COMPARISON OF THE FIRST-PASS METABOLISM OF ETHENZAMIDE AND SALICYLAMIDE IN RATS

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The first-pass metabolism of ethenzamide (ETB), which is considered to be a pro-drug of salicylamide (SAM), was studied in rats in order to compare with that of SAM. The extent of first-pass metabolism after oral ETB estimated by oral and intravenous administration was as much as 82% and remaining 18% ETB entered into systemic circulation. Further, it has been confirmed that the first-pass metabolism of ETB is not due to the intestine but entirely due to the liver in which de-ethylation to SAM occurred at first, followed by exclusive conjugation with sulfuric acid and little SAM enters into the systemic circulation. On the other hand, it was previously demonstrated in rats that *per se* administered SAM is subjected to first-pass metabolism both in the gut and liver as much as about 60% and the remaining 40% entered the systemic circulation. Thus, the behavior of SAM is considerably different depending upon whether it is *per se* administered or formed from ETB through metabolism.

The fact that little SAM appears in the systemic circulation after oral administration of ETB suggests that the pharmacological activity of ETB comes from its own attributes but not from SAM.

Keywords — first-pass metabolism; ethenzamide; salicylamide; salicylamide sulfate; salicylamide glucuronide; prodrug; intestinal metabolism; hepatic extraction ratio; biliary excretion

INTRODUCTION

Some studies on first-pass effect of salicylamide (SAM) have been published previously.^{1–3)} We reported that oral SAM was submitted to first-pass metabolism as much as 95% in rabbits and about 60% in rats, respectively, for which gut wall and liver contribute significantly in both animals.⁴⁾ There were also some studies on the disposition of ethenzamide (ETB) which may be considered as a prodrug of SAM^{5–8)} but no study paying the attention to its first-pass metabolism has been published.

In this study, we attempt to compare the first-pass metabolism of ETB with that of SAM in rats and to obtain some information evaluating ETB as a prodrug of SAM. Overall first-pass metabolism of ETB was estimated by the blood levels of ETB following oral and intravenous ad-

ministration. Further, each contribution of intestine and liver to first-pass metabolism of ETB was investigated by using respective techniques involving surgical procedures.

MATERIALS AND METHODS

Animals — Male Wistar rats weighing 270–330 g were used. The animals were fasted for 18 h prior to the experiments but had free access to water.

Materials — ETB and SAM were of pharmaceutical grade. ETB was purchased from Kongo Kagaku Co., Ltd. (Toyama, Japan) and SAM from Yoshitomi Seiyaku Co., Ltd. (Osaka, Japan). All other chemicals were of reagent grade.

Intravenous and Oral Experiments — ETB solution for intravenous and oral administration

was prepared by dissolving 200 mg ETB in 10 ml of the mixture of propylene glycol, polyethylene glycol 400, and water (3:3:4), which was applied to animals as much as 0.5 ml. The procedures for drug administration, blood sampling, and urine collection were the same as described previously.⁴⁾

Intestinal Experiments — The procedures were the same as reported previously⁴⁾ except that closed intestinal sac was employed as intestinal preparation. Both sides of midileal portion of intestine about 5 cm long were carefully cut and ligated to make a closed sac, in which 2 ml of 10 mg% ETB solution (ETB was dissolved in the pH 7.2 buffer⁹⁾ consisted of the following salts, in mmol per 1: NaCl 145, KCl 4.56, CaCl₂ 1.25, Na₂HPO₄ 1.33 and NaH₂PO₄ 0.33) were injected.

Hepatic Experiments — Simultaneous sampling of portal and hepatic venous blood in the rat following administration of ETB and SAM was performed by the surgical method reported by Suzuki *et al.*¹⁰⁾

Bile Collection — A rat was anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*) and placed in supine position. By making appropriate incision, a polyethylene cannula (0.97 mm OD, Intramedic) was inserted in the common bile duct approximately 1 cm above its entry into the duodenum, through which bile was collected.

