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Intestinal Absorption of Drugs from Solubilized Systems of Long-Chain Fatty Acids in the Rat

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The effects of long-chain fatty acids solubilized by sodium taurocholate on the absorption of various water-soluble drugs from the rat small intestine were investigated using the *in situ* perfusion technique. The addition of 20 mM sodium taurocholate alone or together with lower concentration of oleic acid increased the absorption of poorly absorbable procaine amide, while with higher concentration of oleic acid decreased the absorption of the drug. The addition of oleic acid and monolein significantly increased the formation of micellar complex of well absorbable 2-allyloxy-4-chloro-N-(2-diethylaminoethyl)benzamide hydrochloride (A.C.D.B.) and inhibited the absorption of it, compared to those produced by 20 mM sodium taurocholate alone. The results suggested that the effects of long-chain fatty acids on the intestinal absorption of drugs in the presence of sodium taurocholate were mediated *via* two mechanisms: modification of the permeability of the membrane and micellar complexation of the drug.

The general features of fat absorption in the intestine are now well known. Dietary triglyceride is hydrolyzed in the intestinal lumen, after which the products of hydrolysis, principally fatty acids and monoglycerides, are solubilized by bile salts in mixed micelles. These products are then absorbed into the intestinal mucosal cell where reesterification of the fatty acids and monoglycerides takes place.[2-4]

On the other hand, several investigations have been concerned with the effect of dietary lipids on the intestinal absorption of drugs. The gastrointestinal absorption of griseofulvin,[5,6] lipid-soluble dyes,[7] and macromolecules such as heparin[8] could be markedly enhanced when they were administered with fatty meals or oil-in-water emulsion dosage forms, compared to appropriate controls. However, little precise information is available on how intraluminal and intracellular events during fat absorption influence the intestinal absorption of water-soluble drugs. In a previous paper the effect of short-chain fatty acids on the absorption of drugs from the perfused rat small intestine was described.[9] The purpose of the present investigation was to study the effect of long-chain fatty acids on the intestinal absorption of water-soluble drugs in sodium taurocholate solution.

Drugs used in this study were procaine amide, metoclopramide, 2-allyloxy-4-chloro-N-(2-diethylaminoethyl)benzamide hydrochloride (A.C.D.B.), sulfanilamide, and sulfadimeth-

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2) J.M. Johnston, "Handbook of Physiology," Section 6, Alimentary Canal, Vol. 3, ed. by C.F. Code, Am. Physiol. Soc., Washington, D.C., 1968, p. 1353.
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oxine. These drugs were chosen for investigation because they have a wide divergence in structure, physicochemical properties, and presumably permeability characteristics.

Experimental

Materials——Metoclopramide and A.C.D.B. were kindly supplied by Fujisawa Pharmaceutical Industry Ltd., Osaka. Procaine amide hydrochloride was the gift from Squibb Institute for Medical Research. Lauric acid, palmitic acid (Nakarai Chemicals, Ltd.), oleic acid, monolein, and oleyl alcohol (Tokyo Kasei Kogyo Co., Ltd.) were used as supplied. Sodium taurocholate was synthesized by the method of Norman[10] and was chromatographycally pure. All other chemicals used were of analytical grade and were obtained commercially.

Perfusion Solutions——The specified amounts of drugs, sodium taurocholate, and lipids were dissolved in pH 6.5 isotonic phosphate buffer.

Procedure of Absorption Experiment——Male Wistar albino rats, weighing 150—180 g and fasted for 16—20 hr, were anesthetized with pentobarbital sodium and prepared as described by Koizumi, Arita, and Kakemi[11] for studying drug absorption from the *in situ* small intestine. The bile duct was ligated in all experiments. The rectal temperature was maintained at $37 \pm 1°$ during the experimental period by heat from an incandescent lamp suspended above the animal. Forty milliliters of perfusion solution was recirculated at a rate of 5 ml/min. After 1 hr, the perfusate was withdrawn as completely as possible, and the lumen was washed with physiological saline. The washings were combined to the perfusate. In some experiments, in order to clarify whether the effects on the drug absorption were caused by the interactions between drugs and additives in the perfusion solution, intraluminal interaction, or by some actions of additives to the membrane permeability characteristics, pretreatment with the solution of sodium taurocholate and fatty acids was carried out. Sodium taurocholate solution containing fatty acids was perfused for 30 min in the small intestine, and the small intestinal contents were washed out with physiological saline, and then the solution of a drug alone was perfused for 1 hr. The decrease in the amount of drug in the perfusion solution during the course of an experiment was determined and the amount absorbed was calculated.

Apparent Partition Coefficients——Five ml of the buffered drug solution (pH 6.5) was added to an equal volume of chloroform in glass stoppered tube, and equilibrated at 37° by vigorous shaking. The separated aqueous phase was analyzed. The apparent partition coefficient of a drug was calculated from the decrease of concentration in the aqueous phase.

