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Estrogen therapy effects on different vasoactive factors in recent postmenopausal healthy women

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Abstract

Background: To evaluate whether the route of estrogen therapy (ET) may affect the levels of different vasoactive factors in healthy recent post-menopausal women.
Methods: We conducted a cross-over study in 20 healthy nonsmoking women in recent postmenopause (1.8±0.1 years). Women received either 1-month oral-ET (O-ET, 2 mg oral micronized 17β estradiol daily) or transdermal-ET regimen (T-ET, 17β estradiol 1.5 mg gel daily) with a 1-month wash-out interval. Blood pressure, plasma levels of endothelin-1 (ET), 6-ketoPGF1a (6-ketoPG, prostacyclin metabolite), nitrite/nitrate (NOx), epinephrine (E) and norepinephrine (NE) and lipid profile were measured at baseline and after each treatment.
Results: Both regimens significantly reduced E ($p<0.01$) and NE levels ($p<0.05$). O-ET reduced low-density lipoproteins (LDL) levels ($p<0.05$) and increased NOx values ($p<0.01$). Neither regimen caused significant changes of ET or 6-ketoPG.
Conclusions: Our results, obtained in healthy women in recent menopause, indicate that the ratio between vasodilator (NOx and prostacyclin) and vasoconstrictor (ET) bioavailability shifted towards the previous ones after O-ET, while it remained unchanged after T-ET; moreover, catecholamines levels were reduced by both treatments already from 1 month of therapy. These changes might represent very early beneficial effects evoked by ET on the cardiovascular system.

Keywords: Postmenopausal women; Estrogen therapy; Nitric oxide; Catecholamines; Endothelin; Prostacyclin; LDL; HDL

1. Introduction

Cardiovascular disease incidence is more prevalent in men as compared to women, with difference most marked at younger ages [1]. In reality, after menopause, the reduced risk is gradually lost in women, suggesting that the loss of endogenous estrogens may contribute to the rapid raise of cardiovascular risk in elderly women [2]. Thus, many researchers have focused their studies on the role of sex hormones in the prediction of adverse coronary outcomes, in the promotion or inhibition of atherogenic events and in the effects on endothelial function [1–3]. To date, hormone replacement therapy has solicited great attention and the ratio between advantages over disadvantages of its use represents now one of the major public health issues, also in consideration of the fact that the average life span has extended to 25–30 years beyond the end of the physiological ovarian function.

Observational studies suggest that estrogen therapy with or without the addition of progesterone (HRT and ET, respectively) reduces significantly the cardiovascular risk [4,5]. However, recent results obtained by clinical studies of combined hormone replacement therapy, such as Heart and Estrogen/Progestin Replacement Study (HERS) and Women's Health Initiative (WHI) Trial, have added controversial data to the institution of HRT in postmenopausal women.

Noteworthy, HERS recruited older women (>65 years) who had generally pre-existing coronary artery disease [6]. In addition, to note, most of the subjects enrolled in the WHI are beyond 65 years of age and began treatment years after menopause [7]. Experimental studies on monkeys in the early stage of atherosclerosis indicate marked beneficial

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effects following estrogen treatment, suggesting that the situation may be different in the setting of advanced atherosclerosis as compared to normal vessels [8]. Other evidence suggests that estrogen treatment inhibits the neointimal response to acute injury in normal blood vessels but not in those with preexisting atherosclerosis [9]. Most importantly, atherosclerotic vessels show a greatly reduced density of estrogen receptors, as showed in diseased human coronary arteries [10]. Consequently, as considering that endothelial reactivity declines with aging, endothelial impairment can account, almost in part, for the findings of the great trials mentioned above [11]. Thus, may be of particular interest to focus the possible cardiovascular benefits of ET in the younger category of postmenopausal women.

Accordingly, in this study, we wanted to investigate the effect of ET (both in the oral and transdermal formulation) on different vasoactive factors (nitrite/nitrate, NOx as index of nitric oxide bioavailability, epinephrine [E], norepinephrine [NE], endothelin-1 [Endo], 6-ketoPGF1α, 6-ketoPG, prostacyclin metabolite and lipid profile) in recent postmenopausal healthy women.

