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PHARMACOGENETICS

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# Oral administration of a low dose of midazolam (75 μg) as an in vivo probe for CYP3A activity

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**Abstract** *Objective*: We investigated whether the oral administration of a low dose (75 μg) of midazolam, a CYP3A probe, can be used to measure the in vivo CYP3A activity.
*Methods*: Plasma concentrations of midazolam, 1′OH-midazolam and 4′OH-midazolam were measured after the oral administration of 7.5 mg and 75 μg midazolam in 13 healthy subjects without medication, in four subjects pretreated for 2 days with ketoconazole (200 mg b.i.d.), a CYP3A inhibitor, and in four subjects pretreated for 4 days with rifampicin (450 mg q.d.), a CYP3A inducer.
*Results*: After oral administration of 75 μg midazolam, the 30-min total (unconjugated + conjugated) 1′OH-midazolam/midazolam ratios measured in the groups without co-medication, with ketoconazole and with rifampicin were (mean ± SD): 6.23 ± 2.61, 0.79 ± 0.39 and 56.1 ± 12.4, respectively. No side effects were reported by the subjects taking this low dose of midazolam. Good correlations were observed between the 30-min total 1′OH-midazolam/midazolam ratio and midazolam clearance in the group without co-medication ($r^2 = 0.64$, $P < 0.001$) and in the three groups taken together ($r^2 = 0.91$, $P < 0.0001$). Good correlations were also observed between midazolam plasma levels and midazolam clearance, measured between 1.5 h and 4 h.
*Conclusion*: A low oral dose of midazolam can be used to phenotype CYP3A, either by the determination of total 1′OH-midazolam/midazolam ratios at 30 min or by the determination of midazolam plasma levels between 1.5 h and 4 h after its administration.

**Keywords** CYP · Phenotyping · Midazolam

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## Introduction

Because of its abundance in the intestine and liver, CYP3A, a term that in adults reflects the collective activity of CYP3A4 and CYP3A5, plays a central role in the metabolism of a wide variety of therapeutic compounds [1]. Considering the large inter-individual and intra-individual variability in CYP3A activity, a method allowing the assessment of its activity in vivo is valuable. Several methods for phenotyping CYP3A activity have been proposed that include the administration of midazolam (MID), $^{14}$C-labelled erythromycin, dapsone, alfentanil, nifedipine or lidocaine, or that measure the hydroxylation of endogenous cortisol (for a review see [1, 2, 3]). The most widely accepted and tested probes are erythromycin and MID [1, 2, 3], both of which have their own advantages and limits. The erythromycin breath test involves the administration of a $^{14}$C isotope of the drug, it does not measure the activity of the isozyme CYP3A5, and as it is given IV, intestinal CYP3A activity cannot be assessed [1, 2]. In contrast, MID is a substrate of both CYP3A4 and CYP3A5 [1, 2, 3], and its oral administration allows to measure both intestinal and hepatic CYP3A activity. The simultaneous administration of oral and intravenous MID has been proposed as a means to examine the contributions of intestinal and hepatic CYP3A [4]. Finally, erythromycin is a P-glycoprotein substrate [5], while discrepant results have been published on MID [5, 6].

Following oral administration, MID is oxidised to 1′-OH midazolam (1OHMID) and 4′-OH midazolam (4OHMID) [1]. The validity of using MID as a CYP3A phenotyping probe has been demonstrated in several studies. For example, the total clearance of MID correlated highly with hepatic CYP3A in vitro content measured in ten liver transplant patients ($r = 0.93$, $P < 0.001$) [7]. Several studies examined the possibility of using a single blood sampling to estimate MID clearance. One study found that a single sampling of 1OHMID or 1OHMID/MID plasma ratios (hereafter indicated as MR or metabolic ratio) cannot predict MID clearance in healthy adults, one of the possible reasons for this negative result being the inclusion of some obese subjects [8]. In another study, the plasma MR measured 30 min after an IV administration of MID correlated well with the hepatic CYP3A content ($n = 17$; $r = 0.87$, $P < 0.001$) [9]. However, data from non-induced donors ($n = 11$) showed a weaker correlation ($r = 0.55$, $P < 0.05$) [9]. However, a study with eight healthy male volunteers receiving a single oral dose of 7.5 mg MID, found that the 30-min MR was highly correlated with plasma MID clearance ($r = 0.89$, $P < 0.0068$) [10]. In another study with ten healthy male individuals receiving a single oral dose of 7.5 mg MID, a significant correlation was found between plasma MID clearance and the MR measured 1 h after blood intake ($r = 0.70$, $P < 0.05$) [11]. A significant correlation ($r = 0.67$, $P < 0.001$) has also been found between total (unconjugated + conjugated) MR and cyclosporin clearance, a CYP3A substrate, in 26 liver-transplant recipients [12]. Finally, a retrospective analysis of data from 224 healthy volunteers found that the concentrations of MID measured after an IV or oral dose explained 80% and 91% of the constitutive interindividual variability in MID AUC, respectively [13].

The quantification limit of standard analytical methods requires the use of sufficiently high, pharmacologically active doses of MID (about 2–7.5 mg) with the major disadvantage of causing drowsiness, sedation and even amnesic effects in some subjects. Therefore, we developed and validated a very sensitive gas chromatography—mass spectrometry—negative chemical ionisation analytical procedure [14], which allows to measure low levels of MID and OHMID, i.e. levels reached after the administration of 75 μg MID (one hundredth of the usual dose), which is not expected to produce any central nervous effects. The aim of the present study was to determine whether such a low dose, with the determination of the MR, could be used as an in vivo probe to assess CYP3A activity. For this purpose, the kinetics of oral doses of 7.5 mg and of 75 μg MID were measured in 21 healthy subjects. Subjects were either free of drugs known as being CYP3 inducers or inhibitors (group 1, $n = 13$) or received ketoconazole (group 2, $n = 4$) or rifampicin (group 3, $n = 4$), a strong CYP3A inhibitor and inducer, respectively.

