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VORICONAZOLE AND FLUCONAZOLE INCREASE THE EXPOSURE TO ORAL DIAZEPAM

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ABSTRACT

Objective: We assessed the effect of voriconazole and fluconazole on the pharmacokinetics and pharmacodynamics of diazepam.

Methods: Twelve healthy volunteers took 5 mg of oral diazepam in a randomized order on three study sessions: without pretreatment, after oral voriconazole at 400 mg twice daily on the first day and 200 mg twice daily on the second day, or after oral fluconazole 400 mg on the first day and 200 mg on the second day. Plasma concentrations of diazepam and N-desmethyldiazepam were determined for up to 48 h. Pharmacodynamic variables were measured for 12 hours.

Results: In the voriconazole phase, the area under the plasma concentration-time curve ($AUC_{0-\infty}$) of diazepam was increased (geometric mean ratio) 2.2-fold ($p < 0.05$; 90% confidence interval [CI] 1.56 to 2.82). This was associated with the prolongation of the mean elimination half-life ($t_{½}$) from 31 h to 61 h ($p < 0.01$) after voriconazole. In the fluconazole phase, the $AUC_{0-\infty}$ of diazepam was increased 2.5-fold ($p < 0.01$; 90% CI 1.94 to 3.40) and the $t_{½}$ was prolonged from 31 to 73 h ($p < 0.001$). The peak plasma concentration of diazepam was practically unchanged by voriconazole and fluconazole. The pharmacodynamics of diazepam were changed only modestly.

Conclusion: Both voriconazole and fluconazole considerably increase the exposure to diazepam. Recurrent administration of diazepam increases the risk of clinically significant interactions during voriconazole or fluconazole treatment because the elimination of diazepam is impaired significantly.

Keywords: diazepam, voriconazole, fluconazole, cytochrome P450, drug interaction, pharmacokinetics

INTRODUCTION

Diazepam is a lipid soluble benzodiazepine, which is widely used for sedation, hypnosis, and anxiolysis and also as an anticonvulsant. Oral bioavailability of diazepam is almost 100% [1] and it is extensively metabolized by cytochrome (CYP) P450 enzymes, mainly by CYP2C19 and CYP3A4 [2-5]. The demethylation of diazepam to N-desmethyldiazepam is mediated mainly by a genetically polymorphic CYP2C19, and 3-hydroxylation of diazepam to temazepam by CYP3A4 [4, 5]. Both of these metabolites are pharmacologically active [6].

Voriconazole is a novel triazole antifungal agent used to treat invasive fungal infections both intravenously and orally [7, 8]. Voriconazole is rapidly and almost completely absorbed from the gastrointestinal tract [9], and similar to the other triazole antifungals, voriconazole undergoes extensive oxidative metabolism involving the CYP enzymes CYP2C9, CYP2C19 and to a lesser extent CYP3A4 [10]. Voriconazole inhibits several CYP enzymes, namely CYP3A4, CYP2C9 and CYP2C19 [11, 12, 8] and we have previously shown that voriconazole has strong interactions with CYP3A substrates, midazolam and alfentanil [13, 14]. Fluconazole is another triazole antimycotic, which also inhibits CYP2C9 and CYP2C19, and to a lesser extent CYP3A [15-17]. It has clinically significant pharmacokinetic interactions with, e.g., midazolam, triazolam and alfentanil [18-20].

It has been demonstrated that CYP2C19 inhibitors omeprazole and fluvoxamine affect the pharmacokinetic disposition of diazepam [21, 22]. Previous studies have shown that the strong inhibitors of CYP3A4, erythromycin and itraconazole, have only a minor effect on the pharmacokinetics of diazepam [23, 24]. The relative contribution of CYP2C19 to the metabolism of diazepam can be increased during impaired metabolism by CYP3A4. Because voriconazole and fluconazole inhibit both CYP2C19 and CYP3A4, it is possible that they could have a greater effect

on the pharmacokinetics of diazepam. We, therefore, found it important to study the possible effect of voriconazole and fluconazole on the pharmacokinetics and pharmacodynamics of oral diazepam.

MATERIALS AND METHODS

Subjects and ethics. The study protocol was approved by the Ethics committee of the Hospital District of Southwest Finland, as well as by the Finnish National Agency for Medicines, and was conducted according to the revised Declaration of Helsinki (http://www.wma.net/e/ethicsunit/helsinki.htm). A power analysis was performed before the study to calculate the number of subjects sufficient to show a 30% change in AUC of diazepam with a power of 80% and a significance level of $p<0.05$. Written informed consent was obtained from 12 healthy volunteers, 9 men and 3 women (Table 1). Before entering the study, the volunteers were ascertained to be in good health by medical history, clinical examination and standard haematological and blood chemistry tests. None of the volunteers was receiving any continuous medication, including contraceptive steroids, or natural products, nor was anyone a smoker.

Study design. We used an open, three-phase crossover study design, at intervals of 4 weeks. Before diazepam dosing, the volunteers were given in a randomized order either no pretreatment (control phase), oral voriconazole (voriconazole phase) for two days or oral fluconazole (fluconazole phase) for two days. The dose of voriconazole (Vfend 200 mg tablet, Pfizer Ltd, Sandwich, Great Britain) was 400 mg every 12 hours for one day and then 200 mg every 12 hours for one additional day. The dose of fluconazole (Fluconazol Copypharm 100 mg tablet, Copypharm A/S, Odense, Denmark) was 400 mg once a day for one day and then 200 mg for one additional day. The last doses of voriconazole and fluconazole were given at 8 a.m. with 150 ml of water by the investigators in the research facility and those volunteers not receiving any pretreatment were given 150 ml of water. The volunteers had been instructed to take the pretreatment at home with a meal and the adherence with the drug dosing schedule was assessed by using mobile phone short message service.

