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Effects of vaginally administered high estradiol doses on hormonal pharmacokinetics and hemostasis in postmenopausal women

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Objective: To study the effect of high doses of estradiol released from vaginal rings on the pharmacokinetics of hormones, and the long-term effect on hormones and hemostasis in postmenopausal women.

Design: A pilot, nonrandomized study.

Setting: Healthy volunteers in an academic research environment.

Patient(s): Postmenopausal women.

Intervention(s): Eight women were treated with 17 β-estradiol from three vaginal rings, releasing 7.5 μg per ring for a total of 22.5 μg over 24 hours. The rings were changed every morning for 14 days.

Main Outcome Measure(s): Hemostatic changes were recorded.

Result(s): Estradiol was rapidly absorbed, and statistically significant increases in the levels were found after 15 minutes; C_{max} was obtained after 1 hour and a steady state after 24 hours. No statistically significant changes were found in the levels of coagulation factors V, von Willebrand factor, and activated factor VII; nor were any changes observed for activated protein C resistance, coagulation inhibitors protein C, protein S, or plasminogen activator inhibitor-1. No indication of increased thrombin formation was demonstrated by analyses of prothrombin fragment 1+2, fibrin D-dimer, and soluble fibrin.

Conclusion(s): No statistically significant changes in hemostasis were observed from the vaginal administration of estradiol using a dose equivalent to transdermal administration with a release rate of 100 μg per 24 hours. (Fertil Steril® 2002;78:1172–7. ©2002 by American Society for Reproductive Medicine.)

Key Words: Vaginal, estradiol, pharmacokinetics, hemostasis, coagulation factors, APC resistance, coagulation inhibitors, PAI-1, hormone replacement therapy

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In Europe, hormone replacement therapy (HRT) for climacteric symptoms and prevention of osteoporosis is mostly given as oral 17 β-estradiol. Transdermal administration (patch and gel) is also used and has the advantages of relatively constant circulating estrogen levels during 24 hours and avoidance of the first hepatic passage (1). For treatment of urogenital symptoms, low-dose local estrogen regimens have been developed, such as cream, vaginal suppositories, and a vaginal ring (2). From pharmacokinetic studies of 17 β-estradiol released from a vaginal ring, it is known that this treatment is associated with an initial burst, with high levels of circulating estradiol for 12 hours after insertion, after which time a steady state is reached with a low, almost zero-order release (3). Because administration of 17 β-estradiol by vaginal rings has turned out to be well tolerated as a long-term method for hormonal treatment of urogenital symptoms (4, 5), the idea arose that it might be worthwhile to develop a ring with a higher release of 17 β-estradiol for treatment of systemic climacteric symptoms.

The aim of the present study was to investigate the pharmacokinetics from three vaginal rings, each releasing 7.5 μg (for a total of 22.5 μg per 24 hours) of 17 β-estradiol in postmenopausal women for a period of 24 hours, as well as the long-term effect on hormonal levels and hemostasis of 17 β-estradiol.

MATERIALS AND METHODS

Study Group

Eight healthy postmenopausal women, aged 46 to 55 years, with a diastolic blood pressure below 90 mm Hg and a follicle-stimulating hormone (S-FSH) level above 30 IU/L (57–125 IU/L) were included in the study. All of the women were considered to be menopausal. Seven women were nonsmokers and one smoker abstained from smoking 1 month before joining the study.

Before the study, a clinical evaluation was performed including medical history, general and gynecologic examination, and measurements of blood pressure, and liver, kidney, and thyroid function. The women also answered a questionnaire regarding climacteric symptoms such as sweating and hot flushes. The exclusion criteria were treated breast or gynecologic cancer, acute or chronic liver disease, earlier venous thrombosis, or untreated coronary artery disease.

The study was approved by the ethics committee of the Karolinska Hospital. Each individual consented to being included in the study.

Study Design

Three vaginal rings of 17 β-estradiol (Oestring 7.5 μg/24 hours/ring; Pharmacia & Upjohn, Stockholm, Sweden), releasing a total of 22.5 μg of the hormone over 24 hours, were inserted by the women daily. The women changed to three new vaginal rings every morning at 8 AM. Blood sampling was performed for hormone analyses immediately before and at 15, 30, and 60 minutes and 2, 4, 8, 12, and 24 hours after insertion of the first rings and thereafter every morning before changing of the rings. In addition, samples for analyses of hemostatic variables were taken before the study began, and after 7 and 14 days of treatment (on days 8 and 15). The women were fat fasting, and they rested sitting for 20 minutes before blood was drawn for the hemostatic tests.

