EFFECTS OF CIMETIDINE AND DIETHYLAMINOETHYL 2,2-DIPHENYLVALERATE HCL (SKF 525-A) ON TRIMETHADIONE METABOLISM IN THE RAT

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The effects of cimetidine and diethylaminoethyl 2,2-diphenylvalerate (SKF 525-A) on trimethadione (TMO) metabolism were examined in the rat. The pretreatment of rats with cimetidine (100 mg/kg, *i.p.*) or SKF 525-A (40 mg/kg, *i.p.*) resulted in a prolongation of half-life ($T_{1/2}$), an increase in the area under the curve (*AUC*) and a decrease in the total body clearance (*CL*) of TMO. However, the apparent volume of distribution (V_d) of TMO was not changed. The activities of hepatic cytochrome P-450-dependent drug-oxidizing enzymes such as aminopyrine and TMO N-demethylase, and aniline hydroxylase activities were decreased by pretreatment of rats with cimetidine or SKF 525-A. Cytochrome P-450 contents were not changed. The inhibition of aminopyrine and TMO N-demethylase activities in the rat pretreated with cimetidine or SKF 525-A was noncompetitive. These results suggest that cimetidine and SKF 525-A inhibited TMO metabolism in a similar manner to the rat pretreated with their doses.

Keywords — trimethadione; dimethadione; cimetidine; SKF 525-A; hepatic drug-oxidizing capacity; enzyme inhibition

INTRODUCTION

Cimetidine, an antagonist of a histamine H_2 -receptor, is known to inhibit cytochrome P-450-mediated metabolism of a number of drugs in both, *in vitro* and *in vivo*.^{1,2)} Hajek and associates³⁾ evidenced that imidazole interacts with hem iron, however they reported indirect evidence that imidazole interacted with a second site, namely a site different from the substrate binding site. In addition, Hajek and Novak⁴⁾ reported that chronic imidazole administration to rabbits may induce hepatic microsomal cytochrome P-450. Cimetidine reversibly inhibits hepatic microsomal metabolism in either a competitive or a noncompetitive manner depending on the substrates.^{5–8)} Similar to the reports with other imidazoles, Rendic and colleagues^{7,9)} indicate that cimetidine coordinates with the iron of cytochrome P-450 isozymes; however, this remains to be confirmed. Pelkonen and Puurunen⁶⁾

also suggested that cimetidine has an enzyme specificity since cimetidine inhibited uninduced benzo[*a*]pyrene hydroxylation in liver microsomes but not 3-methylcholanthrene-induced one. Cimetidine has also been shown to inhibit the aminopyrine (cytochrome P-450 mediated) and caffeine (cytochrome P-448 mediated) breath tests in the rat, but it did not alter the phenacetin breath test, which is also believed to utilize cytochrome P-448.¹⁰⁾ The precise mechanism(s) by which cimetidine inhibits drug metabolism is not known. However, concerned with the inhibition mechanism of cimetidine, it was speculated that the interaction of cytochrome P-450 and H_2 -antihistamine is complex, and that the ability to inhibit microsomal oxidation metabolism is not determined simply by the affinity of the interaction. And it was also indicated that cimetidine is a potent but reversible inhibitor of cytochrome P-450-mediated drug

metabolism both *in vivo* and *in vitro*.⁸⁾ However, the clearance of drugs which are eliminated after glucuronidation is unaffected by cimetidine.¹¹⁾

We have already reported a useful method for estimation of hepatic drug-oxidizing capacity. Serum concentration ratios of dimethadione (DMO) to trimethadione (TMO) after oral administration of TMO are well correlated to the drug-oxidizing enzyme activities in the rat pretreated with some chemicals such as hepatotoxic agents and the inducers of the enzyme system.¹²⁻¹⁵⁾ Based on these facts, the present study was carried out to examine whether cimetidine and diethylaminoethyl 2,2-diphenylvalerate (SKF 525-A),¹⁶⁾ well known inhibitors of oxidative drug metabolism, could have any effects on TMO metabolism in the rat.

MATERIALS AND METHODS

Materials — TMO was purified from commercially available powder containing 66.7% TMO (Mino-Aleviatin®; Dainippon, Osaka; Japan). Cimetidine and SKF 525-A were kindly provided by Smith Kline & French Lab. (Philadelphia, Pennsylvania, USA). All other chemicals were of reagent grade.

Animals — Male Wistar rats (Japan Laboratory Animals, Tokyo, Japan), weighting 220–240 g, were used throughout the study and allowed free access to water and food. The animals were fed with a commercially available diet (Sankyo Laboservices, Tokyo, Japan). The rats were starved for 15 h before the start of experiments.

Treatments — Rats were pretreated with cimetidine (100 mg/kg, *i.p.*) or SKF 525-A (40 mg/kg, *i.p.*) 0.5 and 1 h, respectively, prior to the oral administration of TMO (100 mg/kg). Rats were fixed on a plate (CFK Lab., Tokyo, Japan) only at the time of blood sampling from the jugular vein at 0.5, 1, 2, 4, 8, 12, 24 and 48 h after oral administration of TMO without anesthesia. The serum samples prepared by centrifugation were stored at –20 °C until used for the determination of TMO and DMO levels.

