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Effect of route of administration of fluconazole on the interaction between fluconazole and midazolam

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Abstract *Objective*: Midazolam is a short-acting benzodiazepine hypnotic extensively metabolized by CYP3A4 enzyme. Orally ingested azole antimycotics, including fluconazole, interfere with the metabolism of oral midazolam during its absorption and elimination phases. We compared the effect of oral and intravenous fluconazole on the pharmacokinetics and pharmacodynamics of orally ingested midazolam.
Methods: A double-dummy, randomized, cross-over study in three phases was performed in 9 healthy volunteers. The subjects were given orally fluconazole 400 mg and intravenously saline within 60 min; orally placebo and intravenously fluconazole 400 mg; and orally placebo and intravenously saline. An oral dose of 7.5 mg midazolam was ingested 60 min after oral intake of fluconazole/placebo, i.e. at the end of the corresponding infusion. Plasma concentrations of midazolam, α-hydroxymidazolam and fluconazole were determined and pharmacodynamic effects were measured up to 17 h.
Results: Both oral and intravenous fluconazole significantly increased the area under the midazolam plasma concentration-time curve (AUC_{0-3}, AUC_{0-17}) 2- to 3-fold, the elimination half-life of midazolam 2.5-fold and its peak concentration (C_{max}) 2- to 2.5-fold compared with placebo. The AUC_{0-3} and the C_{max} of midazolam were significantly higher after oral than after intravenous administration of fluconazole. Both oral and intravenous fluconazole increased the pharmacodynamic effects of midazolam but no differences were detected between the fluconazole phases.
Conclusion: We conclude that the metabolism of orally administered midazolam was more strongly inhibited by oral than by intravenous administration of fluconazole.

Key words Midazolam, Fluconazole, CYP3A4, interaction, pharmacokinetics, pharmacodynamics

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Introduction

Midazolam is a widely used short-acting hypnotic which undergoes extensive first-pass metabolism with the oral bioavailability being less than 50% [1]. The hydroxylation of midazolam is mediated by CYP3A4 [2] and midazolam can be used as a model drug for CYP3A activity [3]. CYP3A4 is inhibited by many substances in vitro [4, 5]. In humans it has been shown that the orally administered macrolide antibiotic erythromycin [6], the calcium antagonists verapamil and diltiazem [7] and the azole antimycotics ketoconazole, itraconazole [8] and fluconazole [9] increase the plasma concentrations of oral midazolam. These changes result from an increase in oral bioavailability and a decrease in plasma clearance of midazolam. The increase in bioavailability is due to the decreased presystemic metabolism of midazolam. The inhibitory effect of oral fluconazole on CYP3A4-mediated drug metabolism is dose dependent [10].

In addition to the liver, some drugs have been shown to undergo a significant first-pass metabolism in the intestine [11, 12]. If an inhibitor of CYP3A4 is given orally, the concentrations of the inhibitor are probably higher in the cells of the intestinal wall than can be reached by administering the same dose intravenously. We therefore found it important to study the effect of the route of administration of an inhibitor of CYP3A4 on the pharmacokinetics and pharmacodynamics of an orally ingested substrate of CYP3A4. Fluconazole was chosen as the inhibitor and midazolam as the substrate.

Materials and methods

Study design

After obtaining institutional approval and informed written consent, 4 women (1 using contraceptive steroids) and 5 men, aged 19–25 years and weighing 52–92 kg, were studied. Before entering the study, the subjects were ascertained to be healthy by clinical examination. Apart from contraceptive use, none of the volunteers was on any continuous medication.

A randomized, double-dummy, placebo-controlled, cross-over study in three phases was used, at intervals of 4 weeks. The pretreatment, given at 13:00 h, consisted of oral fluconazole 400 mg (Diflucan, Pfizer, Amboise, France) and intravenous saline infused within 60 min (phase 1); of oral placebo and intravenous fluconazole 400 mg (Diflucan) infused within 60 min (phase 2); or of oral placebo and intravenous saline infused within 60 min (phase 3). An oral dose of 7.5 mg midazolam (Dormicum, Hoffmann-La Roche, Basel, Switzerland) was ingested with 150 ml water 60 min after the administration of oral fluconazole/placebo, i.e. at the end of the intravenous infusion of fluconazole/placebo. The volunteers fasted for 3 h before administration of midazolam and had a light standard meal 4 h afterwards. Ingestion of alcohol, coffee, tea and cola beverages was not allowed during the test day, nor was smoking permitted.