## Subjects and methods

### Study design

The study population consisted of 21 healthy volunteers (19 Caucasian, 1 North African, 1 black African; 8 male; mean ± SD, range: age 33 ± 9 years, 23–55 years; weight 62 ± 10 kg, 50–78 kg) who were all free of drugs with the exception of a subject with an oral contraceptive. The study was approved by the ethics committee of the University Department of Psychiatry, and all subjects gave their written informed consent to participate in the study. They received an indemnity of SF 600 (Swiss francs). Subjects had normal hepatic and renal functions, as assessed by standard clinical laboratory tests (ALT, ASP, AP, GGT, urea, creatinine) and were asked not to drink grapefruit juice at least 1 week prior to the study, during and between the blood sampling sessions. The use of alcohol and caffeine-containing beverages was not allowed during the session.

Subjects were separated into three groups. In the first group ($n = 13$), the kinetics of MID and its metabolites were measured during two sessions, separated by an interval of at least 1 week and at the most 3 weeks. They received orally 7.5 mg MID in one session and 75 μg in the other. They were randomly assigned to start either with the 75-μg dose (seven subjects) or the 7.5-mg dose (six subjects) for the first session. In a second group ($n = 4$), the kinetics of oral MID (75 μg) and metabolites were measured during two sessions, separated by an interval of time of at least 1 week and at the most 3 weeks. Two days before the second session, and during two days, the subjects came to the centre (to ensure compliance) in order to take ketoconazole (200 mg b.i.d.). In a third group ($n = 4$), the kinetics of oral MID (75 μg) and metabolites were measured during two sessions, separated by an interval of at least 1 week and at the most 3 weeks. Four days before the second session, the subjects came to the centre (to ensure compliance) to take rifampicin (450 mg q.d.). At the end of the second session, after the last blood sampling, subjects received a fifth dose of rifampicin. The day following the second session, the kinetics of oral 7.5 mg MID was measured.

### Blood sampling

During each session, at approximately 0800 hours, after an overnight fast, an intravenous catheter was inserted into a forearm vein, and a blood sample was taken into a heparinised tube (time 0). The subjects were asked to drink 100 ml water containing either 0.075 mg or 7.5 mg MID, prepared by diluting 75 μl of a 1-mg/ml MID solution into 100 ml of water or 1.5 ml of a 5-mg/ml MID solution into 100 ml of water, respectively. The subjects then drank an additional 100 ml water to rinse the glass. After ingestion of 75 μg

of MID, blood samples were taken at 0.5, 1, 1.5, 2, 3, 4 and 6 h. After ingestion of 7.5 mg MID, supplemental blood samples were taken at 8 h and 24 h (the catheter was removed after the blood sampling at 8 h, the subjects returned home but were asked to come back to the centre for the 24-h blood sample). All blood samplings were performed with subjects in the supine position. The subjects were given a breakfast and a meal, if awake, approximately 1.5 h and 4.5 hours, respectively, after ingestion of the drug. Subjects with the standard dose of MID were asked to lie on a bed until the sedative effects of MID became inconsequential. All subjects were asked to refrain from walking for at least 90 min. After collection, the blood samples were centrifuged within 1 h, and the plasma samples stored at −20°C until analysis.

### Pharmacodynamic measurements

The attention-altering effects of the low and therapeutic dose of MID on pharmacodynamics were measured at the time of blood samplings using the Digit Symbol Substitution Test (DSS) [15]. All volunteers were trained to perform the test prior to the beginning of the study. In this test, the number of digits correctly substituted for by simple symbols in 3 min was recorded (when asleep, subjects were scored zero).

### Determinations of MID and metabolites

Determination of unconjugated MID, 1OHMID and 4OHMID, and of total 1OHMID and 4OHMID (unconjugated + conjugated) was performed by means of gas chromatography—negative chemical ionisation mass spectrometry [14]. Total concentrations were obtained by enzymatic hydrolysis of the glucuronic conjugates. The limits of quantification, as defined by the concentration for which the mean value of replicate determination ($n = 8$) was within 20% of the actual value, the coefficient of variation less than 20%, and which gave a signal-to-noise ratio of at least 10, were found to be 10 pg/ml for the three substances. Intra- and interday coefficients of variation determined at three concentrations (100 pg/ml, 500 pg/ml, 2 ng/ml) ranged from 1% to 8% for MID, from 2% to 13% for 1OHMID, and from 1% to 14% for 4OHMID [14]. The percent theoretical concentrations, which represent the accuracy of the method, were within ±8% for MID and 1OHMID, within ±9% for 4OHMID at 500 pg/ml and 2 ng/ml, and within ±28% for 4OHMID at 100 pg/ml [14]. No interference from ketoconazole or rifampicin was noted during the MID assay (data not shown). MID ratios were calculated with unconjugated and with total concentrations separately. Ratios were then multiplied by 325.8 and divided by 341.8 (the former and the latter values being the molecular weights of MID and OHMID, respectively).

