Thrombosis Research 136 (2015) 1022–1026

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Thromboelastography Identifies Cyclic Haemostatic Variations in Healthy Women Using Oral Contraceptives

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ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form 11 August 2015
Accepted 16 August 2015
Available online 22 August 2015

Keywords:
Thromboelastography
Haemostasis
Oral contraceptives
Menstrual cycle

ABSTRACT

Using thromboelastography (TEG) and standard laboratory haemostatic tests we examined the influence of the menstrual cycle and monophasic oral contraceptive (OC) use on haemostasis in healthy women. Tests were performed on citrated whole-blood and plasma (respectively) collected from 33 healthy non-pregnant women (18 non-OC users and 15 OC users) during menses, the follicular phase and the luteal phase of non-OC users, and the placebo, early-medicated phase, and late-medicated phase of OC users. Results for various coagulation parameters determined by TEG and standard laboratory haemostatic tests were compared within and between groups. TEG detected significantly increased coagulability in OC users during the late-medicated phase when compared to the placebo and early-medicated phases, whereas standard laboratory haemostatic tests failed to reveal significant differences in haemostasis within the OC steroid medication cycle. Neither TEG nor standard laboratory haemostatic tests detected significant differences in haemostasis within the menstrual cycle in non-OC users. When compared to non-OC users, TEG revealed that OC users had significantly increased coagulability only during the late-medicated phase; whereas standard laboratory haemostatic tests detected significant differences between all individual phases of the steroid medication cycle of OC users and the combined phases of the menstrual cycle in non-OC users. In conclusion, TEG provides additional insight into haemostatic function not identifiable using standard laboratory haemostatic tests.

1. Introduction

Women with increased sex hormone levels due to pregnancy or exogenous hormone administration experience an increased risk of venous thromboembolic events, suggesting a role of female sex hormones in coagulation and fibrinolysis [1]. The menstrual cycle is characterised by cyclic fluctuations of female sex hormones throughout menses, the follicular phase and the luteal phase. The influence of cyclic variation on coagulation and fibrinolysis has been widely studied, however results have been inconsistent and varied [2]. Reasons for this inconsistency may be due to the poor sensitivity of standard laboratory haemostatic tests, including prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen concentration. These tests use platelet-poor plasma to assess specific components of the coagulation cascade, ignoring the interaction between clotting factors, platelets and fibrinogen. For this reason, standard laboratory haemostatic tests are unable to measure global haemostasis.

Thromboelastography (TEG) is an *in vitro* tool that addresses this limitation by determining the viscoelastic properties of whole blood during coagulation and fibrinolysis. It is a point-of-care assay that measures clotting time and kinetics of clotting, as well as clot strength and stability. Compared with standard laboratory haemostatic tests, there is growing evidence that TEG reflects more accurately the overall haemostatic status and correlates better with clinical outcomes [3,4]. In the present study we used TEG and standard laboratory haemostatic tests to determine whether menstrual cycle phase and oral contraceptive (OC) use influence haemostasis in a healthy, non-pregnant population.

2. Materials and Methods

2.1. Subjects

This study was approved by the Queen's University Faculty of Health Sciences Research Ethics Board. Following informed written consent, 33

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http://dx.doi.org/10.1016/j.thromres.2015.08.011
0049-3848/

healthy, non-pregnant women were recruited from the general population of Kingston, Ontario. Subjects were divided in two groups based on their contraceptive use: 1) 15 women using monophasic combined OCs and 2) 18 women not using hormonal contraceptives. Exclusion criteria included having irregular menstrual cycles, intrauterine device use, a history of thromboembolic disorders, hypertension, diabetes, cardiovascular disease, other chronic medical conditions, smoking and a body mass index (BMI) greater than 30 kg/m^2. To remove potential variability associated with physiological adjustment of OC use/discontinuation, non-OC users who had stopped using hormonal contraceptives within four months and OC users who had been using their OC for less than four months were excluded from the study.

At recruitment, non-OC women self-reported whether they had a 26-, 28- or 30-day cycle. Menses was defined as day 2–4 of the cycle and the follicular and luteal phases were defined depending on cycle length as follows: follicular phase = days 8–12, 10–14, 12–16 and luteal phase = days 18–22, 20–24, 22–28 for 26, 28 and 30 day cycles, respectively. Only OC users using monophasic combination pills were included to control for daily dosage variations of progesterone and ethinyl estradiol found in multiphasic combination pills. The majority of OCs used by participants in this study had a seven-day placebo phase followed by a 21-day steroid medication phase. However, two participants used an OC with a four-day placebo phase and a 24-day steroid medication phase. OC users were assessed once during menses (days 2–4 after spotting), corresponding with the placebo phase of their OC regimen. OC users were further assessed twice over the course of their medicated monthly cycles to correspond with the time points of assessment used for non-OC users; the early-medicated phase (days 10–14) and the late-medicated phase (days 20–24), with day one being the day the first placebo pill was taken.

