# Pharmacokinetic Analysis of the Absorption Enhancing Action of Decanoic Acid and Its Derivatives in Rats

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The enhancing action of decanoic acid (C10) and its derivatives on mucosal absorption of phenolsulfonphthalein (PSP) in the jejunum or colon was analyzed using pharmacokinetics in rats. After administration of a solution containing PSP and an enhancer [C10, 2-hydroxydecanoic acid (2-OHC10), or 3-hydroxydecanoic acid (3-OHC10)] into the jejunal or colonic loop, the amounts of PSP and enhancer remaining in the loop and/or plasma PSP concentration were determined periodically. 2-OHC10 exhibited a greater absorption enhancing potency than C10, while 3-OHC10 was less effective. Disappearance of residual PSP from the loop ceased after complete absorption of the enhancer. The enhancer-induced disappearance rate constant of PSP correlated well with the product of the enhancer disappearance rate and its capacity to sequester calcium ions. In conclusion, the enhancement of PSP mucosal absorption by C10 and its derivatives is consistent with a pharmacokinetic model, assuming that the enhanced membrane permeability of PSP depends on the enhancer disappearance kinetics from the loop and its calcium ion sequestration capacity.

**KEY WORDS:** absorption enhancer; decanoic acid; 2-hydroxydecanoic acid; 3-hydroxydecanoic acid; phenolsulfonphthalein; mucosal absorption; jejunum; colon; pharmacokinetic analysis; calcium ion sequestration capacity.

## INTRODUCTION

Decanoic acid (C10) has been studied as a potential enhancer of the mucosal absorption of poorly absorbable compounds (1,2) and is now clinically used in Japan as an effective enhancing adjuvant for ampicillin and ceftizoxime suppositories. In the present study, first, we compared the enhancing potencies of 2-hydroxydecanoic acid (2-OHC10), 3-hydroxydecanoic acid (3-OHC10), and C10 on the mucosal absorption of phenolsulfonphthalein (PSP), a poorly absorbed model compound, by employing an *in situ* rat jejunal or colonic loop technique. Second, the absorption enhancing action of C10 and its derivatives was analyzed pharmacokinetically to study the mechanism of their absorption enhancing action.

## MATERIALS AND METHODS

### Materials

2-OHC10 and 3-OHC10 were gifts from Wako Pure Chemicals Co., Ltd. (Osaka, Japan). C10 and PSP were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo). ADAM (9-anthryldiazomethane) was used for the analysis of fatty acid and was obtained from Funakoshi Yakuhin Co., Ltd. (Tokyo). All other reagents and solvents were of reagent grade.

### Animal Studies

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

In Situ *Loop Study*. A 4-cm-long jejunal or colonic loop was closed after the contents were washed out. The jejunal loop was made by ligating the intestine 20 cm below the pyrolus and 4 cm distal to the ileum. A colonic loop was made by ligating the intestine just below the cecum and 4 cm distal to the end. After bile was drained by cannulation of the common bile duct with polyethylene tubing (PE 10, Clay Adams), a solution of PSP (20 m*M*) containing one of the absorption enhancers (0–100 m*M*) was introduced into the loop at a volume of 2 mL/kg. PSP and the enhancer were dissolved in a pH 7.4, 0.05 *M* Tris–HCl buffer solution and the solution was adjusted to 280 mOsm/kg $H_2O$ with NaCl. Blood was collected from the jugular vein at designated time intervals for analysis of PSP. After the final sampling of blood (3 hr after administration), the ligated loops were resected. Contents in the loop were removed by washing with a sufficient amount of distilled water, and washings were collected to determine the PSP concentration. In separate experiments, the ligated loops was removed at designated times (5, 15, 30, or 180 min) after administration of PSP solution with or without the absorption enhancer to determine PSP and the enhancer amounts retained in the loop.

