Oral contraception does not alter single dose saquinavir pharmacokinetics in women

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Aims

Women experience more adverse drug reactions (ADR) to antiretroviral therapy than men. This may be attributed to higher plasma concentrations of protease inhibitors due to pharmacokinetic interactions with hormonal preparations. Thus, in the present study we aimed to investigate the influence of oral contraceptives (OC) on the pharmacokinetics of the protease inhibitor saquinavir.

Methods

Saquinavir was administered in a hard gelatin capsule formulation (Invirase®) to rule out confounding by pharmaceutical aids of the more frequently used soft gelatin capsule. After an overnight fast, eight healthy female participants ingested a single oral dose of 600 mg saquinavir immediately before and after the 19th dose of a combined, low dose OC (0.03 mg ethinylestradiol, 0.075 mg gestodene) in a prospective, fixed sequence study design. The first saquinavir application was scheduled on day 1, 2, or 3 of the individual menstrual cycle. Plasma concentrations of saquinavir and relative concentrations of its M2&M3-hydroxy metabolites were determined by LC/MS/MS for 48 h.

Results

Intake of OC resulted in a significant decrease in morning serum concentrations (before intake of OC, compared to day 19 of OC therapy) of 17 β -estradiol by -23.4 pg ml^{-1} (57%, 95%CI: -76% to -37.4%); progesterone by -0.25 ng ml^{-1} (33%, 95%CI: -45.3% to -21.5%); follicle-stimulating hormone by -4.06 U l^{-1} (82%, 95%CI: -96.5% to -67.7%); and luteinizing hormone by -3.49 U l^{-1} (74%, 95%CI: -93 to -54.6%). Conversely, sexual hormone binding globulin serum concentrations increased by 83.6 nmol l^{-1} (205%, 95%CI: 32.2% to 377%). Pharmacokinetic parameters of saquinavir (AUC, C_{max} , t_{max} , $t_{1/2}$, CL_R) were not affected by OC, nor was the relative metabolic ratio of saquinavir/M2&M3-hydroxy saquinavir. Furthermore, there was no association of serum hormone concentrations or *MDR1*-polymorphisms (C3435T and G2677T) with pharmacokinetic parameters of saquinavir.

Conclusions

There was no effect of OC on saquinavir pharmacokinetics. Thus, pharmacokinetic interactions of synthetic sexual steroids with saquinavir are not likely to account for the increased ADR to antiretroviral therapy seen in women.

Introduction

Adverse drug reactions (ADR) are more frequent in women than in men, and this difference is particularly evident in the 15- to 44-year age group [1]. One factor by which the sex difference can be explained is a disproportionate rate of medication use in women [1]. In Germany 34% of women in reproductive years are current users of oral contraceptives (OC), and the cumulative incidence of ever taking OC is 91% [2]. OC may contribute to poly pharmacotherapy, mainly in the above mentioned age group, and so increase the risk of drug interactions. Accordingly, several studies have shown that women experience more ADR to antiretroviral therapy [3–5], including protease inhibitors [6], such as saquinavir. However, none of these studies reported on the use of OC in affected women.

Controlled studies and clinical case reports provide evidence for interactions of OC with the pharmacokinetics of other drugs. Among others, cytochrome-P450 (CYP) 3A-substrates, such as midazolam [7], nifedipine [8], ciclosporin [9], and prednisolone [10] are involved. But with regard to midazolam – a frequently used probe drug for CYP3A – there are also reports, which failed to show significant interactions [11]. Combined OC usually contain ethinylestradiol and a progestin, which by themselves are metabolized by CYP3A [12, 13]. Synthetic steroids with acetylenic groups, such as ethinylestradiol and gestodene, have been shown to be irreversible inhibitors of CYP3A *in vitro* [14–17]. The enzyme inactivation is mechanism-based [16, 17] and thus merely affects the enzyme involved in the biotransformation of the compound. More recently the drug-transporter P-glycoprotein (PGP) has been recognized as another target of clinically relevant drug interactions (e.g. digoxin-quinidine) [18]. Several progestins are competitive inhibitors of PGP *in vitro* [19], however, just as for the mechanism-based inactivation of CYP3A, it is unclear whether local concentrations of synthetic steroids achieved *in vivo* are sufficient to induce the phenomena described *in vitro*.

