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THE ROLE OF INTESTINAL FLORA IN METABOLISM OF PHENOLIC SULFATE ESTERS

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Abstract—Arylsulfotransferase activity was found in the feces of human (14.74 ± 2.674) and rat (7.37 ± 1.126 μmol/hr/g wet feces). In the case of the rat, arylsulfotransferase activity was markedly and rapidly decreased by the treatment with antibiotics mixture, but restored to the original activity 3 weeks after stopping the administration of antibiotics. The excretion of the sulfate esters derived from *p*-nitrophenylsulfate was enhanced by the administration of acetaminophen but not by the treatment with antibiotics. Furthermore, in rats treated with antibiotics, inorganic sulfate excretion was severely decreased. When only acetaminophen was administered, the excretion of acetaminophen-*O*-sulfate showed a 10–15% decrease in rats treated with antibiotics compared with conventional rat.

Baumann [1] first showed that the formation of sulfate esters takes place in dogs fed on a diet containing phenol, and advanced the view that exogenous sulfate could augment the excretion of phenol as phenylsulfate. Dodgson *et al.* [2, 3] observed that these exogenous sulfates, such as PNS,* are eliminated via the biliary route, and PNS excreted into intestine was not decomposed by the intestinal flora. It has been shown that administration of sulfur-containing compounds such as cysteine, sodium sulfate and DMSO with phenolic compounds markedly increases excretion of the ester forms of sulfate into the urine [4]. Related to the metabolism of these phenolic compounds, the liver and intestinal epithelial cells are generally regarded as the target organs concerned.

However, we [5] recently discovered *Eubacterium* A-44, one of the predominent bacteria in human intestine, which produces arylsulfotransferase and reported the purification and characterization of the enzyme [6]. The bacterial arylsulfotransferase is found to be quite different in the substrate specificity from what had been reported from mammalian organs. Aside from the liver and extrahepatic tissues, therefore, intestinal flora may play an important role in the metabolism of phenolic compounds. The present study was undertaken to investigate the effects of intestinal flora on the formation and excretion of phenolic sulfate esters.

MATERIALS AND METHODS

Chemicals. (^{35}S) labeled PNS was synthesized according to the method of our previous report [6]. Acetaminophen was purchased from Wako Pure Chem. ACS II was from Amersham (U.S.A.). All other chemicals were of analytical reagent grade.

Animals. Male rats (SDD Wistar, body weight 180–220 g) were maintained on pellet food (Nippon Clea CE-2) and tap water *ad lib.* as one group of three to five rats. PNS (2 mg/rat) was injected intraperitoneally and acetaminophen (80 mg/kg rat) was orally administered. In the case of animals administered acetaminophen with PNS, acetaminophen was administered orally 30 min after the intraperitoneal injection of PNS. In the case of rats treated with antibiotics, an antibiotics mixture (chloramphenicol 17.5 mg, nystatin 5000 units, streptomycin 20 mg, erythromycin 10 mg and penicillin 2000 units) was administered orally once a day for 3 days before the experiments. The animals were placed in the metabolic cages that permitted separate collection of urine and feces.

Urine samples were collected 6, 11, 24 and 48 hr, and feces 24 and 48 hr, after the administration. The collected urine samples were made up to a known volume with distilled water. The feces samples were made up to a known volume with distilled water and homogenized. Each sample was immediately assayed for radioactivity; otherwise, it was stored in a freezer (−20°).

Assay of enzyme activity. Arylsulfotransferase activity was assayed under the following conditions; 0.1 ml of the enzyme solution was added to the assay mixture containing 0.29 ml of 20 mM tyramine, 30 μl of 50 mM PNS and 0.21 ml of 0.1 M Tris–HCl buffer, pH 8.0, and the mixture was incubated at 37° for 1 hr. The reaction was stopped by the addition of 1 N NaOH (0.4 ml) and the absorbance at 405 nm was measured. Arylsulfatase activity was measured under the assay condition of the above arylsulfotransferase activity without the addition of tyramine. Control was carried under the same assay condition as described above except that the substrate was added after stopping the reaction.

The enzyme solution was prepared as follows. Feces samples (0.5 g) of humans and rats were directly collected in test tubes. Then, each sample

* Abbreviations used: PNS, *p*-nitrophenylsulfate; DMSO, dimethylsulfoxide; MUS, 4-methylumbelliferylsulfate.

was made up to 5 ml with 0.1 M Tris–HCl buffer, pH 7.0, and homogenized. The homogenate was centrifuged at 1000 rpm. The resulting supernatant fluid was used as a crude enzyme solution.

Measurement of metabolite and radioactivity. Urine samples were analyzed by TLC (Kiesegel $60F_{254}$, Merck, F.R.G.) and HPLC (Gilson system, France). TLC was developed ascendingly with $BuOH/AcOH/H_2O$,25/4/10 (v/v), and the plate was cut at the intervals of 0.5 cm and mixed with 10 ml of ACS II. An aliquot (0.5 ml) of urine and feces homogenate was also mixed with 10 ml of ACS II. The radioactivity was determined by a liquid scintillation counter (Aloka LSC 671).

