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SEX DIFFERENCE IN RESPONSIVENESS TO AZTREONAM OF MONOOXYGENASE SYSTEM IN LIVER MICROSOMES FROM RATS

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Abstract—Effect of successive administration of Aztreonam on microsomal monooxygenase system was investigated in male and female Sprague–Dawley rats. The activities of benzphetamine *N*-demethylase, aminopyrine *N*-demethylase, *p*-nitroanisole *O*-demethylase and aniline hydroxylase in liver microsomes from male rats were decreased dose-dependently by Aztreonam. On the contrary, the activities in liver microsomes from female rats were slightly increased rather than decreased by the administration of Aztreonam. In addition, Aztreonam was found to decrease the specific content of microsomal cytochrome P-450 in male rats but not in female rats. The decreases in the activities observed in male rats were accompanied by a parallel decrease in the specific content of cytochrome P-450. Furthermore, the results of quantitation of P-450 (M-1), one of the male specific forms of cytochrome P-450, indicated that the administration of Aztreonam resulted in a dose-dependent decrease in the content of P-450 (M-1) in liver microsomes from male rats.

Aztreonam is a monobactam antibiotic product developed by E. R. Squibb and Sons, Inc. [1]. Different from conventional bicyclic penicillin and cephalosporin skeletons, Aztreonam has a new skeleton in which the position-1 of the lactam ring is substituted with a sulfonic group.

In a previous 5-week subcutaneous toxicity study of Aztreonam in rats, liver weights increased in male rats which received 300 mg/kg and over, and in female rats which received 1200 mg/kg and over. Furthermore, histopathological examination revealed hypertrophic hepatocytes in female rats which received 1200 mg/kg and both male and female rats which received 2400 mg/kg [2].

A variety of structurally unrelated foreign compounds including drugs and environmental chemicals are known to induce the hepatic microsomal monooxygenase [3–5]. Therefore, the present study was conducted to study whether the increases in liver weights observed after multiple doses of Aztreonam were accompanied by an increase in the activities of microsomal drug oxidation. We report herein that the responsiveness to Aztreonam of the liver microsomal monooxygenase system in male rats was considerably different from that in female rats and male dogs and monkeys.

MATERIALS AND METHODS

Chemicals. NADP, glucose 6-phosphate and glucose 6-phosphate dehydrogenase were purchased from the Oriental Yeast Co. Emulgen 913 and benzphetamine were provided by Kao Atlas Co., and the Upjohn Co., respectively. Aztreonam was donated from E. R. Squibb and Sons, Inc. Anti-P-450 (M-1) was a generous gift from Dr. T. Omura, Kyushu University. All other reagents were of the highest purity commercially available.

Animals and pretreatment. Male and female Sprague–Dawley rats were obtained at four weeks of age from the Sizuoka Laboratory Animal Breeders' Co., and were maintained on a commercial rat chow (CE-2 Nippon Clea Co., Japan). Rats received subcutaneous injection of Aztreonam dissolved in physiological saline once a day for 7 successive days. The concentrations of Aztreonam injected are as specified in the tables and figures. Last injections were conducted 24 hr prior to sacrifice. Rat liver microsomes were prepared as described elsewhere [6]. Portions of the liver were promptly removed upon necropsy of beagles in a 26-week i.v. toxicity study. A 40% Aztreonam solution was prepared by dissolving Aztreonam and arginine in distilled water for injection in a ratio of 1:0.78. Different volumes were administered to different groups once daily for 26 weeks. A 5.56% NaCl solution was given to the control. Male crab eating monkeys were pretreated with Aztreonam at doses specified in the figures, for 4 weeks (i.v.). For monkeys, a 25% Aztreonam solution was prepared and administered as in the case of dogs. The liver samples were processed in the same way as in the case of rats.

Incubations. Reaction mixtures contained 0.4–0.7 mg of microsomal protein, NADPH-generating system (0.33 mM NADP, 8 mM glucose 6-phosphate, 0.1 unit glucose 6-phosphate dehydrogenase and 6 mM $MgCl_2$), 0.1 mM ethylenediaminetetraacetic acid and 0.1 M K-phosphate

Table 1. Effect of Aztreonam on liver microsomal monooxygenase activities in male and female rats

