Decanoic Acid Induced Enhancement of Rectal Absorption of Hydrophilic Compounds in Rats

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The enhancing effect of decanoic acid (C10) on the rectal absorption of phenolsulfonphtalein (PSP) was analyzed with a pharmacokinetic model. The transfer index of PSP from the rectal lumen to the epithelial layer in the presence of C10 was proportional to the product of the C10 disappearance rate from the rectal lumen and its calcium ion sequestration capacity. The enhancement of rectal PSP absorption by different doses of C10 was also predictable by using a permeability index, Pa, of PSP, which was defined as a proportionality constant relating transfer index and the product value described above. The Pa values of various hydrophilic compounds with different molecular weights were also estimated from their rectal bioavailability in the presence of C10. A linear relationship was observed between Pa values and reciprocal values of the square root of individual molecular weight. These findings suggest that the Pa index is related to the permeant's diffusion coefficient through paracellular aqueous openings formed by C10.

KEY WORDS: absorption enhancer: decanoic acid; rectal absorption; hydrophilic compound; molecular weight; diffusion coefficient; pharmacokinetic analysis; mechanism.

INTRODUCTION

Decanoic acid (C10) enhances the mucosal absorption of poorly absorbable compounds (1), and it is now clinically used in Japan as an effective enhancing adjuvant for ampicillin and ceftizoxime suppositories. The absorption enhancing action of C10 and 2-hydroxydecanoic acid on the jejunal and colonic absorption of phenolsulfonphtalein (PSP) was evaluated in a pharmacokinetic model (2). The transfer index (TI) of PSP from lumen to the epithelial layer was assumed to be proportional to the product of the enhancer disappearance rate from the lumen and its calcium ion sequestration capacity. The obtained proportionality constant, Pa, of PSP was independent of the enhancer used and their doses, and it was termed as the permeability index. The permeability index was expected to reflect the free diffusion coefficient of the permeant through aqueous openings in the paracellular route possibly formed by an enhancer (3).

In the present study, the previously proposed pharmacokinetic model was evaluated for predicting the C10 effect on PSP rectal absorption. Further, the permeability index, Pa, was investigated in relation to the molecular weight of permeants.

MATERIALS AND METHODS

Materials

C10, PSP, p-aminobenzoic acid (PABA), and trypan blue were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Fluorescein isothiocyanate-dextranes with molecular weight of 4,400 (FD-4) and 9,000 (FD-10s) were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.). 9-Anthryldiazomethane (ADAM) for the analysis of decanoic acid was obtained from Funakoshi Yakuhin Co., Ltd. (Tokyo, Japan). All other reagents and solvents were of reagent grade.

Animal Study

Male Wistar rats weighing 200–250 g were fasted 16 h prior to experiments, but water was given freely.

Intravenous Administration: Anesthetized rats (Nembutal^R, 30 mg/kg, i.p.) were held supine on a surface kept at 37°C. PSP, PABA, trypan blue, FD-4 or FD-10s dissolved in saline were administered through the tail vein at a dose of 50 (PSP, PABA and trypan blue) or 10 (FD-4 and FD-10s) μmol/ml/kg. Blood was collected from a jugular vein with a heparinized syringe at appropriate time intervals for the analysis of drug plasma levels, except for PABA. In the case of PABA, each rat was housed in a metabolic cage for 24 h after intravenous administration of PABA to collect urine for the analysis of PABA metabolites, such as p-acetylaminobenzoic acid, p-aminohippuric acid, and p-acetylaminohippuric acid (4,5).

Pharmacokinetic Model Evaluating The Enhanced Rectal Absorption of PSP: PSP (50 mM) and C10 (50 mM) were dissolved in a pH 7.9, 0.05 M Tris-HCl buffer solution (6). The osmolarity of the solution (Osmostat OM-6020, Kyoto Daiichi Kagaku Co., Ltd.) was adjusted to 560 mOsm/kg H_2O by adding NaCl, because the osmolarity of the added PSP solution containing 200 mM C10 was higher than isotonic. Anesthetized rats were held supine on a surface kept at 37°C. The PSP solution was introduced into the 3 cm-long rectal loop prepared as reported previously at a volume of 1 ml/kg (1). The ligated loop was resected 5, 10, 15, 30 or 180 min after administration of PSP solution. Luminal contents of the loop were removed by washing with 50 ml saline. The washings were subjected to the analysis of PSP and C10 amounts retained in the loop. In separate experiments, blood was collected from a jugular vein at appropriate time intervals for the analysis of PSP in plasma after administration of PSP solution containing 50 mM C10.

