Pharmacokinetic Analysis of *in Vivo* Metabolism of Amino Acid or Dipeptide Conjugates of Salicylic Acid in Rabbit Intestinal Microorganisms

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We analyzed the pharmacokinetics of salicylic acid (SA)–amino acid (alanine, glutamic acid, methionine, and tyrosine) or SA–dipeptide (glycylglycine) conjugates in rabbits, by using a model that takes into account the metabolism of prodrug to SA by intestinal microorganisms and, also, by model-independent analysis. The blood concentration profiles of these prodrugs and released SA following intracecal and oral administration to rabbits were obtained previously (Nakamura *et al.*, *J. Pharm. Pharmacol.*, 44, 295–299, 1992; *Chem. Pharm. Bull.*, 40, 2164–2168, 1992; *Int. J. Pharm.*, 87, 59–66, 1992; *J. Pharm. Pharmacol.*, 44, 713–716, 1992). First, the overall *in vivo* behavior was evaluated by statistical moment analysis. Next, the blood concentration profiles of prodrug and SA following intracecal and oral administration were simultaneously fitted to the above model. In general, good agreement was observed between fitted lines and experimental data for every prodrug, suggesting the validity of this model. The obtained parameters characterized the difference in the rate of metabolism and absorption among the prodrugs. Lower absorbability and enhanced hydrolysis rate of the prodrug lead to prolonged blood concentration of SA.

KEY WORDS: pharmacokinetic analysis; salicylic acid; prodrug; metabolism; rabbit; intestinal microorganism.

INTRODUCTION

In the previous studies, we prepared salicylic acid (SA)–alanine (S-Ala) (1), SA–glutamic acid (S-Glu) (2), SA–methionine (S-Met) (3), SA–tyrosine (S-Tyr) (3), and SA–glycylglycine (S-Glygly) (4) conjugates as prodrugs having various physicochemical properties, and we studied the effect of metabolism by intestinal microorganisms, using a pharmacokinetic analysis of the released SA in rabbits.

Previous work on pharmacokinetic analysis of drug metabolism in the intestinal microorganisms employed a simplified model (5,6).

In the present study, we analyzed the metabolism of SA prodrugs in the intestinal microorganisms, by developing a more detailed pharmacokinetic model and, also, by a model-independent approach.

MATERIALS AND METHODS

In Vivo Experiment

Following oral, intravenous, and intracecal administration of prodrug or SA (434, 72, and 36 μmol/kg, respectively) to male albino rabbits (2–3 kg), blood was collected at appropriate time intervals from an ear vein. Prodrug and SA in blood were analyzed by HPLC and were reported previously (1–4).

Statistical Moment Analysis

The area under the blood concentration curve (AUC) and mean residence time (MRT) following intracecal and oral administration of prodrug or SA were calculated by numerical integration using a linear trapezoidal formula and extrapolation to infinite time based on a monoexponential equation (7).

Pharmacokinetic Model

The pharmacokinetic model was constructed based on the following assumptions: (i) orally administered prodrug is partly absorbed, and unabsorbed prodrug is transported to the cecum, containing a large amount of intestinal microorganisms; (ii) prodrug absorption is continuous throughout a segment of the gastrointestinal tract; (iii) prodrug in the cecum is metabolized to SA, followed by SA absorption from the cecum; (iv) absorbed prodrug and SA in the systemic circulation eliminate biexponentially; and (v) the availability of orally administered prodrug (F_{po}) is the ratio of the total prodrug absorbed, as prodrug itself and as SA, to the administration dose.

The pharmacokinetic models following intravenous, intracecal, and oral administration of SA or prodrug are depicted schematically in Fig. 1. Mass balance equations in each model are given in Table I. Absorption of SA from the cecum is governed by the first-order rate constant k_a (Model 2). The first-order rate constant K_a' and k_m determine the absorption of prodrug from the cecum and the metabolism of prodrug to SA in the cecum, respectively (Model 3). Absorption of orally administered prodrug is assumed to be continuous throughout a segment of the gastrointestinal tract and is governed by the first-order rate constant K_a, and the first-order rate constant k_t is used to describe the drug transfer from the administered gut compartment to the cecum compartment (Model 4). In this model, t_0 is the lag time during which drug is available for transfer to the cecum compartment.

