.3 [g,h]	0.3 ± 0.1 [g,h]	0.8 ± 0.0 [g,h]	177.9 ± 7.3 [g,h]	63.8 ± 2.6 [g,h]
With HPE-101 and HP-β-CyD (10% w/v)	180.6 ± 10.2 [g,h]	0.3 ± 0.1 [g,h]	0.8 ± 0.0 [g,h]	200.3 ± 6.7 [g,h]	71.9 ± 2.4 [g,h]
With HPE-101 and HP-β-CyD (15% w/v)	148.4 ± 15.0 [g,h]	0.4 ± 0.1 [g,h]	0.9 ± 0.0 [g,h]	176.4 ± 11.6 [g,h]	63.3 ± 4.2 [g,h]
With HPE-101 and HP-β-CyD (20% w/v)	76.6 ± 10.3 [g]	0.5 ± 0.0 [g,h]	1.0 ± 0.0 [g]	96.5 ± 11.8 [g]	34.6 ± 4.2 [g]

[a] Each value represents the mean ± S.E. of four to seven rats.
[b] Maximum plasma level.
[c] Time required to reach the maximum plasma level.
[d] Mean residence time in plasma.
[e] Area under the plasma level-time curve up to 2 h post-administration.
[f] Extent of bioavailability compared with the AUC value of BLA administered intravenously (0.1 mg/kg).
[g] $p < 0.01$ vs BLA alone.
[h] $p < 0.01$ vs with HPE-101.

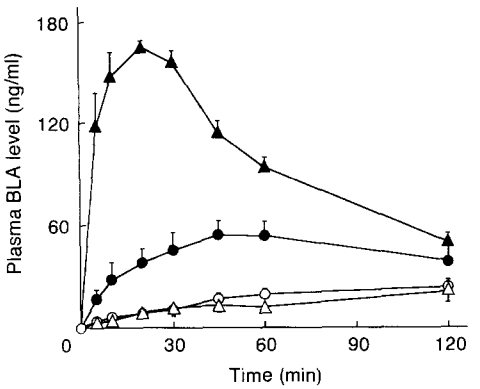

Fig. 2. Plasma levels of BLA after nasal administration of BLA (0.1 mg/kg) with HPE-101 (1% w/v) and HP-β-CyD (10% w/v) to rats: (○) BLA alone, (●) with HPE-101, (△) with HP-β-CyD, (▲) with HPE-101 and HP-β-CyD. Each point represents the mean ± S.E. of four to seven rats.

ministration of HPE-101 and HP-β-CyD (5–15% w/v) provided an approx. 6-fold increase in the AUC of BLA. Interestingly, the combination of HPE-101 (1% w/v) and HP-β-CyD (10% w/v) achieved the highest nasal bioavailability (about 72% of the AUC value for intravenous administration). The enhanced nasal absorption of BLA can be ascribed to the increase in the effective concentration of HPE-101 through water-soluble complex formation with HP-β-CyD. Thus, the dissolved HPE-101 could readily access the nasal membrane surface, and act as an absorption enhancer in the aqueous phase rather than the oily state. However, there seemed to exist an optimal combination ratio of HPE-101 and HP-β-CyD: the mixtures of HPE-101 (1% w/v) and HP-β-CyD (5–15% w/v) demonstrated an almost equal enhancing effect, while the higher concentration of HP-β-CyD (20% w/v) decreased the plasma BLA levels. Maximum penetration of HPE-101 into the nasal mucosa may be achieved when just enough HP-β-CyD is used to maintain all the HPE-101 in solution (Irie et al., 1992b), which is consistent with recent findings described by Loftsson et al. (1994). This phenomenon can be explained on the basis of the complexation equilibrium between host and guest molecules in aqueous solution, since the complexed form of the guest molecule is generally less permeable through biological membranes due to its bulky and highly hydrophilic nature (Uekama et al., 1991). Based on the phase solubility diagram of the HPE-101-HP-β-CyD system (Irie et al., 1992b), the minimum concentration of HP-β-CyD necessary for solubilization of 1% w/v HPE-101 was estimated to be 13.1% w/v. Thus, by the use of a large excess of HP-β-CyD (20% w/v), HPE-101 can be converted completely to the complex, as expected from the large stability constant, which may be no longer effective for deriving the absorption enhancing ability. Gill et al. (1994) have described similar findings in which the interaction of CyDs with absorption enhancers such as glycodeoxycholate and L-α-lysophosphatidylcholine was associated with considerable loss in absorption promoting activity of such enhancers along with reduced membrane damage.

