The Effect of H₂-Receptor Antagonists on Antipyrine and Pentobarbital Metabolism in Male and Female Rats

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Abstract—Pretreatment of male and female rats with cimetidine decreased the amount of 3-hydroxymethylantipyrine in the 24-hr urine, but urinary antipyrine and 4-hydroxyantipyrine were increased compared to that of the corresponding control rats. On the other hand, the amount of norantipyrine and the total amount of antipyrine and its metabolites were not changed by cimetidine, ranitidine and famotidine. These data suggest that ranitidine and famotidine have little effect on the microsomal mixed function oxidase system in male and female rats.

Histamine H₂-receptor antagonists are among the most widely used drugs and are very likely to be frequently coadministered. Hence, the potential of drug interactions was reported and needs to be investigated. Cimetidine is an imidazole derivative and is a microsomal enzyme inhibitor in rats (1–7) and humans (8–11). However, the structurally different ranitidine (furan ring) also binds to hepatic cytochrome P-450 in the liver where it appears to exert inhibitory effects, but to a lesser extent than cimetidine (4, 6).

Thus, it is important to evaluate the drug-drug interactions during development of a new H₂-receptor antagonist, famotidine (thiazole ring). We have already reported that cimetidine treatment inhibited the metabolism of trimethadione, a marker drug of hepatic oxidizing activity in the rat (4, 7).

In the present study, we compared the effects of cimetidine, ranitidine and famotidine on urinary excretion of antipyrine and its metabolites, and the pentobarbital-induced sleeping time in male and female rats, another *in vivo* parameter of the hepatic microsomal mixed function oxidase activity.

Antipyrine (AP) and pentobarbital were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Dainabott

Co., Ltd. (Osaka, Japan), respectively. Norantipyrine (NORA), 4-hydroxyantipyrine (4OHA), and 3-hydroxymethylantipyrine (3HMA) were obtained from Chiko Co., Ltd. (Tokyo, Japan).

Male and female Wistar rats weighing 210–250 g were used in this study.

In the AP study, AP was administered by an intraperitoneal injection (i.p.) at 50 mg/kg, 30 min after i.p. injection of cimetidine (60 or 120 mg/kg), ranitidine (160 mg/kg) or famotidine (160 mg/kg). The doses chosen for famotidine and ranitidine were equimolar to 120 mg/kg of cimetidine. For collection of urine after antipyrine administration, rats were individually placed in metabolic cages and were given water only and no food, and 24-hr urine samples were collected. The samples were frozen and stored at –80°C until analyzed.

The amounts of AP and its metabolites in the urine were measured by the HPLC procedure with modifications as described by Teunissen et al. (12) and Blyden et al. (13).

In the pentobarbital (PEB)-induced sleeping time study, PEB sodium (50 mg/kg) was injected i.p. 30 min after an i.p. injection of cimetidine (60 and 120 mg/kg), ranitidine (160 mg/kg) or famotidine (160 mg/kg).

The duration of sleeping time was determined as the time period between the onset of loss and return of the righting reflex. Righting reflex was defined as the ability of the rat to place both forefeet on the ground upon application of pressure to the tail with forceps. This stimulation was applied at 2 min intervals. For statistical analyses, Student's *t*-test was used.

Excretion data of AP and its main metabolites in the 24-hr urine of the four groups are summarized in Table 1, where the results are expressed as percentage of the administered dose (50 mg/kg, i.p.) of AP.

Treatment with 60 and 120 mg/kg of cimetidine in male and female rats decreased the amount of 3HMA in the 24-hr urine, but urinary AP and 4OHA were increased compared to the corresponding controls. On the other hand, the amount of NORA and the total amount of antipyrine and metabolites (conjugated+free) were not changed. Ranitidine or famotidine did not affect AP metabolism even at the high dose of 160 mg/kg.

Figure 1 shows the effects of the three H₂-receptor antagonists on PEB-induced sleeping time in male and female rats. Treatment with 60 and 120 mg/kg of cimetidine in male rats prolonged the mean PEB-induced sleep-

ing time from 54±4 (mean±S.D.) min to 102±6 min and 153±6 min, respectively, in a dose-dependent manner. Treatment with 60 and 120 mg/kg of cimetidine also prolonged the sleeping time from 206±5 min to 285±9 min and 352±12 min, respectively, in female rats.

However, in the rats pretreated with ranitidine (male: 64±7 min, female: 210±11 min) and famotidine (male: 60±5 min, female: 203±8 min), the mean sleeping time was not changed as compared to the controls.

