Eur J Clin Pharmacol (2010) 66:1189–1197
DOI 10.1007/s00228-010-0906-2

CLINICAL TRIAL

Systemic uptake of miconazole during vaginal suppository use and effect on CYP1A2 and CYP3A4 associated enzyme activities in women

Mia Birkhøj Kjærstad · Flemming Nielsen · Lene Nøhr-Jensen · Stine Zwisler · Kim Brøsen · Helle Raun Andersen

Received: 5 July 2010 / Accepted: 17 September 2010 / Published online: 6 October 2010

Abstract
Purpose To investigate if the ordinary use of a vaginal suppository containing miconazole results in systemic absorption that is sufficient to affect the activities of CYP1A2 and CYP3A4, which are major drug- and steroid-metabolising enzymes.
Methods In 20 healthy non-pregnant women aged 18–45 years, the serum concentration of miconazole was determined following the use of a vaginal suppository containing 1,200 mg miconazole. Enzyme activities of CYP1A2 and CYP3A4 were determined as metabolic ratios of caffeine (CMR=(AFMU+1MU+1MX)/17DMU) and quinidine (QMR=3-hydroxy-quinidine/quinidine) respectively before and 34 h after insertion of the suppository. Miconazole was analysed by LC-MS/MS, while caffeine and metabolites were analysed by HPLC-UV and quinidine and hydroxy-quinidine were analysed by HPLC fluorescence.
Results All 20 women had measurable concentrations of miconazole in serum (mean ± SD: 12.9±5.6 μg/L; range: 3.5–24.6 μg/L). Although not statistically significant, an association between the serum concentrations of miconazole and the inhibition of CYP1A2 activity was indicated. No relation was observed between the CYP3A4 activity and the miconazole serum concentration.
Conclusions Miconazole is absorbed via the vaginal mucosa to the systemic circulation in measurable concentrations. Our data indicate a concentration-dependent inhibition of CYP1A2, but the effect is negligible compared with the variation in the activity of CYP1A2 and is regarded to be of no clinical significance to the women. However, further studies on the ability of miconazole to be transferred across the placenta or to interfere with the placental function are warranted to secure safe use during pregnancy.

Keywords Miconazole · Antifungal · CYP1A2 · CYP3A4 · Vaginal uptake

Clinical trial registry: www.clinicaltrials.gov

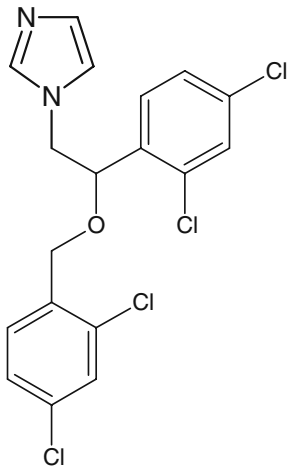
Registration number: NCT00668538

M. B. Kjærstad (✉) · F. Nielsen · H. R. Andersen
Department of Environmental Medicine,
Institute of Public Health, University of Southern Denmark,
JB Winsløwsvej 17,
5000 Odense C, Denmark
e-mail: mbirkhoej@health.sdu.dk

F. Nielsen · L. Nøhr-Jensen · S. Zwisler · K. Brøsen
Clinical Pharmacology, Institute of Public Health,
University of Southern Denmark,
JB Winsløwsvej 19,
5000 Odense C, Denmark

Introduction

Miconazole (Fig. 1) is a pharmaceutical antifungal. It inhibits the fungal enzyme lanosterol 14-α-demethylase (CYP51), which regulates ergosterol synthesis essential for formation of the fungal cell membrane. Miconazole is used for the treatment of oral candidiasis in infants and vaginal mycoses in pregnant women and may reach the systemic circulation of the child and fetus if it is able to cross the mucous membrane and the placental barrier. However, the systemic bioavailability of miconazole following absorption via the vagina and the gut were reported to be low, 1.4% and 27% respectively [1–3], and orally and vaginally absorbed miconazole were eliminated with half-lives between 2 and 57 h [3–5]. Nevertheless, drug interactions between oral gel or vaginally used miconazole and the



Miconazole

Fig. 1 Chemical structure of miconazole, a pharmaceutical imidazole fungicide

anticoagulant warfarin have been reported, indicating a systemic concentration high enough to affect biotransformation [6, 7].

In a panel of *in vitro* test systems, miconazole showed the potential to disrupt the endocrine system by several mechanisms [8]. Miconazole inhibited testosterone biosynthesis and the estrogen synthesising enzyme aromatase (CYP19) and it was a weak estrogen receptor antagonist. In other experimental test systems, miconazole had unspecific effects on the humane cytochrome P450 (CYP) system and has been reported to inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP17, CYP19 and CYP51 activities [9–14]. However, the inhibitory potency of miconazole differed among different CYPs. CYP17 and CYP19 are involved in steroid synthesis whereas CYP1A2 and CYP3A4 are major drug-metabolising enzymes, but they are also involved in estrogen metabolism [15, 16]. Disturbance of these enzymatic pathways may affect homeostasis. The developing fetal and infantile endocrine and reproductive systems are particularly susceptible to endocrine disrupting substances [17–19]. Other conazoles which like miconazole, have been reported to inhibit CYP19 and testosterone synthesis *in vitro*, and have been shown to disturb steroid synthesis in rat fetuses after prenatal exposure [20, 21]. Consequently, there is a concern that miconazole after ordinary use orally or in the vagina could result in systemic concentrations high enough to disturb the endocrine balance of the infant, the pregnant woman, or the fetus.