Analytical Method — ETB, SAM, SAM glucuronide (SAMG), and SAM sulfate (SAMS) were separately quantitated from blood and bile by the fluorometric assay method. The latter three compounds were estimated as described in the previous paper.⁴⁾ SAM and ETB were together extracted from the biological samples mixed with 0.1 M acetate buffer (pH 5.0) into ethylene dichloride. After reextracting SAM into 0.1 M carbonate buffer (pH 11), ETB was estimated from ethylene dichloride layer. The organic layer was evaporated to dryness and the resulting residue was extracted into aqueous solution saturated with sodium chloride by shaking. After centrifugation, the fluorescence of ETB in

aqueous solution was determined at the activation and emission wavelengths of 295 and 355 nm, respectively. Sodium chloride was proved to be of use for preventing the solution from a milky turbidity possibly due to lipid components in the blood by its salting-out effect. With respect to urine samples following administration of ETB, only SAMG and SAMS were determined since ETB and SAM were negligibly small.

Calculation of AUC — The AUC was calculated by trapezoidal rule for the observed blood levels from 0 to 7 h after administration. The AUC from 7 h to infinity was negligible.

RESULTS

Overall First-Pass Metabolism

In our previous works,⁴⁾ overall first-pass metabolism of SAM in rats was investigated in doses of 10 and 30 mg/head. In this work, ETB disposition was observed under the dose of 10 mg/head for the comparison of SAM. AUC of ETB, SAM, SAMG, and SAMS and urinary recoveries of the conjugates for 7 h were listed in Table I and the comparison of time course of ETB blood levels after intravenous and oral administration are shown in Fig. 1. Among the conjugates excreted in the urine, SAMS was exclusively large. In the blood, ETB and SAMS were the major drugs recovered and SAM and SAMG were much less. It is noticed that urinary recoveries are below one-half of the administered drug in both routes of administration. When urine collections were made up to 48 h, the recoveries were still about 50% in common with the both routes of administration. This may indicate that intestinal absorption of ETB after oral administration is rather good and in addition, the results of intestinal experiments described below present another evidence of good intestinal absorption of ETB. Thus, the extent of first-pass metabolism (f) could be estimated by the following Eq. 1:

$$f = 1 - \frac{AUC_{po}}{AUC_{iv}} \quad (1)$$

TABLE I. *AUC and Urinary Recoveries for Ethenzamide (ETB), Salicylamide (SAM), Salicylamide Sulfate (SAMS), and Salicylamide Glucuronide (SAMG) after Intravenous and Oral Administration of 10 mg/head Dose of Ethenzamide in Rats^{a)}*

Route	<i>AUC (mg% · min)^{b)}</i>				<i>Urinary recovery^{b)}</i>		
	ETB	SAM	SAMS	SAMG	SAMS (mg)	SAMG (mg)	% of dose
<i>i.v.</i>	402.3 ± 72.1	2.4 ± 1.3	48.2 ± 17.3	2.4 ± 1.0	4.4 ± 0.3	0.2 ± 0.0	45.5 ± 3.2 ^{c)}
<i>p.o.</i>	72.8 ± 20.1	0.2 ± 0.1	41.0 ± 8.9	1.4 ± 0.8	3.6 ± 0.4	0.1 ± 0.0	36.8 ± 4.5 ^{c)}

a) Results are expressed as the mean ± S.E. from four animals. SAM, SAMS, and SAMG are expressed in term of ETB.

b) From 0 to 7 h.

c) Not different significantly.

FIG. 1. *The Concentrations of ETB in Blood after Intravenous and Oral Administration of 10 mg/head Dose of ETB in Rats*

—○—: intravenous, —●—: oral.

Each point represents the mean of four animals and the vertical bar indicates the standard error.

where AUC_{po} and AUC_{iv} are AUC for ETB after oral and intravenous administration, respectively. Value of f estimated for ETB was 82%. This presystemic loss, contrary to our expectation, was larger than 60% for SAM which was determined in the previous studies.⁴⁾

Intestinal Metabolism

The appearance of ETB and its metabolites into the mesenteric blood was measured after injecting ETB solution into middle portion of the intestine and the data are shown in Table II. About 75% of the drug injected appeared unchanged within 90 min and other metabolites were not detected indicating that ETB is well absorbed and de-ethylation in gut wall is negligible, if any.

