activity was measured at a 5'-AMP concentration of 200 μM. Secondly, the potency of alkylxanthines to stimulate lipolysis did not correlate with their inhibitory potency on the nucleotidase (Table 1). IBMX and 8-phenyltheophylline were at least equipotent to theophylline in stimulating lipolysis but showed no inhibition of the 5'-nucleotidase at a substrate concentration equivalent to the K_m of the non-inhibited enzyme. Xanthine itself significantly inhibited 5'-AMP hydrolysis but was devoid of lipolytic efficacy. Thirdly, the stimulation of lipolysis could be completely antagonized with PGE_2 (1 μg/ml) (Fig. 1), whereas the prostaglandin did not alter the inhibition of the nucleotidase by the methylxanthine (Fig. 1). Therefore, on the basis of the present experiments it seems unlikely that inhibition of 5'-nucleotidase may contribute to the lipolytic action of xanthines.

In summary, the present results show that a cell surface form of rat adipocyte 5'-nucleotidase is inhibited by alkylxanthines. The most potent inhibitors were 3-methylxanthine, theophylline and theobromine. The inhibition of the enzyme was predominantly competitive. The potency of alkylxanthines to inhibit the hydrolysis of 5'-AMP did not correlate with their lipolytic activity.

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REFERENCES

1. B. B. Fredholm, P. Hedqvist and L. Vernet, *Biochem. Pharmac.* **27**, 2845 (1978).
2. J. Tsuzuki and R. W. Newburgh, J. *Neurochem.* **25**, 895 (1975).
3. A. C. Newby, J. P. Luzio and C. N. Hales, *Biochem. J.* **146**, 625 (1975).
4. M. Rodbell, *J. biol. Chem.* **239**, 375 (1964).
5. J. Gliemann, K. Østerlind, J. Vinten and S. Gammeltoft, *Biochim. biophys. Acta* **286**, 1 (1972).
6. M. K. Gentry and R. A. Olsson, *Analyt. Biochem.* **64**, 624 (1975).
7. M. Eggstein and F. H. Kreutz, *Klin. Wschr.* **44**, 262 (1966).
8. C. S. Hanes, *Biochem. J.* **26**, 1406 (1932).
9. I. Bihler and B. Jeanrenaud, *Biochim. biophys. Acta* **202**, 496 (1970).
10. J. N. Fain, *Biochim. biophys. Acta* **573**, 510 (1979).

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Comparative effects of famotidine and cimetidine on trimethadione metabolism in the rat

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A histamine H_2-receptor antagonist, cimetidine, is known to inhibit the metabolism of many drugs that are metabolized by the hepatic mixed function oxidase system [1, 2]. There is evidence suggesting that some H_2-receptor antagonists (e.g. furan derivative ranitidine) do not share this property.

Famotidine (YM 11170) is a newly developed selective H_2-receptor antagonist having the chemical structure of amidine-substituted thiazole [3]. We have reported a useful method for estimation of hepatic drug-oxidizing capacity. Serum concentration ratios of dimethadione (DMO), which is the only metabolite of trimethadione (TMO), to unchanged TMO after oral administration of TMO are well correlated to drug-oxidizing enzyme activities in rats pretreated with chemicals such as hepatotoxic agents [4–8] and inducers [9] of the enzyme system.

In the present study, we compared the effects of cimetidine and famotidine on the metabolism of TMO, a marker drug of hepatic oxidizing activity in the rat.

