Effect of a Triphasic Oral Contraceptive on Drug-Metabolizing Enzyme Activity as Measured by the Validated Cooperstown 5+1 Cocktail

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The effects of a common oral contraceptive preparation on the activity of 7 drug-metabolizing enzymes were investigated using the validated Cooperstown 5+1 Cocktail. In a randomized crossover fashion, 10 premenopausal women received caffeine, dextromethorphan, omeprazole, intravenous midazolam, and warfarin + vitamin K with and without a triphasic oral contraceptive (ethinyl estradiol 35 μg) and varying doses of daily norgestimate (0.18, 0.215, and 0.25 mg). Bioequivalence testing showed nonequivalence in drug versus no-drug treatment on the activity of drug-metabolizing enzymes (as reflected by metabolite ratios following probe drug administration); the activity of CYP1A2, CYP2C19, and NAT-2 decreased following the oral contraceptive, whereas the activity of CYP2C9 and CYP2D6 increased. No effects on xanthine oxidase or hepatic CYP3A were seen. Application of a nonparametric statistical testing approach revealed a significant difference only for CYP1A2 and CYP2C19. This triphasic oral contraceptive may have a clinically significant effect on the activity of some drug-metabolizing enzymes.

Keywords: *Oral contraceptives; cocktail; cytochrome P450; drug-metabolizing enzymes*

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Knowledge of, and the ability to predict, drug interactions is of great clinical importance. Many drug-drug interactions occur because of changes in the activity of drug-metabolizing enzymes that are subject to control by intrinsic factors (eg, genetics, neuroendocrine modulation, disease expression) and extrinsic factors (eg, environment, concomitant therapy).[1] Patients who take multiple medications are at increased risk for complications caused by drug interactions that can occur either as a consequence of drug toxicity or reduced pharmacologic activity.

Estrogens and progesterones are hormones occurring naturally in humans. In women, supratherapeutic doses of these agents are used frequently as a method of contraception. In many oral contraceptive preparations, a combination of estrogen and progesterone is used to achieve effective contraception (mediated by the progesterone component) while balancing the adverse effects of the progesterone (estrogen component). Newer contraceptive agents have included fixed doses of ethinyl estradiol while varying the weekly dose of progesterone (triphasic oral contraceptives). Also, progesterones with less androgenic activity (eg, norgestimate) are now incorporated into oral contraceptive products in an attempt to further reduce adverse effects.[2]

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Much clinical research has focused on the influence of other medications on the effectiveness of oral contraceptives.[3] Some human studies have investigated the influence of oral contraceptives on the metabolism of other medications or pharmacologic probe substrates designed to reflect the activity of specific cytochrome P450 (CYP) isoenzymes.[4-37] However, only a few of these studies have used women as their own controls.[4-12] Rather, many have assessed differences between groups of participants receiving or not receiving oral contraceptives.[13-37] Such an approach produces substantial variability in the results because of normal, but substantial, intersubject variability in the activity of specific CYPs (ie, the result of genetic [gene-dose effect] and environmental differences). Although some crossover studies have suggested that oral contraceptives may affect the activity of some drug-metabolizing enzymes,[4-12] no broad screen of the influence of oral contraceptives on drug-metabolizing enzyme activity has been performed in a single group of women.

We have previously validated the use of a 5-drug phenotyping cocktail (Cooperstown 5+1 Cocktail) to simultaneously measure the activity of 7 drug-metabolizing enzymes.[38] The cocktail consists of caffeine, dextromethorphan (DM), omeprazole, and warfarin plus vitamin K, all given by the oral route, and intravenous midazolam. By quantitating either the parent drug:metabolite ratio or estimating drug clearance, the Cooperstown 5+1 Cocktail provides a surrogate assessment of the activity of the following clinically important drug-metabolizing enzymes: CYP1A2 (caffeine), CYP2C9 (warfarin), CYP2C19 (omeprazole), CYP2D6 (dextromethorphan), hepatic CYP3A (midazolam), N-acetyltransferase, and xanthine oxidase (XO; caffeine).

The specific aim of this study was to assess the effect of a triphasic oral contraceptive on drug-metabolizing enzyme activity in healthy adult women as measured by the Cooperstown 5+1 Cocktail, controlling for expected pharmacogenetic variability by using each participant as her own control.

METHODS

This study was approved by the Institutional Review Board of Bassett Healthcare, and written informed consent was obtained from all participants prior to performance of any study procedures.

Participants and Eligibility

Women were recruited from a list of healthy volunteers who had previously participated in clinical trials conducted at Bassett Healthcare. Women were premenopausal, nonsmoking, and at least 18 years of age. None of the women used hormonal contraception for 1 year prior to the study. They were not permitted to take any medications, herbals, or supplements during the study period; they were required to adhere to dietary restrictions (no caffeine-containing or theobromine-containing products, no grapefruit, grapefruit juice, Seville oranges, or cruciferous vegetables for 48 hours before and 24 hours after the administration of the Cooperstown 5+1 Cocktail); and they had to be nonbinge drinkers (less than one 12-oz beer or equivalent daily). The women agreed to practice nonhormonal methods of contraception during the study period. A negative qualitative urine pregnancy test result was required at baseline and immediately before each study phase. Participants were excluded from the study if they had clinically significant abnormal findings by history or physical examination, abnormal laboratory tests (alanine aminotransferase, aspartate aminotransferase, or gamma glutamyl transferase >2 times above normal limits; total bilirubin >1.3 mg/dL; international normalized ratio [INR] >1.2; serum creatinine >1.1 mg/dL), or a history of an allergy or serious drug reaction to any of the medications used in the phenotyping cocktail.

