The effects of an oral contraceptive containing ethinyloestradiol and norgestrel on CYP3A activity

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Aims To examine the effects of an oral contraceptive containing ethinyloestradiol and norgestrel on intestinal and hepatic CYP3A activity using midazolam as a probe substrate.

Methods In a nonblinded sequential study, nine healthy women received simultaneous doses of intravenous midazolam (0.05 mg kg^{-1}) and oral $^{15}N_3$-midazolam (3 mg) on days 0, 4, 6, 8, and 14. On study day 5, Ovral™ (50 μg ethinyloestradiol/500 μg norgestrel) was administered for 10 days. Serum and urine samples were assayed for midazolam, $^{15}N_3$-midazolam and metabolites by liquid chromatography-mass spectrometry. A Digit Symbol Substitution Test (DSST) was used to assess changes in the pharmacodynamic activity of midazolam.

Results Moderate (% CV 26–46) interindividual variability in the pharmacokinetics of midazolam were observed. Compared with baseline, $AUC(0,\infty)_{iv}$ ratios (95% CIs) after 2, 4, and 10 days treatment with OC were 89% (79, 101), 96% (85, 109), and 88% (77, 99), respectively. The $AUC(0,\infty)_{oral}$ ratios (95% CIs) were 101% (82, 125), 105% (85, 130), and 114% (92, 141), respectively, after 2, 4, and 10 days OC treatment compared with baseline. Concomitant administration of the oral contraceptive, Ovral™ for 2, 4 or 10 days did not significantly alter the area under the curve, clearance, or half-life of midazolam after either oral or intravenous administration. No alterations in pharmacodynamic effects of midazolam were observed between treatment days. Mean DSST scores strongly correlated with mean total midazolam blood concentrations ($r = -0.936$).

Conclusions Administration of Ovral™ for 10 days had no impact on intestinal or hepatic CYP3A activity as determined by midazolam metabolism.

Keywords: CYP3A, ethinyloestradiol, midazolam, norgestrel

Introduction

The cytochrome P450 3A (CYP3A) subfamily is comprised of CYP3A4, CYP3A5, CYP3A7, and CYP3A43. As the most abundant cytochrome P450 in the liver and small intestines, CYP3A4 participates in the metabolism of approximately 50% of drugs currently on the market [1]. CYP3A5 protein is detected in only 30% of human livers [2, 3] and is the principal CYP3A isoform expressed in renal tissue [4]. CYP3A7 is expressed almost exclusively in fetal liver and is thought to play an important role in the 16-α-hydroxylation of dehydroepiandrosterone-sulphate [5]. The newest member of the CYP3A family to be identified, CYP3A43 is expressed in the liver, kidney, pancreas and prostate [6]. At this point, the catalytic activity and relative abundance of CYP3A43 have not been defined. The CYP3A subfamily is known to metabolize a large number of structurally diverse compounds including nifedipine, diltiazem, lignocaine, lovastatin, erythromycin, troleandomycin, cyclosporin and steroids such as oral contraceptives (OC) [7].

Approximately 60–70 million women worldwide take an OC. The major oestrogenic component of OC is 17α-ethinyloestradiol (EE_2). About 65% of EE_2 undergoes first-pass metabolism in the gut wall to EE_2-sulphate [8]. In humans, the dominant oxidative reaction of EE_2 is aromatic hydroxylation at the 2-position with

