Hemostatic changes associated with menopausal hormone therapy. Comparison of transdermal and oral administration

Zmiany hemostazy związane z terapią hormonalną wieku menopauzalnego. Porównanie doustnej i przezskórnej drogi podania

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Summary

Hormone therapy (HT) is a popularly recommended treatment for eliminating or alleviating the symptoms of menopause. It is established that combined preparations containing estrogen and progestin are related with a small, but clinically significantly increased risk of arterial and venous thrombosis, may increase myocardial infarction and stroke in women. The aim of our study was to confront the hemostatic effects of taking oral and transdermal HT in postmenopausal Polish women compared with non-users (controls) matched for age *for 6 months*. The concentration of thrombin-activatable fibrinolysis inhibitor (TAFI) was measured using ELISA kit Imclone Tafi's produced by American Diagnostica GmbH. Generated thrombin was measured according to the method *described by Lau el al.* Plasmin formation in plasma was determined by chromogenic substrate (Chromozym PL). The activity of generated thrombin was statistically higher in plasma of women after o-HT (72.6 ±8.5 mOD/min) than in patients with t-HT (53.7 ±10.1 mOD/min) and controls (51.2 ±10 mOD/min). Amidolytic plasmin activity was the highest in controls (84.5 ±10.2 mOD/min). The value of plasmin activity in women after o-HT treatment was lower (61.9 ±7.9 mOD/min) compared to patients taking t-HT (77.7 ±14.5 mOD/min). The highest level of TAFI was observed in patients after oral hormones (80.38 ±8.23%); women on transdermal HT had 61.58 ±9.81% and the lowest concentration of TAFI was noted in the control group (44.70 ±10.16). The influence of HT on hemostasis has been largely attributed to the estradiol part of the preparation, although progestogen can also affect observed changes. The effect we observed may in part be explained by the dose and type of progestogen.

Key words: menopausal hormone therapy, hemostatic changes.

Streszczenie

Terapia hormonalna (*hormone therapy* – HT) jest często zalecana w celu eliminacji lub zmniejszenia objawów menopauzy. Udowodniono, że złożone preparaty zawierające estrogeny i progestageny są związane z niewielkim, ale klinicznie istotnym wzrostem ryzyka zakrzepicy tętniczej i żylnej, zwiększają również ryzyko zawału serca i udaru. Celem badania było porównanie wpływu na hemostazę 6-miesięcznej HT doustnej i przezskórnej stosowanej u kobiet pomenopauzalnych, w odniesieniu do grupy kontrolnej kobiet niestosującej terapii w porównywalnym wieku. Stężenie inhibitora fibrynolizy aktywowanego trombiną (*thrombin activatable fibrinolysis inhibitor* – TAFI) było oceniane metodą ELISA przy użyciu Imclone Tafi's kit (American Diagnostica GmbH). Trombina oznaczana była metodą wg Lau i wsp. Plazmina w osoczu była oznaczana za pomocą barwnego substratu (Chromozym PL). Aktywność wytworzonej trombiny była statystycznie większa w osoczu kobiet stosujących terapię doustną (72,6 ±8,5 mOD/min) niż u pacjentek stosujących terapię przezskórną (53,7 ±10,1 mOD/min) i z grupy kontrolnej (51,2 ±10 mOD/min). Aktywność plazminy była największa w grupie kontrolnej (84,5 ±10,2 mOD/min), a u pacjentek przyjmujących terapię doustną była mniejsza (61,9 ±7,9 mOD/min) w porównaniu z pacjentkami stosującymi terapię przezskórną (77,7 ±14,5 mOD/min). Największe stężenia TAFI były obserwowane u kobiet po terapii doustnej (80,38

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±8,23%); stężenie TAFI w grupie kobiet stosujących terapię przezskórną wynosiło 61,58 ±9,81%, a najmniejsze stężenie odnotowano w grupie kontrolnej (44,70 ±10,16). Wpływ terapii hormonalnej na hemostazę zależał przede wszystkim od komponenty estrogenowej, aczkolwiek progestagen miał również wpływ na opisywane zmiany. Obserwowane efekty mogły być również częściowo wynikiem różnych progestagenów i różnych ich dawek.

Słowa kluczowe: terapia hormonalna, menopauza, zmiany hemostazy.