Blood for genotyping was obtained from all participants prior to phenotyping. Participants who were genotypically poor metabolizers of CYP2C9, CYP2C19, and CYP2D6 (those possessing 2 reduced or null-activity alleles or a combination of these alleles) were excluded from the study. Thus, the extensive metabolizer phenotype was included so as to enable hypothesis testing within the phenotype reflecting the greatest frequency for each of the drug-metabolizing enzymes evaluated.

Study Procedures

The study consisted of 2 phases. Each participant was assigned to undergo study phases in a random order. A 2-month washout period was allowed between the study phases. Each participant was phenotyped twice: once while not on the oral contraceptive and once at the end of the third week of the second month of oral contraceptive (Ortho-Tricyclen; Ortho McNeil, Raritan, NJ) dosing. The oral contraceptive was taken as follows: week 1, norgestimate 0.18 mg plus ethinyl estradiol 0.035 mg (7 days); week 2, norgestimate 0.215 mg plus ethinyl estradiol 0.035 mg (7 days); week 3, norgestimate 0.25 mg plus ethinyl estradiol 0.035 mg (7 days); and week 4, placebo tablets (7 days).

Subjects fasted from midnight the night before each study phase. The study phases began between 6:00 and 10:00 AM. Prior to the administration of the phenotyping cocktail, each participant was instructed to completely empty her bladder. The participants received simultaneous dosing with oral caffeine (2 mg/kg rounded to the nearest 50 mg), oral DM (30 mg), oral omeprazole (40 mg), intravenous midazolam (0.025 mg/kg infused over 1 minute), and oral warfarin plus oral vitamin K (10 mg each). For the first 2 hours after midazolam administration, participants remained in a sitting position with vital signs checked at least every 15 minutes. Continuous oxygen saturation was monitored via pulse oximetry for the first hour.

For caffeine and DM phenotyping, women first emptied their bladders and then collected all urine for 12 hours after cocktail administration, emptying their bladders 12 hours after the dose. Three grams of ascorbic acid were added to urine collection containers to stabilize caffeine metabolites. For omeprazole phenotyping, one 15-mL blood sample was obtained 2 hours after the oral omeprazole dose. For midazolam phenotyping, 7 blood samples (10 mL each) were obtained immediately before midazolam administration (0 min) and then at 5, 30, 60, 120, 240, 300, and 360, minutes. Blood samples (10 mL) for warfarin were obtained at 0, 3, 6, 12, 24, 36, 48, 72, and 96 hours following warfarin administration. INR was obtained at 48 hours and if >1.7, vitamin K (5 mg) was administered by mouth daily until the INR was <1.2. All blood samples were collected in EDTA-containing tubes and centrifuged for 15 minutes at 2800 rpm and 4°C, after which a 3.0-mL sample was removed by manual aspiration. For urine collections, the total volume and pH were determined, and a 15-mL aliquot was retained for analysis. Plasma and urine samples were frozen at –80°C until analysis.

Assays

Genotype analysis. CYP2C9, CYP2C19, and CYP2D6 genotyping was performed on all participants. A single 7-mL blood sample was collected into an acid citrate dextrose Vacutainer (Becton Dickson & Co, Franklin Lakes, NJ) and stored at 4°C until DNA was extracted, which was accomplished with a QIAamp blood DNA isolation kit (Qiagen, Chatsworth, Calif).

CYP2C9 genotyping comprised testing for CYP2C9*2 and *3 alleles, employing methods described by Stubbins et al[39] and Sullivan-Klose et al,[40] respectively. To detect CYP2C9*2, PCR products were digested with AvaII. Wild type–derived PCR fragments were cut once (115 bp and 75 bp), whereas CYP2C9*2-derived PCR fragments remained uncut (190 bp). To detect CYP2C9*3, PCR fragments were cut with NsiI. Wild type–derived PCR products were digested into 140-bp and 30-bp fragments, whereas CYP2C9*3-carrying PCR products remained intact at 170 bp. All CYP2C9*3 alleles were confirmed by a second assay that allowed its identification by KpnI restriction digestion.

Procedures to genotype CYP2C19*2 and *3 allelic variants were adapted from Goldstein and Blaisdell,[41] with some modifications in the primer sequences. CYP2C19*2 was detected by SmaI restriction digestion of a 322-bp-long PCR product that left the *2 allele uncut, whereas wild-type PCR products were cut (110 bp and 212 bp). Similarly, CYP2C19*3 was identified by digestion with BamHI that cut wild-type PCR products (170 bp and 96 bp) but not CYP2C19*3 (166 bp).

CYP2D6 genotyping was carried out as described previously.[42] Briefly, the CYP2D6 gene was amplified with specific primers by long PCR to discriminate against the CYP2D7 and CYP2D8 pseudogenes. Subsequently, the CYP2D6 long PCR product served as a template to perform PCR and restriction digestions to identify nucleotide variations associated with CYP2D6*2, *3, *4, *6, *7, *9, *10, *17, and *41 alleles. In addition, long PCR was performed to detect the presence of CYP2D6*5 (gene deletion) and *1×2, *2×2, and *4×2 gene duplications.

PCR fragments were separated by agarose gel electrophoresis (3% gelTWIN matrix, JT Baker, Phillipsburg, NJ), stained with ethidium bromide, and documented with a Kodak CT440 Imaging System.

Phenotyping assays and pharmacokinetic analysis. Determination of 1-methylurate (1U), 1-methylxanthine (1X), 1,7-dimethylurate (17U), and 5-acetylamino-6-formylamino-3-methyluracil (AFMU) in urine was accomplished using a modification of high-performance liquid chromatography (HPLC) according to the methods of Evans et al[43] with modification.[38] Data outputs were normalized to the internal standard, and the molar amount was determined using standard curves prepared daily in drug-free urine specimens.