One hour after the last doses of voriconazole or fluconazole or water during the control phase, all volunteers ingested 5 mg diazepam (Stesolid 5 mg tablet, Dumex-Alpharma A/S, Copenhagen, Denmark) with 150 ml water. The volunteers fasted for 12 hours before the administration of diazepam, and they were given standard meals 4 hours and 8 hours after diazepam administration. The drinking of grapefruit juice, alcohol, coffee, tea or cola was forbidden on the test days and for 2 days prior to the study.

Sampling. For each session, an intravenous catheter was placed in a cubital vein for blood sampling. A baseline venous blood sample was drawn into EDTA tube just before the last dose of pretreatment and timed blood samples (10 ml each) were drawn 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 and 48 hours after diazepam administration. Plasma was separated within 30 minutes and stored at -40 ºC until analysis.

Bioanalysis of diazepam and N-desmethyldiazepam. Plasma concentrations of diazepam and N-desmethyldiazepam were quantified by high performance liquid chromatography (HPLC) with UV detection as previously described [25]. The temazepam concentrations were below the limit of quantification and we could not reliably quantify plasma concentrations of temazepam. The limit of quantification for both diazepam and N-desmethyldiazepam was 1 ng/ml. The interday coefficient of variation (CV) for diazepam was 10.4%, 2.3%, and 1.1% at 7.0 ng/ml, 70 ng/ml, and 700 ng/ml, respectively (n = 13) and for N-desmethyldiazepam 20.2%, 5.9% and 4.5% at 7.0 ng/ml, 70 ng/ml, and 700 ng/ml, respectively (n = 13).

Bioanalysis of voriconazole and fluconazole. After a solid-phase extraction of voriconazole from plasma, its concentrations were determined by HPLC with UV detection at 255 nm by using a fluconazole analog (UK 54373, Pfizer Ltd) as the internal standard [26, 27]. The limit of

quantification was 20 ng/ml for voriconazole and the interday CV was 3.5%, 0.6% and 0.8% at 50 ng/ml, 1000 ng/ml and 10000 ng/ml (n = 4), respectively. The plasma fluconazole concentrations were determined after a solid-phase extraction by HPLC with UV detection at 210 nm by using UK 54373 as the internal standard [28]. The limit of quantification for fluconazole was 0.2 mg/l. The CV was 1.3 % and 3.0% at 3 mg/l and 18 mg/l (n = 3), respectively. Trough concentrations (C_{trough}) of voriconazole and fluconazole were determined from the baseline samples.

Genotyping. Genotyping for CYP2C19 was carried out by a polymerase chain reaction method with specific primers used as described earlier by de Morais et al [29]. As all subjects were Finnish Caucasians, they were tested for the *CYP2C19*2* allele, and not for the *CYP2C19*3*, which is present in Asians. We did not analyze for the *CYP2C19*17* allele, which gives slightly increased activity of the encoded enzyme [30], as this should have no major effect in the present study.

Pharmacokinetic analysis. The peak plasma concentrations (C_{max}) and corresponding peak plasma concentration times (t_{max}) were observed directly from the data. C_{48h} refers to the highest observed value for N-desmethyldiazepam plasma concentrations. For each subject, the terminal log-linear phase of the plasma diazepam concentration-time curve was identified visually and the elimination rate constant (k_{el}) was determined by regression analysis on the basis of at least 6 time points. The elimination half-life ($t_{½}$) was then calculated from the equation: $t_{½} = \ln 2 / k_{el}$. The areas under the diazepam and N-desmethyldiazepam plasma concentration-time curves (AUC_{0-48} and $AUC_{0-\infty}$) were calculated using the trapezoidal method. $AUC_{0-\infty}$ was extrapolated to infinity by use of the respective k_{el} value. We used the linear trapezoidal rule while successive concentration values were increasing and the logarithmic trapezoidal rule for successive descending concentration values. The apparent oral clearance (CL/F) and the apparent volume of distribution of diazepam during elimination (V_z/F) were also calculated with noncompartmental methods based on statistical moment theory. The

pharmacokinetic data were analyzed by use of the pharmacokinetic program WinNonlin (version 4.1, Pharsight Corporation, California, USA).

Pharmacodynamic measurements. The effects of diazepam were assessed with digit symbol substitution test (DSST), the Maddox wing test and three visual analog scales (VAS) just before administration of the last dose of voriconazole, fluconazole or water and at the time of blood sampling up to 12 hours after diazepam administration. The DSST was used to quantify the pharmacodynamic response associated with the administration of oral diazepam [31]. Subjects were asked to draw the appropriate matched symbol beneath each presented digit. The number of symbols correctly drawn in 3 minutes was noted. Because the code can be learned, multiple versions of the DSST were developed for this study. The Maddox wing was used to measure the effect of diazepam on the coordination of the extraocular muscles, and the result was given in diopters [32]. Subjective effects (no effects of the drug to very strong effects of the drug, alert to drowsy, very good performance to very poor performance) were recorded with 100-mm horizontal visual analog scales. [33] For each pharmacodynamic variable, the area under the response-time curve was determined by the trapezoidal rule for 12 hours.