2.2. Blood collection

Subjects were asked to avoid consuming caffeine, acetylsalicylic acid (ASA) and large meals prior to blood collection scheduled in the morning. Venous blood was drawn from an antecubital vein into 4.5-ml Vacutainer tubes (Becton Dickinson, USA) containing 3.2% sodium citrate (0.105 M). Within two hours of collection, blood samples were analyzed by TEG by one of two trained operators, and then underwent centrifugation at 1,600 *g* for 20 minutes to prepare platelet-poor plasma. Platelet-poor plasma was frozen at −80 °C until further analysis.

2.3. Thromboelastography

Thromboelastography was performed in strict accordance to the manufacturer's guidelines using a TEG 5000 Thromboelastograph Haemostasis System (Haemoscope Corporation, USA) and TEG Analytical Software (TAS) Version 4.2.3 (Haemoscope Corporation, USA). Electronic quality tests were performed each day prior to analysis. For sample analysis, 340 μl of citrated native blood was re-calcified with 20 μl of 0.2 M $CaCl_2$ in a disposable plastic cup at 37 °C. A pin is suspended in an oscillating cup by a torsion wire that allows the motion of the pin in the oscillating cup to be electrically transduced to a computer. Initially a straight line is produced, however as the clot develops, its elasticity and strength changes the rotation of the pin in the cup creating a characteristic tracing (Fig. 1).

Six TEG parameters were analyzed. Reaction time (R time, minutes) is the time from the start of the test to initial clot formation, and is the point at which all other plasma clotting assays, such as PT and APTT, are stopped. Kinetics time (K time, minutes) represents the time until a fixed level of clot firmness is reached (20 mm). Alpha angle (α angle, degrees) is the angle between the horizontal line of the tracing and the line tangent to the curve. Both K time and α angle provide information about the kinetics of clot formation (fibrin build up). Maximum amplitude (MA, mm) is a measure of the maximum strength of the clot

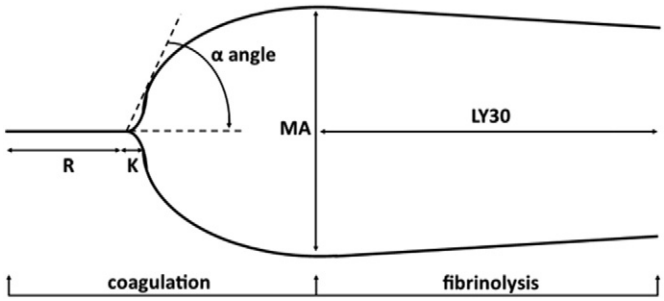

Fig. 1. Schematic standard TEG tracing. R = reaction time, time to initial clot formation (min); K = kinetics time, time to defined clot formation (sec); α angle = description of rate of clot formation; MA = maximum amplitude, absolute clot strength (mm); LY30 = fibrinolysis (%) at 30 min after MA.

and also provides information about platelet function. Coagulation index (CI) is a description of overall coagulation incorporating R time, K time, α angle and MA. Reduced R and K time, and greater values for α angle, MA and CI indicate greater whole blood coagulability. LY30 (percent) is a measure of the percentage of clot fibrinolysis 30 minutes after MA is achieved and provides information about clot lysis and fibrinolytic activity.

2.4. Standard laboratory haemostatic tests

Frozen samples were thawed to room temperature. PT, a measure of the extrinsic pathway of coagulation; APTT, a measure of the intrinsic and common coagulation pathways; and plasma fibrinogen concentrations were measured using a Sysmex CA-500 coagulation analyzer and the listed reagents or kits in parentheses: PT (Dade Innovin, Siemens Healthcare Diagnostics Products Gmbh, Germany); APTT (Dade Actin Activated Cephaloplastin Reagent, Siemens Healthcare Diagnostics Products Gmbh, Germany); and fibrinogen concentration (Multifibren* U, Siemens Healthcare Diagnostics Products Gmbh, Germany). All standard laboratory haemostatic tests were performed by one of two trained operators.

2.5. Statistical analysis

Statistical analysis was performed using Prism GraphPad version 6. To assess differences in demographic characteristics between OC and non-OC users, an unpaired two-tailed Student's *t*-test was performed for each characteristic. A repeated measures one-way ANOVA and *post hoc* multiple comparisons test was used to analyze the influence of phase on each TEG parameter and standard laboratory haemostatic test results in non-OC users and OC users. To analyze the influence of OC use on TEG parameters and standard laboratory haemostatic tests, the mean value for the three phases of each parameter was determined for each non-OC user and a two-tailed Student's *t*-test was performed on these values against each phase for OC users. When data were not normally distributed, non-parametric tests (Mann–Whitney U) were used. A P value < 0.05 was considered statistically significant.

3. Results

There were no significant differences in demographic characteristics between OC and non-OC users (Table 1), and in TEG parameters or standard laboratory haemostatic test results between menstrual cycle phases of non-OC users (Table 2). However, during the late-medicated phase, OC users had a significantly shorter R time and K time, when compared to the placebo ($P < 0.001$ and <0.01, respectively) and the early-medicated phases ($P < 0.01$ for both; Fig. 2, a and b). Furthermore, α angle and CI were significantly greater during the late-medicated phase when compared to the placebo ($P < 0.05$ and <0.01, respectively) and early-medicated phases ($P < 0.05$ and <0.01, respectively; Fig. 2, c