Calculation of Pharmacokinetic Parameters

First, the mean blood concentration profiles of prodrug or SA following intravenous administration of them were fitted to the biexponential equation by the nonlinear least-squares method (MULTI) (8). Then the volume of the central compartment (V_c) and first-order rate constants (K_{12}, K_{21}, and K_{el}) were calculated from hybrid parameters. These parameters were substituted into the following mass balance equations (Table I).

In the same way, the mean blood concentration profile of SA following its intracecal administration was fitted in the two-compartment model with first-order input and output (Model 2), by use of the MULTI program. Then k_a was

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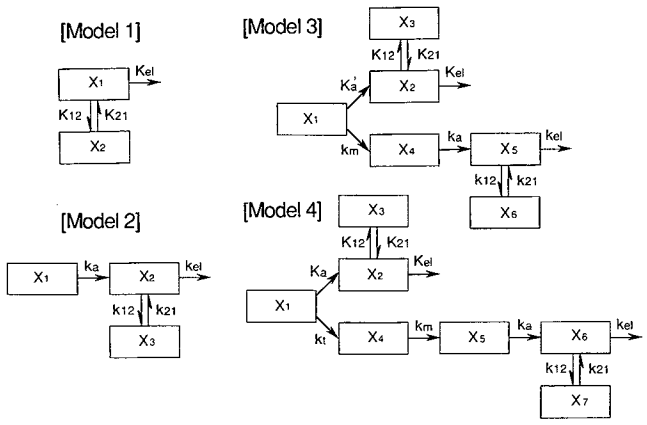

Fig. 1. Pharmacokinetic model used for the analysis of blood concentration profiles of prodrug and SA following intravenous administration (Model 1), intracecal administration of SA (Model 2) or prodrug (Model 3), and oral administration of prodrugs (Model 4). Model 1: X_1, drug in central compartment; X_2, drug in peripheral compartment; K_{12} and K_{21}, transfer rate constant; K_{el}, elimination rate constant. Model 2: X_1, SA in cecum compartment; X_2, SA in central compartment; X_3, SA in peripheral compartment; k_a, absorption rate constant of SA from cecum; k_{12} and k_{21}, transfer rate constant of SA; k_{el}, elimination rate constant of SA. Model 3: X_1, prodrug in cecum compartment; X_2, prodrug in central compartment; X_3, prodrug in peripheral compartment; X_4, SA in cecum compartment; X_5, SA in central compartment; X_6, SA in peripheral compartment; K_a', absorption rate constant of prodrug from cecum; K_{12} and K_{21}, transfer rate constant of prodrug; K_{el}, elimination rate constant of prodrug; k_m, metabolism rate constant of prodrug; k_a, absorption rate constant of SA from cecum; k_{12} and k_{21}, transfer rate constant of SA; k_{el}, elimination rate constant of SA. Model 4: X_1, prodrug in gut compartment; X_2, prodrug in central compartment; X_3, prodrug in peripheral compartment; X_4, prodrug in cecum compartment; X_5, SA in cecum compartment; X_6, SA in central compartment; X_7, SA in peripheral compartment; K_a, absorption rate constant of prodrug; K_{12} and K_{21}, transfer rate constant of prodrug; K_{el}, elimination rate constant of prodrug; k_t, transfer rate constant of prodrug; k_m, metabolism rate constant of prodrug; k_a, absorption rate constant of SA from cecum; k_{12} and k_{21}, transfer rate constant of SA; k_{el}, elimination rate constant of SA. Lag time (t_0) exists in the transfer process from X_1 to X_4 compartment.

Table II. Pharmacokinetic Parameters of SA and Prodrugs Following Intravenous Administration to Rabbits

Compound	V_c (L/kg)	AUC_{iv} ($\mu g/mL \cdot hr$)	K_{12} (hr^{-1})	K_{21} (hr^{-1})	K_{el} (hr^{-1})
SA	0.17	252	1.0	3.6	0.23
S-Ala	0.15	16	1.0	4.3	4.2
S-Glu	0.18	13	4.0	7.9	4.6
S-Met	0.12	21	1.5	3.5	3.9
S-Tyr	0.15	14	1.5	3.2	4.9
S-Glygly	0.28	9.0	2.2	3.6	3.9

determined and substituted into the following mass balance equations (Table I).

