Effect of the ethinylestradiol/levonorgestrel combined oral contraceptive on the activity of cytochrome P4503A in obese women

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Obesity is known to suppress CYP3A4/5 activity.
- Use of combined oral contraceptives (COC) has been shown to suppress CYP3A4/5 activity in non-obese women.
- However, the effect of COC on CYP3A4/5 activity is unknown in obese women who constitute 50% of women of reproductive age.

WHAT THIS STUDY ADDS

- Although we hypothesized that obesity exposure might amplify the impact on CYP3A4/5 activity in COC users, we found that this was not the case.
- This finding is reassuring regarding potential additional drug–drug interactions in obese COC users as CYP3A4/5 is a major player in the metabolism of many marketed drugs.

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While it is known that CYP3A4/5 activity is decreased with combined oral contraceptive (COC) use and obesity suppresses CYP expression, the combined effects of obesity and COC use on CYP3A4/5 activity are unclear. Therefore, our aim was to examine the effect of COC usage on CYP3A4/5 activity in obese women.

METHODS

Thirty-four, obese (body mass index, BMI > 30 kg m⁻²) women of reproductive age (18–35 years old) were placed on a COC pill containing 20 µg ethinylestradiol/100 µg levonorgestrel for 21 days starting at the onset of menses. A midazolam pharmacokinetic study was conducted prior to initiation and after 21 days of COC treatment. Serial blood samples were collected and plasma concentrations of midazolam were measured using liquid chromatography tandem mass spectrometry. Pharmacokinetic parameters were estimated using a non-compartmental method.

RESULTS

Midazolam clearance, a surrogate measure of CYP3A4/5 activity, was significantly decreased upon COC use (63.3 l h⁻¹ vs. 53.9 l h⁻¹, $P < 0.05$). A median decrease of 5.6 l h⁻¹ (95% CI –4.1, 13.3 l h⁻¹) was observed. However, the magnitude of change was similar to that reported in women with normal BMI.

CONCLUSIONS

Although we hypothesized that obesity might amplify the impact on CYP3A4/5 activity in COC users, we found that this was not the case. This finding is reassuring regarding potential additional drug–drug interactions in obese COC users as CYP3A4/5 is a major enzyme in the metabolism of many marketed drugs.

Introduction

Combined oral contraceptives (COC) have been in use for about 50 years and are one of the most popular forms of contraception worldwide [1]. COCs are comprised of a synthetic estrogen and progestin. Ethinylestradiol (EE) is the most common estrogenic component but there are a number of different progestin types with levonorgestrel (LNG) dominating the market [2–4].

Both LNG and EE undergo metabolism resulting in plasma concentrations that fluctuate based on the activity of various drug metabolizing enzymes [5, 6]. However, COCs also affect these enzymes [7–10]. *In vitro*, EE demonstrates inhibition of both phase I and phase II metabolizing enzymes including a major cytochrome P450 (CYP) enzyme, CYP3A4/5 [9, 10]. The effect of LNG on CYP3A activity is less clear as reports are conflicting [7, 8]. This close interaction between COCs and drug metabolizing enzymes makes the pharmacokinetics (PK) and subsequent pharmacotherapy of COCs vulnerable to other factors that influence drug metabolism [6].

Obesity, a low grade chronic inflammatory condition [11], is known to suppress the expression of CYP3A4/5 activity [12]. Research has demonstrated altered PK of midazolam, a known marker for CYP3A4/5 activity, in obese subjects [13]. The interaction of obesity and COC exposure on the CYP pathway is unknown but critically important as impaired COC therapeutics could result in an unplanned pregnancy, a potentially devastating event for this already high risk population [1, 14]. As obese women constitute 50% of the population of reproductive age [15, 16] and COCs are widely used in these women for their contraceptive and non-contraceptive benefits [17, 18], the potential public health impact is significant. Additionally, the interaction of obesity and COC use could compromise the pharmacotherapy of co-administered drugs as a result of COC induced drug–drug interactions. Our objective was to examine the *in vivo* activity of CYP3A4/5 using midazolam as a probe for CYP3A4/5 in obese users of COC containing LNG, the most widely used progesterone.

Methods

Study subjects

A randomized, prospective study was conducted at the Oregon Health and Science University (OHSU) in Portland, OR. The OHSU Institutional Review Board approved the study protocol and all subjects gave written informed consent.

Thirty-four, obese (body mass index, BMI >30 kg m⁻²) women of reproductive age (18–35 years old), not currently using hormonal contraception but seeking to initiate COC use, were recruited. Additional eligibility criteria included demonstration of regular menstrual cycles with proven ovulation (luteal phase progesterone ≥3 ng ml⁻¹),

not actively seeking weight gain or loss, no evidence of anaemia (haematocrit ≥36%), no contraindications to hormonal contraception, no use of tobacco or drugs known to interfere with the metabolism of sex steroids and no overt clinical features of or prior treatment for metabolic disorders (i.e. polycystic ovarian syndrome).