Blood sampling

Blood samples were drawn immediately before the administration of midazolam and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 17 h after administration. Plasma was separated within 30 min and stored at −40 °C until analysed.

Determination of drugs in plasma

The concentrations of midazolam and its active metabolite α-hydroxymidazolam were determined by high-performance liquid chromatography (HPLC) [13]. The sensitivity of the method for midazolam was 1 $ng \cdot ml^{-1}$ and for α-hydroxymidazolam 0.3 $ng \cdot ml^{-1}$. The coefficients of day-to-day variation (CV) for midazolam were 5.8% at 3.1 $ng \cdot ml^{-1}$ ($n = 14$) and 2.1% at 59.5 $ng \cdot ml^{-1}$ ($n = 14$). The CV for α-hydroxymidazolam were 8.0% at 3.0 $ng \cdot ml^{-1}$ ($n = 14$) and 4.7% at 61.3 $ng \cdot ml^{-1}$ ($n = 14$). Fluconazole was quantified by HPLC [14, 15]. The sensitivity of the method was 0.1 $mg \cdot l^{-1}$ and the CV was 3.7% at 5.4 $mg \cdot l^{-1}$ ($n = 10$).

Pharmacokinetic analysis

Peak midazolam concentrations (C_{max}) and the corresponding times (t_{max}) were picked up directly from the data. For each subject the terminal log-linear phase of the plasma concentration-time curve was identified visually. The elimination rate constant (k_{el}) was determined by regression analysis of the log-linear part of the curve. The elimination half-life was calculated from $t_{½} = ln2/k_{el}$. The area under the drug plasma concentration-time curve values (AUC_{0-3}, AUC_{0-17} and $AUC_{0-\infty}$) were calculated using the logarithmic trapezoidal method. For $AUC_{0-\infty}$ extrapolation to infinity was done by dividing the last measured concentration (17 h) by the corresponding k_{el} value. The AUC_{0-17} and $t_{½}$ were also calculated for α-hydroxymidazolam.

The AUC_{1-18} and the C_{max} values for oral and intravenous fluconazole were determined. The AUC_{1-18} of fluconazole refers to the time between 0 and 17 h after the ingestion of midazolam, i.e. between 1 and 18 h after administration of oral fluconazole or start of the fluconazole infusion. Furthermore, the ratio of the AUC_{1-18} of oral fluconazole to that of intravenous fluconazole was determined.

Pharmacodynamic methods

The effects of midazolam on psychomotor performance were assessed at the time of blood sampling by using the digit symbol substitution test (DSS), critical flicker fusion test (CFF), alert-drowsy visual analogue scale (VAS) and Maddox wing test (MW) as described previously [7]. For each pharmacodynamic variable the area under the response-time curve was determined by the trapezoidal rule for 0–3 h and for 0–17 h (DSS_{AUC}, CFF_{AUC}, VAS_{AUC}, MW_{AUC}). The maximum effects over 0–17 h (DSS_{min}, CFF_{min}, VAS_{max}, MW_{max}) were also recorded.

Statistical analysis

Results are expressed as mean values (SEM). Analysis of variance with repeated measures was used; a posteriori testing was done with Fisher's least significant difference test. Differences were regarded statistically significant if $P < 0.05$. All the data were analysed by use of the Systat for Windows program, version 5.0 (Systat, Evanston, IL, USA).

Results

Pharmacokinetics of midazolam and α-hydroxymidazolam

A single dose of 400 mg oral or intravenous fluconazole increased the AUC_{0-3}, AUC_{0-17} and $AUC_{0-\infty}$ of midazolam 2- to 3-fold ($P < 0.001$), the $t_{½}$ of midazolam 2.5-fold ($P < 0.001$) and its C_{max} 2- to 2.5-fold ($P < 0.01$) compared with placebo. The AUC_{0-3} and the C_{max} of midazolam were higher ($P < 0.05$) after oral than after intravenous administration of fluconazole (Fig. 1, Table 1).

Both oral and intravenous fluconazole increased the $t_{½}$ of α-hydroxymidazolam 2.5-fold ($P < 0.001$). The AUC_{0-17} of α-hydroxymidazolam was smaller ($P < 0.01$) during the placebo than during the oral and intravenous fluconazole phases. However, the ratio of the AUC_{0-17} of α-hydroxymidazolam to the AUC_{0-17} of midazolam was significantly ($P < 0.001$) higher during the placebo phase (mean 0.39) than during the oral fluconazole phase (mean 0.17) or the intravenous fluconazole phase (mean 0.18; Table 1).