*Single-Perfusion Study*. Bile was drained via cannulated polyethylene tubing. Whole small intestines from the duodenum to the ileocecum were washed with 50 mL saline. A solution of PSP (1 m*M*) with or without C10 (50 m*M*) was perfused in a single perfusing manner at a flow rate of 2 mL/min. The effluent was collected serially at 10-min intervals to determine PSP and C10 concentrations. The disappearance clearance ($CL_{disapp}$) of PSP or C10 was calculated using the following equation; $CL_{disapp} = [(Q_{in} \times C_{in}) - (Q_{out} \times C_{out})]/C_{in}$, where $Q_{in}$ and $Q_{out}$ represent the flow rate of the influent (2 mL/min) and the effluent ($Q$ mL/min), respectively. $C_{in}$ and $C_{out}$ represent the concentration of PSP or C10 in the influence and the effluent, respectively. The effluent flow rate was calculated from the weight of the effluent in 10 min.

### Calcium Ion Sequestration Capacity *in Vitro*

*Dye Indicator Method*. The calcium ion sequestration capacity of an enhancer was determined as reported previously (3).

*Loop Method*. One end of the isolated jejunum was ligated. An isotonic pH 7.4 buffer solution or a solution containing one of the enhancers (50 m*M*) was introduced inside the sac (0.4 mL/loop). The other end was also ligated to make a 4-cm-long sac and the sac was immersed in a 50-mL incubation medium (isotonic pH 7.4 buffer solution) under

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bubbling with 95% $CO_2$–5% $O_2$. Incubation was at 37°C for 15 min. The sac was then removed and the concentrations of calcium ion and enhancer in the medium were determined.

### Analytical Method

The concentration of PSP in the plasma and washings of the luminal contents was determined spectrophotometrically at 560 nm after dilution with appropriate amounts of 1 *N* NaOH. The concentration of C10 or its derivatives in the luminal washings was determined by HPLC equipped with a fluorometric detector after derivatization with the ADAM reagent. Briefly, C10 or its derivatives in the luminal washings was extracted with benzene under acidic conditions. After the benzene was evaporated, residues were dissolved in 200 μL methanol containing an internal standard (octanoic, 2-hydroxydodecanoic, or 3-hydroxydodecanoic acid for C10, 2-OHC10, or 3-OHC10 sample, respectively). Then 200 μL of ADAM reagent-methanol solution (0.1%, w/v) was added. After standing for 60 min at ambient temperature, an aliquot of the solution was injected onto an HPLC column (a TSK-Gel reverse phase column, ODS-80TM, Tosoh). The flow rate of the mobile phase (a mixture of acetonitrile and water (9:1, v/v)) was 1 mL/min. C10 or its derivatives and an internal standard were detected at wavelengths of 365 and 412 nm for excitation and emission, respectively.

For determination of the concentration of calcium ion in the medium outside the sac, C10 or its derivatives in the incubation medium were extracted with benzene under acidic conditions. The aqueous layer was separated and lyophilized. The residues were dissolved in 50 μL of water and subjected to determination of the calcium ion concentration with the Calcium C Test (Wako Pure Chemicals, Osaka, Japan).

## RESULTS

### Disappearance of PSP and Decanoic Acid or Its Derivatives from the Jejunal or Colonic Loop

As a typical example, the time course of the disappearance of PSP and an enhancer from the colonic loop is shown in Fig. 1. No further loss of PSP was observed after disappearance of the enhancer. Hence, the disappearance rate of PSP varied with time, depending on the disappearance rate of the enhancer itself. Similar results were also observed in the jejunum.

Each absorption enhancer disappeared from the colonic loop according to first-order kinetics as follows: C10, $\log X = 1.991 - 0.0393t$, $r = 0.999$; 2-OHC10, $\log X = 1.988 - 0.0240t$, $r = 0.996$; and 3-OHC10, $\log X = 1.969 - 0.0234t$, $r = 0.977$, where $X$ represents the amount of enhancer remaining in the loop at time $t$ after administration of a concentration of 50 m*M*. Disappearance rate constants at different enhancer concentrations and disappearance percentage of PSP from the loop during 3 hr are summarized in Tables I and II, respectively. 2-OHC10 exhibited a greater absorption enhancing potency than C10, while 3-OHC10 was less effective.