Group	(mg/kg)	Benzphetamine *N*-demethylation	Aminopyrine *N*-demethylation	*p*-Nitroanisole *O*-demethylation	Aniline hydroxylation
		nmole/mg of protein/15 min			
Male	Control	59.41 ± 3.99	113.31 ± 6.87	26.02 ± 1.76	20.08 ± 0.42
	40	47.96 ± 1.78	119.30 ± 3.68	25.22 ± 0.50	20.17 ± 1.09
	150	51.93 ± 1.71	131.74 ± 2.50	23.75 ± 1.01	17.18 ± 1.14
	600	41.39 ± 2.12*	101.11 ± 6.41*	21.44 ± 1.93	15.72 ± 1.12*
	1200	31.65 ± 2.41†	63.77 ± 1.9†	13.16 ± 0.40†	9.40 ± 0.72†
Female	Control	18.35 ± 0.98	55.61 ± 4.49	10.42 ± 0.91	10.66 ± 1.04
	40	20.07 ± 1.84	56.12 ± 6.18	11.28 ± 1.18	13.65 ± 2.19
	150	33.21 ± 3.58†	81.06 ± 4.80†	15.16 ± 0.55†	17.60 ± 1.38†
	600	21.71 ± 2.60	62.31 ± 5.22	15.18 ± 0.81**	17.25 ± 1.89*
	1200	24.65 ± 1.95*	68.86 ± 1.14*	14.77 ± 0.76*	16.40 ± 0.99†

The activities of benzphetamine N-demethylase, aminopyrine *N*-demethylase, *p*-nitroanisole *O*-demethylase and aniline hydroxylase were measured as described in Materials and Methods. Rats were given different doses of Aztreonam specified in the table. Each value represents mean ± SE of four animals.

* $P < 0.05$, † $P < 0.01$.

(pH 7.4) in a final volume of 1.0 ml. The concentrations of benzphetamine, aminopyrine and *p*-nitroanisole used were 1 mM, 5 mM and 4 mM, respectively. Reactions were initiated by the addition of NADPH-generating system which had been preincubated for 5 min at 37° and were carried out aerobically for 15 min at 37°.

Assays. The activities of benzphetamine *N*-demethylase and aminopyrine *N*-demethylase were measured by the method of Nash [7]. Aniline hydroxylase activity was estimated by determining *p*-aminophenol formed by the method of Imai *et al.* [8]. *p*-Nitroanisole *O*-demethylase activity was estimated by measuring the production of *p*-nitrophenol as described elsewhere [9]. 7-Ethoxycoumarin *O*-deethylase activity was measured by the method of Aitio [10]. Cytochrome P-450 was determined according to the method of Omura and Sato [11] except that microsomes were diluted with 0.1 M K-phosphate (pH 7.25) containing 20% glycerol and 0.2% Emulgen 913 prior to measurement. Spectrophotometric measurement was carried out using a Hitachi two wavelength recording spectrophotometer (Model 356). The amount of cytochrome P-450 was calculated from the difference in absorbance between 450 nm and 490 nm using a molar extinction coefficient of 91 $mM^{-1}cm^{-1}$. The amount of cytochrome b_5 was calculated from the difference in absorbance between 410 nm and 424 nm using a molar extinction coefficient of 185 $mM^{-1}cm^{-1}$. Microsomal protein was estimated according to the method of Lowry *et al.* using bovine serum albumin as a standard [12]. Quantitation of P-450 (M-1) [13] in liver microsomes from male rats was conducted by Western blot PAP-staining. This cytochrome is considered to be the same as P-450 male [14] based on a minimal molecular weight, carbon monooxide difference spectrum and metabolism of several drug substrates. Electrophoresis and staining were carried out essentially as described previously [15–17]. Densitometry was carried out with the nitrocellulose sheets by using a Shimazu thin-layer chromatoscanner CS-930.

Statistical analysis. Levels of statistical significance were assessed using Student's *t*-test between two means for unpaired data. All results are expressed as mean ± SE.

RESULTS

There was no significant difference in gain of body weight between control and Aztreonam-treated groups of both male and female rats throughout these studies (data not shown). The effects of Aztreonam on drug oxidations in male and female rats were shown in Table 1. Dose-dependent decreases in the activities of benzphetamine *N*-demethylase, aminopyrine *N*-demethylase, *p*-nitroanisole *O*-demethylase and aniline hydroxylase were observed in liver microsomes from male rats. On the contrary, in microsomes from female rats, these activities were increased with increasing the dose of Aztreonam. The significant decreases in the activities of all of drug oxidations tested were observed in male rats treated with Aztreonam at a dose of 1200 mg/kg/day whereas the slight but significant increases in the activities were observed in female rats treated with Aztreonam at a same dose as in the male rats. As can be seen in Table 2, the effects of successive administration of Aztreonam on cytochrome P-450 content in male rats were also different from those observed in female rats. The specific contents of spectrally determined cytochrome P-450 in liver microsomes from male rats was dose-dependently decreased and was significantly decreased by Aztreonam at a dose of 600 or 1200 mg/kg/day. On the contrary, cytochrome P-450 in liver microsomes from female rats was found to be virtually unaffected by Aztreonam even at a dose of 1200 mg/kg/day. The specific content of cytochrome b_5 in male rat was also significantly decreased by Aztreonam pretreatment at a dose of 600 or 1200 mg/kg/day. On the other hand, in female rat it showed a tendency to decrease the content of cytochrome b_5, but the decrease was not significant even at a dose of 1200 mg/kg/day. Figure 1 shows the effect of

