Effect of fluconazole dose on the extent of fluconazole-triazolam interaction

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- 1 Azole antimycotics interact with the short acting hypnotic triazolam. The effect of fluconazole dose on the extent of fluconazole-triazolam interaction was investigated in a double-blind, randomized cross-over study of four phases.
- 2 Eight healthy volunteers received either 50 mg, 100 mg or 200 mg (400 mg on day 1) of fluconazole or placebo orally once a day for 4 days. On day 4, they took a 0.25 mg oral dose of triazolam, after which plasma samples were collected and pharmacodynamic effects measured for 18 h.
- 3 The mean area under the triazolam concentration-time curve (AUC) was increased 1.6-, 2.1- and 4.4-fold ($P < 0.001$) by fluconazole 50 mg, 100 mg and 200 mg, respectively. The increase in the elimination half-life of triazolam ($t_{1/2,z}$) varied from 1.3-fold (fluconazole 50 mg, $P < 0.05$) to 2.3-fold (fluconazole 200 mg, $P < 0.001$). The peak concentration of triazolam was also increased significantly during all fluconazole phases; more than twofold by fluconazole 200 mg ($P < 0.001$).
- 4 The pharmacodynamic effects of triazolam were increased significantly ($P < 0.05$) by fluconazole 100 mg and 200 mg.
- 5 Even a small 50 mg daily dose of fluconazole can interact with triazolam and the extent of the interaction increases with increasing fluconazole dose. When triazolam is used concomitantly with fluconazole 50–200 mg, the dose of triazolam should be reduced, accordingly. Simultaneous use of triazolam with higher fluconazole doses should be avoided.

Keywords triazolam fluconazole interaction dose dependent

Introduction

The short acting benzodiazepine triazolam is metabolized during its first-pass and elimination phases by CYP3A4 [1]. Azole antimycotics are inhibitors of CYP3A4 and can markedly increase the hypnotic effect of triazolam. Fluconazole affects the metabolism of triazolam less than either ketoconazole or itraconazole [2, 3].

The present study aimed to investigate the effect of different doses of fluconazole on the pharmacokinetics and pharmacodynamics of triazolam.

Methods

Subjects and study design

The study protocol was approved by the Ethics Committee of the Department of Clinical Pharmacology, University of Helsinki, and Finnish National Agency for Medicines. Eight healthy volunteers (five females and three males; age range 20–32 years; weight range 53–83 kg) signed written informed consent to participate in the investigation. None of the subjects was receiving continuous medication. Volunteers who had received medication within 3 weeks before the commencement of the study were excluded.

A randomized, double-blind, cross-over study design of four phases was used. The phases were separated by

an interval of at least 2 weeks. The subjects were given orally a 4-day-premedication with either fluconazole 50 mg, fluconazole 100 mg, fluconazole 400 mg on the first day and 200 mg on the next 3 days (referred as fluconazole 200 mg in the text) (Diflucan, Pfizer, Amboise, France) or placebo once a day at 13.00 h. On day 4, a 0.25 mg tablet of triazolam (Halcion, Upjohn, Kalamazoo, Michigan, USA) was ingested with 150 ml water at 14.00 h i.e., 1 h after the last dose of fluconazole or placebo. The volunteers fasted for 3 h before administration of triazolam and had a light standard meal 4 h afterwards. Alcohol, coffee, tea or cola and tobacco were forbidden during the test period.

Blood sampling and determination of plasma triazolam and fluconazole

On day 4, a forearm vein was cannulated with a plastic cannula and blood samples (10 ml) were collected into EDTA-tubes immediately before the administration of triazolam and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 18 h after it. Plasma was separated within 30 min and stored at -40°C until assay of triazolam and fluconazole. Plasma triazolam concentrations were determined by gas chromatography with use of a modified method of de Kroon *et al.* [4] and Gaillard *et al.* [5]. A Hewlett-Packard (Hewlett-Packard Company, Little Falls, Del.) fused silica capillary column (50% phenyl), with a length of 30 m and an internal diameter of 0.53 mm, was used. Column temperature was raised up to 285°C . Methoxy-diazepam was used as an internal standard and an electron-capture detector for detection. The sensitivity of the method was 0.1 ng ml^{-1} and the coefficient of day-to-day variation was 6.1% at 0.28 ng ml^{-1} ($n=15$) and 3.6% at 1.88 ng ml^{-1} ($n=16$). Fluconazole concentrations were also determined by gas chromatography, using nitrogen-phosphorus-detector [6]. The sensitivity of the fluconazole method was 100 ng ml^{-1} and the coefficient of day-to-day variation was 2.1% at $5.3\text{ }\mu\text{g ml}^{-1}$ ($n=12$).