Next the Laplace transforms of the mass balance equations for blood concentration of prodrug (C_2) and appeared SA (C_5) in Model 3 were simultaneously fitted to the mean blood concentration profiles of prodrug itself and appeared SA following intracecal administration of prodrug with the aid of MULTI(FILT) (9), a nonlinear least-squares regression computer program based on a fast inverse Laplace transform algorithm. This program is written in MS-FORTRAN and run on a personal computer (NEC PC-9801 VX). In this case, the availability of intracecally administered prodrug (F_{ic}) is the ratio of the total prodrug absorbed, as prodrug itself and as SA, to the dose. Then F_{ic}, k_m, and K_a' were calculated, by simultaneous curve fitting, and k_m was substituted into the following mass balance equations (Table I).

Experimental data following oral administration of prodrugs were treated in the same way. The Laplace transforms of the mass balance equations for the blood concentration of prodrug (C_2) and appeared SA (C_6) in Model 4 are simultaneously fitted to the mean profiles of prodrug and appeared SA following oral administration of prodrug, by using MULTI(FILT) program. Finally, F_{po}, K_a, k_t, and t_0 were determined.

Table I. Mass Balance Equations for Each Compartment in the Pharmacokinetic Models Shown in Fig. 1

Model 1	$dX_1/dt = K_{21} \cdot X_2 - K_{12} \cdot X_1 - K_{el} \cdot X_1$	(1)
	$dX_2/dt = K_{12} \cdot X_1 - K_{21} \cdot X_2$	(2)
Model 2	$dX_1/dt = -k_a \cdot X_1$	(3)
	$dX_2/dt = k_a \cdot X_1 + k_{21} \cdot X_3 - k_{12} \cdot X_2 - k_{el} \cdot X_2$	(4)
	$dX_3/dt = k_{12} \cdot X_2 - k_{21} \cdot X_3$	(5)
Model 3	$dX_1/dt = -(K_a' + k_m) \cdot X_1$	(6)
	$dX_2/dt = K_a' \cdot X_1 + K_{21} \cdot X_3 - K_{12} \cdot X_2 - K_{el} \cdot X_2$	(7)
	$dX_3/dt = K_{12} \cdot X_2 - K_{21} \cdot X_3$	(8)
	$dX_4/dt = k_m \cdot X_1 - k_a \cdot X_4$	(9)
	$dX_5/dt = k_a \cdot X_4 + k_{21} \cdot X_6 - k_{12} \cdot X_5 - k_{el} \cdot X_5$	(10)
	$dX_6/dt = k_{12} \cdot X_5 - k_{21} \cdot X_6$	(11)
Model 4	$dX_1/dt = -(K_a + k_t) \cdot X_1$	(12)
	$dX_2/dt = K_a \cdot X_1 + K_{21} \cdot X_3 - K_{12} \cdot X_2 - K_{el} \cdot X_2$	(13)
	$dX_3/dt = K_{12} \cdot X_2 - K_{21} \cdot X_3$	(14)
	$dX_4/dt = k_t \cdot X_1 - k_m \cdot X_4$	(15)
	$dX_5/dt = k_m \cdot X_4 - k_a \cdot X_5$	(16)
	$dX_6/dt = k_a \cdot X_5 + k_{21} \cdot X_7 - k_{12} \cdot X_6 - k_{el} \cdot X_6$	(17)
	$dX_7/dt = k_{12} \cdot X_6 - k_{21} \cdot X_7$	(18)

Table III. Moment Parameters of SA and Prodrugs Following Intracecal and Oral Administration to Rabbits

Compound	Prodrug		SA		Prodrug		SA	
	AUC_{ic} (μg/mL · hr)	MRT_{ic} (hr)	AUC_{ic} (μg/mL · hr)	MRT_{ic} (hr)	AUC_{po} (μg/mL · hr)	MRT_{po} (hr)	AUC_{po} (μg/mL · hr)	MRT_{po} (hr)
SA	—	—	145	8.8	—	—	nt[a]	nt
S-Ala	0	—	126	19	62	3.8	420	21
S-Glu	0	—	119	14	0	—	1526	27
S-Met	6.6	3.8	89	20	96	7.7	148	23
S-Tyr	4.6	4.4	56	22	56	11	619	27
S-Glygly	0	—	130	10	0	—	1246	25

[a] Not tested.

