

EFFECTS OF CYP3A4 INHIBITION BY DILTIAZEM ON PHARMACOKINETICS AND DYNAMICS OF DIAZEPAM IN RELATION TO CYP2C19 GENOTYPE STATUS

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ABSTRACT:

Diazepam is metabolized by CYP2C19 and CYP3A4 in the liver. CYP2C19 shows genetic polymorphism associated with the poor metabolizer (PM) and extensive metabolizer (EM) phenotypes. The aim of this study was to assess the effect of diltiazem, a CYP3A4 inhibitor, on pharmacokinetics and dynamics of diazepam in relation to CYP2C19 genotype status. Thirteen healthy volunteers (eight EMs and five PMs) were given placebo or diltiazem (200 mg) orally for 3 days before and for 7 days after the oral 2-mg dose of diazepam in a double-blind, randomized, crossover manner. The pharmacokinetics and pharmacodynamics of diazepam were assessed with and without diltiazem. Plasma concentrations and area under the plasma concentration-time curves (AUCs) of diazepam and *N*-desmethyldiazepam were significantly greater in the PM compared with the EM group during the placebo phase. Diltiazem significantly increased AUC and prolonged elimination $t_{1/2}$ of diazepam in both the PM and EM groups. These pharmacokinetic changes, however, caused no significant difference in the pharmacodynamics between the two trial phases. Diltiazem affects the pharmacokinetics of diazepam in the PM and EM groups of CYP2C19. Inhibition of CYP3A4 by a concomitant substrate drug like diltiazem may cause a pharmacokinetic interaction with diazepam irrespective of CYP2C19 genotype status, but whether this interaction would reflect a pharmacodynamic change of diazepam remains unconfirmed by our study.

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Diazepam, which is widely used as a muscle relaxant, sedative, anxiolytic, and anticonvulsant, is metabolized to *N*-desmethyldiazepam by a genetically determined enzyme, *S*-mephenytoin 4'-hydroxylase (CYP2C19) (Bertilsson et al., 1989). It has been reported that omeprazole (Sohn et al., 1992a; Chang et al., 1995; Ieiri et al., 1996) and fluvoxamine (Xu et al., 1996; Jeppesen et al., 1997), known as a substrate/inhibitor of CYP2C19, affects the pharmacokinetic disposition of diazepam (Gugler and Jensen, 1984, 1985; Perucca et al., 1994).

The pharmacogenetic entity of CYP2C19 has shown a marked interethnic difference in the incidence of poor metabolizers (PMs¹); the PM frequency is much greater in Japanese (18–23%) (Nakamura et al., 1985; Horai et al., 1989; Kubota et al., 1996) than in American or European white populations (3–5%) (Nakamura et al., 1985; Wilkinson et al., 1989). At least two different mutations associated

with the PM genotypes of CYP2C19 were determined by de Morais et al. (1994a,b). CYP2C19 has a wild-type (wt) gene and mutations at two sites, i.e., CYP2C19 m1 in exon 5 (m1) and CYP2C19 m2 in exon 4 (m2), and combination of both mutations leads to a reduced activity of the enzyme (de Morais et al., 1994a,b). Individuals with homozygous m1/m1 and m2/m2 or heterozygous m1/m2 are PMs, whereas those with heterozygous m1/wt and m2/wt are hetero-type extensive metabolizers (EMs), and those with homozygous wt/wt are homo-type EMs. CYP2C19 m1 and CYP2C19 m2 allele variants account for the reported Japanese PMs (de Morais et al., 1994a). The detection of m1 and m2 concordantly predicts the phenotypes of CYP2C19 in a Japanese population (Kubota et al., 1996).