Pharmacokinetics

Peak concentrations (C_{max}) and concentration peak times (t_{max}) were registered directly from the original data. The elimination rate constant (λ_z) was determined for each subject from the log-linear part of the individual plasma triazolam curve by regression analysis. The elimination half-life ($t_{1/2,z}$) was calculated from $t_{1/2,z} = \ln 2 / \lambda_z$. Area under the triazolam plasma concentration-time curves were calculated by use of the trapezoidal rule [AUC(0,18 h)] and with extrapolation to infinity (AUC) by dividing the 18 h concentration by λ_z . The AUC(0,18 h) of fluconazole on the day 4 refers to the time between 0 and 18 h after the ingestion of triazolam, i.e., between 1 and 19 h after the last dose of fluconazole.

Pharmacodynamic measurements

The pharmacodynamic effects of triazolam were measured immediately after each blood sample by four tests: subjective drowsiness using a 100 mm long visual analogue scale (VAS), the digit symbol substitution test

(DSST), measuring the number of digits correctly substituted in 3 min, the Maddox wing test measuring the coordination of extraocular muscles and the critical flicker fusion test (CFF) measuring discrimination of the fusion of flickering red light, as described earlier [3]. The subjects were properly trained to do the tests before the trial. For each pharmacodynamic variable, the areas under the response-time curves were determined by the trapezoidal rule for 0 to 7 h [AUC(0,7 h)]. The maximum effects were also recorded.

Statistical analysis

The results are expressed as mean values with s.e. mean and 95% confidence intervals also given (C_{max} , AUC values and $t_{1/2,z}$), or as median and range (t_{max}). The pharmacokinetic and pharmacodynamic data during the placebo and fluconazole phases were compared using the analysis of variance and *a posteriori* testing with the Tukey's test. If necessary to ensure the validity of the modelling assumptions, the data were transformed before the analysis. The t_{max} values were compared with non-parametric Friedman's two-way ANOVA and the Wilcoxon signed-rank test. Pearson's product-moment correlation coefficient was used in correlation studies. The statistical program Systat for Windows, version 5.0 (Systat, Evanston, Illinois) was used for the analysis. The chosen significance level was $P < 0.05$.

Results

Pharmacokinetics

Plasma concentrations of triazolam and fluconazole during various phases are shown in Figure 1. The C_{max} of triazolam was raised in seven of the eight subjects by fluconazole 50 mg ($P < 0.05$) and fluconazole 100 mg ($P < 0.05$) and in every subject by fluconazole 200 mg ($P < 0.001$), when compared with the placebo phase. Fluconazole 200 mg increased the mean C_{max} more than twofold.

The mean AUC of triazolam was increased 1.6-fold ($P < 0.01$) by fluconazole 50 mg, 2.1-fold ($P < 0.001$) by fluconazole 100 mg and 4.4-fold ($P < 0.001$) by fluconazole 200 mg when compared with the placebo phase. The individual increases in the AUC of triazolam ranged from 1.3-fold to 4.3-fold by fluconazole 100 mg and from 2.5-fold to 16.0-fold by fluconazole 200 mg.

The $t_{1/2,z}$ of triazolam was increased in seven of the eight subjects by fluconazole 50 mg, but in every subject by fluconazole 100 mg and 200 mg. The individual increase in the $t_{1/2,z}$ of triazolam caused by fluconazole 100 mg ranged from 1.4-fold to 2.8-fold and by fluconazole 200 mg from 2.0-fold to 3.5-fold (Figure 2). The mean $t_{1/2,z}$ of triazolam was prolonged from a control value of $3.1 \pm 0.4\text{ h}$ 1.3-fold (to $4.0 \pm 0.3\text{ h}$, $P < 0.05$), 1.8-fold (to $5.5 \pm 0.7\text{ h}$, $P < 0.001$), and 2.3-fold (to $7.0 \pm 0.4\text{ h}$, $P < 0.001$) by fluconazole 50 mg, 100 mg and 200 mg, respectively (Table I).

