

Effects of Genetically Determined S-Mephenytoin 4-Hydroxylation Status and Cigarette Smoking on the Single-Dose Pharmacokinetics of Oral Alprazolam

Koichi Otani, M.D., Ph.D., Norio Yasui, M.D., Sunao Kaneko, M.D., Ph.D., Tadashi Ohkubo, Ph.D., Takako Osanai, B.S., Kazunobu Sugawara, Ph.D., Kunihiro Hayashi, Ph.D., Kan Chiba, Ph.D., and Takashi Ishizaki, M.D., Ph.D.

This study examines the effects of genetically determined S-mephenytoin 4-hydroxylation capacity and cigarette smoking on the single-dose pharmacokinetics of oral alprazolam in 12 healthy male volunteers. Six subjects each were extensive metabolizers (EMs) and poor metabolizers (PMs) of S-mephenytoin 4-hydroxylation. Seven subjects were smokers (>10 cigarettes/day), and five were nonsmokers, according to their self-reports. Each subject took a single oral dose of 0.8 mg of alprazolam, and blood samples were collected up to 48 hours postdose. Psychomotor function was assessed at times of blood samplings using the Digit Symbol Substitution Test (DSST), Visual Analog Scale (VAS), and UKU Side Effect Rating Scale. Plasma alprazolam concentrations were measured by a high-performance liquid chromatography

assay. None of the mean pharmacokinetic parameters was significantly different between the EM and PM phenotype groups. Although the mean elimination half-life was significantly shorter in the smoker group ($p < .01$) than in the nonsmoker group (13.1 ± 2.9 vs. 20.0 ± 2.7 hours, mean \pm SD), other pharmacokinetic parameters did not differ significantly between the two groups. Psychomotor function parameters did not differ significantly either between the EM and PM groups or between the nonsmoker and smoker groups. The present study thus suggests that neither S-mephenytoin 4-hydroxylation status nor self-reports of extensive cigarette smoking has a major impact on the metabolism of alprazolam in humans. © 1997 American College of Neuropsychopharmacology [Neuropsychopharmacology 16:8-14, 1997]

KEY WORDS: Alprazolam; Metabolism; S-mephenytoin 4-hydroxylation; Smoking; Cytochrome P450 (CYP) isoforms.

From the Department of Neuropsychiatry (KO, NY, SK) and Department of Pharmacy (TO, KS), Hirosaki University Hospital, Hirosaki; the Department of Pharmacy (Takako O), Goshogawara City Hospital, Goshogawara; the Biostatistics Department (KH), Yamanouchi Pharmaceutical, Tokyo; and the Department of Clinical Pharmacology (KC, TI), Research Institute, International Medical Center of Japan, Tokyo, Japan.

Address correspondence to: Koichi Otani, M.D., Ph.D., Department of Neuropsychiatry, Hirosaki University Hospital, Hon-cho 53, Hirosaki 036, Japan.

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The triazolobenzodiazepine alprazolam has been widely used as an anxiolytic and antipanic agent (Greenblatt and Wright 1993). It has been shown that a significant concentration-response relationship exists in the treatment of panic disorder; optimal reduction of anxiety occurs in the plasma concentration range of 20 to 40 ng/mL, while central nervous system-depressing side effects increase progressively at higher plasma concentrations (Greenblatt and Wright 1993). Alprazolam is metabolized primarily by hepatic microsomal oxidation, yielding 4- and α -hydroxyalprazolam as its principal metabolites (Greenblatt and Wright 1993).

The metabolism of several psychotropic drugs has been shown to be catalyzed by two cytochromes P450, CYP2D6

(Dahl and Bertilsson 1993; Spina and Caputi 1994; Yasui et al. 1995) and CYP2C19 (Bertilsson et al. 1989; Sohn et al. 1992a; Chiba et al. 1994; Koyama et al. 1994) designated as debrisoquin 4-hydroxylase and S-mephenytoin 4-hydroxylase, respectively. These isoenzymes are the sources of genetically determined oxidation polymorphisms that result in pronounced interindividual variability in the metabolism of antidepressants and neuroleptics (Dahl and Bertilsson 1993; Spina and Caputi 1994).