RESULTS AND DISCUSSION

Intravenous Administration of SA or Prodrug

Pharmacokinetic parameters for SA and prodrugs following intravenous administration are listed in Table II. Every prodrug was rapidly eliminated compared to SA, judging from the K_{el} value.

Statistical Moment Analysis

Moment parameters calculated from the profiles of prodrug and SA following intracecal and oral administration are summarized in Table III. The difference between MRT_{ic} value of SA following its intracecal administration and that of prodrug might correspond to the mean time value for metabolism of prodrug in the cecum. This difference value

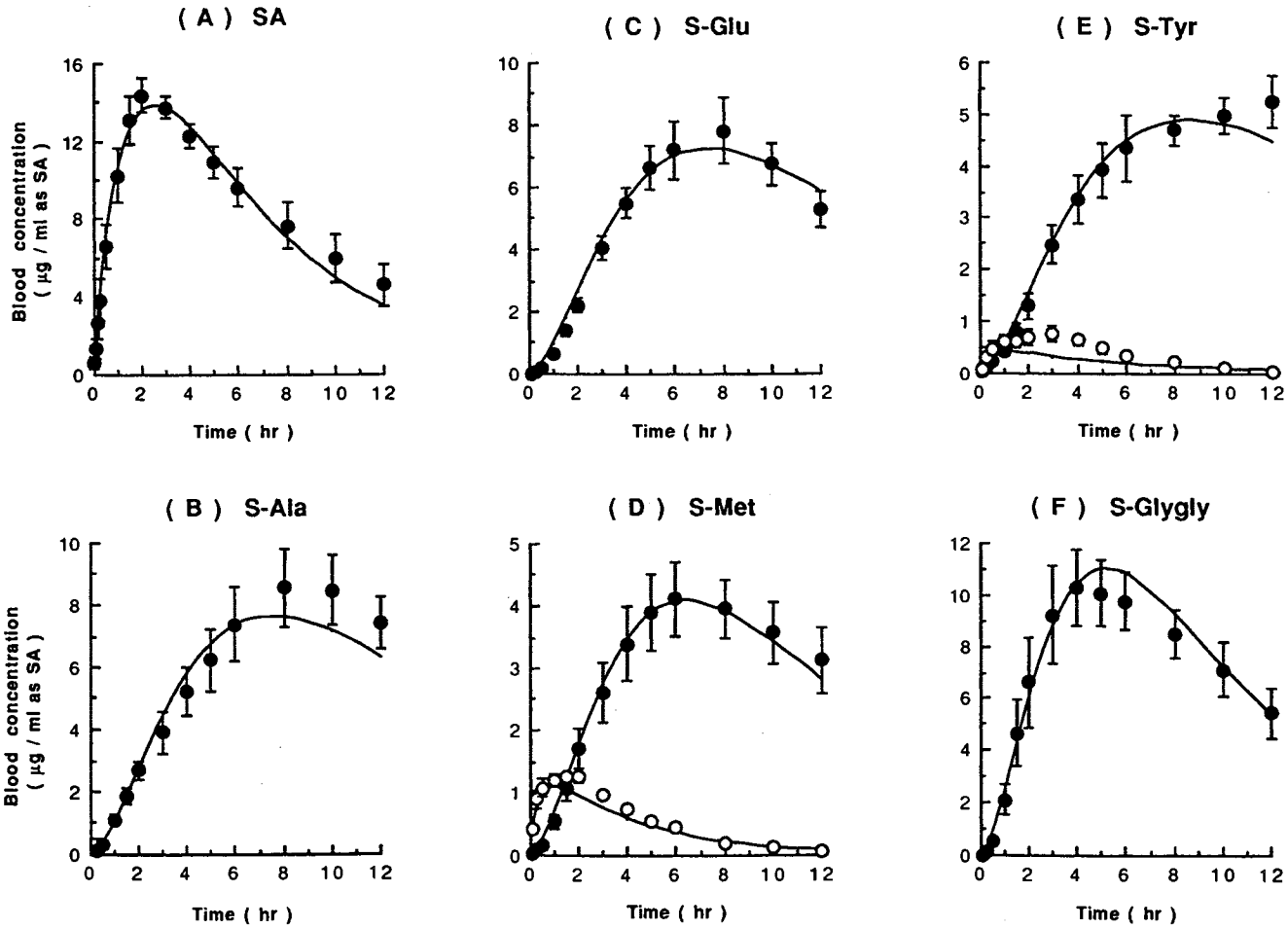

Fig. 2. Blood concentration profiles of prodrug (○) and SA (●) following intracecal administration of SA (A), S-Ala (B), S-Glu (C), S-Met (D), S-Tyr (E) and S-Glygly (F) (36 μmol/kg) to rabbits. Each point represents the mean ± SE of at least four experiments. Curves show simulated functions obtained based on the parameters shown in Tables II and IV.