In some [20], but not all reports [21], a higher saquinavir exposure has been reported for women, compared to men, which may account for their increased risk of ADR to protease inhibitors. Saquinavir is both, a substrate for CYP3A [22] and PGP [23], and inhibition of CYP3A and PGP increases saquinavir concentrations [24]. Thus, in the present study we intended to evaluate the effect of a combined OC, containing 0.03 mg ethinylestradiol and 0.075 mg gestodene, on the pharmacokinetics of saquinavir in young healthy females, and to assess the potential contribution of CYP3A and PGP.

Methods

The study protocol was approved by the Ethics Committee of the Medical Faculty, University of Heidelberg and conducted in accordance with the Declaration of Helsinki, as amended in Somerset West 1996. Written informed consent was obtained from each participant before entering the study.

Participants

Eight healthy, nonsmoking female individuals, with no menstrual cycle abnormalities were included into the study. All were of Caucasian origin. Mean ($\pm$ s.d.) age was 23.8 ($\pm$ 2.25) years, and mean body mass index 21.1 ($\pm$ 1.82) kg/m². All participants were ascertained to be healthy by physical examination; gynaecological examination, comprising a PAP smear; electrocardiography; and routine laboratory testing. Pregnancy was excluded by a qualitative β -human chorionic gonadotropin (β HCG)-test in urine. None of the participants received any regular drug treatment, including OC and herbal products, within the last 2 months before entering the study. Alcoholic and caffeinated beverages were not allowed from 12 h before until 48 h after saquinavir administration.

Study design

The study was performed in a prospective, open, fixed sequence design (Figure 1). After an overnight fast, all participants received 600 mg saquinavir (3×200 mg Invirase[®] capsules, F. Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany), along with 200 ml water, at approximately 08:00 a.m. on days 1 and 22 of the individual study period. During the first 4 h following drug administration the participants were asked to stay in a semirecumbent position in bed. A standardized meal

Figure 1

Study design: The first evaluation of saquinavir pharmacokinetics was scheduled on day 1, 2, or 3 of the individual menstrual cycle of each participant (study days 1–3), and evaluation of saquinavir pharmacokinetics was repeated on day 19–21 of therapy with the OC (study days 22–24)

was served 4 and 8 h after saquinavir administration. From study day 4, participants received a single tablet of a low-dose combination OC, containing 0.03 mg ethinylestradiol and 0.075 mg gestodene (Minulet®, Wyeth-Pharma GmbH, Münster, Germany) once daily for 21 days. The beginning of the study period (day 1) was scheduled on the first three days of the women's menstrual cycle. On day 22 the 19th OC dose was administered along with 100 ml water 15–30 min before saquinavir intake.

Venous blood samples were drawn through an intravenous catheter into ammonium-heparin coated 7.5 ml tubes. Blood samples were obtained before, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12, 24, and 48 h after saquinavir administration. They were immediately centrifuged at 3000 g for 10 min at 4 °C, and the plasma aliquoted and stored at –20 °C until analysis. On study days 1 and 22 additional serum samples were taken for hormone and SHBG analysis, and EDTA blood was taken for RNA and DNA isolation. Urine was collected in consecutive fractions 12, 24, and 48 h after administration of saquinavir. After measurement of the urine volume, a 3 ml aliquot of each fraction was stored at –20 °C until analysis.

