Enhanced effect of triazolam with diltiazem

Kazuhiro Kosuge, Masahiko Nishimoto, Masahiko Kimura, Kazuo Umemura,¹ Mitsuyoshi Nakashima¹ & Kyoichi Ohashi

Department of Clinical Pharmacology and ¹Department of Pharmacology, Hamamatsu University School of Medicine, Hamamatsu, Japan

Aims Triazolam, a triazolobenzodiazepine hypnotic agent, is metabolized by CYP3A4. Diltiazem is an inhibitor of this isozyme. The aim of this study was to determine if diltiazem affects plasma concentrations of triazolam in humans.

Methods We investigated the interaction between triazolam and diltiazem in a randomized, three-phase crossover study. Seven healthy male volunteers received orally either a single 0.25 mg dose of triazolam, a 0.25 mg dose of triazolam after a 3-day treatment of diltiazem (180 mg day⁻¹), or a placebo. Plasma samples were collected to determine triazolam concentration over a 24 h period. The pharmacodynamic effects of triazolam were investigated using the peak saccadic velocity of eye movements (PSV), electroencephalogram (EEG), and visual analogue scale (VAS) through 8 h.

Results Diltiazem pretreatment significantly increased the area under the triazolam concentration–time curve (8.0 ± 2.4 to 18.2 ± 3.1 ng ml⁻¹ h; $P < 0.001$; mean $\pm$ s.d.). Peak triazolam concentration was increased (2.1 ± 0.7 to 3.6 ± 1.0 ng ml⁻¹, $P < 0.05$) and the elimination half-life prolonged (4.1 ± 2.1 to 7.6 ± 1.9 h; $P < 0.01$). The PSV, EEG, and VAS of the triazolam plus diltiazem group revealed significant differences from the triazolam alone group or the control placebo group.

Conclusions Diltiazem markedly affects the pharmacokinetics of triazolam and increases the intensity of its sedative effects. Inhibition of CYP3A isozyme by diltiazem may explain the observed pharmacokinetic interaction. Therefore, triazolam should be avoided when patients are using diltiazem.

Keywords: triazolam, diltiazem, drug interaction, pharmacokinetics

Introduction

The calcium channel blocker diltiazem is widely used for treatment of hypertension, angina pectoris, arrhythmias, and other cardiovascular diseases. Diltiazem has been reported to inhibit hepatic enzyme metabolism in combination with many therapeutic agents that are metabolized by the CYP3A isozyme, for example, nifedipine, carbamazepine, propranolol and cyclosporine [1]. Ohashi *et al.* [2, 3] showed that the metabolism of nifedipine, a substrate for CYP3A4, was affected by the concomitant use of diltiazem, revealing that the effect was dose-dependent and was maintained on a plateau during repeated administration.

Triazolam is a short-acting hypnotic, widely used for treatment of insomnia. Triazolam is metabolised during its absorption and elimination phases by CYP3A4 isozyme [4]. A recent study [5] has reported that metabolism of triazolam was inhibited potently by ketoconazole and itraconazole. Midazolam, which resembles triazolam in its chemical structure, is metabolized via hydroxylation by CYP3A4. In other reports, the metabolism of midazolam was inhibited by ketoconazole, itraconazole [6], diltiazem and verapamil [7]. However, there have been only limited reports on the interaction between triazolam and diltiazem [8]. Some

reported cases in which triazolam use caused amnesia may have involved this drug interaction. Therefore, we intended to investigate the interaction between triazolam and diltiazem in terms of the pharmacokinetics and pharmacodynamics of triazolam.