The primary aim of this study was therefore to investigate whether the ordinary use of a vaginal suppository containing 1,200 mg of miconazole resulted in systemic absorption. The secondary aim was then to investigate if the systemic concentration of miconazole was high enough to affect enzyme activities associated with CYP1A2 and CYP3A4.

Materials and methods

Subjects

Twenty healthy female subjects were recruited in the period May to August 2008. The participants were mainly students at the University of Southern Denmark or staff at Odense University Hospital. Characteristics of the study subjects are shown in Table 1. After oral and written information about the study and their rights, 30 subjects gave their written consent and a written authority giving relevant personnel from the GCP Unit and the Danish Medicines Agency access to documents and data of importance for the study. Out of these 30, 4 subjects regretted their enrolment and withdrew their consent. Four individuals were excluded because of use of medication, and 2 were excluded since their electrocardiogram (ECG) revealed irregularities. Hence, 20 subjects completed the study.

Inclusion and exclusion criteria

Inclusion criteria were healthy, non-pregnant (evaluated by no detection of human chorionic gonadotrophin in a urine sample) and non-breastfeeding women aged between 18 and 45 years. They were ascertained to be in good health based on clinical examination including blood pressure, medical history and evaluation of a standard 12-lead ECG

Table 1 Characteristics of the study subjects

Characteristic	Data
Number of subjects completing the study	20
Age, years (range)	26 (23–43)
Weight, kg (range)	65 (52–95)
Height, cm (range)	169 (158–180)
BMI, kg/m^2 (range)	22.2 (18.5–31.0)
Race and nationality	
Caucasian—Danish	16
Caucasian—Norwegian	3
Unknown—Danish	1
Work	
Students	14
Hospital workers	5
Maternity leave	1
Number of subjects menstruating during the study	9
Contraceptives	
None	3
Condom	10
Intrauterine device made of copper	7
Non-smokers	18

For continuous variables, data represent median (range)

done by the physician responsible for the study. Subjects with daily alcohol or medical use including oral contraceptives or known allergy to miconazole, caffeine, quinidine, sodium methyl 4-hydroxybenzoate (E219), or sodium propyl 4-hydroxybenzoate (E217) were excluded. Smokers were not excluded since smoking is known to have an inducing effect on CYP1A2, not an inhibitory effect, and since the subjects were their own controls and exclusion of smokers might hamper the recruitment of subjects.

Ethics

The study was approved by The Regional Scientific Ethical Committee for Southern Denmark (record number: S-20080022), the Danish Medicines Agency (record number: 2612-3691), and the Danish Data Protection Agency (record number: 2008-41-2011). The study was performed according to the Helsinki-II-Declaration and in accordance with guidelines of Good Clinical Practice (ICH-GCP) as monitored by the GCP Unit, Odense University Hospital (record number: 07.035).

Study design and procedures

Initially, all participants answered a questionnaire regarding vegetable and fruit intake, smoking status, job function and use of antifungals in the homes including personal hygiene products such as shampoo or cream.

For each subject the study lasted 11 days (Fig. 2). It was a two-period study with a wash-out period of 2 days before each caffeine and quinidine administration period and of 5 days after insertion of the miconazole-containing vaginal suppository.

The subjects were asked to abstain from ingesting quinine-, quinidine- and methylxanthine-containing food and beverages (specific items were pointed out) as well as alcohol and medication, from 48 h before caffeine and quinidine administration and until the last blood sampling at day 8. To avoid pregnancy in the study period the subjects were asked to use a condom when having intercourse from the time of the pregnancy test until the insertion of the vaginal suppository. Thereafter, sexual abstinence was required until the end of the trial at day 11.

Each subject ingested 200 mg of caffeine (batch number 2151631) and 200 mg of quinidine (batch number 51313011), both supplied by the Central Pharmacy, Odense University Hospital (Denmark), at 11 pm on day 3. Previous studies indicate that simultaneous administrations of low doses of these two compounds do not influence the outcome of their respective metabolic ratios [22–24]. Morning urine was collected after a minimum of 3 h. Ten millilitres of the urine was poured into a conical tube containing 300 μl 1 M hydrochloric acid (HCl) for the CYP1A2 activity analysis. Forty millilitres was transferred into each of two sterile empty test tubes for the miconazole analysis. The urine samples were brought to the laboratory and stored at −20°C until analysis. At 9–10 h after quinidine administration, blood samples (5×10 ml) were collected by vein puncture at the laboratory for the CYP3A4 activity and miconazole analysis. Blood sampling was done by either the technician or the physician involved in the study. Twenty millilitres was collected in heparin tubes and 30 ml in dry tubes. All blood samples were centrifuged for 10 min at 3,500 rpm and plasma and serum were transferred to sterile cryogenic vials and kept frozen at –20°C until analysis.

On day 6 at 11 pm the vaginal suppository was inserted and the subjects were instructed to lie down afterwards for the night. Twenty-four hours later caffeine and quinidine were administered again for the evaluation of CYP1A2 and CYP3A4 activity after miconazole exposure. The urine and blood sampling procedures were repeated.