Saquinavir determination

Plasma and urine concentrations of saquinavir were assessed by a validated LC/MS/MS method, as described previously [25]. Intraassay variability (%CV) obtained by analysing replicate samples (6 replicates in each of 3 batches) at three concentrations varied between 0.10% and 9.50% for plasma and 0.04% to 12.0% for urine, and interassay variability (%CV) varied between 1.70% and 7.70% for plasma. Lower limits of quantification (LLOQ) for saquinavir were 0.1 ng ml^{–1} for both, plasma and urine. Due to the lack of reference material saquinavir monohydroxylated metabolites M2&M3 [26] were measured indirectly using their theoretical tandem mass spectroscopic transitions and expressed as the signal ratio of metabolites to saquinavir, assuming that the mass spectroscopic response of M2&M3 is nearly equal to saquinavir. Since both metabolites reveal the same retention time and mass transition, M2 and M3 cannot be discriminated. Thus, M2&M3 refers to a combined signal response.

Hormone and SHBG determination

Serum concentrations of 17β-estradiol, progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were determined on days 1 and 22 by routine laboratory electrochemiluminescence immunoassays (ECLIA) (Elecsys Systeme®, Roche Diagnos-

tics GmbH, Mannheim, Germany), and sexual hormone binding globulin (SHBG) serum concentration was measured by means of a routine laboratory enzyme immunoassay (ELISA) (DRG Instruments GmbH, Marburg, Germany).

MDR1 genotyping

C3435T polymorphisms in exon 26 of the multidrug resistance gene 1 (*MDR1*) was detected by rapid-cycle polymerase chain reaction (PCR) and fluorescent melting point analysis using fluorogenic hybridization probes on the LightCycler™ (Roche Applied Sciences, Mannheim, Germany) [27]. G2677T polymorphism in exon 21 of the *MDR1* gene was genotyped by PCR-restriction fragment length polymorphism (RFLP) according to Cascorbi *et al.* [28].

Pharmacokinetic analysis

The peak plasma concentrations of saquinavir (C_{max}) and the time to reach C_{max} (t_{max}) were taken from the raw data. All other pharmacokinetic parameters were determined by noncompartmental analysis using WinNonlin 3.1 (Pharsight Corporation, Mountain View, CA, USA). The area under the plasma concentration time curve during a time interval t_1 – t_2 (AUC_{t_1,t_2}) was calculated by using the linear trapezoidal rule. The slope of the terminal part of the plasma concentration-time curve ($1z$) was obtained by linear regression after semilogarithmic transformation. The terminal plasma half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln 2 \cdot 1z^{-1}$. Renal clearance (CLR) of saquinavir was calculated as amount excreted into urine (A_e) during the time interval t_1 – t_2 divided by the respective $AUC_{(t_1,t_2)}$ interval. The relative metabolic ratio of saquinavir and its hydroxy metabolites M2&M3 in plasma was calculated as $AUC_{SQV(0-48)}/AUC_{M2\&M3(0-48)}$, and in urine as $Ae_{SQV(0-48)}/Ae_{M2\&M3(0-48)}$.

Statistical analysis and sample size

The results are expressed as median (range) in the table. Because of the highly asymmetrical distribution of pharmacokinetic parameters we decided to use nonparametric analyses throughout the study. Differences in serum hormone and SHBG concentrations and pharmacokinetic parameters of saquinavir in women with and without OC were compared by the nonparametric Wilcoxon signed rank test for paired data. Spearman rank correlation analyses were performed between hormone levels and pharmacokinetic parameters of saquinavir. Pharmacokinetic parameters of saquinavir in the different *MDR1* genotype groups CC vs CT + TT for the C3435T polymorphism [29], and GG vs GT + TT for the G2677T polymorphism [30] were compared by the non-

parametric Wilcoxon rank sum test (Mann–Whitney *U*-test) for unpaired data. A *P*-value < 0.05 was considered statistically significant.

A sample size of 16 was determined to achieve a 90% power of demonstrating a statistically significant difference of 33% in saquinavir AUC at the 5% alpha level. The data are based on estimates of intraindividual variability of the AUC of saquinavir (40%) observed in our previous study in healthy males [31]. The present study was early terminated because an interim analysis did not reveal a trend towards different saquinavir concentrations before and after therapy with OC. A post hoc power calculation from the present data (*N* = 8, intraindividual variability = 29.7%) resulted in a power of 87% of detecting a statistically significant difference of 33% in saquinavir AUC at the 5% alpha level.