### Data analysis

The concentrations of unconjugated MID, 1OHMID and 4OHMID determined after each administration were plotted against time, both individually, and as geometrical means and standard deviations according to condition (dosage level, co-medication with ketoconazole or rifampicin). The individual curves were then characterised by their peak level ($C_{max}$), time to peak ($t_{max}$), terminal rate constant estimated by log-linear regression ($\lambda_z$), and area under the curve calculated by log-trapezoidal rule with extrapolation to infinity ($AUC_{inf}$). The terminal half-life ($t_{1/2}$) was taken as $\ln(2)/\lambda_z$, the apparent clearance ($CL'$ or $CL/F$) as dose/$AUC_{inf}$, and the apparent volume during the terminal phase ($V_z'$ or $V_z/F$) as $CL'/\lambda_z$. For MID, the term "apparent" denotes that the estimate is the ratio of the true clearance or volume over the oral bioavailability; for metabolites, it was further divided by the fraction of the dose metabolised along the corresponding pathway. All calculations were performed using the non-compartmental method implemented in the program Kinetica (version 4.0, Innaphase, Buckinghamshire, UK). For correlation between MID ratios or MID plasma levels with MID clearance, the latter values were those calculated during the session with the therapeutic dose, in the group of 13 subjects without co-medications and in the four subjects with rifampicin, as the terminal phase was better characterised with this higher dose and as the aim of the study was to validate the use of MID ratios or MID plasma levels measured after the low dose against pharmacokinetic parameters determined with the usual therapeutic dose. In the group of four subjects with ketoconazole, clearance values used for the correlations were those calculated during the 75-μg dose session (subjects in that group did not receive the higher dose). Correlations were done with the Spearman test. The parameter values obtained in the same subjects under different conditions were compared using Friedman nonparametric two-way analysis of variance, while comparisons between different groups of subjects were performed using Kruskall-Wallis test (Statistix version 7, Analytical Software, Tallahassee, FL). The differences were appreciated considering a significance level of $P < 0.05$.

## Results

The mean plasma concentrations of MID and its metabolites after oral administration of 75 μg or 7.5 mg MID are shown in Fig. 1. Pharmacokinetic parameters of MID, 1OHMID and 4OHMID, according to dose and co-medication with either rifampicin or ketoconazole are provided in Table 1, Table 2 and Table 3, respectively. The pharmacokinetic profile associated with the administration of the 75-μg dose was similar to the profile observed after 7.5 mg. Both sets of concentration curves displayed a slightly bi-exponential

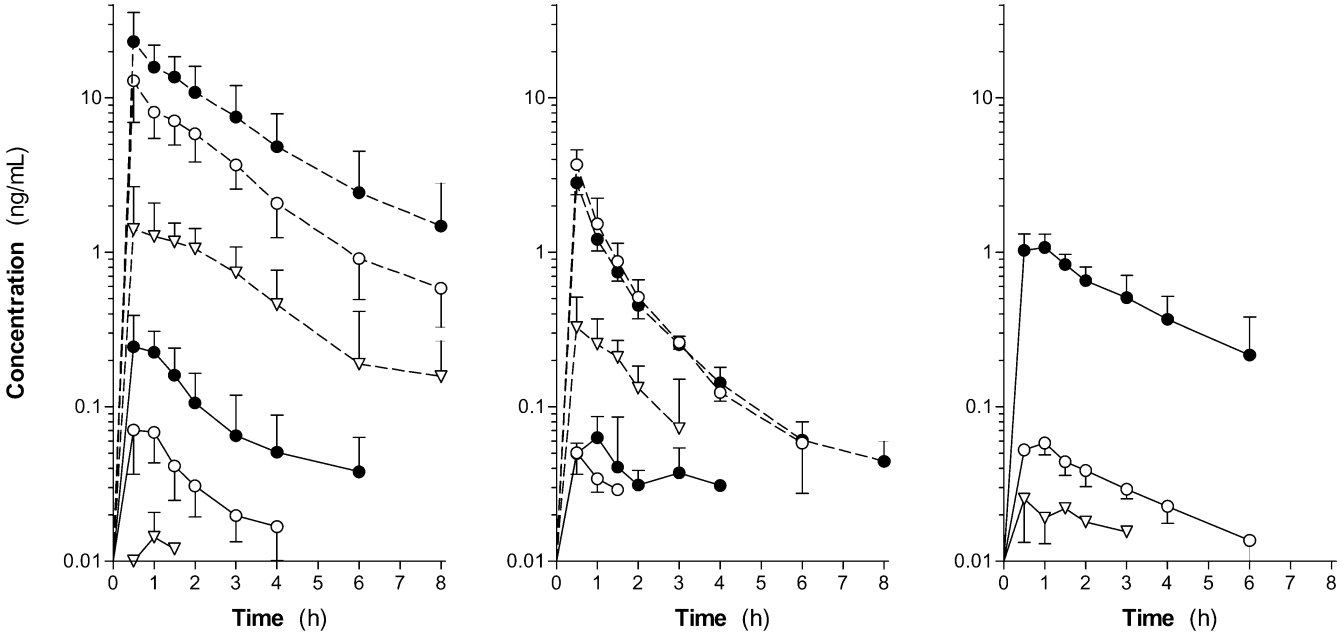


**Fig. 1** Concentration profile of midazolam (*closed circles*), 1-OH-midazolam (*open circles*) and 4-OH-midazolam (*open triangles*) after oral administration of 75 μg (*continuous lines*) or 7.5 mg (*dashed lines*) midazolam in healthy volunteers without pre-treatment (*left*), after rifampicin (*middle*) and after ketoconazole pre-treatment (*right*; the 7.5-mg dose was not given with ketoconazole). Geometric means and standard deviations are shown

pattern. As expected, the terminal phase was better characterised after the higher dose, which produced residual concentrations still measurable 24 h after administration (not shown). This probably explains the somewhat longer half-life values calculated under 7.5 mg (2.2 h versus 1.7 h, n.s.), and the corresponding impact on the estimated apparent volume of distribution (383 l versus 276 l, $P = 0.005$). A similar problem related to detection limit probably also explains the longer half-life calculated for 1OHMID after the higher dose (2 h versus 1.3 h, $P = 0.002$), while for this metabolite it was the apparent clearance that changed accordingly (258 l/h versus 516 l/h, $P = 0.0003$).