The time schedule was chosen based on knowledge from caffeine [25] and quinidine [23] kinetics studies and the findings of Daneshmend (1986) [3], which showed a continuously maximal miconazole concentration in plasma from 6 to 48 h after insertion of a 1,200-mg miconazole-containing suppository.

Determination of miconazole in serum

The serum concentration of miconazole was quantified by liquid chromatography coupled to a Thermo TSQ Quantum

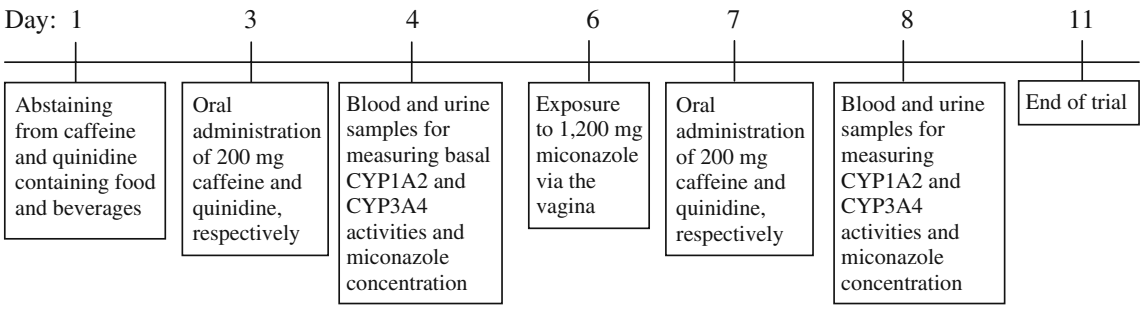


Fig. 2 Time schedule of the trial

Ultra triple quadrupole mass spectrometer (LC-HESI-MS/MS system; Thermo Scientific, Waltham, MA, USA). The extraction procedure was as follows: a volume of 250 μl of the serum sample, 60 μl 0.2 M sodium hydroxide and 50 μl of internal standard (0.5 ng/ml tebuconazole in ethanol) was briefly vortex mixed in a 10-ml centrifugation tube. Then, 1 ml *tert*-butyl methylether was added and the samples were mixed horizontally for 20 min at 200 rpm followed by 15 min centrifugation at 3,000*g*. The supernatant was transferred to a new extraction tube. The extraction procedure was repeated. The two supernatants were combined and evaporated to accurate dryness at 40°C under a gentle stream of nitrogen. The dried extract was dissolved in 125 μl acetonitrile (ACN) followed by 125 μl buffer solution (2 mM ammonium acetate and 2 mM acetic acid, pH 4.6). The sample was centrifuged for 10 min at 3,000*g*, transferred to a 300-μl sample vial, and injected (30 μl) onto the analytical system.

The separation was performed on a Phenomenex C12 MAX-RP column (4 μm, 150×2 mm) kept at 20°C using a two-solvent gradient system: (2 mM ammonium acetate and 2 mM acetic acid), pH 4.6 : ACN (95:5) (A) and (5:95) (B). The gradient profile was: 0 to 4.37 min: 40% to 100% B; 4.38 to 10.00 min: 100% B; 10.01 to 11.50 min: 100% to 40% B; 11.51 to 15.00 min: 40% B. The flow rate was 0.3 ml/min. The MS/MS system was operated with positive polarity in SRM mode with a scan width of 0.2 m/z and a scan time of 0.250 s. The spray voltage was 3,500 V, the vaporiser temperature 350°C, the sheath gas pressure 60 AU, the auxiliary gas pressure 45 AU and the capillary temperature 315°C. Miconazole was quantified with a parent mass of 416.86 m/z fragmented to a product mass of 160.95 m/z, and was detected at a retention time of 8.66 min. Tebuconazole was quantified with a parent mass of 308.1 m/z to the product masses of 124.8 and 70.0 m/z and was detected at a retention time of 5.91 min.

Quantitation of miconazole was based on calibration curves obtained after the addition of known concentrations of the compound to blank serum and water samples and then extracted and analysed as described above. The recovery of the extraction method was > 90%. The linearity was investigated in a range from 0.1 to 50.0 ng/ml (R^2>0.98). The interday repeatability (n=3) was < 11 %. The accuracy for the quality control samples, spiked to a concentration of 5 ng/ml, was −4%. The limit of quantification (LOQ) for miconazole was 0.10 ng/ml and the limit of detection (LOD) was 0.05 ng/ml.

Determination of CYP1A2 activity

Four metabolites of caffeine: 5-acetylamino-6-formylamino-3-methyluracil (AFMU), 1-methyluric acid (1MU), 1-methylxanthine (1MX) and 1,7-dimethyluric acid (17DMU) were determined in morning urine samples conserved with 1M HCl by high-performance liquid chromatography with UV detection [26]. The CYP1A2 activity was estimated by the caffeine metabolic ratio (CMR)=(AFMU+1MU+1MX)/17DMU.

Determination of CYP3A4 activity

Quinidine and 3-hydroxy-quinidine in plasma samples collected 9–10 h after ingestion of 200 mg quinidine were analysed using high-performance liquid chromatography, as described previously [27]. The CYP3A4 activity was estimated by the quinidine metabolic ratio (QMR)=3-hydroxy-quinidine/quinidine as suggested by Damkier and Brosen [23].