Methods

Study design

Seven healthy male volunteers participated in a randomized, double-blind, three-phase crossover study at intervals of at least 1 week. The subjects, having given written informed consent, ranged in age from 20 to 22 years and weighed 71.1 ± 13.2 (mean $\pm$ s.d.) kg. This study was approved by the Institutional Review Board of Hamamatsu University School of Medicine. No subject was taking any medication. The subjects received two different pretreatments: 60 mg diltiazem (two 30 mg tablets, Tanabe Pharmaceutical Company, Ltd) or a matched placebo three times daily for 3 days and 1 h before triazolam administration (in a total of ten doses). Subjects were unaware of declining blood pressure, pulse rate, and other physiological responses during the administration of diltiazem. The subjects took the three treatments in a randomized a cross-over manner: (1) placebo with placebo pretreatment (control); (2) triazolam with placebo pretreatment (placebo + triazolam); and (3) triazolam

Correspondence: Dr Kazuhiro Kosuge, Department of Clinical Pharmacology, Hamamatsu University, School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan

with diltiazem pretreatment (diltiazem + triazolam). The subjects took each dose of 0.25 mg triazolam (0.25 mg tablet, Upjohn, Ltd) or placebo with 150 ml of tap water and remained fasting for 3 h after dosing. They were not allowed to smoke or ingest alcohol, coffee, tea, or other caffeine containing drinks on the study days.

Blood sampling and determination of triazolam

Blood samples were drawn into heparinized tubes before(0) and at 0.5, 1, 2, 3, 4, 6, 8 and 24 h after the drug was taken. The plasma was immediately separated and stored at -30°C until the assay.

Plasma triazolam concentration was measured by gas-chromatography mass-spectrometry [9] using diazepam as an internal standard. The sensitivity of the method was 0.2 ng ml^{-1} and the coefficient of variation was 6.3, 5.3, and 1.4% at 0.5, 2.0, and 10.0 ng ml^{-1} , respectively.

Pharmacokinetics analysis

After oral administration of triazolam, peak concentrations (C_{max}) and time to reach the C_{max} (t_{max}) were read from the actual observed data. Elimination half-life ($t_{1/2}$) was obtained by log-linear regression of the terminal phase of the concentration-time curve. The area under the triazolam plasma concentration-time curve (AUC(0,8 h)) was calculated by the trapezoidal rule.

Pharmacodynamics analysis

Peak saccadic velocity of eye movements (PSV), as described by Van *et al.* [10] was measured before and at 0.5, 1, 2, 3, 4, 5, 6, and 8 h postdose in each of the three trial phases, and the stimulated horizontal displacement (SLE-5100: Nihon Koden Corporation, Tokyo, Japan) at red lights of $50 (\pm 25)$ degrees, at random intervals, was recorded. The subjects, as quickly as possible, responded to and followed a signal for 30 s. A NeuroPack 5880 (Nihon Koden Corporation, Tokyo, Japan) with high cut filter at 100 Hz was used for the signal collection and analysis.

Electroencephalogram (EEG) registrations were made using silver-silver chloride electrodes at Fp_1 , Fp_2 , O_1 , and O_2 with a common ground electrode at Fp_z (international 10–20 method). Electrode resistances were kept below 10 k Ω . During recordings, the subjects were seated in a chair with their eyes closed for 3 min. The signals were obtained from NeuroPack 5880 with high cut filter at 100 Hz, and recorded on a 16 channel digital audio recorder (PC216: SONY, Tokyo, Japan) with a sampling frequency of 6 kHz. For fast-Fourier transform, EEG data were collected using a signal-processor (DP1200A: Nihon Denki Sanei, Tokyo, Japan), and analysed by summation of 24 sessions of 5 s each. The first-Fourier transform was performed to obtain the sum of amplitude at the delta (2.0–4.0 Hz), theta (4.0–8.0 Hz), α_1 (8.0–10.0 Hz), α_2 (10.0–13.0 Hz), β_1 (13.0–20.0 Hz), and β_2 (20.0–30.0 Hz) frequency ranges.

Visual analogue scale (VAS) was employed with use of the 16 questions with a 100 mm-long line. The sides of the lines signed the question in the subject's native language.

Recordings were made before and at 0.5, 1, 2, 3, 4, 5, 6 and 8 h after taking the dose.