A previous study (Bertilsson et al. 1988) has shown that alprazolam inhibited neither the 10-hydroxylation of nortriptyline (a substrate for CYP2D6) *in vivo* nor the 2-hydroxylation of desipramine (a substrate for CYP2D6) *in vitro* (Dahl and Bertilsson 1993; Spina and Caputi 1994) suggesting that CYP2D6 is not involved in alprazolam metabolism. On the other hand, despite the fact that CYP2C19 is involved in the metabolism of diazepam (Bertilsson et al. 1989; Sohn et al. 1992a), the association of this isoenzyme with the metabolism of alprazolam has, to our knowledge, never been studied.

Therefore, we intended to assess the possible effect of S-mephenytoin 4-hydroxylation capacity on the pharmacokinetics of alprazolam. In addition, the effect of habitual smoking on the kinetics of alprazolam was reexamined, although two previous studies (Smith et al. 1983; Fleishaker and Hulst 1994) have shown a negligible effect. We reexamined this relationship because two studies (Fleishaker and Hulst 1994; von Moltke et al. 1995) have suggested that CYP1A2, which is an isoenzyme inducible by smoking (Bock et al. 1994), was involved in the metabolism of alprazolam.

MATERIALS AND METHODS

Subjects

Twelve unrelated, healthy male subjects (age, 25 to 41 years; weight, 52 to 68 kg) participated in the study. Their mean (\pm SD) age and weight were 31.6 ± 5.6 years and 61.3 ± 5.5 kg, respectively. Seven subjects were habitual smokers (smoking more than 10 cigarettes per day for at least 9 years), and the remaining five were nonsmokers, according to their self-reports. This study was approved by the Institutional Ethics Committee of the Hiroaki University Hospital, and each subject gave his written informed consent before the study.

Phenotyping

According to the method reported from our laboratory (Horai et al. 1989; Sohn et al. 1992a; Koyama et al. 1994), each of the subjects had previously been phenotyped for his capacity to 4-hydroxylate S-mephenytoin. Briefly, each subject took an oral dose of 100 mg of racemic mephenytoin (Mesantoin[®], Sandoz Inc., East Hanover, NJ, USA), and urine was collected up to 8 hours postdose. Urinary 4-hydroxymephenytoin (4-HM) was measured

by gas chromatography, and the \log_{10} percentage of urinary excretion of 4-HM per the dose administered as racemic mephenytoin (\log_{10} 4-HM % dose) was calculated. A subject with a \log_{10} 4-HM % dose under 0.3 was defined as a poor metabolizer (PM), and one with a value above 0.3 as an extensive metabolizer (EM). Six of the twelve subjects were identified as EMs and PMs, respectively. The \log_{10} 4-HM % dose ranged from 1.49 to 1.60 in the EM group and from -0.581 to 0.088 in the PM group.

Protocol

After an overnight fast each subject took a single oral dose of 0.8 mg of alprazolam as the tablet formulation (Solanax[®], Japan Upjohn, Tokyo, Japan) with a cup of tap water at 9:00 A.M. No food was allowed until noon. Blood samples (10 mL each) were collected into heparinized tubes from an antecubital vein before and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, and 48 hours after the dosing. At times of blood samplings, psychomotor function was evaluated using the Digit Symbol Substitution Test (DSST) adapted from the Wechsler Adult Intelligence Scale in 3 minutes, the Visual Analog Scale (VAS) of Mood and Subjective States employed in pharmacodynamic assessment study of chlordiazepoxide (Greenblatt et al. 1977), and the item Sleepiness of the UKU Side Effect Rating Scale (Lingjaerde et al. 1987). The smokers were allowed to smoke cigarettes as usual throughout the study.