The pre-treatment of rifampicin induced an impressive drop of MID concentration levels, both after the 75-μg and 7.5-mg doses. Thus, for the 7.5-mg dose, rifampicin decreased the $C_{max}$ by 89%: (2.9 ng/ml versus 26 ng/ml, $P = 0.003$) and the AUC by 95% (3.5 h×ng/ml versus 67 h×ng/ml, $P = 0.003$). Rifampicin had almost no influence on the $t_{1/2}$, as observed after the high dose (2.3 h versus 2.2 h, n.s.). The $t_{1/2}$ could be reliably determined with the high dose of MID even with rifampicin, as MID was still measurable 8 h (two subjects) or 24 h (two subjects) after MID intake. No effect of rifampicin on MID $t_{1/2}$ could be reliably assessed after the low dose, due to the detection-limit problem [MID was already undetected 2 h (one subject), 4 h (two subjects) and 6 h (one subject) after MID intake]. Rifampicin had a major influence on MID apparent clearance and distribution volume, both after the high dose (2179 l/h versus 129 l/h, $P = 0.003$; respectively 7149 l versus 383 l, $P = 0.003$) and the low dose (1089 l/h versus 128 l/h, $P = 0.009$; respectively 1101 l versus 276 l, $P = 0.009$). The pre-treatment by ketoconazole markedly decreased the apparent clearance by 85% (19 l/h versus 128 l/h, $P = 0.003$) and distribution volume by 76% (67 l versus 276 l, $P = 0.003$), and prolonged the $t_{1/2}$ of the marker drug (2.7 h versus 1.7 h, $P = 0.01$).

Total MR varied only moderately over 0.5–6 h after administration, whatever the pre-treatment condition (Fig. 2 for the 75-μg dose after 30 min without pretreatment: mean ± SD, geometric mean, range 6.23 ± 2.61, 5.66, 1.88–11.0). It could hardly be determined at the last plasma sampling times after administration of the lower dose under rifampicin, due to plasma concentrations of MID below the limit of quantification (Fig. 2). The MR increased markedly under rifampicin, both for the low and the higher dose (for the 75-μg dose after 30 min: mean ± SD, geometric mean, range 56.1 ± 12.4, 55.5, 47.3–64.9; $P < 0.05$). It decreased drastically after 75 μg MID under ketoconazole (for the 75-μg dose after 30 min: mean ± SD, geometric mean, range 0.79 ± 0.39, 0.71, 0.37–1.20). The pharmacodynamic effects of 75 μg and 7.5 mg MID, with and without ketoconazole, and with and without rifampicin, are shown in Fig. 3. As the 75-μg dose of MID was not tested against placebo, the true pharmacodynamic effect of this low dose cannot be assessed. However, no particular side effects, including drowsiness, were reported by the subjects taking this low dose, with or without ketoconazole. As expected, with 7.5 mg MID, all 13 subjects slept for a variable period of time, which resulted in significantly lower scores of the DSS than after the 75-μg dose ($P < 0.05$ at times 1, 2, 3, 4 and 6 h). With 75 μg MID, the pre-administration of ketoconazole did not result in a significant decrease of the scores of the DSS. Finally, with 7.5 mg MID, the

**Table 1** Pharmacokinetic parameters of midazolam according to dose and co-medication with either rifampicin or ketoconazole (see text for parameters definition). Means ± SD and medians (range). Differences between rifampicin or ketoconazole and no co-medication at the same dose level

| | Dose 75 μg ($n=21$) | Dose 75 μg rifampicin ($n=4$) | | Dose 75 μg ketoconazole ($n=4$) | | Dose 7.5 mg ($n=13$) | | Dose 7.5 mg rifampicin ($n=4$) | |
|---|---|---|---|---|---|---|---|---|---|
| $C_{max}$ (ng/ml) | 0.31 ± 0.09, 0.30, (0.16–0.52) | 0.07 ± 0.02, 0.07, (0.04–0.10) | * | 1.16 0.21, 1.10, (0.99–1.44) | * | 26 ± 11, 24, (15–55) | § | 2.9 ± 0.5, 2.9, (2.2–3.4) | *§ |
| $t_{max}$ (h) | 0.8 ± 0.3, 0.5, (0.5–1.5) | 1.1 ± 0.3, 1, (1–1.5) | | 0.8 ± 0.3, 0.75, (0.5–1) | | 0.7 ± 0.4, 0.5, (0.5–2) | | 0.5 ± 0.02, 0.5, (0.5–0.5) | § |
| $AUC_{inf}$ (h·ng/ml) | 0.68 ± 0.25, 0.71, (0.23–1.17) | 0.30 ± 0.11, 0.36, (0.17–0.37) | * | 4.4 ± 1.6, 4.2, (3.0–6.3) | * | 67 ± 30, 61, (35–135) | § | 3.5 ± 0.2, 3.6, (3.1–3.6) | * |
| $t_{1/2}$ (h) | 1.7 ± 0.8, 1.7, (0.6–3.7) | 3.1 ± 1.7, 2.7, (1.6–5.0) | | 2.7 ± 1.0, 2.6, (1.9–3.9) | * | 2.2 ± .5, 2.2, (1.5–3.1) | | 2.3 ± 1.1, 2.1, (1.2–3.7) | |
| CL′ (l/h) | 128 ± 60, 103, (64–330) | 1089 ± 1619, 322, (202–3513) | * | 19 ± 7, 19, (12–25) | * | 129 ± 44, 124, (56–216) | | 2179 ± 148, 2111, (2091–2400) | * |
| $V_z'$ (l) | 276 ± 94, 254, (137–526) | 1101 ± 341, 1030, (800–1472) | * | 67 ± 10, 68, (55–79) | * | 383 ± 101, 404, (223–504) | § | 7149 ± 3309, 6716, (3738–11430) | * |

*$P < 0.05$: differences at the same dose under different conditions (i.e. with and without co-medication)
§$P < 0.05$: differences between 7.5 mg and 75 μg under same condition