Materials and methods

Male Wistar rats (Doken, Ibaraki, Japan) weighing 221–250 g were used throughout the study and allowed free access to water and food. In a single administration study, these rats were injected intraperitoneally (i.p.) with cimetidine (100 mg/kg) or famotidine (100, 150 or 200 mg/kg) 0.5 hr prior to the oral administration of TMO (100 mg/kg). In the short-term administration study, these rats were injected i.p. with cimetidine (100 mg/kg) or famotidine (200 mg/kg) twice daily for 5 days. In the pharmacokinetic study, blood samples were obtained from the jugular vein after oral administration of TMO. Procedures for preparing liver samples were described previously [8]. Microsomal cytochrome P-450 contents were determined by the method of Omura and Sato [10]. The activity of TMO (2–20 mM) *N*-demethylase was measured by the procedure of Cochin and Axelrod [11]. Experiments to determine the K_m values for cimetidine and famotidine were performed by using control rat hepatic microsome fraction as the enzyme preparation. Four concentrations (2, 5, 10 and 20 mM) of substrates were used for each determination of K_m. Kinetic constants were determined from graphically depicted data as described by Lineweaver and Burk. Serum TMO and DMO levels were determined by a gas–liquid chromatographic method as described previously [4]. Concentration–time curves for serum TMO and DMO levels were drawn on semilogarithmic scales. The half-life ($t_{\frac{1}{2}}$) and elimination rate constant (K_{el}) were calculated by linear regression analysis. The apparent volume of distribution (V_d) was calculated from the ratio of the administered dose to the concentration extrapolated to time zero.

Total body clearance (CL) was calculated according to the equation $CL = 0.693V_d/t_{\frac{1}{2}}$. Famotidine was obtained from Yamanouchi Pharmaceutical Co. (Tokyo, Japan). The results were statistically analysed by the Student's *t*-test.

Results and discussion

The effects of a single dose of cimetidine and famotidine on TMO metabolism in the rat *in vivo* are shown in Fig. 1. The serum DMO/TMO ratios at 2 hr after oral administration of TMO in cimetidine-treated rats were significantly decreased by 32.8% compared to the controls. However, famotidine did not significantly decrease this ratio from control levels in a dose of not only 100 mg/kg but also 150 and 200 mg/kg. In the pharmacokinetic study, the pretreatment of rats with cimetidine significantly prolonged the TMO serum $t_{\frac{1}{2}}$ (1.61 ± 0.14 vs 2.11 ± 0.10 hr, mean ± SEM, $P < 0.05$) and decreased CL (0.32 ± 0.03 vs 0.281 ± 0.051/kg/hr, $P < 0.05$), while in the rats pretreated with famotidine in a dose of 100, 150 or 200 mg/kg, these parameters were not changed. V_d values were

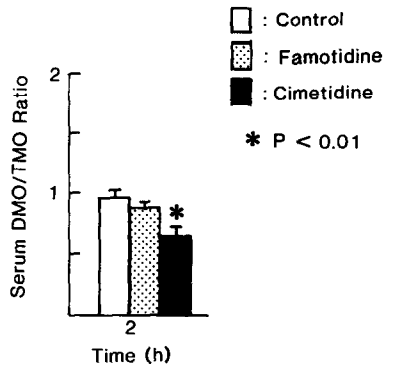

Fig. 1. The serum DMO/TMO ratios at 2 hr after oral administration of TMO (100 mg/kg) in a single cimetidine (100 mg/kg) or famotidine (200 mg/kg) pretreated rats. Values are expressed means ± S.E.M. (N = 5).

Table 2. Kinetics of TMO *N*-demethylase activity in cimetidine and famotidine-pretreated rats

Treatment	TMO *N*-demethylase	
	K_m (mM)	V_{max} (nmol/mg protein/min)
Control	5.44 ± 0.30	2.47 ± 0.18
Cimetidine	5.24 ± 0.41	1.31 ± 0.11*
Famotidine	5.50 ± 0.37	2.41 ± 0.20

* $P < 0.01$. Values are expressed means ± S.E.M. (N = 5).

The activity of TMO *N*-demethylase (substrate concentrations = 2, 5, 10 and 20 mM) measured by the procedure of Cochin and Axelrod [11]. Cimetidine (5 mM) or famotidine (10 mM) were preincubated with enzyme preparation in the medium for 5 min before addition of the substrate.