Caffeine demethylation ratios were used to express CYP1A2, NAT-2, and XO activity as follows:

$$\text{CYP1A2 activity} = \text{AFMU} + 1\text{X} + 1\text{U}/17\text{U} \quad (1)$$

$$\text{NAT-2 activity} = \text{AFMU}/(1\text{X} + 1\text{U}) \quad (2)$$

$$\text{XO activity} = 1\text{U}/1\text{X} + 1\text{U} \quad (3)$$

Urine aliquots were assayed for concentrations of DM and dextrorphan (DX) using a modification of the

methods of Park et al[44] and Lam and Rodriguez.[45] Data outputs were normalized to the internal standard, and the molar amount was determined using standard curves prepared daily in drug-free urine specimens. The DM/DX molar ratio was used as a reflection of CYP2D6 activity.

Omeprazole and its metabolites, 5'-hydroxyomeprazole (5OH) and omeprazole sulphone, were quantitated from a single plasma sample with a modification of the method of Lagerstrom and Persson.[46] The ratio of omeprazole/5OH omeprazole was used as a biomarker of CYP2C19 activity.

Validated analytical methods were used to quantitate midazolam[47] and R/S warfarin[48] from plasma.

Plasma S-warfarin and midazolam data were analyzed using noncompartmental analysis with WinNonlin, version 3.1 (Pharsight Corp, Mountain View, Calif). The plasma clearance (Cl) of midazolam was used as the surrogate to assess hepatic CYP3A activity and was calculated as $Cl = dose/AUC_{0-\infty}$, where $AUC_{0-\infty}$ is the area under the plasma concentration-versus-time curve extrapolated to infinity using the slope of the apparent elimination phase (λ_z) and determined using the linear trapezoidal rule. The apparent plasma clearance (Cl/F) of S-warfarin was used to assess CYP2C9 activity and was calculated for each participant as follows: $Cl/F = dose/AUC_{0-\infty}$, where F represents the fraction of the dose absorbed into the systemic circulation (ie, extent of bioavailability).

Statistical Analyses

All statistical analyses were performed using the SAS software system (Version 8, SAS Institute, Cary, NC) running on an ALPHA VMS host. Data are presented as the median and the mean ± SD.

With 80% power and an $\alpha = .05$, a sample size of 10 was needed to detect a 20% difference in the phenotyping measures for caffeine (CYP1A2), omeprazole, midazolam, and S-warfarin. For DM, a sample size of 10 was expected to detect a 65% change in the molar ratio of DM/DX.

Two methods were used to determine whether oral contraceptive treatment altered the apparent activity of the drug-metabolizing enzymes evaluated. Equivalence testing was performed using methods outlined in the 1998 Food and Drug Administration (FDA) Guidance for Bioequivalence Studies.[49] With this technique, the 90% confidence intervals (CIs) for the geometric mean ratios of the AUC data from the 2 study phases are compared. The oral contraceptive treatment phase was considered the test treatment, and the treatment phase with no oral contraceptive was considered the reference. If the 90% CIs are 0.8 to 1.25, equivalence is assumed, and it is concluded that no difference in drug-metabolizing enzyme activity occurred.[49] In contrast, values outside the 0.8 to 1.25 range are considered indicative of an interaction between oral contraceptive treatment and the specific drug-metabolizing enzyme (potentially clinically significant). In addition, significance testing was performed for all pharmacologic probe substrates using the Wilcoxon signed-rank test of the variables (eg, AUC or plasma concentration ratio data). A significance limit of $\alpha = .05$ was applied to all statistical analyses.

RESULTS

Sixteen white women were enrolled in the protocol, and 10 women completed all study phases; thus, paired data for 10 women were used for analysis. Reasons for study discontinuation included leaving the geographic area (n = 2), becoming menopausal (n = 1), an adverse reaction to the oral contraceptive (n = 1), voluntary withdrawal from the study (n = 1), and administrative removal (n = 1). The average age and weight of the 10 women completing the study were 37.4 ± 4.9 years and 84.6 ± 12.7 kg, respectively. All women carried at least 1 normal-activity allele for CYP2C9, CYP2C19, and CYP2D6.

Figures 1 to 7 illustrate the individual data for each pharmacologic probe substrate as well as the mean ± SD (histogram) and geometric mean value for each treatment phase (ie, control vs oral contraceptive). Summary data are presented in the table, with the results for statistical comparisons indicated. When examined using significance testing (Wilcoxon signed-rank test), CYP1A2 and CYP2C19 activity differed significantly ($P < .05$) between the control versus oral contraceptive phase of the study.

With bioequivalence testing (using the 0.8-1.25 90% CI range), differences were seen for CYP1A2, CYP2C19, and NAT-2 (all decreased activity during the oral contraceptive phase). The activities of CYP2C9 and CYP2D6 appeared to increase as a result of oral contraceptives. No effect on hepatic CYP3A or XO activity was observed.

No subject required additional vitamin K for an elevated INR after warfarin administration. During midazolam administration, all subjects experienced mild sedation, but no one had a significant change in vital signs (respiratory rate, heart rate, blood pressure) or oxygen saturation (data not shown).