All study subjects were placed on a combination monophasic oral contraceptive pill containing 20 µg EE/100 µg LNG (Alesse, Wyeth, Madison, NJ, USA) at the onset of menses following the pretreatment cycle. The medication was dosed in a cyclical fashion (21 days active pill with a 7 day hormone-free interval). Women were instructed to take the pill at 09.00 h daily. Self-reported compliance with the medication was recorded on a calendar, and women were asked to contact investigators if there was a delay in time of pill ingestion and/or two or more missed pills in one cycle. Two or more delayed and/or missed pills prompted study withdrawal (women were not informed of this consequence but were educated regarding the importance of compliance).

Study design

Subjects were admitted to the Oregon Health and Science University's Oregon Clinical and Translational Research Institute to collect blood samples for determining PK data prior to initiation and after 21 days of COC treatment. Venous blood samples were collected at 15 and 30 min and 1, 2, 4, 5, 6, 12 and 24 h following 2 mg midazolam orally. Plasma was isolated and stored at –80°C for long term storage prior to analysis.

Plasma concentrations of midazolam were measured using liquid chromatography tandem mass spectrometry (LC/MS/MS). Briefly, midazolam was extracted from plasma samples (0.1 ml) with *t*-butylmethylether (5 ml) after addition of 0.5 ng d₄-midazolam as internal standard as described by Hebert *et al.* [19]. Dried extracts were resuspended and analyzed by LC/MS/MS using electrospray ionization in the positive mode using a modified method of Zhang *et al.* [20] with a Kinetex C18 column (2.6 µ, 100 Å 50 × 2.1 mm) and a Javelin Betabasic C18 guard column. The mobile phase consisted of 20 mM ammonium formate/0.1% formic acid and methanol with 20 mM ammonium formate/0.1% formic acid (25:75) at a flow rate of 0.3 ml min⁻¹. The total run time was 10 min. Midazolam and d₄-midazolam were monitored using selective reaction monitoring of the mass transitions of *m/z* = 326 →291.2 and *m/z* = 330 →295.2, respectively. A Thermo TSQ Quantum with an Accela HPLC system was used for samples with concentrations from 1 to 20 ng ml⁻¹ midazolam and a AB Sciex QTRAP 5500 with a Shimadzu Prominence HPLC system was used for samples with concentrations from 0.01–1 ng ml⁻¹. Calibration curves were generated by spiking naïve plasma with 0.5 ng–20 ng ml⁻¹ midazolam for the TSQ instrument and 0.1 ng–5 ng ml⁻¹ for the QTRAP 5500. Least squares linear regression of the peak area ratio (midazolam normalized to

d₄-midazolam) vs. concentration of midazolam was used for quantification. Slopes of the standard curves varied by <10% over 22 different runs with the TSQ Quantum and <5% with five different runs with the QTRAP 5500.

The assay limit of detection was 0.01 ng ml⁻¹. The accuracy of lower (0.05 ng ml⁻¹) and upper (20 ng ml⁻¹) limits of quantitation was 90% and 99%, respectively. The precision of lower and upper limits of quantitation was 13.7% and 1% relative SD ($n = 12$), respectively.

Pharmacokinetic analysis

Midazolam PK data were analyzed by non-compartmental methods using WinNonLin (v 5.2; Pharsight, Mountain View, CA, USA).

Statistical analysis

Demographic data were analyzed using descriptive statistics. Wilcoxon signed-rank test ($\alpha = 0.05$) was used to assess statistical differences in the pre and post treatment PK

parameters of midazolam using SigmaPlot software (v 11.0; Systat Software, Inc., San Jose, CA).

Results

All 34 recruited women completed the study. Thirty of them were non-hispanic, Caucasians. The mean age of study participants was 29.1 ± 4.6 (mean $\pm$ SD) years with a mean BMI of 39.7 ± 6.8 kg m⁻².

The plasma concentrations of midazolam were consistently higher after COC use compared with pre-treatment values (Figure 1). The PK parameters, both estimated and observed, were not normally distributed (Table 1). Maximum plasma concentration ($C_{\max}$) and time to maximum concentration ($t_{\max}$), were not affected by 3 weeks of COC use in these obese women. The elimination half-life ($t_{1/2}$) was found to be slightly longer after the use of COCs. However, the difference failed to reach statistical significance [median 7.5 h (95% CI 7.0, 9.6) pre treatment vs. 7.9 h (95% CI 7.4, 8.7) post treatment]. With COC use, apparent volume of distribution (V_d) and apparent oral clearance (CL) significantly decreased [V_d median 701 l (95% CI 660, 844) to 657 l (95% CI 609, 812), $P < 0.05$; CL median 63.3 l h⁻¹ (95% CI 59.7, 74.6) to 53.9 l h⁻¹ (95% CI 53.5, 71.7), $P < 0.05$]. Conversely, COC use increased the median AUC(0,∞) from 31.6 ng ml⁻¹ h (95% CI 29.3, 37.3) to 37.1 ng ml⁻¹ h (95% CI 32.2, 42.9), $P < 0.01$.