Data analysis

The mean miconazole absorption was tested by a paired *t* test comparing the mean miconazole concentrations in serum 34 h after and before the start of miconazole exposure. The differences in metabolic ratios are expressed as the relative differences, e.g. $(CMR_{after}–CMR_{before})/CMR_{before}$. The mean relative differences in CMR and QMR during and before exposure to miconazole were analysed by one-sample *t* tests comparing the relative differences with zero. The assumptions of normal distribution were considered fulfilled after evaluation by Q-Q plot (not shown) of the differences. The variations were roughly constant along the x-axis. Relative difference (Bland–Altman) plots of CMR and QMR were used as supplements to analyse the deviation between the metabolic ratios during and before exposure to miconazole.

The association between the relative difference in CMR or QMR and the miconazole concentration in serum was examined by linear regression.

P values<0.05 were considered statistically significant. The statistical analyses were performed using Stata/SE 9.0 for Windows (Stata, College Station, TX, USA). Sample size calculations were based on the primary outcome, absorption of miconazole. As the background concentration of miconazole was zero and the LOQ=0.10 μg/L we consider a concentration of at least 1 μg/L to be an indication of absorption. Twenty women were considered suitable to detect miconazole in serum, which would give a power of >90% at a significance level of 5%.

Results

Subjects

None of the subjects had adverse drug reactions. Seventeen subjects had one or more side effects. The side effects that occurred were expected: headache, trouble sleeping, diar-

rhoea, palpitations and vaginal itching/discomfort. All side effects were followed up and all subjects were well again after the study. Three subjects had no side effects.

Miconazole

The mean serum concentration of absorbed miconazole in the 20 women after 34 h use of a vaginal suppository containing 1,200 mg miconazole was 12.9±5.6 μg/L (mean ± SD; range: 3.5–24.6 μg/L; Fig. 3). Background serum concentrations of miconazole were below the limit of detection. The absorption of miconazole was not associated with menstruation during the study, age, or BMI (data not shown). The miconazole concentration in the urine samples was very high because of leakage from the vagina and therefore the urine concentration could not be used as an indication of the absorbed concentration of miconazole.

CYP1A2 and CYP3A4 activity

During the chromatographic detection of caffeine and metabolites, interference from the urine sample hindered the separation of AFMU in six samples. Consequently, only 14 out of 20 subjects were included in the calculations of CYP1A2 activity. One subject had trouble swallowing the quinidine capsule and therefore 19 out of 20 subjects were included in the CYP3A4 activity determination. The urine sampling time after caffeine administration differed between the two administrations with more than 1 h for 5 subjects, while for the rest of the subjects the difference was close to zero.

Data of CMR are presented as a scatter plot showing the relative difference in CMR between the two exposure periods against the average of CMR of the two periods (Fig. 4a). The mean relative CMR difference (the horizontal unbroken line) was shifted a little below zero, but this was not statistically significant (Table 2). One subject had high CMRs compared with the other subjects and a relatively large change in CMR (the dot furthest to the right).

The QMR data are presented in Fig. 4b. The mean relative QMR difference (the horizontal unbroken line) was shifted a little above zero, but this was not statistically significant (Table 2). One subject (not the same as the one who had high CMRs above) had a relatively large change in quinidine metabolism.

The associations between the relative difference in CMR or QMR and serum miconazole concentrations are shown in Fig. 5. A low, non-statistically significant decrease (p=0.33) in relative CMR difference with increased concentration of miconazole in serum was indicated. There was no concentration-dependent association (p=0.89) between QMR and miconazole.

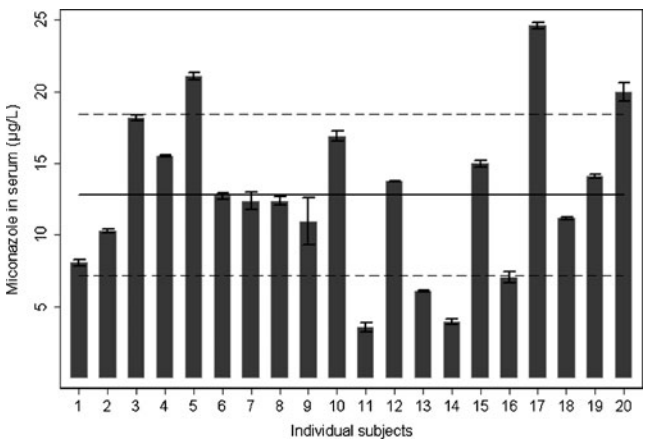


Fig. 3 Miconazole concentration measured in serum after 34 h use of a 1,200-mg miconazole-containing suppository. Each bar represents one subject. Background concentrations of serum miconazole were zero. The *horizontal unbroken line* represents the mean (n=20) and the two *horizontal dashed lines* represent ±SD of the mean