Assay

We measured plasma alprazolam concentrations in duplicate by a high-performance liquid chromatographic (HPLC) method developed in our laboratory. Alprazolam and estazolam as an internal standard were kindly supplied by the Upjohn Company (Kalamazoo, MI, USA). All solvents were of HPLC grade (Wako Pure Chemical Industries, Osaka, Japan). All reagents were purchased from Waco Pure Chemical Industries or Nakarai Tesque (Kyoto, Japan). Ten nanogram of estazolam in 10 μ L of methanol was added to 1 mL of plasma sample. The plasma sample was diluted with 5 mL of 1 M NaCl, and the solution was briefly mixed. The mixture was applied to Sep-Pak[®] CN cartridge (Millipore Co., Bedford, MA, USA), which had previously been activated with 5 mL of acetonitrile and water. The cartridge was then washed with 10 mL of water. The fraction desired was eluted with 5 mL of 20% acetonitrile. The eluate was evaporated to dryness in vacuum at 60°C. The residue was dissolved in 50 μ L of methanol and 100 μ L of mobile phase, and the sample was injected onto the HPLC system. The HPLC system consisted of a Rheodyne Model 7120 injector (Rheodyne, Cotati, CA, USA), a stainless-steel column (150 \times 4.6 mm ID) packed with Develosil C₈-5 stationary phase (5 μ m, Nomura Chemical, Seto, Japan), a Jasco Model PU-880 chromatography pump (Jasco, Tokyo, Japan), and a Jasco Uvidec 980 ul-

traviolet detector (Jasco, Tokyo, Japan). The wavelength was set at 230 nm. The mobile phase consisted of 0.5% KH_2PO_4 -acetonitrile (pH 4.5; 70:30, v/v). The flowrate was 1 mL/minute at ambient temperature. Retention times for alprazolam and estazolam were 17.5 and 14.3 minutes, respectively. The lowest limit of detection was 0.5 ng/mL, and the coefficient of variation (both intra- and interassay) was less than 7.8%.

Data Analysis

The elimination rate constant (k) of alprazolam was estimated from the nonlinear least-squares regression analysis of the terminal log-linear concentration data, and the elimination half-life ($t_{1/2}$) was calculated as $0.693/k$. The area under the plasma concentration-time curve from 0 to 48 hours [$\text{AUC}_{(0-48)}$] was calculated by the trapezoidal rule. The AUC from 0 hour to infinity [$\text{AUC}_{(0-\infty)}$] or total AUC, was calculated as $\text{AUC}_{(0-48)} + C_{48}/k$, where C_{48} is the plasma concentration at 48 hours postdose. The apparent oral clearance (CL_{oral}) and volume of distribution (V_d) were estimated from $\text{CL}_{\text{oral}} = \text{dose}/\text{total AUC}$ and $V_d = \text{CL}_{\text{oral}}/k$, respectively.

Statistical analyses were performed using the Student's t -test and Wilcoxon rank-sum test, where appropriate. A p value of <0.05 was considered statistically significant. As the subjects were split twice, once for S-mephenytoin 4-hydroxylation and once for cigarette smoking, the level of significance was corrected by the Bonferroni method.

RESULTS

Pharmacokinetic Assessment

The mean plasma alprazolam concentration-time data observed in the EM and PM groups of S-mephenytoin 4-hydroxylation are shown in Figure 1, and those data in relation to smoking status are shown in Figure 2. The mean plasma alprazolam concentration-time data shown in Figures 1 and 2 gave no statistically significant differences at all time points between the respective two different groups. When the 12 subjects were subdivided into the four groups [i.e., EMs/nonsmokers ($n = 3$), EMs/smokers ($n = 3$), PMs/nonsmokers ($n = 2$), and PMs/smokers ($n = 4$)], the mean plasma alprazolam concentration-time data obtained from these small numbers of the subdivided groups were found to be similar to those shown in Figures 1 and 2. However, statistical comparisons of the mean alprazolam concentration-time data were not made among the four subdivided groups because of the small number of the subdivided subjects in each group.

The mean (\pm SD) pharmacokinetic parameters of alprazolam in relation to the S-mephenytoin 4-hydroxylation or smoking status are summarized in Table 1.