Rectal Absorption of Hydrophilic Compounds: PSP (50 mM), PABA (50 mM), trypan blue (50 mM), FD-4 (10 mM) or FD-10s (10 mM) were dissolved in a pH 7.9, 0.05 M Tris-HCl buffer solution containing C10 (25, 50, 100, 150 or 200 mM). The osmolarity of the solution was adjusted to 560 mOsm/kg H_2O. The solution was introduced into the 3 cm-long rectal loop at a volume of 1 ml/kg. In case of trypan blue, FD-4 or FD-10s, blood was collected from a jugular vein for the anal-

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ysis of the permeant in plasma. Plasma samples were obtained by centrifugation at 3,000 r.p.m. for 5 min immediately after the blood collection and stored at −30°C until analysis. In case of PABA, each rat was housed in an individual metabolic cage for 24 h after administration of PABA solution to collect urine for the analysis of metabolites. The absorbed percentage of PSP was estimated from the difference of dose and the amount of PSP retained in the rectal loop 3 h after administration. Absorbed percentages of trypan blue, FD-4, FD-10s or PSP were estimated by comparing the area under the plasma concentration—time curve (AUC) extrapolated to the infinite with that after intravenous administration at the same dose. Absorbed percentage of PABA was estimated by comparing the total amounts of PABA metabolites (p-acetylaminobenzoic acid, p-aminohippuric acid, and p-acetylaminohippuric acid) excreted into the urine with those after intravenous administration.

Analytical Method

The concentration of PSP in plasma and in the luminal washings was spectrophotometrically determined as reported previously (2). The concentration of trypan blue in plasma was spectrophotometrically determined at 575 nm after diluting the plasma sample with distilled water. The concentration of FD-4 and FD-10s in plasma was fluorometrically determined after diluting with pH 7.4 Tris-HCl buffer solution at 495 nm and 516 nm for excitation and emission, respectively. The concentration of C10 in the luminal washings was determined by HPLC equipped with a fluorometric detector after derivatization with the ADAM reagent (2). The concentration of metabolites of PABA in the urine was fluorometrically determined by HPLC as follows. Urine was diluted at least 50 times with pH 3.5 phosphate buffer solution. One ml of the solution was vigorously mixed with 5 ml of ethylacetate. After centrifugation at 3,000 r.p.m. for 5 min, 4 ml of the organic layer was removed and the solvent was evaporated to dryness under reduced pressure. The residues were dissolved with 0.5 ml of a mobile phase (acetonitrile: pH 3.5, 0.05 M phosphate buffer solution = 15: 100 v/v) containing sulfanilamide as an internal standard. Ten μl of the solution was injected onto a HPLC reverse phase column (TSK-Gel ODS-80TM, Tosoh, Tokyo). Flow rate of the mobile phase was 1 ml/min. Detection of the metabolites and sulfanilamide was made at 280 nm and 340 nm for excitation and emission, respectively.

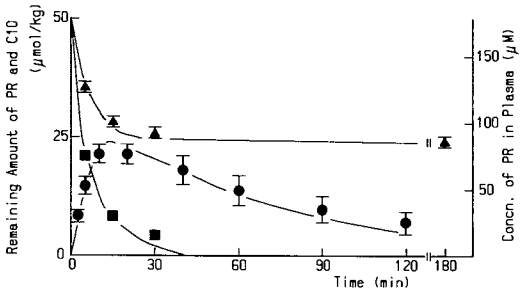

Fig. 1 Disappearance of PSP and C10 from Rectal Loop and Plasma Concentration of PSP After Administration into Rectal Loop in Rats Solution of PSP (50 mM) containing 50 mM C10 was administered into rectal loop (1 ml/kg). (●), concentration of PSP in plasma; (▲), remaining amount of PSP in the loop; (■), remaining amount of C10 in the loop. Solid lines represent the curve fittings. Each point represents the mean ± S.E. of 3–4 trials. S.E. is included within the symbol in case of without error bar.

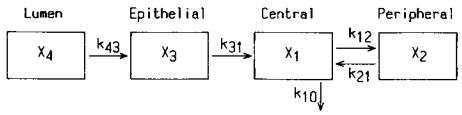

Fig. 2 Pharmacokinetic Model Used in The Present Study. Differential equations based on mass balance are given in previous report (2).

