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COMMUNICATIONS

Evaluation of permeability enhancement of hydrophilic compounds and macromolecular compounds by bile salts through rabbit corneas in-vitro

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The effect of bile salts, sodium taurocholate (TC-Na) and sodium taurodeoxycholate (TDC-Na), on the permeability of hydrophilic compounds and macromolecular compounds through the rabbit cornea in-vitro was examined. 6-Carboxyfluorescein and glutathione were used as low molecular weight hydrophilic model compounds and FITC-dextran (mol. wt 4000) and insulin were used as relatively macromolecular model compounds. TC-Na (2 and 10 mM) marginally increased the corneal permeabilities to hydrophilic compounds and macromolecular compounds. TDC-Na (2 and 10 mM) markedly increased the corneal permeabilities of these compounds.

The most common ophthalmic dosage form, the eye-drop, does not provide an optimal drug delivery system to the intraocular tissues. Ophthalmic drugs are absorbed into the eye mainly through the cornea (Ahmed & Patton 1985). This is a unique tissue which has an aqueous middle region (stroma) between two hydrophobic layers, thus restricting the corneal permeability of hydrophilic and macromolecular compounds. Absorption promoters have been shown to enhance the absorption of poorly absorbed drugs through the small intestine, the rectum and other mucosal membranes (Murakami et al 1984; Morimoto et al 1985). However, there have been few reports of applications of these materials for topical ophthalmic drug delivery (Singh & Mezei 1983, 1984). In the present study, the promoting effects of the trihydroxy bile salt, sodium taurocholate and the dihydroxy bile salt, sodium taurodeoxycholate on the in-vitro corneal permeability of hydrophilic compounds and macromolecular compounds were examined. 6-Carboxyfluorescein (CF) and glutathione (GSH) which are hydrophilic and of small molecular weight, were used as model compounds. FITC-dextran (mol. wt 4000) and insulin (mol. wt 5500) were used as relatively macromolecular model compounds.

Materials and methods

Materials. Glutathione (L-glutamyl-L-cysteinylglycine: GSH) was purchased from Kojin Co. Ltd, Tokyo, Japan; 6-carboxyfluorescein (CF) from Eastman Kodak Co., Rochester, NY, USA, fluorescein isothiocyanate (FITC)-dextran (mean mol. wt 4100), bovine crystalline insulin (26·5 iu mg^{-1}), sodium taurocholate (TC-Na) and taurodeoxycholate (TDC-Na) from Sigma Chemical Co. St Louis, MO, USA. All other chemicals were reagent grade.

Preparation of drug solutions. 2% w/v GSH solution, 0·05% w/v CF solution, 0·05% w/v FITC-dextran solution and 1 u mL^{-1} insulin solution were prepared in isotonic buffer solution (pH 7·4). The constituents of isotonic buffer solution were NaCl (0·9% w/v) 100 mL, KCl (1·15% w/v) 4·0 mL, $CaCl_2$ (1.22% w/v) 3·0 mL, $MgSO_4 \cdot 7H_2O$ (3·79% w/v) 1·0 mL, KH_2PO_4 (2·09% w/v) 1·0 mL, $NaHCO_3$ (1·29% w/v) 21 mL, glucose (5·54% w/v) 1·0 mL; total 131·0 mL. The adjuvants, TC-Na and TDC-Na were dissolved in the drug solutions at 2 and 10 mM. The final pH (pH 7·4) of drug solution was adjusted by addition of NaOH.

Permeability test. Corneas from adult albino rabbits (2·0–2·5 kg) were used. Rabbits were killed with Na-pentobarbitone and the eyes were removed. Corneas with a 2 to 3 mm scleral rim were isolated from the eyes and immediately clamped into the chamber as indicated in Fig. 1. The lower chamber (receptor phase) contained 5 mL isotonic buffer solution (pH 7·4) at 37 °C, and was stirred at approximately 500 rev min^{-1} with a magnetic stirring bar. The upper chamber (donor phase) contained 0·5 mL drug solution. At appropriate intervals, a 0·2 mL sample of receptor phase was withdrawn and replaced with an equal volume of isotonic buffer to keep a constant volume in the receptor phase.