Statistical analysis

Results are expressed as mean values $\pm$ s.d. Data were analyzed with the statistical programme StatView for Macintosh, version 4.5 (Abacus Concepts, Inc, Berkeley, CA, U.S.A.). The paired *t*-test was used for pharmacokinetic parameters of triazolam to compare each condition. On the pharmacodynamic parameters, the initial analysis with ANOVA with repeated measures was used and *a posteriori* testing was done to each trial phase group by one-way ANOVA.

Results

Pharmacokinetics

Diltiazem pretreatment increased the mean AUC of triazolam by about 2.3-fold (from 8.0 ± 2.4 to $18.2 \pm 3.1\text{ ng ml}^{-1}\text{ h}$; $P < 0.001$) and the mean C_{max} by about 1.7-times (from 2.1 ± 0.7 to $3.6 \pm 1.0\text{ ng ml}^{-1}$; $P < 0.05$). In addition, the pretreatment prolonged the mean half-life of triazolam by around 1.8 times (from 4.1 ± 2.1 to $7.6 \pm 1.9\text{ h}$; $P < 0.01$) in comparison to the triazolam trial without diltiazem pretreatment (Figure 1; Table 1). The kinetic parameters ranged interindividually: 1.3 to 3.4 times

Figure 1 Plasma concentration of triazolam (mean $\pm$ s.d.) after an oral dose of 0.25 mg triazolam following pretreatment with oral diltiazem 60 mg (●) or placebo (○) three times daily for 3 days in seven healthy volunteers.

Table 1 The pharmacokinetic variables of triazolam 0.25 mg (mean $\pm$ s.d.) following pretreatment with oral diltiazem 60 mg or placebo three times daily for 3 days in seven healthy volunteers.

	Triazolam	Triazolam + diltiazem
$t_{1/2,\alpha}$ (h)	4.1 ± 2.1	$7.6 \pm 1.9^{***}$
t_{max} (h)	1.6 ± 1.2	1.9 ± 1.7
AUC ($\text{ng ml}^{-1}\text{ h}$)	8.0 ± 2.4	$18.2 \pm 3.1^{***}$
C_{max} (ng ml^{-1})	2.1 ± 0.7	$3.6 \pm 1.0^*$

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 2 Peak velocity of saccadic eye movements test (mean $\pm$ s.d.) after an oral dose of 0.25 mg triazolam following pretreatment with oral diltiazem 60 mg (●) or placebo (○) and control (Δ) three times daily for 3 days in seven healthy volunteers. Significant (*: $P < 0.05$, **: $P < 0.01$) different from the placebo control. Significant (#: $P < 0.05$, ##: $P < 0.01$) different from the placebo pretreatment.

for AUC (0,8h), 0.8 to 3.8 times for C_{max} , and 1.2 to 3.0 times for $t_{1/2}$. Diltiazem caused no statistical change in the t_{max} for triazolam. At 24 h after the administration of triazolam, a plasma concentration of triazolam was detected in the five subjects who had the diltiazem pretreatment, but in none of the subjects who have received the placebo pretreatment.

Pharmacodynamics

PSV remained to be at baseline levels throughout the control day. PSV was significantly decreased by the triazolam administration and was observed to be more effective with the diltiazem pretreatment compared with the control (i.e., placebo + placebo) or placebo + triazolam trial phase (Figure 2). A statistically significant difference between the subjects pretreated with diltiazem + triazolam and those given the control phase ($P < 0.01$), and between pretreatment of diltiazem + triazolam and placebo + triazolam ($P < 0.05$). The mean PSV as indicative of the triazolam pharmacodynamic effects was decreased to 70.6% at 1 h for the subjects with placebo + triazolam and to 52.8% at 2 h for those with diltiazem + triazolam. The effect of triazolam on PSV was significantly increased with the diltiazem pretreatment compared with the placebo pretreatment ($P < 0.05$). The decreased PSV with the diltiazem pretreatment returned to the basal levels with time, but without a complete restoration at 8 h after the administration of triazolam.