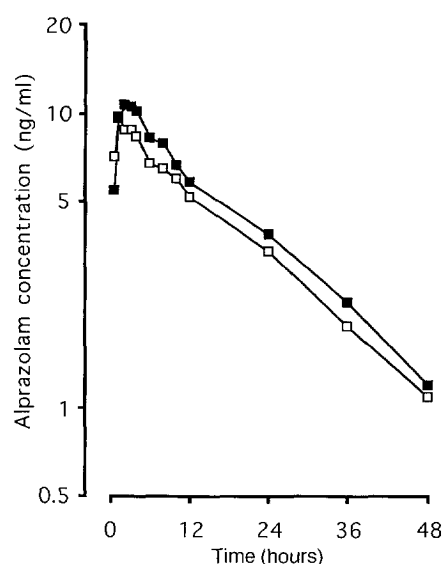


Figure 1. Mean plasma alprazolam concentration-time data after a single oral dose of 0.8 mg of alprazolam. Open squares, extensive metabolizer group ($n = 6$); solid squares, poor metabolizer group ($n = 6$) of S-mephenytoin 4-hydroxylation.

There was no significant difference in any kinetic parameters between the EM and PM groups. The mean elimination half-life of alprazolam was significantly ($p < .01$) shorter in the smoker group than in the nonsmoker group, although other pharmacokinetic parameters did not differ significantly between the two groups (Table 1).

Pharmacodynamic Assessment

The mean psychomotor function parameters assessed by the DSST, VAS, and the item Sleepiness of the UKU

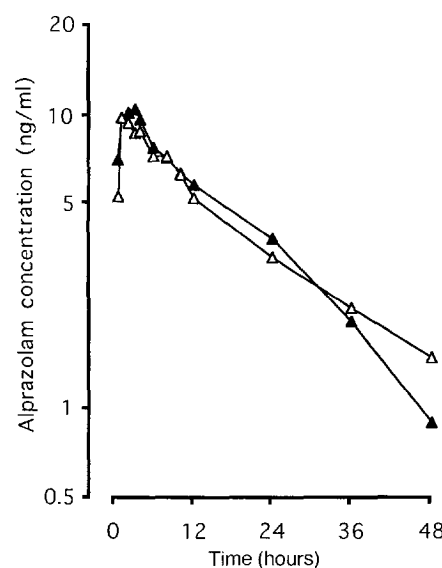


Figure 2. Mean plasma alprazolam concentration-time data after single oral dose of 0.8 mg of alprazolam. Open triangles, nonsmoker group ($n = 5$); solid triangles, smoker group ($n = 7$).

Table 1. Pharmacokinetic Parameters of Alprazolam in the Extensive Metabolizer (EM) and Poor Metabolizer (PM) groups of S-Mephenytoin 4-Hydroxylation and in the Nonsmoker and Smoker Groups

Parameters	EMs (n = 6)	PMs (n = 6)	Nonsmokers (n = 5)	Smokers (n = 7)
C _{max} (ng/mL)	11.4 ± 4.1	12.6 ± 2.0	11.0 ± 2.8	12.7 ± 3.4
t _{max} (hr)	1.3 ± 1.0	1.6 ± 0.9	1.3 ± 0.7	1.6 ± 1.1
AUC ₍₀₋₄₈₎ (ng · hr/mL)	186 ± 49	215 ± 33	195 ± 45	205 ± 44
AUC _(0-∞) (ng · hr/mL)	213 ± 58	245 ± 46	237 ± 54	224 ± 55
CL _{oral} (mL/min/kg)	1.09 ± 0.38	0.95 ± 0.23	1.03 ± 0.38	1.00 ± 0.28
V _d (L/kg)	1.52 ± 0.65	1.23 ± 0.39	1.79 ± 0.61	1.09 ± 0.19
Elimination half-life (hr)	16.3 ± 3.9	15.6 ± 5.4	20.0 ± 2.7	13.1 ± 2.9*

Data are given as mean ± SD.

*p < .01 compared with the nonsmoker group.