Statistical analysis. Pharmacokinetic variables were compared with the analysis of variance for repeated measures, and *a posteriori* testing was performed using Tukey's test. The tests were used to determine the contributions of treatment, gender, genotype, phase and sequence to overall variance. t_{max} was analyzed by Friedman's test, and the Wilcoxon signed rank test was used for pairwise comparisons. In addition, we used Student's t-test for independent samples to compare the data between different genotypes of CYP2C19. Differences were regarded statistically significant if $p < 0.05$. Since pharmacokinetic drug interactions can also be assessed statistically by the same methods that are standard for investigation of bioequivalence, we calculated the geometric mean

ratios with 90% confidence intervals (CIs). Bioequivalence (i.e., the lack of an interaction) was concluded if the 90% CI of the geometric mean ratios for pharmacokinetic variables were within the acceptance limit of 0.8 to 1.25. Pearson's product-moment correlation coefficient was used to investigate the possible relationship between the ratio of the $AUC_{0-\infty}$ of diazepam during the voriconazole or fluconazole phase to the $AUC_{0-\infty}$ of diazepam during the control phase and the AUC_{0-24} of voriconazole or fluconazole, respectively. The results are expressed as mean ± standard deviation (SD). All data were analyzed with the statistical program Systat for Windows, version 10.2 (Systat Software, Richmond, California, USA).

RESULTS

Diazepam pharmacokinetics. Mean plasma concentrations of diazepam and N-desmethyldiazepam as a function of time during different phases of the study are shown in the Fig. 1. During the first 4 h following diazepam ingestion, the concentrations of diazepam did not differ between the phases. However, both voriconazole and fluconazole increased the mean $AUC_{0-\infty}$ of oral diazepam, by 2.2-fold ($p < 0.05$) and 2.5-fold ($p < 0.01$), respectively. Voriconazole and fluconazole increased both the $AUC_{0-\infty}$ and $t_{½}$ of oral diazepam in all subjects (Fig. 2). Voriconazole prolonged the mean $t_{½}$ of diazepam from 30.9 h to 61.1 h ($p < 0.01$) and fluconazole from 30.9 h to 72.8 h ($p < 0.001$). The values of C_{max} and V_z/F did not differ between the phases (Fig. 2, Table 2). During the voriconazole and fluconazole phases, the geometric mean ratios with 90% CI for all calculated pharmacokinetic variables for diazepam were outside the bioequivalence acceptance limits, except for C_{max} after voriconazole pretreatment (Table 2).

N-desmethyldiazepam pharmacokinetics. Voriconazole and fluconazole decreased the C_{48h} and AUC_{0-48} of N-desmethyldiazepam significantly ($p < 0.001$; Fig. 1, Table 2). The ratio of N-desmethyldiazepam AUC_{0-48} to diazepam $AUC_{0-\infty}$ was significantly ($p < 0.001$) lower during the control phase as compared to the voriconazole or fluconazole phases (Table 2).

Plasma voriconazole and fluconazole. The C_{trough} of voriconazole and fluconazole, before their last doses, were 1.17 mg/l (range 0.20 to 2.77) and 4.14 mg/l (range 3.05 to 6.58), respectively (Fig. 3). The C_{trough}-values did not differ significantly between study phases and there was no significant linear correlation between the change in the $AUC_{0-\infty}$ of diazepam and the AUC_{0-24} of voriconazole ($r = 0.43$; $p = 0.16$) or fluconazole ($r = 0.05$; $p = 0.83$).

CYP2C19 genotyping. Four of the subjects had the *CYP2C19*1/*2* genotype (3 men, 1 woman) and the other eight subjects were homozygous wild type (*1/*1) for CYP2C19 (Table 1). There were no statistically significant differences in any of the pharmacokinetic variables of diazepam between these two genotypes during the control phase or after antimycotic pretreatments. The AUC_{0-24} of voriconazole was significantly higher in subjects carrying *CYP2C19*1/*2* genotype ($p < 0.05$).

Pharmacodynamics. There was a linear correlation between plasma diazepam concentration and effect in all pharmacodynamic variables ($p < 0.001$). The AUC_{0-12} for DSST during the fluconazole phase differed significantly ($p < 0.05$) from that of the control phase but no statistically significant differences were seen in any of the other pharmacodynamic variables between the voriconazole and control or fluconazole and control phases (Fig. 4).

Adverse effects. All volunteers completed the study according to the protocol. Five of the twelve volunteers reported visual adverse events during the voriconazole pretreatment. Transient altered perception of light, chromatopsia and photophobia were experienced shortly after taking voriconazole. No other clinically relevant adverse effects were observed or reported during the study.

DISCUSSION

Both voriconazole and fluconazole markedly affected the pharmacokinetics of oral diazepam. While the absorption of diazepam was unchanged, voriconazole and fluconazole caused a considerable delay in the elimination of diazepam. The $t_{½}$ as well as the exposure to diazepam were approximately doubled but no effect was seen in the C_{max} of diazepam, and the pharmacological effects remained largely unchanged. Unaltered C_{max} of diazepam after its single dose and the reduction of the formation of its active metabolite(s) [6] by voriconazole and fluconazole can explain the minor pharmacodynamic differences between the study phases. However, since no double-blind study design was used, and the study subjects were young healthy volunteers, the pharmacodynamic findings should be interpreted cautiously but they do support the observed changes in pharmacokinetics.

During the first 4 hours following the administration of diazepam, its concentrations were essentially similar in all phases. This is in accordance with the practically complete oral bioavailability of diazepam [1]. The differences in the pharmacokinetics of diazepam did not become evident until 4 hours post-ingestion, i.e., during its elimination phase. Drugs which have a lower oral bioavailability than diazepam and which are metabolized by CYP enzymes during absorption and elimination from body, are more prone to have forceful interactions with inhibitors and inducers of CYP enzymes, because the interactions occur not only during elimination but also during the first pass extraction [18, 34, 35].

Diazepam is extensively metabolized by CYP2C19 and CYP3A4, which account for 80 % of the biotransformation of diazepam to its metabolites [2-5]. The demethylation of diazepam to N-desmethyldiazepam is mediated by a genetically polymorphic CYP2C19 [2], and the 3-

hydroxylation of diazepam to temazepam by mediated CYP3A, predominantly by CYP3A4 [4, 5]. In the present study both voriconazole and fluconazole caused a significant delay and decrease in the formation of N-desmethyldiazepam which is in good agreement with the available information on the interaction potential of voriconazole and fluconazole *in vitro* and *in vivo* [8, 15, 17].