Several previous studies have reported that the pharmacokinetic and pharmacodynamic differences of diazepam were observed between the CYP2C19-related EM and PM groups (Andersson et al., 1990; Sohn et al., 1992b; Ishizaki et al., 1995; Wan et al., 1996). These studies seemed to focus mainly on the association between the pharmacokinetics of diazepam and CYP2C19 genotype status, although CYP2C19 and CYP3A contribute to microsomal *N*-desmethylation of diazepam at low-substrate concentrations, and CYP3A is a major enzyme of 3-hydroxylation to temazepam (Yasumori et al., 1993, 1994; Andersson et al., 1994; Jung et al., 1997; Yang et al., 1999). Meanwhile, grapefruit juice, a potent inhibitor of CYP3A4 in the small intestine, has been reported to increase the bioavailability of diazepam (Ozdemir et al., 1998). Thus, CYP3A4 might play an important role in the absorption or presystemic disposal phase of diazepam. For this reason, in the PMs of CYP2C19, the main metabolic

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¹ Abbreviations used are: PM, poor metabolizer; wt, wild-type; m1, mutation in exon 5; m2, mutation in exon 4; EM, extensive metabolizer; HPLC, high-pressure liquid chromatography; AUC, area under the plasma concentration-time curve.

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pathway of diazepam might be shifted from CYP2C19 to CYP3A4 because diazepam is *N*-desmethylated by CYP2C19 but 3-hydroxylated to temazepam by CYP3A4, which is not genetically determined in humans. Therefore, a drug interaction is assumed to occur when diltiazem, which has been reported to inhibit the metabolic activity of CYP3A4 (Kosuge et al., 1997; Sutton et al., 1997), is coadministered with diazepam.

With the background mentioned above, we intended to assess whether diltiazem would really affect the pharmacokinetic disposition of diazepam, thereby causing a possible pharmacodynamic interaction of diltiazem with diazepam in relation to the CYP2C19 genotype status.

Materials and Methods

Subjects. Thirteen healthy subjects (ranging from 22–36 years old and weighing from 55–85 kg) participated in this study. The subjects had not taken any drugs at least 1 week before and during the study. The written informed consent had been obtained from each of the subjects before participation in the study. The Ethical Committee of Hamamatsu University School of Medicine, Hamamatsu, Japan, approved this study.

Study Design. This study was conducted according to a randomized crossover and two-period design. Each trial was performed at an interval of at least 1-month washout. After the oral administration of 200 mg of diltiazem (Herbesser R, sustained release capsule; Tanabe Pharmaceutical Co., Ltd., Osaka, Japan) or a matched placebo once daily for 3 days, each of the subjects received a single-oral dose of 2 mg of diazepam (Cercine; Takeda Chemical Industries, Ltd., Osaka, Japan) with 150 ml of tap water and fasted for 3 h. The oral dose of 200 mg of diltiazem or the placebo was administered for 7 days after the diazepam dosing (i.e., in total, 10 doses of diltiazem or placebo). Subjects were not allowed to smoke or ingest alcohol, coffee, tea, cola, or grapefruit (including juice) during the study phases.

Venous blood samples for determining plasma concentrations of diazepam and *N*-desmethyldiazepam were obtained before and at 2, 4, 8, 12, 24, 48, 72, 96, 144, 192, 264, 336, and 408 h after the administration of diazepam. Three psychomotor tests, a subjective test, a critical flicker fusion test, and a postural sway test, as described by Spiegel and Aebi (1981), were performed at each blood-sampling time and at 1, 3, and 6 h after diazepam dosing.

Genotyping Procedure for CYP2C19. Genotyping procedures for identifying the CYP2C19 wild-type gene and two mutated alleles, CYP2C19 m1 in exon 5 and CYP2C19 m2 in exon 4, were performed by a polymerase chain reaction-restriction fragment length polymorphism method, as reported by Kubota et al. (1996).

Determination of Diazepam and Its Metabolite Concentrations in Plasma. Blood samples were drawn into heparinized tubes. The plasma was immediately separated from blood cells. Each sample was stored at -30°C until the assay. Plasma concentrations of diazepam and its metabolite (*N*-desmethyldiazepam) were measured by the high-pressure liquid chromatographic (HPLC) method (Sohn et al., 1992b) with minor modifications, as described below.