Pharmacokinetics of fluconazole

No differences were detected in the AUC_{1-18} or the C_{max} of fluconazole when 400 mg fluconazole was given orally or intravenously within 60 min (Table 1). At the time of midazolam ingestion, the plasma fluconazole concentration was about twice as high ($P < 0.01$) after the intravenous infusion (mean 8.2 $mg \cdot l^{-1}$) as after oral ingestion (mean 4.4 $mg \cdot l^{-1}$) of fluconazole (Fig. 1). The ratio of the AUC_{1-18} of oral fluconazole to that of intravenous fluconazole was 1.0 (0.02).

Pharmacodynamics

The AUC_{0-17} of the DSS ($P < 0.01$) as well as the AUC_{0-3} of the DSS, CFF and VAS ($P < 0.05$) were

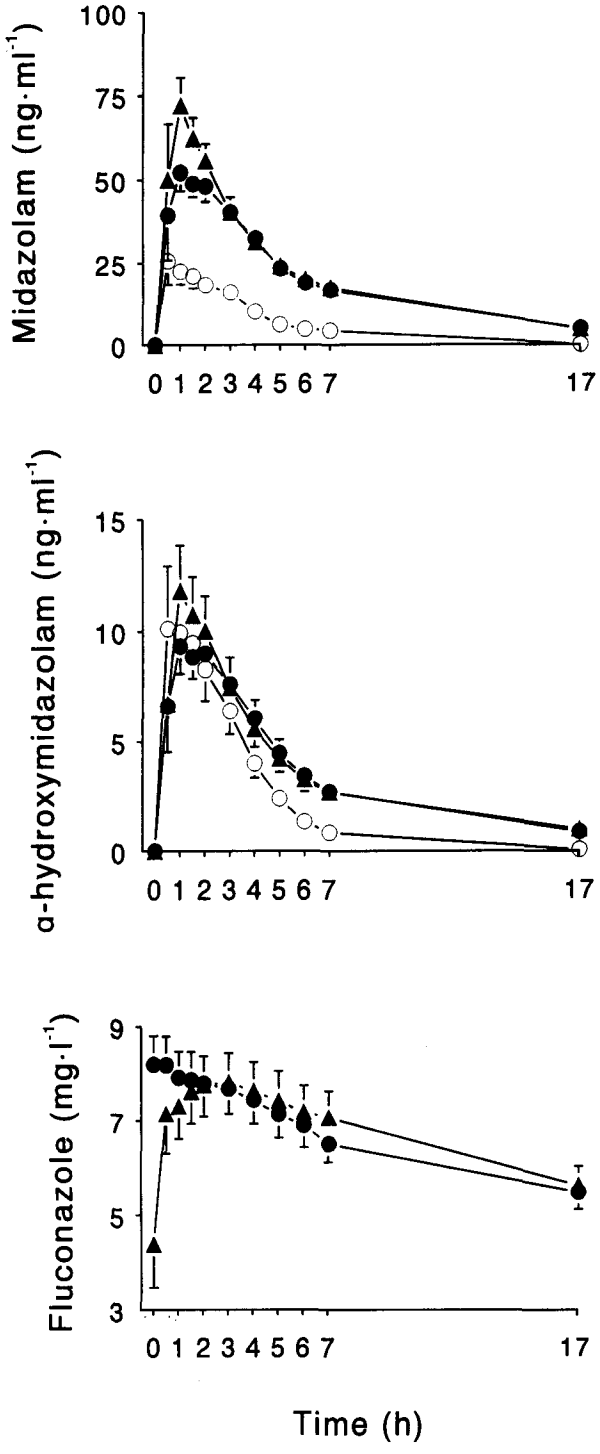

Fig. 1 Concentrations of midazolam (*top panel*) and α-hydroxymidazolam (*middle panel*) in plasma [means (SEM)] in nine subjects after an oral dose of 7.5 mg midazolam following pretreatment with placebo (○) or oral (▲) or intravenous fluconazole (●). *Bottom panel*: Concentrations of fluconazole in plasma after an oral (▲) or intravenous (●) dose of 400 mg fluconazole (*bottom panel*: time 0 h refers to the time of midazolam administration, i.e. 1 h after the intake of oral fluconazole or start of the fluconazole infusion)