**Table 2** Pharmacokinetic parameters of 1-OH-midazolam according to dose and co-medication with either rifampicin or ketoconazole (see text for parameters definition). Means ± SD and medians (range). Differences between rifampicin or ketoconazole and no co-medication at the same dose level

| | Dose 75 μg ($n=21$) | Dose 75 μg, rifampicin ($n=4$) | | Dose 75 μg, ketoconazole ($n=4$) | | Dose 7.5 mg ($n=13$) | | Dose 7.5 mg, rifampicin ($n=4$) | |
|---|---|---|---|---|---|---|---|---|---|
| $C_{max}$ (ng/ml) | 0.10 ± 0.04, 0.08 (0.04–.016) | 0.05 ± 0.02, 0.05 (0.03–0.07) | * | 0.06 ± 0.01, 0.06 (0.05–0.07) | * | 16.1 ± 8.5, 13.1 (5.8–32.2) | § | 3.7 ± 0.8, 3.9 (2.7–4.6) | *§ |
| $t_{max}$ (h) | 0.7 ± 0.3, 0.5 (0.5–1.5) | 0.5 ± 0.01, 0.5 (0.5–0.5) | * | 0.9 ± 0.3, 1 (0.5–1) | | 0.7 ± 0.5, 0.5 (0.5–2) | | 0.5 ± 0.02, 0.5 (0.5–0.5) | |
| $AUC_{inf}$ (h·ng/ml) | 0.18 ± 0.09, 0.15 (0.06–0.40) | 0.09 ± 0.2, 0.09 (0.08–0.11) | * | 0.24 ± 0.07, 0.22 (0.18–0.35) | | 32 ± 11, 31 (18–55) | § | 4.8 ± 2.1, 4.1 (3.2–7.7) | *§ |
| $t_{1/2}$ (h) | 1.3 ± 0.7, 1.1 (0.4–3.0) | 1.1 ± 0.5, 1.2 (0.6–1.6) | | 2.8 ± 1.4, 2.2 (1.8–4.8) | * | 2.0 ± 0.7, 1.8 (1.3–3.7) | § | 6.1 ± 8.6, 2.3 (0.8–18.9) | |
| CL′ (l/h) | 516 ± 250, 510 (187–1235) | 839 ± 125, 880 (660–937) | * | 325 ± 82, 335 (216–413) | | 258 ± 91, 244 (135–429) | § | 1760 ± 610, 1847 (969–2375) | *§ |
| $V_z'$ (l) | 878 ± 560, 690 (274–2597) | 1349 ± 514, 1290 (837–1978) | | 1195 ± 276, 1213 (850–1503) | * | 713 ± 263, 677 (335–1231) | | 10413 ± 10854, 6337 (2512–26469) | *§ |

*$P < 0.05$: differences at the same dose under different conditions (i.e. with and without co-medication)
§$P < 0.05$: differences between 7.5 mg and 75 μg under same condition

**Table 3** Pharmacokinetic parameters of 4-OH-midazolam according to dose and co-medication with either rifampicin or ketoconazole (see text for parameters definition). Means ± SD and medians (range)

| | Dose 75 µg ($n=21$) | Dose 75 µg, rifampicin ($n=4$) | Dose 75 µg, ketoconazole ($n=4$) | Dose 7.5 mg ($n=13$) | Dose 7.5 mg, rifampicin ($n=4$) | |
|---|---|---|---|---|---|---|
| $C_{max}$ (ng/ml) | Not assessable | | 0.03 ± 0.01, 0.03 (0.02–0.04) | 1.81 ± 0.69, 1.67 (0.89–3.38) | 0.36 ± 0.17, 0.31 (0.22–0.60) | * |
| $t_{max}$ (h) | | | 1.0 ± 0.5, 1 (0.5–1.5) | 0.9 ± 0.6, 0.5 (0.5–2) | 0.6 ± 0.3, 0.5 (0.5–1) | |
| $AUC_{inf}$ (h·ng/ml) | | | 0.11 ± 0.08, 0.12 (0.03–0.18) | 5.45 ± 2.25, 5.0 (3.2–11.0) | 0.66 ± 0.21, 0.61 (0.46–0.94) | * |
| $t_{1/2}$ (h) | | | 2.8 ± 2.4, 3.18 (0.3–5.0) | 1.8 ± 0.5, 1.84 (1.0–2.9) | 1.0 ± 0.3, 1.0 (0.7–1.4) | * |
| CL′ (l/h) | | | 1211 ± 1197, 626 (419–2588) | 1551 ± 499, 1496 (683–2373) | 12340 ± 3726, 12540 (8018–16263) | * |
| $V_z'$ (l) | | | 2376 ± 998, 2869 (1228–3031) | 3722 ± 983, 3980 (2087–5444) | 18588 ± 8353, 17300 (10462–29289) | * |

*$P < 0.05$: differences between rifampicin and no comedication after 7.5 mg

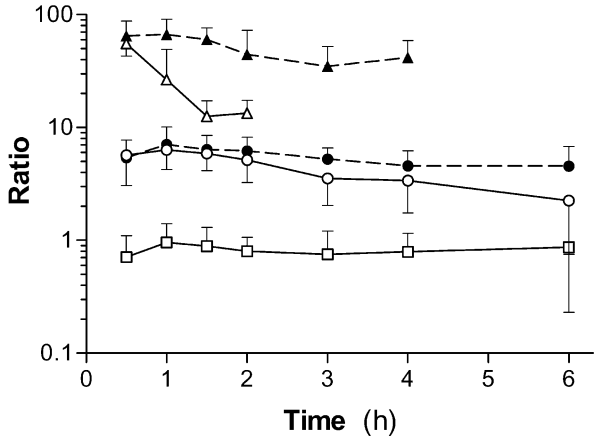


**Fig. 2** Total (unconjugated + conjugated) 1-OH midazolam/midazolam ratios in function of time after oral administration of 75 µg (*open symbols*, *continuous lines*) or 7.5 mg (*closed symbols*, *dashed lines*) midazolam in healthy volunteers without pre-treatment (*circles*), after rifampicin (*triangles*) and after ketoconazole pre-treatment (*squares*; the 7.5-mg dose was not given with ketoconazole). Geometric means and standard deviations are shown