Preparation of the Liver Sample — Rats were

sacrificed 2 h after TMO administration. Then the livers were perfused *in situ* with cold 0.9% NaCl solution *via* the portal vein, removed and homogenized in 4 volumes of 1.15% KCl by using a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at $9000 \times g$ for 20 min. The resulting supernatant fraction was recentrifuged at $105000 \times g$ for 60 min. The microsomal pellet was washed once and resuspended in 0.05 M phosphate buffer (pH 7.6) containing 1 mM ethylenediaminetetraacetic acid (EDTA).

Enzyme Assays — Microsomal cytochrome P-450 content was determined by the method of Omura and Sato.¹⁷⁾ The activities of aminopyrine (10 mM) and TMO (20 mM) N-demethylase were measured by the procedure of Cochin and Axelrod,¹⁸⁾ whereas the activity of aniline (80 mM) hydroxylase was determined by measuring the formation of *p*-aminophenol by the method of Imai *et al.*¹⁹⁾ Protein concentration was determined by a modified method of Lowry *et al.*²⁰⁾ using bovine serum albumin as the standard.

Determination of Michaelis Constants — Studies to determine the K_m values for cimetidine and SKF 525-A were performed utilizing control rat hepatic microsome, and cimetidine or SKF 525-A rat hepatic microsome as enzyme preparations. Three or four concentrations of substrates were utilized for each determination of K_m . Kinetic constants were determined from graphic treatment of data as described by Lineweaver and Burk.

Serum TMO and DMO Assay — Serum TMO and DMO levels were determined by a gas-liquid chromatographic method using maleic acid as the internal standard, as previously described.¹²⁾

Pharmacokinetic Studies — Concentration-time curves for serum TMO and DMO levels were drawn on semilogarithmic scales. The half-life ($T_{1/2}$) 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. The area under the curve (*AUC*) of TMO was calculated according to the log-linear trapezoidal rule including extrapolation to infinity. The total body clearance (*CL*) of TMO was calculated according to the following equation:

$$CL = \frac{1.43 \cdot V_d}{T_{1/2}}$$

Statistical Analysis — The results were statistically analyzed by the Student's *t*-test.

RESULTS AND DISCUSSION

We have studied the effect of a single dose pretreatment with cimetidine or SKF 525-A on TMO metabolism in the rat, *in vivo* and *in vitro*. The serum concentration of TMO in control rats reached its peak at 0.5–1 h, and DMO reached

its peak at around 10 h and gradually decreased. The serum concentration of TMO in cimetidine- and SKF 525-A-treated rats reached its peaks at 0.5–1 h and 0.5–1.5 h, respectively, while DMO production in both cimetidine- and SKF 525-A-treated rats was reduced as compared to controls (Fig. 1).

In pharmacokinetic study, pretreatment with cimetidine or SKF 525-A in the rat prolonged *T*_{1/2}, increased *AUC*, and decreased *CL* of TMO. However, *V*_d values of TMO were not changed as compared to controls (Table I).

As shown in Table II, aminopyrine and TMO *N*-demethylase activities, and aniline hydroxylase activity in the rat pretreated with cimetidine or SKF 525-A were significantly decreased

FIG. 1. Time Course of Serum TMO (A) and DMO (B) Levels Following the Oral Administration of TMO to Rats Pretreated with Cimetidine and SKF 525-A. Values are Means $\pm$ S.E. (*n*=5). $\circ$, control; $\bullet$, cimetidine; $\triangle$, SKF 525-A.

TABLE I. Pharmacokinetic Parameters Following the Oral Administration of TMO to Rats Pretreated with Cimetidine and SKF 525-A

Treatment	<i>T</i> _{1/2} , h	<i>V</i> _d , l/kg	<i>CL</i> , l/kg/h	<i>AUC</i> , μ g/ml/h
Control	1.58 $\pm$ 0.12	0.632 $\pm$ 0.051	0.306 $\pm$ 0.019	354 $\pm$ 9.6
Cimetidine	2.01 $\pm$ 0.08 ^{b)}	0.652 $\pm$ 0.101	0.281 $\pm$ 0.051 ^{b)}	396 $\pm$ 8.1 ^{b)}
SKF 525-A	1.58 $\pm$ 0.16 ^{a)}	0.612 $\pm$ 0.062	0.250 $\pm$ 0.037 ^{a)}	416 $\pm$ 9.6 ^{a)}

a) *p* < 0.01. b) < 0.05. *T*_{1/2} = half-life; *V*_d = apparent volume of distribution; *CL* = total body clearance; *AUC* = area under the curve. Cimetidine (100 mg/kg, i.p.) and SKF 525-A (40 mg/kg, i.p.) were administered 0.5 and 1 h, respectively, prior to the oral administration of TMO (100 mg/kg). Values are means $\pm$ S.E. (*n*=5).

as compared to controls. However, the contents of cytochrome P-450 were not changed as compared to controls. Pharmacokinetic parameters of TMO in cimetidine- or SKF 525-A-treated rats *in vivo* coincided well to the changes in hepatic drug-oxidizing enzyme activities *in vitro*.