Measurement of Micellar Interactions——Interactions between drugs and additives were determined by the molecular sieve method of Ashworth and Heard.[12]

Analytical Methods——Procaine amide, metoclopramide, sulfanilamide, and sulfadimethoxine were diazotized, coupled with 2-diethylaminoethyl-1-naphthylamine oxalate, and the colored materials were extracted with isopenthyl alcohol by the addition of sodium chloride. The organic phases were determined spectrophotometrically at 562, 541, 550, and 550 nm, respectively. In case of A.C.D.B., the sample solution was alkalinized with 1 ml of 1N NaOH, and extracted with 5 ml of benzene by the addition of 0.5 g of sodium chloride. The organic phase was determined spectrophotometrically at 294 nm.

Results and Discussion

The effects of concomitant administration of 20 mM sodium taurocholate alone and 6 mM oleic acid with 20 mM sodium taurocholate on the intestinal absorption of various drugs at pH 6.5 are summarized in Table I, which also lists the chloroform-to-pH 6.5 phosphate buffer partition coefficients of the drugs. A significant increase in the absorption of procaine amide, a poorly absorbable drug, in the presence of sodium taurocholate alone was observed, and similar effect in the presence of oleic acid with sodium taurocholate was also found. In contrast, there was no significant change of the intestinal absorption of A.C.D.B., with a higher partition coefficient, in the presence of sodium taurocholate, but a significant decrease of its absorption was observed in sodium taurocholate solution containing oleic acid. Little or no effect on the absorption of metoclopramide and sulfanilamide was obtained when the intraluminal environmental conditions were modified by the addition of these adjuvants.

A previous report showed that short-chain fatty acids such as butyric acid enhanced the absorption of water in the rat small intestine, and the pH of perfusate containing short-chain

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Table I. Effect of Sodium Taurocholate (STC) and Oleic Acid (OA) on the Absorption of Drugs from Rat Small Intestine

Drug	Conc. (mM)	P.C.[a]	% absorbed in 60 minutes[b]		
			Control	STC	STC+OA
Procaine amide	0.1	0.04	9.6±1.5	15.8±2.0	15.7±1.8
Metoclopramide	0.1	2.8	24.4±2.7	25.7±2.3	23.2±0.9
A.C.D.B.	1.0	430	43.1±2.8	42.3±1.4	27.4±2.6
Sulfanilamide	0.1	0.03	43.6±5.0	39.5±3.0	39.4±2.0
Sulfadimethoxine	0.1	11	73.4±0.9	72.6±2.8	62.1±1.8

concentration of sodium taurocholate=20 mM
concentration of oleic acid=6 mM
a) $CHCl_3$-to-pH 6.5 phosphate buffer partition coefficients
b) Each value is the mean ± S.D. of 4—6 animals.

fatty acids rose after experiments.[9] Long-chain fatty acids, however, did not show such physiological effects in these experimental conditions. Therefore, the effect of sodium taurocholate-fatty acid system on the intestinal absorption of drugs could be the result of other mechanisms. Possible mechanisms include an interaction between the drug and the adjuvants and/or a direct effect of the adjuvants on the intestinal membrane with a resultant change in drug absorption.

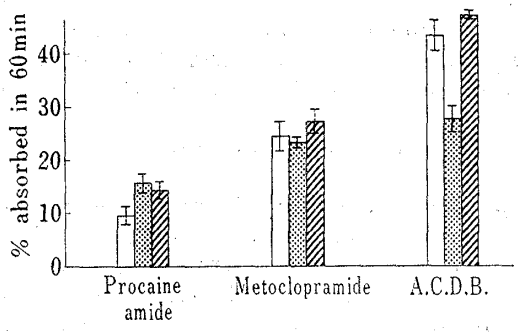

Fig. 1. Effect of Pretreatment with Sodium Taurocholate and Oleic Acid on the Absorption of Drugs from Rat Small Intestine

concentration of sodium taurocholate=20 mM
concentration of oleic acid=6 mM
□: control
⊞: coexistence with sodium taurocholate and oleic acid
▨: pretreatment with sodium taurocholate and oleic acid
Each column represents the mean±S.D. in at least 4 animals.

Table II. Effect of Sodium Taurocholate (STC) and Oleic Acid on the Absorption of Procaine Amide from Rat Small Intestine

Adjuvant	% absorbed in 60 minutes
Control	9.6±1.5
STC	15.8±2.0
STC+ 6 mM Oleic acid	15.7±1.8
STC+12 mM Oleic acid	17.3±2.3
STC+18 mM Oleic acid	10.5±2.2

concentration of sodium taurocholate=20 mM
Each value is the mean±S.D. of 4 animals.

Fig. 1 shows the effect of pretreatment with 20 mM sodium taurocholate and 6 mM oleic acid on the intestinal absorption of drugs. The absorption of procaine amide was increased by the pretreatment with sodium taurocholate solution containing oleic acid. The degree of enhancement observed was similar to that produced by coexistence of the adjuvants. The pretreatment of the small intestine with sodium taurocholate and oleic acid did not produce significant inhibition in the absorption of A.C.D.B. compared to that of coexistence of them.

It seems likely, therefore, that the increase of procaine amide absorption is attributed to a direct effect of the adjuvants on the intestinal membrane, while the decrease of A.C.D.B. absorption is attributed to micellar entrapment of the drug molecules.