Discussion

We found that COC use in obese individuals affected the PK of midazolam including clearance, a measure of the functional status of CYP3A4/5. The magnitude of difference found in obese COC users was similar to changes reported in women of normal BMI [21, 22]. Although we hypothesized that obesity might amplify the impact on CYP3A4/5 activity in COC users, we found that this was not the case. This finding is reassuring regarding potential additional drug-drug interactions in obese COC users as

Figure 1

Pharmacokinetic profile of midazolam before (◆) and after (◇) COC treatment. Each data point represents mean of 34 subjects with one sided error bar indicating 1 SD

Table 1

Pharmacokinetic parameters of midazolam in obese women prior to and after treatment with a combined oral contraceptive containing levonorgestrel and ethinylestradiol

PK parameter	Pre treatment	Post treatment	Difference
$C_{\max}$ (ng ml ⁻¹)	6.74 (6.26, 8.30)*	6.68 (6.53, 8.50)	-0.52 (-1.12, 0.65)
$t_{\max}$ (h)	0.53 (0.67, 0.97)	1.00 (0.71, 1.16)	0.00 (-0.39, 0.16)
$t_{1/2}$ (h)	7.5 (7.0, 9.6)	7.9 (7.4, 8.7)	-0.45 (-0.90, 1.27)
V_d (l)	701 (660, 844)	657 (609, 812)†	80 (-37, 119)
CL (l h ⁻¹)	63.3 (59.7, 74.6)	53.9 (53.5, 71.7)†	5.6 (-4.1, 13.3)
AUC(0,∞) (ng ml ⁻¹ h)	31.6 (29.3, 37.3)	37.1 (32.2, 42.9)†	-5.2 (-8.8, 0.2)

*Median (95% CI). †Denotes a significant difference between pre and post treatment pharmacokinetic parameter.

CYP3A4/5 is a major enzyme in the metabolism of many marketed drugs.

We employed midazolam clearance as a specific probe for CYP3A4/5 activity. Midazolam is an intermediate extraction drug [23], and therefore the determinants of clearance are (a) intrinsic clearance, (b) the fraction unbound in plasma, and (c) blood flow to the liver. In most studies including the current one, changes in the clearance of midazolam are assumed to be due to alterations in intrinsic clearance of midazolam mostly mediated by CYP3A4/5. The inherent assumption in such a conclusion is that the hepatic blood flow and unbound fraction of midazolam are unaltered. While the lack of haemodynamic effects of COCs has been clearly demonstrated [24], the status of protein binding of midazolam is unclear.

Midazolam is highly (96–98%) bound to human serum albumin and α_1 -acid glycoprotein [25]. While the effect of COCs on serum albumin concentrations is inconclusive [26–28], the concentrations of other serum proteins including α_1 -acid glycoprotein increase upon exposure to COCs [27, 28]. Furthermore, it is well known that COCs, especially their estrogen component, greatly increase the serum concentrations of sex hormone binding globulin. However, binding of midazolam to sex hormone binding globulins has not been reported. In addition to the amount of serum proteins, the binding affinity of drugs to the serum proteins plays a vital role in the circulation of the bound or unbound form. The binding of midazolam to albumin was shown, using *in vitro* incubations, to increase in the presence of a high ionic plasma concentration [29]. The use of COCs has been shown to affect the renal handling of electrolytes [30]. While estrogens promote sodium retention, progestins are known to promote natriuresis. Since COCs have more estrogenic than progestagenic activity [30], it is expected that the combined effect of COCs would be sodium retention leading to an increase in ionic concentration in plasma. The above changes in serum proteins could potentially alter the unbound fraction of a drug, including midazolam.

The volume of distribution of a drug is directly related to plasma volume, tissue volume and fraction unbound in plasma, and inversely related to fraction unbound in tissues. Data on tissue binding is limited due to the inaccessibility of this body compartment. Since the volume of distribution was compared before and after 3 weeks of COC use in the same obese subjects, not actively looking to lose weight during the study duration, we assumed that the tissue volumes were unchanged over this period. For a highly lipophilic drug like midazolam, the role of plasma volume could be considered to be insignificant. In this study, volume of distribution of midazolam was slightly, but significantly, decreased (~7%) after 3 weeks of COC use. Since there is a direct relationship between volume of distribution and fraction unbound in plasma, we deduced that the unbound fraction of midazolam was also decreased after COC use. Therefore, the change in

midazolam clearance after COC treatment was a cumulative effect of decreased CYP3A4/5 activity and increased protein binding. Though the overall decrease of ~15% in midazolam clearance was statistically significant, the clinical significance of such a minor alteration is questionable.

The PK of drugs co-administered with COCs has been shown to be altered in many studies [6], although most of the reported drug interactions with COCs are non-CYP3A4/5 mediated [6, 8]. However, it is unclear if that is true in women with obesity which alters the expression of a host of enzymes including CYP3A4/5 [12, 31]. We hypothesized that COCs in the presence of obesity would cause an additive, if not greater, alteration in the activity of CYP3A4/5. The current study findings reveal lack of such an effect in obese women.

Competing Interests

AE has been a trainer for Merck and received royalties from UpToDate.com. There are no other competing interests to declare.

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