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Effect of Sodium Taurodihydrofusidate on Nasal Drug Delivery: Differences in Its Concentration and Penetrant Molecular Weight

Ken-ichi Hosoya[1], Hiroyuki Kubo[1], Takashi Akutsu[1], Hideshi Natsume[1], Kenji Sugibayashi[1,2] and Yasunori Morimoto[1,2]

[1]*Department of Pharmaceutics, Faculty of Pharmaceutical Sciences and* [2]*Life Science Research Center, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-02, Japan*

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The effect of sodium taurodihydrofusidate (STDHF) on drug permeation across nasal mucosa was studied *in vitro* using Ussing type diffusion chamber. Disodium cromoglicate (DSCG, M.W. 512.3) and fluorescein isothiocyanate-dextran (FD) of different molecular weights (M.W. 4400~71200) were used as model drugs. Permeation profiles of DSCG and FDs showed a typical pseudo steady-state curve with short lag time. The permeability coefficient of FD (M.W. 9400) sigmodially increased with increasing STDHF concentration. It also enhanced the DSCG permeation. Interestingly the enhancement efficacy was independent of molecular weight of penetrants.

Key words: Absorption enhancer, Sodium taurodihydrofusidate, Ussing chamber, Nasal mucosa, Nasal delivery

INTRODUCTION

An increasing number of peptide and protein drugs have been produced because of developing of gene technology. The drugs have been administered mostly by the parenteral route which often causes pain. Alternative routes have been intensively studied to improve quality of life and patient's compliance. Nasal route is considered to be promising due to avoidance first pass effect and to lowering of protein degradation than oral route. A few dosage forms using the nasal route have already been marketed. Most macromolecule protein drugs, however, require absorption enhancers to assure their adequate absorption. Many enhancers such as bile salts (Gordon *et al.*, 1985; Duchateau *et al.*, 1986), surfactants (Hirai *et al.*, 1981), chelating agents (Aungst and Rogers, 1988) and fatty acid derivatives (Mishima *et al.*, 1987) have been investigated with regard to facilitating the nasal absorption of peptide and protein drugs, but they generally cause damage and irritation to the nasal mucosa as well as absorption enhancement.

Sodium taurodihydrofusidate (STDHF) has been reported as a good nasal absorption enhancer with lower irritation (Longenecker *et al.*, 1987). STDHF improved the extent of bioavailability of intranasally administered insulin (Longenecker *et al.*, 1987) and phenol red (Duchateau *et al.*, 1987). However, little is known about the enhancement efficacy of STDHF and its molecular weight-dependency.

In this study, we studied the enhancing effect of STDHF on the *in vitro* nasal membrane permeation of disodium cromoglicate (DSCG) and fluorescein isothiocyanate-dextran (FD) of different molecular weight. The rabbit nasal mucosa was used in all animal experiments.

Correspondence to: Y. Morimoto, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences and Life Science Research Center, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-02, Japan

MATERIALS AND METHODS

Materials

DSCG and FD of different molecular weights, 4400 (FD-4), 9400 (FD-10), 36500 (FD-40) and 71200 (FD-70) were obtained commercially (Sigma Chemicals, MO, USA). STDHF was generously supplied by Leo Pharmaceuticals (Bullerup, Denmark). All other reagents were of reagent grade and obtained commercially.

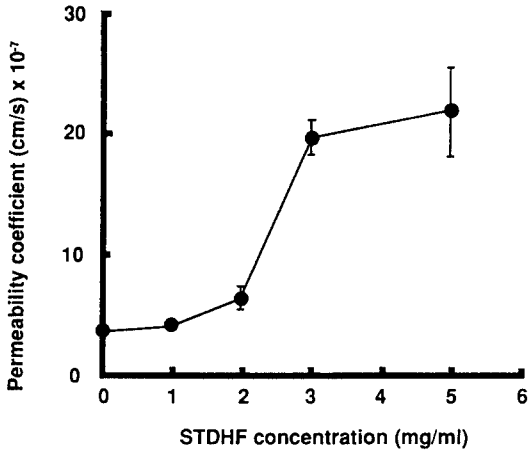

Fig. 1. Effect of STDHF concentration on permeability of FD-10. Each point represents the mean± S.E. of at least 3 experiments.

Tissue Preparations

Male Japanese white rabbits (Tokyo Laboratory Animals, Tokyo, Japan) weighing 2.5~3.0 kg were used in this study. The nasal mucosa was prepared and mounted in a Ussing type diffusion chamber as previously reported (Hosoya *et al.*, 1993). The effective diffusion area of the mucosa was 0.5 cm^2. Both sides of the mucosa were filled with 11 ml of standard Ringer solution (Hosoya *et al.*, 1993) and bubbled with 95% O_2-5% CO_2 to maintain tissue viability and to circulated the solution. The chambers were placed in a temperature-controlled box to maintain the solution temperature at 37℃.