Figure 1 a) The mean plasma concentrations ($\pm$ s.e. mean) of triazolam after an oral dose of 0.25 mg triazolam following pretreatment with fluconazole 50 mg ($\bullet$), fluconazole 100 mg ($\blacktriangle$), fluconazole 200 mg ($\blacksquare$) or placebo ($\circ$) once daily for 4 days in eight healthy volunteers. b) Plasma concentrations of fluconazole on day 4. The time zero refers to the administration of triazolam (i.e., 1 h after administration of fluconazole).

The plasma concentrations of fluconazole on the day of administration of triazolam are shown in Figure 1. The highest plasma concentrations of fluconazole on day 4, after administration of triazolam, were 2.2 ± 0.1 mg l^{-1} for fluconazole 50 mg, 4.4 ± 0.3 mg l^{-1} for fluconazole 100 mg and 10.6 ± 0.4 mg l^{-1} for fluconazole 200 mg. The highest fluconazole concentrations, the concentrations of fluconazole at the time of administration of triazolam, and the AUC of fluconazole correlated significantly ($P < 0.001$) with the changes of AUC values of triazolam caused by fluconazole.

Pharmacodynamics

The pharmacodynamic effects of triazolam during the different fluconazole phases and during the placebo phase are shown in Figure 3. The maximum effects and the AUC(0,7 h) of the CFF, subjective drowsiness (VAS), the Maddox wing test and the DSST during the fluconazole 200 mg phase differed significantly ($P < 0.05$) from the placebo phase. Fluconazole 100 mg increased significantly ($P < 0.05$) the maximum effects and the AUC(0,7 h) of DSST and CFF. Fluconazole 50 mg did not change significantly any of the tests.

Discussion

The present results indicate that the extent of the fluconazole-triazolam interaction is clearly dependent on the dose of fluconazole. The 50 mg daily dose of fluconazole caused significant changes in the pharmacokinetics of triazolam, without any effect on the pharmacodynamics. On the other hand, higher doses of 100 and 200 mg resulted in more pronounced pharmacokinetic changes and also increased the effects of triazolam in a dose dependent manner. There were large interindividual differences in the extent of the interaction at each fluconazole dose level, but the correlation between, for example, the increase of the triazolam AUC and the AUC of fluconazole was good. In our earlier study fluconazole 100 mg changed the pharmacokinetics of triazolam to the same extent as fluconazole 100 mg in the present study, i.e. the AUC of triazolam was more than doubled and the $t_{1/2}$ was nearly doubled by fluconazole 100 mg, when compared with the placebo phase [2].

Fluconazole has also been shown to interact with other substrates of CYP3A, e.g. cyclosporine and terfenadine [7–9]. The reports about these interactions are controversial, however, and the discrepancies may reflect the dose-dependent nature and large interindividual variation of the fluconazole interactions. In some case reports, fluconazole 100 mg and 200 mg changed the pharmacokinetics of cyclosporine, while in other reports no such change was noticed [7]. In one patient there was a sharp rise in cyclosporine trough concentration when the patient was taking fluconazole 300 mg, but no such increase occurred with fluconazole 100 mg [7].

The antihistamine terfenadine has a clinically significant interaction with the antimycotics ketoconazole and itraconazole [10, 11]. In the study of Honig *et al.* [8], patients were given fluconazole 200 mg day^{-1} simultaneously with terfenadine. Unmetabolized terfenadine was not detected in plasma in any subject, and cardiac depolarisation was not significantly changed. However, high dose fluconazole, 800 mg day^{-1} , increased mean AUC of terfenadine by 52% [9]. Fluconazole 800 mg day^{-1} was also associated with an increase in Q_{tc} . Our present and previous results indicate that triazolam and midazolam may be more sensitive than terfenadine to interact with fluconazole [12].