Using VAS scores, two-way ANOVA showed a statistically significant difference between the diltiazem + triazolam and placebo + triazolam in the time course ($P < 0.05$). Although no statistically significant difference was obtained in drowsiness from triazolam between the subjects pretreated with and without diltiazem (Figure 3), the mean drowsiness with diltiazem + triazolam tended to increase as compared with placebo + triazolam.

Figure 3 Subjective drowsiness of visual analogue scale test (mean $\pm$ s.d.) after an oral dose of 0.25 mg triazolam following pretreatment with oral diltiazem 60 mg (●) or placebo (○) and control (Δ) three times daily for 3 days in seven healthy volunteers. Significant (*: $P < 0.05$, **: $P < 0.01$) different from the placebo control. Significant (#: $P < 0.05$, ##: $P < 0.01$) different from the placebo pretreatment.

Table 2 Alpha and beta power (mean $\pm$ s.d.) of electroencephalogram before an oral dose of triazolam or placebo.

		Control	Triazolam	Triazolam + diltiazem
Alpha	Fp1	12.2 $\pm$ 2.5	11.2 $\pm$ 1.5	12.0 $\pm$ 1.9
	Fp2	11.6 $\pm$ 2.4	11.5 $\pm$ 1.6	12.2 $\pm$ 1.8
	O1	14.5 $\pm$ 5.0	14.2 $\pm$ 2.3	13.8 $\pm$ 2.3
	O2	17.0 $\pm$ 6.3	14.4 $\pm$ 1.6	15.5 $\pm$ 2.5
Beta	Fp1	5.4 $\pm$ 0.6	5.5 $\pm$ 0.8	5.7 $\pm$ 0.8
	Fp2	5.8 $\pm$ 0.8	5.8 $\pm$ 1.0	6.0 $\pm$ 1.0
	O1	6.2 $\pm$ 2.2	6.3 $\pm$ 2.3	6.4 $\pm$ 2.7
	O2	6.7 $\pm$ 2.4	6.2 $\pm$ 1.7	6.5 $\pm$ 2.1

The range of alpha- and beta-wave powers in EEG did not differ significantly among the three treatment conditions in the predose phase (Table 2). The change in the mean percentage of alpha-wave range EEG activity after the administration of triazolam differed significantly in all electrode positions ($P < 0.01$) between diltiazem + triazolam and placebo + triazolam (Figures 4 and 5). In contrast, beta-wave power was increased in Fp₁ and Fp₂ ($P < 0.05$; between control and diltiazem + triazolam). After administration of triazolam, these significant changes of alpha- and beta-wave powers in subjects with diltiazem + triazolam were seen for 8 h after the administration of triazolam.

Discussion

In this study, we assessed the kinetic and dynamic aspects of the interaction between triazolam and diltiazem in young adult volunteers. Pretreatment with diltiazem three times daily for 3 days increased the blood concentration of triazolam in the study subjects. The difference in mean plasma concentrations of triazolam with and without

Figure 4 Alpha and beta power of electroencephalogram (mean $\pm$ s.d.) at Fp1 and Fp2 after an oral dose of 0.25 mg triazolam following pretreatment with oral diltiazem 60 mg (●) or placebo (○) and control (Δ triangles) three times daily for 3 days in seven healthy volunteers. Significant (*: $P < 0.05$, **: $P < 0.01$) different from the placebo control. Significant (#: $P < 0.05$, ##: $P < 0.01$) different from the placebo pretreatment.