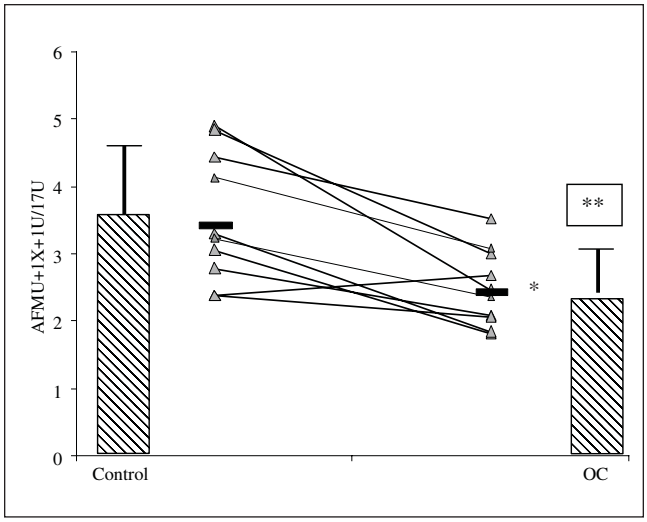

*Figure 1. Comparison of CYP1A2 ratio for control versus oral contraceptive (OC) after administration of 5+1 Cocktail. Solid bars indicate geometric means. Boxes indicate mean ± SD. AFMU, 5-acetylamino-6-formylamino-3-methyluracil; 17U, 1,7-dimethylurate; 1U, 1-methylurate; 1X, 1-methylxanthine. *OC phase not bioequivalent to control phase. **P = .01.*

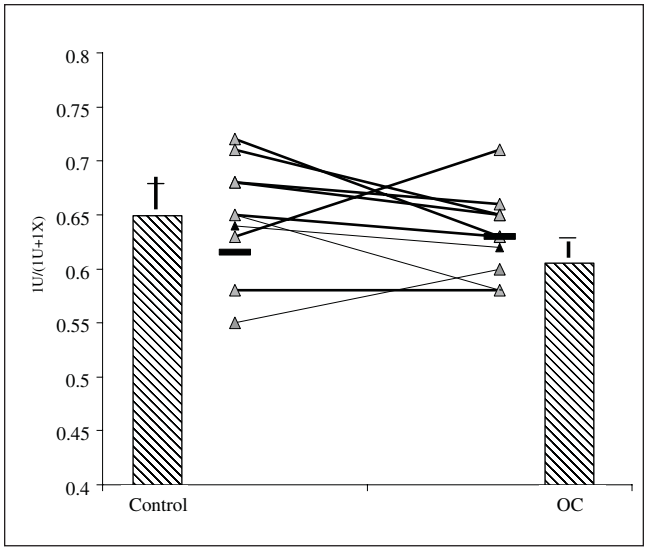

Figure 2. Comparison of xanthine oxidase ratio for control versus oral contraceptive (OC) after administration of 5+1 Cocktail. 1U, 1-methylurate; 1X, 1-methylxanthine. Solid bars indicate geometric mean values. Boxes indicate mean ± SD.

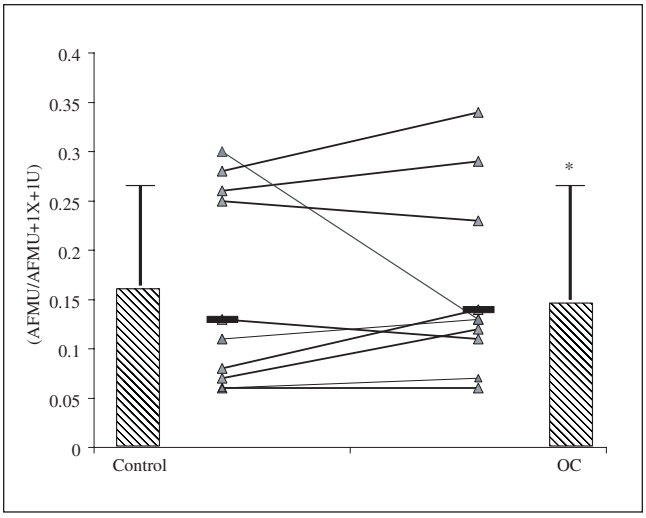

*Figure 3. Comparison of N-acetyltransferase-2 ratio for control versus oral contraceptive (OC) after administration of 5+1 Cocktail. Solid bars indicate geometric mean values. Boxes indicate mean ± SD. AFMU, 5-acetylamino-6-formylamino-3-methyluracil; 1U, 1-methylurate; 1X, 1-methylxanthine. *OC phase not bioequivalent to control phase.*

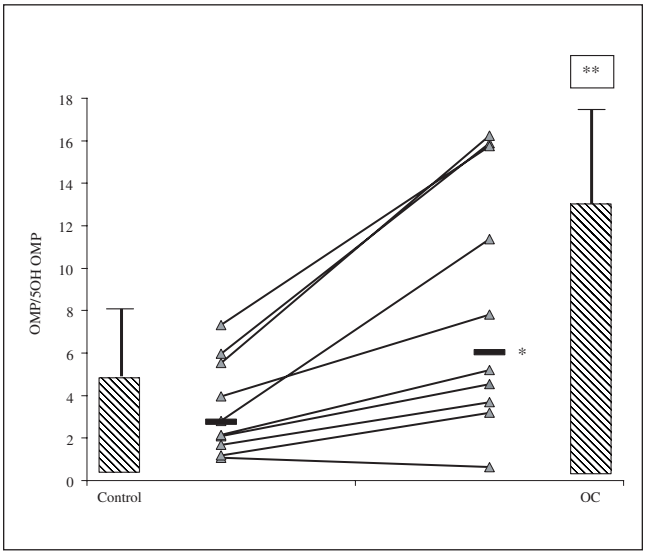

*Figure 4. Comparison of CYP2C19 ratio for control versus oral contraceptive (OC) after administration of 5+1 Cocktail. Solid bars indicate geometric mean values. Boxes indicate mean ± SD. 5OH, 5'-hydroxyomeprazole; OMP, omeprazole. *OC phase not bioequivalent to control phase. **P = .005.*