Analytical methods. The concentration of GSH was determined by fluorescence measurement as described by Takahashi et al (1981). The determination involved two steps, (i) disulphides were reduced to thiols by addition of KCN, (ii), the released thiols were reacted

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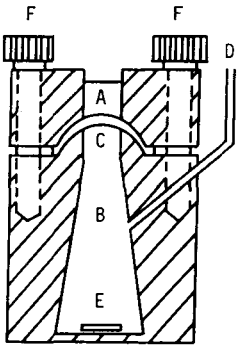

FIG. 1. Apparatus for in-vitro corneal permeability test: A, donor phase (drug solution); B, receptor phase; C, mounted rabbit cornea; D, sampling site; E, magnetic stirrer; F, adjusting screw.

with *N*-(9-acridinyl) maleimide and the fluorescence intensities were measured at an excitation wavelength of 360 nm and emission wavelength of 435 nm. The concentrations of CF and FITC-dextran were determined by fluorescence measurement at excitation wavelengths of 490 nm and 495 nm, respectively, and emission wavelengths of 520 nm and 521 nm, respectively. The concentration of insulin was determined by radioimmunoassay based on the double antibody technique (Daiichi Radioisotope Labs., Tokyo, Japan).

Results and discussion

The effects of TC-Na and TDC-Na on the permeability of low molecular weight and hydrophilic compounds through the cornea are shown in Figs 2 and 3. CF scarcely permeated through the cornea in the absence of bile salt. However, its corneal permeability increased slightly after a short lag time in the presence of 2 and 10 mM TC-Na, and increased rapidly and markedly with 2 and 10 mM TDC-Na. The total amount of CF

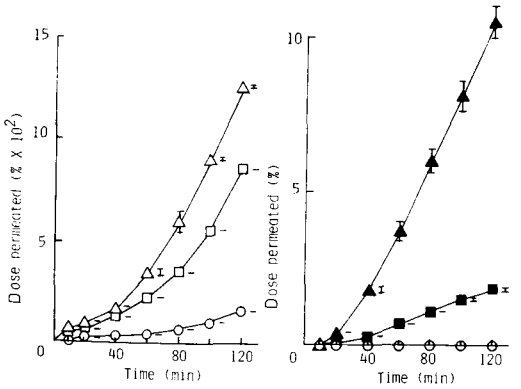

FIG. 2. Effects of taurocholate and taurodeoxycholate on the permeability of 6-carboxyfluorescein through rabbit cornea in-vitro: (○) in the absence of bile salt, (□) 2 mM and (△) 10 mM sodium taurocholate, (■) 2 mM and (▲) 10 mM sodium taurodeoxycholate. Each point is the mean ± s.e. of at least four experiments.

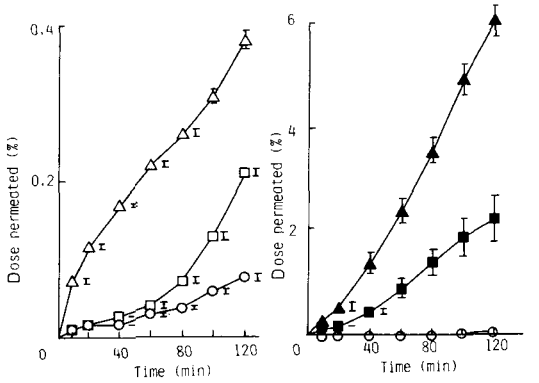

FIG. 3. Effects of taurocholate and taurodeoxycholate on the permeability of glutathione through rabbit cornea in-vitro: (○) in the absence of bile salt, (□) 2 mM and (△) 10 mM sodium taurocholate, (■) 2 mM and (▲) 10 mM sodium taurodeoxycholate. Each point is the mean ± s.e. of at least four experiments.

permeated over the duration of the experiment (120 min) was increased 7·18 times with 10 mM TC-Na and 593 times with 10 mM TDC-Na compared with controls. The permeation of GSH showed a similar trend, with little GSH detectable in the receptor phase in the absence of bile salts. The corneal permeability of GSH was increased with TC-Na and TDC-Na (2 and 10 mM). The total amount of GSH transferred to the receptor compartment over the 120 min exposure was 5·03 times with 10 mM TC-Na and 80·6 times with 10 mM TDC-Na compared with controls.

The effects of TC-Na and TDC-Na on the permeability of macromolecular compounds, FITC-dextran and insulin through corneas are shown in Figs 4 and 5. FITC-dextran showed little permeation but this was slightly increased with 2 and 10 mM TC-Na, and greatly increased with 2 and 10 mM TDC-Na. The total amount of permeated FITC-dextran over 120 min was increased 30·9 times with 2 mM TDC-Na and 61·5 times with 10 mM TDC-Na compared with control values.