Diazepam was purchased from Wako Pure Chemical Industries (Osaka, Japan) and *N*-desmethyldiazepam was supplied by the Tanabe Seiyaku (Osaka, Japan). Nitrazepam was used as an internal standard, and all solvents for the HPLC grade were purchased from Wako Pure Chemical Industries. The HPLC system consisted of AS-8020 auto-sampler, DP-8020 pump, UV-8020 ultraviolet detector, CO-8020 column oven, and TSK-GEL ODS-80Ts column (150 × 4.6-mm internal diameter) (TOSOH, Tokyo, Japan).

Nitrazepam (10 $\mu\text{g}/\text{ml}$ in methanol) in 50 μl and 1 ml of saturated trisodium phosphate was added to 1-ml plasma sample. The sample was shaken with 5 ml of dichloromethane for 20 min. The organic layer was evaporated to dryness with nitrogen gas at 40°C . The residue was dissolved in 250 μl of the mobile phase, and 90 μl of the sample was injected into the HPLC system, as described above. The wavelength was set at 240 nm. The mobile phase consisted of methanol, 50 mM sodium phosphate, pH 5.3, and triethylamine (60/40/0.1). The flow rate was 1.0 ml/min at 40°C . The lowest detectable limits of both diazepam and *N*-desmethyldiazepam were 3 ng/ml. Retention times were 5.2 min for nitrazepam, 9.4 min for *N*-desmethyldiazepam, and 11.1 min for diazepam.

Pharmacokinetics Analysis. The elimination half-life ($t_{1/2}$) of diazepam was obtained by the log-linear regression of the terminal phase of the concentration-time data for at least four points. The area under the plasma concentration-time curves (AUCs) of diazepam and *N*-desmethyldiazepam were calculated by the trapezoidal rule. Diazepam apparent oral clearance was calculated as $\text{dose}/\text{AUC}_{0-\infty}$, where the dose is 2 mg of the base, and the $\text{AUC}_{0-\infty}$ was calculated as AUC_{0-144} plus $\text{AUC}_{144-\infty}$. The $\text{AUC}_{144-\infty}$ was determined as C/λ , where C is the last assayed concentration, and λ is the elimination rate constant.

Pharmacodynamics Analysis. The pharmacodynamic tests were conducted according to the methods described in a textbook by Spiegel and Aebi (1981) as follows: subjective effects were evaluated with a visual analog scale that was employed with use of the 16 questions, and subjects had to mark on the 100-mm-long line to show the degree of their feeling. The discrimination of the fusion of a flickering red light was measured in the critical flicker fusion test. The value of measurement used for the fusion time was flicks per second. Data were obtained from a digital flicker (DF-1; Sibata Chemical, Tokyo, Japan). The postural sway was measured by a swaymeter (G5500; Anima, Tokyo, Japan) for the 60-s period with eyes closed. The moving length for 60 s was used as the test result.

Statistical Analysis. Results of pharmacokinetics parameters are expressed as mean \pm S.E. Data were analyzed with the statistical program StatView for Macintosh, version 4.5 (Abacus Concepts, Inc., Berkeley, CA). The paired t test was used for the mean pharmacokinetic parameters of diazepam to compare the data between the two trial phases, and the unpaired t test was used to compare the data between the PM and EM groups of CYP2C19. For evaluating the mean pharmacodynamic parameters, analysis of variance was used with the repeated measures. Values of $p < 0.05$ were taken to indicate statistical significance.

Results

No clinically important adverse events, including changes in blood pressure, pulse rate, and other physiological responses, were recognizable throughout the study phases. All subjects completed the study following the protocol.

Among the 13 subjects, three were homozygous EMs (wt/wt), five were heterozygous EMs (wt/m1 or wt/m2), one was homozygous PM (m1/m1), and four were heterozygous PMs (m1/m2). These subjects were arbitrarily classified into the two groups as follows: the extensive metabolizer group (wt/wt, wt/m1, and wt/m2; $n = 8$) and the poor metabolizer group (m1/m2 and m1/m1; $n = 5$). The mean (\pm S.E.) age and weight in the EM and PM groups were 24.4 ± 1.8 (range, 22–36) and 26.8 ± 1.7 (range, 23–31) years and 68.6 ± 3.9 (range, 55–85) and 75.0 ± 2.7 (range, 67–83) kg, respectively. These mean demographic data did not significantly differ between the two groups.