Results

Intake of OC resulted in a statistically significant decrease in 17 β -estradiol, progesterone, FSH, and LH serum concentrations, and in a significant increase in SHBG serum concentrations (Table 1).

Despite the highly standardized study procedure, pharmacokinetic parameters of saquinavir revealed a high interindividual variability. Furthermore, the distribution of the pharmacokinetic parameters is highly asymmetrical which accounts for the large differences seen between mean and median relative changes of the variables during OC relative to before OC. Treatment with OC had no effect on saquinavir pharmacokinetics, and there was no difference in the relative ratio of saquinavir and its M2&M3-hydroxy metabolites, indicating no effect of OC on CYP3A activity (Table 2).

In Figure 2 concentration-time curves of saquinavir

and its M2&M3-hydroxy metabolites in women without and with OC are shown. Saquinavir was absorbed rapidly, and a second smaller peak occurred after 4 h, reflecting the known influence of food intake on the bioavailability of the substance [32].

MDR1-genotypes were distributed as follows: C3435T: CC 1/8, CT 6/8, TT 1/8; G2677T: GG 2/8, GT 6/8, TT 0/8. There was no association of *MDR1*-polymorphisms or hormone concentrations with pharmacokinetic parameters of saquinavir (data not shown).

The concentration-time curves of participant #2 are depicted separately, due to the large deviation of the curves from the others. Her saquinavir concentrations were roughly five-fold higher (C_{max} : 19.9 ng ml⁻¹ without OC and 35.2 ng ml⁻¹ with OC) and increased largely only after the meal. The relative ratios of saquinavir to its M2&M3-hydroxy metabolites in plasma were, compared to the 7 other participants, on average 11-fold higher (ratio: 2158 without OC and 1403 with OC), indicating decreased CYP3A activity which may account for reduced saquinavir clearance and higher saquinavir concentrations. Her *MDR1*-genotype was CT for the C3435T polymorphism and GT for the G2677T polymorphism. Statistical evaluation of the group without participant #2 was similar to the results of the group as a whole (Table 2): (Mean relative change (95%CI) and *P*-value (Wilcoxon signed rank test for paired data) for t_{max} : -67.4% (-235% to 100%), *p* = 0.73; C_{max} : -49% (-199% to 101%), *p* = 0.87; $t_{1/2}$: -7.8% (-53.4% to 37.9%), *p* = 0.5; AUC₀₋₄: -32.8% (-157% to 91.6%), *p* = 0.74; AUC₀₋₄₈: -1.77% (-18% to 21.5%), *p* = 0.74; AUC_{0-∞}: -3% (-15.9% to 21.5%), *p* = 0.61; CL_R(0-24): -26% (-93.7% to 41.6%), *p* = 0.75, and Saquinavir/M3&M3 Plasma: 18.5% (-33.4% to 17.4%), *p* = 0.35;

Table 1

Median (range) of sexual hormones and sexual hormone binding globulin (SHBG) in eight women before and during therapy with oral contraceptives (OC)

Variable	Before OC	During OC	P*	Relative change†	
				Mean (±SD)	Median (95% CI, %)
17 β -estradiol [pg/ml]	31 (20-65)	14 (8-19)	0.012	-56.7 (±23.1)	-63.8 (-76 to -37.4)
Progesterone [ng/ml]	0.65 (0.3-1)	0.5 (0.2-0.6)	0.011	-33.4 (±14.2)	-33.3 (-45.3 to -21.5)
FSH [U/l]	5.25 (0.7-8.1)	0.5 (0.1-3.5)	0.012	-82.1 (±17.3)	-87.8 (-96.5 to -67.7)
LH [U/l]	4.3 (0.2-7.9)	0.35 (0.1-4.5)	0.011	-73.8 (±23.1)	-81.4 (-93 to -54.6)
SHBG [nmol/l]	54 (19-79)	151.5 (47-190)	0.012	205 (±207)	130 (32.2 to 377)

P*: Wilcoxon signed rank test for paired data, comparing women not taking OC with women taking OC.