Cimetidine, ranitidine and famotidine are histamine H₂-receptor antagonists and have imidazole, furan and thiazole rings, respectively, in their structures. In vivo experiments showed that the imidazole derivative cimetidine inhibits the metabolisms of a number of other drugs in rats (1–7) and humans (8–11). This inhibition was explained by a reversible binding of the imidazole ring of cimetidine to cytochrome P-450 (14).

In the present study, we used two methods of drug metabolism investigation: one was a widely used procedure for in vivo assessment of hepatic microsomal mixed function oxidase activity, and the other was measurement of the PEB-induced sleeping time. Possible sex difference was also investigated.

Table 1. Excretion of antipyrine and its metabolites expressed as percentage of dose in the 24-hr urine in male and female rats pretreated with three H₂-receptor antagonists

Treatment	Sex	3-Hydroxy-methylantipyrine	Norantipyrine	Antipyrine	4-Hydroxy-antipyrine	Total
Control	M	25.8±0.5	6.9±0.1	2.3±0.1	16.2±0.6	51.2±0.8
	F	14.9±0.3	7.1±0.2	4.7±0.1	22.8±0.8	49.4±0.8
Cimetidine						
60 mg/kg	M	21.4±0.8**	5.7±0.4	3.7±0.5**	19.5±0.7**	50.3±1.1
	F	11.3±0.5**	6.6±0.6	6.2±0.3*	26.2±1.2*	50.3±1.6
120 mg/kg	M	18.7±0.4*	4.7±0.7	5.1±0.5*	21.5±1.7*	50.0±2.1
	F	10.6±0.3*	5.1±0.5	7.4±0.3*	29.3±1.1*	52.4±1.7
Ranitidine						
160 mg/kg	M	22.1±0.9	6.8±0.3	3.3±0.2	17.5±0.6	49.7±1.2
	F	15.2±1.0	6.8±0.3	4.1±0.2	22.6±0.4	48.7±1.2
Famotidine						
160 mg/kg	M	22.9±0.5	8.6±0.9	3.1±0.4	16.9±0.9	51.5±1.3
	F	14.7±0.6	7.5±0.3	4.1±0.3	23.5±0.7	49.8±1.1

Antipyrine (50 mg/kg) was injected i.p. 30 min before the i.p. injection of three H₂-receptor antagonists. M: Male, F: Female. Each value represents the mean±S.D. of 5 rats. *Different from the control at *P*<0.05. **Different from the control at *P*<0.01.

Fig. 1. Effect of cimetidine, ranitidine and famotidine on pentobarbital-induced sleeping time in male and female rats. (1): Control; (2): Cimetidine, 60 mg/kg; (3): Cimetidine, 120 mg/kg; (4): Ranitidine, 160 mg/kg; (5): Famotidine, 160 mg/kg. Pentobarbital sodium (50 mg/kg) was injected i.p. 30 min after the i.p. injection of three H_2 -receptor antagonists. Values represent the mean $\pm$ S.D. of 5 rats. *Significantly different from the control at $P < 0.01$.

Cimetidine, but not ranitidine or famotidine, decreased urinary excretion of 3HMA selectively, and it increased urinary 4OHA and did not change urinary NORA and antipyrine (Table 1). This finding was qualitatively and quantitatively comparable between males and females; and furthermore, this finding is similar to those obtained by Shaw et al. (5), suggesting that cimetidine selectively inhibits only a certain form of cytochrome P-450. However, a recent study in man has also reported non-selective inhibition of cimetidine (15). This discrepancy suggests that there are different properties of cytochrome P-450 in relation to AP metabolism in rats and humans. In the control experiment without histamine H_2 -receptor antagonists, urinary excretion of 3HMA was about 40% smaller in females, and those of 4OHA and AP were about 40% and 100%, respectively, larger in females than in males. Recently, Nakagawa et al. (16) reported that only cytochrome P-450 male, a male specific form of cytochrome P-450, extensively metabolized AP to give all three major metabolites in an in vitro study. However, P-450-female did not hydroxylate AP to give 4OHA or 3HMA, as demonstrated with cytochrome P-450-male. The discrepancy from our findings might be related to in vivo and in vitro differences, strain differences, sex difference or some other factors due to the rats used.

The pentobarbital-induced sleeping time

was also significantly prolonged in both male and female rats only by cimetidine as reported by Serlin et al. (17) and Honma et al. (18) (Fig. 1). Sleeping caused by another hypnotic hexobarbital is also prolonged by pretreatment with cimetidine (1, 6).

In conclusion, the results of the present study suggest that cimetidine, but not ranitidine or famotidine, inhibits the hepatic microsomal mixed function oxidase system.