The mean plasma concentration-time data on diazepam and *N*-desmethyldiazepam after the single-oral 2-mg dose of diazepam during the placebo or diltiazem phase in the EM and PM groups are shown in Fig. 1. The mean pharmacokinetic parameters of diazepam and *N*-desmethyldiazepam are summarized in Table 1. In the placebo trial, mean plasma concentrations (Fig. 1) and AUC_{0-144} of diazepam (Table 1) were significantly higher ($p < 0.05$) in the PM group compared with those in the EM group. The mean AUC_{0-408} of *N*-desmethyldiazepam, a major metabolite of diazepam, was also significantly higher ($p < 0.05$) in the PM compared with in the EM group (Table 1). Coadministration of diltiazem significantly increased ($p < 0.05$) the AUC_{0-144} , prolonged the elimination $t_{1/2}$, and decreased the oral clearance of diazepam both in the EM and PM groups (Table 1). Diltiazem significantly increased ($p < 0.05$) the AUC_{0-408} of *N*-desmethyldiazepam in the PM group. This trend was also observed, but not significantly, in the EM group (Table 1).

Diltiazem did not produce any statistically significant pharmacodynamic changes after the oral administration of diazepam in the EM or PM group (Fig. 2).

Discussion

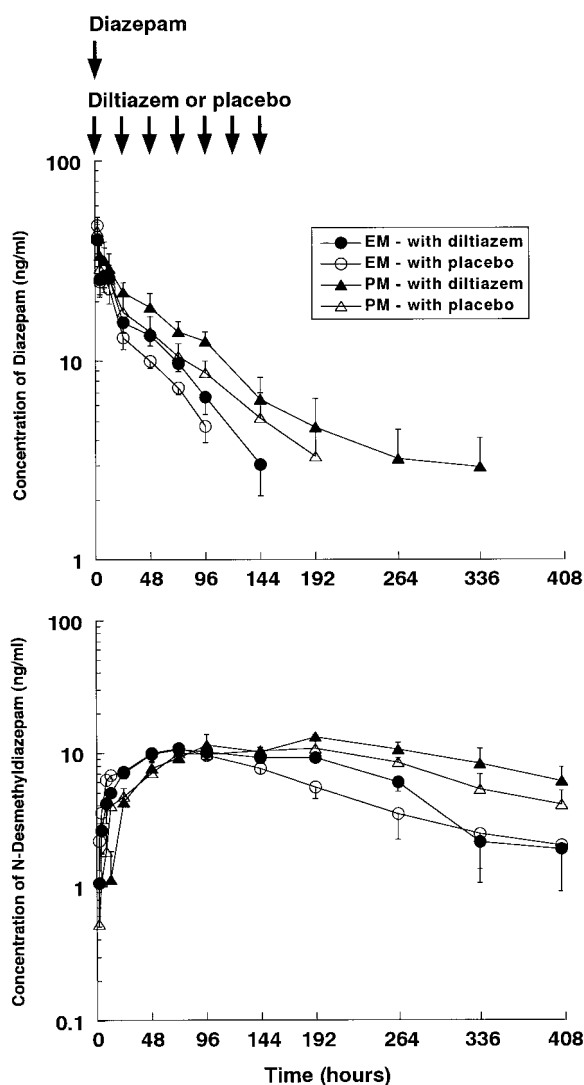


Fig. 1. Mean concentrations of diazepam and *N*-desmethyldiazepam after an oral diazepam.

Mean (\pm S.E.) concentrations of diazepam (upper panel) and *N*-desmethyldiazepam (lower panel) after an oral 2-mg dose of diazepam are shown during the placebo and diltiazem trial phases in the EM (circles) and PM (triangles) groups. An oral, once-daily regimen of placebo or diltiazem (200 mg) was given for 3 days before the diazepam dosing (not shown in Fig.), and the same regimen was repeatedly conducted for 7 days after the diazepam dosing as illustrated.