†: Percentage ratio of the means during OC (study day 22, after the 19th OC dose) relative to before OC (study day 1).

Table 2

Median (range) of saquinavir after a single oral 600 mg dose in eight women before and during therapy with oral contraceptives (OC)

Variable	Before OC	During OC	P*	Relative change†	
				Mean (±SD)	Median (95% CI, %)
$t_{\max}$ [h]	1.75 (0.75–6)	1.5 (0.75–5)	0.48	–61.5 (±182)	–10 (–312 to 90.6)
$C_{\max}$ [ng/ml]	5.42 (1.49–19.9)	5.98 (1.54–35.2)	0.48	–37.4 (±165)	10.3 (–175 to 100)
$t_{1/2}$ [h]	19.9 (11–26.3)	20.1 (5.89–34.6)	0.58	–10.7 (±50)	7.23 (–52.4 to 31.0)
AUC_{0-4} [ng x h/ml]	6.6 (2.36–15)	7.19 (2.46–17)	0.67	–27.6 (±135)	10.2 (–140 to 85.2)
AUC_{0-48} [ng x h/ml]	23.2 (15.9–98.6)	25.4 (17.1–101)	0.89	1.8 (±21.3)	–5.65 (–16.0 to 19.6)
$AUC_{0-\infty}$ [ng x h/ml]	24.5 (18.1–121)	27.5 (19.7–111)	0.40	1.56 (±20.8)	–6.46 (–15.8 to 18.9)
$CL_R (0-24)$ [ml/min]	12.8 (7.16–14.2)	11.1 (5.56–13.7)	1	–20 (±66.4)	2.76 (–81.5 to 41.5)
Saquinavir/M2&M3 _{Plasma}	140 (47.5–2158)	198 (52.8–1403)	0.92	6.43 (±51.1)	3.92 (–47.2 to 60.1)
Saquinavir/M2&M3 _{Urine}	36.2 (19.7–67.1)	35.7 (14.7–75.4)	0.61	–16.9 (±28)	–12 (–42.8 to 9.10)

P*: Wilcoxon signed rank test for paired data, comparing women not taking OC vs. women taking OC.

†: Relative change of the variables during OC (study day 22, after the 19th OC dose) relative to before OC (study day 1).

Figure 2

Concentration-time curves of saquinavir (dots) and its M2&M3-hydroxy metabolites (squares) in women without (open symbols) and with (solid symbols) OC. Concentration-time curves of participant #2 are depicted separately (insert). M2&M3 plasma concentrations are described as relative units (see Methods)

Saquinavir/M2&M3 Urine: –12.5% (–39.2% to 14.3%), $p = 0.92$).

Discussion

Saquinavir is a specific HIV protease inhibitor, which is widely used in highly active antiretroviral therapy

(HAART). Its therapeutic [33] effects are concentration-dependent and – although generally well tolerated – also adverse effects [34] appear to be linked to systemic concentrations; thus, an altered bioavailability may necessitate dose-adjustments. Due to their extensive phase I metabolism by CYP3A in the liver and gut, protease inhibitors are prone to drug interactions. Inhibitors of CYP3A such as ritonavir, nelfinavir, indinavir, ketoconazole, clarithromycin [24, 35], and grapefruit juice [36] increase the AUC of saquinavir up to 50-fold. There is an intriguing overlapping substrate specificity of CYP3A and PGP [37] and many of the above mentioned CYP3A-inhibitors such as ritonavir, ketoconazole, and grapefruit juice also inhibit PGP. Indeed, recent data of our group demonstrated an up to 5-fold increase of the AUC of saquinavir, when coadministered with the pharmaceutical aid cremophor EL [30]. The effect of cremophor EL on saquinavir pharmacokinetics occurred during the absorption phase, indicating an effect of the pharmaceutical aid on gastrointestinal dissolution or inhibition of intestinal first pass metabolism, e.g. by inhibition of mucosal PGP [38]. Thus, these data support, although they do not prove, a contribution of PGP to drug interactions with saquinavir.