Previous studies have shown that diazepam is susceptible to drug interactions [36-38], some of which are also clinically important [36, 38]. However, erythromycin and itraconazole, both strong inhibitors of CYP3A4, have only a minor effect on the pharmacokinetics of diazepam. They caused a 10-30% increase in the AUC of diazepam with no change in N-desmethyldiazepam [23, 24]. The authors concluded, that the increase in the AUC of diazepam was most likely due to the inhibition of CYP3A4 leading to a decreased formation of temazepam. Both voriconazole and fluconazole are inhibitors of CYP2C19 and CYP3A4. Thus, it is plausible, that the interaction between diazepam and voriconazole or fluconazole is stronger than that between diazepam and erythromycin or itraconazole. Unfortunately, in the present study, where only a small single dose (5 mg) of diazepam was given to healthy volunteers, we could not reliably quantify plasma concentrations of temazepam and we can not draw conclusions on the relative importance of the inhibition of CYP2C19 and CYP3A4 on the voriconazole-diazepam and fluconazole-diazepam interaction.

There is a notable variation in the CYP2C19 activity between subjects carrying different CYP2C19 alleles yielding ultrarapid, extensive, intermediate and poor metabolizer genotypes [29, 30, 39]. Several previous studies have reported differences in the diazepam pharmacokinetics and pharmacodynamics between the CYP2C19 poor and extensive metabolizers [40-42]. It has been recently shown that CYP2C19 genotype affects the emergence from general anaesthesia in patients who have been given oral diazepam for premedication [43]. We therefore determined the CYP2C19 genotype in the subjects participating in our study. Four of our 12 subjects expressed

*CYP2C19*1/*2* genotype while the rest had the wild type *CYP12C19*1/*1* genotype. In accordance with the previous results [42, 43], the pharmacokinetic variables of diazepam did not differ between subjects having *CYP2C19*1/*2* or *CYP12C19*1/*1* genotype. It has also been suggested that women at fertile age have a slightly reduced CYP2C19 activity [44]. This was shown to be correlated with the use of oral contraceptives. However, none of the female volunteers participating in this study were on oral contraceptives.

Our study can be criticized for having a too short period of blood sampling for the full characterization of the pharmacokinetics of diazepam. Therefore, the pharmacokinetic parameters could not be determined with such reliability as usual. However, the observed $t_{½}$ values are similar to those reported earlier [45]. Furthermore, the calculation of elimination rate constants was based on at least 6 time points. We therefore believe that no bias is caused by the relatively short time interval of blood sampling as compared to the observed values of $t_{½}$.

In conclusion, both voriconazole and fluconazole decreased the elimination clearance of diazepam significantly. When single doses of diazepam are administered during voriconazole or fluconazole treatment, dose adjustment of diazepam is most likely not needed, because the initial diazepam plasma concentrations are not affected. If diazepam is given repeatedly, the dosage of diazepam should be reduced, since the accumulation of diazepam and its active metabolites may prolong and increase the effects of diazepam. We also want to emphasize that our study was done in healthy young volunteers. The results may not be extrapolated uncritically to the elderly patients or patients with critical illness.

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REFERENCES

1. Divoll, M, Greenblatt, DJ, Ochs, HR, Shader, RI (1983) Absolute bioavailability of oral and intramuscular diazepam: effects of age and sex. Anesth Analg 62 (1): 1-8

2. Bertilsson, L, Henthorn, TK, Sanz, E, Tybring, G, Sawe, J, Villen, T (1989) Importance of genetic factors in the regulation of diazepam metabolism: relationship to S-mephenytoin, but not debrisoquin, hydroxylation phenotype. Clin Pharmacol Ther 45 (4):348-355

3. Andersson, T, Miners, JO, Veronese, ME, Birkett DJ (1994) Diazepam metabolism by human liver microsomes is mediated by both S-mephenytoin hydroxylase and CYP3A isoforms. Br J Clin Pharmacol 38 (2):131-137

4. Jung, F, Richardson, TH, Raucy, JL, Johnson, EF (1997) Diazepam metabolism by cDNA-expressed human 2C P450s: identification of P4502C18 and P4502C19 as low K(M) diazepam N-demethylases. Drug Metab Dispos 25 (2):133-139

5. Yang, TJ, Krausz, KW, Sai, Y, Gonzales, FJ, Gelboin, HV (1999) Eight inhibitory monoclonal antibodies define the role of individual P-450s in human microsomal diazepam, 7-ethoxycoumarin, and imipramine metabolism. Drug Metab Dispos 27 (1):102-109

6. Caccia, S., and S. Garattini. 1990. Formation of active metabolites of psychotropic drugs: an updated review of their significance. Clin Pharmacokinet 18 (6):434-459

7. Boucher, HW, Groll, AH, Chiou, CC, Walsh, TJ (2004) Newer systemic antifungal agents. Pharmacokinetics, safety and efficacy. Drugs 64 (18):1997-2020

8. Theuretzbacher, U, Ihle, F, Derendorf, H (2006) Pharmacokinetic/pharmacodynamic profile of voriconazole. Clin Pharmacokinet 45 (7):649-663

9. Purkins, L, Wood, N, Greenhalgh, K, Allen, MJ, Oliver, SD (2003) Voriconazole, a novel wide spectrum triazole: oral pharmacokinetics and safety. Br J Clin Pharmacol 56 Suppl 1:10-16

10. Hyland, R, Jones, BC, Smith, DA (2003) Identification of the cytochrome P450 enzymes involved in the N-oxidation of voriconazole. Drug Metabol Dispos 31 (5):540-547