RESULTS AND DISCUSSION

Pharmacokinetic Analysis of The Rectal Absorption of PSP and Enhancement by C10

Following administration of PSP solution (50 mM) containing 50 mM C10 into a rectal loop, the amounts of PSP and C10 retained in the loop and/or concentration of PSP in plasma were determined (Fig. 1). C10 disappeared from the rectal loop according to first order kinetics ($\log X = 1.990 - 0.0499\ t$, $r = 0.993$, where X represents the amount of C10 retained in the loop at time t after administration and r the correlation coefficient). The loss of C10 from the loop was almost completed within 45 min. After disappearance of C10, no further loss of PSP was observed, even though rather high concentration of PSP remained in the loop. Hence, the disappearance rate of PSP varied with time, depending on the disappearance rate of C10, as observed previously in the jejunum and the colon (2). Thus, the C10 effect on PSP rectal absorption was analyzed via a previously proposed pharmacokinetic model shown in Fig. 2 (2). Parameters estimated with a nonlinear least squares regression program, MULTI (RUNGE) (7) are summarized in Table I. In the analysis, parameters of PSP plasma disposition were assumed to be the same as those obtained by two-compartmental pharmacokinetic analysis after intravenous administration of PSP. The value of calcium ion sequestration capacity of C10 (CS) was the same as that reported previously (2). Curve fittings in Fig. 1 shown as solid lines agree well with the measured concentrations (amounts) of PSP and C10 in plasma and/or rectal loop.

To test the validity of the obtained parameters, the percentage of PSP disappeared from the rectum in the presence of different doses of C10 was predicted by the use of Eq. (1) and Eq. (2), which were given in the previous study (2).

Table I. Pharmacokinetic Parameters for Phenolsulfonphthalein Rectal Absorption Enhanced by Decanoic Acid

Parameter	Unit	Value	Parameter	Unit	Value
Pa	$\mu mol^{-1} \cdot kg$	0.0619[a]	k_{31}	min^{-1}	0.0348[a]
ka_{PA}	min^{-1}	0.115[b]	k_{21}	min^{-1}	0.193[b]
Ca	mol/mol	0.250[b]	k_{12}	min^{-1}	0.282[b]
V_1	l/kg	0.938[b]	k_{10}	min^{-1}	0.0417[b]

[a] Estimated by pharmacokinetic analysis.
[b] Separately determined and fixed for pharmacokinetic analysis.

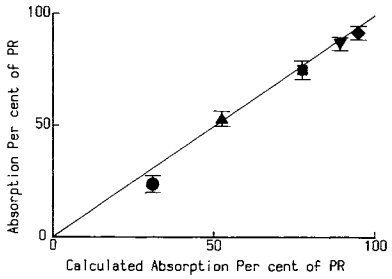

Fig. 3 Relationship between the Predicted and Observed Percentages of Rectal Absorption of PSP Enhanced by C10 in Rats After administration of PSP (50 mM) with different concentrations of C10 into rectal loop (1 ml/kg), remaining amount of PSP in the loop 3 h was determined. Concentration of C10 (mM): (●), 25; (▲), 50; (■), 100; (▼), 150; (♦), 200. Each point of observed value represents the mean ± S.E. of 3–4 trials.

$$TI = Pa \times Ka_{FA} \times Dose_{FA} \times \exp(-Ka_{FA} \times t) \times CS \quad (1)$$

$$dX4/dt = -TI \times X4 \quad (2)$$

Doses of C10 were 25, 100, 150 and 200 μmol/kg. The amount of PSP retained in the loop (X4) 3 hr after the administration of 50 μmol PSP/kg with different doses of C10 was calculated by Runge-Kutta-Gill method. On the other hand, the absorbed percentage of PSP in the presence of different doses of C10 was experimentally determined from the difference between dose and the amount of PSP retained in the loop 3 hr after the administration. As shown in Fig. 3, calculated percentages of absorbed PSP were similar to the observed values.

Relationship Between the Permeability Index and Molecular Weight of Permeants

Rectal absorption of several hydrophilic compounds in the presence of C10 was determined by comparing AUCs of the dosed compound or urinary excreted amount of metabolites after intravenous and rectal administrations. Results are summarized in Table II. The permeability index of each permeant was also estimated using Eq. (1) and (2). In the use of these equations, the absorbed percentages of each compound shown in Table II was assumed to be the same as the amount disappeared from the rectal loop. Estimated permeability indices (Pa values) are also summarized in Table II. The permeability index is a value specific to each permeant irrespective of different doses of C10.

