GYNECOLOGY

Estimating systemic exposure to ethinyl estradiol from an oral contraceptive

Carolyn L. Westhoff, MD; Malcolm C. Pike, PhD; Rosalind Tang, MS; Marianne N. DiNapoli, MD; Monica Sull, MPH; Serge Cremers, PharmD, PhD

OBJECTIVE: This study was conducted to compare single-dose pharmacokinetics of ethinyl estradiol in an oral contraceptive with steady-state values and to assess whether any simpler measures could provide an adequate proxy of the "gold standard" 24-hour steady-state area under the curve (AUC) value. Identification of a simple, less expensive measure of systemic ethinyl estradiol exposure would be useful for larger studies that are designed to assess the relationship between an individual's ethinyl estradiol exposure and side-effects.

STUDY DESIGN: We collected 13 samples over 24 hours for pharmacokinetic analysis on days 1 and 21 of the first cycle of a monophasic oral contraceptive that contained 30 μg ethinyl estradiol and 150 μg levonorgestrel in 17 nonobese healthy white women. We also conducted an abbreviated single-dose 9-sample pharmacokinetic analysis after a month washout. Ethinyl estradiol was measured by liquid chromatography-tandem mass spectrometry. We compared results of a full 13-sample steady-state pharmacokinetic analysis with results that had been calculated with the use of fewer samples (9 or 5) and after the single doses. We calculated Pearson correlation coefficients to evaluate the relationships between these estimates of systemic ethinyl estradiol exposure.

RESULTS: The AUC, maximum, and 24-hour values were similar after the 2 single oral contraceptive doses (AUC; $r = 0.92$). The steady-state 13-sample 24-hour AUC value was correlated highly with the average 9-sample AUC value after the 2 single doses ($r = 0.81$; $P = .0002$). This correlation remained the same if the number of single-dose samples was reduced to 4, taken at time 1, 2.5, 4, and 24 hours. The 24-hour value at steady-state was correlated highly with the 24-hour steady-state AUC value ($r = 0.92$; $P < .0001$). The average of the 24-hour values after the 2 single doses was also correlated quite highly with the steady-state AUC value ($r = 0.72$; $P = .0026$).

CONCLUSION: Limited blood sampling, including results from 2 single doses, gave highly correlated estimates of an oral contraceptive user's steady-state ethinyl estradiol exposure.

Key words: ethinyl estradiol, oral contraceptive, pharmacokinetics, single-dose, steady-state

Cite this article as: Westhoff CL, Pike MC, Tang R, et al. Estimating systemic exposure to ethinyl estradiol from an oral contraceptive. Am J Obstet Gynecol 2015;212:614.e1-7.

Oral contraceptives (OCs) are prescribed with a general approach of prescribing the lowest effective dose. Most currently used OCs contain 20-35 μg of ethinyl estradiol (EE2) along with 1 of several progestins. Venous thromboembolism is the main reason to avoid higher EE2 doses[1-3]; however, the lowest EE2 doses are associated with more breakthrough bleeding.[4] These associations have been defined through studies that have evaluated the administered dose. The dose in each daily tablet maybe a poor indicator of a particular woman's systemic exposure. Numerous pharmacokinetic studies demonstrate that the steady-state levels of EE2 vary widely among women who use the same OC.[5-7] These between-woman differences are larger than the dose differences between current and older OC formulations.

Among women who use an OC, individual-level systemic exposure to EE2 could be related to the frequency of

From the Department of Obstetrics and Gynecology and Epidemiology (Dr Westhoff), the Department of Obstetrics and Gynecology (Dr DiNapoli and Ms Tang and Ms Sull), and Pathology and Cell Biology (Dr Cremers), Columbia University Medical Center, and Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center (Dr Pike), New York, NY.

Received July 25, 2014; revised Oct. 29, 2014; accepted Dec. 8, 2014.

This pilot study was funded by the Howard Solomon Research Fund of the Department of Obstetrics and Gynecology, Columbia University Medical Center (CUMC), and the Irving Institute for Clinical and Translational Research Collaborative and Multidisciplinary Pilot Research Award from CUMC. The CUMC Biomarkers Core Laboratory supported the ethinyl estradiol assays. This publication was supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through grant number UL1 TR000040, formerly the National Center for Research Resources, grant number UL1 RR024156.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

C.L.W. is a paid consultant to Merck and Bayer, which manufactures oral contraceptives, not the oral contraceptive evaluated in this study. The remaining authors report no conflict of interest.

Corresponding author: Carolyn L. Westhoff, MD. clw3@columbia.edu

0002-9378/$36.00 • • http://dx.doi.org/10.1016/j.ajog.2014.12.007

side-effects, and such measures can indicate important drug interactions.[8,9] To evaluate the possible relationships between individual systemic exposure to EE2 and OC side-effects generally would require studies much larger than the typical 15-20 participants in a detailed pharmacokinetic study and would be prohibitively expensive. Small intensive pharmacokinetic studies are necessary during drug development; however, they are not useful tools for pharmacoepidemiologic studies of drug effects. Studies of side-effects and individual systemic exposure have not been done with an OC.