Table IV. Pharmacokinetic Parameters of SA and Prodrugs Following Intracecal and Oral Administration to Rabbits

Compound	k_a (hr^{-1})	K_a' (hr^{-1})	k_m (hr^{-1})	F_{ic}	K_a (hr^{-1})	k_t (hr^{-1})	t_0 (hr)	F_{po}
SA	0.68	—	—	1	—	—	—	—
S-Ala	(0.68)	0	0.16	1	0.35	0.11	0.37	1
S-Glu	(0.68)	0	0.15	0.92	0	0.17	1.1	1
S-Met	(0.68)	0.14	0.11	1	0.22	0.036	3.4	0.70
S-Tyr	(0.68)	0.028	0.089	1	0.054	0.041	0.0041	1
S-Glygly	(0.68)	0	0.44	1	0	0.10	2.5	0.77

ranged from 1.6 to 13.0 hr among prodrugs, indicating that metabolism rates of prodrugs differ greatly in the cecum.

In the case of S-Glu and S-Glygly, AUC_{po} values for the prodrug were zero, while those for SA were larger than for the other prodrugs. This result suggests that S-Glu and S-Glygly are potent prodrugs of SA. The AUC_{po} value for S-Met was the largest among the prodrugs, reflecting its high lipophilicity compared to other prodrugs (partition coefficient between chloroform and 0.1 *N* HCl: 5.5). Partition coefficient values for S-Ala, S-Glu, S-Tyr, and S-Glygly were determined to be 0.46, 0.03, 0.13, and 0.05, respectively. Accordingly, the overall absorption and metabolism processes can be evaluated stochastically with moment parameters. In particular, AUC_{po} and MRT_{po} for the profile of SA following oral administration of prodrug can be appropriate parameters for roughly evaluating the usefulness of prodrugs.

Analysis of the Blood Concentration Profile Based on a Pharmacokinetic Model

The mean blood concentration profile of SA following its intracecal administration was well fitted to Model 2 (Fig. 2A). k_a was calculated to be 0.675 hr^{-1} (Table IV).