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29–64% of an oral dose being converted to 2-hydroxy-ethinyloestradiol [8]. The 2-hydroxylation of EE_2 is mediated primarily by CYP3A [9]. This metabolite can undergo further oxidation with subsequent sulphation and glucuronidation prior to excretion [9]. Not only is EE_2 hydroxylated at the 2-position by CYP3A, but it also can undergo oxidation at the 17α-acetylenic bond resulting in mechanism-based inhibition of CYP3A [10]. In addition, studies in human liver microsomes have shown that progestins containing the 17α-acetylenic moiety, which are commonly used with EE_2 in OC formulations, are also mechanism-based inactivators of CYP3A *in vitro* [9]. Thus as a result of mechanism-based inhibition, women on OCs would appear to have an elevated risk for experiencing drug–drug interactions with CYP3A substrates. In fact, a number of case reports describe an increase in cyclosporin concentrations after the initiation of OC therapy suggesting an inhibition of cyclosporin metabolism [11, 12]. In addition, women are known to have higher CYP3A activity compared with men [13]. However as demonstrated by a recent study, women taking OCs had midazolam clearance values similar to those obtained in men [14]. The extent of CYP3A modulation by OCs is unclear and thus additional studies examining this issue are warranted.

The goal of the current study was to evaluate the effects of OC dosing on hepatic and intestinal CYP3A catalytic activity. Midazolam is selectively hydroxylated at the 1′ and 4-positions by CYP3A and was employed in the current study as an *in vivo* probe for CYP3A hepatic and intestinal activity [15].

Methods

This was a nonblinded, sequential study comprised of five periods evaluating the effect of an OC Ovral™ (50 μg EE_2 and 500 μg norgestrel) on CYP3A activity. Ovral™ was chosen because of its high EE_2 content (50 μg) and because EE_2 and norgestrel (levonorgestrel is the active isomer of norgestrel) combination products are the most commonly prescribed OC [16]. The study was conducted at the Lilly Laboratory for Clinical Research in Indianapolis, Indiana. The protocol and informed consent documents were approved by Indiana University – Purdue University at Indianapolis Institutional Review Board. After giving written informed consent, participants were enrolled into the study. Volunteers were determined to be in good health on the basis of medical histories, physical examinations, vital signs, electrocardiograms and laboratory evaluations. Exclusion criteria included any clinically relevant abnormality identified at the physical examination or laboratory screening, the use of any medication within 14 days before the first drug administration, blood donation within 60 days before the start of the study and a documented history of drug allergy.

Starting on day 1, participants were served a restricted diet avoiding foods such as grapefruit juice, oranges, orange juice, limes, mandarin oranges, ethanol containing beverages, charbroiled meat and cruciferous vegetables, which may alter cytochrome P450 activity. During the inpatient study days, subjects were not allowed to drink caffeine containing beverages or smoke tobacco. On each dosing day, subjects fasted from midnight until approximately four hours after dosing. Participants were simultaneously administered 0.05 mg kg^{-1} intravenous midazolam (infused over 30 min) and 3 mg of $^{15}N_3$-midazolam oral solution on days 0, 4, 6, 8, and 14. On day 5, Ovral™ therapy was initiated. Serial blood samples (7 ml) were collected before and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, and 10 h following midazolam and $^{15}N_3$-midazolam administration. All blood samples were drawn into heparinized tubes. Plasma was separated by centrifugation, transferred to polypropylene tubes and stored at −70° C until analysis. Each participant emptied her bladder just before the start of the urine collection period. Urine was collected over the following intervals 0–10 and 10–24 h post dose and frozen at −70° C until assayed for 1′-hydroxymidazolam.

Plasma samples were processed with the use of a liquid-liquid extraction technique [14]. The internal standard, desmethyldiazepam, was added to each 0.5 ml plasma sample before extraction. The sample residue was reconstituted with 200 μl mobile phase (acetonitrile, methanol and 20 mM ammonium acetate, pH 7.4; 40 : 20 : 40) and a portion was injected onto a h.p.l.c. column. Chromatographic separation of the analytes and internal standard was accomplished with a Phenomenex Luna C-18 column (5 μM × 4.6 mm i.d. × 150 mm) and a Brownlee RP-18 guard column. The mobile phase was delivered isocratically at a flow rate of 1 ml min^{-1}. The effluent was delivered to a mass spectrometer (Navigator™, Finnigan, San Jose, CA) interfaced with a Hewlett Packard 1100 binary pump equipped with a HP1100 autosampler. The APCI probe was run in the positive ion mode with source and probe temperatures of 200° C and 550° C, respectively. Midazolam, $^{15}N_3$-midazolam, 1′-hydroxymidazolam, 1′-hydroxy-$^{15}N_3$-midazolam and desmethyldiazepam were detected in the selected ion recording mode at m/z 326, 331, 342, 347, and 271, respectively. The limit of quantification was 0.25 ng ml^{-1} for midazolam, $^{15}N_3$-midazolam, 1′-hydroxymidazolam and 1′-hydroxy-$^{15}N_3$-midazolam. The accuracy and precision for midazolam and $^{15}N_3$-midazolam at 1.4, 10, and 40 ng ml^{-1} was less than 5% and 6%, respectively. In the case of 1′-hydroxymidazolam the accuracy and precision at 1.5 ng ml^{-1} and 3 ng ml^{-1} was less than 2% and 6%,