Our study revealed that diltiazem, an inhibitor of CYP3A4 (Kosuge et al., 1997; Sutton et al., 1997; Jones et al., 1999; Mayhew et al., 2000), significantly increased the AUC and prolonged the elimination $t_{1/2}$ of diazepam in both the PM and EM groups classified in terms of the CYP2C19 genotypic status. Human liver microsomal studies have indicated that diazepam was mainly metabolized by CYP3A4 to temazepam and partly by CYP3A4 and CYP2C19 to *N*-desmethyldiazepam (Yasumori et al., 1993, 1994; Andersson et al., 1994; Jung et al., 1997; Yang et al., 1999). Recently, grapefruit juice, which is known as an inhibitor of CYP3A4 in enterocytes, has been reported to markedly increase the bioavailability of diazepam (Ozdemir et al., 1998), suggesting that CYP3A4 is involved in the presystemic disposal of diazepam at the gut site. Our results indicated that the metabolic pathway mediated via CYP3A4 contributed to the overall metabolism of diazepam not only in the PM group but also in the EM group to a substantial extent, although the kinetic disposition of diazepam has been known to differ between the healthy CYP2C19-related EM and PM individuals (Bertilsson et al., 1989; Sohn et al., 1992b; Ishizaki et al., 1995; Qin et al., 1999).

In the mean pharmacokinetic parameters of diazepam with placebo, the AUC_{0-144} and $t_{1/2}$ in the PM group were significantly greater than those in the EM group. The mean AUC_{0-408} of *N*-desmethyldiazepam in the PM group was also significantly greater than that in the EM group. These results were in agreement with previous studies in which the pharmacokinetic profiles of diazepam depend on the activity of CYP2C19 enzyme (Bertilsson et al., 1989; Ishizaki et al., 1995; Qin et al., 1999).

Coadministration of diltiazem significantly increased the AUC_{0-408} of *N*-desmethyldiazepam, but not the AUC_{0-144} , in the PM group. This trend was also observed in the EM group (Table 1). This observation can be explained by the assumption that further metabolism of *N*-desmethyldiazepam may be mediated via CYP3A4 of which the enzymatic activity might have been inhibited by diltiazem, and *N*-desmethyldiltiazem accumulated during the repeated doses of diltiazem. This assumption appears to be supported by the previous findings that 1) *N*-desmethyldiltiazem is a stronger inhibitor of CYP3A4 than diltiazem (Sutton et al., 1997), and 2) the clearance of diltiazem is decreased by *N*-desmethyldiltiazem, and diltiazem itself, during a prolonged treatment of diltiazem (Abernethy and Montamat, 1987; Montamat and Abernethy, 1987). Furthermore, the two recent studies have revealed one of the inhibitory mechanisms of diltiazem; the metabolite of diltiazem formed a metabolite intermediate complex with CYP3A4, which is an inactive enzyme form, thereby leading to

TABLE 1

Mean pharmacokinetic parameters of diazepam and *N*-desmethyldiazepam in EM and PM groups with diltiazem or placebo

Values represent the mean \pm S.E.

Parameters	EMs (n = 8)		PMs (n = 5)	
	With Diltiazem (A)	With Placebo (B)	With Diltiazem (C)	With Placebo (D)
Diazepam				
AUC_{0-144} (ng · h/ml)	1620 \pm 141 ^{a,c}	1302 \pm 92	2333 \pm 275 ^e	1858 \pm 237 ^f
CL/F (ml/min/kg)	0.146 \pm 0.015 ^{a,c}	0.184 \pm 0.023	0.093 \pm 0.016 ^e	0.119 \pm 0.020
$t_{1/2}$ (h)	65.0 \pm 20.5 ^{b,c}	45.6 \pm 12.9	103.8 \pm 40.2 ^e	77.0 \pm 28.6 ^f
<i>N</i>-Desmethyldiazepam				
AUC_{0-144} (ng · h/ml)	1277 \pm 140	1259 \pm 84	1159 \pm 111	1144 \pm 69
AUC_{0-408} (ng · h/ml)	2714 \pm 156 ^d	2275 \pm 263	3794 \pm 245 ^e	3203 \pm 274 ^f

^a $p < 0.05$ in A versus B.

^b $p < 0.01$ in A versus B.