Introduction

The advent of the menopause is associated with an increased risk of cardiovascular disease. Women after menopause have higher levels of known risk factors for cardiovascular disease, such as fibrinogen and factor VII, compared with younger women [1]. Hormone therapy (HT) is a popularly recommended treatment for eliminating or alleviating the symptoms of menopause [2, 3]. It is established that combined hormone therapy containing estrogen and progestin is related with a small, but clinically significantly increased risk of arterial and venous thrombosis, may increase myocardial infarction and stroke in older women [4, 5]. During the treatment with oral hormone therapy (o-HT) the risk of venous thromboembolic complications increases 2- to 3-fold, particularly within the first 6 months of therapy [6, 7]. The hormonal treatment also affects the structure and metabolism of proteins involved in coagulation and fibrinolysis [8-10]. However, the effects of HT on hemostasis may depend on the dose and route of administration. In oral hormone therapy, a high dose of estrogens is necessary because oral formulations have bioavailability problems, such as intestinal and hepatic first-pass effects, and thus stimulate production of coagulation factors, increasing the risk of venous thrombosis (VTE). The transdermal route of administration has little influence on coagulation factors and consequently decreases the risk of VTE [11, 12]. The purpose of our study was to assess the effects of oral and transdermal hormone therapy on hemostasis determining such parameters as: fibrinogen concentration, plasma level or thrombin inhibitor of fibrinolysis (TAFI) *and compare two other parameters: amidolytic activity of generated thrombin and plasmin* in plasma. Our research was undertaken to confront the hemostatic effects of taking combined estrogen preparations containing two different progestogens in oral and transdermal HT in postmenopausal Polish women compared with non-users (controls) matched for age [12]. *In the present study, the effect of HT on hemostatic factors was studied for 6 months.*

Material and methods

Study participants

Fifty postmenopausal Polish women who visited the Department of Gynecology and Menopausal Disorders, Polish Mother's Memorial Hospital – Research Institute in Lodz were examined. All of them were Caucasian living in the industrial area of central Poland. None of the women had a history of thrombosis, hypertension, diabetes or any metabolic disorders affecting the coagulation system. None of them smoked, used alcohol or had a thyroid or liver disease. They did not take any cholesterol-lowering drugs, antioxidant-vitamin supplements or any medication that might affect the metabolism of sex steroids before the present study. All patients were divided into three groups according to treatment: group I (mean age 55.6 years, n = 18): oral hormone therapy; 17β-estradiol (1 mg daily) and dydrogesterone (5 mg daily), group II (mean age 52.2 years, n = 14): transdermal hormone therapy; 17β-estradiol (50 µg daily) 19-norethisterone (250 µg daily), group III (mean age 54.6 years, n = 18): controls, women without HT treatment. The study was approved by the Bioethics Committee at the Polish Mother's Memorial Hospital – Research Institute in Lodz (no. RNN/57/06/KE). All subjects gave written informed consent.

Laboratory assays

Prior to the study, the following examinations were performed: gynecological examination with cervical cytological analysis, transvaginal ultrasound assessment of the reproductive organs, palpative breast examination and mammography. Blood samples were collected from an antecubital vein of the overnight-fasting subjects between 8.00 and 9.00 to avoid diurnal variation. All studies related to coagulation factors and fibrinolytic components were performed on platelet-free plasma obtained from centrifugation (2500xg, 15 min, 20°C) of 4.5 ml of blood mixed with 0.5 ml of 3.8% sodium citrate solution. Concentrations of glucose, fibrinogen (Fg) estimated by a method of functional fibrinogen determination using a Multifibren U kit (Dade-Behring Inc), platelet count (PLT) – using a Baker 810 platelet analyzer, activated partial thromboplastin time (APTT) – using a Pathromtin SL reagent, (Dade-Behring Inc) and triglyceride levels in blood of all patients were estimated in the Laboratory of the Polish Mother's Memorial Hospital – Research Institute in Lodz.

Levels of thrombin inhibitor of fibrinolysis (TAFI)

The concentration of thrombin-activatable fibrinolysis inhibitor (TAFI) was measured using ELISA kit Imclone Tafi's produced by American Diagnostica GmbH.

Measurement of thrombin generation by subsampling method

Plasma was mixed with fibrin polymerization inhibitor Sigma® T1895 and incubated with rabbit thromboplastin. Using S-2238 chromogenic substrate amidolytic activity of generated thrombin was measured according to a method described by Lau et al. [13].