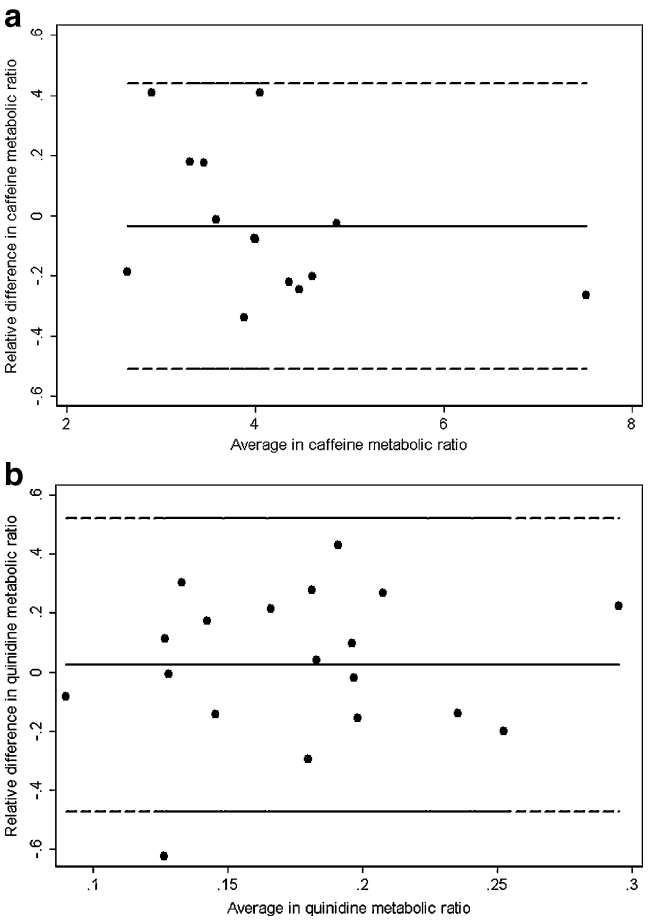


Fig. 4 Relative difference (Bland–Altman) plot of **a**) CYP1A2 activity determined by the caffeine metabolic ratio (CMR=(AFMU+1MU+1MX)/17DMU) and of **b**) CYP3A4 activity determined by the quinidine metabolic ratio (QMR=3-hydroxy-quinidine/quinidine). The plots help to analyse the deviation between the metabolic ratios determined 34 h after and before exposure to miconazole started. The *horizontal unbroken line* represents the mean relative CMR or QMR difference. The *horizontal dashed lines* represent the limits of agreement (±1.96*SD)

Table 2 Serum concentration of miconazole and the caffeine (*CMR*) and quinidine (*QMR*) metabolic ratio in women before and 34 h after the start of exposure to miconazole

	Mean ± SD	Range	95% confidence interval	*t* test (*p* value)
Miconazole (μg/L; *n*=20)	12.9±5.6	3.6–24.6	10.3–15.5	<0.0001
CMR_{before} (*n*=14)	4.3±1.5	2.4–8.7	3.4–5.2	
CMR_{after}	3.9±0.9	2.4–6.4	3.4–4.5	
Relative $CMR_{difference}$	-0.035±0.24	-0.34–0.41	-0.17–0.11	0.60
QMR_{before} (*n*=19)	0.18±0.05	0.09–0.27	0.15–0.20	
QMR_{after}	0.18±0.06	0.07–0.32	0.15–0.21	
Relative $QMR_{difference}$	0.024±0.25	-0.63–0.43	-0.098–0.15	0.68

Note: Miconazole before exposure was below the limit of detection. The *t* test was performed on the difference (after–before)

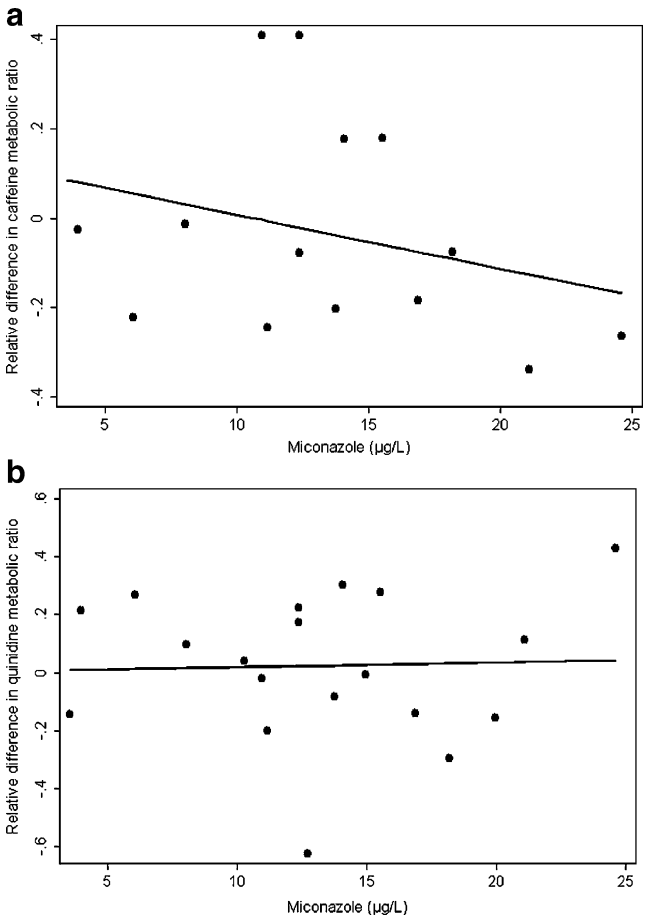


Fig. 5 Correlation between **a**) CYP1A2 activity determined by the relative difference in caffeine metabolic ratio or **b**) CYP3A4 activity determined by the relative difference in the quinidine metabolic ratio and miconazole concentration in serum. In plot **a**) the estimated association is described by y=-0.0121×[miconazole]+0.128 with R^2=0.0738 (*p*=0.33). In plot **b**) no association was indicated as y=0.0016×[miconazole]+0.003 with R^2=0.0013 (*p*=0.89)