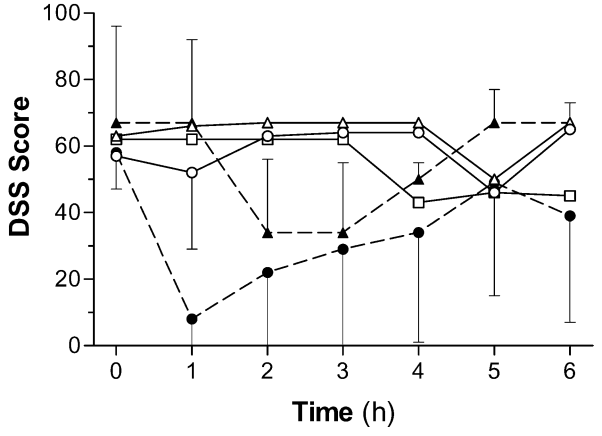


**Fig. 3** Results of the Digit Symbol Substitution (DSS) test as a function of time after oral administration of 75 µg (*open symbols*, *continuous lines*) or 7.5 mg (*closed symbols*, *dashed lines*) midazolam in healthy volunteers without pre-treatment (*circles*), after rifampicin (*triangles*) and after ketoconazole pre-treatment (*squares*; the 7.5-mg dose was not given with ketoconazole). Means and standard deviations are shown (for clarity, only selected standard deviations bars are represented)

pre-administration of rifampicin resulted in significantly higher scores when compared with the test with 7.5 mg MID without rifampicin ($P < 0.01$), but only 1 h after the oral administration of MID.

Table 4 and Table 5 list the correlations measured between total (conjugated and non-conjugated) MR and MID clearance, determined after oral administration of 75 µg and 7.5 mg MID, respectively, at various time points, in the group of subjects with or without pre-treatment. Thus, 30 min after oral administration of the low dose, a good correlation ($r^2 = 0.64$, $P < 0.001$) was calculated in the group of 13 subjects without

co-medication, while excellent correlations were calculated when including subjects with induced and/or inhibited CYP3A ($r^2 = 0.91$, $P < 0.0001$ for 13 subjects without co-medication + 4 subjects with ketoconazole + 4 subjects with rifampicin; Table 4). Although being significant when using total MR calculated after administration of the higher dose of MID (Table 5), the correlations with MID clearance were lower under administration of the lower dose. Interestingly, both after 75 μg and after 7.5 mg MID, much lower correlations were obtained between MID clearance and MR calculated with unconjugated concentrations of 1OH-MID. Significant correlations were thus observed only when including subjects with inhibited and/or induced CYP3A activity, but not when including subjects without co-medication, and that at all times (data not shown).

We also tried to determine whether MID plasma levels, determined at various times after administration of the low and higher dose of MID, can be used to assess MID clearance (Table 6 and Table 7). It can be seen

**Table 4** Spearman correlation coefficients between total 1-OH midazolam/midazolam ratios (determined after oral administration of 75 μg midazolam), and midazolam clearance at various time points and with different groups of subjects

| | 0.5 h | 1 h | 1.5 h | 2 h | 3 h | 4 h | 6 h |
|---|---|---|---|---|---|---|---|
| Subjects without co-medication ($n = 13$) | | | | | | | |
| Spearman correlation | 0.80 | 0.76 | 0.54 | 0.68 | 0.57 | 0.25 | 0.46 |
| Coefficient of determination ($r^2$) | 0.64 | 0.58 | 0.30 | 0.46 | 0.32 | 0.06 | 0.21 |
| $P$ level | < 0.001 | < 0.005 | NS ($P = 0.055$) | < 0.05 | < 0.05 | NS | NS |
| Subjects without co-medication ($n = 13$) + subjects with ketoconazole ($n = 4$) | | | | | | | |
| Spearman correlation | 0.91 | 0.89 | 0.80 | 0.86 | 0.80 | 0.66 | 0.70 |
| Coefficient of determination ($r^2$) | 0.83 | 0.80 | 0.63 | 0.73 | 0.65 | 0.44 | 0.50 |
| $P$ level | < 0.0001 | < 0.0001 | < 0.0005 | < 0.0001 | < 0.0005 | < 0.005 | < 0.005 |
| Subjects without co-medication ($n = 13$) + subjects with rifampicin ($n = 4$) | | | | | | | |
| Spearman correlation | 0.91 | 0.88 | 0.75 | 0.82 | 0.71 | 0.51 | 0.46 |
| Coefficient of determination ($r^2$) | 0.83 | 0.77 | 0.56 | 0.67 | 0.51 | 0.26 | 0.21 |
| $P$ level | < 0.0001 | < 0.0001 | < 0.001 | < 0.0005 | < 0.005 | NS ($P = 0.051$) | NS |
| Subjects without co-medication ($n = 13$) + subjects with ketoconazole ($n = 4$) + subjects with rifampicin ($n = 4$) | | | | | | | |
| Spearman correlation | 0.95 | 0.94 | 0.87 | 0.91 | 0.86 | 0.76 | 0.70 |
| Coefficient of determination ($r^2$) | 0.91 | 0.87 | 0.76 | 0.82 | 0.74 | 0.57 | 0.50 |
| $P$ level | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0005 | < 0.005 |