not changed by either of the drugs as compared to the controls. Effects of short-term administration of cimetidine and famotidine on hepatic microsomal drug-oxidizing enzymes in rats *in vivo* are shown in Table 1. TMO *N*-demethylase activities in the cimetidine-treated rats were significantly decreased as compared to the controls. However, their cytochrome P-450 contents were not changed. On the other hand, TMO *N*-demethylase activities and cytochrome P-450 contents were not changed by pretreatment with famotidine. Kinetic parameters of TMO *N*-demethylase in rats *in vitro* pretreated with cimetidine and famotidine are shown in Table 2. The apparent V_{max} value of TMO *N*-demethylase in the cimetidine-treated rats was significantly decreased as compared to the controls, but their apparent K_m value was not changed appreciably. In the famotidine-treated rats, neither of the parameters were changed as compared to controls. This result shows that TMO metabolism was inhibited by cimetidine in a noncompetitive manner. Wilkinson *et al.* [12] reported that the imidazole portion of cimetidine molecule may be responsible for its effects, as many imidazole derivatives are known to inhibit drug oxidation. However, ranitidine with furan ring in its structure did not decrease metabolic clearance of TMO [13]. In our results, famotidine, having the chemical structure of amidine-substituted thiazole, did not inhibit the clearance of TMO, a marker drug of hepatic oxidizing activity in the rat. These results suggest that the difference in affinity for cytochrome P-450 and in the structure of side chain are important for the difference in the effects of cimetidine and famotidine on TMO metabolism [2].

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REFERENCES

1. A. Somogi and R. Gugler, *Clin. Pharmacokinet* **7**, 23 (1982).
2. J. R. Powell and K. H. Donn, *J. clin. Gastroenterol.* **5**, 95 (1983).
3. T. Takagi, M. Takeda and H. Maeno, *Archs int. Pharmacodyn.* **256**, 49 (1982).
4. E. Tanaka, H. Kinoshita, T. Yamamoto, Y. Kuroiwa and E. Takabatake, *J. Pharm. Dyn.* **4**, 576 (1981).

Table 1. Effects of *in vivo* short-term administration of cimetidine and famotidine pretreatment on hepatic microsomal drug-oxidizing enzymes in the rat

Treatment	Content of cytochrome (nmol/mg protein) P-450	Enzyme activity (nmol/mg protein/min) TMO N-demethylase
Control	0.69 ± 0.11	2.18 ± 0.20
Cimetidine	0.68 ± 0.12	1.45 ± 0.13*
Famotidine	0.69 ± 0.08	2.16 ± 0.12

* $P < 0.05$. Values are expressed means ± S.E.M. (N = 5).

Rats were injected i.p. cimetidine (100 mg/kg) or famotidine (200 mg/kg) twice daily for 5 days and sacrified 0.5 hr after the last administration, and then their microsomes were prepared. Microsomal cytochrome P-450 contents were determined by the method of Omura and Sato [10]. The activity of TMO *N*-demethylase (substrate concentration = 20 mM) was measured by the procedure of Cochin and Axelrod [11].

5. E. Tanaka, H. Kinoshita, T. Yoshida and Y. Kuroiwa, *J. Pharm. Dyn.* **4**, 961 (1981).
6. E. Tanaka, H. Kinoshita, T. Yoshida and Y. Yuroiwa, *Japan. J. Pharmac.* **32**, 1182 (1982).
7. E. Tanaka, H. Kinoshita, T. Yoshida and Y. Kuroiwa, *Pharmacology* **25**, 22 (1982).
8. E. Tanaka, T. Nakamura, S. Misawa, T. Yoshida and Y. Kuroiwa, *Res. Commun. Chem. Path. Pharmac.* **45**, 137 (1984).
9. E. Tanaka, H. Kinoshita, T. Yoshida and Y. Kuroiwa, *J. Pharm. Dyn.* **5**, 162 (1982).
10. T. Omura and R. Sato, *J. biol. Chem.* **82**, 70 (1959).
11. J. Cochin and J. Axelrod, *J. Pharmac. exp. Ther.* **125**, 105 (1959).
12. C. F. Wilkinson, K. Hetnarski and L. J. Hicks, *Pest. Biochem. Physiol.* **4**, 299 (1974).
13. E. Tanaka and S. Misawa, *Res. Commun. Chem. Path. Pharmac.* **45**, 137 (1984).