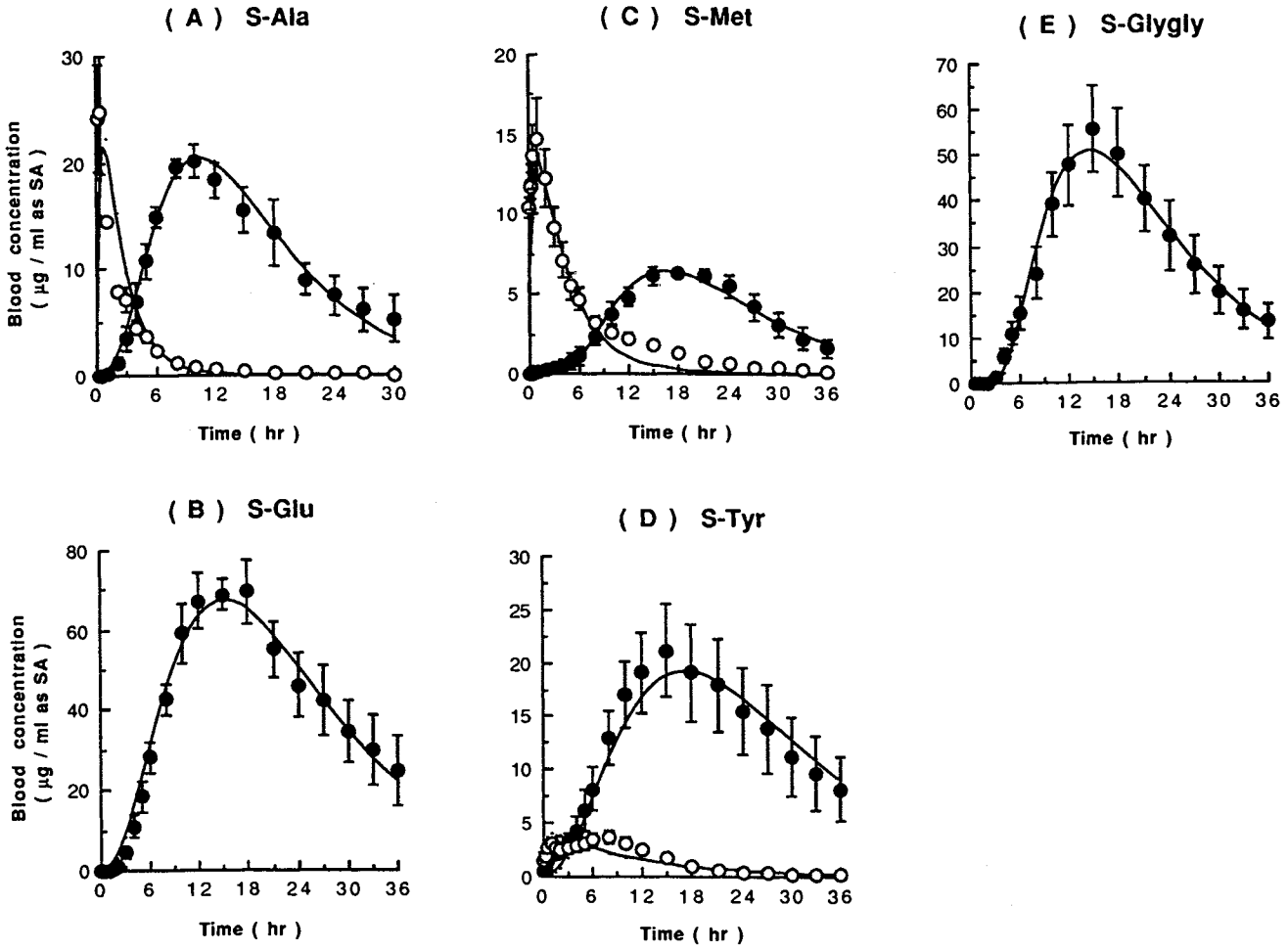

Fig. 3. Blood concentration profiles of prodrug (○) and SA (●) following oral administration of S-Ala (A), S-Glu (B), S-Met (C), S-Tyr (D), and S-Glygly (E) (434 μmol/kg) to rabbits. Each point represents the mean ± SE of at least four experiments. Curves show simulated functions obtained based on the parameters shown in Tables II and IV.

The mean blood concentration profiles of prodrug itself and released SA following intracecal administration of prodrug were simultaneously fitted to the Laplace-transformed equations derived from Model 3. As shown in Figs. 2B–F, the fitted lines agreed well with the experimentally observed data for every prodrug, suggesting the validity of this model.

Calculated pharmacokinetic parameters are given in Table IV. For S-Ala, S-Glu, and S-Glygly, unchanged prodrug absorption was undetectable, i.e., $K_a' = 0$. The K_a' value of S-Met was the largest among the prodrugs, partly because of its relatively high lipophilicity. On the other hand, the k_m value was the largest for S-Glygly, as expected from the high hydrolytic activity of cecal contents against S-Glygly to SA measured *in vitro* (1–4). Because prodrug hydrolysis to SA was inhibited in rabbits pretreated with kanamycin sulfate, the intestinal microorganisms were thought to account for the biotransformation of these prodrugs (1,2,10). Therefore, the k_m might correspond to the metabolic rate constant of prodrug conversion to SA caused by cecal microorganisms.

We previously distinguished the species differences in the ability of intestinal microorganisms to hydrolyze salicyluric acid (SA–glycine conjugate; SU) to SA, by its incubation with normal feces from rats, rabbits, and humans (6). Recently, SU-hydrolyzing enzyme purified from rabbit intestinal microorganisms was reported to catalyze the hydrolysis of *N*-benzoyl amino acids and their derivatives (11), suggesting that other amino acid or dipeptide conjugates of SA were also hydrolyzed to SA in humans.