### Relationship of the Disappearance of PSP and Enhancer

In a single perfusion study, the disappearance clearance of PSP was constant as long as the concentration of C10 in the perfusate or the disappearance clearance of C10 remained constant (data not shown). This finding indicates that the disappearance kinetics of PSP from the lumen depend on that of the enhancer. As a possible mechanism for the absorption enhancing action of C10 and its derivatives, a role of the calcium ion sequestration capacity has been proposed (1,3–5). The calcium ion sequestration capacities ($Ca^{2+}$ mol/mol enhancer) of C10, 2-OHC10, and 3-OHC10 determined by the dye indicator method *in vitro* were 0.250, 0.476, and 0.133, respectively. The sequestration capacity was also examined employing the *in situ* jejunal loop method (Table III). These results show that calcium ions within the membrane are sequestered and released to the serosal side concurrent with the penetration of C10 or its derivatives. The molar ratio of the amount of calcium ion removed by the enhancer was comparable to the value determined by the dye indicator method. These findings suggest that the difference between C10 and 2-OHC10 in terms of their absorption enhancing potencies can be attributed to the difference in their calcium ion sequestration capacities. If correct, the initial disappearance rate constant of PSP in the presence of enhancers, which was obtained by dividing the amount of PSP lost from the loop during 5 min by the initial concentration of PSP in the dosing solution, must be related to the product of the enhancer disappearance rate and its calcium ion sequestration capacity. As shown in Fig. 2, a single linear relationship

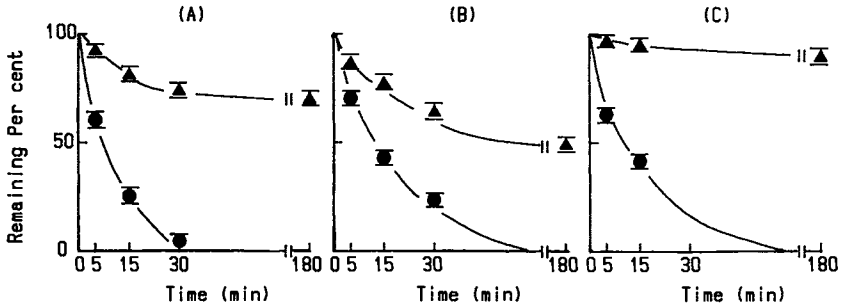


Fig. 1. Disappearance of PSP and C10 or its derivatives from the colonic loop. (▲) PSP; (●). A, C10; B, 2-OHC10; C, 3-OHC10. A solution of PSP (20 m*M*) containing an enhancer (50 m*M*) was administered into a 4-cm-long loop at a volume of 2 mL/kg. Each point represents the mean ± SE of four to six trials.

**Table I.** Disappearance Rate Constants of Decanoic Acid and Its Derivatives from the Jejunal or Colonic Loop in Rats[a]

| Enhancer | Dose (μmol/kg) | Jejunum ($min^{-1}$) | Colon ($min^{-1}$) |
|---|---|---|---|
| C10 | 50 | 0.117 ± 0.022 | 0.103 ± 0.007 |
| | 100 | 0.119 ± 0.010 | 0.091 ± 0.003 |
| | 200 | 0.108 ± 0.020 | 0.092 ± 0.007 |
| 2-OHC10 | 50 | 0.088 ± 0.009 | 0.058 ± 0.001 |
| | 100 | 0.090 ± 0.007 | 0.056 ± 0.003 |
| 3-OHC10 | 100 | 0.085 ± 0.008 | 0.056 ± 0.008 |

[a] Each value represents the mean ± SE ($n$ = 3–4).

was obtained regardless of the enhancer used and its dose. These findings suggest that the amount of calcium ion which was sequestered by the enhancer and had penetrated through the membranes is an important factor of the enhanced PSP membrane permeability.