11. Romero, AJ, Le Pogamp, P, Nilsson, L-G, Wood, N (2002) Effect of voriconazole on the pharmacokinetics of cyclosporine in renal transplant patients. Clin Pharmacol Ther 71 (4):226-234

12. Hynninen, VV, Olkkola, KT, Leino, K, Lundgren, S, Neuvonen , PJ, Rane, A, Valtonen, M, Vyyrylainen, H, Laine, K (2006) Effects of the antifungals voriconazole and fluconazole on the pharmacokinetics of s-(+)- and R-(-)-Ibuprofen. Antimicrob Agents Chemother 50 (6):1967-1972

13. Saari, TI, Laine, K, Leino, K, Valtonen, M, Neuvonen, PJ, Olkkola, KT (2006). Effect of voriconazole on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam. Clin Pharmacol Ther 79 (4):362-370

14. Saari, TI, Laine, K, Leino, K, Valtonen, M, Neuvonen, PJ, Olkkola, KT (2006) Voriconazole, but not terbinafine, markedly reduces alfentanil clearance and prolongs its half-life. Clin Pharmacol Ther 80 (5):502-508

15. Morita, K, Konishi, H, Shimakawa, H (1992) Fluconazole: a potent inhibitor of cytochrome P-450-dependent drug-metabolism in mice and humans in vivo. Comparative study with ketoconazole. Chem Pharm Bull (Tokyo) 40 (5):1247-1251

16. Wienkers, LC, Wurden, CJ, Storch, E, Kunze, KL, Rettie, AE, Trager, WF (1996). Formation of (R)-8-hydroxywarfarin in human liver microsomes. A new metabolic marker for the (S)-mephenytoin hydroxylase, P4502C19. Drug Metab Dispos 24 (5):610-614

17. Niwa, T, Shiraga, T, Takagi, A (2005) Effect of antifungal drugs on cytochrome P450 (CYP) 2C9, CYP2C19, and CYP3A4 activities in human liver microsomes. Biol Pharm Bull 28(9):1805-1808

18. Olkkola, KT, Ahonen, J, Neuvonen, PJ (1996) The effects of the systemic antimycotics, itraconazole and fluconazole, on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam. Anesth Analg 82 (3):511-516

19. Varhe, A, Olkkola, KT, Neuvonen, PJ (1996) Fluconazole, but not terbinafine, enhances the effects of triazolam by inhibiting its metabolism. Br J Clin Pharmacol 41 (4):319-323

20. Palkama, VJ, Isohanni, MH Neuvonen, PJ, Olkkola, KT (1998) The effect of intravenous and oral fluconazole on the pharmacokinetics and pharmacodynamics of intravenous alfentanil. Anesth Analg 87 (1):190-194

21. Gugler R, Jensen, JC (1985) Omeprazole inhibits oxidative drug metabolism. Studies with diazepam and phenytoin in vivo and 7-ethoxycoumarin in vitro. Gastroenterology 89 (6): 1235-1241

22. Perucca, E, Gatti, G, Cipolla, G, Spina, E, Barel, S, Soback, S, Gips, M, Bialer, M (1994) Inhibition of diazepam metabolism by fluvoxamine: a pharmacokinetic study in normal volunteers. Clin Pharmacol Ther 56 (5): 471-476

23. Luurila, H, Olkkola, KT, Neuvonen, PJ (1996) Interaction between erythromycin and the benzodiazepines diazepam and flunitrazepam. Pharmacol Toxicol 78 (2):117-122

24. Ahonen, J, Olkkola, KT,Neuvonen, PJ (1996) The effect of the antimycotic itraconazole on the pharmacokinetics and pharmacodynamics of diazepam. Fundam Clin Pharmacol 10 (3):314-318

25. Stebler, T, Guentert, TW (1991) Determination of diazepam and nordazepam in milk and plasma in the presence of oxazepam and temazepam. J Chromatogr 564 (1):330-337

26. Gage, R, Stopher, DA (1998) A rapid HPLC assay for voriconazole in human plasma. J Pharm Biomed Anal 17 (8):1449-1453

27. Pennick, GJ, Clark, M, Sutton, DA, Rinaldi, MG (2003) Development and validation of HPLC assay for voriconazole. Antimicrobial Agents and Chemotherapy 47 (7):2348-2350

28. Inagaki, K, Takagi, J, Lor, E, Okamoto, MP, Gill, MA (1992). Determination of fluconazole in human serum by solid-phase extraction and reversed-phase high-performance liquid chromatography. Ther Drug Monit 14 (4):306-311

29. De Morais, SM, Wilkinson, GR, Blaisdell, J, Nakamura, K, Meyer, UA, Goldstein JA (1994) The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. J Biol Chem 269 (22):15419-15422

30. Sim, SC, Risinger, C, Dahl, ML, Aklillu, E, Christensen, E, Bertilsson, L, Ingelman-Sundberg, M (2006) A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. Clin Pharmacol Ther 79(6):103-113

31. Stone, BM (1984) Pencil and paper tests--sensitivity to psychotropic drugs. Br J Clin Pharmacol 18 Suppl 1:15S-20S

32. Hannington-Kiff JG (1970) Measurement of recovery from outpatient general anaesthesia with a simple ocular test. Br Med J 3(5715):132-5

33. Bond, A, M Lader (1974) The use of analogue visual scales in rating subjective feelings. Br J Med Psychol. 47:211-8

34. Backman, JT, Kivistö, KT, Olkkola, KT, Neuvonen, PJ (1998) The area under the plasma concentration-time curve for oral midazolam is 400-fold larger during treatment with itraconazole than with rifampicin. Eur J Clin Pharmacol 54 (1):53-58