diltiazem pretreatment was about two-fold. Therefore, we assume that the resulting increase in the mean pharmacokinetic parameters such as AUC(0,8h), $C_{\max}$, and $t_{1/2}$ of triazolam led to the significantly increased effects of hypnotic action we measured in the study. A previous study with midazolam, which is extensively metabolized in the liver by CYP3A4, has shown that its pharmacokinetic parameters were affected by diltiazem and verapamil, and that the AUC for the subjects pretreated with these Ca-antagonizing drugs was increased by about 4 and 3 times, respectively, in comparison with the placebo pretreatment. In our study, the AUC of triazolam was increased by about 2.3 times, and the $t_{1/2}$ was prolonged by 1.8 times with diltiazem pretreatment. Thus, our results resembled these reported by Backman *et al.* [7] and Varhe *et al.* [8]. Furthermore, previous studies [4, 11] have suggested that diltiazem increased the bioavailability of midazolam, and decreased the clearance caused by inhibiting the hepatic CYP3A isozymes. A recent report [12] has indicated that CYP3A4 in the wall of the small intestine plays an important role in the first-pass

metabolism of triazolam. If diltiazem inhibits the CYP3A isozyme both in the liver and in the wall of the small intestine, it should be associated with a decrease in the first-pass effect thereby increasing the $C_{\max}$ and AUC. Because we observed a prolonged half-life of triazolam, the hepatic metabolism of triazolam appears to be impaired by diltiazem. However, whether either of CYP3A existing in the gut wall or in the liver would be involved preferentially in the metabolism of triazolam remains unclear.

Diltiazem has been reported to affect the pharmacokinetics of nifedipine depending on the dosage [2], and the period of pretreatment [3]. There was no difference reported between 3 days and 6 days of treatment. We therefore decided to make the period of pretreatment with diltiazem 3 days. The previous study showed that the AUC of nifedipine was altered (increased 2.4 times) and $t_{1/2}$ (prolonged 1.3 times) with diltiazem [13]. Since the inhibiting effects of diltiazem in that study were the same or weaker than those seen in our study, we presume that both nifedipine and triazolam are metabolized by the same

Figure 5 Alpha power of electroencephalogram (mean $\pm$ s.d.) at O1 and O2 after an oral dose of 0.25 mg triazolam following pretreatment with oral diltiazem 60 mg (●) or placebo (○) and control (△) three times daily for 3 days in seven healthy volunteers. Significant (*: $P < 0.05$, **: $P < 0.01$) different from the placebo control. Significant (#: $P < 0.05$, ##: $P < 0.01$) different from the placebo pretreatment.

CYP3A isozyme. A recent report showed no ethnic difference in the metabolism of triazolam between Caucasians and southern Asians [14], although another report suggested that the metabolism of nifedipine exhibits ethnic difference [15]. These findings suggest that the activity or character of CYP3A is not generally defined by a single substrate such as nifedipine, triazolam, or other similar drugs. Therefore, a study of the interaction between drugs is needed to reveal whether the inhibition of CYP3A activity is due to differences in ethnicity, or to the drugs.

In our study, these pharmacokinetic changes were accompanied with increased pharmacodynamic tests using PSV and EEG parameters derived by fast-Fourier transform (FFT), and subjective parameters by VAS. Previous studies have used PSV and EEG to assess wake-sleep transitions in subjects taking benzodiazepines [16]. FFT analysis of EEG revealed that changes in alpha- and beta-wave amplitudes were related to the effects of the benzodiazepine hypnotic [17]. In our study, PSV and EEG was significantly altered by triazolam, and the mean alteration of PSV and EEG-wave power corresponded to the mean maximum plasma concentration of triazolam. The statistical depression of PSV and alpha-wave power in EEG occurred up to 8 h after triazolam with diltiazem pretreatment.

Drowsiness with diltiazem + triazolam tended to increase as compared with placebo + triazolam, although no significant difference was observed. Backman *et al.* reported that midazolam with diltiazem pretreatment significantly increased the VAS of drowsiness [7]. The limited number of subjects were employed in our study.

In conclusion, diltiazem significantly increased the plasma concentration of orally ingested triazolam and prolonged its half-life. Physicians need to take special care in prescribing triazolam, or other drugs metabolized by CYP3A4, to patients already using diltiazem.