In the previous report, we discussed that the permeability index of each permeant may reflect the apparent diffusion constant of the permeant in the aqueous openings in the membrane formed by an enhancer (2). Steward calculated the expected paracellular fluxes of various non-electrolytes with the assumption that their free-solution diffusion coefficients, Dn, are related to their molecular weights, M, by the relationship $Dn \times M^{0.5} = \text{constant}$ (8). If the permeability index, Pa, obtained in the present study is related to the diffusion coefficient of each permeant, the Pa value must be related with the reciprocal value of $M^{0.5}$. As shown in Fig. 4, plotting the estimated Pa values of each permeant against the reciprocal value of $M^{0.5}$ resulted in a good linear relationship.

The enhancing potency of C10 on the rectal absorption of hydrophilic compounds in rats was reported to correlate well with the permeability of these compounds through cellulose membranes or their diffusion constants (11). The restricted permeation of compounds by pore size and pore abundance is also reported in rabbit gall-bladder epithelium (8), colonic membrane of the rat (12), confluent monolayers of bovine cerebrovascular cells in primary culture (13), and in iontophoretic transdermal permeation across the excised human skin (14). Thus, the finding that the estimated Pa value of permeants relates to the free-solution diffusion coefficient of the permeants indicates that the extent of the rectal absorption of a permeant enhanced by C10 must be limited by the diffusion coefficient of the permeant itself as long as the aqueous pores are kept open. The duration of aqueous openings is determined by the disappearance kinetics of the enhancer and its calcium ion sequestration capacity at the site of administration. Present results also suggest that C10 forms large aqueous openings through which a com-

Table II. Rectal Absorption of Hydrophilic Compounds in the Presence of Decanoic Acid in Rats and Estimated Permeability Index, Pa

Compounds	Dose of Compound μmol/kg	Dose of C10 μmol/kg	Absorption Percent	Pa × 10^2
P-Aminobenzoic acid	50	50	70.11 ± 1.52	9.737 ± 0.406
	50	100	80.49 ± 1.67	6.606 ± 0.354
Phenolsulfonphtalein	50	25	23.81 ± 3.85	6.601 ± 0.499
	50	50	52.95 ± 3.65	6.184 ± 0.804
	50	100	75.22 ± 4.16	5.841 ± 0.785
	50	150	87.30 ± 2.08	5.850 ± 0.848
	50	200	91.89 ± 3.34	5.187 ± 0.427
Trypan Blue	50	50	26.57 ± 2.61	2.494 ± 0.283
	50	100	39.66 ± 6.97	2.211 ± 0.701
FITC-Dextran-4	10	50	12.11 ± 1.17	1.044 ± 0.224
	10	100	16.30 ± 2.38	0.729 ± 0.232
FITC-Dextran-10s	10	50	9.31 ± 2.83	0.728 ± 0.310
	10	100	12.65 ± 3.01	0.546 ± 0.137

Each value represents the mean ± S.E. of 3–5 trials.

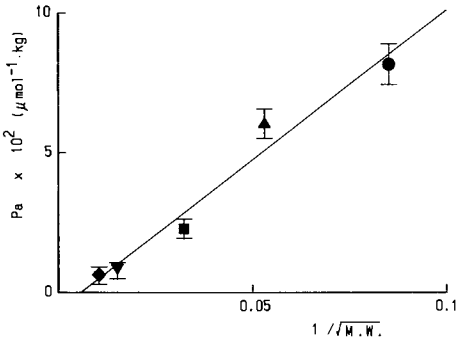

Fig. 4 Relationship between The Permeability Index, Pa and Reciprocal Value of Square Root of Molecular Weight of Compounds (●), PABA; (▲), PSP; (■), trypan blue; (▼), FD-4; (◆), FD-10s. Each point for Pa represents the mean ± S.E. in Table II.

pound of molecular size less than 10,000 D can diffuse freely (the intercept on x-axis is less than 0.01). Also, the dose-dependent enhancing effect of C10 can be accounted for by differences in pore abundance, or fractional pore area in the membrane.

In conclusion, C10 effects on PSP rectal absorption can be pharmacokinetically modeled. The model permits the prediction of the enhancing effect of C10 at different doses, if the permeability index, Pa, of the permeant is provided.

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