Permeation Studies

The nasal mucosa in the chamber was equilibrated with the standard Ringer solution for 120 min (Kubo *et al.*, 1994) and then DSCG or FD solution (5.1 or 2.5 mg/ml standard Ringer solution, respectively) was introduced to the mucosal phase in a control permeation experiment. After 120 min, the entire test solution was drained from the mucosal phase and replaced with the STDHF solution containing DSCG (5.1 mg/ml) or FD (2.5 mg/ml) to measure the effect of STDHF. Different STDHF concentration (1~5 mg/ml) was used to check the effect of its concentration (Fig. 1) (5 mg/ml was used when no explanation). Serosal sample (1 ml) was taken intermittently and the same volume of fresh buffer was replaced to keep the volume constant. The permeability coefficient was calculated from the steady-state flux (80~120 min without STDHF, 200~240 min with STDHF) of plot of the cumulative amount of drug permeated versus time.

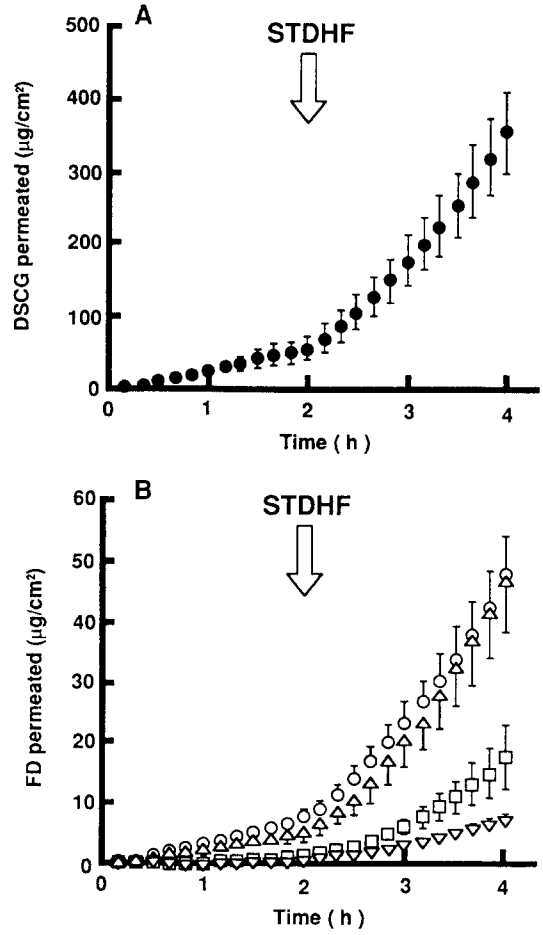

Fig. 2. Time course of DSCG (A) and FD (B) permeation across the nasal mucosa.
Key: A; DSCG, B; FD, FD-4 (○), FD-10 (△), FD-40 (□), FD-70 (▽). Each point represents the mean± S.E. of at least 3 experiments.

Analysis

DSCG concentration was determined using a high-performance liquid chromatography as previously reported (Kubo *et al.*, 1994). Fluorescence intensity of FD was measured by a spectrofluorometer (RF-5000, Shimadzu) at an excitation wavelength of 495 nm and an emission wavelength of 515 nm.

RESULTS AND DISCUSSIONS

Figure 1 shows the effect of STDHF concentration on the permeability coefficient of FD-10. The permeability coefficient gradually increased with increasing STDHF concentration until 2 mg/ml, markedly increased from 2 to 3 mg/ml and reached plateau about 3~5 mg/ml. Longenecker *et al.* (1987) reported that STDHF enhanced insulin absorption from the nasal mucosa. And their data for the relationship between

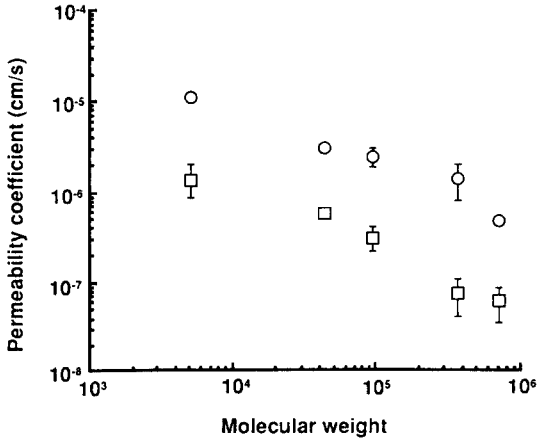

Fig. 3. Relationship between permeability and molecular weight.
Key: without STDHF (□), with STDHF (○). Each point represents the mean± S.E. of at least 3 experiments.

AUC after intranasal administration of insulin and STDHF concentration was similar to ours except a little difference in sharp break point. They suggested that the mechanism of enhanced insulin absorption was solubilization of insulin monomers or dimers and prevention of aggregation of insulin by STDHF. FD-10 is unlikely to associate or aggregate, so our results suggest direct interaction of STDHF with the nasal mucosa.

Figure 2 shows the time course of DSCG and FDs that permeated across the nasal mucosa. Permeation profiles of both DSCG and FDs showed a typical pseudo steady-state curve with a short lag time less than 120 min. Then, STDHF was applied at 120 min on the mucosa to observe the enhancing effects on all drugs.

The relationship between the permeability coefficient with or without STDHF and molecular weight is shown in Fig. 3. The permeability coefficient decreased with increasing molecular weight independent of the absence or presence of STDHF. Interestingly enhancement efficacy of STDHF (ratio of permeability coefficient of presence against absence) was independent of molecular weight. These results suggest that the enhancing effect of STDHF on the nasal mucosa is not size-discriminant and STDHF is a good enhancer to deliver macromolecule drugs from the nasal mucosa.

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