Because we followed changes in hemostatic variables in each individual woman, we used the first sample drawn as a control sample.

Laboratory Methods

The hormone assays for estradiol (E_2) determinations were performed using the commercial radioimmunoassay kits, Eir Ria 25 and Eir Ria 155 (Radioisotopic Service, Warehingen, Switzerland). For determination of S-FSH, commercial kits were also used (Diagnostic Products, Los Angeles, CA). For the estrone (E_1) and estrone sulphate (E_1S) measurements, we used the methods described by Aedo et al. (6).

The blood samples for hemostatic analyses were taken in three vacuum tubes containing 0.129 mmol/L of trisodium citrate (pH 7.4, 1 part + 9 parts of blood), and then they were immediately centrifuged for 20 minutes at 3000 $\times$ *g*. The plasma contents from two citrate tubes were aliquoted into eight Ellerman tubes and frozen at −70°C until analyzed. To be used for APC resistance analyses, the plasma from the third tube was centrifuged once more for 20 minutes (3000 $\times$ *g*), and the plasma was thereafter aliquoted into two Ellerman tubes and frozen at −70°C.

Amidolytic methods, employing chromogenic peptide substrates, were used to assay P-protein C (7) and P-soluble fibrin (8) with kits from Chromogenix (Mölndal, Sweden), as well as P-tissue plasminogen activator inhibitor-1 (PAI-1) (9), using the reagent Chromolize PAI-1 (Biopool, Umeå, Sweden). Soluble fibrin of a few samples was reanalyzed with another, newer kit from Chromogenix. Enzyme linked immunosorbent assay (ELISA) was used for measuring P-prothrombin fragment 1+2 (Fr 1+2) (10) with a kit from Behringwerke (Marburg, Germany), P-fibrin-D-dimers with the TintElize D-dimer kit from Biopool, and P-free protein S and P-VWF (von Willebrand factor) antigen (11) with kits from Stago (Asnières, France).

Resistance to activated protein C was analyzed with a kit from Chromogenix, and the results were correlated to those of a pooled reference plasma. Activated P-FVII (FVIIa), was measured with the reagent StaClot VIIa-rTF (Stago). The measurement of P-fibrinogen was performed with a modified polymerization time method (12).

Statistical Methods

For statistical evaluation, the descriptive statistics of arithmetic mean, standard deviation, and percentiles were used. The time required to reach peak concentration was calculated directly from the plasma profiles. Differences between concentrations were calculated using analysis of variance (ANOVA).

RESULTS

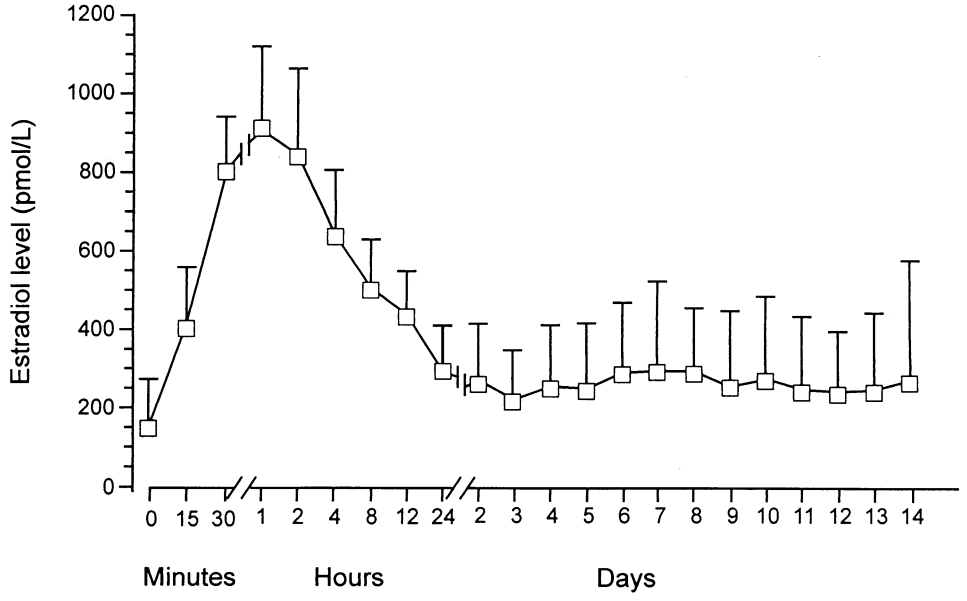
All eight women completed the study and reported relief from climacteric symptoms within 5 days. As expected, FSH decreased after ring insertion and reached a steady state after 5 days (mean 41 ± 14; range: 23–59 IU/L). In five women the levels continued to decrease until the end of the study (mean 36 ± 20; range: 7–63 IU/L).