respectively. The same analytical procedure was used to determine the 1′-hydroxymidazolam and 1′-hydroxy-$^{15}N_3$-midazolam in urine after treatment of the samples with β-glucuronidase. The lower limit of quantification for 1′-hydroxymidazolam in the urine was 0.075 μg ml^{-1}. The accuracy and precision for 1′-hydroxymidazolam in urine was less than 10% and 9% at concentrations of 0.4 and 8 μg ml^{-1}, respectively. The midazolam concentration in the infusate was determined by h.p.l.c. [17].

Blood concentrations were determined as previously described [14]. The pharmacokinetic evaluation of midazolam and $^{15}N_3$-midazolam blood concentration-time data employed noncompartmental methods of analysis using WinNonlin (Version 3, Pharsight Corp., Mountain View, CA). The maximum blood concentration (C_{max}) and the corresponding time of the maximum concentration (t_{max}) were identified by visual inspection of the data. The elimination rate constant (λ_z) was determined as the slope of the linear regression for the terminal log-linear portion of the concentration-time curve. A terminal half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$. The area under the blood concentration-time curve (AUC from zero to the final detectable midazolam plasma concentration) after intravenous and oral administration was determined by a combination of linear and logarithmic trapezoidal methods with extrapolation to infinity (AUC$(0,\infty)$). The percent area extrapolated after intravenous administration ranged from 3.58 to 29.0% with a mean value of 12.9 ± 7.21% for all treatment periods. For oral administration, the percent area extrapolated ranged from 1.81 to 25.9% with a mean value of 12.6 ± 5.81% for all treatment periods. Volume of distribution (V_d), systemic clearance (CL_{iv}), oral clearance (CL_{oral}) and oral availability (F_{oral}) were determined using standard methods. The intravenous dose of midazolam was estimated as the infused solution concentration multiplied by the rate of infusion and the duration of infusion.

Psychomotor performance was assessed using the Digit Symbol Substitution Test (DSST) [18]. Participants were presented with a code in which the numbers 1 through 9 were matched with symbols. Prior to the administration of midazolam a DSST was administered to subjects for a minimum of three 90 s practice sessions in order to minimize the effect of learning. The DSST was administered before and at 0.5, 1, 2, 3, 4, and 24 h after midazolam and $^{15}N_3$-midazolam dosing. The pharmacodynamic end-point was the number of correctly completed matches in 90 s. A decrease in DSST score is indicative of psychomotor impairment. During periods of complete sedation, a DSST score of zero was recorded.

Summary statistics (arithmetic mean and percent CV) were calculated for the derived pharmacokinetic parameters. The pharmacokinetic parameters were subjected to statistical analysis by using a cross-over analysis of variance (ANOVA) model. The effects due to subject and treatment were extracted. The C_{max} and AUC parameters were evaluated based on log-transformed data and were expressed as the ratio of the mean parameter of the treatment (days 4, 6, 8, and 12) to the control (day 0). Ninety-five percent confidence intervals (95% CI) for the C_{max} and AUC ratios were calculated using the pooled residual error and associated degrees of freedom from the ANOVA.