3.2. Effects of HPE-101 and HP-β-CyD on enzymatic degradation of BLA

Since the enzymatic degradation of peptides is one of the critical absorption barriers during passage of the mucosal membrane, inhibition of the enzymatic degradation of BLA may contribute to improvement of the nasal absorption of BLA (Raehs et al., 1988). Subsequently, the effects of HPE-101 and HP-β-CyD on the enzymatic degradation of BLA in rat nasal membrane homogenates were examined. As shown in Fig. 3, the degradation of BLA was inhibited by both HPE-101 (1% w/v) and HP-β-CyD (10% w/v). This stabilizing effect of HP-β-CyD did not reflect the absorption enhancement of BLA displayed in Fig. 2, probably due to the limited contribution of the stability factor to the overall absorption enhancement and/or the formation of a less membrane permeable complex of BLA with HP-β-CyD (Uekama et al., 1994). The stabilizing effect of the combined system was significantly greater than that of each component, and was much more effective than that of aprotinin (0.65% w/v). Although the superior stabilizing effect observed for the combined system was not clearly explained, each component may synergistically participate in a different manner. For example, HPE-101, which shows intense surface activity,

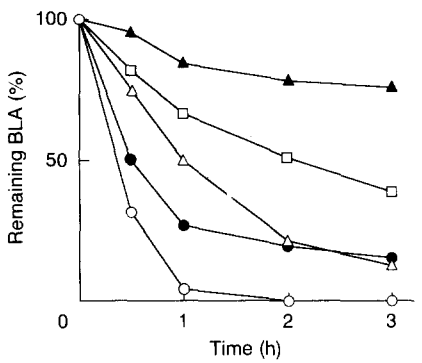

Fig. 3. Effects of HPE-101 (1% w/v), HP-β-CyD (10% w/v) and aprotinin (0.65% w/v) on degradation of BLA (0.01% w/v) in rat nasal homogenates in isotonic phosphate buffer (pH 7.4) at 37° C: (○) BLA alone, (●) with HPE-101, (△) with HP-β-CyD, (▲) with HPE-101 and HP-β-CyD, (□) with aprotinin. Each point represents the mean of three experiments.

may interact with the mucosal or cytosolic peptidase, and hence reduce the peptidase activity, possibly by denaturing the enzyme and preventing the formation of the enzyme-substrate complex (Lee and Yamamoto, 1990). In addition, HP-β-CyD may partially include some hydrophobic amino acid residues in the BLA molecule or proteolytic enzymes, which may restrict the formation of the catalytic complex of enzyme with BLA. In fact, the phenomena of the partial inclusion of CyDs with hydrophobic amino acid residues (tryptophan, tyrosine, and *t*-butyl-D-serine) in BLA were recently confirmed by NMR and fluorescence techniques (Irie et al., 1992c). Therefore, these interactions may be responsible for the stabilization of the BLA molecule against enzymatic degradation.

3.3. Effects of HPE-101 and HP-β-CyD on nasal membrane permeability of inulin

Since membrane permeability is one of the most important factors in the absorption of peptides, the effects of HPE-101 and HP-β-CyD on nasal membrane permeability were evaluated in rats, using [^{3}H]inulin (Mol. Wt 5500), an inert and non-permeable polysaccharide (Irie et al., 1992a). As shown in Fig. 4A, no appreciable change in plasma levels of radioactivity after the nasal administration of [^{3}H]inulin was observed over the concentration ranges of HPE-101 employed (0.2–1.5% w/v), demonstrating a dose-independent penetration enhancing effect of HPE-101 in emulsions. In sharp contrast, coadministration of HPE-101 and HP-β-CyD (10% w/v) showed a marked increase in nasal membrane permeation of inulin with increasing concentration of HPE-101 (Fig. 4B). Therefore, this perme-