### Pharmacokinetic Analysis of the Enhancing Action of Decanoic Acid and Its Derivatives

On the basis of enhancer disappearance kinetics and its corresponding calcium ion sequestration capacity, a pharmacokinetic model was constructed (Fig. 3) with the assumption that (a) enhanced membrane permeability to PSP varies with time, depending on the disappearance rate of the enhancer, and (b) the enhancer effect is proportional to the degree of calcium ion sequestration caused by the enhancer during penetration through the epithelial membranes. The differential equations for PSP absorption based on mass balance are

$$dX4/dt = \mathrm{TI} \times X4 \quad (1)$$

$$dX3/dt = \mathrm{TI} \times X4 - k_{31} \times X3 \quad (2)$$

$$dX1/dt = k_{31} \times X3 + k_{21} \times X2 - (k_{21} + k_{10}) \times X1 \quad (3)$$

$$dX2/dt = k_{12} \times X1 - k_{21} \times X2 \quad (4)$$

where $X4$, $X3$, $X1$, and $X2$ denote the amount of PSP in the lumen, epithelial compartments, central compartments, and peripheral compartments, respectively. $X1$ was assumed to be the product of the plasma concentration of PSP ($C_1$) and distribution volume of PSP in the central compartment ($V_1$).

**Table II.** Enhanced Disappearance Percentage of Phenolsulfonphtalein in the Presence of Decanoic Acid, 2-Hydroxydecanoic Acid, or 3-Hydroxydecanoic Acid in Rats[a]

| Enhancer | Dose (μmol/kg) | Jejunum | Colon |
|---|---|---|---|
| None | 0 | 4.4 ± 1.0 | 6.4 ± 1.3 |
| C10 | 50 | 7.7 ± 1.0* | 13.3 ± 1.3* |
| | 100 | 25.3 ± 3.6** | 30.0 ± 0.9** |
| | 200 | 52.6 ± 2.3** | 49.5 ± 3.1** |
| 2-OHC10 | 25 | 5.1 ± 0.4 | 11.3 ± 1.1* |
| | 50 | 28.0 ± 9.8** | 26.7 ± 2.1** |
| | 100 | 62.2 ± 3.3** | 50.9 ± 1.1** |
| 3-OHC10 | 100 | 7.7 ± 1.0* | 10.6 ± 2.6 |

[a] Disappearance of PSP from the loop was determined 3 hr after administration. Each value represents the mean ± SE ($n$ = 3–4).
* Significantly different from no enhancer, $P < 0.05$.
** Significantly different from no enhancer, $P < 0.01$.

**Table III.** Released Amount of Calcium Ion and Penetrated Amount of Decanoic Acid and Its Derivatives from the Jejunal Loop

| Enhancer (20 μmol) | (a) Observed released $Ca^{2+}$ (nmol)[a] | (b) Increased $Ca^{2+}$ (nmol)[b] | (c) Penetrated enhancer (nmol)[a] | Ratio $Ca^{2+}$/ enhancer[c] |
|---|---|---|---|---|
| Control | 137.8 ± 21.7 | — | — | — |
| C10 | 321.8 ± 8.5 | 184.0 | 788.9 ± 172.8 | 0.233 |
| 2-OHC10 | 351.0 ± 46.8 | 213.2 | 407.0 ± 71.3 | 0.524 |
| 3-OHC10 | 201.5 ± 21.7 | 63.7 | 522.7 ± 48.6 | 0.122 |

[a] Determined 15 min after administration. Each value represents the mean ± SE ($n$ = 3–4).
[b] Amount of $Ca^{2+}$ released by the presence of an enhancer.
[c] Estimated by dividing the value of b by c.

The transfer index of PSP to the epithelial compartment from the lumen, TI, which depends on the disappearance kinetics of the enhancer, was defined by Eq. (5) on the basis of experimental results (Fig. 2).

$$\mathrm{TI} = P_a \times K_{a,FA} \times \mathrm{Dose}_{FA} \times \exp(-K_{a,FA} \times t) \times \mathrm{CS} \quad (5)$$