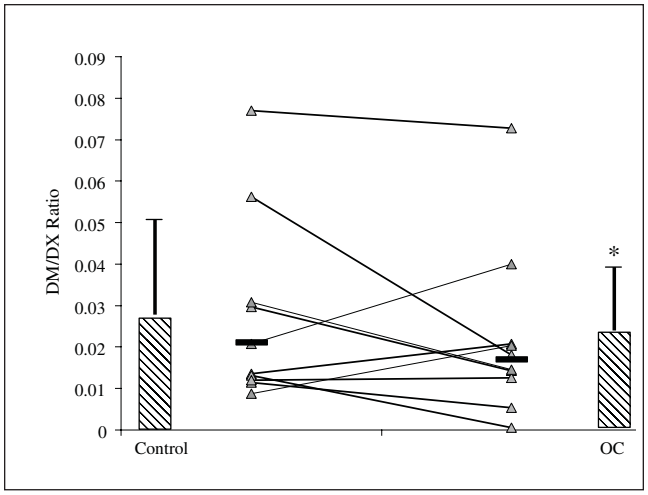

*Figure 5. Comparison of dextromethorphan/dextrorphan (DM/DX) ratio for control versus oral contraceptive (OC) after administration of 5+1 Cocktail. Note: a decrease in the DM/DX ratio indicates an increase in enzyme activity. Solid bars indicate geometric mean values. Boxes indicate mean ± SD. *OC phase not bioequivalent to control phase.*

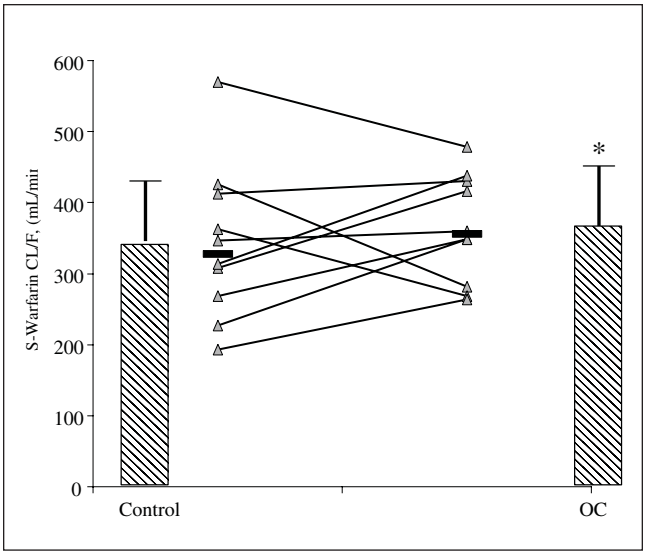

*Figure 7. Comparison of S-warfarin plasma clearance versus the fraction of the dose absorbed into systemic circulation (CL/F) for control versus oral contraceptive (OC) after administration of 5+1 Cocktail. Solid bars indicate geometric mean values. Boxes indicate mean ± SD. *OC phase not bioequivalent to control phase.*

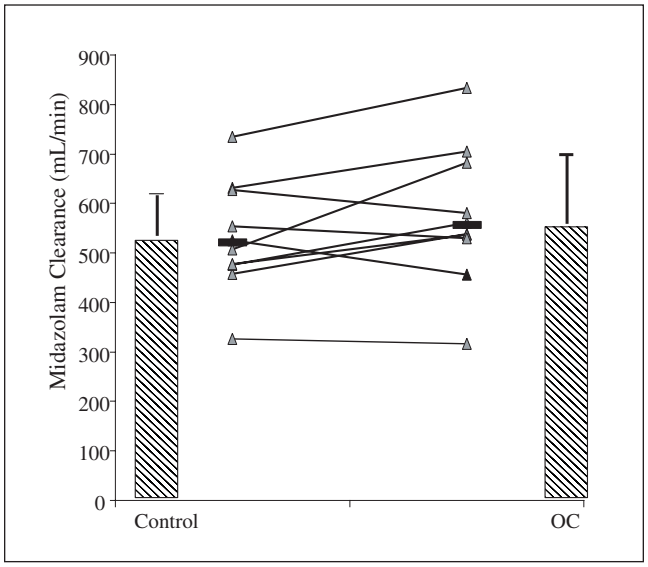

Figure 6. Comparison of midazolam clearance for control versus oral contraceptive (OC) after administration of 5+1 Cocktail. Solid bars indicate geometric mean values. Boxes indicate mean ± SD.

DISCUSSION

The effect of female sex steroids on drug-metabolizing activity has been reported as being variable.[4-37] This can be partially explained by the in vitro data of Laine et al[50] that suggest the effect of estrogens and progesterones on drug-metabolizing enzyme activity differs based on the preparation studied. One limitation of the Laine et al study is that only single concentrations of sex steroids were studied.

The present study found that the activity of some drug-metabolizing enzymes differed when women were taking triphasic oral contraceptives. When evaluated by standard bioequivalence testing (using the 90% CI range of 0.8-1.25), the activity of CYP2C9 and CYP2D6 appeared to be increased by oral contraceptives. The activities of CYP1A2, CYP2C19, and NAT-2 were decreased. No effect was seen on hepatic CYP3A or XO activity. These findings are in contrast to our previous data, which found no effect of the menstrual cycle (midfollicular vs midluteal phases when endogenous progesterone concentrations differ substantially but are not supratherapeutic) on the drug-metabolizing enzymes CYP1A2, CYP2D6, CYP2C19, hepatic CYP3A, XO, and NAT-2.[51-54] When significance testing was used for evaluation, oral contraceptives significantly reduced the activity of CYP1A2 and CYP2C19. These disparate assessments make it important to determine the clinical relevance of these findings.

Current data suggest that there may be a different effect of supratherapeutic doses of sex steroids as compared to endogenous hormonal changes seen during the menstrual cycle.[51-54] Interpretation of the literature on the effect of sex steroid exposure on drug-

metabolizing enzyme activity is confusing because many comparisons are of different women on and off oral contraceptives (not using a woman as her own control and therefore not controlling for genetic influences),[13-37] use of nonspecific probes of drug-metabolizing activity, or unvalidated measures of drug-metabolizing activity in crossover studies.[4-7,9] Use of nonspecific or unvalidated probes can lead to erroneous interpretations of collected data.[55]

When considering only crossover studies that used appropriate probes of CYP enzymes, our data confirm previous studies and suggest a relatively small and clinically insignificant effect on hepatic CYP3A activity[10,11] and a potentially clinically significant inhibition of CYP2C19[12] and CYP1A2.[56] Crossover studies examining the effect of oral contraceptives on CYP2C9 and CYP2D6 were not found.