As shown in Table III and Fig. 2, the apparent $V_{\max}$ values of aminopyrine and TMO N-demethylase activity of rats pretreated with cimetidine or SKF 525-A were significantly decreased as compared to controls. On the other hand, the apparent K_m values were not appreciably changed. These results indicate that aminopyrine

and TMO metabolism were inhibited by cimetidine and SKF 525-A in competitive manner and the potencies of metabolic inhibition are weaker in cimetidine than in SKF 525-A-pretreated rats (Table I and II). These differences may be that there are multiple forms of cytochrome P-450 exhibiting different catalytic activities towards substrates of range of substrates.²¹⁾ In recent study, Parrott and Christensen²²⁾ reported that pretreatment of rats with cimetidine (50 mg/kg, *i.p.*) or SKF 525-A (50 mg/kg, *i.p.*) prolonged $T_{1/2}$ and increased AUC of ibuprofen (50 mg/kg, *p.o.*). These findings were similar to our results

TABLE II. *Effects of Cimetidine and SKF 525-A Pretreatment on Hepatic Microsomal Drug-Oxidizing Enzymes in the Rat*

Treatment	Content of cytochrome P-450 (nmol/mg protein)	Enzyme activity (nmol/mg protein/min)		
		Aminopyrine N-demethylase	TMO N-demethylase	Aniline hydroxylase
Control	0.73 ± 0.05	5.75 ± 0.31	2.21 ± 0.16	0.62 ± 0.07
Cimetidine	0.75 ± 0.23	3.56 ± 0.23 ^{b)}	1.61 ± 0.09 ^{b)}	0.50 ± 0.17 ^{b)}
SKF 525-A	0.71 ± 0.08	2.34 ± 0.24 ^{a)}	1.02 ± 0.11 ^{a)}	0.31 ± 0.05 ^{a)}

a) $p < 0.01$. b) $p < 0.05$. Values are means ± S.E. ($n=5$). The activities of aminopyrine (10 mM) and TMO (20 mM) N-demethylase were measured by the procedure of Cochine and Axelrod,¹⁸⁾ whereas the activity of aniline (80 mM) hydroxylase was measured by the procedure of Imai *et al.*¹⁹⁾ Cimetidine (2.5 mM) or SKF 525-A (0.1 mM) were preincubated in medium for 5 min before addition to the substrate.⁵⁾

TABLE III. *Kinetic Constants of Aminopyrine and TMO N-Demethylase Activity in Cimetidine and SKF 525-A Pretreated Rats*

Treatment	Aminopyrine N-demethylase		TMO N-demethylase	
	K_m (mM)	$V_{\max}$ (nmol/mg protein/min)	K_m (mM)	$V_{\max}$ (nmol/mg protein/min)
Control	2.33 ± 0.24	9.26 ± 0.41	5.65 ± 0.32	2.50 ± 0.16
Cimetidine	2.11 ± 0.19	5.17 ± 0.39 ^{b)}	5.21 ± 0.31	1.50 ± 0.21 ^{a)}
SKF 525-A	2.34 ± 0.21	4.11 ± 0.31 ^{a)}	5.32 ± 0.28	1.31 ± 0.20 ^{a)}

a) $p < 0.01$. b) $p < 0.05$. Values are means ± S.E. ($n=5$). The activities of aminopyrine (1–10 mM) and TMO (2–20 mM) N-demethylase were measured by the procedure of Cochine and Axelrod.¹⁸⁾ Cimetidine (2.5 mM) or SKF 525-A (0.1 mM) were preincubated in medium for 5 min before addition to the substrate.⁵⁾ K_m and $V_{\max}$ values are calculated from Lineweaver-Burk plots with three or four substrates concentrations.

FIG. 2. *Lineweaver-Burk Plots of TMO (A) and Aminopyrine (B) N-Demethylation Obtained with Rat Hepatic Microsomes in Absence and Presence of Various Concentrations of Cimetidine and SKF 525-A*
Each point represents the mean of duplicate determinations. ○, control; ●, cimetidine; △, SKF 525-A.

indicating decreases in the activities of microsomal oxidizing enzymes. Although cimetidine may also inhibit epoxidation and hydroxylation, it apparently uniformly inhibits N-demethylation.^{1,2,5-7,23} It is, of course, difficult to extrapolate the findings in this study to man, but the possibility that a similar interaction took place in the patient we observed merits consideration.¹² In both preliminary *in vivo* and *in vitro* studies performed in our laboratory, cimetidine and SKF 525-A produced a dose dependent reduction on TMO, aminopyrine and aniline metabolism. The patterns in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of hepatic microsomal protein from the rats pretreated with cimetidine and SKF 525-A were not changed as compared to control (unpublished data). These results suggest, together with the previous findings, that inhibition of TMO metabolism by cimetidine and SKF 525-A in the rat are produced by a similar mechanism.

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