Results

Pharmacokinetics

Nine women ranging from 24 to 39 years of age participated in the study. Eight women completed all aspects of the study. After finishing four of the five study periods, one subject withdrew from the study for personal reasons. For all study days a higher percentage of the administered dose was excreted into the urine as 1′-hydroxymidazolam after oral administration compared to the amount of 1′-hydroxymidazolam recovered after intravenous administration ($P < 0.05$) indicating complete intestinal absorption of $^{15}N_3$-midazolam (Tables 1 and 2).

Mean midazolam and $^{15}N_3$-midazolam blood concentration-time profiles for study days 0, 4, 6, 8, and 14 are presented in Figure 1. To examine potential confounding dietary influences on CYP3A activity, the results from day 0 (regular diet) and day 4 (restricted diet) were compared. The midazolam and $^{15}N_3$-midazolam blood concentration-time profiles for study days 0 and 4 showed comparable characteristics. Mean midazolam and $^{15}N_3$-midazolam pharmacokinetic parameters for days 0 and 4 are summarized in Tables 1 and 2. For both routes of administration, these parameters were not significantly changed with diet. When comparing diet effects (day 4 to day 0) on midazolam's pharmacokinetics, the $AUC(0,\infty)_{iv}$ ratio was 95% (95% CI: 84, 107); $AUC(0,\infty)_{oral}$ ratio was 104% (95% CI: 84, 129); the C_{max} ratio was 105% (95% CI: 80, 139). The mean t_{max} value following oral administration on day 4 (1.44 h) was similar to that on day 0 (1.53 h). The values of $t_{1/2}$, V_d, CL_{iv}, CL_{oral} and F_{oral} for intravenous or oral midazolam were essentially unchanged by diet treatment. Overall, the mean pharmacokinetic parameters suggest that the diets of the participants on days 0 and 4 had a comparable effect on the hepatic or intestinal CYP3A. As a result, day 0 was employed as baseline.

As demonstrated in Figure 1, the midazolam and $^{15}N_3$-midazolam blood concentration-time profiles for study days 0, 6, 8, and 14 were nearly superimposable, indicating no differences in the pharmacokinetics of midazolam and $^{15}N_3$-midazolam, with or without

Table 1 Arithmetic mean (% CV) pharmacokinetic parameters of intravenous midazolam in eight healthy females following a restricted diet and oral contraceptive (OC) therapy.

	Day 0 (Control)	*Day 4 (Diet)*	*Day 6 (2 days OC)*	*Day 8 (4 days OC)*	*Day 14 (10 days OC)*
Intravenous dose (mg)	3.26 (24)	3.29 (24)	3.26 (24)	3.25 (24)	3.26 (24)
$AUC(0,\infty)_{iv}$ ($\mu g\ l^{-1}\ h$)	90.8 (28)	86.5 (29)	81.7 (29)	87.4 (28)	80.8 (34)
Ratio; 95% CI[a]	–	95%; 84, 107	89%; 79, 101	96%; 85, 109	88%; 77, 99
$t_{1/2,iv}$ (h)	3.96 (31)	3.65 (39)	3.82 (31)	4.01 (28)	3.99 (37)
V_d (l)	208 (33)	207 (42)	225 (31)	218 (30)	242 (38)
CL_{iv} ($l\ h^{-1}\ kg^{-1}$)	0.588 (27)	0.627 (28)	0.663 (32)	0.608 (27)	0.675 (32)
% dose excreted in urine as 1′-hydroxymidazolam	71.4 (8)	68.0 (13)	60.3 (26)	58.4 (29)	55.1 (27)

$AUC(0,\infty)_{iv}$, intravenous area under the concentration-time curve; $t_{1/2,iv}$, elimination half-life after intravenous administration; V_d, volume of distribution; CL_{iv}, total blood clearance after intravenous administration.
[a]Ratio and 95% confidence intervals based on log-transformed data.

Table 2 Arithmetic mean (% CV) pharmacokinetic parameters of oral $^{15}N_3$-midazolam in eight healthy females following a restricted diet and oral contraceptive (OC) therapy.