In Eq. (5), $K_{a,FA}$, $\mathrm{Dose}_{FA}$, CS, and $P_a$ represent the disappearance rate constant from the loop, the dose, the calcium ion sequestration capacity of C10 and its derivatives, and a proportionality constant, respectively. The proportionality constant, $P_a$, was named temporarily as a permeability index. The value of $K_{a,FA} \times \mathrm{Dose}_{FA} \times \exp(-K_{a,FA} \times t)$ represents the disappearance rate of enhancer at time $t$. Also, the value of $K_{a,FA} \times \mathrm{Dose}_{FA} \times \exp(-K_{a,FA} \times t) \times \mathrm{CS}$ represents the rate of calcium ion sequestration caused by an enhancer which has penetrated through the jejunal or colonic membrane. The parameters of $k_{12}$, $k_{21}$, and $k_{10}$ were intended to be the same as those obtained by the two-compartment pharmacokinetic analysis in plasma disposition of PSP after intravenous administration in the present study. Thus, $P_a$ and $k_{31}$ were estimated by the use of a nonlinear least-squares regression program, MULTI (RUNGE) (6), with

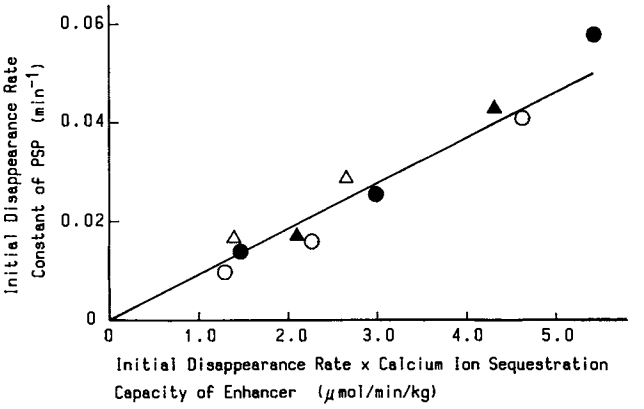


Fig. 2. Relationship between the enhanced initial disappearance rate constant of PSP and the product of the initial disappearance rate and the calcium ion sequestration capacity of the enhancer. C10: (●) jejunum; (○) colon. 2-OHC10: (▲) jejunum; (△) colon. Each point represents the mean of four to six trials.

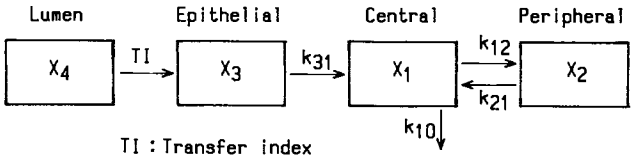


Fig. 3. Pharmacokinetic model used in the present study.

slight modifications. Estimated parameters and other fixed parameters for calculation are summarized in Table IV.

Time profiles of plasma PSP concentration, luminal PSP amount, and luminal enhancer amount can be predicted using the above equations and estimated parameters. Curve fittings shown as solid lines in Fig. 4 are a typical example and are in good agreement with the measured concentrations (or amounts) of PSP and enhancers in plasma and colon. Similar levels of goodness of fit were observed in the jejunum and 2-OHC10.

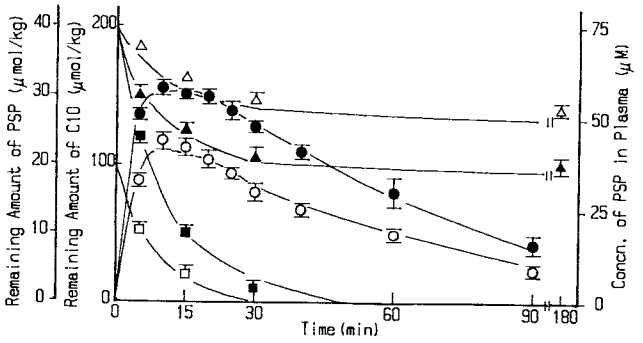


Fig. 4. Time profile of enhanced plasma concentration of PSP and disappearance profile of PSP and C10 from the colonic loop. A solution of PSP (20 m*M*) containing C10 (open symbols, 50 m*M*; filled symbols, 100 m*M*) was administered into a 4-cm-long loop at a volume of 2 mL/kg. (○, ●) Concentration of PSP in plasma; (△, ▲) amount of PSP remaining in the loop; (□, ■) amount of C10 remaining in the loop. Each point represents the mean ± SE of four to six trials.