^c $p < 0.05$ in A versus C.

^d $p < 0.01$ in A versus C.

^e $p < 0.05$ in C versus D.

^f $p < 0.05$ in B versus D.

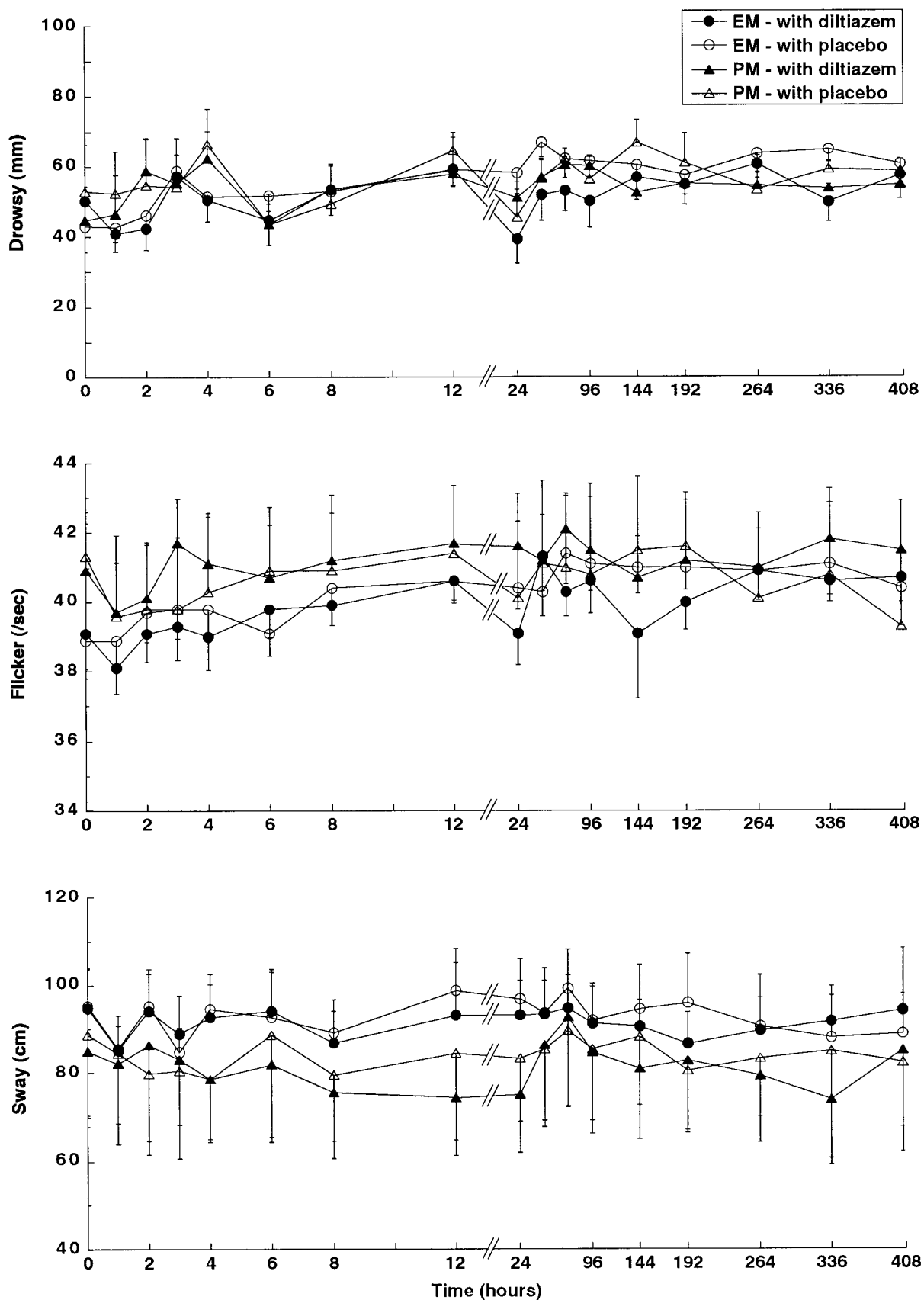


FIG. 2. Time courses of three pharmacodynamic assessment parameters.