In vitro data indicated a potent inhibition of both, CYP3A and PGP, particularly by gestodene and to a lesser extent by ethinylestradiol [14–17]. Hence, in the present study we investigated the pharmacokinetic interaction of an ethinylestradiol/gestodene combination OC with saquinavir. This may be of particular practical

relevance when HIV infected women on saquinavir therapy start OC treatment. In all participants a clear decrease in LH, FSH, 17β -estradiol, and progesterone and an increase in SHBG concentrations during the 21-day period were observed, indicating reliable intake of OC. However, therapy with OC had no effect on any of the parameters of saquinavir pharmacokinetics, and there was also no effect on the relative ratio of saquinavir to its M2&M3-hydroxy metabolites, a biotransformation step in which CYP3A is largely involved [26]. Thus, no relevant inhibition of either CYP3A or PGP occurred *in vivo*.

In general saquinavir pharmacokinetics revealed a high interindividual variability, and particularly the AUC of one participant showed a large deviation from the others. Compared to the other participants, in this individual the relative ratio of saquinavir to its M2&M3-hydroxy metabolites was on average 11-fold higher, indicating decreased CYP3A activity. CYP3A activity is regulated by transcriptional control of its expression [39], and it is known that up to 400-fold variations in CYP3A expression and activity contribute to the interindividual differences in the bioavailability and systemic clearance of CYP3A substrates [40], including protease inhibitors [41], which could explain this finding.

As known from other studies with saquinavir administration in the fasted state [31, 36], maximal plasma concentrations (C_{max}) of saquinavir were quite low also in the present study, although they were just within the IC₅₀ values of HIV protease inhibition of 1–10 nmol l⁻¹ (~0.67–6.7 ng ml⁻¹) reported *in vitro* [42]. Saquinavir was administered in the fasted state to lower the known inter- and intraindividual variability in pharmacokinetics. Furthermore, despite a more than 3-fold higher bioavailability of saquinavir from the soft gelatin capsule (SGC) [43], in the present study we deliberately decided to administer saquinavir in a hard gelatin (HGC) preparation, because we could not rule out confounding of the results by pre-existent PGP-inhibition by pharmaceutical aids of the SGC [31]. Saquinavir HGC is still used in boosted regimens in which its tolerability seems to be more favourable than that of the SGC [34, 44]. Unlike to the present study, in this setting saquinavir is usually combined with lopinavir or ritonavir which largely increase its bioavailability [45]. Thus, application of a saquinavir HGC without ritonavir is unlikely to occur in clinical practice which potentially limits the generalisability of our results. However, we assumed that this design would have the best chances for detecting an OC–saquinavir interaction, because we selected a progestin with a high interaction potential to which participants were exposed for a suf-

ficiently long period of time (19 days). Concurrently we minimized the influence of other factors which possibly affect the bioavailability of saquinavir (food [32], pharmaceutical aids [31], ritonavir [45]). Under these conditions the achieved systemic saquinavir concentrations (~15 nmol l⁻¹) were quite similar to those expected with gestodene (~13–41 nmol l⁻¹) [13], thus qualifying this design for the evaluation of pharmacokinetic drug interactions.

In vitro both, competitive inhibition [46] and a potent mechanism-based inhibition of CYP3A – particularly by synthetic steroids with an acetylenic group at the C17 position [17] – have been described. Two studies [16, 47] reported an inhibition of the microsomal nifedipine oxidation by different steroids, such as gestodene, 3-keto-desogestrel, 17α -ethinylestradiol, desogestrel, levonorgestrel, 11-CH₂- Δ_{15} -norethisterone, norethisterone, and 11-CH₂-norethisterone, in descending order of potency. Gestodene (K_i = 4.8 μ M) exhibited a 3–8 fold greater inhibitory effect on CYP3A than other synthetic steroids, including ethinylestradiol (K_i ~30 μ M) [48]. This order of inhibitor potency was confirmed in experiments assessing the effects of these steroids on the 2-hydroxylation of 17α -ethinylestradiol, but this reaction was generally less affected. In another study inhibition of CYP by progestins was confirmed, however, gestodene revealed similar inhibitor potency, compared to the other progestins [48]. Besides CYP inhibition, progestins, such as progesterone and medroxyprogesterone acetate are also inhibitors of PGP-mediated vinblastine efflux [19], with a potency comparable to that of verapamil. Concentrations of steroids in these *in vitro* studies were 30–100 μ M which is approximately 1000–10000-fold higher than plasma concentrations reported *in vivo* [13]. However, one may expect higher concentrations at the site of absorption (intestinal epithelium) and the major site of metabolism (liver), the place where the interaction is most likely to occur.