35. Palkama, VJ, Neuvonen, PJ Olkkola, KT (1999) Effect of saquinavir on the pharmacokinetics and -dynamics of oral and intravenous midazolam. Clin Pharmacol Ther 66 (1):33-39

36. Klotz, U, Reinman, I (1981) Elevation of steady-state diazepam levels by cimetidine. Clin Pharmacol Ther 30 (4):513-517

37. Klotz, U, Reinman, I (1984) Pharmacokinetic and pharmacodynamic interaction study of diazepam and metoprolol. Eur J Clin Pharmacol 26 (2):223-226

38. Greenblatt, DJ, Abernethy, DR, Morse, DS, Harmatz, JS, Shader, RI (1984) Clinical importance of the interaction of diazepam and cimetidine. N Engl J Med 310 (25):1639-1643

39. Goldstein, JA (2001) Clinical relevance of genetic polymorphisms in the human CYP2c subfamily. Br J Clin Pharmacol 52 (4):349-355

40. Sohn, DR, Kobayashi, K, Chiba, K, Lee, KH, Shin, SG, Ishizaki T (1992) Disposition kinetics and metabolism of omeprazole in extensive and poor metabolizers of S-mephenytoin 4'-hydroxylation recruited from an Oriental population. J Pharmacol Exp Ther 262 (3):1195-1202

41. Ishizaki, T, Chiba, K. Manabe, K, Koyama, E, Hayashi, M, Yasuda, S, Horai, Y, Tomono, Y, Yamato, C, Toyoki, T (1995) Comparison of the interaction potential of a new proton pump

inhibitor, E3810, versus omeprazole with diazepam in extensive and poor metabolizers of S-mephenytoin 4'-hydroxylation. Clin Pharmacol Ther 58 (2):155-164

42. Qin, XP, Xie, HG, Wang, W, He, N, Huang, SL, Xu, ZH, Ou-Yang, DS, Wang, YJ, Zhou ,HH (1999) Effect of the gene dosage of CYP2C19 on diazepam metabolism in Chinese subjects. Clin Pharmacol Ther 66 (6):642-646

43. Inomata, S, Nagashima, A, Itagaki, F, Homma, M, Nishimura, M, Osaka, Y, Okuyama, K, Tanaka, E, Nakamura, T, Kohda, Y, Naito, S, Miyabe, M, Toyooka, H (2005) CYP2C19 genotype affects diazepam pharmacokinetics and emergence from general anesthesia. Clin Pharmacol Ther 78 (6):647-655

44. Laine, K, Tybring, G, Bertilsson, L (2000) No sex-related differences but significant inhibition by oral contraceptives of CYP2C19 activity as measured by the probe drugs mephenytoin and omeprazole in healthy Swedish white subjects. Clin Pharmacol Ther 68 (2):151-159

45. Herman, RJ, Wilkinson, GR (1996) Disposition of diazepam in young and elderly subjects after acute and chronic dosing. Br J Clin Pharmacol. 42 (2):147-155.

TABLE 1. Characteristics of the subjects and the AUC_{0-24} values of voriconazole and fluconazole after pretreatment with oral voriconazole or fluconazole, respectively.

Subject No.	Age (y)	Gender	Weight (kg)	CYP2C19 genotype	Voriconazole AUC_{0-24} ($mg \cdot ml^{-1} \cdot h$)	Fluconazole AUC_{0-24} ($mg \cdot ml^{-1} \cdot h$)
1	23	Male	68	*1/*2	52.1	139.7
2	23	Male	78	*1/*1	8.6	116.5
3	21	Male	76	*1/*2	75.8	123.2
4	26	Male	70	*1/*1	19.0	125.5
5	24	Male	78	*1/*1	9.3	126.7
6	21	Female	75	*1/*2	19.9	115.8
7	22	Male	68	*1/*2	53.1	148.8
8	21	Female	53	*1/*1	26.6	159.1
9	20	Female	61	*1/*1	14.08	181.1
10	21	Male	108	*1/*1	3.8	100.7
11	24	Male	84	*1/*1	20.6	108.9
12	30	Male	113	*1/*1	5.6	107.4

TABLE 2. Pharmacokinetic parameters of diazepam and N-desmethyldiazepam after oral administration of 5 mg diazepam to 12 healthy volunteers without pretreatment (control) or following pretreatment with fluconazole, or oral voriconazole.

Parameter	Value for:			Geometric mean ratio (90% CI) for	
	Control	Fluconazole	Voriconazole	Fluconazole/ control	Voriconazole/ control
Diazepam					
C_{max} (ng · ml^{-1}) % of control (range)	131 ± 37 100	143 ± 35 125 (87-243)	129 ± 35 102 (80-143)	1.10 (1.08, 1.32)	1.02 (0.81, 1.20)
t_{max} (h)	1.0 (0.5-3.0)	0.5 (0.5-1.5)	1.0 (0.5-2.0)		
AUC_{0-48} (ng · ml^{-1} · h) % of control (range)	1800 ± 500 100	2720 ± 920* 150 (109-191)	2500 ± 830*** 138 (86-181)	1.50 (1.16, 1.94)	1.37 (1.06, 1.78)
$AUC_{0-\infty}$ (ng · ml^{-1} · h) % of control (range)	2760 ± 800 100	7550 ± 4610** 254 (144-401)	6140 ± 2930*** 221 (110-343)	2.57 (1.94, 3.40)	2.10 (1.56, 2.82)
CL/F (ml · min^{-1} · kg^{-1}) % of control (range)	32 ± 9.0 100	13 ± 4.6* 44 (25-69)	17 ± 8.2** 52 (29-91)	0.39 (0.29, 0.52)	0.48 (0.35, 0.64)
V_z/F (l · kg^{-1}) % of control (range)	93± 59 100	81± 40 92 (71-109)	79 ± 28 92 (52-119)	1.11 (0.67, 1.21)	1.10 (0.69, 1.19)
$t_{½}$ (h) % of control (range)	31 ± 7.3 100	73 ± 25* 226 (136-327)	61 ± 23** 195 (111-257)	2.31 (1.90, 2.81)	1.90 (1.53, 2.38)
N-Desmethyldiazepam					
C_{48h} (ng · ml^{-1}) % of control (range)	18.1 ± 5.6 100	7.1 ± 4.2* 36 (16-59)	9.4 ± 4.5* 49 (30-77)	0.35 (0.25, 0.50)	0.50 (0.36, 0.68)
AUC_{0-48} (ng · ml^{-1} · h) % of control (range)	640 ± 210 100	190 ± 110* 29 (10-56)	230 ± 110** 33 (17-61)	0.33 (0.24, 0.45)	0.26 (0.18, 0.38)
AUC ratio % of control (range)	0.35 ± 0.08 100	0.10 ± 0.13* 19 (8-40)	0.10 ± 0.04* 25 (13-50)	0.51 (0.45, 0.56)	0.54 (0.48, 0.60)