	Day 0 (Control)	*Day 4 (Diet)*	*Day 6 (2 days OC)*	*Day 8 (4 days OC)*	*Day 14 (10 days OC)*
Oral dose (mg)	3.03 (3)	2.97 (3)	3.06 (3)	2.94 (3)	3.02 (3)
$AUC(0,\infty)_{oral}$ ($\mu g\ l^{-1}\ h$)	25.5 (46)	25.6 (32)	24.3 (27)	25.7 (29)	28.1 (30)
Ratio; 95% CI[a]	–	104%; 84–129	101%; 82–125	105%; 85–130	114%; 92–141
C_{max} ($ng\ ml^{-1}$)	8.67 (42)	9.24 (45)	7.32 (32)	7.98 (40)	9.81 (26)
Ratio; 95% CI[a]	–	105%; 80, 139	87%; 66, 115	93%; 70, 122	118%; 90, 156
t_{max} (h)	1.53 (57)	1.44 (77)	1.66 (70)	1.63 (54)	1.13 (56)
$t_{1/2,oral}$ (h)	1.85 (17)	2.04 (35)	2.62 (93)	1.91 (25)	1.81 (33)
CL_{oral} ($l\ h^{-1}\ kg^{-1}$)	2.15 (37)	2.01 (32)	2.04 (13)	1.92 (26)	1.85 (34)
F_{oral}	0.310 (51)	0.336 (39)	0.329 (32)	0.325 (26)	0.380 (29)
% dose excreted in urine as 1′-hydroxymidazolam	93.5 (15)	90.7 (10)	73.4 (28)	72.1 (31)	73.0 (29)

$AUC(0,\infty)_{oral}$, oral area under the concentration-time curve; C_{max}, maximum blood concentration; t_{max}, time of the maximum concentration; $t_{1/2,oral,}$ elimination half-life after oral administration; CL_{oral}, oral clearance; F_{oral}, oral availability.
[a]Ratio and 95% confidence intervals based on log-transformed data.

coadministration of Ovral™. The effects of Ovral™ administration on midazolam pharmacokinetics following intravenous and oral dosing are presented in Tables 1 and 2. No mean significant differences were observed in the pharamacokinetic parameters for midazolam and $^{15}N_3$-midazolam between the four treatment periods. Midazolam $AUC(0,\infty)_{iv}$ ratios for days 6, 8, and 14 relative to day 0 were 89% (95% CI: 79, 101), 96% (95% CI: 85, 109), and 88% (95% CI: 77, 99), respectively (Table 1). For $^{15}N_3$-midazolam, $AUC(0,\infty)_{oral}$ ratios for days 6, 8, and 14 relative to day 0 were 101% (95% CI: 82, 125), 105% (95% CI: 85, 130), and 114% (95% CI: 92, 141), respectively; C_{max} ratios for 87% (95% CI: 66, 115), 93% (95% CI: 70, 122), and 118% (95% CI: 90, 156), respectively (Table 2). The wide 95% CI for $AUC(0,\infty)_{iv}$, $AUC(0,\infty)_{oral}$, and C_{max} suggests that there may be individuals with differences between treatments. The mean t_{max} values following oral administration of $^{15}N_3$-midazolam with OC therapy (day 6 h, 1.66; day 8, 1.63 h; day 14, 1.13 h) were similar to control (day 0, 1.53 h) (Table 2). Plasma elimination half-life was calculated from λ_z after both routes of administration. Among the four treatments, mean $t_{1/2}$ for midazolam ranged from 3.65 to 4.01 h and for $^{15}N_3$-midazolam from 1.81 to 2.62 h. As expected, the mean calculated values for V_d, CL_{iv}, CL_{oral}, and F_{oral} were unchanged by OC therapy (Tables 1 and 2). These results indicate that the doses of EE_2 and norgestrel found in Ovral™ do not alter intestinal or hepatic CYP3A activity.