Abbreviations: C_{max}, peak plasma concentration; t_{max}, time to C_{max}; AUC₍₀₋₄₈₎, area under the plasma concentration-time curve from 0 to 48 hours; AUC_(0-∞), area under the plasma concentration-time curve from 0 hour to infinity; CL_{oral}, apparent oral clearance; V_d, apparent volume of distribution.

Scale in the EM and PM groups are shown in Figure 3, and those in the nonsmoker and smoker groups in Figure 4. None of the psychomotor function parameters differed significantly between the EM and PM groups (Figure 3) or between the nonsmoker and smoker groups (Figure 4).

DISCUSSION

In the largest single-dose pharmacokinetic study of alprazolam involving 22 subjects (Friedman et al. 1991),

the coefficient of variation in clearance was 40%. Assuming that a 50% difference in clearance is clinically important, the power to detect a difference of 0.545 mL/min/kg (50% of the mean CL_{oral} of the EM group) between the EM and PM groups is calculated as 0.77 (Zar 1996). Similarly, the power to detect a difference of 0.515 mL/min/kg (50% of the mean CL_{oral} of the nonsmoker group) between the nonsmoker and smoker groups is calculated as 0.68. Although these relatively low powers are drawbacks, this study still provides important information on the metabolism of alprazolam.

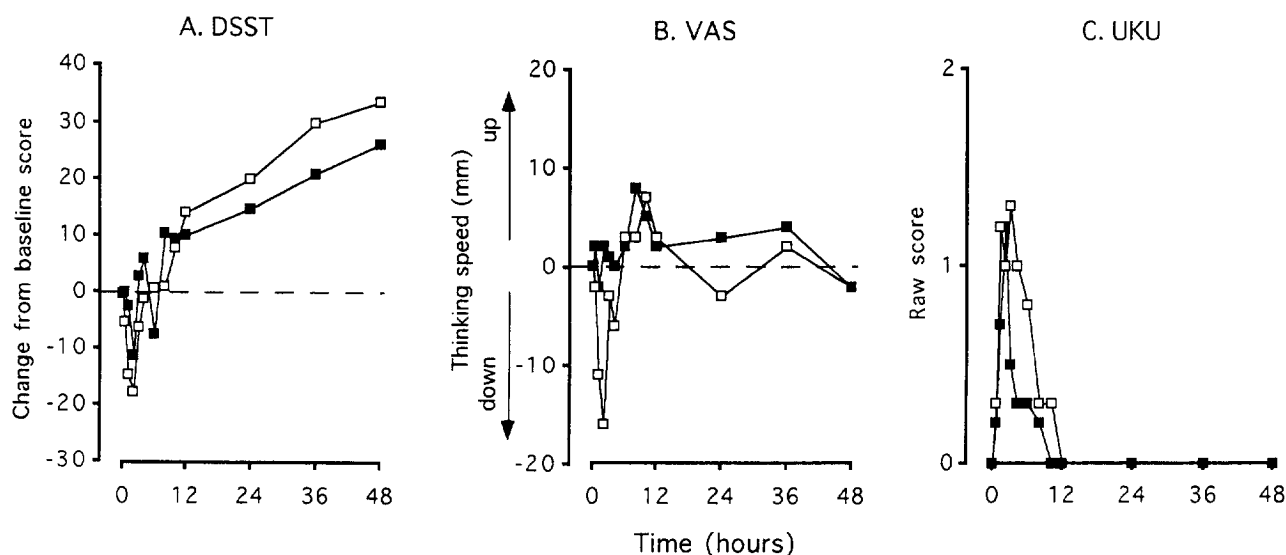


Figure 3. Mean psychomotor function parameters after a single oral dose of 0.8 mg of alprazolam. Open squares, extensive metabolizer group (n = 6); solid squares, poor metabolizer group (n = 6) of S-mephenytoin 4-hydroxylation. (A) Scores on the Digit Symbol Substitution Test (DSST). (B) Scores on the Visual Analog Scale (VAS) (only the item Thinking Speed for brevity). (C) Scores on the UKU Side Effect Rating Scale (the item Sleepiness) are shown in A, B, and C. For the DSST and VAS each time point is the mean increase or decrease over the predose baseline score at the corresponding postdose times, and for the UKU Scale it is the mean raw score. There were no significant differences in any parameters between the two groups.