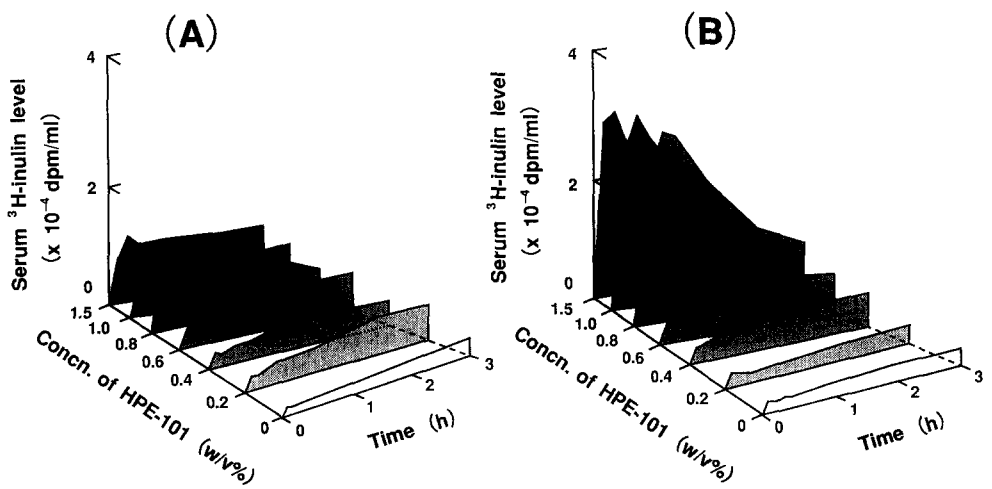

Fig. 4. Serum levels of [^{3}H]inulin after nasal administration of inulin (2.5 μCi/body) containing various concentrations of HPE-101 with or without HP-β-CyD (10% w/v) to rats. (A) HPE-101 alone; (B) HPE-101 with HP-β-CyD. Each point represents the mean of three rats.

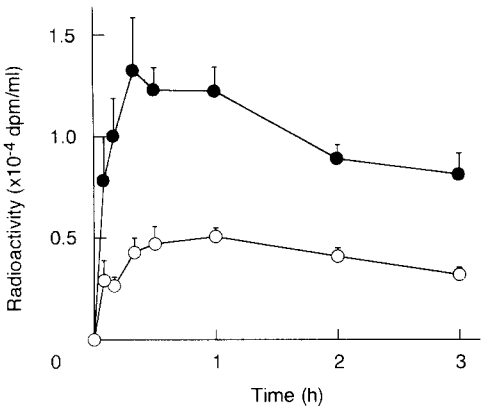

Fig. 5. Plasma levels of radioactivity after nasal administration of [^{14}C]HPE-101 (2 μCi/body) with or without HP-β-CyD (10% w/v) to rats: (○) HPE-101 alone, (●) with HP-β-CyD. Each point represents the mean ± S.E. of four to seven rats.

ation enhancing effect may be at least partly due to the solubilization of HPE-101 by HP-β-CyD.

3.4. Transition of HPE-101 after nasal administration

As mentioned above, HP-β-CyD was found to be useful for enhancing the absorption promoting ability of HPE-101, probably assisting the transmucosal penetration of the enhancer into the nasal epithelium, a site of action and subsequently its entry into the systemic circulation. Because of the oily nature of HPE-101 and rapid penetration into the nasal mucosa, it is difficult to determine the exact amount of HPE-101 penetrating into the nasal mucosal epithelium, where its action is most desired. Thus, in order to estimate the efficacy of transmucosal penetration of HPE-101, we pursued the transition of radioactivity corresponding to [^{14}C]HPE-101 and its metabolites from the nasal cavity to systemic circulation after the nasal administration of [^{14}C]HPE-101 in the presence and absence of HP-β-CyD. As shown in Fig. 5, the plasma level of [^{14}C]HPE-101 was significantly increased by the addition of HP-β-CyD (10% w/v), where an increase in absorption rate of HPE-101 was also observed.

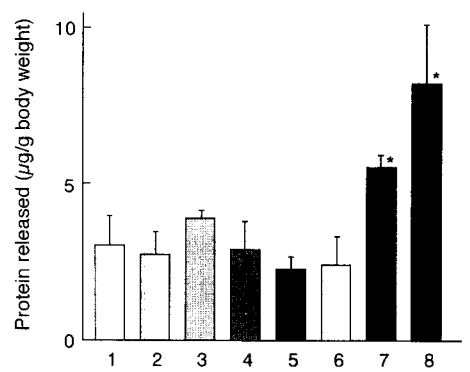

Fig. 6. Effects of HPE-101 (1% w/v), HP-β-CyD, laureth-9 (1% w/v) and SDC (1% w/v) on protein release from nasal mucosal membrane into perfusate in isotonic phosphate buffer (pH 7.4) at 37° C, Each value represents the mean ± S.D. of three to five rats. $^{*}p < 0.01$ vs buffer control. (1) Buffer control, (2) with HPE-101, (3) with HPE-101 and HP-β-CyD (10% w/v), (4) with HPE-101 and HP-β-CyD (15% w/v), (5) with HPE-101 and HP-β-CyD (20% w/v), (6) with HP-β-CyD (20% w/v), (7) with laureth-9 (1% w/v), (8) with SDC (1% w/v, pH 8.5).