Plasmin generation activity assays

Plasmin formation in plasma was determined by chromogenic substrate (Chromozym PL). For the activation, plasma was diluted 10 times in 50-mM phosphate buffer, pH 7.4 and incubated with streptokinase for 30 minutes, at 37°C. Assays were performed at 25°C in 96 well-polystyrene flat-bottom plates, using the kinetic protocol in a microplate reader at 415nm (Bio-Rad Microplate Reader, model 550) [14].

Tab. I. Characteristics of patients (mean ± SD)

	o-HT mean ± SD	t-HT mean ± SD	Controls mean ± SD
Patients (*n*)	18	14	18
Age (years)	55.6 ±5.10	52.2 ±5.40	54.6 ±5.50
Years after menopause	5.3 ±3.62	4.9 ±3.22	5.1 ±4.13
Body mass index (kg/m^2)	25.9 ±2.29	26.3 ±3.17	26.7 ±2.82

Tab. II. Characteristics of some laboratory assays (mean ± SD)

	o-HT mean ± SD	t-HT mean ± SD	Controls mean ± SD	*p*
Glucose [mg/dl]	94 ±12.30	93 ±11.50	95 ±12.80	0.15
Fibrinogen [g/l]	4.14 ±1.28	3.6 ±2.18	3.12 ±1.12	< 0.001
Platelet count	234,000 ±60.10	294,000 ±57.30	252,000 ±70.10	0.163
APTT [s]	29.08 ±3.91	28.16 ±2.34	27.11 ±2.36	0.282
Triglycerides [mmol/l]	1.97 ±0.59	1.84 ±0.69	1.75 ±0.41	0.05

Tab. III. Variables of plasmin and thrombin activity in postmenopausal women after hormone therapy

Hemostatic parameter	o-HT *n* = 18 mean ± SD	t-HT *n* = 14 mean ± SD	Controls *n* = 18 mean ± SD
Thrombin activity [mOD/min]	72.6 ±8.5	53.7 ±10.1	51.2 ±10
Amidolytic plasmin activity [mOD/min]	61.9 ±7.9	77.7 ±14.5	84.5 ±10.2

Tab. IV. Levels of TAFI in patients' plasma

	o-HT mean ± SD	t-HT mean ± SD	Controls mean ± SD
Patients (n)	18	14	18
TAFI level [%]	80.38 ±8.23	61.58 ±9.81	44.70 ±10.16

Statistical analysis

All the values in this study were expressed as mean values ± SD using StatSoft Inc. "Statistica" v. 7.0. To check normality of sample distribution the data were analyzed with a Shapiro-Wilk test. When the distribution was normal, the statistical analysis of differences between the control plasma and plasma treated with o-HT and t-HT was done with a paired Student's t-test using StatSoft Inc. "Statistica" v. 7.0. When the distribution was not normal, the non-parametric Mann-Whitney U test was used.

Results

The patients' characteristics are presented in Table I. There were no significant differences in terms of their mean age, years after menopause, body mass index (BMI) among the three groups (Table I). The clinical and biochemical characteristics of postmenopausal women who received oral and transdermal HT and patients did not take any hormones are summarized in Table II. We observed that after 6 months of HT, the level of fibrinogen was higher than in the control group (Fg 3.12 g/l vs. 4.14 g/l (o-HT); 3.6 g/l (t- HT); $p < 0.001$). There were no significant differences in platelet count, APTT and triglycerides between the three groups of patients. Variables of plasmin and thrombin activity are presented in Table III. The activity of generated thrombin was statistically higher in plasma of women after o-HT (72.6 ±8.5 mOD/min) than in patients with t-HT (53.7 ±10.1 mOD/min) and controls (51.2 ±10 mOD/min). There were no statistically significant differences between controls and women on t-HT treatment ($p = 0.430$). Amidolytic plasmin activity was the highest in controls (84.5 ±10.2 mOD/min). The value of plasmin activity in women after o-HT treatment was lower (61.9 ±7.9 mOD/min) compared to patients taking t-HT (77.7 ±14.5 mOD/min). There were no statistically significant differences between controls and women on t-HT treatment ($p = 0.132$). The concentration of TAFI in patients' plasma is shown in Table IV. The highest level of TAFI was observed in patients after oral hormones (80.38 ±8.23%); women on transdermal HT had 61.58 ±9.81% and the lowest concentration of TAFI was noted in the control group (44.70 ±10.16).

