

## Title

### Report

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

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## Abstract

We analyzed the pharmacokinetics of salicylic acid (SA)-amino acid (alanine, glutamic acid, methionine and tyrosine) or -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 behaviour 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) conjugate 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 mmol/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 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 denoted 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 that 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 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 MULTI program. Then  $k_a$  was 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 personal computer (NEC PC-9801 VX). In this case, the availability of intracecally administered prodrug ( $F_{ic}$ ) is the ratio of 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.

## RESULTS AND DISCUSSION

**Intravenous Administration of SA or Prodrug** Pharmacokinetic parameters of 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  $MRT_{ic}$  values of SA following its intracecal administration and that of prodrug might correspond to the mean time value for metabolism process of prodrug in the cecum. This difference value 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 of SA were larger than those of 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 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 of 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 evaluating roughly the usefulness of prodrugs.

**Analysis of 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. 2 (A)).  $k_a$  was calculated to be  $0.675 \text{ hr}^{-1}$  (Table 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 Fig. 2(B)-(F), the fitted lines agreed well with the experimentally observed data for every prodrug, suggesting the validity of this model.

Calculated pharmacokinetic parameters are shown in Table IV. As to 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 accord for the biotransformation of these prodrugs (1,2,10). Therefore,  $k_m$  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 faeces from rats, rabbits and man (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 was also hydrolyzed to SA in man.

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 of other prodrugs (Table III), a reduction of  $K_a$  leads to the enhancement of 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  $MRT_{ic}$  values of SA following its intracecal administration and that of prodrug, and the difference between  $MRT_{po}$  and  $MRT_{iv}$  values of prodrug, respectively. In contrast to statistical moment analysis, this pharmacokinetic model could allow us to discuss the in vivo behaviour 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 the 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).

## Figure captions

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). Explanation of symbols are as follows:

Model 1: X1, drug in central compartment; X2, drug in peripheral compartment; K12 and K21, transfer rate constant; Kel, elimination rate constant

Model 2: X1, SA in cecum compartment; X2, SA in central compartment; X3, SA in peripheral compartment; ka, absorption rate constant of SA from cecum; k12 and k21, transfer rate constant of SA; kel, elimination rate constant of SA

Model 3: X1, prodrug in cecum compartment; X2, prodrug in central compartment; X3, prodrug in peripheral compartment; X4, SA in cecum compartment; X5, SA in central compartment; X6, SA in peripheral compartment; Ka', absorption rate constant of prodrug from cecum; K12 and K21, transfer rate constant of prodrug; Kel, elimination rate constant of prodrug; km, metabolism rate constant of prodrug; ka, absorption rate constant of SA from cecum; k12 and k21, transfer rate constant of SA; kel, elimination rate

constant of SA

Model 4: X1, prodrug in gut compartment; X2, prodrug in central compartment; X3, prodrug in peripheral compartment; X4, prodrug in cecum compartment; X5, SA in cecum compartment; X6, SA in central compartment; X7, 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 X1 to X4 compartment.

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 mmol/kg) to rabbits. Each point represents the mean  $\pm$  S.E. of at least four experiments. Curves show simulated functions obtained based on the parameters shown in Tables II and 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 mmol/kg) to rabbits. Each point represents the mean  $\pm$  S.E. of at least four experiments. Curves show simulated functions obtained based on the parameters shown in Tables II and IV.