At 15 minutes after insertion of the rings, circulating E_2 already had risen significantly (mean 402 ± 153; range: 160–548 pmol/L) from baseline (mean 151 ± 118; range: 73–362 pmol/L; $P<.001$), as shown in Figure 1. Maximum levels (mean 915 pmol/L ± 202; range: 697–1307) were observed after 1 hour ($P<.001$) and a steady state was reached after 24 hours (mean levels 297 ± 104; range: 194–518 pmol/L).

Estrone (E_1) rose slowly after ring insertion; a statistically significant increase from baseline was observed after 48 hours (mean 418 ± 50; range: 256–706 pmol/L; $P<.01$) and a plateau was reached after 6 to 7 days (mean 486 ± 199;

FIGURE 1

Circulating levels of estradiol before and after the first insertion of three vaginal rings, each releasing 7.5 μg estradiol over 24 hours, and thereafter before changing rings every 24 hours. □ = mean + SD.



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range: 284–878 pmol/L) (Fig. 2).

Estrone sulphate (E_1S) also rose slowly and a plateau was found at 6 to 7 days (mean 10.0 ± 5.4; range: 5.0–19.3 nmol/L) (Fig. 3).

No statistically significant changes were seen in any of the analyzed hemostatic factors and inhibitors. Neither was

FIGURE 2

Circulating levels of estrone before and after insertion of three vaginal rings. □ = mean + SD.