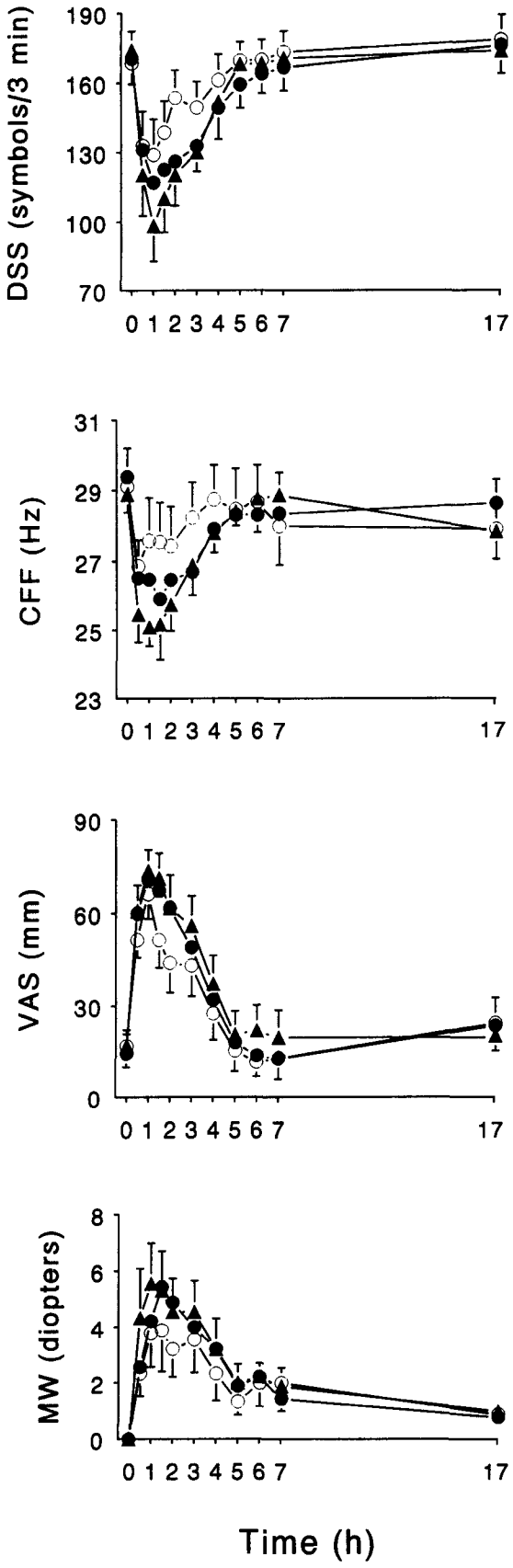

Fig. 2 Results [means (SEM)] of the digit symbol substitution (*DSS*) test, critical flicker fusion (*CFF*) test, subjective drowsiness (visual analogue scale, *VAS*) and Maddox wing test in nine subjects after oral intake of 7.5 mg midazolam following pretreatment with placebo (○) or oral (▲) or intravenous fluconazole (●)

significantly higher during the oral and intravenous fluconazole phases than during the placebo phase (Fig. 2). No differences were detected between the fluconazole phases.

Table 1 Pharmacokinetic parameters of midazolam, α-hydroxymidazolam and fluconazole [mean (SEM) except median for t_{max}] after ingestion of 7.5 mg midazolam following a single dose of 400 mg oral or intravenous fluconazole or placebo in nine healthy volunteers

Parameter/phase	Placebo	I.v. fluconazole	Oral fluconazole
Midazolam			
C_{max} ($ng \cdot ml^{-1}$)	37.4 (3.9)	66.8 (8.0)[a]	85.9 (9.8)[a,b]
t_{max} (h)	0.5	1.0	1.0
AUC_{0-3} ($ng \cdot ml^{-1} \cdot h$)	56.1 (7.2)	126 (11.4)[c]	153 (15.5)[b,c]
AUC_{0-17} ($ng \cdot ml^{-1} \cdot h$)	112 (15.6)	345 (30.2)[c]	382 (35.4)[c]
$AUC_{0-\infty}$ ($ng \cdot ml^{-1} \cdot h$)	113 (16.4)	389 (37.2)[c]	421 (42.1)[c]
$t_{½}$ (h)	2.2 (0.2)	4.9 (0.4)[c]	4.9 (0.5)[c]
α-Hydroxymidazolam			
C_{max} ($ng \cdot ml^{-1}$)	14.7 (1.5)	11.9 (1.7)	13.3 (1.9)
AUC_{0-17} ($ng \cdot ml^{-1} \cdot h$)	41.2 (5.1)	61.8 (8.0)[a]	64.3 (9.7)[a]
$t_{½}$ (h)	1.9 (0.4)	4.6 (0.6)[c]	4.9 (0.4)[c]
AUC ratio[d]	0.39 (0.4)	0.18 (0.2)[c]	0.17 (0.2)[c]
Fluconazole			
C_{max} ($mg \cdot l^{-1}$)		8.4 (0.6)	8.2 (0.6)
AUC_{1-18} ($mg \cdot l^{-1} \cdot h$)		118 (7.5)	121 (9.5)