**Table 5** Spearman correlation coefficients between total 1-OH midazolam/midazolam ratios (determined after oral administration of 7.5 mg midazolam) and midazolam clearance at various time points and with different groups of subjects

| | 0.5 h | 1 h | 1.5 h | 2 h | 3 h | 4 h | 6 h |
|---|---|---|---|---|---|---|---|
| Subjects without co-medication ($n = 13$) | | | | | | | |
| Spearman correlation | 0.51 | 0.64 | 0.63 | 0.37 | 0.21 | 0.29 | 0.60 |
| Coefficient of determination ($r^2$) | 0.26 | 0.41 | 0.40 | 0.14 | 0.04 | 0.09 | 0.35 |
| $P$ level | NS ($P = 0.07$) | < 0.05 | < 0.05 | NS | NS | NS | NS |
| Subjects without co-medication ($n = 13$) + subjects with ketoconazole ($n = 4$) | | | | | | | |
| Spearman correlation | 0.78 | 0.84 | 0.84 | 0.72 | 0.64 | 0.71 | 0.87 |
| Coefficient of determination ($r^2$) | 0.61 | 0.71 | 0.71 | 0.51 | 0.42 | 0.51 | 0.76 |
| $P$ level | < 0.0005 | < 0.0001 | < 0.0001 | < 0.005 | < 0.01 | < 0.005 | < 0.0005 |
| Subjects without co-medications ($n = 13$) + subjects with rifampicin ($n = 4$) | | | | | | | |
| Spearman correlation | 0.78 | 0.82 | 0.80 | 0.65 | 0.48 | 0.57 | 0.60 |
| Coefficient of determination ($r^2$) | 0.61 | 0.68 | 0.64 | 0.42 | 0.23 | 0.32 | 0.35 |
| $P$ level | < 0.0005 | < 0.0001 | < 0.0005 | < 0.01 | NS ($P = 0.07$) | < 0.05 | NS |
| Subjects without co-medication ($n = 13$) + subjects with ketoconazole ($n = 4$) + subjects with rifampicin ($n = 4$) | | | | | | | |
| Spearman correlation | 0.88 | 0.91 | 0.90 | 0.82 | 0.74 | 0.80 | 0.87 |
| Coefficient of determination ($r^2$) | 0.78 | 0.82 | 0.81 | 0.67 | 0.55 | 0.64 | 0.76 |
| $P$ level | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0005 | < 0.0005 | < 0.0005 |

**Table 6** Spearman correlation coefficients between midazolam plasma levels (determined after oral administration of 75 μg midazolam) and midazolam clearance at various time points and with different groups of subjects

| | 0.5 h | 1 h | 1.5 h | 2 h | 3 h | 4 h | 6 h |
|---|---|---|---|---|---|---|---|
| Subjects without co-medication ($n=13$) | | | | | | | |
| Spearman correlation | 0.12 | −0.66 | −0.85 | −0.81 | −0. 83 | −0.83 | −0.63 |
| Coefficient of determination ($r^2$) | 0.01 | 0.43 | 0.73 | 0.66 | 0.69 | 0.68 | 0.40 |
| *P* level | NS | < 0.05 | < 0.0005 | < 0.001 | < 0.0005 | < 0.005 | NS |
| Subjects without co-medication ($n=13$) + subjects with ketoconazole ($n=4$) | | | | | | | |
| Spearman correlation | −0.50 | −0.84 | −0.93 | −0.91 | −0.92 | −0.93 | −0.91 |
| Coefficient of determination ($r^2$) | 0.25 | 0.70 | 0.86 | 0.84 | 0.85 | 0.86 | 0.82 |
| *P* level | < 0.05 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0005 |
| Subjects without co-medication ($n=13$) + subjects with rifampicin ($n=4$) | | | | | | | |
| Spearman correlation | −0.27 | −0.77 | −0.90 | −0.87 | −0.83 | −0.83 | −0.63 |
| Coefficient of determination ($r^2$) | 0.07 | 0.59 | 0.82 | 0.76 | 0.69 | 0.68 | 0.40 |
| *P* level | NS | < 0.001 | < 0.0001 | < 0.0001 | < 0.0005 | < 0.005 | NS |
| Subjects without co-medications ($n=13$) + subjects with ketoconazole ($n=4$) + subjects with rifampicine ($n=4$) | | | | | | | |
| Spearman correlation | −0.64 | −0.88 | −0.95 | −0.94 | −0.92 | −0.93 | −0.91 |
| Coefficient of determination ($r^2$) | 0.41 | 0.77 | 0.90 | 0.88 | 0.85 | 0.86 | 0.82 |
| *P* level | < 0.005 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0005 |

**Table 7** Spearman correlation coefficients between midazolam plasma levels (determined after oral administration of 7.5 mg midazolam) and midazolam clearance at various time points and with different groups of subjects

| | 0.5 h | 1 h | 1.5 h | 2 h | 3 h | 4 h | 6 h |
|---|---|---|---|---|---|---|---|
| Subjects without co-medication ($n=13$) | | | | | | | |
| Spearman correlation | −0.08 | −0.51 | −0.87 | −0.91 | −0.87 | −0.96 | −0.86 |
| Coefficient of determination ($r^2$) | 0.01 | 0.26 | 0.75 | 0.83 | 0.76 | 0.91 | 0.74 |
| *P* level | NS | NS ($P=0.07$) | < 0.0005 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0005 |
| Subjects without co-medication ($n=13$) + subjects with rifampicin ($n=4$) | | | | | | | |
| Spearman correlation | −0.59 | −0.76 | −0.93 | −0.94 | −0.91 | −0.96 | 0.11 |
| Coefficient of determination ($r^2$) | 0.35 | 0.58 | 0.86 | 0.89 | 0.84 | 0.93 | 0.01 |
| *P* level | < 0.05 | < 0.0005 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | NS |

that, after both doses, excellent correlations were observed between 1.5 h and 4 h for the low doses (r between −0.81 and −0.85) and between 1.5 h and 6 h for the higher dose (r between −0.87 and −0.96), in the subjects without co-medication. As expected, better correlations were also observed when including the subjects with ketoconazole and/or rifampicin.

