DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 17(12), 1625-1634 (1991)

INFLUENCE OF BILE SALTS ON THE PERMEABILITY THROUGH THE NASAL MUCOSA OF RABBITS OF INSULIN IN COMPARISON WITH DEXTRAN DERIVATIVES

Naoki Uchida, Yoshie Maitani* Yoshiharu Machida, Masayuki Nakagaki, and Tsuneji Nagai

Faculty of Pharmaceutical Sciences, Hoshi University,

Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan

ABSTRACT

The nasal drug absorption and the effect of absorption promoters have been studied in rabbits. Nasal mucosa excised from rabbits was mounted as a flat sheet in an *in vitro* chamber. The result indicates that the change in the porosity of the membrane by pretreatment with bile salts increased the permeability coefficient of sodium chloride in the nasal membrane. The permeabilities of dextran derivatives were enhanced by pretreatment with sodium glycocholate (GC). The permeability coefficient (P) of fluorescein isothiocyanate diethylaminoethyl dextran

*To whom correspondence should be addressed.

(FITC-dextran, DE) was higher than that of FITC-dextran (DT), and P of FITC dextran sulfate (DS) was lower than that of DT with the same molecular weight. Comparing insulin with dextran that is the same molecular weight as insulin, P of insulin induced by the pretreatment with GC was higher than that of hydrophilic dextran. The partition coefficient seemed to have better effect on the nasal membrane transport than the molecular weight of the penetrant.

INTRODUCTION

The nasal mucosa is negatively charged and the charge densities of the nasal membrane are not changed by pretreatment with 1 % bile salt solutions on the mucosal side (1). In nasal absorption of peptide, bile salts are used as absorption promoters (2,3). However, the mechanism by which bile salts enhance nasal membrane permeability is not known clearly. The bile salts might affect the nasal membrane and create temporal pore (4). Therefore, the effect of bile salt on the nasal membrane was evaluated by enhancement of the membrane permeability coefficient of sodium chloride caused by the pretreatment with bile salts. Also, the permeabilities of dextran derivatives with molecular weights ranging from 3860 to 40,500 were measured to investigate the action of bile salt on the pore size of the membrane examined.

In addition, the bile salt might transiently modify membrane charges and increase transport of the charged compound. Therefore, the permeabilities of dextran derivatives which represent neutral, positive and negative charges and insulin which has an isoelectric point were also investigated.

MATERIALS AND METHODS

Materials

The chemicals used were as follows: fluorescein isothiocyanate (isomer-1, FITC) was obtained from Wako Pure Chem. Ind. LTD.; sodium cholate (C) from Tokyo Kasei Ind. Ltd.; ethylenediamine tetraacetic acid, disodium salt (EDTA) from Iwai Chem. Co.; sodium deoxycholate (DC) and glycocholate (GC) from Nakarai Chem. Co.; dextran (mw.6000, 9000), FITC-dextran (DT, mw. 3860, 9000, 17,500, 40,500), dextran sulfate (mw. 5000, 8000), crystalline bovine pancreas insulin (24.5 IU per mg; zinc content, approx. 0.5 %), sodium glycodeoxycholate (GDC), taurocholate (TC) and taurodeoxycholate (TDC) were purchased from Sigma Chem. Co. (U.S.A).

Diethylaminoethyl dextran (DEAE-dextran) was synthesized according to the method reported by McKernan and Ricktte (5). Fluorescein isothiocyanate-labeled DEAE-dextran (DE) and dextran sulfate (DS) were synthesized from DEAE-dextran and dextran sulfate, respectively, according to the method reported by Belder and Granath (6).

Methods

The nasal mucosa used in these experiments was obtained from male New Zealand White rabbits (Saitama Experimental Animal Supply Co.). Nasal mucosa was separated from just anterior orbits of the junction of the nasal bone with the dosal pariental cartilage. After being rinsed in distilled water, a piece of nasal mucosa was mounted as a flat sheet on a circular window with 15.2 mm^2. Two glass chambers are tightly set to prevent solution leakage in the chambers with the aid of a silicone O-ring.

The permeability of sodium chloride was obtained by measuring conductivity of serosal bathing solution to calculate the concentration of NaCl. The permeabilities of dextran derivatives were obtained by measuring their fluorescence intensities with a fluorescence spectrophotometer (Shimadzu, model RF-530) at an emission wavelength of 516 nm and an excitation wavelength of 480 nm.

The insulin concentration was measured by using enzyme immunoassay with a commercially available kit (Insulin EIA kit, Dainabot Co, Ltd.).