Interestingly, double-peaks in $^{15}N_3$-midazolam blood concentration-time profiles were observed in six of the eight individuals for day 8, which is indicative of delayed absorption. This phenomena has been described with

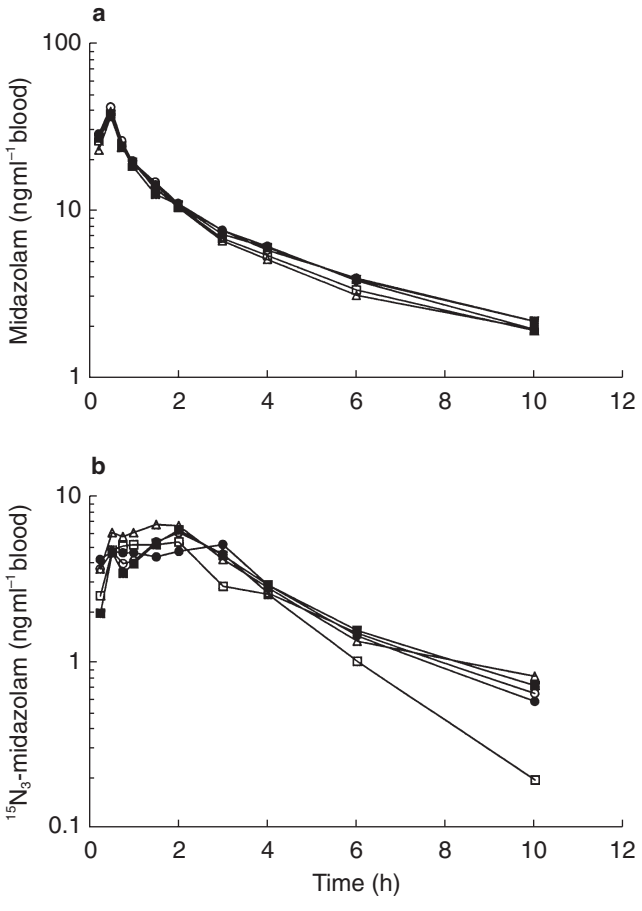

Figure 1 Mean blood concentration *vs* time curve of (a) midazolam and (b) $^{15}N_3$-midazolam after simultaneous intravenous and oral administration. ○ represents day 0 (control); ● represents day 4 after a restricted diet; □ represents day 6 after oral administration of oral contraceptive for 2 days; ■ represents day 8 after oral administration of oral contraceptive for 4 days; △ represents day 14 after oral administration of oral contraceptive for 10 days.

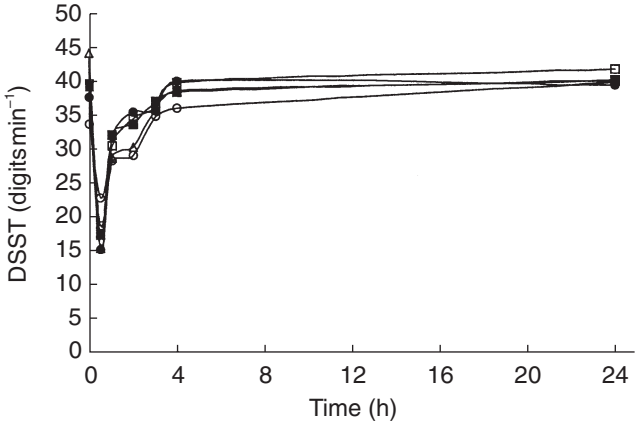

Figure 2 Mean digit symbol substitution test (DSST) scores *vs* time curve after simultaneous administration of intravenous and oral midazolam. ○ represents day 0 (control); ● represents day 4 after a restricted diet; □ represents day 6 after oral administration of oral contraceptive for 2 days; ■ represents day 8 after oral administration of oral contraceptive for 4 days; △ represents day 14 after oral administration of oral contraceptive for 10 days.