All of the women who participated in this study were white. Significant interindividual variability in drug-metabolizing activity was evident, as can be seen in the figures. The crossover design of the study was important because allowing women to serve as their own controls decreased the variability in the study by controlling for genetic factors.

Using bioequivalence testing, the activity of CYP2D6 increased with oral contraceptive use in this study. Data have suggested that pregnancy, a time when female sex steroids increase significantly compared to the nonpregnant state, increases the activity of CYP2D6 and decreases CYP1A2 and NAT-2 activity.[57-58] These data are consistent with our findings in women who are not pregnant but who are receiving supraphysiological (or pharmacological) doses of hormones. However, there are large intraindividual differences in CYP2D6 activity. Our findings of an increase of 20% in the metabolic ratio may not be clinically significant.

The differences in findings in this study when using different statistical methods (significance testing vs equivalence testing) raise questions about how to interpret this type of drug-interaction data. Recent data by Gorski et al[59] used the 2 statistical approaches that we used—that is, equivalence testing and significance testing. Bioequivalence testing using the 90% CI range of 80% to 125% (based on a 20% difference between treatment and reference groups) was used in the study of Gorski et al to determine clinically significant differences when examining a drug interaction. As in their study, we used the same 90% CI range, thereby suggesting that a 20% difference could be clinically significant. In fact, given the difference in the therapeutic-to-toxic ratios for drugs, in some instances this 20% difference could translate into a significant drug interaction, and in other instances may not be important. Shadle et al[60] recently tried to modify the standard approach to bioequivalence testing for drug interactions by increasing the 90% CI range to 0.7 to 1.43—a 30% difference between baseline and treatment. In addition, for enzymes such as CYP2D6, where large intraday variability in activity can be seen,[53] it is unclear if a clinically significant change, as defined by a 30% change, is due to a drug interaction or the underlying variability in enzyme activity. In our review of the literature, there does not appear to be a consensus concerning what change in biomarker quantitation constitutes a clinically significant effect. We believe that the FDA and the scientific community should address this issue and attempt to reach a consensus. In fact, based on the extent of intersubject and intrasubject variability in activity for a given enzyme, the target interval for a clinically significant change may vary between enzymes.

This is the first study examining the activity of multiple drug-metabolizing enzymes using the validated Cooperstown 5+1 Cocktail as a broad drug-interaction screening tool. Using this approach in the drug development process could reduce costs associated with the use of individual drug-interaction trials as well as reduce the time required to generate such information.

Differences in bioequivalence were noted for CYP1A2, CYP2C9, CYP2C19, NAT-2, and CYP2D6. No differences in XO or hepatic CYP3A activity were found. Based on significance testing of the data, we suggest that the studied triphasic oral contraceptive may cause a clinically significant decrease in CYP2C19 and CYP1A2 activity. Knowledge of the effect of oral contraceptives on drug-metabolizing enzymes will prove important in future clinical and research applications.

REFERENCES

1. Rogers JF, Nafziger AN, Bertino JS Jr. Pharmacogenetics affects dosing, efficacy, and toxicity of cytochrome P450-metabolized drugs. *Am J Med*. 2002;113:746-750.

2. Loose-Mitchell DS, Stancel GM. Estrogens and progestins. In: Hardman JG, Limbird LE, eds. *Goodman and Gilman's The Pharmacologic Basis for Therapeutics*. 10th ed. New York, NY: McGraw Hill; 2001:1597-1634.

3. Fotherby K. Interactions with oral contraceptives. *Am J Obstet Gynecol*. 1990;163:2153-2159.

4. Carter DE, Goldman JM, Bressler R, Huxtable RJ, Christian CD, Heine MW. Effect of oral contraceptives on drug metabolism. *Clin Pharmacol Ther*. 1974;15:22-31.

5. Ferandez-Pol JA, Zaninovich AA. Effects of administration of estrogen or diphenylhydantoin on the kinetics of dipehnylhydantoin in man. *J Nucl Med*. 1975;16:305-308.

6. Gupta KC, Joshi JV, Pohujani SM, Sayoskar RS. Effect of low estrogen combination oral contraceptive on metabolism of aspirin and phenylbutazone. *Int J Clin Pharmacol*. 1982;20:511-513.

7. Joshi JV, Gupta KC, Hazari KT, Gokral J, Pohujani S, Satoskar R. Salivary antipyrine half-life during injectable progestagen contraception. *Clin Pharmacokinet.* 1986;11:171-175.

8. Balogh A, Gessinger S, Svarovsky U, et al. Can oral contraceptive steroids influence the elimination of nifedipine and its primary pyridine metabolite in humans? *Eur J Clin Pharmacol.* 1998;54:729-734.

9. Tsunoda SM, Harris RZ, Mroczkowski PJ, Benet LZ. Preliminary evaluation of progestins as inducers of cytochrome P450 3A4 activity in postmenopausal women. *J Clin Pharmacol.* 1998;38:1137-1143.

10. Palovaara S, Kivisto KT, Tapanainen P, Manninen P, Neuovonen PJ, Laine K. Effect of an oral contraceptive preparation containing ethinylestradiol and gestodene on CYP3A4 activity as measured by midazolam 1-hydroxylation. *Br J Clin Pharm.* 2000;50:333-337.

11. Belle DJ, Callaghan JT, Gorski JC, et al. The effects of an oral contraceptive containing ethinyloestradiol and norgestrel on CYP3A activity. *Br J Clin Pharmacol.* 2002;53:67-74.