Discussion

In this study we demonstrated that the use of a vaginal suppository containing 1,200 mg miconazole resulted in a mean serum concentration of miconazole of 12.9±5.6 μg/L (*n*=20) after 34 -h use. No substantial mean differences in CYP1A2 and CYP3A4 activities were observed after exposure to miconazole (exposure range: 3.5–24.6 μg/L serum). However, a weak negative and statistically non-significant association between the serum concentrations of miconazole and the relative difference in CMR was seen, indicating inhibition of CYP1A2. Because of considerable interindividual variations in the metabolic ratios and in the absorbed miconazole concentration only marked effects on enzyme activities can be detected in this study. Post hoc calculations using the standard deviation obtained from this study show that a true relative difference of at least 0.2 could be demonstrated for both CMR (*n*=14) and QMR (*n*=19) activities at a significance level of 5% and with a power of 80%. The association should therefore be interpreted with caution.

The large variation in serum miconazole absorption between subjects is in accordance with a previous study (mean serum concentration: 10.4±4.2 μg/L, *n*=11) [3]. The reason for this variation is not clarified, but might be due to differences in the vaginal environment and physical activity. In our study, neither menstruation during the study nor the duration of vaginal miconazole leakage (subjective evaluation of vaginal discharge) seemed to influence the systemic concentration of miconazole. The subjects were not examined for vaginal infections, but a damaged mucous membrane could probably absorb more miconazole, similar to when slightly damaged skin increased the penetration of pesticides in an *in vitro* skin model [28]. Also, genetic variations in the biotransformation of miconazole might play a role [29]. Other formulations of vaginal miconazole, i.e. vaginal cream, are not expected to give higher or lower

systemic levels as the application is repeated every day for 2 weeks, resulting in a similar total local dose to that with the suppository.

The mean CMR (CYP1A2 activity) estimated before the exposure to miconazole was 4.3±1.5. This was very similar to the values obtained in two other studies with non-smoking Caucasian women who did not use oral contraceptives [26, 30]. In a study with Faroese residents the overall mean (not adjusted for confounders such as gender, smoking, oral contraceptives) was 7.8±5.8 (n=307) [31], which was statistically significantly different from a Danish twin study with a mean value of 5.9±3.4 (n=378) [30], indicating that genetic or environmental factors or both should be considered when measuring and comparing CYP1A2 activity.

None of the women in our study used oral contraceptives or other medical products. All women were Caucasian except for one whose genetic background was unknown. This woman smoked occasionally and she was the only smoker included in the determination of CYP1A2 activity. No obvious deviation from the results of the other women was observed for this subject. Intraindividual variability may derive from difference in morning urine sampling time before and during miconazole exposure as the time interval differed by up to 4 h. It could also be speculated that the menstrual cycle might influence the activities of the enzymes and add to the variability, although studies investigating the effect of the menstrual cycle on CYP1A2 and CYP3A4 activities did not report such an effect [32–34].

The mean CYP3A4 activity (based on QMR) was not affected by exposure to miconazole. The wide interindividual variability was in accordance with other studies determining CYP3A4 based on the urinary 6β-hydroxycortisol/cortisol ratio or formation clearance of 3-hydroxyquinidine [23, 35]. One woman had a decreased CYP3A4 activity during the use of the miconazole vaginal suppository, but it was not the woman with the highest concentration of absorbed miconazole. The cause has not been identified, as the subjects were instructed to refrain from known CYP3A4 inhibitors. Miconazole and quinidine are both known inhibitors of P-glycoprotein and also likely substrates for this efflux transporter [36–39]. Intake of quinidine during miconazole exposure could be speculated to reduce the hepatic elimination of miconazole causing an elevation of the systemic concentration of miconazole. By inhibiting P-glycoprotein, miconazole use during pregnancy might also increase the placental transfer of other exogenous compounds that are P-glycoprotein substrates. In placenta, P-glycoprotein is expressed both at the membrane facing maternal circulation and at the membrane facing fetal circulation [40]. If not metabolised in the placenta, miconazole concentration may increase in this tissue when pregnant women are treated with miconazole.

Miconazole has been reported to inhibit several CYP enzymes in human liver or lymphoblast microsomes *in vitro*, including CYP1A2 and CYP3A4, with an inhibition constant (K_i) of 3.2 and 0.028 μM [14] and a half maximal inhibitory concentration (IC_{50}) of 2.9 and 0.074 μM [41, 42] respectively. This indicates that miconazole in the mean absorbed concentration of 12.9 μg/L serum corresponding to 0.03 μM might also have an effect on the enzymes *in vivo*.