References

- 1 Pelkonen, O. and Puurunen, J.: The effect of cimetidine on in vitro and in vivo microsomal drug metabolism in the rat. *Biochem. Pharmacol.* **29**, 3075–3080 (1980)
- 2 Fruncillo, R.J., Digregorio, G.J. and Soll, A.: Effect of cimetidine on the pharmacokinetics of quinidine and lidocaine in the rat. *J. Pharm. Sci.* **72**, 826–828 (1983)
- 3 Parrott, K.A. and Christensen, J.M.: Influence of cimetidine on the disposition of ibuprofen in the rat. *Res. Commun. Chem. Pathol. Pharmacol.* **43**, 369–380 (1984)
- 4 Tanaka, E. and Misawa, S.: Effects of cimetidine and ranitidine on trimethadione metabolism in the rat. *Res. Commun. Chem. Pathol. Pharmacol.* **47**, 461–464 (1985)
- 5 Shaw, P.N., Tseti, J., Warburton, S., Adedoyin, A. and Houston, J.B.: Inhibition of antipyrine metabolite formation. *Commun. Chem. Pathol. Pharmacol.* **14**, 271–276 (1986)
- 6 Lin, J.H., Cocchetto, D.M., Yen, K.C. and Duggan, D.E.: Comparative effects of H_2 -receptor antagonists on drug interaction in rats.

- Drug Metab. Dispos. **14**, 649–654 (1986)
- 7 Tanaka, E., Misawa, S. and Kuroiwa, Y.: Comparative effects of famotidine and cimetidine on trimethadione metabolism in the rat. *Biochem. Pharmacol.* **35**, 869–871 (1986)
 - 8 Serlin, M.J., Sibeon, R.G., Mossman, S., Breckenridge, A.M., Williams, J.R.B., Atwood, J.L. and Willoughby, J.M.T.: Cimetidine: Interaction with oral anticoagulants in man. *Lancet* **2**, 317–319 (1979)
 - 9 Roberts, R.K., Grice, J., Wood, L., Petroff, V. and McGuffie, C.: Cimetidine impairs the elimination of theophylline and antipyrine. *Gastroenterology* **81**, 19–21 (1981)
 - 10 Hetzel, D.J., Bochner, F., Hallpike, J.F. and Shearman, D.J.C.: Cimetidine interaction with phenytoin. *Br. Med. J.* **282**, 1512 (1981)
 - 11 Feely, J., Wilkinson, G.R. and Wood, A.J.J.: Reduction of liver blood flow and propranolol metabolism by cimetidine. *N. Engl. J. Med.* **304**, 692–695 (1981)
 - 12 Teunissen, M.W.E., Meerburg-van der Torren, J.E., Vermeulen, N.P.E. and Breimer, D.D.: Automated high-performance liquid chromatographic determination of antipyrine and its main metabolites in plasma, saliva and urine, including 4,4'-dihydroxyantipyrine. *J. Chromatogr.* **278**, 367–378 (1983)
 - 13 Blyden, G.T., LeDuc, B.W. and Greenblatt, D.J.: Large theophylline requirements due to high theophylline clearance: Verification by the antipyrine test. *Pharmacology* **32**, 226–231 (1986)
 - 14 Wilkinson, C.F., Hetnarski, K. and Hicks, L.J.: Substituted imidazoles as inhibitors of microsomal oxidation and insecticide synergists. *Pestic. Biochem. Physiol.* **4**, 299–312 (1974)
 - 15 Dossing, M., Pilsgaard, H., Rasmussen, B. and Poulsen, H.E.: Time course of phenobarbital and cimetidine mediated changes in hepatic drug metabolism. *Eur. J. Clin. Pharmacol.* **25**, 215 (1983)
 - 16 Nakagawa, A., Nakamura, K., Maeda, K., Kamataki, T. and Kato, R.: Studies on in vitro antipyrine metabolism by ^{13}C , ^{15}N double labeled method. *Life Sci.* **41**, 133 (1987)
 - 17 Serlin, M.J., Challiner, M., Park, B.K., Turcan, P.A. and Breckenridge, A.M.: Cimetidine potentiates the anticoagulant effect of warfarin by inhibition of drug metabolism. *Biochem. Pharmacol.* **29**, 1971–1972 (1981)
 - 18 Honma, S., Akutsu, R., Kambegawa, A., Iwamura, S. and Tsukamoto, K.: Metabolic fate of 2-acetoxy-N-[3-[m-(1-piperidiny)methyl]phenoxy]propyl]acetamide hydrochloride (TZU-0460), a new H_2 -receptor antagonist. (6) Effect of hepatic drug metabolizing enzyme. *Pharmacometrics* **30**, 357–366 (1985)