Hepatic Metabolism

ETB and its metabolites in the blood samples collected simultaneously from the portal and hepatic vein at various intervals after intravenous and oral administration of ETB were determined. The patterns of their time course following intravenous and oral administration were similar except that the concentrations after oral administration were much lower than those after intravenous administration. Results after intravenous administration were shown in Fig. 2. ETB levels in the hepatic venous blood were always lower than those in the portal venous blood. Among the metabolites, SAMS was only detected significantly, being the levels in the hepatic venous blood consistently higher than those in the portal venous blood. These facts indicate that the liver is responsible not only for deethylation of ETB but also for successive sulfo-conjugation of SAM. Recently, high activity of

the enzyme for *in vitro* de-ethylation of ETB has been demonstrated in the rat liver microsomes.⁷⁾ Here, hepatic extraction ratios (E_H) of ETB were calculated by the following Eq. 2:

$$E_H = \frac{C_p - C_h}{C_p} \quad (2)$$

were C_p and C_h are the concentrations in the portal and hepatic venous blood, respectively. E_H values decreased appreciably with increasing C_p as shown in Fig. 3. The f value of 82% for ETB described above corresponds possibly to the E_H values of 60–90% at C_p less than 10 $\mu\text{g/ml}$, since the blood concentrations of ETB after oral administration of 10 mg/ head ETB are consistently below 10 $\mu\text{g/ml}$.

For the comparison, the function of liver was also examined after intravenous administration of 10 mg/ head SAM in rats. SAM, SAMS, and SAMG were detectable in the portal venous and hepatic venous blood as shown in Fig. 4. The facts that C_h is lower than C_p in SAM and the reverse is in SAMS indicate finely that SAM is conjugated with sulfuric acid to form SAMS in

the liver. E_H values for SAM calculated by Eq. 2 are ranged from 40 to 60% showing a somewhat dependency on C_p . In the previous paper⁴⁾ extents of first-pass metabolism in the intestine (f_i) and liver (f_h) after oral administration of 10 mg/head SAM in rats were estimated as 0.2 and 0.49, respectively, in which blood levels of SAM were consistently below 10 $\mu\text{g/ml}$. E_H can be calculated also by the following Eq. 3:

$$E_H = \frac{f_h}{1 - f_i} \quad (3)$$

E_H value for SAM estimated by Eq. 3 becomes 61%, which is in accord with E_H obtained directly by the present hepatic experiments. SAMG levels in both venous blood were also shown in Fig. 4. Within the samples collected at the same time, C_h is rather lower than C_p , suggesting the possibility that the elimination of SAMG surpasses the formation in the liver. Biliary excretion might be the most probable explanation for this hepatic decrease of SAMG. Concurrently, the increase of SAMG levels was also recognized in both venous blood from 5 to 6 min

TABLE II. Amounts of Ethenzamide (ETB), Salicylamide (SAM), Salicylamide Sulfate (SAMS), and Salicylamide Glucuronide (SAMG) Appearing in the Mesenteric Blood in a Rat Intestinal Preparation Filled with 2 ml of 10 mg% ETB Solution^{a)}

Collection time (min)	Amounts (μg) of			
	ETB	SAM	SAMS	SAMG
0–10	5.3	0	0	0
10–20	32.3	0	0	0
20–30	30.0	0	0	0
30–40	28.6	0	0	0
40–50	16.4	0	0	0
50–60	14.3	0	0	0
60–75	13.6	0	0	0
75–90	6.3	0	0	0
Total	146.8	0	0	0
Remainder in intestine	17.5	0	0	0
Total recovery	164.3	0	0	0

a) Contains 200 μg of ETB.

after administration. This may be considered as an evidence supporting the fact that SAMG is formed in organs other than liver. Such hepatic behaviors of SAMG would be invisible after administration of ETB, since then, the formation of SAMG is too small.

Biliary Excretion

In order to assess the behavior of SAMG in the liver, biliary excretion in the rats receiving

oral ETB and SAM was investigated and the results are shown in Table III. Total amount of biliary excretion is very poor for both drugs (below 1% of the doses), which seems likely from a consideration of their small molecular weights. However, the excretion of SAMG following administration of SAM is notably much, being consistent with the hepatic movement of SAMG described above.