2. Materials and methods

2.1. Study population and design

Twenty non-obese, healthy normotensive sedentary postmenopausal non-smoker women (age: 50±0.6 years, mean±S.E.) were enrolled. They were randomized to start O-ET (2 mg oral micronized 17β estradiol daily) or T-ET (17β estradiol 1.5 mg gel/daily) for 1 month, with a 1-month wash-out interval; the treatment was crossed over for the second month of therapy (Fig. 1). The rationale for adopting a cross-over design is that the effect of a number of treatments may be assessed in a small group of subjects, with the same power as that of a larger parallel study. In fact, the total number of subjects needed for a parallel trial resulted fourfold of that required for a two-period cross-over trial [12]. In addition, recent investigations on the estrogen receptor, the genome and cellular cofactors have demonstrated a heterogeneous molecular response to hormones, which implies a heterogeneous response among subjects [13]. Thus, we designed our study so to allow each patient to act as her own control, averaging out the many biases which may affect the final results.

Cardiovascular or other major diseases (e.g. diabetes, hypertension, hypercholesterolemia, etc.) were excluded on the bases of physical examination, ECG and routine laboratory tests. Subjects were excluded if they were on any pharmacological treatment. All women enrolled had similar dietary habits that were characteristic of the Mediterranean diet which was prescribed and checked at regular intervals by a dietician, as we previously reported [14]. In addition, all participants fasted for at least 12 h over the study period were requested to adhere to a low-nitrate diet (namely, exclusion of food containing a high concentration of nitrate such as processed meat, fruits and, in particular, leafy green vegetables) for 72 h before collection of blood samples. None had ever received hormone replacement therapy.

Fully informed consent was obtained from each subject entering the study and the experimental protocol was approved by the local Hospital Ethics Committee.

2.2. Recording of blood pressure, blood sampling and analytical methods

Each subject was allowed to rest in a supine position for at least 15 min before recording blood pressure, which was carried out by means of a Dinamap automatic recorder (Critikon, USA). Accordingly, blood pressure values reported in the present study, represent the average of at least three recordings, allowing 3-min intervals between single readings. Once blood samples were collected, they were immediately centrifuged at 3000×*g* at 4 °C for 10 min and stored at −80 °C for less than 2 weeks before subsequent analysis.

Serum samples were obtained for follicle-stimulating hormone (FSH) and estradiol (E_2) evaluation, determined by fully automated laboratory methods (Access, Beckman).

Plasma concentrations of total cholesterol, HDL cholesterol and triglycerides were determined by standard laboratory methods. The concentration of LDL cholesterol was calculated using the Friedewald equation.

Plasma E and NE levels were measured by an automated HPLC analyser based on fluorescence detector (HLC-725, Eurogenetics-Italia, Torino).

To perform NOx assay, plasma samples were ultrafiltered through 30 kDa molecular weight cut-off filters and centrifuged at 1000×*g* for 60 min. NOx concentration in different dilutions of plasma ultrafiltrate was determined by a colorimetric assay kit (Cayman, Ann Arbor, USA) based on Griess reaction. The limit of quantification was 1 μmol/l. Intra- and inter-assay coefficient of variations was always lower than 5% [15].

Concentrations of endothelin-1 in plasma samples were determined by means of a kit for competitive enzyme-linked immunoassay of endothelin-1 (Cayman Chemicals, Ann Arbor, MI, USA). Before the assay procedures, samples were subjected to purification by means of Supelclean LC-18 columns (Supelco, Bellefonte, PA, USA), according to manufacturer's instructions. The limit of quantification was 0.5 pg/ml. Intra- and inter-assay coefficient of variations was always lower than 4%.

The concentration of 6-keto prostaglandin $F_{1\alpha}$ was assayed in plasma samples by means of a kit for competitive enzyme-linked immunoassay of prostacyclin (Cayman Chemicals, Ann Arbor, MI, USA). Before the assay procedures, samples were subjected to purification by means of Supelclean LC-18 columns (Supelco, Bellefonte,