Plasma Levels of Activated Factor VII Decrease during the Menstrual Cycle

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Summary

Men have an increased cardiovascular risk as compared to women, which is largely ascribed to the cardioprotective effects of female sex steroids. We hypothesised that this may be reflected by differences in the activation status of the coagulation system. Hence the aim of this study was to compare plasma levels of activated factor VII (FVIIa) in men and women, and to study the influence of the menstrual cycle on FVIIa levels.

In a prospective study we investigated 20 healthy young women and 20 men. Men had significantly higher levels of activated factor VII (60 mU/ml, CI: 52 to 67) than women during all phases of the menstrual cycle. In women FVIIa was higher during the follicular phase (41 mU/ml, CI: 33 to 50) than during midcycle (34 mU/ml, CI: 24 to 45; p = 0.022 vs. follicular phase) and during the luteal phase (33 mU/ml, CI: 24 to 42; p = 0.006 vs. follicular phase). Prothrombin fragment (F1 + 2) levels decreased from 0.86 nmol/l (CI: 0.51-1.21) by -23% (-39% to -8%; p = 0.011) during midcycle and by -25% (CI: -51% to 1%; p = 0.023) during the luteal phase.

These data support the contention that plasma levels of FVIIa, a key enzyme of the coagulation cascade, may be down-regulated by endogenously produced female sex hormones during the menstrual cycle. This may at least partially explain the marked gender differences found in FVIIa.

Introduction

Men have an increased cardiovascular risk as compared to women, which is largely ascribed to the cardioprotective effects of female sex steroids. This is mainly supported by the finding that this gender difference is lost soon after menopause and that the administration of oestrogens to postmenopausal women decreases the risk for atherosclerosis and coronary artery disease (1-4).

It is well known that patients at risk for coronary artery disease present with disturbances of blood coagulation (5) or fibrinolysis (6). The extent of factor VII activation determines the basal functional state of the coagulation system (7). Recently, elevated levels of FVIIa were found in diseases associated with thrombotic vascular complications, e.g. atherosclerosis (8) or associated with cardiovascular risk factors (9-11), and FVII coagulant activity (FVII:c) is long known to predict cardiovascular risk (12,13). Recently, it has been shown, that the FVII:c/FVII:Ag ratio and the FVIIa levels are lower in premenopausal women than in men, and FVIIa levels were increased in postmenopausal as compared to premenopausal women (11, 14). Further, parenteral estradiol substitution was associated with lower FVIIa levels in postmenopausal women (11, 15).

Against this background of evidence, we hypothesised that naturally occurring increases of female sex steroids during the menstrual cycle may lower FVIIa. Hence, we compared plasma levels of FVIIa between healthy young men and women, and investigated the fluctuations of FVIIa on three occasions during the menstrual cycle.

Subjects and Methods

Study Design

This study was performed in 20 healthy men and 20 healthy women. The study protocol was approved by the local Ethics Committee and written consent was obtained from all subjects prior to inclusion.

Subjects

Twenty healthy male volunteers, aged 19-37 years (29 ± 5 years, mean ± 1 SD) and 20 healthy female volunteers, aged 21-38 years (27 ± 5 years, mean ± 1 SD) were studied. All subjects were recruited by advertising at the university campus and consisted mainly of students or nurses. All women reported regular menstrual cycles and none of them had been taking oral contraceptives for at least 3 cycles. Health was determined by a battery of clinical and laboratory tests as described elsewhere (16, 17).

Experimental Protocol

Fasting blood was sampled with the subjects in seated position after a 30 minutes resting period, men donating blood only once. The female volunteers were requested to report at the Department of Clinical Pharmacology on day 7 of their menstrual cycle between 8:00 and 9:00 am after an overnight fast. Thereafter, the volunteers were instructed to use a commercially available test (Epignost Ovulationstest®, Sigma-Braumapharm, Vienna, Austria) for daily screening of their urine for the LH-surge at home. A positive test result was confirmed by a second test (Clearplan®, Much Pharma GmbH, Vienna, Austria). The volunteers were asked for additional blood sampling on the day after the test turned positive and again 9 days thereafter, or to report for blood sampling, if tests were not positive until the third morning after the expected ovulation, as estimated on the basis of their last three cycles.

Laboratory Methods

Fasting blood samples were obtained at 8:00 a.m. by venipuncture through a 21-gauge needle into Vacutainer, tubes containing 0.5 ml of 0.129 mol trisodium citrate. All plasma samples were centrifuged immediately at 2000 g for 15 min at room temperature and stored at -80 °C until analysis; all samples were run in duplicates. The three blood samples of each female volunteer...
were run in the same assay. Samples were coded in order to blind the analyst regarding gender, the sampling day and ovulatory status.

Activated factor VII was determined by Sta clot VII-rTF on a STA analyser (Diagnostica Stago, Asnieres-Sur-Seine, France), using a recombinant soluble tissue factor mutant which abolishes activation of factor VII, but not cofactor function for factor VIIa in the activation of factor X. The normal range for activated factor VII is 27.1-103.2 mU/ml. As specified by the instruction manual, the standard containing freeze-dried recombinant soluble tissue factor and phospholipids has been calibrated against a secondary standard of the first international standard 89/688 established in 1993. Equivalence between mU and ng units has been established and found to be approximately 30 mU/ng (18).

Factor VII clotting activity was determined by a one step clotting assay (factor VII deficient plasma and PT reagent Thromborel S were from Behring, Marburg, Germany) on a KC-10 coagulometer (Amelung, Lengo-1, Liene, Germany). The normal range for factor VII:c is 75-130%.