Miconazole is used for treatment of vaginal mycoses, even in pregnant women, and of oral candidiasis in infants as these treatments are regarded as being effective and safe with regard to an alleged negligible absorption [2, 3]. The mean serum miconazole concentration of 12.9 μg/L was indeed low, but remained so for several days [3]. The biological effect measured in individuals using the miconazole-containing suppository was negligible. However, orally administered miconazole has been reported to interact with the CYP2C9 substrate warfarin *in vivo* [7, 43–45]. Hence, if miconazole is present with sufficient systemic concentration, a biological effect *in vivo* of miconazole is possible. Therefore, the potential adverse effects on the developing fetus and infant after miconazole exposure should be investigated further *in vivo*. Furthermore, it should be investigated whether miconazole is transferred to the fetus or if miconazole concentration is increased in the placenta with the possibility of disturbing the development of the fetus or the placental function respectively.

In conclusion, miconazole is absorbed via the vaginal mucosa to the systemic circulation in measurable concentrations. Our data indicate a concentration-dependent inhibition of CYP1A2, but the effect is negligible compared with the variation in the activity of CYP1A2 and is regarded to be of no clinical significance to the women. However, further studies on the ability of miconazole to be transferred across the placenta or to interfere with the placental function are warranted to ensure safe use during pregnancy.

Acknowledgements This work was supported by Clinical Pharmacology and the Department of Environmental Medicine at the University of Southern Denmark and with grants from Hartmann Fonden (number A1461), Beckett Fonden (number 80530), and Kong Christian den tiendes Fond (number 75/2008).

We are indebted to Vibeke Kvist Pedersen and Stine Rosendahl for excellent technical assistance.

Conflict of interest The authors declare that there are no conflicts of interest.

References

1. Boelaert J, Daneels R, Van Landuyt H, Symoens J (1976) Miconazole plasma levels in healthy subjects and in patients with impaired renal function. Chemotherapy 6:165–169