To clarify the mechanisms of these varying effects of sodium taurocholate-oleic acid system, the intestinal absorption of procaine amide and A.C.D.B. were further investigated.

Absorption of Procaine Amide

The effect of 20 mM sodium taurocholate solution containing various concentrations of oleic acid on the procaine amide absorption from rat small intestine is summarized in Table II. The result shows a significant increase in the absorption of procaine amide in the presence of 20 mM sodium taurocholate alone or together with 6 and 12 mM oleic acid. In the case of the addition of 18 mM oleic acid to the perfusion solution containing 20 mM sodium taurocholate, however, the increased absorption of procaine amide produced by sodium taurocholate returned to the control level. In another experiment, no significant increase in the drug-micelle interaction by the addition of oleic acid was observed.

The bile salts were found to produce an increase in the permeability characteristics of the intestinal membrane.[13,14] On the other hand, Feldman and Gibaldi[15] reported that the addition of lecithin and fat digestion products to the solution containing sodium taurodeoxycholate produced a pronounced decrease in the permeability of the everted rat intestine to salicylate. The incorporation of 18 mM oleic acid in the sodium taurocholate micelle may therefore reduce the ability of the bile salt micelle to solubilize lipid components of the intestinal membrane, resulting in a decrease in the absorption-enhancing effect of sodium taurocholate on procaine amide.

Absorption of A.C.D.B.

Table III shows the intestinal absorption of A.C.D.B. from the perfusion solution containing oleic acid, oleyl alcohol, and monolein in the presence of 20 mM sodium taurocholate and

Table III. Effect of Oleic Acid, Oleyl Alcohol, and Monolein with Sodium Taurocholate (STC) on the Intestinal Absorption of A.C.D.B. and Their Micellar Complex Formation

Adjuvant	% absorbed in 60 minutes[a]	Micellar/Free[b]
Control	43.1±2.8	0.00
STC	42.3±1.4	0.43
STC+ 6 mM Oleic acid	27.4±2.6	2.72
STC+12 mM Oleic acid	24.7±1.5	2.76
STC+ 6 mM Oleic acid 3 mM Monolein	30.3±1.4	2.99
STC+ 6 mM Oleyl alcohol 3 mM Monolein	33.0±2.1	1.69

concentration of sodium taurocholate=20 mM
a) Each value is the mean±S.D. in at least 4 animals.
b) ratio of micellar to free fraction of A.C.D.B. Each value is the mean of 3 experiments.

their micellar complex formation. The addition of oleic acid, oleyl alcohol, and monolein significantly increased the formation of micellar complex of A.C.D.B. and inhibited the absorption of the drug, compared to those produced by 20 mM sodium taurocholate alone.

It is recognized that bile salts form mixed micelles with a variety of other soluble and insoluble lipidic substances and these mixed micelles incorporate very appreciable amounts of lipophilic molecules.[16,17] The concentration of A.C.D.B. in the mixed micelles could be

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17) M.C. Carey and D.M. Small, *Arch. Intern. Med.*, **130**, 506 (1972).

higher than in the aqueous surroundings, resulting in a higher partitioning tendency between the hydrophobic part of the micelle and its environment. Therefore, the above results could be interpreted in terms of the loss of thermodynamic activity of A.C.D.B. due to the interaction between mixed micelles and the drug.

Table IV. Effect of Various Fatty Acids with Sodium Taurocholate (STC) on the Intestinal Absorption of A.C.D.B. and Their Micellar Complex Formation

Adjuvant	% absorbed in 60 minutes[a]	Micellar/Free[b]
Control	43.1±2.8	0.00
STC	42.3±1.4	0.43
STC+0.5 mM Lauric acid	36.6±3.2[c]	0.57
STC+0.5 mM Palmitic acid	35.9±1.0[d]	0.67
STC+0.5 mM Oleic acid	37.8±2.5[e]	0.64

concentration of sodium taurocholate=20 mM

a) Each value is the mean±S.D. in at least 4 animals.
b) ratio of micellar to free fraction of A.C.D.B. Each value is the mean of 3 experiments.
c) significantly different from STC alone, $p < 0.02$
d) significantly different from STC alone, $p < 0.001$
e) significantly different from STC alone, $p < 0.05$

Table IV shows the effect of various fatty acids in the presence of 20 mM sodium taurocholate on the intestinal absorption of A.C.D.B. and their micellar complex formation. The relative water-insolubility of lauric and palmitic acids prevented their being used at higher concentrations. There were no significant differences between the absorption of A.C.D.B. observed in the presence of various fatty acids. However, in the addition of fatty acids, a small but significant inhibitory effect on the intestinal absorption of A.C.D.B. and increased micellar interaction were noted. In contrast, short-chain fatty acid such as butyric acid did not increase the micellar complexation of A.C.D.B.

On the basis of the absorption and micellar interaction studies, it is suggested that the effects of long-chain fatty acids on the intestinal absorption of drugs in the presence of sodium taurocholate were mediated *via* two mechanisms: modification of the permeability of the membrane and micellar complexation of the drug.