Factor VII antigen was measured by an ELISA Asserachrom VII:Ag (Diagnostica Stago), normal range 76-123% (19). Prothrombin fragment F1 + 2 was measured with an enzyme immunoassay (Behring) (20).

Serum levels of estradiol and progesterone were measured by radioimmunoassay (RIA) (Coat-A-Count® Estradiol or Progestrone Diagnostic Products Corporation, Los Angeles, CA, USA). Normal progesterone levels are <4.77 and >9.54 nmol/l for the follicular and the luteal phase, respectively (17, 21).

**Statistical Analyses**

Data are presented as means and the 95% confidence intervals (CI). As data were non-normally distributed, non parametric tests were used for statistical comparison. To test variations of measured endpoints during the menstrual cycle for significance the Friedman ANOVA and the Wilcoxon signed ranks test for post hoc comparisons were used. The Mann Whitney U-test was used for comparisons between sexes. A two tailed p-value of <0.025 was considered significant.

**Results**

Three out of 20 female volunteers discontinued prematurely for personal reasons. Men had significantly higher plasma levels of FVIIa (60 mU/ml; CI: 52-67; Fig. 1) than women during all time points (follicular phase: 41 mU/ml; CI: 33-50; p = 0.006). Factor VIIa levels decreased even further from the follicular phase (midcycle: -18%; CI: -31 to -6 p = 0.022 and luteal phase: -20%; CI: -34 to -7% p = 0.006). Parallel changes in FVII:Ag and FVII:c were also observed (Fig. 2). The FVIIa/FVII:Ag ratio did not change (follicular: 0.28 [CI: 0.23-0.33]; luteal: 0.27 [0.20-0.34]; p > 0.05). However, F1+2 levels decreased from 0.86 nmol/l (CI: 0.51-1.21) by -23% (-39% to-8%; p = 0.011) during midcycle and by -25% (CI: -51% to 1%; p = 0.023) in the luteal phase.

**Changes of Sex-hormone Levels during the Menstrual Cycle**

Progesterone serum concentrations increased 17-fold in the luteal phase and estradiol serum levels increased 2.8 fold in the luteal phase (Fig. 2). Eight subjects had no positive LH-test, but there were no differences in the changes of female sex hormones or FVII status from follicular phase to the luteal phase between those subjects with and without a positive LH-test (p > 0.05).

**Correlations**

There were no correlations between changes of FVIIa, FVIIc, FVII:Ag, and the FVIIa/FVII:Ag ratio from the follicular phase to the luteal phase and the changes of estradiol and progesterone. All correlations were below r² = 0.25 and p > 0.05. Also no correlation was observed between changes in FVIIa and F1+2 levels.

**Discussion**

Based on the well known sex difference in cardiovascular disease we hypothesised that men may exhibit a higher activation status of the coagulation system than women. The aim of the current investigation was to study the impact of gender on plasma levels of FVIIa and to evaluate whether the naturally occurring increase in female sex steroid hormones during the menstrual cycle is associated with a decrease in FVIIa. Our results support the notion that young men have substantially lower FVIIa levels than age-matched women. In addition, we demonstrate that plasma levels of FVIIa decline simultaneously with decreasing estradiol and progesterone when concentrations of female sex hormones increase during the menstrual cycle.

Our findings are in general agreement with other prospective trials demonstrating that very high endogenous oestrogen levels, induced by ovarian hyperstimulation, decreased FVIIa:Ag and FVII:c levels (22). Estradiol together with a synthetic progesterone also decreased FVIIa and FVII:Ag (23), and transdermal estradiol was associated with lower
FVII:Ag and FVIIa in postmenopausal women (11, 15). However, another study demonstrated that transferally administered estradiol and conjugated oestrogens increased FVIIa: c and FVII:Ag (24). Yet, oral contraceptives have been found to increase FVIIa, FVIIa: c, and FVII:Ag in healthy women (25–27). Finally, FVII:Ag and FVIIa increase during pregnancy (28). Hence, there may be differences in the way natural and synthetic oestrogens affect FVII levels or its activation. Alternatively, effects of oestrogens may depend on whether they are produced endogenously or administered exogenously.

What may cause the decline in FVIIa during the menstrual cycle? As depicted in Figure 2 FVII:Ag decreased simultaneously with FVIIa. Thus, the decreased plasma levels of FVIIa may be due to a decrease in “the substrate” FVII:Ag. However, when comparing men and women during the follicular phase we found no difference in FVII:Ag levels, while FVIIa levels were substantially lower in women. This would point towards decreased generation of FVIIa from FVII in women. Recently, an ex-vivo study demonstrated a reduction of monocyte tissue factor, the main activator of FVII, after initiation of hormone replacement therapy in postmenopausal women (29). This would point to a role of sex steroid hormones in the regulation of the main activator of the coagulation cascade, which could partially account for the observed sex differences of FVIIa plasma levels. However, similar to the diurnal changes in FVIIa levels which are accompanied by circadian changes in F1+2 levels (19), the decrease in FVIIa was accompanied by changes in F1+2 levels of similar magnitude. Although no direct correlation could be observed between changes of those two coagulation markers, FVIIa levels conceivably determine thrombin generation of healthy premenopausal women during their menstrual cycle.

In conclusion, our data support the notion that plasma levels of FVIIa are critically dependent on gender and may be down-regulated by endogenously produced female sex hormones during the menstrual cycle. Hence, the phase of the menstrual cycle is an important factor to take into account when interpreting the laboratory results of factor VII levels in women. Suchindran CM, Tyroler HA, Rifkind BM. Estrogen use and all-cause mortality. Thromb Haemost 1996; 75: 772-7.


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