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Clotting factor changes during the first cycle of oral contraceptive use☆

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Abstract

Objectives: The risk of venous thromboembolism (VTE) is highest during the initial months of oral contraceptive (OC) use. We sought to evaluate the extent of hemostatic variable changes during the initial OC cycle and if such changes are related to systemic ethinyl estradiol (EE2) exposure.

Study design: Participants provided multiple blood samples during a 21-day OC cycle (30 mcg EE2; 150 mcg levonorgestrel) and after a single dose following a washout period. Analytes included D-dimer, factor VIII activity, protein C total antigen and the hepatic proteins corticosteroid-binding globulin (CBG) and sex-hormone-binding globulin (SHBG). EE2 pharmacokinetic analyses related to the 24 h after the first OC tablet (OC1) and at steady state (OC21).

Results: Seventeen women completed the study. D-dimer more than doubled by OC6 (p=.013) and remained elevated at OC21 (p=.012). D-dimer levels within women varied widely from day to day. Factor VIII increased 27% by OC2 (p<.001) but declined to a 9% increase by OC21. Protein C increased only 6%. EE2 steady-state area-under-the-curve ranged from 488 to 1103 pg·h/mL; higher levels were not correlated with greater increases in clotting variables. CBG and SHBG increased significantly but were not significantly correlated with levels of EE2 or with the hemostatic variables.

Conclusions: D-dimer increases during the first OC cycle were at least as great as increases seen with longer OC use. These results provide support for the increased VTE risk during initial OC use. The extreme variability in D-dimer levels may be an important component of this risk.

Implications: This study showed that increases in D-dimer are clearly evident in the first cycle of OC use and may be larger than are seen after a longer duration of use and thus provide biological support for the increased VTE risk during initial OC use found in epidemiological studies.

Keywords: Oral contraceptives; Venous thromboembolism; D-dimer; Factor VIII; Protein C

1. Introduction

The risk of venous thromboembolism (VTE) within the first 3 months of oral contraceptive (OC) use may be more than double the risk after the first year, with the risk gradually decreasing between the first 3 months and 1 year [1–3], although this has not been invariably found [4]. Despite this, hemostatic variable changes before 3 months of use have not been reported. We therefore designed the study reported here to measure hemostatic changes during the first OC treatment cycle.

Numerous studies have assessed the effects of OC use on the coagulation system [5–7]. The large 'Seven OC Study', measured 24 hemostatic variables after 3 and 6 OC cycles in 707 women [6]. D-dimer concentration, a global marker of fibrinolysis associated with future VTE risk [8,9], increased approximately 50% after 3 and 6 cycles of all OC regimens [6]. Factor VIII activity, another independent risk factor for

☆ Conflicts of Interest: Dr. Westhoff receives honoraria from Merck and Bayer as a data safety and monitoring board member, both of which produce oral contraceptives, however, not the oral contraceptive studied here. None of the other authors have any conflicts to report.

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VTE [10–15], increased approximately 20% after 3 and 6 cycles [6]. Neither ethinyl estradiol (EE2) dose nor progestin type had a clear effect on these increases. The significance of the observed changes in D-dimer and factor VIII to the increased VTE risk among healthy OC users has not been studied. We chose to measure D-dimer concentration and factor VIII activity levels due to their association with risk of future VTE and their change with OC use.

OCs may also disequilibrate the coagulation system through increased synthesis of hepatic proteins. Protein C, a hepatic clotting factor, increased ~15% after 3 and 6 OC cycles [6]. We chose to measure protein C total antigen as our representative hepatic clotting factor, even though it is an anticoagulant, as its short half-life (6–7 h) [16,17] may facilitate detection of short-term changes. We also studied how changes in these measures correlated with corticosteroid-binding globulin (CBG) and sex-hormone-binding globulin (SHBG) [18,19].

Epidemiological studies show that higher OC doses of EE2 are associated with a greater increase in VTE risk [20–22]. We, therefore, also explored whether a woman's systemic EE2 level during the first OC cycle was related to the magnitude of her clotting system changes.