2. Daneshmend TK, Warnock DW (1983) Clinical pharmacokinetics of systemic antifungal drugs. Clin Pharmacokinet 8:17–42
3. Daneshmend TK (1986) Systemic absorption of miconazole from the vagina. J Antimicrob Chemother 18:507–511
4. Mannisto PT, Mantyla R, Nykanen S, Lamminsivu U, Ottoila P (1982) Impairing effect of food on ketoconazole absorption. Antimicrob Agents Chemother 21:730–733
5. Stevens DA, Levine HB, Deresinski SC (1976) Miconazole in coccidioidomycosis. II. Therapeutic and pharmacologic studies in man. Am J Med 60:191–202
6. Murty M (2001) Miconazole-warfarin interaction: increased INR. CMAJ 165:81–86
7. Pemberton MN, Oliver RJ, Theaker ED (2004) Miconazole oral gel and drug interactions. Br Dent J 196:529–531
8. Kjaerstad MB, Taxvig C, Nellemann C, Vinggaard AM, Andersen HR (2010) Endocrine disrupting effects in vitro of conazole antifungals used as pesticides and pharmaceuticals. Reprod Toxicol. doi:10.1016/j.reprotox.2010.07.009
9. Ayub M, Levell MJ (1987) Inhibition of testicular 17 alpha-hydroxylase and 17, 20-lyase but not 3 beta-hydroxysteroid dehydrogenase-isomerase or 17 beta-hydroxysteroid oxidoreductase by ketoconazole and other imidazole drugs. J Steroid Biochem 28:521–531
10. Ayub M, Levell MJ (1989) Inhibition of human adrenal steroidogenic enzymes in vitro by imidazole drugs including ketoconazole. J Steroid Biochem 32:515–524
11. Mason JI, Carr BR, Murry BA (1987) Imidazole antimycotics: selective inhibitors of steroid aromatization and progesterone hydroxylation. Steroids 50:179–189
12. Trosken ER, Fischer K, Volkel W, Lutz WK (2006) Inhibition of human CYP19 by azoles used as antifungal agents and aromatase inhibitors, using a new LC-MS/MS method for the analysis of estradiol product formation. Toxicology 219:33–40
13. Trosken ER, Adamska M, Arand M et al (2006) Comparison of lanosterol-14 alpha-demethylase (CYP51) of human and Candida albicans for inhibition by different antifungal azoles. Toxicology 228:24–32
14. Zhang W, Ramamoorthy Y, Kilicarslan T et al (2002) Inhibition of cytochromes P450 by antifungal imidazole derivatives. Drug Metab Dispos 30:314–318
15. Shou M, Korzekwa KR, Brooks EN et al (1997) Role of human hepatic cytochrome P450 1A2 and 3A4 in the metabolic activation of estrone. Carcinogenesis 18:207–214
16. Yamazaki H, Shaw PM, Guengerich FP, Shimada T (1998) Roles of cytochromes P450 1A2 and 3A4 in the oxidation of estradiol and estrone in human liver microsomes. Chem Res Toxicol 11:659–665
17. Grandjean P, Bellinger D, Bergman A et al (2008) The Faroes statement: human health effects of developmental exposure to chemicals in our environment. Basic Clin Pharmacol Toxicol 102:73–75
18. Skakkebaek NE, Rajpert-De Meyts E, Main KM (2001) Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 16:972–978
19. Swan SH, Main KM, Liu F et al (2005) Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect 113:1056–1061
20. Taxvig C, Hass U, Axelstad M et al (2007) Endocrine-disrupting activities in vivo of the fungicides tebuconazole and epoxiconazole. Toxicol Sci 100:464–473
21. Vinggaard AM, Hass U, Dalgaard M et al (2006) Prochloraz: an imidazole fungicide with multiple mechanisms of action. Int J Androl 29:186–192
22. Christensen M, Andersson K, Dalen P et al (2003) The Karolinska cocktail for phenotyping of five human cytochrome P450 enzymes. Clin Pharmacol Ther 73:517–528
23. Damkier P, Brosen K (2000) Quinidine as a probe for CYP3A4 activity: intrasubject variability and lack of correlation with probe-based assays for CYP1A2, CYP2C9, CYP2C19, and CYP2D6. Clin Pharmacol Ther 68:199–209
24. Frye RF, Matzke GR, Adedoyin A, Porter JA, Branch RA (1997) Validation of the five-drug "Pittsburgh cocktail" approach for assessment of selective regulation of drug-metabolising enzymes. Clin Pharmacol Ther 62:365–376
25. Rost KL, Roots I (1994) Accelerated caffeine metabolism after omeprazole treatment is indicated by urinary metabolite ratios: coincidence with plasma clearance and breath test. Clin Pharmacol Ther 55:402–411
26. Rasmussen BB, Brosen K (1996) Determination of urinary metabolites of caffeine for the assessment of cytochrome P4501A2, xanthine oxidase, and N-acetyltransferase activity in humans. Ther Drug Monit 18:254–262
27. Nielsen F, Nielsen KK, Brosen K (1994) Determination of quinidine, dihydroquinidine, (3S)-3-hydroxyquinidine and quinidine N-oxide in plasma and urine by high-performance liquid chromatography. J Chromatogr B Biomed Appl 660:103–110
28. Nielsen JB (2005) Percutaneous penetration through slightly damaged skin. Arch Dermatol Res 296:560–567
29. Duret C, Daujat-Chavanieu M, Pascussi JM et al (2006) Ketoconazole and miconazole are antagonists of the human glucocorticoid receptor: consequences on the expression and function of the constitutive androstane receptor and the pregnane X receptor. Mol Pharmacol 70:329–339
30. Rasmussen BB, Brix TH, Kyvik KO, Brosen K (2002) The interindividual differences in the 3-demethylation of caffeine alias CYP1A2 is determined by both genetic and environmental factors. Pharmacogenetics 12:473–478
31. Petersen MS, Halling J, Damkier P et al (2006) Caffeine N3-demethylation (CYP1A2) in a population with an increased exposure to polychlorinated biphenyls. Eur J Clin Pharmacol 62:1041–1048
32. Kashuba AD, Bertino JS Jr, Kearns GL et al (1998) Quantitation of three-month intraindividual variability and influence of sex and menstrual cycle phase on CYP1A2, N-acetyltransferase-2, and xanthine oxidase activity determined with caffeine phenotyping. Clin Pharmacol Ther 63:540–551
33. Kharasch ED, Russell M, Garton K et al (1997) Assessment of cytochrome P450 3A4 activity during the menstrual cycle using alfentanil as a noninvasive probe. Anesthesiology 87:26–35
34. Zaigler M, Rietbrock S, Szymanski J et al (2000) Variation of CYP1A2-dependent caffeine metabolism during menstrual cycle in healthy women. Int J Clin Pharmacol Ther 38:235–244
35. Petersen MS, Halling J, Damkier P et al (2007) Polychlorinated biphenyl (PCB) induction of CYP3A4 enzyme activity in healthy Faroese adults. Toxicol Appl Pharmacol 224:202–206
36. Chang C, Bahadduri PM, Polli JE, Swaan PW, Ekins S (2006) Rapid identification of P-glycoprotein substrates and inhibitors. Drug Metab Dispos 34:1976–1984
37. Elsby R, Surry DD, Smith VN, Gray AJ (2008) Validation and application of Caco-2 assays for the in vitro evaluation of development candidate drugs as substrates or inhibitors of P-glycoprotein to support regulatory submissions. Xenobiotica 38:1140–1164
38. Fromm MF, Kim RB, Stein CM, Wilkinson GR, Roden DM (1999) Inhibition of P-glycoprotein-mediated drug transport: a unifying mechanism to explain the interaction between digoxin and quinidine [see comments]. Circulation 99:552–557
39. Sakaeda T, Iwaki K, Kakumoto M et al (2005) Effect of micafungin on cytochrome P450 3A4 and multidrug resistance protein 1 activities, and its comparison with azole antifungal drugs. J Pharm Pharmacol 57:759–764

40. Vahakangas K, Myllynen P (2009) Drug transporters in the human blood-placental barrier. Br J Pharmacol 158:665–678
41. 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 28:1805–1808
42. Niwa T, Inoue-Yamamoto S, Shiraga T, Takagi A (2005) Effect of antifungal drugs on cytochrome P450 (CYP) 1A2, CYP2D6, and CYP2E1 activities in human liver microsomes. Biol Pharm Bull 28:1813–1816
43. Ariyaratnam S, Thakker NS, Sloan P, Thornhill MH (1997) Potentiation of warfarin anticoagulant activity by miconazole oral gel. BMJ 314:349
44. O'Reilly RA, Goulart DA, Kunze KL et al (1992) Mechanisms of the stereoselective interaction between miconazole and racemic warfarin in human subjects. Clin Pharmacol Ther 51:656–667
45. Silingardi M, Ghirarduzzi A, Tincani E, Iorio A, Iori I (2000) Miconazole oral gel potentiates warfarin anticoagulant activity. Thromb Haemost 83:794–795