Data is given as mean ± SD, except for t_{max} data, which is given as median and range. % of control was calculated individually for each subject; mean and range of these individual values are reported. AUC ratio, ratio of N-desmethyldiazepam AUC_{0-48} to diazepam $AUC_{0-\infty}$.

*Significantly ($p < 0.001$) different from control.

**Significantly ($p < 0.01$) different from control.

***Significantly ($p < 0.05$) different from control.

FIGURE LEGENDS

FIG. 1. Plasma concentrations (mean ± SD) of diazepam and N-desmethyldiazepam in 12 healthy volunteers after a single oral dose of 5 mg of diazepam without pretreatment (open circles) or following pretreatment with oral voriconazole (solid circles) or oral fluconazole (solid triangles). The inset depicts the same data on a semilogarithmic scale.

FIG. 2. Individual values for CL/F, $AUC_{0-\infty}$, and $t_{½}$ in 12 healthy volunteers after a single oral dose of 5 mg of diazepam without pretreatment (Control) or following pretreatment with oral voriconazole (Vori) or oral fluconazole (Fluco). Subjects with CYP2C19*1/*1 (●) and CYP2C19*1/*2 (○) genotype are indicated.

FIG. 3. Plasma concentrations (mean ± SD) of with oral voriconazole (solid circles) or oral fluconazole (solid triangles) in 12 healthy volunteers during the oral voriconazole and fluconazole phases, respectively. The arrow denotes the time when 5 mg of oral diazepam was administered. P refers to the time point before the administration of the last doses of voriconazole or fluconazole.

FIG. 4. Results (mean ± SD) of Maddox wing test, DSST and the recordings of subjective drug effect, drowsiness, and performance from visual analog scales (VAS) in 12 healthy volunteers after a single oral dose of 5 mg of diazepam without pretreatment (open circles) or following pretreatment with oral voriconazole (solid circles) or oral fluconazole (solid triangles).

Fig 1.

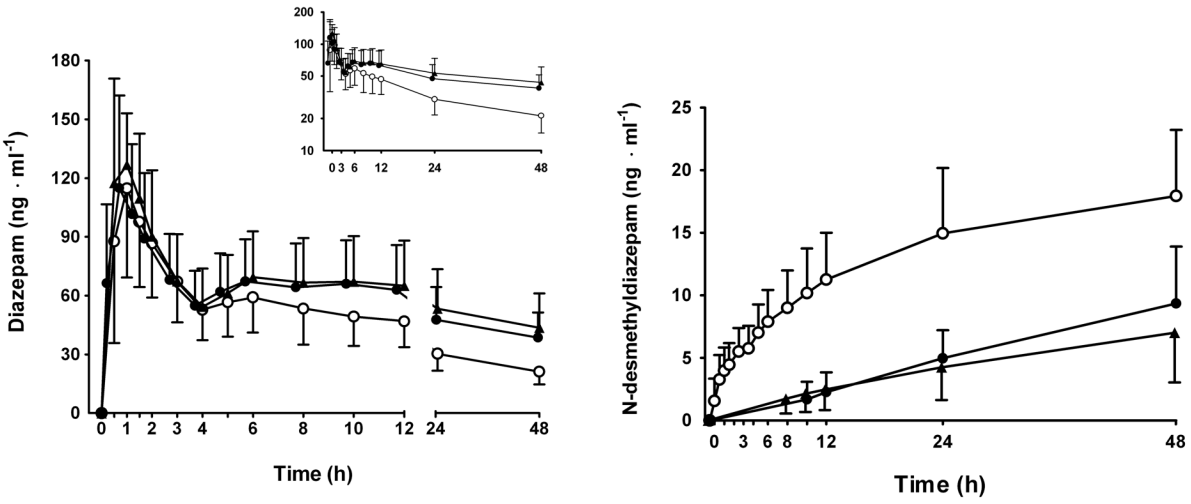

Diazepam (ng · ml-1)
Time (h)
N-desmethyldiazepam (ng · ml-1)
Time (h)

Fig 2.