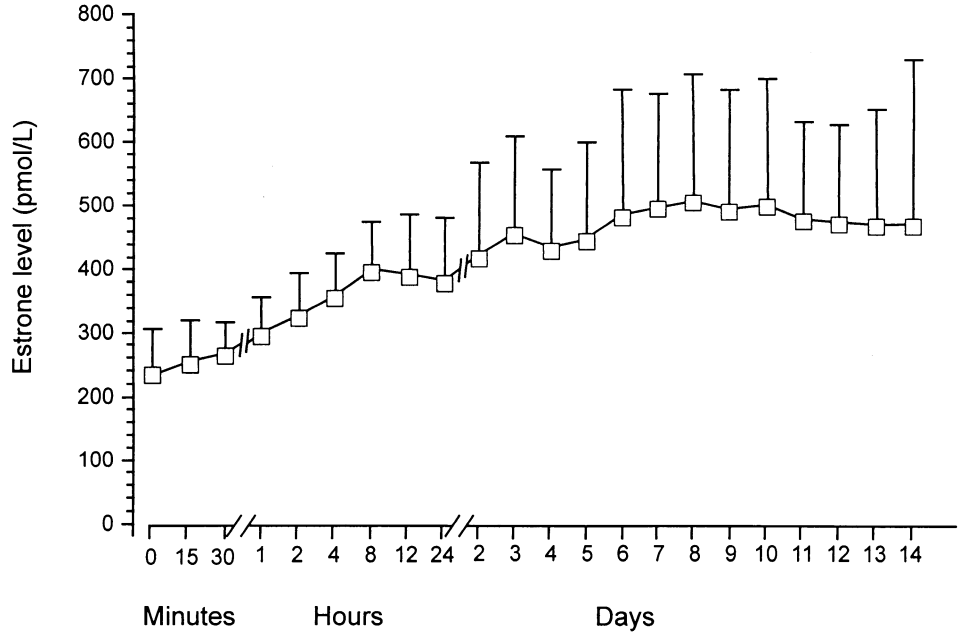

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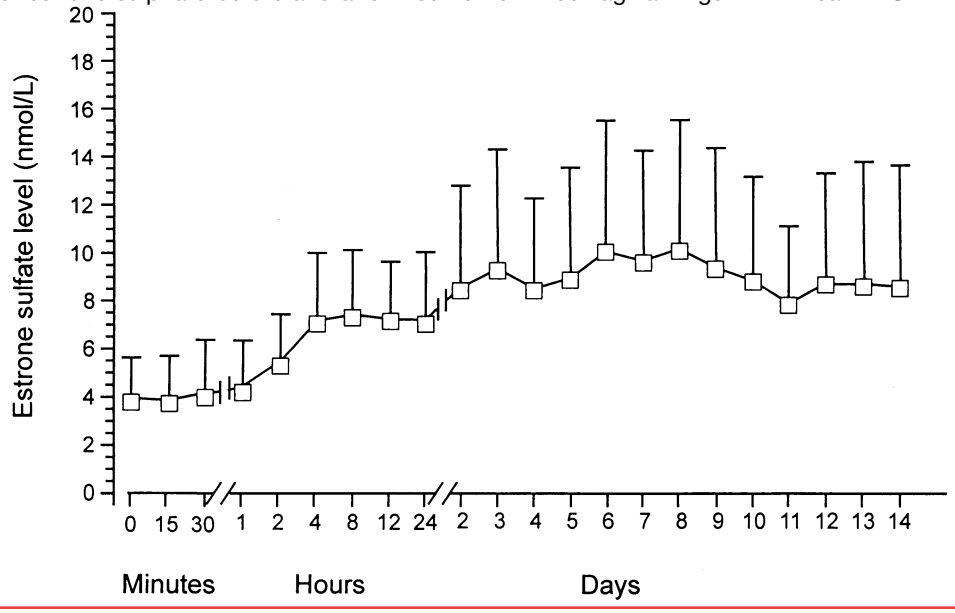
FIGURE 3

Circulating levels of estrone sulphate before and after insertion of three vaginal rings. □ = mean + SD.



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there any indication of increased thrombin generation; that is, there were no statistically significant changes in the levels of any of the thrombin markers: prothrombin fragment 1+2 (Fr 1+2), fibrin D-dimers, and soluble fibrin (Table 1).

However, in one woman the Fr 1+2 levels were elevated and the VWF levels were low (close to the low end of the range in our reference population) on all three occasions. The levels of the fibrinolytic inhibitor PAI-1 were high on all occasions in one woman, and the APC ratio was low in another. As a decreased APC ratio most often stems from the 11691 G-A mutation in coagulation factor V (also called the Leiden mutation), a DNA sample was drawn from the latter woman for analysis, but the mutation was not found.

One woman had a somewhat increased fibrinogen level in the first sample, but this had normalized by the second occasion. A few samples showed a slight increase in soluble fibrin levels. When the samples from these women were

TABLE 1

Hemostatic variables in the eight postmenopausal women before and after 8 and 14 days when treated with three vaginal rings releasing a total of 22.5 μg 17 β-estradiol/24 hours.

	Before			Day 8			Day 15		
Hemostatic variable	25 thp	50 thp	75 thp	25 thp	50 thp	75 thp	25 thp	50 thp	75 thp
Fibrinogen g/L	2.59	3.14	3.89	2.70	3.41	3.80	2.70	3.33	3.80
VWF, IU/mL	0.79	1.01	1.18	0.60	1.11	1.19	0.81	1.02	1.15
APC ratio	0.87	1.03	1.09	1.03	1.19	1.31	0.98	1.10	1.30
Factor VIIa, IU/mL	39.00	43.50	52.00	36.50	47.50	57.00	40.50	49.00	54.50
Protein C, IU/mL	1.01	1.08	1.19	1.01	1.12	1.14	0.96	1.10	1.22
Protein S free U/mL	0.25	0.27	0.32	0.26	0.27	0.28	0.25	0.27	0.29
Fr1+2, nmol/L	0.61	0.84	1.08	0.62	0.79	0.95	0.55	0.77	1.63
Soluble fibrin nmol/L	8.00	9.50	13.00	9.00	11.00	16.50	11.00	12.00	17.50
D-dimer ng/mL	11.00	15.50	21.50	11.50	14.50	34.50	12.30	22.00	28.80
PAI-1 IU/mL	3.00	3.00	10.00	1.00	3.00	15.00	2.00	3.50	6.00

