An Ethinyl Estradiol-Levonorgestrel containing oral contraceptive does not alter cytochrome P4502C9 in vivo activity

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List of Nonstandard Abbreviations: CYP, Cytochrome P450; OC, Oral Contraceptives; LNG, Levonorgestrel.
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 cytochrome P4502C9 (CYP2C9). While in vitro studies refuted this interaction, there are no confirmatory in vivo studies. In the current study, we have examined the phenotypic status of CYP2C9 using low dose (125mg) Tolbutamide, pre- and post- oral contraceptive use in reproductive age women. Blood was collected 24 hr post Tolbutamide oral dose, plasma isolated, and Tolbutamide concentration (C24) was measured using liquid chromatography- mass spectrometry. The natural logarithm of Tolbutamide C24, a metric for CYP2C9 phenotype, was found to be equivalent (within 80-125% equivalency boundaries) between pre- and post- oral contraceptive use. In conclusion, levonorgestrel containing oral contraceptives, the most commonly used form of oral contraception, does not affect the status of CYP2C9 enzyme. This suggests that it is safe to co-administer levonorgestrel containing oral contraceptives and CYP2C9 substrates which includes a wide array of drugs.
**Introduction:** Cytochrome P450 (CYP) 2C9 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 inter individual variability (Yasar et al., 2002). While genetic polymorphisms are known to contribute to this variability (Goldstein, 2001), non-genetic factors may also contribute. Sandberg et al., reported that the use of oral contraceptives (OCs) contributes to inter individual variability of CYP2C9 among women (Sandberg et al., 2004).

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., LNG administered as a post-coital emergency oral contraceptive was implicated in drug-drug interaction with warfarin, a known substrate of CYP2C9 (Ellison et al., 2000); however, this report was contradicted by post-marketing 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 pre- and post-OC use.
**Materials and Methods:** Thirty-four, 18–35 years old women (body mass index >30 kg/m²) women, seeking to initiate OCs were recruited. Additional eligibility criteria included: regular menstrual cycles with proven ovulation (luteal phase progesterone ≥3 ng/mL), hematocrit ≥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). The Oregon Health & Science University (OHSU) Institutional Review Board approved the study protocol, and all subjects underwent written informed consent.

All study subjects (n=34), at the onset of menses, were placed on a monophasic OC containing 20 mcg ethinyl estradiol /100 mcg LNG (Alesse; Wyeth, Madison, NJ, USA) dosed in a cyclic fashion (21 days active pill 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 the OHSU’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 following 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 (Battaile et al., 2001) and genotyped for CYP2C9 alleles (*1, *2, and *3) using melt curve analysis (Hill et al., 2006).

Demographic data was analyzed using descriptive statistics. Natural logarithm of Tolbutamide concentration at 24 hours (C₂₄) was used as a metric of CYP2C9 status, based on the prior work by Jetter et al (Jetter et al., 2004). Equivalency of CYP2C9 status pre and post treatment was tested based on the guidelines of Food & Drug Administration (FDA) and
European Medicines Agency (EMA) (FDA-, 2003; EMA-, 2010) using SigmaPlot software (v 11.0; Systat Software, Inc., San Jose, CA).
Results & Discussion: All 34 recruited women completed the study; 30/34 were non-hispanic, Caucasian. The mean age of study participants was 29.1 ± 4.6 (mean ± S.D.) years with a body mass index of 39.7 ± 6.8 kg/m². Five of the study participants were *1/*2 allele expressors, 1 was *1/*3 expressor, and the rest were homozygous wild type expressors.

CYP2C9 status was unchanged after 3 weeks of OC use (Figure 1). The pre-dose Tolbutamide $C_{24}$ was 1538 (469, 6331) ng/ml (median; range). The post-dose $C_{24}$ was 1446 (485, 6294) ng/ml. The geometric mean of the differences in pre and post $C_{24}$ was 0.9978, and the 90% confidence interval of the mean was 0.9781 – 1.0174. The confidence interval is well within the acceptable the equivalency boundaries of 80-125% recommended by regulatory agencies (FDA-, 2003; EMA-, 2010). This study is consistent with the lack of change in CYP2C9 status reported by Gainer E using in vitro systems (Gainer, 2003). However Sandberg et al observed a significantly lower CYP2C9 activity in females taking OCs compared to naïve women. Interestingly, the type of progestin was not recorded in their study, and 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 where triphasic OCs containing norgestimate as source of progestin was 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 CYP 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-micro molar concentration of ethinyl estradiol. The maximum plasma concentrations of ethinyl estradiol achieved by women in the current study were ~100pg/ml (~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 OC user women.

Earlier we have demonstrated that OC use suppresses CYP3A4 activity in women (Edelman et al., 2012). In the current study, we observed lack of effect of OCs on CYP2C9
activity suggesting that OCs have isozyme specific effects. Furthermore, studies including the current one suggest that the type of progestin influences the overall effect of OCs on CYP enzyme status in women. In conclusion, LNG containing OCs, the most commonly used form of OC, does not affect the status of CYP2C9 enzyme. This suggests that it is safe to co-administer LNG containing OC and CYP2C9 substrates which includes a wide array of drugs.
Authorship Contributions:

Participated in research design: Cheral, Edelman.

Conducted experiments: Cheral, Pearson, Edelman.

Contributed new reagents or analytic tools: Maslen

Performed data analysis: Pearson, Cheral.

Wrote or contributed to the writing of the manuscript: Cheral, Pearson, Edelman.
References:


Footnotes:

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**Figure Legend:**

**Figure 1: Oral contraceptive use and CYP2C9 clearance.** (A) Each box represents 25 to 75 percentile data with median (solid line) and mean (broken line), and whiskers depicting 5-95% percentile data; n=34. (B) Each of the 34 subjects was depicted with their respective pre and post dose CYP2C9 clearance surrogate marker.
Figure 1

(A)

Ln (Tolbutamide C24)

6.0   6.5   7.0   7.5   8.0   8.5   9.0

Predose Postdose

(B)

Ln Tolbutamide (C24)

6.0   6.5   7.0   7.5   8.0   8.5   9.0

Predose Postdose