RESULTS AND DISCUSSION

The influence of bile salts on the nasal absorption of polypeptides was estimated from the viewpoint of drug permeation. The change in the membrane constant (f) was evaluated by means of the change in the permeability of NaCl with the pretreatment of bile salts. Figure 1 shows the

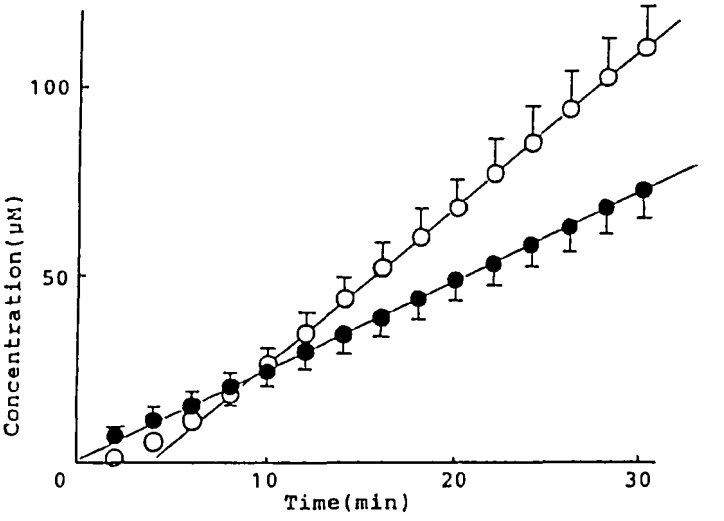

Fig. 1. Relation between Time and Concentration of NaCl in the Receiving Cell at 36°C
Data are given as mean±S.E. (n=4).
●, before pretreatment; ○, after pretreatment by 10 mM solution of DC.

relation between the permeation time and the concentration of NaCl in the serosal bathing solution.

The ratios of the membrane permeability coefficients of NaCl before pretreatment (P^*) and after pretreatment (P^*_t) by bile salts or EDTA are shown in Table 1. Membrane permeability of NaCl was not changed by the pretreatment of saline solution ($P^*_t/P^*=1.00$), which was used as a control. DC and GDC showed a remarkable effect on the membrane permeability than did C and GC. The values of P^*_t/P^* reflect the change in the porosity of the membrane (f_t/f) by the bile salts. Therefore, the value of ($\beta_t \cdot \Phi_t/\beta \cdot \Phi$) will be evaluated by measuring the permeabilities of dextran derivatives which have neutral, positive and negative

Table 1. Influence of Bile Salts and EDTA on Permeability Coefficient in the Nasal Mucosa for NaCl In Vitro

Agent added	$P^* \times 10^7$ (cm^2/sec)	$P^*t \times 10^7$ (cm^2/sec)	P^*t / P^*
Saline a)	6.05±1.83	6.21±2.14	1.00±0.04
C a)	5.85±1.30	7.25±1.53	1.24±0.04
DC a)	6.01±0.96	10.89±1.51	1.83±0.07 #
GC b)	5.20±2.20	8.44±3.45	1.66±0.05 #
GDC b)	5.43±1.55	10.27±2.54	1.92±0.16 #
TC b)	5.75±1.23	10.59±2.47	1.87±0.20 #
TDC a)	5.39±2.31	9.08±2.95	1.87±0.31 #
EDTA c)	6.25±1.56	9.34±1.99	1.64±0.15

Concentration of bile salts and EDTA were 10 mM.
Data are given as mean±S.E.
: $P<0.05$ (conpared to the control (saline)).
a) n=4, b) n=3, c) n=7.

charges and various molecular weights becuause P is equal to the product of f, partition coefficient (β) and diffusion coefficient (Φ).

Table 2 shows the values of P, P_t and P_t/P in the case of various molecular weights of DT, DE and DS. The value P of dextran derivatives decreased with an increase of their molecular weights as diffusion coefficients of these compounds with high molecular weights are low. The membrane permeability was enhanced by the pretreatment with GC. The value of P for DE was higher than that for DT, and the value of P for DS was lower than that for DT which has the same molecular weight as DE and DS. The data of dextran derivatives suggest that the negatively charged

Table 2. Influence of Sodium Glycocholate on Permeability Coefficient for Dextran Derivatives In Vitro

Dextran Derivatives	$P \times 10^8$ (cm^2 sec)	$Pt \times 10^8$ (cm^2 sec)	Pt / P
DT(mw. 3860)	15.91±4.31	26.95± 5.60	1.69
DT(mw. 9000)	9.79±4.33	11.60± 3.20	1.18
DT(mw.17,500)	7.92±4.05	8.81± 1.16	1.11
DT(mw.40,500)	4.20±1.85	7.27± 1.63	1.73
DE(mw. 6000)	20.03±2.69	31.56±12.01	1.58
DE(mw. 9000)	5.61±1.30	19.16± 4.15	3.42
DS(mw. 5000)	8.67±0.40	13.08± 5.28	1.51
DS(mw. 8000)	6.07±2.93	4.65± 1.05	0.77

Concentration of sodium glycocholate was 10 mM.
Data are given as mean±S.E. (n=3 or 4).

compounds (DS) are repellent and the positively charged compounds (DE) give an access for drug diffusion into the membrane bearing a negative charge.