[a]Significantly different from placebo ($P < 0.01$)
[b]Significantly different from intravenous fluconazole ($P < 0.05$)
[c]Significantly different from placebo ($P < 0.001$)
[d]Ratio of the AUC_{0-17} of α-hydroxymidazolam to the AUC_{0-17} of midazolam

Discussion

In the present study both oral and intravenous fluconazole significantly increased the C_{max}, the $t_{½}$, and the AUC values of midazolam, as well as its pharmacodynamic effects. The lower ratio between plasma concentrations of α-hydroxymidazolam and midazolam during the fluconazole phases compared with the placebo phase indicates inhibition of midazolam hydroxylation by oral and intravenous fluconazole. During the first 2 h after midazolam administration, the plasma fluconazole concentrations were higher after intravenous than after oral administration of fluconazole. However, the C_{max} and the AUC_{0-3} values of midazolam were higher after oral than after intravenous administration of fluconazole. During the intestinal absorption of midazolam, the concentration of fluconazole in the intestinal wall, as well as in the liver, is obviously much higher after oral than after intravenous administration of the antimycotic. Consequently, the inhibition of the intestinal and hepatic CYP3A4 is more effective by oral than by intravenous fluconazole. There is a marked interpatient heterogeneity in the intestinal [16] and hepatic [17] expression of CYP3A4. Furthermore, the intestinal and hepatic CYP3A4 activities do not correlate well within individuals [16]. In the present study, the crossover design was preferred to minimize the impact of interindividual differences in CYP3A4 activity. Our study design, however, does not allow conclusions concerning the role of the intestinal wall versus the liver in the fluconazole-midazolam interaction.

Although the pharmacodynamic effects of midazolam were significantly increased by oral and intravenous fluconazole compared with placebo, no significant differences were detected in the pharmacodynamic effects of midazolam between the oral and intravenous fluconazole phases. Due to the log-linear relationship of the concentration versus response, however, modest differences in midazolam concentrations between the oral and intravenous fluconazole phases did not result in significant changes of the pharmacodynamic effects of midazolam.

During the last few years the extrahepatic biotransformation of drugs has gained growing interest. Biotransformation in the gastrointestinal wall is of particular interest since some drugs have been shown to undergo a significant first-pass metabolism in the intestine [11, 12]. Duodenal epithelium, as well as the intestinal flora, has the capacity to metabolize several substrates [18]. Studies with an antibody against human CYP3A protein and a rat CYP3A cDNA probe show that CYP3A isoforms account for the majority of total P450 present in human jejunal mucosa [19, 20].

Cyclosporine, a particularly well studied CYP3A4 substrate, is quite extensively metabolized in the small intestine [11]. A recent report suggests that after oral intake of cyclosporine, its metabolism is more significant in the gut than in the liver and the inhibition of CYP3A enzymes is more effective in the gut than in the liver [21]. The intestinal CYP3A4 can also be induced by rifampicin [22].

The first step in midazolam metabolism is hydroxylation by CYP3A4; the metabolites formed are α-hydroxymidazolam and 4-hydroxymidazolam. α-Hydroxymidazolam is at least as potent as the parent compound and may contribute significantly to the effects of the parent drug when present in sufficiently high concentrations. 4-Hydroxymidazolam is quantitatively unimportant [23]. In addition to the liver, midazolam is metabolized also at extrahepatic sites. This has been demonstrated by the discovery of metabolites following intravenous injection of midazolam during the anhepatic period of liver transplantation [24]. Our results suggest that the intestinal CYP3A4 is of importance in the presystemic metabolism of midazolam. However, the

role of the intestinal CYP3A4 in both the first-pass metabolism and elimination of midazolam requires further evaluation.

In conclusion, single doses of both orally and intravenously administered fluconazole inhibit the metabolism of orally ingested midazolam. However, the interaction is somewhat stronger when fluconazole is given orally than intravenously, thus suggesting the role of intestinal drug metabolism in the interaction.

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