Insulin in the absence of bile salts was not detected in the receptor phase over 120 min of exposure, however, a small amount was detected after the application of insulin solution with 2 and 10 mM TC-Na and more was detected with 2 and 10 mM TDC-Na.

Since the cornea consists of a hydrophobic epithelium and endothelium and a hydrophilic middle region, the stroma, differential solubility has been the major theory used to describe transcorneal drug permeation. The corneal endothelium is far more permeable than the epithelium. In this study, the hydrophilic compounds, CF and GSH and macromolecular compounds, FITC-dextran and insulin, did not permeate significantly through the rabbit cornea. However, the permeation of these compounds was markedly facilitated by bile salts. An increased enhancement in absorption of these compounds was noted in the presence of TDC-Na, the dihydroxy bile salt, which was greater than the adjuvant

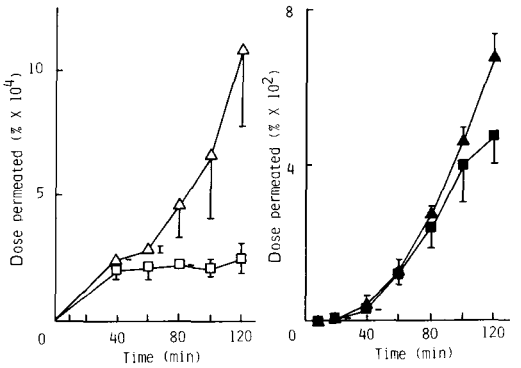

FIG. 4. Effects of taurocholate and taurodeoxycholate on the permeability of FITC-dextran through rabbit cornea in-vitro: (○) in the absence of bile salt, (□) 2 mM and (△) 10 mM sodium taurocholate, (■) 2 mM and (▲) 10 mM sodium taurodeoxycholate. Each point is the mean ± s.e. of at least four experiments.

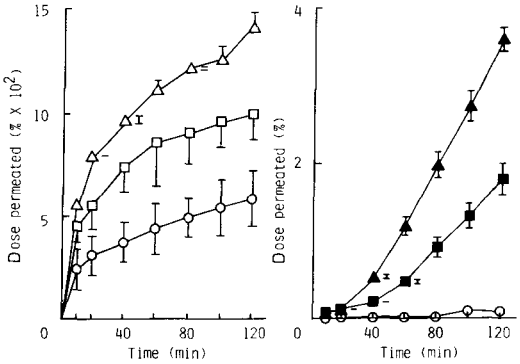

FIG. 5. Effects of taurocholate and taurodeoxycholate on the permeability of insulin through rabbit cornea in-vitro: (□) 2 mM and (△) 10 mM sodium taurocholate, (■) 2 mM and (▲) 10 mM sodium taurodeoxycholate. Each point is the mean ± s.e. of at least four experiments.

effect of the trihydroxy bile salt, TC-Na.

Bile salts have been reported to produce changes in the permeability to many drugs through the small intestine, rectum and other mucosal membranes. Murakami et al (1984) also reported that the absorption-promoting effect was observed with dihydroxy bile salts, whereas trihydroxy bile salts did not enhance the rectal absorption of sodium ampicillin. Kimura et al (1985) reported that enhancement or inhibitory effect on the absorption of various model drugs by TDC-Na and TC-Na, and magnitude by TDC-Na was greater than that by TC-Na. The differences in the physicochemical properties of bile salts, e.g. solubilizing activity, lipophilicity and calcium ion sequestration capacity would relate to their permeability enhancing effects. The critical micellar concentration (CMC) of dihydroxy bile salts is generally lower, and their aggregation numbers larger than those of trihydroxy salts (Nair & Kritcheusky 1971). Thus the solubilizing activity of TDC-Na is higher than that of TC-Na. The lipophilicity and calcium ion sequestration activity of TDC-Na are also higher than that of TC-Na (Murakami et al 1984). These higher physicochemical activities of TDC-Na would be expected to cause loosening of the corneal epithelium barrier. The mechanism of enhancement of corneal permeability of hydrophilic and macromolecular compounds by bile salts is still unclear. However, the considerable permeability of these compounds may indicate that it is mainly through a pore-like route, such as intercellular channels, rather than through partition to the cell membrane.

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