Table 2. Effect of Aztreonam on the content of cytochromes P-450 and b_5 in liver microsomes from male and female rats

Group (mg/kg)		Cytochrome P-450	Cytochrome b_5
		(nmole/mg of protein)	
Male	Control	1.11 ± 0.04 (100)	0.38 ± 0.03 (100)
	40	1.15 ± 0.06 (104)	0.34 ± 0.04 (89)
	150	1.11 ± 0.06 (100)	0.39 ± 0.03 (103)
	600	0.84 ± 0.09* (76)	0.28 ± 0.03* (74)
	1200	0.52 ± 0.04† (47)	0.12 ± 0.01† (32)
Female	Control	0.48 ± 0.04 (100)	0.29 ± 0.03 (100)
	40	0.40 ± 0.04 (83)	0.24 ± 0.02 (83)
	150	0.49 ± 0.04 (102)	0.28 ± 0.01 (97)
	600	0.47 ± 0.04 (98)	0.22 ± 0.02 (76)
	1200	0.45 ± 0.05 (94)	0.21 ± 0.02 (72)

Cytochromes P-450 and b_5 were spectrally determined as described in Materials and Methods. Figures in parentheses are presented as per cent of control. Each value represents mean ± SE of four animals. * $P < 0.05$, † $P < 0.01$.

Aztreonam on the contents of cytochrome P-450 in liver microsomes from male and female dogs. No sex difference in the specific content of cytochrome P-450 was observed. In addition, the contents of cytochrome P-450 were virtually unchanged by Aztreonam-treatment for 26 days except that at a dose of 600 mg/kg/day, a significant decrease in the content of cytochrome P-450 was observed in liver microsomes from female dogs. As can be seen in Fig. 2, the specific content of cytochrome P-450 in liver microsomes from male crab eating monkeys was significantly decreased by Aztreonam-treatment for 4 weeks. However, none of the activities of drug oxidations studied were changed by Aztreonam. Rabbit antiserum raised to purified P-450 (M-1) recognized a protein with an identical electrophoretic mobility in microsomes prepared from Aztreonam-treated and untreated male rats. However, the protein band which was recognized by the antibodies, was found to be decreased with increasing the dose of Aztreonam administered. Therefore, an antiserum raised to purified P-450 (M-1) was used to quantitate the amounts of P-450 (M-1) in liver microsomes from male rats. The amounts of P-450 (M-1) immunochemically determined in microsomes of untreated male rats accounted for about 30% of total cytochrome P-450 spectrally determined as has been reported elsewhere [13]. As can be seen in Table 3, the amount of P-450 (M-1) was found to be dose-dependently decreased with increasing dose of Aztreonam injected.

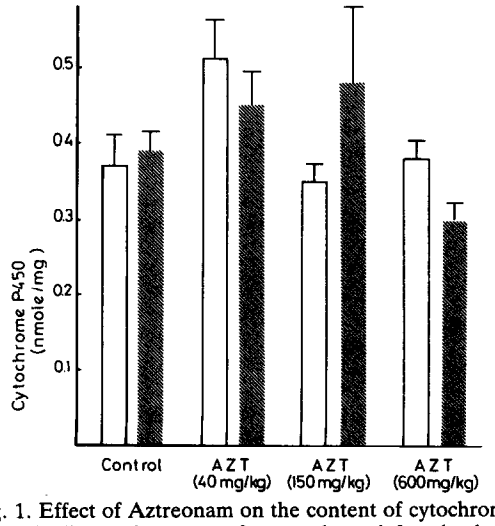

Fig. 1. Effect of Aztreonam on the content of cytochrome P-450 in liver microsomes from male and female dogs. Cytochrome P-450 in liver microsomes from male (□) and female (■) dogs was measured as described in Materials and Methods. Each value represents mean ± SE of four animals. * $P < 0.05$.