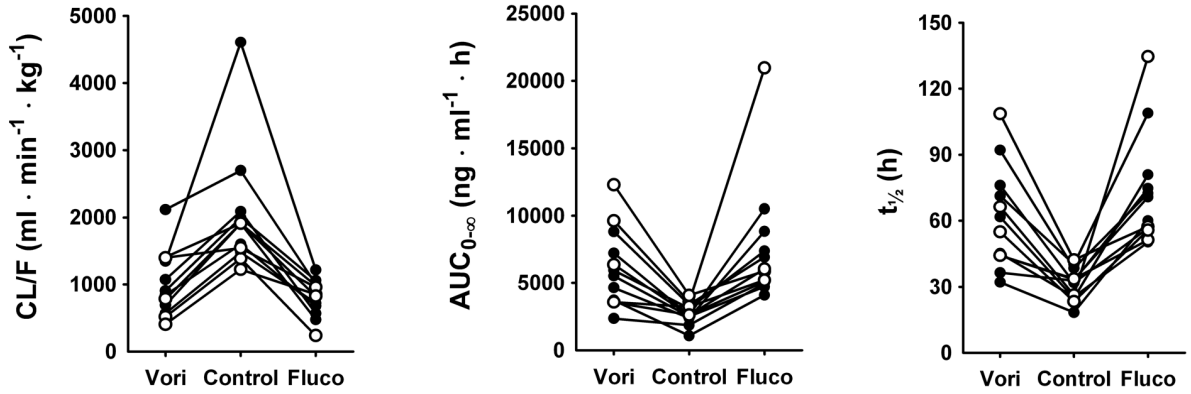

CL/F (ml · min-1 · kg-1)
AUC0-∞ (ng · ml-1 · h)
t½ (h)
Vori Control Fluco
Vori Control Fluco
Vori Control Fluco

Fig 3.

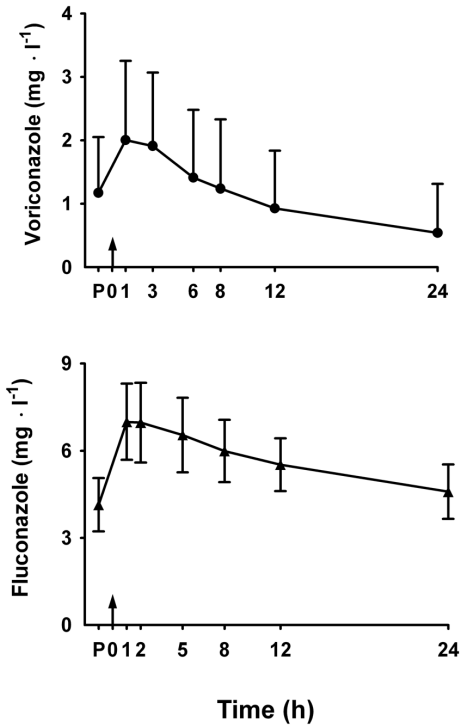
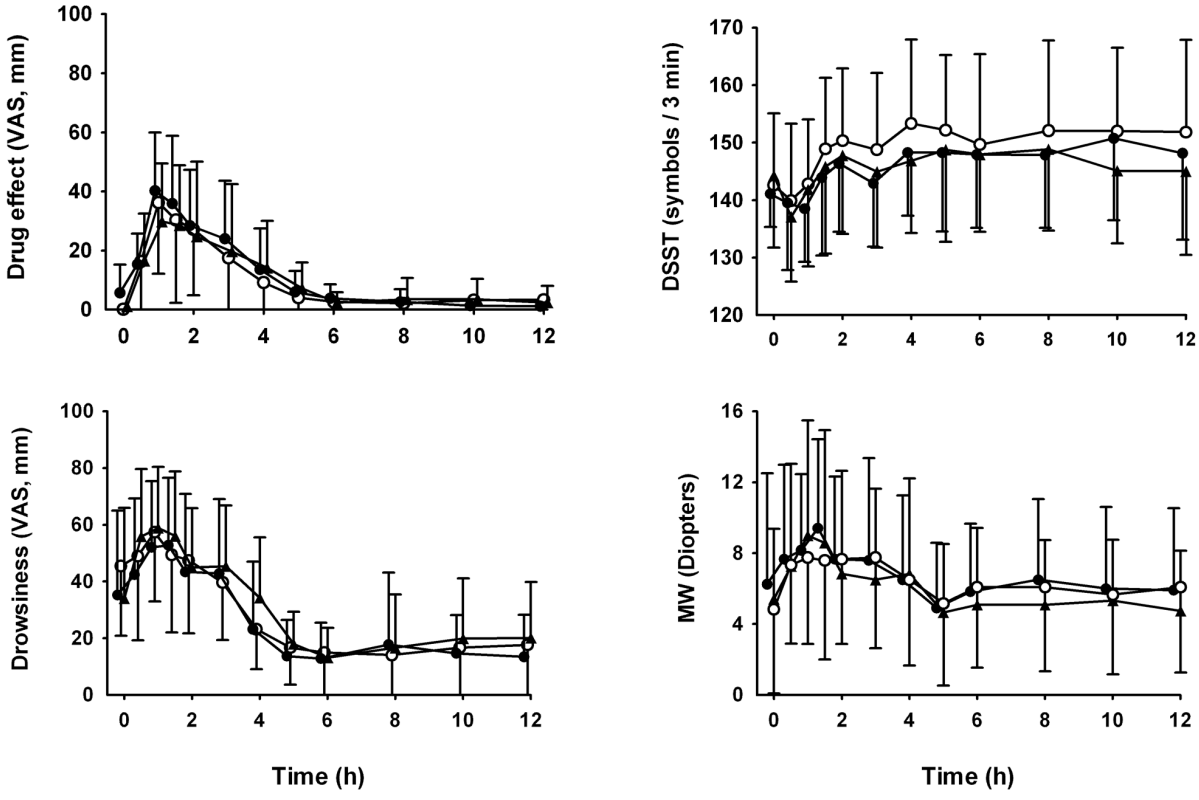

Voriconazole (mg · l-1)
Fluconazole (mg · l-1)
P 0 1 3 6 8 12 24
P 0 1 2 5 8 12 24
Time (h)

Fig 4.


Drug effect (VAS, mm)
DSST (symbols / 3 min)
Drowsiness (VAS, mm)
MW (Diopters)
Time (h)
Time (h)