## Discussion

The aim of the present study was to determine whether MID ratios or MID concentrations measured after the administration of a low dose of MID (75 μg) can be used to assess CYP3A activity. MID ratios and MID concentrations measured after the administration of the low dose were thus correlated with clearance of MID determined after administration of the therapeutic dose (7.5 mg) in 13 healthy subjects. Both doses of MID produced fairly similar pharmacokinetic profiles and, although pharmacokinetic parameters could be quantified more accurately with the 7.5-mg dose, they were also calculated for the 75-μg dose.

To assess whether the 75-μg dose can also be used to reflect intra-individual changes of CYP3A activity, the pharmacokinetic profile of 75 μg MID was determined in two groups of four subjects, before and after a pre-treatment with rifampicin and ketoconazole. Rifampicin is a strong CYP3A inducer, which has previously been shown to markedly decrease the blood concentrations and/or pharmacodynamic effects of several drugs metabolised by CYP3A, such as MID [16], alprazolam [17], zolpidem [18], buspirone [19], triazolam [20] or zopiclone [21]. Ketoconazole is a strong CYP3A inhibitor which has been shown to increase the blood concentrations and/or pharmacodynamic effects of several drugs metabolised by CYP3A, such as MID [22], triazolam [23, 24] or alprazolam [24].

The pre-treatment by rifampicin had a major influence on its apparent clearance and distribution volume.

This is first explained by a pronounced negative effect on MID bioavailability, which increases the denominator of the apparent value of both those parameters. Rifampicin increases CYP3A4 activity both in the liver and in the bowel wall [16]. The absence of change in MID terminal $t_{1/2}$ with the therapeutic dose in response to rifampicin cannot be explained by a too-short period during which MID was quantified. On the contrary, the early disposition of MID was characterised by a steeper slope after rifampicin pretreatment; this observation is in line with authors having reported a shortening of MID $t_{1/2}$ under rifampicin (from 3.1 h to 1.3 h), using a less sensitive assay and a shorter period of blood collection [16]. In the present study, the slope of the terminal part of the disposition curve was not significantly influenced by rifampicin. One could imagine that this late phase reflects a slow redistribution of MID from a deep compartment. It must also be mentioned that a higher dose of rifampicin (600 mg/day) was used in the previous [16] than in the present study (450 mg/day). Changes observed in the apparent pharmacokinetic parameters of 1OHMID and 4OHMID are more difficult to interpret. These values are under the dependence of the oral bioavailability, but they also reflect changes induced by rifampicin in the metabolic fraction corresponding to the respective pathway. This is illustrated by the important increase in the plasma ratio of 1OHMID over MID.

The effect of ketoconazole on MID kinetics was opposed to that of rifampicin as reported in a previous study [22]. The decrease of the apparent clearance and distribution volume is mainly mediated by an increase of MID bioavailability. As the relative decrease in apparent distribution volume was less than in apparent clearance, this suggests an alteration of the true clearance in addition to the effect on bioavailability. The prolongation of MID $t_{1/2}$ by ketoconazole observed in the present study is also in agreement with previously published results [22]. However, a much stronger increase (5.5-fold increase versus 1.6-fold) was found in the latter study [22], which could tentatively be explained by a longer blood sampling period (24 h). The $t_{1/2}$ of 1OHMID and 4OHMID appeared prolonged too, but this is probably an indirect effect, the disposition of these metabolites being driven by their production.

The present study shows that the 30 min MR can be reliably used as an indicator of MID clearance and, thus, of CYP3A activity. In the group of 13 subjects with non-induced and non-inhibited CYP3A activity, the MR accounts for 64% of the variability of MID clearance. In the group of 17 subjects including either four subjects with induced or four subjects with reduced CYP3A4 activity, the MR accounts for 83% of its variability, while in the group of 21 subjects, including four subjects with induced and four subjects with reduced CYP3A activity, the 30-min MR accounts for 91% of its variability. This result confirms those of previously published studies, although in the latter only the higher dose of MID was used [7, 9, 10, 11, 12]. In fact, unexpectedly, better correlations were obtained with the lower than with the higher dose, which however needs to be confirmed by other studies. The present study also shows that MR calculated with total concentrations (unconjugated + conjugated) is a better indicator of CYP3A activity than MR calculated with unconjugated concentrations. This result can most probably be explained by the fact that the use of total concentrations, determined after enzymatic hydrolysis of the glucuronic conjugates, allows removal of the interindividual variability of glucuroconjugation from the MR [9].

The present study also shows that MID plasma levels can be used as an indicator of MID presystemic metabolism, after both the lower and higher doses. However, only values measured from 90 min up to 4–6 h can be used reliably. This result is in agreement with those of another study that attempted to validate the use of alprazolam as a CYP3A test probe, and that found excellent correlations between alprazolam plasma concentrations measured 6, 8, 10 and 24 h after oral administration of a therapeutic dose of alprazolam (i.e. 1 mg) and AUC of this drug [17]. It is also in agreement with those of a recent retrospective study suggesting that, following an IV or oral MID administration, the optimal single sampling time to predict AUC was between 3 h and 4 h [13]. The question arises whether only intestinal or both intestinal and hepatic CYP3A are assessed by the use of such a low dose of MID; the fact that good correlations are observed between MID clearance measured after the 7.5-mg dose and the 30-min MR or the MID plasma levels measured after the 75-μg dose is an argument supporting the latter hypothesis.

In summary, the present study shows that a low oral dose of MID (75 μg) can be reliably used to assess CYP3A activity. This can be performed either by the determination of the 30-min MR calculated with total (unconjugated + conjugated) concentrations or by measuring MID plasma levels between 1.5 h and 4 h after oral intake. Due to the lack of central nervous system side effects induced by this low dose, even in subjects with a low CYP3A activity, and due to its short duration (in particular when using the MR), this test can easily be used for large-scale phenotyping tests.

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