12. Palovaara S, Tybring G, Laine K. The effect of ethinyloestradiol and levonorgestrel on the CYP2C19 mediated metabolism of omeprazole in healthy female subjects. *Br J Clin Pharmacol.* 2003;56: 232-237.

13. Patwardhan RV, Desmond PV, Johnson RF, Schenker S. Impaired elimination of caffeine by oral contraceptive steroids. *J Lab Clin Med.* 1980;95:603-608.

14. Kendall MJ, Quartman CP, Jack DB, Belley J. Metoprolol pharmacokinetics and the oral contraceptive pill. *Br J Clin Pharmacol.* 1982; 14:120-122.

15.. Tornatore KM, Kanarkowski R, McCarthy TL, Gardner MJ, Yurchak AM, Jusko WJ. Effect of chronic oral contraceptive steroids on theophylline disposition. *Eur J Clin Pharmacol.* 1982;23:129-134.

16. Abernethy DR, Divoll M, Ochs HR, Ameer B, Greenblatt DJ. Increased metabolic clearance of acetaminophen with oral contraceptive use. *Obstet Gynecol.* 1982;60:338-341.

17. Boekenoogen SJ, Szefler SJ, Jusko WJ. Prednisolone disposition and protein binding in oral contraceptive users. *J Clin Endocrin Metab.* 1983;56:702-709.

18. Roberts RK, Grice J, McGuffie C, Heilbronn L. Oral contraceptive steroids impair the elimination of theophylline. *J Lab Clin Med.* 1983; 101:821-825.

19. Gardner MJ, Tornatore KM, Kanarkowski R, Jusko WJ. Effects of tobacco smoking and oral contraceptive use on theophylline disposition. *Br J Clin Pharmacol.* 1983;16:271-280.

20. Abernethy DR, Todd EL. Impairment of caffeine clearance by chronic use of low-dose oestrogen-containing oral contraceptives. *Br J Clin Pharmacol.* 1985;28:425-428.

21. Koren G, Chin TF, Correia J, Tesoro A, MacLeod SM. Theophylline pharmacokinetics in adolescent females following coadministration of oral contraceptives. *Clin Invest Med.* 1985;8:222-226.

22. Kroboth PD, Smith RB, Stoehr GP, Julh RP. Pharmacodynamic evaluation of the benzodiazepine-oral contraceptive interaction. *Clin Pharmacol Ther.* 1985;38:525-532.

23. Gustavson LE, Legler UF, Benet LZ. Impairment of prednisolone disposition in women taking oral contraceptives or conjugated estrogens. *J Clin Endocrin Metab.* 1986;62:234-237.

24. Scavone JM, Greenblatt DJ, Blyden GT. Antipyrine pharmacokinetics in women receiving conjugated estrogens. *J Clin Pharmacol.* 1988;28:463-466.

25. Holazo AA, Winkler MB, Patel IH. Effects of age, gender and oral contraceptives on intramuscular midazolam pharmacokinetics. *J Clin Pharmacol.* 1988;28:1040-1045.

26. Scavone JM, Greenblatt DJ, Locniskar A, Shader RI. Alprazolam pharmacokinetics in women on low-dose oral contraceptives. *J Clin Pharmacol.* 1988;28:454-457.

27. Wanwimolruk S, Kaewvichit S, Tanthayaphinant O, Suwannarach C, Oranratnachai A. Lack of effect of oral contraceptive use on the pharmacokinetics of quinine. *Br J Clin Pharmacol.* 1991; 31:179-181.

28. Slayter KL, Ludwig EA, Lew KH, Middleton E Jr, Ferry JJ, Jusko WJ. Oral contraceptive effects of methylprednisolone pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther.* 1996;59:321-321.

29.. Scavone JM, Greenblatt DJ, Abernethy DR, Luna BG, Harmatz JS, Shader RI. Influence of oral contraceptive use and cigarette smoking, alone and together, on antipyrine pharmacokinetics. *J Clin Pharmacol.* 1997;37:437-441.

30. Tamminga WJ, Wemer J, Oosterhuis B, et al. CYP2D6 and CYP2C19 activity in a large population of Dutch healthy volunteers: indications for oral contraceptive-related differences. *Eur J Clin Pharmacol.* 1999;55:177-184.

31. Laine K, Palovaara S, Tapanainen P, Manninen P. Plasma tacrine concentrations are significantly increased by concomitant hormone replacement therapy. *Clin Pharmacol Ther.* 1999;66:602-608.

32. Pollock BG, Wylie M, Stack JA, et al. Inhibition of caffeine metabolism by estrogen replacement therapy in postmenopausal women. *J Clin Pharmacol.* 1999;39:936-940.

33. Seidegard J, Simonsson M, Edsbacker S. Effect of an oral contraceptive on the plasma levels of budesonide and prednisolone and the influence of plasma cortisol. *Clin Pharmacol Ther.* 2000;67:373-381.

34. Laine K, Tybring G, Bertilsson L. No sex-related differences but significant inhibition by oral contraceptives of CYP2C19 activity as measured by the probe drugs mephenytoin and omeprazole in healthy Swedish white subjects. *Clin Pharmacol Ther.* 2000;68:151-159.

35. McCune JS, Lindley C, Decker JL, et al. Lack of gender differences and large intrasubject variability in cytochrome P450 activity measured by phenotyping with dextromethorphan. *J Clin Pharmacol.* 2000;41:723-731.

36. Hagg S, Spigset O, Dahlqvist R. Influence of gender and oral contraceptives on CYP2D6 and CYP2C19 activity in healthy volunteers. *Br J Clin Pharmacol.* 2001;51:169-173.