## DISCUSSION

Pretreatment of the mucous membrane with EDTA enhances its permeability to poorly absorbable compounds, although the enhancing potency is less than observed with enhancer cotreatment (7). However, in the present study, the disappearance of PSP from the loop was negligible after completion of the absorption of C10 or its derivative (Fig. 1). These findings indicate that C10, or its derivative, enhances the membrane permeability to PSP only during cotreatment with the permeant. 2-OHC10 disappeared from the lumen with a rate constant similar to that of 3-OHC10 but less than that of C10 (Table I). However, 2-OHC10 showed the greatest effect on PSP disappearance (Fig. 1, Table II). As a possible mechanism of the enhancement effect by medium-chain fatty acid, the membrane perturbation was proposed to be caused by the interaction between the enhancer and membrane proteins or lipids (8), resulting in an increase in the equivalent pore radius in the paracellular route (9). Further, the dose-dependent enhancing effects of *N*-acylamino acids can be accounted for by the extent of calcium ion sequestered by the enhancer within the membrane (5). Calcium ions maintain the integrity of cell membrane and intercellular junctions (7,10–12). As shown in Fig. 2, the enhancing effects of C10 and 2-OHC10 are related to both the calcium ion sequestration capacities and the enhancer disappearance kinetics.

The permeability index obtained in Eq. (5), $P_a$, for PSP was independent of the enhancers used and their doses (Table IV). The $P_a$ value may represent the apparent diffusion constant in the membrane when aqueous openings are formed by an enhancer (9). If correct, the $P_a$ value may vary depending on the molecular size of the permeant.

It was observed that the time to reach the peak plasma concentration ($T_{max}$) of PSP was longer from the jejunum than from the colon, although the disappearance rate constant of the enhancers from the jejunum was greater than that from the colon (Table I). The difference in $k_{31}$ between jejunum and colon may reflect differences in the $T_{max}$ of PSP between jejunum and colon.

In conclusion, on the basis of the enhancer disappearance kinetics and their calcium ion sequestration capacity, the absorption enhancing action of C10 and its derivatives on PSP mucosal absorption can be accounted for via the pharmacokinetic model.

**Table IV.** Pharmacokinetic Analysis of Enhancing Effect of Decanoic Acid Derivatives for Jejunal or Colonic Absorption of Phenolsulfonphtalein in Rats[a]

| Segment | Enhancer | Dose (μmol/kg) | $P_a \times 10^2$ ($\mu mol^{-1} \cdot kg$) | $K_{31}$ ($min^{-1}$) |
|---|---|---|---|---|
| Jejunum | C10 | 100 | 1.68 ± 0.25 | 0.663 ± 0.037 |
| | | 200 | 1.44 ± 0.10 | 0.323 ± 0.028 |
| | 2-OHC10 | 50 | 1.77 ± 0.12 | 0.294 ± 0.048 |
| | | 100 | 1.63 ± 0.14 | 0.237 ± 0.035 |
| Colon | C10 | 100 | 1.53 ± 0.09 | 1.909 ± 0.431 |
| | | 200 | 1.71 ± 0.08 | 2.432 ± 0.173 |
| | 2-OHC10 | 50 | 1.84 ± 0.14 | 2.173 ± 0.376 |
| | | 100 | 1.77 ± 0.07 | 1.882 ± 0.427 |

[a] The value of each parameter fixed for the calculation using Eqs. (1)–(5) was as follows: $k_{12} = 0.3184\ min^{-1}$, $k_{21} = 0.2277\ min^{-1}$, $k_{10} = 0.0534\ min^{-1}$, and $V_1 = 0.0986$ L/kg. CS of enhancer: C10, 0.250; 2-OHC10, 0.476. $K_{a,FA}$ of enhancer ($min^{-1}$): C10, 0.114, and 2-OHC10, 0.0900, in jejunum; and C10, 0.0913, and 2-OHC10, 0.0571, in colon. Dose of PSP was 40 μmol/kg. The drug solution containing enhancer was administered into the jejunal or colonic loop (4 cm). Each value represents the mean ± SE ($n$ = 4–6).

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