Discussion

Postmenopausal women are often prescribed estrogen-replacement therapy to treat menopausal

symptoms and to prevent osteoporosis, but there is increasing recognition that it is also associated with important health risks [11, 15]. Harmful effects of HT include breast cancer and venous thromboembolism (VTE). Hormone treatment might also increase the risk of coronary heart disease and stroke [16]. In our study, HT with estrogen combination of two different progestogens, dydrogesterone and 19-norethisterone showed significant differences in hemostatic parameters. We compared the effects of two preparations of 17β-estradiol/progestogen, one oral and one transdermal, on markers of coagulation and fibrinolysis. Both preparations are low-dose continuous combined HT products, thus the data allowed us to estimate the impact of the route of administration. We found that the transdermal route of hormones administration appears to have a less marked effect overall on the hemostatic system compared to the oral form and non-users. Levels of fibrinogen did not change significantly in the transdermal group compared to controls, whereas a considerable increase was observed in the oral group. The unchanged fibrinogen concentration after transdermal HT has been also found in other studies [17, 18]. Apart from being an acute phase reactant, fibrinogen has been considered a major cardiovascular risk factor and its level seems to be important during HT treatment. Enhanced thrombin activity in Polish women on oral hormone therapy compared to the transdermal group and non-HT users has been observed by us and other groups of investigators assessing the use of estrogen therapy [19]. In most previous studies, investigators suggest that oral, but not transdermal hormones increase plasma concentrations of prothrombin fragment 1+2, which is a marker for in-vivo thrombin generation. In postmenopausal HT treatment, estrogens are usually given orally, but such a delivery route has drawbacks, including intestinal and hepatic first-pass effects. Oral estrogen administration leads to high hormone levels in the liver and promotes hepatic protein synthesis [20]. A study of pharmacokinetics of oral and transdermal estradiol showed a dose-dependent increase in serum estradiol exposure. The route of administration significantly affected fibrinolysis in this study. TAFI (thrombin activatable fibrinolysis inhibitor) plays an important role in regulation of this process. TAFI is activated by thrombin and protects the fibrin clot against lysis [21]. We found increased TAFI antigen levels in oral HT patients and this could be associated with a long time of fibrinolysis. We showed that the oral route of hormone administration appears to have a marked effect overall on the fibrinolytic system compared to the transdermal form and non-users. In accordance with these results, much lower amidolytic plasmin activity was found on oral treatment in our study, compared with transdermal HT and controls. We choose TAFI and plasminogen to measure because both are expressed in the liver and changes in these proteins may also influence plasmin production. Our studies show a relationship between high activity of thrombin generated and the level of TAFI in the plasma of patients taking oral hormone preparations. High concentrations of thrombin are required for the activation of TAFI and contrast the small amounts of thrombin that are sufficient for fibrin formation [22]. Our data suggest that oral hormone therapy might impair the balance between procoagulant factors and antithrombotic mechanisms, whereas transdermal treatment seems to have a little or no effect on hemostasis. Transdermal administration has the benefit of avoiding first-pass effect in the liver where many of the coagulation factors are synthesized. In addition to the influence of delivery route on hemostatic changes in HT patients, the dose and composition of each treatment can impact results. The oral HT used in our study was low-dose (1 mg daily) estradiol preparation combined with dydrogesterone (5 mg daily). It may explain a little the differences between our results and those found in similar ravages using higher-dose HT. The influence of hormone therapy on hemostasis has been largely attributed to the estradiol part of the preparation, although progestogen can also affect observed changes. The effect we observed may in part be explained by the dose and type of progestogen. Recent studies suggest that progestogens in oral combined postmenopausal therapy have differential effects on the fibrinolytic system and can change the prothrombotic impact of estradiol [23]. The clinical significance of our studies might be important for women at high risk of VE who need short-term HT for severe menopausal symptoms. We propose that the goal of selecting HT for climacteric complaints with optimal hemostatic safety should be replaced by that of finding a preparation with minimal changes in coagulation and fibrinolysis markers. Both efficacy and safety can accordingly be evaluated in future experimental studies.

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