37. Olubodun JO, Ochs HR, Truten V, et al. Zolpidem pharmacokinetic properties in young females: Influence of smoking and oral contraceptive use. *J Clin Pharmacol.* 2002;42:1142-1146.

38. Chainuvati S, Nafziger A, Leeder JS, et al. Combined phenotypic Assessment of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A, NAT2 and XO with the Cooperstown 5+1 Cocktail. *Clin Pharmacol Ther.* 2003;74:437-447.

39. Stubbins MJ, Harries LW, Smith G, Tarbit MH, Wolf CR. Genetic analysis of the human cytochrome P450 CYP2C9 locus. *Pharmacogenetics.* 1996;6:429-439.

40. Sullivan-Klose TH, Ghanayem BI, Bell DA, et al. The role of the CYP2C9-Leu359 variant in the tolbutamide polymorphism. *Pharmacogenetics.* 1996;6:341-349.

41. Goldstein JA, Blaisdell J. Genetic tests which identify the principal defects in CYP2C19 responsible for the polymorphism in mephenytoin metabolism. *Methods Enzymol.* 1996;272:210-217.

42. Gaedigk A, Gotschall RR, Forbes NS, Simon SD, Kearns GL, Leeder JS. Optimization of cytochrome P4502D6 (CYP2D6) phenotype assignment using a genotyping algorithm based on allele frequency data. *Pharmacogenetics.* 1999;9:669-682.

43. Evans WE, Relling MV, Petros WP, Meyer WH, Mirro J Jr, Crom WR. Dextromethorphan and caffeine as probes for simultaneous determination of debrisoquin-oxidation and *N*-acetylation phenotypes in children. *Clin Pharmacol Ther.* 1989;45:568-573.

44. Park YH, Kullberg MP, Hinsvark ON. Quantitative determination of dextromethorphan and three metabolites in urine by reverse-phase high-performance liquid chromatography. *J Pharm Sci.* 1984;73:24-29.

45. Lam YW, Rodriguez SY. High-performance liquid chromatography determination of dextromethorphan and dextrorphan for oxidation phenotyping by fluorescence and ultraviolet detection. *Ther Drug Monit.* 1993;15:300-304.

46. Lagerstrom PO, Persson BA. Determination of omeprazole and metabolites in plasma and urine by liquid chromatography. *J Chrom.* 1984;309:347-356.

47. Rogers JF, Morrison AL, Nafziger AN, Jones CL, Rocci ML Jr, Bertino JS Jr. Flumazenil reduces midazolam-induced cognitive impairment without altering pharmacokinetics. *Clin Pharmacol Ther.* 2002;72:711-717.

48. Henne KR, Gaedigk, A, Gupta G, Leeder JS, Rettie AE. Chiral phase analysis of warfarin enantiomers in patient plasma in relation to CYP2C9 genotype. *J Chromatogr.* 1998;710:143-148.

49. Williams RL, Chen M, Hauck WW. Equivalence approaches. *Clin Pharmacol Ther.* 2002;72:229-237.

50. Laine K, Yasar U, Widen J, Tybring G. A screening study of eight different female sex steroids to inhibit CYP2C9, 2C19 and 3A4 activities in human liver microsomes. *Pharmacol Toxicol.* 2003;93:77-81.

51. Kashuba ADM, Bertino JS Jr, Rocci ML Jr, Kulawy RW, Beck DJ, Nafziger AN. Quantitation of 3-month intraindividual variability and influence of sex and menstrual cycle phase on CYP3A activity as measured by phenotyping with intravenous midazolam. *Clin Pharmacol Ther.* 1998;64:269-277.

52. Kashuba ADM, Bertino JS Jr, Kearns GL, et al. Quantitation of three-month intraindividual variability and the influence of sex and menstrual cycle phase, on CYP1A2, N-acetyltransferase-2, and xanthine oxidase activities as determined by caffeine phenotyping. *Clin Pharmacol Ther.* 1998;63:540-551.

53. Kashuba ADM, Nafziger AN, Kearns GL, et al. Quantitation of long-term intraindividual variability and the influence of menstrual cycle phase on CYP2D6 activity as measured by dextromethorphan phenotyping. *Pharmacogenetics.* 1998;8:403-410.

54. Kim MJ, Bertino JS Jr, Gaedigk A, Zhang Y, Sellers EM, Nafziger AN. Effect of sex and menstrual cycle phase on cytochrome P450 2C19 activity with omeprazole used as a biomarker. *Clin Pharmacol Ther.* 2002:72:192-199.

55. Streetman DS, Bertino JS Jr, Nafziger AN. Phenotyping of drug-metabolizing enzymes in adults: a review of in-vivo cytochrome P450 phenotyping probes. *Pharmacogenetics.* 2000;10:187-216.

56. Balogh A, Klinger G, Henschel L, Borner A, Vollanth R, Kuhnz W. Influence of ethinylestradiol-containing combination oral contraceptives with gestodene or levonorgestrel on caffeine elimination. *Eur J Clin Pharmacol.* 1995;48:161-166.

57. Wadelius M, Darj E, Frenne G, Rane A. Induction of CYP2D6 in pregnancy. *Clin Pharmacol Ther.* 1997;62:400-407.

58. Tsutsumi K, Kotegawa T, Matsuki S, et al. The effect of pregnancy on cytochrome P4501A2, xanthine oxidase, and N-acetyltransferase activities in humans. *Clin Pharmacol Ther.* 2001;70:121-125.

59. Gorski JC, Huang SM, Pinto A, et al. The effect of echinacea (*Echinacea purpurea* root) on cytochrome P450 activity in vivo. *Clin Pharmacol Ther.* 2004;75:89-100.

60. Shadle CR, Lee Y, Majumdar AK, et al. Evaluation of potential inductive effects of aprepitant on cytochrome P450 3A4 and 2C9 activity. *J Clin Pharmacol.* 2004;44:215-223.