3.5. Evaluation of nasal membrane damage

Since the absorption enhancing effect is generally associated with membrane irritation, a more effective and safer formulation is desired for trans-nasal administration. In order to evaluate the local tissue irritancy of the combination of HPE-101 and HP-β-CyD, therefore, the release behavior of the total protein and the morphological changes in the nasal mucosa were examined after exposure to sample solutions by means of the in situ recirculating perfusion technique. As shown in Fig. 6, when isotonic phosphate buffer (pH 7.4) was given as a control, the amount of protein recovered from the rat nasal membrane in the perfusate was very small. When laureth-9 and SDC were given as a positive control, approx. 2- and 3-fold increases in protein release were observed, respectively, both of which may exert some nasal membrane irritation. On the other hand, the amount of proteins released for the exposure to HPE-101 with or without HP-β-CyD was almost comparable to that of control. These results suggest that HPE-101-HP-β-CyD system may not cause the serious irritation in nasal administration.

Moreover, morphological evaluation of nasal membrane damage after 10 min exposure to sample solutions was carried out by means of scanning electron microscopy. Using the method of Ennis et al. (1990), the degree of irritation was scored in the three categories (see section 2) and the results are summarized in Table 2. The tissue damaging effect of each preparation appears to be much more pronounced than that estimated form the protein release study (Fig. 6), since the entire nasal cavity was flooded with the preparation under dorsal recumbency which inhibited mucociliary drainage. Single administration of HPE-101 (1% w/v) did not cause appreciable membrane damage, and the morphological scores were almost comparable to those of the control (isotonic phosphate buffer). In this condition, laureth-9 was used as a positive control, since it is well known as a typical irritative surfactant (Ennis et al., 1990). This surfactant caused severe membrane irritation as indicated by morphological scores above 4 in all categories. On the other hand, the morphological scores for the combined systems of HP-β-CyD and HPE-101 were largely dependent on the concentration of HP-β-CyD, and there seemed to exist a favorable concentration of HP-β-CyD. When HP-β-CyD was added at 5, 15, and 20% w/v, their irritation scores showed slightly higher value compared to the control, while the 10% w/v HP-β-CyD system elevated the irritation scores in all categories compared to HPE-101 alone. These increased tissue damaging effects may arise from maximum penetration of HPE-101 into the nasal mucosa mediated by HP-β-CyD at the critical concentration necessary for the solubilization of the enhancer.

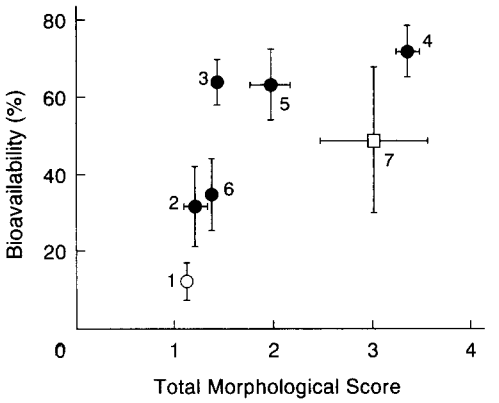

Fig. 7. Relationship between nasal membrane morphological scores and bioavailability after nasal administration of BLA (0.1 mg/kg) with HPE-101 (1% w/v) and HP-β-CyD to rats. (1) BLA alone, (2) with HPE-101, (3) with HPE-101 and HP-β-CyD (5% w/v), (4) with HPE-101 and HP-β-CyD (10% w/v), (5) with HPE-101 and HP-β-CyD (15% w/v), (6) with HPE-101 and HP-β-CyD (20% w/v), (7) with DM-β-CyD (10.7% w/v) (taken from published data (Irie et al., 1992c)). Each point represents the mean ± S.D. of four to seven rats.