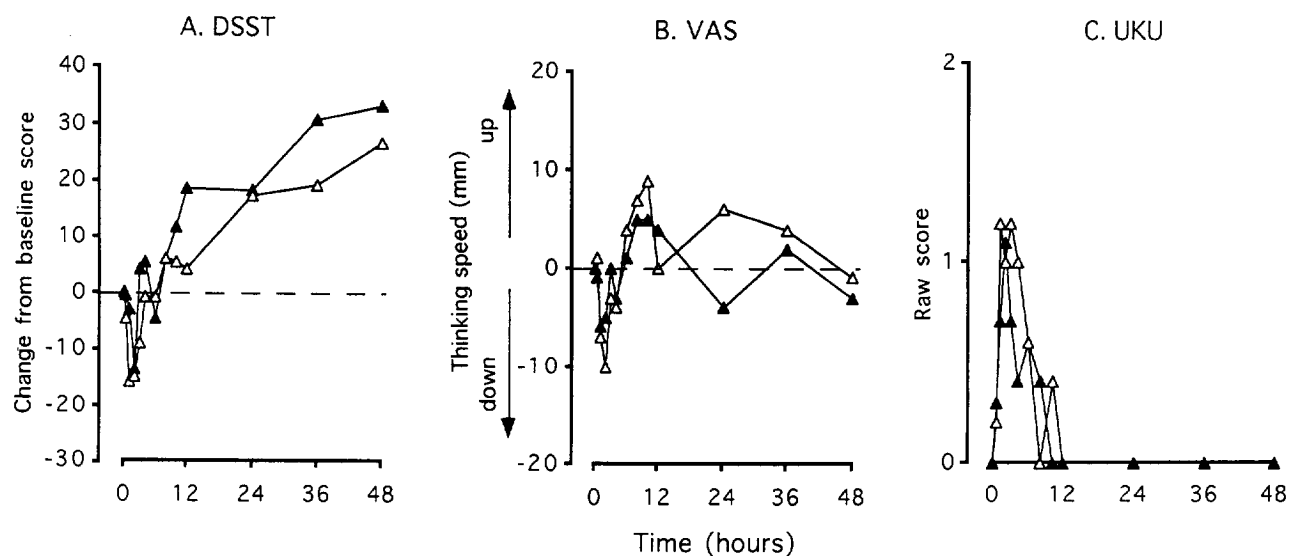


Figure 4. Mean psychomotor function parameters after a single oral dose of 0.8 mg of alprazolam. *Open triangles*, nonsmoker group ($n = 5$); *solid triangles*, smoker group ($n = 7$). **(A)** Scores on the Digit Symbol Substitution Test (DSST). **(B)** Scores on the visual Analog Scale (VAS) (only the item Thinking Speed for brevity). **(C)** Scores on the UKU Side Effect Rating Scale (the item Sleepiness) are shown in A, B, and C. For the DSST and VAS each time point is the mean increase or decrease over the predose baseline score at the corresponding postdose times, and for the UKU Scale it is the mean raw score. There were not significant differences in any parameters between the two groups.

The PM phenotype of S-mephenytoin 4-hydroxylation resulting from deficient CYP2C19 activity is inherited as an autosomal recessive trait (de Morais et al. 1994; Goldstein and de Morais 1994) and occurs with a frequency of about 13% to 23% in Asian populations (Horai et al. 1989; Wilkinson et al. 1989; Sohn et al. 1992a; Goldstein and de Morais 1994) and about 3% to 5% in Caucasian populations (Küpfer and Preisig 1984; Wilkinson et al. 1989; Alvan et al. 1990). The gene encoding for CYP2C19 is located in the long arm of chromosome 10 (de Morais et al. 1994; Goldstein and de Morais 1994), and CYP2C19_{m1} and CYP2C19_{m2} have been reported as the mutant genes causing deficient isoenzyme activity (Goldstein and de Morais 1994). Representative drugs for which metabolism cosegregates with genetically determined CYP2C19 status include diazepam (Bertilsson et al. 1989; Sohn et al. 1992a), imipramine (Dahl and Bertilsson 1993; Chiba et al. 1994; Koyama et al. 1994; Spina and Caputi 1994), omeprazole (Sohn et al. 1992b; Chiba et al. 1993), mephobarbital (Wilkinson et al. 1989; Goldstein and de Morais 1994) and propranolol (Wilkinson et al. 1989; Goldstein and de Morais 1994).