RESULTS AND DISCUSSION

Arylsulfotransferase and arylsulfatase activity in the feces of humans and rats

In the preliminary experiment, arylsulfotransferase and arylsulfatase activities in human and rat feces were assayed (Table 1). Both activities were present in all cases. These enzyme activities were higher and more deviated in human feces than in rat's, and arylsulfatase activity of rats was very low. These deviations in human feces seem to be due to two causes: one is the difference in these enzymes-producing flora of each individual, and the other is the dietary difference in the amounts of acceptor substrates of arylsulfotransferase.

Alteration of arylsulfotransferase activity by the administration of antibiotics

Arylsulfotransferase and arylsulfatase activities were assayed in rat feces after the administration of the antibiotics mixture (Fig. 1). Arylsulfotransferase activity was markedly decreased and arylsulfatase activity was diminished to undetectable amounts by the treatment. Water contents of feces of antibiotics-treated rats increased only 10% over those of conventional rats, and diarrhea was not observed in any rat treated with antibiotics. However, arylsulfotransferase activity increased daily and restored to the original value 3 weeks after the cease of the administration of antibiotics. Arylsulfatase activity restored in parallel with arylsulfotransferase activity.

Table 1. Arylsulfotransferase and arylsulfatase activities in human and rat feces

No.	Activity (μmol/hr/g wet feces)	
	Arylsulfotransferase	Arylsulfatase
Human feces		
1	18.1	1.87
2	16.2	2.65
3	15.9	0.70
4	13.0	1.43
5	10.5	0.57
Rat feces		
1	8.4	0.16
2	7.9	0.16
3	5.8	0.16

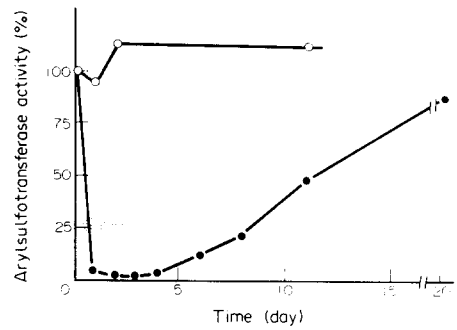

Fig. 1. Alternation of arylsulfotransferase in rat feces by the treatment with antibiotics. ▒ indicates the period of antibiotics administration; control group (N = 3), —○—; antibiotics-treated group (N = 3), —●—.

Effect of acetaminophen on urinary excretion of the sulfate esters

After the intraperitoneal injection of 2 mg of (^{35}S)PNS per rat, urine and feces were collected and their radioactivities were measured (Fig. 2). Radioactivity of (^{35}S)PNS administered was mostly excreted into urine and scarcely excreted into feces. When only PNS was administered, rats treated with antibiotics excreted more radioactivity into urine over the conventional rats but the difference was not significant. Since the number of intestinal bacteria decreased markedly by the administration of antibiotics, PNS excreted in the gut via the biliary route seems to be rapidly reabsorbed from intestine without decomposition and was excreted more into urine. Furthermore, when PNS was administered with acetaminophen, 94% of total radioactivity administered was excreted into urine of the conventional rats, whereas 62% was excreted in antibiotics-treated rats. That is, by the administration of a phenolic compound, urinary excretion of sulfate esters was significantly enhanced, probably because of functions of intestinal flora. Dodgson and Tudball [2]

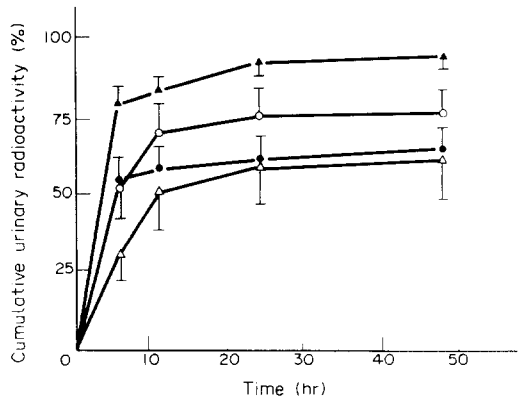

Fig. 2. Urinary excretion of (^{35}S) radioactivity recovery after the administration of (^{35}S)PNS with or without acetaminophen. The average area was expressed as mean ± SD. With acetaminophen (N = 5): control group, —▲—; antibiotics-treated group, —△—. Without acetaminophen (N = 3): control group, —●—; antibiotics-treated group, —○—.

showed that 72.6% of total radioactivity of (^{35}S)PNS administered was excreted within 48 hr, but our result obtained was 62.5%. The difference between them seems to be caused by the differences in amounts of some phenolic compounds containing in diet as well as amounts of PNS administered. Urinary excretion of radioactivity was not significantly affected by acetaminophen in antibiotics-treated rats. Urinary excretion of radioactivity was significantly increased in conventional rats administered PNS with acetaminophen than the other groups. Particularly, arylsulfotransferase activity in human feces might be influenced by phenolic compounds containing in diet, acceptor substrates of the enzyme as well as by the difference of microorganisms among individuals. The amounts of radioactivity excreted into feces were less than 1% of that administered. Dodgson and Tudball [2] also showed that the negligible amounts of radioactivity were observed in feces. Rhodes *et al.* [7] assayed fecal arylsulfatase activity, by using MUS as a substrate. However, our findings suggest that the sulfatase activity detected by Rhodes *et al.* is probably arylsulfotransferase activity, determined actually by using MUS as a donor substrate and some phenolic compounds containing in feces as acceptor substrates.