We would like to thank Takashi Ishizaki, MD, PhD, International Medical Center of Japan, who provided the suggestion for this manuscript.

References

- Schlantz K, Myre S, Bottorff M. Pharmacokinetic interactions with calcium channel antagonists (Part I). *Clin Pharmacokin* 1991; **21**: 344–356.
- Tateishi T, Ohashi K, Sudo T, *et al.* Dose dependent effect of diltiazem on the pharmacokinetics of nifedipine. *J Clin Pharmacol* 1989; **29**: 994–997.
- Ohashi K, Sudo T, Sakamoto K, *et al.* The influence of pretreatment periods with diltiazem on nifedipine kinetics. *J Clin Pharmacol* 1993; **33**: 222–225.
- Kronbach T, Mathys D, Umeno M, Gonzales F, Meyer U. Oxidation of midazolam and triazolam by human liver cytochrome P450III4. *Mol Pharmacol* 1989; **36**: 89–96.
- Varhe A, Olkkola KT, Neuvonen PJ. Oral triazolam is potentially hazardous to patients receiving systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther* 1994; **56**: 601–607.
- Olkkola KT, Backman JT, Neuvonen PJ. Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther* 1994; **55**: 481–485.
- Backman JT, Olkkola KT, Aranko K, Himberg JJ, Neuvonen PJ. Dose of midazolam should be reduced during diltiazem and verapamil treatments. *Br J Clin Pharmacol* 1994; **37**: 221–225.
- Varhe A, Olkkola KT, Neuvonen PJ. Diltiazem enhances the effects of triazolam by inhibiting its metabolism. *Clin Pharmacol Ther* 1996; **59**: 369–375.
- Cairns ER, Dent BR, Ouwerkerk JC, Porter LJ. Quantitative analysis of alprazolam and triazolam in hemolysed whole blood and liver digest by GC/MS/NICI with deuterated internal standards. *J Anal Toxicol* 1994; **18**: 1–6.
- Van S, AL, Kroon J, Schoemaker H, Pieters P, Breimer D, Cohen A. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analogue lines to the effects of incremental doses of temazepam in healthy volunteers. *Clin Pharmacol Ther* 1991; **50**: 172–180.
- Allonen H, Ziegler G, Klots U. Midazolam kinetics. *Clin Pharmacol Ther* 1981; **30**: 653–661.
- Robin DW, Lee M, Hasan SS, Wood AJ. Triazolam in

- cirrhosis: pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther* 1993; **54**: 630–637.
- 13 Ohashi K, Tateishi T, Sudo T, *et al.* Effects of diltiazem on the pharmacokinetics of nifedipine. *J Cardiovasc Pharmacol* 1990; **15**: 96–101.
- 14 Kinirons MT, Lang CC, He HB, Ghebreselasie K, Shay S, Robin DW, Wood AJJ. Triazolam pharmacokinetics and pharmacodynamics in Caucasians and southern Asians: ethnicity and CYP3A activity. *Br J Clin Pharmacol* 1996; **41**: 69–72.
- 15 Ahsan CH, Renwick AG, Waller DG, Challenor VF, George CF, Amanullah M. The influences of dose and ethnic origins on the pharmacokinetics of nifedipine. *Clin Pharmacol Ther* 1993; **54**: 329–338.
- 16 Van SA, Mandema JW, Tuk B, *et al.* A comparison of the concentration–effect relationships of midazolam for EEG-derived parameters and saccadic peak velocity. *Br J Clin Pharmacol* 1993; **36**: 109–115.
- 17 Mandema JW, Danhof M. Electroencephalogram effect measures and relationships between pharmacokinetics and pharmacodynamics of centrally acting drugs. *Clin Pharmacokinet* 1992; **23**: 191–215.

(Received 10 June 1996,
accepted 12 December 1996)