Finally, the mean blood concentration profiles of prodrug itself and appeared SA following oral administration of prodrugs were simultaneously fitted to the Laplace-transformed equations derived from Model 4. In general, good agreement was observed between fitted lines and experimentally observed data in every prodrug as shown in Fig. 3, suggesting that this pharmacokinetic model and its analysis are appropriate. Pharmacokinetic parameters of the prodrugs (Table IV) differed considerably among each other. In the case of S-Glu and S-Glygly, absorption of prodrug itself from the gastrointestinal tract was negligible ($K_a = 0$), similar to intracecal administration. Since the AUC_{po} values for the profile of released SA following oral administration of S-Glu and S-Glygly were larger than those for other prodrugs (Table III), a reduction in K_a leads to enhanced bioavailability of SA.

In the present compartment model analysis, k_m and K_a are considered the most important parameters for evaluating the usefulness of a prodrug depending on metabolism in the intestinal microorganisms, because these parameters clearly characterize the difference in the rate of metabolism and absorption of prodrugs. Furthermore, k_m and K_a might correspond to the difference between the MRT_{ic} value for SA following its intracecal administration and that for prodrug and the difference between MRT_{po} and MRT_{iv} values for prodrug respectively. In contrast to statistical moment analysis, this pharmacokinetic model could allow us to dissect the *in vivo* behavior of prodrugs into multiple-component processes with parameters reflecting each of them.

In conclusion, this approach for the pharmacokinetic properties of prodrugs could improve the design of more potent prodrugs depending on metabolism in the intestinal microorganisms.

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REFERENCES

1. J. Nakamura, C. Tagami, K. Nishida, and H. Sasaki. Development of a prodrug of salicylic acid, salicylic acid-L-alanine conjugate, utilizing hydrolysis by rabbit intestinal microorganisms. *J. Pharm. Pharmacol.* 44:295–299 (1992).
2. J. Nakamura, K. Asai, K. Nishida, and H. Sasaki. A novel prodrug of salicylic acid, salicylic acid-glutamic acid conjugate utilizing hydrolysis in rabbit intestinal microorganisms. *Chem. Pharm. Bull.* 40:2164–2168 (1992).
3. J. Nakamura, M. Kido, K. Nishida, and H. Sasaki. Hydrolysis of salicylic acid-tyrosine and salicylic acid-methionine prodrugs in the rabbit. *Int. J. Pharm.* 87:59–66 (1992).
4. J. Nakamura, K. Asai, K. Nishida, and H. Sasaki. A novel prodrug of salicylic acid, salicylic acid-glycylglycine conjugate, utilizing the hydrolysis in rabbit intestinal microorganisms. *J. Pharm. Pharmacol.* 44:713–716 (1992).
5. H. G. Boxenbaum, G. S. Jodhka, A. C. Ferguson, S. Riegelman, and T. R. MacGregor. The influence of bacterial gut hydrolysis on the fate of orally administered isonicotinuric acid in man. *J. Pharmacokinet. Biopharm.* 2:211–237 (1974).
6. J. Shibasaki, Y. Inoue, K. Kadosaki, H. Sasaki, and J. Nakamura. Hydrolysis of salicyluric acid in rabbit intestinal microorganisms. *J. Pharmacobio-Dyn.* 8:989–995 (1985).
7. K. Yamaoka, T. Nakagawa, and T. Uno. Statistical moments in pharmacokinetics. *J. Pharmacokinet. Biopharm.* 6:547–558 (1978).
8. K. Yamaoka, Y. Tanigawara, T. Nakagawa, and T. Uno. A pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharmacobio-Dyn.* 4:879–885 (1981).
9. Y. Yano, K. Yamaoka, and H. Tanaka. A nonlinear least squares program, MULTI(FILT), based on fast inverse Laplace transform for microcomputers. *Chem. Pharm. Bull.* 37:1035–1038 (1989).
10. J. Nakamura, M. Kido, K. Nishida, and H. Sasaki. Effect of oral pretreatment with antibiotics on the hydrolysis of salicylic acid-tyrosine and salicylic acid-methionine prodrugs in rabbit intestinal microorganisms. *Chem. Pharm. Bull.* 40:2572–2575 (1992).
11. S. Ogushi, H. Watanabe, M. Nakayama, and D. Tsuru. Salicylurate-hydrolyzing enzyme from intestinal bacterium in rabbit: Purification and characterization. *Chem. Pharm. Bull.* 36:4539–4546 (1988).