2. Materials and methods

This single-arm, open-label pilot study took place at Columbia University Medical Center (CUMC) after institutional review board approval. Participants provided written informed consent prior to enrolment. Women were eligible if aged 18–35 years and self-identified as white. We excluded women with any medical contraindication to use of OCs [23]. Additional exclusion criteria included use of medications known to affect the CYP450 system, use of injectable contraception in the past 6 months or use of other hormonal contraceptives within the past month, pregnancy within the past 6 weeks, smoking and a body mass index ≥ 30.0 kg/m^2. We instructed participants to abstain from ibuprofen, aspirin and grapefruit juice throughout the study; alcohol within 24 h; and caffeine within 1 h of study visits as suggested by the European Concerted Action on Thrombosis Manual [24].

The study OC contained 30 mcg EE2 and 150 mcg levonorgestrel packaged with 21 active and 7 placebo tablets (Portia®, Teva Pharmaceuticals, Philadelphia, PA). Treatment began within 7 days of the start of menses [25]. Participants selected a particular time to take her daily OC, and we directly observed OC intake at this particular time on study visit days. Participants underwent multiple blood draws to measure hemostatic variables over 4 weeks immediately before each OC was taken on days 1 ($OC1_0$), 2 ($OC1_{24}$), 3 ($OC2_{24}$), 4 ($OC3_{24}$), 7 ($OC6_{24}$) and 21 ($OC21_0$) and at the same time on days 22 ($OC21_{24}$) and 28. After completing the OC pack, each participant returned for a single OC pill at her next spontaneous menses and we collected blood samples over the following 4 days (noted as days 60–63). Participants sat quietly for 30 min prior to each blood draw using a 21-gauge butterfly needle in the antecubital vein. Each participant was admitted for 24 h on days 1 and 21 to collect 14 timed samples for pharmacokinetic (PK) analyses, as previously described [25]. At each visit, participants answered questions about use of concomitant medications, caffeine/alcohol intake and adverse events since the last visit. All study visits were conducted in winter 2012–2013, to minimize seasonal variation in hemostatic variables [26].

Samples for clotting factor analyses were collected in a citrated vacutainer and centrifuged at 3000 rpm at 4°C for 10 min; plasma was transferred and frozen in 1 mL aliquots at −80°C until analysis in batches. The ARUP National Reference Laboratories (Salt Lake City, UT) measured D-dimer concentration, factor VIII activity and protein C total antigen. D-dimer was measured by immunoturbidimetric assay using the STA Compact analyzer (Diagnostica Stago Inc., Parsippany, NJ), factor VIII activity was measured by a clotting assay using the STA-R analyzer (Diagnostica Stago Inc., Parsippany, NJ) and protein C total antigen was measured by an enzyme-linked immunosorbent assay (ELISA) using EIA Reader 520 (ARUP, Salt Lake City, UT). The within-run precision for each assay was 1.9% for D-dimer at levels around 2 mcg/mL and was 5.8% and 3% for factor VIII and protein C, respectively. The between-run precision for each assay was 0.9% for D-dimer at levels around 2 mcg/mL and was 4.6% and 5.0% for factor VIII and protein C, respectively. The lower limits of detection were 0.2 mcg/mL, 1% and 10%, respectively. We set D-dimer results that were below the detection limit as 0.2 mcg/mL for analysis; this produces a conservative bias in the measurement of increases in low-level D-dimer concentration with OC use.

The CUMC Biomarkers Core Laboratory performed CBG radioimmunoassays (IBL-America, Minneapolis, MN) and SHBG chemiluminescence immunoassays on an automated immunochemistry analyzer (Immulite 1000; Siemens Healthcare Diagnostics Inc., Deerfield, IL) from serum collected at baseline and on days 21 and 28 and after the washout period. We measured EE2 serum concentrations using liquid chromatography–tandem mass spectrometry and conducted standard PK analyses [25].

The D-dimer, factor VIII and protein C measures were log-transformed for analysis, but all results are presented in the original units for ease of interpretation. We normalized factor VIII activity and protein C total antigen measurements to mean baseline values of 100% [6]. To reduce random variation at steady state, we averaged the values of the hemostatic variables immediately before and 24 h after OC21, except in Figs. 1 and 2 where we show these values separately. We summarized the levels of hemostatic variables and binding globulins using descriptive statistics. We conducted matched-pairs t tests to evaluate changes over time in D-dimer, factor VIII and protein C. We used linear regression to assess the relationship between (untransformed) steady-state 24-h EE2 area under the curve (EE2-AUC21) and the change in hemostatic variables from baseline to OC21