DISCUSSION

In the present study, we found that the effect of Aztreonam on liver microsomal monooxygenase system in male rats was considerably different from that in female rats. A slight but significant increase in the activities of benzphetamine *N*-demethylase, aminopyrine *N*-demethylase, *p*-nitroanisole *O*-demethylase and aniline hydroxylase was observed when female rats were pretreated with Aztreonam at a dose of 1200 mg/kg. Conversely, in male rats, Aztreonam produced significant decrease in all the activities studied at a dose of 1200 mg/kg. Since no significant quantitative and qualitative difference in the Aztreonam metabolites formed *in vivo* was observed between male and female rats, the difference in responsiveness to Aztreonam of monooxygenase between male and female rats may not be due to the sex difference in the metabolism Aztreonam.

The decrease in the activities was found to be of a roughly similar extent to that observed in total cytochrome P-450 in liver microsomes from male rats. The P-450 (M-1) content was also dramatically decreased by Aztreonam, suggesting that the decrease in the content of P-450 (M-1) may be at

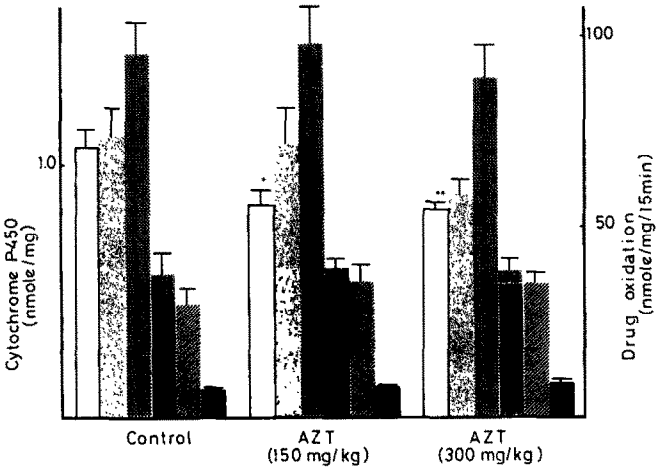

Fig. 2. Effect of Aztreonam on the content of cytochrome P-450 and the activities of drug oxidations in liver microsomes from male crab eating monkeys. Cytochrome P-450 (□) and the activities of benzphetamine *N*-demethylase (▨), aminopyrine *N*-demethylase (■), *p*-nitroanisole *O*-demethylase (■), 7-ethoxycoumarin *O*- deethylase (■) and aniline hydroxylase (■) were measured as described in Materials and Methods. Each value represents mean ± SE of four animals. * $P < 0.05$, ** $P < 0.01$.

least, in part, responsible for the decrease in the amount of total cytochrome P-450 and drug oxidations due to Aztreonam in male rats. On the other hand, Aztreonam produced no effect on cytochrome P-450 content in liver microsomes from male dogs. In addition, the activities of benzphetamine *N*-demethylase, aminopyrine *N*-demethylase, *p*-nitroanisole *O*-demethylase, 7-ethoxycoumarin *O*-deethylase and aniline hydroxylase in liver microsomes from male crab eating monkeys were unaffected by Aztreonam administration at a dose of 300 mg/kg/day for 4 weeks. Although the cytochrome P-450 content based on grammes of liver in male rat was also found to be decreased by Aztreonam-treatment for 7 days, the cytochrome P-450 content based on grammes of liver in male monkey was unchanged by Aztreonam-treatment for 4 weeks. From these results, the difference in rats appeared to be attributable to the effect of Aztreonam on a cytochrome P-450 isozyme peculiar to male rats. The mechanism by which Aztreonam produced decrease in the content of cytochrome P-450, especially P-450 (M-1), is not known at present and is now under investigation. However, the activity of liver microsomal monooxygenase in rats has been shown to be regulated by steroid hormones [18]. The possibility, therefore, might be considered that the effects of Aztreonam and/or its metabolite(s) on liver microsomal monooxygenase system in rats are directly or indirectly associated with the steroid hormone regulations.

Table 3. Immunochemical determination of cytochrome P-450, P-450 (M-1), with liver microsomes from male rats

Group (mg/kg)	P-450 M-1 (nmole/mg of protein)
Control	0.364 ± 0.028 (100)
40	0.193 ± 0.059 (53)
150	0.253 ± 0.057 (70)
600	0.143 ± 0.025 (39)
1200	0.028 ± 0.011 (8)

The amount of P-450 (M-1), one of the male specific forms of cytochrome P-450 in rat liver microsomes, were immunochemically determined as described in Materials and Methods. Each value represents mean ± SD of two animals.

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