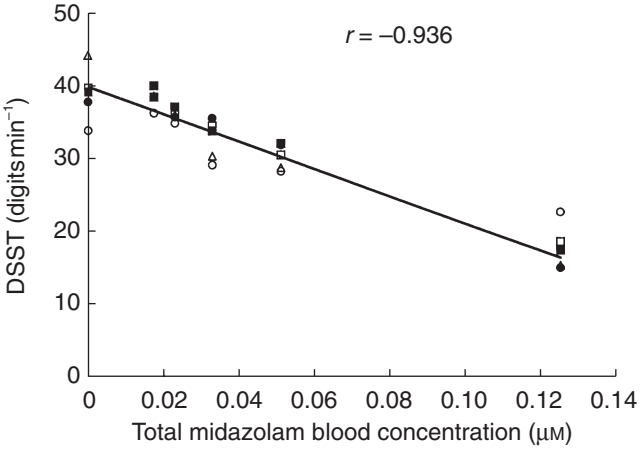

Figure 3 Relationship of total blood midazolam concentration (μM) to the digit symbol substitution test (DSST) score. ○ represents day 0 (control); ● represents day 4 after a restricted diet; □ represents day 6 after oral administration of oral contraceptive for 2 days; ■ represent day 8 after oral administration of oral contraceptive for 4 days; △ represents day 14 after oral administration of oral contraceptive for 10 days. Each point represents the mean value.

other benzodiazepines and is thought to be a result of reduced gastric motility caused by muscle relaxant effect of benzodiazepines [19].

Pharmacodynamics

Co-treatment with Ovral™ did not alter the pharmacodynamics of midazolam. The mean DSST score-time profiles for the 5 treatment days are illustrated in Figure 2. The lowest DSST score achieved by subjects corresponded well with the respective t_{max} for intravenous administration of midazolam. Maximum pharmacodynamic response occurred 30 min after dosing with DSST scores returning to baseline values by 4 h. In all groups, a strong negative correlation ($r = -0.936$) between DSST scores and total blood midazolam (midazolam plus $^{15}N_3$-midazolam) concentrations was observed (Figure 3).

Discussion

The liver contains high levels of CYP3A and thus it was thought that the relatively small amounts of norgestrel and EE_2 present in birth control pills may have little

effect on the systemic clearance of substrates of CYP3A. Although CYP3A4 represents a large portion of the CYP present in the small intestine, its absolute levels are small compared with the liver [20]. Yet, the intestinal CYP3A4 has been shown to play an important role in the first pass metabolism of a number of compounds, including midazolam [21, 22]. Therefore the consequence of modulating intestinal CYP3A activity may be pronounced and exposure to OC treatment was predicted to effect intestinal CYP3A activity.

The CYP3A isoforms participate in the metabolism of midazolam and EE_2 [9, 17]. The biotransformation of midazolam involves oxidation to 1′-hydroxymidazolam, 4-hydroxymidazolam and 1′,4-dihydroxymidazolam with urinary recovery of 1′-hydroxymidazolam accounting for 70% of the administered dose [23]. The main oxidative pathway of metabolism for EE_2 is aromatic hydroxylation at the 2-position [9]. Most combination OC preparations contain both EE_2 and a progestin, such as norgestrel. Acetylenic steroids such as EE_2 and norgestrel have been shown to be mechanism-based inhibitors of CYP3A mediated metabolism *in vitro* [9, 10, 24]. However since steroids such as these may also induce CYP3A levels *in vivo*, it is possible that these agents can cause an initial inhibition followed by an offsetting-induction of CYP3A [7].