In vitro fluconazole is a competitive inhibitor of CYP2C whereas the inhibition of CYP3A mediated metabolism (of midazolam) did not appear to be competitive. Fluconazole is a considerably less potent inhibitor of CYP3A mediated metabolism than either ketoconazole or itraconazole *in vitro*, with a K_i greater than 80 μ M (ketoconazole 3 μ M, itraconazole 6 μ M) [13]. *In vivo* therapeutic plasma concentrations of fluconazole are about 100-fold higher than those of itraconazole. Furthermore, about 90% of fluconazole in plasma is present as free drug, whereas the free fraction of itraconazole is of the order of 1% [14, 15]. With daily doses of fluconazole 100 mg, the steady-state plasma concentration of fluconazole is about 5–10 mg l^{-1} (15–35 μ mol l^{-1}), i.e. less than the K_i of 80 μ M. The

Figure 2 The individual AUC and $t_{1/2}$ values of triazolam during the control phase and three fluconazole phases (i.e. fluconazole 50, 100 and 200 mg) in eight subjects.

Table 1 The pharmacokinetic variables of triazolam 0.25 mg (mean $\pm$ s.e. mean or median and range) following pretreatment with different daily doses of fluconazole or placebo for 4 days in eight healthy volunteers. 95% confidence intervals for the ratio of the fluconazole and placebo phases are presented

Variable	Placebo (Control)	Fluconazole 50 mg	Fluconazole 100 mg	Fluconazole 200 mg
$C_{\max}$ (ng ml^{-1}) (95% CI)	1.5 ± 0.2	$2.2 \pm 0.3^{\text{a,d}}$ (108%, 207%)	$2.1 \pm 0.1^{\text{a,d}}$ (94%, 234%)	$3.5 \pm 0.4^{\text{b}}$ (133%, 444%)
$t_{\max}$ (h)	1.3 (0.5, 3.0)*	1.5 (0.5, 4.0)*	2.5 (1.0, 4.0)*	2.0 (0.5, 3.0)*
AUC(0, 18 h) ($\text{ng ml}^{-1} \text{h}$) (95% CI)	9.2 ± 1.6	$14.6 \pm 1.6^{\text{b,d}}$ (125%, 235%)	$18.3 \pm 1.4^{\text{c,d}}$ (138%, 347%)	$33.6 \pm 4.0^{\text{c}}$ (195%, 798%)
AUC ($\text{ng ml}^{-1} \text{h}$) (95% CI)	9.2 ± 1.7	$15.0 \pm 1.7^{\text{b,d}}$ (130%, 234%)	$18.9 \pm 1.6^{\text{c,d}}$ (142%, 357%)	$40.7 \pm 4.8^{\text{c}}$ (238%, 949%)
$t_{1/2,z}$ (h) (95% CI)	3.1 ± 0.4	$4.0 \pm 0.3^{\text{a,d}}$ (102%, 170%)	$5.5 \pm 0.6^{\text{c,d}}$ (140%, 229%)	$7.0 \pm 0.4^{\text{c}}$ (167%, 323%)

* Median (range). ^aSignificantly ($P < 0.05$) different from the placebo phase. ^bSignificantly ($P < 0.01$) different from the placebo phase. ^cSignificantly ($P < 0.001$) different from the placebo phase. ^dSignificantly ($P < 0.05$) different from the fluconazole 200 mg phase.

concentration of fluconazole in the gut wall and liver can be considerably higher than in plasma, especially during absorption; this increases the probability of a significant interaction between fluconazole and triazolam. Liver:plasma partition coefficients for fluconazole in rat are concentration dependent ranging from 30 to 2 [16]. At fluconazole concentrations of 15–35 $\mu\text{mol l}^{-1}$ the partition coefficient is approximately 2–4.

In conclusion, although fluconazole has a smaller risk than either itraconazole or ketoconazole to interact with triazolam, daily fluconazole doses of 50 mg or more may cause a significant interaction. There are interindividual differences between subjects in the extent of the interaction, but this increases along with the dose and plasma concentrations of fluconazole. If used simultaneously with fluconazole 100 mg or 200 mg day^{-1} , the

Figure 3 Results of the digit symbol substitution test (DSST), subjective drowsiness (VAS), the critical flicker fusion test (CFF) and the Maddox wing test after an oral 0.25 mg dose of triazolam following pretreatment with fluconazole 50 mg (●), 100 mg (▲), 200 mg (■) or placebo (○). Mean values of eight subjects.

dose of triazolam, for example, should be reduced by 50–75%. The concomitant use of triazolam and high doses of fluconazole cannot be recommended.

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