In summary, these *in vitro* data give evidence for an inhibitory interaction of progestins with CYP3A mediated biotransformation and for an inhibition of active drug transport by PGP. Because gestodene was found to be one of the most potent CYP mechanism-based inactivators ever described [16], we decided to select a gestodene containing OC for our study protocol.

In contrast to the present study other clinical studies reported significant interactions of OC with substrates of CYP3A. For example, the $t_{1/2}$ of i.v. prednisolone was reported to be 107% longer in women on OC [10], and a case report described doubled ciclosporin plasma-concentrations after introduction of an OC [9] which caused typical ADR to this substance. Moreover, there

was a slight, but nonsignificant increase of the nifedipine AUC after intake of OC for 21 days, and the AUC of its hydroxymethyl metabolite decreased significantly by 25% [8]. Finally, the AUC of orally administered alprazolam was 35% higher [49], and the AUC of orally administered midazolam was 21% higher [7] in women taking OC, compared to women not taking OC. With respect to the *in vitro* data it is remarkable that a further study failed to show a significant interaction of OC and midazolam [11]. Interestingly, in the positive interaction study [7] an OC containing gestodene as the progestagenic component was used, whereas in the negative study norgestrel was administered which is roughly 5 times less potent an inhibitor of CYP3A *in vitro* [16].

Hence, the available *in vivo* data suggest that substrates with a significant interaction with OC are compounds with a lower affinity to CYP3A than saquinavir ($K_i = 0.7 \mu\text{M}$) [50]. The apparent K_i values of nifedipine are 10–22 μM , of midazolam 40–63 μM , and of ciclosporin 1–37 μM [50]. Furthermore, the dose of saquinavir (600 mg) administered in the present study was rather higher than the dose of midazolam (7.5 mg) [8], nifedipine (10 mg) [7], and prednisolone (0.53 mg kg^{-1}) [10]. Therefore, the concentration of saquinavir, relative to the concentrations of OC steroids at the intestinal epithelium, is higher than in the positive interaction studies, which may limit the likelihood of an interaction during the absorption phase, and thus explain the negative findings in the present study.

In conclusion this study revealed that therapeutic contraceptive doses do not increase saquinavir plasma concentrations. Because the progestin used in this study is the most potent CYP3A inhibitor of all evaluated progestins, OC combination therapy generally appears safe with respect to concentration-dependent ADR of saquinavir in women.

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With the assistance of Dr. M. M. Hoffmann (University of Freiburg, Germany) the whole CYP3A4 gene of participant #2 was sequenced to evaluate whether the substantial pharmacokinetic differences are caused by a polymorphism of this gene. PCR amplification of exons 1 to 13 was carried out using intronic primers flanking each of the CYP3A4 exons. Oligonucleotide sequences

were created based on the published sequence (GenBank Accession No. AF209389), excluding sequences with homology to the genes of CYP3A5, CYP3A7, and CYP3A43. Purified PCR products (20 ng) were directly sequenced in two directions using the amplification primers and the dideoxy chain termination method (DYEnamic ET Kit, Amersham Biosciences, Freiburg, Germany) on a MegaBACE500 sequencer (Amersham Biosciences, Freiburg, Germany). No sequence variations were found thus excluding polymorphisms in the CYP3A4 gene as the underlying cause for the different saquinavir kinetics in participant #2.

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