Fig. 7 shows the relationship between irritation score and nasal bioavailability of BLA (see Table 1). In this plot, the data for DM-β-CyD-BLA system reported previously were also included. It is noteworthy that the bioavailability of the com-

Table 2
Morphological evaluation of nasal mucosal damage after 10 min exposure to HPE-101 (1% w/v), HP-β-CyD and laureth-9 (1% w/v)

System	Irritation score [a]			
	Category 1	Category 2	Category 3	Total score
Buffer control	1.12 ± 0.05	1.17 ± 0.05	1.06 ± 0.04	1.12 ± 0.06
With HPE-101	1.16 ± 0.07	1.35 ± 0.12	1.13 ± 0.06	1.21 ± 0.12
With HPE-101 and	1.43 ± 0.10	1.46 ± 0.19	1.40 ± 0.16	1.43 ± 0.03 [b]
With HPE-101 and HP-β-CyD (10% w/v)	3.49 ± 0.66 [b,c]	3.31 ± 0.49 [b,c]	3.27 ± 0.40 [b,c]	3.36 ± 0.12 [b,c]
WithHPE-101 and HP-β-CyD (15% w/v)	2.15 ± 0.62 [b,c]	2.00 ± 0.64 [b,c]	1.76 ± 0.33 [b,c]	1.97 ± 0.20 [b,c]
With HPE-101 and HP-β-CyD (20% w/v)	1.40 ± 0.17	1.37 ± 0.20	1.33 ± 0.13	1.37 ± 0.04 [b]
With laureth-9	4.08 ± 0.39 [b,c]	4.54 ± 0.24 [b,c]	4.11 ± 0.22 [b,c]	4.24 ± 0.26 [b,c]

[a] Each value represents the mean ± S.D. of four rats.
[b] $p < 0.01$ vs buffer control.
[c] $p < 0.01$ vs with HPE-101.

bined use of HP-β-CyD (10% w/v) and HPE-101 (1% w/v) was almost identical to that of the single application of DM-β-CyD (10.7% w/v), showing the highest irritation. On the other hand, the combined use of 5 or 15% w/v HP-β-CyD with HPE-101 (1% w/v) showed rather high nasal bioavailability of BLA with little membrane irritation. From inspection of Fig. 7, the system containing 5% w/v HP-β-CyD with 1% w/v HPE-101 appeared to be the most preferable formulation to provide a superior absorption enhancing ability of HPE-101 with lesser membrane irritation.

The pharmacokinetic behavior of the enhancer applied nasally may be related not only to the efficacy of the absorption promoting effects but also to the possible tissue damaging effect and its reversibility. 3 h after the nasal administration of [^{14}C]HPE-101, recoveries of radioactivity in the nasal lumen and mucosal tissues were very small; $1.03 \pm 0.31\%$ for HPE-101 alone and $0.41 \pm 0.07\%$ for HPE-101 with HP-β-CyD, respectively. Then, the tissue disposition of HPE-101 was compared with nasal administration of HPE-101 alone, HPE-101 with HP-β-CyD, and intravenous administration of HPE-101. The remaining radioactivity of [^{14}C]HPE-101 and its metabolites in several tissues was determined 3 h after the nasal administration of [^{14}C]HPE-101. The radioactivity was mainly recovered in the kidney and the distribution pattern for the HPE-101-HP-β-CyD system was similar to that of the intravenous administration of HPE-101 (data not shown). Since the dose of HPE-101 applied nasally in this study (1 mg/kg) was much lower than the LD_{50} value reported (> 2 g/kg, i.p.), it would involve no serious toxicological issue, even if it was absorbed completely (Yano and Saita, 1991). These results suggest that the combination of HPE-101 with HP-β-CyD may not be a serious obstacle against their safe use.

4. Conclusion

The present results clearly suggested that the nasal absorption of BLA was significantly improved by the coadministration of HPE-101 and HP-β-CyD at the appropriate combination ratio. This enhancing effect was ascribable to the lowering of both the enzymatic and membrane permeation barriers, probably through the facilitated transfer of HPE-101 into the nasal mucosa. Since HP-β-CyD was proven to be useful in extending the function of HPE-101 without causing severe local irritation, the combination of the biocompatible solubilizer and lipophilic absorption enhancer will become a valuable tool for designing aqueous trans-mucosal formulations of peptides and protein drugs.

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