DISCUSSION

The inactivity of the intestine on ETB de-ethylation was clarified in the present studies and it was in agreement with the recent findings of Klipper *et al.*¹¹⁾ who found that phenacetin is not de-ethylated in the gut wall of rats. On the other hand, high activity of rat liver for de-ethylation of ETB was demonstrated in the pres-

FIG. 2. Comparison of Portal and Hepatic Venous Blood Concentrations of ETB and SAMS Following Intravenous Administration of 10 mg/head Dose of ETB in Rats

—○—: ETB in portal venous blood,
 - - -●- - -: ETB in hepatic venous blood,
 —□—: SAMS in portal venous blood,
 - - -■- - -: SAMS in hepatic venous blood.

Each point represents the mean of three animals and the vertical bar indicates the standard error.

FIG. 3. Plots of Hepatic Extraction Ratio of ETB versus ETB Concentration in Portal Venous Blood Following Administration of Various Doses of ETB in Rats

The doses of ETB used in this series of experiments were intravenous 2 and 10 mg/head and oral 10 mg/head.

ent studies, which is in accord with *in vitro* high activity of rat liver microsomes reported by Lin *et al.*⁷⁾ The lack of hepatic glucuronidation for SAM and the existence of biliary excretion of SAMG in rats which had been predicted in our previous studies⁴⁾ were more directly demonstrated by the present studies.

Comparisons of urinary recoveries after administration of ETB and SAM⁴⁾ administration are summarized in Fig. 5. It is noticed that SAMG formation is extremely smaller after ETB administration than after SAM administration and the same tendency is also observed in the comparison of *AUC*. These facts might support the consideration that ETB is de-ethylated only in the liver regardless of the routes of administration and SAM thus formed is completely conjugated in the same organ without moving

elsewhere and consequently, formed little SAMG, while *per se* administered SAM, in contrast, might have chance to contact not only with the liver but also with the tissues having activity for glucuronidation. It seems likely that SAM which is formed from ETB is in the site where the enzyme system for sulfonation is also present, while SAM which is administered *per se* and appeared in the liver is apart from the site and the rate of diffusing there is considerably low. One decade ago, we¹²⁾ found the similar behavior in hydroxymethyl-tolbutamide, a metabolite of tolbutamide, depending on whether it is administered *per se* or formed from tolbutamide through metabolism, the latter being more susceptible to further oxidation yielding carboxy-tolbutamide.

Here, we cannot but consider that the pharmacological activity of ETB^{13,14)} comes from its own attribute but not from SAM, since little

FIG. 4. Comparison of Portal and Hepatic Venous Blood Concentrations of SAM, SAMS, and SAMG Following Intravenous Administration of 10 mg/head Dose of SAM in Rats

—○—: SAM in portal venous blood,
 - -●- - -: SAM in hepatic venous blood,
 —□—: SAMS in portal venous blood,
 - -■- - -: SAMS in hepatic venous blood,
 —△—: SAMG in portal venous blood,
 - -▲- - -: SAMG in hepatic venous blood.

Each point represents the mean of three animals and the vertical bar indicates the standard error.

FIG. 5. Schematic Comparison of the Amounts of Urinary SAMG and SAMS Following Intravenous and Oral Administration of 10 mg/head Dose of ETB and SAM in Rats

□: intravenous, ■: oral.

Each column represents the mean $\pm$ S.E. of three to four animals.

TABLE III. *Biliary Excretion Following Oral Administration of 10 mg/head Dose of Ethenzamide (ETB) and Salicylamide (SAM) in Rats^{a)}*

Compounds administered	Amounts excreted					
	ETB (μg)	SAM (μg)	SAMS (μg)	SAMG (μg)	Total (μg)	% of dose
ETB ^{b)}	4.7	0	2.7	0	7.4	0.07
SAM ^{b)}	—	0	13.3	36.2	49.5	0.50

a) Estimated for the bile collected for 2 h after administration. b) Mean from two animals.

SAM enters into the circulation after administration of ETB. In other word, ETB is not a prodrug of SAM pharmacologically, although it is certainly prodrug from the stand point of biotransformation. Similar consideration was earlier presented for phenacetin by Conney *et al.*¹⁵⁾ who stated that antipyretic activity of the drug is not dependent on metabolism to acetaminophen but due to itself.

