Short Communication

An Ethinyl Estradiol-Levonorgestrel Containing Oral Contraceptive Does Not Alter Cytochrome P450C9 In Vivo Activity

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ABSTRACT

Oral contraceptives have been in wide use for more than 50 years. Levonorgestrel, a commonly employed progestin component of combined oral contraceptives, was implicated in drug–drug interactions mediated via CYP2C9. Although in vitro studies refuted this interaction, there are no confirmatory in vivo studies. In the current study, we examined the phenotypic status of CYP2C9 using low-dose (125 mg) tolbutamide before and after oral contraceptive use in reproductive age women. Blood was collected 24 hours after the tolbutamide oral dose was administered, plasma was isolated, and

tolbutamide concentration (C_{24}) was measured using liquid chromatography–mass spectrometry. The natural logarithm of tolbutamide C_{24} , a metric for CYP2C9 phenotype, was found to be equivalent (within 80%–125% equivalency boundaries) before and after oral contraceptive use. In conclusion, levonorgestrel-containing oral contraceptives, the most commonly used form of oral contraception, do not affect the status of the CYP2C9 enzyme. This suggests that it is safe to coadminister levonorgestrel-containing oral contraceptives and CYP2C9 substrates, which include a wide array of drugs.

Introduction

CYP2C9 is a major drug-metabolizing enzyme whose substrate list spans several classes of therapeutic agents (anti-inflammatory, anti-diabetics, and oral anticoagulants) (Miners and Birkett, 1998). CYP2C9-mediated human drug metabolism exhibits large interindividual variability (Yasar et al., 2002). Although genetic polymorphisms are known to contribute to this variability (Goldstein, 2001), nongenetic factors may also contribute. Sandberg et al. (2004) reported that the use of oral contraceptives (OCs) contributes to interindividual variability of CYP2C9 among women.

OCs are one of the most commonly used methods of contraception globally and have been in use for more than half a century (Trussell, 2007). Typically, OCs include both a synthetic estrogen and progestin. Ethinyl estradiol is the most common estrogenic component but there are a number of different progestin types, with levonorgestrel (LNG) dominating the market (Seaman et al., 2003; Dinger et al., 2011). In a case report published by Ellison et al. (2000), LNG administered as a postcoital emergency oral contraceptive was implicated in drug–drug interaction with warfarin, a known substrate of CYP2C9. However, this report was contradicted by postmarketing data and in vitro studies (Gainer, 2003). No conclusive evidence is available in vivo. Hence, our objective was to evaluate in vivo status of CYP2C9 before and after OC use.

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Materials and Methods

We recruited 34 women aged 18–35 years (body mass index >30 kg/m²) who were seeking to initiate OCs. Additional eligibility criteria included the following: regular menstrual cycles with proven ovulation (luteal phase progesterone ≥ 3 ng/ml), hematocrit $\geq 36\%$, 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 of metabolic disorders (i.e., polycystic ovarian syndrome). The Oregon Health & Science University Institutional Review Board approved the study protocol, and all subjects provided written informed consent.

All study subjects ($N = 34$), at the onset of menses, were placed on a monophasic OC containing 20 μ g ethinyl estradiol/100 μ g LNG (Alesse; Wyeth, Madison, NJ) dosed in a cyclic fashion (21 days of active pills with a 7-day hormone-free interval). Women were instructed to take the pill daily at 9:00 AM. Self-reported compliance was recorded and reviewed. Two or more delayed and/or missed pills prompted study withdrawal.

Subjects were admitted to Oregon Health & Science University's Oregon Clinical & Translational Research Institute to collect blood samples for determining CYP2C9 status prior to initiation and after 21 days of OCs. Venous blood samples were collected at 24 hours after administration of 125 mg tolbutamide orally. Plasma was isolated and stored at -80°C for long-term storage prior to analysis using liquid chromatography–mass spectrometry (Jetter et al., 2004). Genomic DNA was extracted from dried blood spots (Battaille et al., 2001) and genotyped for CYP2C9 alleles (*1, *2, and *3) using melt curve analysis (Hill et al., 2006).

Demographic data were analyzed using descriptive statistics. The natural logarithm of tolbutamide concentration at 24 hours (C_{24}) was used as a metric of CYP2C9 status, based on the prior work by Jetter et al. (2004). Equivalency of CYP2C9 status before and after treatment was tested based on the guidelines of US Food and Drug Administration (2003) and the European Medicines Agency (2010) using SigmaPlot software (version 11.0; Systat Software, Inc., San Jose, CA).

Results and Discussion

All 34 recruited women completed the study; 30 of 34 participants were non-Hispanic, Caucasian. The mean age of study participants was

ABBREVIATIONS: LNG, levonorgestrel; OC, oral contraceptive; P450, cytochrome P450.

Fig. 1. OC use and CYP2C9 clearance. (A) Each box represents 25th to 75th percentile data with median (solid line) and mean (broken line), and whiskers depicting 5th to 95th percentile data ($N = 34$). (B) Each of the 34 subjects was depicted with their respective predose and postdose CYP2C9 clearance surrogate marker.

29.1 ± 4.6 years, with a mean body mass index of $39.7 \pm 6.8 \text{ kg/m}^2$. Five of the study participants were $*1/*2$ allele expressors, one participant was a $*1/*3$ expressor, and the rest were homozygous wild-type expressors.

CYP2C9 status was unchanged after 3 weeks of OC use (Fig. 1). The median predose tolbutamide C_{24} was 1538 ng/ml (range, 469, 6331), and the median postdose C_{24} was 1446 ng/ml (range, 485, 6294). The geometric mean of the differences in C_{24} before and after OC use was 0.9978 (90% confidence interval, 0.9781–1.0174). The confidence interval is well within the acceptable equivalency boundaries of 80%–125% recommended by regulatory agencies (US Food and Drug Administration, 2003; European Medicines Agency, 2010). This study is consistent with the lack of change in CYP2C9 status reported by Gainer (2003) using in vitro systems. However, Sandberg et al. (2004) observed significantly lower CYP2C9 activity in women taking OCs compared with women naïve to OC treatment. Interestingly, the type of progestin was not recorded in their study; hence, it can be speculated that the different progestins have different effects on CYP2C9 activity. The above speculation is supported by the findings of a study in which triphasic OCs containing norgestimate as a source of progestin were shown to enhance the activity of CYP2C9 (Shelepova et al., 2005).

Ethinyl estradiol, the estrogenic component of the OC regimen, was shown to inhibit various P450 isozymes, including CYP2C9 (Laine et al., 2003; Chang et al., 2009). Both of these in vitro studies demonstrate that CYP2C9 inhibition occurs at a supra-micromolar concentration of ethinyl estradiol. The maximum plasma concentrations of ethinyl estradiol achieved by women in the current study were approximately 100 pg/ml (approximately 0.33 pM) (Edelman et al.,

2013). Given the 10^6 – 10^9 differential in the plasma concentrations and IC_{50} , it is highly unlikely that ethinyl estradiol contributes to CYP2C9 variability among women who use OCs.

We previously demonstrated that OC use suppresses CYP3A4 activity in women (Edelman et al., 2012). In the current study, we observed a lack of effect of OCs on CYP2C9 activity, suggesting that OCs have isozyme-specific effects. Studies, including ours, further suggest that the type of progestin influences the overall effect of OCs on P450 enzyme status in women. In conclusion, LNG-containing OCs, the most commonly used form of OC, does not affect the status of the CYP2C9 enzyme. This suggests that it is safe to coadminister LNG-containing OCs and CYP2C9 substrates, which include a wide array of drugs.

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