Half-lives: fibrinogen, 4.5 days; VWF, 12–20 hours; FVIIa, 5–6 hours; protein C, 6–8 hours; F1+2, 1.0–1.5 hours; soluble fibrin, 4–6 hours; D-dimers, 15–30 hours; PAI-1, approximately 2 hours.

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reanalyzed with a modified method developed later by the same firm, all levels were within the normal range.

DISCUSSION

Treatment with 17 β-estradiol from three vaginal rings, roughly equivalent to transdermal administration using patches, reduced climacteric symptoms effectively in all eight women.

In our study, the circulating plateau levels of estradiol (mean 290 pmol/L, range: 300–217) after insertion of three rings were somewhat higher than the levels found in the study by Nash et al. (13), which used one vaginal ring releasing 200 μg per day. They also reported relief from climacteric symptoms and an absence of effects on lipoproteins as a result of avoiding the first liver passage.

In our study, a peak value of estradiol up to 1300 pmol/L was obtained after 1 hour, followed by a rapid decrease to values associated with the middle to late follicular phase of the menstrual cycle (200–500 pmol/L). The levels of E_2 were approximately three times those reported for one ring; peak levels of 270 $\pm$ 80 pmol/L declined to $<$120 pmol/L within 24 hours (14).

The absorption of estradiol through the vaginal wall was excellent, with a mean burst effect of 915 pmol/L, range: 697–1307 that lasted for 24 hours. In samples drawn 24 hours after ring exchange, a plateau was reached with circulating E_2 levels (297 pmol/L, range: 194–518) comparable to those observed during either oral treatment with 2 mg 17 β-estradiol daily or transdermal release of 100 μg estradiol over 24 hours (1, 14).

We can therefore assume that the burst (if any) after ring change was much smaller than after insertion of the first rings. This assumption is supported by the circulating estrone and estrone sulphate levels, which reached plateaus after 6 to 7 days. In a study by Englund et al. (15), circulating estradiol levels in the second treatment cycle after ring change were similar to those seen at the end of the first cycle because the absorption of estradiol through the epithelium is reduced due to the increased proliferation of the vaginal mucosa (16, 17).

Because vaginal administration of estradiol avoids the first liver passage, no statistically significant changes in hemostasis were seen in this pilot study with a dose that gave plasma levels associated with a release rate of 100 μg over 24 hours, the equivalent to oral treatment with 2 mg 17 β-estradiol.

Our aim, however, was to study the hormonal influence when a plateau had been reached. The half-life times of hemostatic factors and inhibitors are generally less than 3 days, though fibrinogen has a half-life of 4.4 days in women (18) (see Table 1). Thus, a steady state with regard to the hemostatic factors measured must have been obtained after 2 weeks, when our samples were drawn, except for fibrinogen for which a slight extra effect might have been present. However, we found no increase in the fibrinogen concentration to reflect this.

In 1983, Conard et al. (19) observed no change in the level of antithrombin after transdermal administration of 17 β-estradiol. Scarabin et al. (20) treated women with transdermal 17 β-estradiol as a gel (2.5 mg/day), adding micronized progesterone (200 mg/day) on days 14 to 25. No statistically significant increase in the thrombin activation prothrombin fragment 1+2 or decrease in antithrombin was observed after 6 months of transdermal treatment.

Three recent studies (21–23) have confirmed that, due to avoidance of the liver passage, transdermal 17 β-estradiol does not elicit a tendency toward hypercoagulation in the coagulation and fibrinolytic systems. These data support our findings of no changes when another route of administration (such as vaginally) avoids this passage.

In summary, vaginal administration of 17 β-estradiol in a dosage three times that for urogenital treatment reduced climacteric symptoms effectively. No statistically significant changes in hemostasis were observed, measured at a hormonal plateau level. If rings are to be used for hormone replacement therapy, slow-release rings will be needed to dispense with daily shifts.

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