Time courses of three pharmacodynamic assessment parameters, subjective drowsiness (visual analog scale), critical flicker fusion test, and postural sway (mean \pm S.E.) are shown after an oral 2-mg dose of diazepam in the EM and PM groups during the placebo or diltiazem trial phase.

a drug-drug interaction of diltiazem (Jones et al., 1999; Mayhew et al., 2000).

We have reported that a more than 3-day repeated dosing scheme of diltiazem could cause a substantial CYP3A4 inhibition (Ohashi et al., 1993). We have also reported that a 3-day diltiazem treatment caused a sufficient inhibition of triazolam metabolism (Kosuge et al., 1997). However, the maximum CYP3A4 inhibition by diltiazem occurred at a plateau phase during the 6-day dosing (Ohashi et al., 1993). Furthermore, the inhibitory effect of diltiazem might be maintained for several days after the end of diltiazem dosing. For the reasons discussed above, we evaluated both the AUC values for *N*-desmethyldiazepam during the 0- to 144- and 0- to 408-h postdose periods of the diltiazem phase after the diazepam administration and compared the postdiltiazem *N*-desmethyldiazepam AUCs with the same respective AUC values obtained during the placebo trial period (Table 1).

We observed that any psychomotor function parameters assessed for the pharmacodynamics of diazepam did not differ between the placebo-diazepam and diltiazem-diazepam trial phases (Fig. 2), despite diltiazem increasing plasma diazepam concentrations significantly compared with placebo (Fig. 1; Table 1). This observation is difficult to interpret because the pharmacokinetic interaction effect of diltiazem on diazepam was not reflected by any significant change in the psychomotor function parameters we assessed in the study (Fig. 2). Nevertheless, we wish to offer our assumptions for this observation as follows. First, because we were concerned about the possible side effect(s) (e.g., an excessive sedation, drowsiness) in our volunteers, particularly in the PM individuals, we did not administer the usual initial dose of diazepam (e.g., 5 mg indicated for Japanese patients). Thus, the single-oral 2-mg dose used in our study might have not been sufficient to elicit the pharmacodynamic changes, although the pharmacokinetic changes were successfully detectable (Table 1). Second, another possible explanation is that our pharmacodynamic assessment methods (Fig. 2) may not be sensitive enough to detect any change and/or may not have a sufficient power for the limited sample size (i.e., $n = 13$) in the psychomotor function status. We did not use other psychomotor function tests, which have successfully been used to detect the pharmacokinetic-dynamic relationship of several benzodiazepines in humans (Greenblatt et al., 1989a,b). Nevertheless, it has been shown in a cimetidine-diazepam interaction study that, despite an increase in diazepam concentration by about 60% during the treatment with cimetidine, only minimal changes were observed in the clinical pharmacodynamic effects (Greenblatt et al., 1984). Similarly, in a fluoxetine-diazepam interaction study (Lemberger et al., 1988), despite that the significant increment of plasma diazepam concentration occurred by about 50% with the coadministration of fluoxetine, no psychopharmacological changes were detected using the pharmacodynamic assessment tests similar to those we used. In addition, because benzodiazepines have a wide therapeutic index (Mandelli et al., 1978; Shader and Greenblatt, 1993), an increase of about 25% in the AUC_{0-144} of diazepam by diltiazem (Table 1) appears unlikely to lead to any significant change in the psychomotor function variables assessed, which is apparently compatible with the cimetidine-diazepam (Greenblatt et al., 1984) or fluoxetine-diazepam interaction study (Lemberger et al., 1988).

In closing, our results showed that diltiazem affects the pharmacokinetics, but not the pharmacodynamics, of diazepam both in the PM and EM groups of CYP2C19. Thus, whether a pharmacokinetic diazepam-diltiazem interaction would have any clinical consequence remains unknown based upon our results. Nevertheless, caution may be needed in clinical practice when diazepam is coprescribed with CYP3A4-related substrates/inhibitors like diltiazem irrespective of

the genetically determined CYP2C19-related genotypic or phenotypic polymorphism status.

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