In the current investigation, short and long-term, repeated dosing of 50 μg EE_2 and 500 μg norgestrel produced no effect on the mean pharmacokinetic parameters of intravenous or oral midazolam. The observed disposition characteristics of midazolam were similar to those previously reported in healthy women after intravenous and oral midazolam administration [14, 22]. Several previous *in vivo* studies evaluating the effect of OC use on the metabolism of CYP3A substrates have produced conflicting results. Repeated dosing of oral EE_2 (60 μg) alone did not alter the pharmacokinetic parameters of ^{13}C-EE_2 when administered intravenously [25]. Similarly, the OC combinations of 2 mg dienogest plus 30 μg EE_2 and 125 μg levonorgestrel plus 30 μg EE_2 did not influence the plasma concentration-time curve of the CYP3A substrate nifedipine [26]. In contrast, a recent study demonstrated that OC users (150 μg desogestrel/30 μg EE_2) had a 131% increase in prednisolone steady-state plasma concentrations when compared with control [27]. In a long-term study comparing two OC preparations, Jung-Hoffmann & Kuhl noted that the areas under the EE_2 serum concentration-time curves were 70% higher in women taking 75 μg gestodene plus 30 μg EE_2 compared with women taking 150 μg desogestrel plus 30 μg EE_2 [28]. Since in the majority of these studies the EE_2 dose was identical, it would appear that the variation might reflect the influence of the different progestins on CYP3A.

Several *in vitro* studies have addressed the issue of the effects of the different acetylenic steroids on CYP3A activity. Back *et al.* studied the inhibition of microsomal ethinyl oestradiol 2-hydroxylase activity by coincubation with various progestins [10]. This group demonstrated that with EE_2 as the substrate, the K_i values for gestodene, 3-keto desogestrel, desogestrel, levonorgestrel and norgestimate for competitive inhibition were 99, 93, 54, 40, and 74 μM, respectively [10]. Peak plasma concentration after a 30–50 μg dose of EE_2 range between 0.6 nM–0.7 nM [8, 29]. Additionally, peak plasma concentrations with 250–500 μg dose of levonorgestrel can range between 0.01 μM and 0.05 μM [30]. Thus, it is unlikely that the small dose of EE_2 and norgestrel present in OCs could attain concentrations sufficient to competitively inhibit the metabolism of a second substrate of CYP3A.

Back *et al.* also performed studies examining 17α-acetylenic progestins as mechanism-based inhibitors of CYP3A using cyclosporin as the substrate and rank ordered the progestins according to their inhibitory potency such that gestodene > 3-keto desogestrel > norethisterone > norgestrel [10]. A similar rank order of 17α-acetylenic progestins as mechanism based inhibitors was obtained by Guengerich [24]. In addition, Guengerich found EE_2 to be a mechanism-based inhibitor of relatively similar potency to norgestrel [24]. Thus the differing results of the various *in vivo* studies on the effects of OC on CYP3A activity appear to be related to the choice of progestin. In the current case, norgestrel a relatively poor mechanism-based inhibitor was used with EE_2 and no interaction was observed. However, with the use of more potent mechanism-based inhibitors like gestodene, inhibition may occur [10, 24].

One possible explanation for the lack of CYP3A alteration seen in this study, particularly with EE_2, is that metabolism of these steroids at the 17α-acetylenic position is not a major metabolic pathway. To date, most *in vitro* studies have been performed using EE_2 at micromolar concentrations instead of at clinically relevant concentrations, which are in the nanomolar range. A recent study in human hepatocytes in which the investigators used clinically relevant concentrations of EE_2 (1 nM), suggests that direct conjugation of EE_2 as opposed to EE_2 oxidation represents the predominant elimination pathway for this drug [31]. Because of the predominant role of phase II enzymes in the metabolism of these agents, it is possible that the interactions of EE_2 and norgestel with CYP3A are minimal at therapeutic concentrations and thus alteration of CYP3A activity would be unlikely.

In conclusion, this study did not find a difference in the pharmacokinetics or the pharmacodynamics of midazolam after intravenous or oral administration in women taking Ovral™. These results indicate that the doses of EE_2

and norgestrel contained in this very popular combination OC product did not produce significant inhibition of hepatic or intestinal CYP3A activity. However, drug–drug interaction studies with OCs containing different progestins, such as gestodene, which is a more potent *in vitro* mechanism-based inhibitor than norgestrel may yield a different response.

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