The objective of our study was to estimate systemic exposure to EE2 among a group of healthy white women using standard 13-sample pharmacokinetic techniques and then to assess whether any simpler measures could provide an adequate proxy of the "gold standard" 24-hour steady-state area under the curve (AUC). Identifying a simple, less expensive measure of EE2 exposure would enable the development of large studies to assess the relationship between an individual's EE2 exposure and the side-effects.

Materials and Methods

This single-arm, open-label clinical trial took place at Columbia University Medical Center after Institutional Review Board approval. Participants were 18-35 years old and self-identified as white/caucasian and provided written informed consent before enrollment. We excluded women with medical contraindications to use of combined hormonal contraception.[10] Additional exclusion criteria included hysterectomy or oophorectomy, cycles >35 days or irregular, childbirth within 6 months, breastfeeding, current smoker, body mass index $\geq$30.0 kg/m^2, and use of OCs within 1 month or injectable contraception within 6 months.

After telephone screening, women attended a pretreatment visit for informed consent and full assessment of eligibility. At baseline, we assessed blood pressure, height and weight, urine pregnancy test results, and hemoglobin level to screen for anemia (hemoglobin <10 mg/dL) before study blood draws. We asked participants to abstain from use of acetaminophen, ibuprofen, and aspirin and to avoid grapefruit juice throughout the study, alcohol within 24 hours, and caffeine within 1 hour of study visits.

The study OC contained 30 μg EE2 and 150 μg levonorgestrel packaged with 21 active and 7 placebo tablets (Portia; Teva, Philadelphia, PA). Treatment began within 7 days of the start of menses. After 21 active pills had been used, participants had a 5-week, OC-free wash-out period. On next menses, each participant returned to take a single OC tablet. A study coordinator directly observed OC intake on study visit days and instructed participants to take each OC at the same time with the use of a daily alarm.

Participants made 10 study visits over 9 weeks. The 5 study visits of interest to the results presented here occurred on OC cycle day 1 (referred to here as single-dose 1 [SD1]), cycle days 2 and 3, day 21 (steady-state), and the visit for a single OC tablet at approximately day 60 after study entry (single-dose 2 [SD2]). Participants underwent multiple timed venous blood collections on days 1, 21, and 60 for pharmacokinetic testing, during which they were admitted to the Irving Institute of Clinical and Translational Research at Columbia University Medical Center. With the use of an indwelling catheter in an antecubital vein, 13 samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, and 24 hours (t_0, $t_{0.5}$, … t_{24}) after OC administration on days 1 and 21. On day 60, the first 8 specimens up to 4 hours and the specimen at 24 hours were collected (9 samples); samples were also collected at 48 and 72 hours. OC administration occurred immediately after each t_0 blood draw. Samples were allowed to clot for 30 minutes at room temperature, were centrifuged at 3000 rpm at 4°C for 10 minutes, and were stored in 1 mL aliquots at −80°C. Levels of corticosteroid-binding globulin (CBG) were measured in serum specimens collected at t_0 on days 1, 21, and 60 to monitor treatment compliance.[11]

Laboratory methods

EE2 concentrations were measured with liquid chromatography-tandem mass spectrometry. In short, EE2 was measured in serum after liquid/liquid extraction with tetra-deuterated-EE2 as the internal standard. EE2 was derivatized with dansyl chloride before analysis. The steroids were quantified by positive electrospray ionization in multiple reaction-monitoring mode with the use of the Waters Xevo TQ-S system (Waters, Milford, MA). The method was linear between 2.5 and 100 pg/mL (limit of quantification: 2.5 pg/mL). Intra- and interassay precision were <3.9% and <4.4%, respectively. The EE2 levels that were obtained with this assay are lower than, but highly correlated with ($r = 0.95$), those obtained by the radioimmunoassay that we have used previously among women who used the same OC.[7,12] CBG was measured with the use of a radioimmunoassay kit (IBL-America, Minneapolis, MN).

Pharmacokinetic analysis

The pharmacokinetic analyses were conducted with the use of the noncompartmental analysis procedure, pkexamine, with the trapezoidal rule in the statistical package STATA12 (Stata Corporation, Austin, TX). These analyses were confirmed to give results identical to those obtained with the noncompartmental analysis procedure in WinNonLin (Certara, St. Louis, MO). The AUCs at SD1 and steady-state with all samples are noted as $AUC_{SD1,\ 0\text{-}24}$ and $AUC_{SS,\ 0\text{-}24}$. The AUCs at SD1 and SD2 (ignoring any samples taken at time points >4 hours and <24 hours) are noted as $AUC_{SD,\ 0\text{-}4\text{-}24}$. For SD1, the AUC from 0 to infinity ($AUC_{SD1,\ 0\text{-}inf}$) was calculated in the standard manner by the estimation of the terminal elimination rate (k_{elim}) of EE2 with the EE2 values at 12, 16, and 24 hours with a linear fit to the log EE2 values and then with the fitted equation to "correct" the 12, 16 and 24 hour values and extend the linear fit to infinity.

Statistical methods

We examined results for outliers using a standard modified Z-score approach