The mean pharmacokinetic parameters of alprazolam in our subjects were found to be compatible with those reported previously (Greenblatt and Wright 1993). The very similar kinetic profiles in the EM and PM groups (Table 1) strongly indicate that CYP2C19 is not involved extensively in the metabolism of alprazolam. Therefore, the interindividual variability in the clearance of this triazolobenzodiazepine cannot be explained by the genetically determined CYP2C19 activity. We

also presume that competitive inhibition of alprazolam metabolism by substrates of CYP2C19 is unlikely to occur. In contrast, the metabolism of diazepam cosegregates with that of omeprazole, a substrate for CYP2C19 (Sohn et al. 1992b; Chiba et al. 1993) and is inhibited by omeprazole (Gugler and Jensen 1985; Andersson et al. 1990; Ishizaki et al. 1995).

Fluvoxamine, a selective serotonin reuptake inhibitor, which is a specific inhibitor of CYP1A2 (Brøsen et al. 1993; Rasmussen et al. 1995), inhibits the 4- and α -hydroxylation of alprazolam in vitro (von Moltke et al. 1995) and increases plasma alprazolam concentrations in vivo in humans (Fleishaker and Hulst 1994), suggesting that CYP1A2 is involved in the metabolism of alprazolam. Because this isoform is inducible by smoking (Bock et al. 1994), it was assumed that smokers would show a greater clearance of alprazolam than nonsmokers. However, in agreement with two previous studies (Smith et al. 1983; Fleishaker and Hulst 1994) reporting a negligible effect of smoking on the clearance of alprazolam, the present study showed no significant difference in clearance between the smoker and nonsmoker groups (Table 1). We have no reasonable explanation for the discrepancy between the effects caused by fluvoxamine [an inhibitor of CYP1A2 (Brøsen et al. 1993; Rasmussen et al. 1995)] and cigarette smoke [an inducer of CYP1A2 (Bock et al. 1994)] on the metabolism of alprazolam.

The elimination half-life was significantly shorter in the smoker group than in the nonsmoker group (Table 1). We have no clear explanation for this result, as neither the CL_{oral} nor the V_d , which are the determinants of

half-life [i.e., $t_{1/2} = 0.693 \cdot V_d / CL_{oral}$ (Rowland and Tozer 1995)], differed significantly between the two groups.

The present study thus indicates that neither S-mephenytoin 4-hydroxylation capacity nor self-reports of extensive cigarette smoking has a major impact on the metabolism of alprazolam. These were reflected in the results of pharmacodynamic assessment; there was little difference in the psychomotor function parameters both between the EM and PM groups (Figure 3) and between the nonsmoker and smoker groups (Figure 4).

Thus, the involvement of CYP2C19 in the metabolism of alprazolam is unlikely, and that of CYP1A2 remains inconclusive. However, a recent in vitro study using human liver microsomes (von Moltke et al. 1994) has shown that ketoconazole, a relatively specific inhibitor of CYP3A4 (Watkins 1994), inhibits the 4- and α -hydroxylation of alprazolam, suggesting that CYP3A4 rather than CYP1A2 is involved in the metabolism of this triazolobenzodiazepine in humans. Obviously, to confirm this in vitro finding (von Moltke et al. 1994), an in vivo human study where the metabolism of alprazolam is assessed with and without a candidate substrate or inhibitor [e.g., erythromycin, ketoconazole (Watkins 1994)] is definitely required.

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