The effect of intestinal flora in sulfoconjugation of acetaminophen

Acetaminophen was administered orally to rats treated with or without antibiotics. Since acetaminophen was mainly excreted into urine, urinary acetaminophen-*O*-sulfate was determined by HPLC (Table 2). Acetaminophen-*O*-sulfate was mostly excreted within 11 hr and 72% of total acetaminophen was a sulfoconjugate form. A similar result has been observed in the conventional rats by Miller and Fisher [8] and Galinsky and Levy [9]. Urinary excretion % of acetaminophen-*O*-sulfate was significantly 10–15% higher in the conventional rats than those in antibodies treated rats, which shows that the intestinal flora enhance the excretion of acetaminophen-*O*-sulfate. It was suggested that intestinal flora play an important role in the detoxification of phenolic compounds such as acetaminophen which is known to cause hepatic necrosis.

When acetaminophen and (^{35}S)PNS were administered, the considerable amount of radioactivity excreted was (^{35}S)PNS, which was not metabolized, and (^{35}S)acetaminophen-*O*-sulfate, which was sulfoconjugated by arylsulfotransferase (Fig. 3). The proportion of the urinary PNS and acetaminophen-*O*-sulfate to total urinary radioactivity was lower, though total radioactivities excreted into urine were slightly higher, in conventional rats than in antibiotics treated rats. In the case of conventional rats, radioactivity observed near the origin of TLC was about 40% of that excreted in urine. One half of the radioactivity near the origin was reduced by the previous precipitation of urinary sample with $BaCl_2$. Inorganic sulfate excretion was remarkably decreased, by the treatment of antibiotics. When only PNS was administered, radioactivity detected near the origin, probably due to inorganic sulfate excretion was also decreased by the treatment of antibiotics. The finding that inorganic sulfate was increasingly excreted into urine in the conventional rats suggests phenylsulfate esters were hydrolyzed by arysulfate existing in the metabolic route from intestinal absorption to urinary excretion. Furthermore, these data show that a sulfate group of PNS was transferred to phenolic compounds, for example, to acetaminophen by bacterial arylsulfotransferase and that arylsulfate esters formed were more easily hydrolyzed by arylsulfatase than PNS was. At all events, besides the liver and extrahepatic tissues, intestinal flora producing arylsulfotransferase play the important role in the metabolism of phenolic compounds as sulfoconjugation.

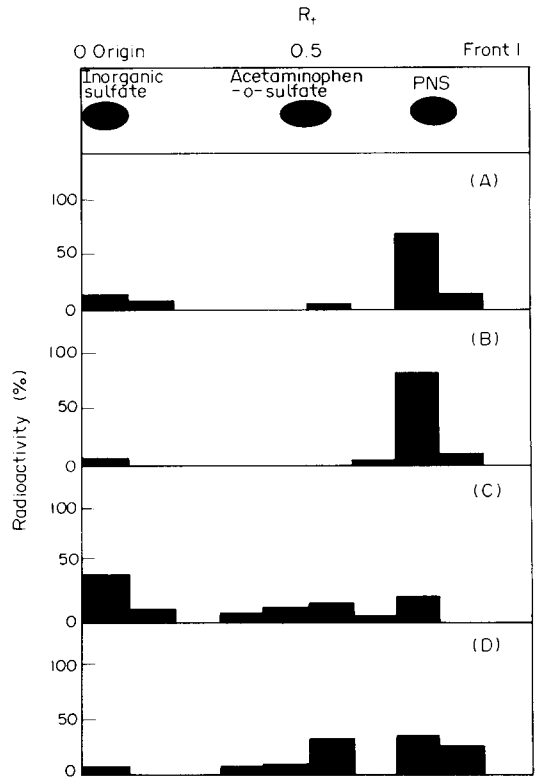

Fig. 3. TLC patterns of radioactivity excreted into urine. PNS only (conventional, (A); antibiotics-treated group, (B)) or PNS and acetaminophen (conventional rats, (C); antibiotics-treated group, (D)) were administered to rats and 11 hr-urine was collected and analyzed.

Table 2. Percentage of urinary acetaminophen-*O*-sulfate after oral administration of acetaminophen to rats

Group	Urinary acetaminophen sulfoconjugate*	
	after 6 hr	after 12 hr
Conventional (N = 3)	55.7 ± 5.72	70.5 ± 4.57
Antibiotics-treated (N = 3)	45.5 ± 2.94	56.6 ± 6.33

* Data are expressed as mean ± SD. Acetaminophen-*O*-sulfate was determined by HPLC (column, μ-MICROSORB C_{18}; solvent, 5% MeOH (0.05% TFA); wavelength, 280 nm).

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