Permeation of insulin was not observed at pH 5.4, even if pretreated with 10 mM solution of GC, as shown in Fig. 2. The isoelectric point of insulin is from pH 5.3 to 5.35. Insulin in saline solution (pH 5.4) is not charged and forms a hexamer or octamer since its apparent molecular weight is 36,000 to 48,000 (7). On the other hand, insulin in saline solution (pH 2.0) is positively charged and forms a monomer, so that it is able to permeate the nasal mucosa which is negatively charged. As shown in Table 3, the value P of insulin at pH 2.0 was 9.95×10^{-7} cm^2/sec, and the permeability coefficient of insulin after pretreatment by 10

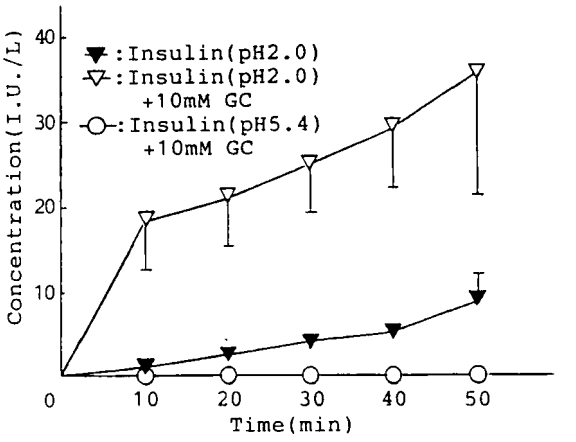

Fig. 2. Concentration versus Time in the Receiving Cell for Insulin at 36°C
Data are given as mean±S.E.

Table 3. Influence of Sodium Glycocholate on Permeability Coefficient for Insulin In Vitro

pH	P x 10^7 (cm^2 sec)	Pt x 10^7 (cm^2 sec)	Pt / P
5.4	0.0	0.0	—
2.0	9.95±1.48	27.91±1.92	2.87±0.21

Concentration of sodium glycocholate was 10 mM.
Data are given as mean±S.E. (n=3).

mM solution of GC (P_t) was 2.79×10^{-6} cm^2/sec. The permeability of insulin was enhanced 2.79 times by the pretreatment of GC.

Since dextran derivatives are homopolymers and have the structure of a random coil, the diffusion coefficients of compounds is generally proportional to the reciprocal of the

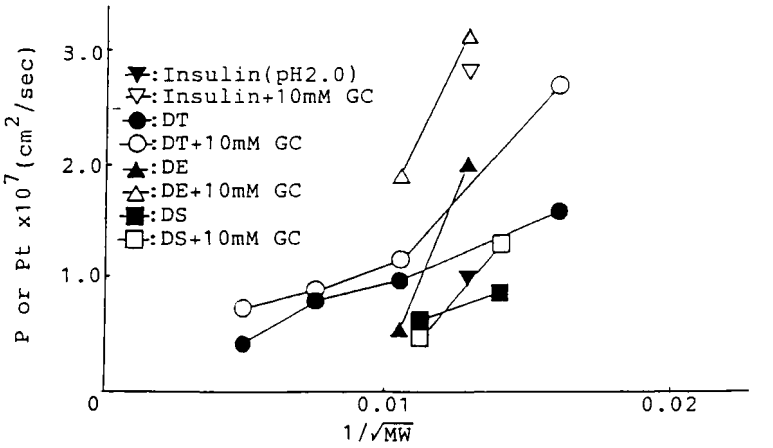

Fig. 3. Correlation between Permeability Coefficients and Molecular Weights for Dextran Derivatives and Insulin

square root of the molecular weight. The correlation between P or P_t and the reciprocal of the square root of the molecular weights of dextran derivatives shows the linearity in Fig. 3, that is, the values of P for DT, DE and DS decrease with increased molecular weight.

ACKNOWLEDGEMENT

The authors are grateful to Mr. Hisao Ohkuki for his assistance with the experimental work.

REFERENCES

1. Y. Maitani, N. Uchida, N. Nakagaki and T. Nagai, submitted.
2. S. Hirai, T. Yashiki and H. Mima, . J. Pharm. Sci., 9, 165 (1981).

3. Y. Maitani, T. Igawa, Y. Machida and T. Nagai, Drug Des. Delivery., 4, 109 (1988).

4. G. S. Gordon, A. C. Moses, R. D. Silver, J. S. Flier and M. C. Carey, Proc. Natl. Acad. Sci., 82, 7419 (1985).

5. W. M. McKernan and C. R. Rickette, Biochem. J., 76, 117 (1960).

6. A. N. Belder and K. Granath, Carbohydro. Res., 30, 375 (1973).

7. E. Heimerhorst and G. B. Stokes, Diabetes, 36, 261 (1987).