Finally, it must be added that the incomplete urinary recoveries after ETB administration is a problem to be more precisely examined. Further, there have been conflicting data in the literature concerning this subject: Davison *et al.*⁵⁾ found incomplete urinary recovery in rats receiving intravenous ETB as in the present studies and they suggested the existence of unidentified metabolites. On the contrary, Lin *et al.*⁷⁾ have recently presented the data indicating almost complete urinary recovery within 24 h after intravenous dose of ETB.

REFERENCES

- 1) S. Riegelman and M. Rowland: Effect of route of administration on drug disposition, *J. Pharmacokinet. Biopharm.*, **1**, 419–434 (1973).
- 2) R. Gugler, P. Lain, and D. L. Azarnoff: Effect of portacaval shunt on the disposition of drugs with and without first-pass effect, *J. Pharmacol. Exp. Ther.*, **195**, 416–423 (1975).
- 3) J. B. Houston and G. Levy: Effect of route of administration on competitive drug biotransformation interaction: Salicylamide-ascorbic acid interaction in rats, *J. Pharmacol. Exp. Ther.*, **198**, 284–294 (1976).
- 4) J. Shibasaki, R. Konishi, M. Koike, A. Imamura, and M. Sueyasu: Some quantitative evaluation of first-pass metabolism of salicylamide in rabbit and rat, *J. Pharm. Dyn.*, **4**, 91–100 (1981).
- 5) C. Davison, J. Wagner, and P. K. Smith: On the metabolism of *o*-ethoxybenzamide, *J. Pharmacol. Exp. Ther.*, **136**, 226–231 (1962).
- 6) H. Nogami, M. Hanano, S. Awazu, and H. Moon: Pharmacokinetic analysis on the disappearance of ethoxybenzamide from plasma: Statistical treatment of data of two compartmental model by a digital computer, *Chem. Pharm. Bull.*, **17**, 2097–2104 (1969).
- 7) J. H. Lin, M. Hayashi, S. Awazu, and M. Hanano: Correlation between *in vitro* and *vivo* drug metabolism rate: Oxidation of ethoxybenzamide in rat, *J. Pharmacokinet. Biopharm.*, **6**, 327–337 (1978).
- 8) K. Kigasawa, M. Hiiragi, and T. Hayashi: Biopharmaceutical studies of drugs. III. Serum concentration level of *o*-ethoxybenzamide and *N*-(hydroxybutyryl)-*p*-phenetidine after simultaneous administration to rabbit, *Yakugaku Zasshi*, **101**, 556–566 (1981).
- 9) L. S. Schanker and D. J. Tocco: Active transport of some pyrimidines across the rat intestinal epithelium, *J. Pharmacol. Exp. Ther.*, **128**, 115–121 (1960).
- 10) T. Suzuki, T. Ohkuma, T. Rikihisa, and S. Isozaki: Improved method for sampling hepatic venous blood in the rat, *J. Pharm. Sci.*, **64**, 895–897 (1975).
- 11) P. J. M. Klippert, R. J. J. Littel, and J. Noordhoek: *In vivo* *O*-de-ethylation of phenacetin in 3-methylcholanthrene-pretreated rats: Gut wall and liver first-pass metabolism, *J. Pharmacol. Exp. Ther.*, **225**, 153–157 (1983).
- 12) J. Shibasaki, R. Konishi, T. Morishita, and T. Ueki: Drug absorption, metabolism, and excretion. IX. Some new aspects of pharmacokinetics on tolbutamide in rabbits, *Chem. Pharm. Bull.*, **21**, 1754–1763 (1973).
- 13) E. M. Bavin, F. J. Macrae, D. E. Seymour, and P. D. Waterhouse: Analgesic and antipyretic properties of some salicylamide derivatives, *J. Pharm. Pharmacol.*, **4**, 872–878 (1952).
- 14) M. Carron, J. Tasbart, and M. Jullien: The analgesic properties of some substituted salicylamide derivatives, *Therapie*, **7**, 27–36 (1952).
- 15) A. H. Conney, M. Sansur, F. Soroko, R. Koston, and J. J. Burns: Enzyme induction and inhibition in studies on the pharmacological actions of a acetophenetidin, *J. Pharmacol. Exp. Ther.*, **151**, 133–138 (1966).