CUTANEOUS MICROVASCULAR EFFECTS OF MID-TERM HORMONE REPLACEMENT THERAPY IN HEALTHY POSTMENOPAUSAL WOMEN

A prospective placebo controlled trial

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ABSTRACT:

Cutaneous microvascular effects of mid-term hormone replacement therapy in healthy postmenopausal women. A prospective placebo controlled trial

Objective: To study the mid-term effects of Hormone Replacement Therapy (HRT) on cutaneous microcirculatory blood flow and reactivity in healthy postmenopausal women.

Design: In a double-blind placebo controlled randomized study, 16 healthy postmenopausal women received either placebo or HRT (micronized estradiol: 1 mg/day, day 1-28, promegestone: 0.25 mg/day, day 14-28). This regimen was completed 6 times.

Cutaneous microcirculatory blood flow was recorded by laser-Doppler velocimetry on the foot dorsum, in the supine and then dependent positions, and after post-ischemic hyperemia.

Results: At day 0, the two groups were similar and none of the following data differed significantly between treated and placebo group: (supine flux: 11.8 ± 1.8 u vs. 13.2 ± 3.9, venoarteriolar reflex: 5.6 ± 1.3 vs. 6 ± 3.3, and post-ischemic hyperemia: 35.2 ± 3.9 vs. 48.3 ± 11). At the end of the study (day 26-28 of 6th cycle), the supine flux was 9.8 ± 2.1 in the HRT group vs. 12.9 ± 6 in the placebo group (NS), the venoarteriolar reflex, 1.2 ± 2 vs. 7 ± 1.7 (p = 0.04), and post ischemic hyperemia, 31.8 ± 5.4 vs. 39.5 ± 4.6 (NS).

Intragroup values did not change significantly for any of the microcirculatory parameters measured, which remained stable throughout the 6 months of the study.

Conclusions: HRT does not impair the resting supine cutaneous microcirculation blood flow or post-ischemic hyperemia. (J Mal Vasc 2003; 28: 190-193)

Key-words: Hormone replacement therapy, Laser-Doppler velocimetry, Microcirculation.

RÉSUMÉ:

Etude à moyen terme des effets microcirculatoires cutanés du traitement hormonal substitutif de la ménopause chez des femmes ménopausées en bonne santé : étude prospective, randomisée, versus placebo

Objectif: Les actions vasculaires du traitement hormonal substitutif de la ménopause ont fait l’objet de nombreux travaux qui ont été étudié le plus souvent la macrocirculation et plus rarement la microcirculation.

Nous avons étudié dans une série de femmes ménopausées en bonne santé, l’action à moyen terme du traitement hormonal substitutif de la ménopause, sur les flux cutanés microcirculatoires et sur la vasoréactivité.

Méthodes: Dans cette étude contrôlée, randomisée, versus placebo, 16 patientes ménopausées en bonne santé ont reçu soit un placebo soit un traitement hormonal substitutif de la ménopause (estradiol micronisé : 1 mg/jour pendant les 28 jours du cycle et promegestone : 0.25 mg/jour entre les 14 et 28ème jours). Cette séquence a été répétée 6 fois.

Les flux cutanés microcirculatoires et la vasoréactivité ont été étudiés à l’aide de la technique laser-Doppler au niveau du dos du pied, en position allongée, assise et après une hyperémie.

Résultats: A J0, les deux groupes (traitement substitutif versus placebo) sont similaires et ne diffèrent pas statistiquement: (flux bas al longé 11.8 ± 1.8 unités vs. 13.2 ± 3.9, réflexe veino-artériolaire 5.6 ± 1.3 vs. 6 ± 3.3, et hyperémie postischémique 35.2 ± 3.9 vs. 48.3 ± 11).

À la fin du sixième cycle (jour 26-28) le flux bas al longé était de 9.8 ± 2.1 dans le groupe traitement substitutif vs. 12.9 ± 6 dans le groupe placebo (non significatif), le réflexe veino-artériolaire 1.2 ± 2 vs. 7 ± 1.7 (p = 0.04), et l’hyperémie postischémique de 31.8 ± 5.4 vs. 39.5 ± 4.6 (non significatif).

Les résultats intragroupes sont restés stables tout au long des 6 mois de l’étude.

Les résultats intergroupes objectivent peu de différences entre les différents paramètres microcirculatoires à l’exception du réflexe veino-artériolaire qui, à la fin de l’étude (j 26-28 du sixième cycle), est plus petit dans le groupe traitement hormonal substitutif que dans le groupe placebo, p = 0.04.

Conclusion: Le traitement hormonal substitutif de la ménopause ne semble pas délétère sur la microcirculation cutanée et ne modifie ni le flux bas al en position allongée ni la réaction d’hyperémie. (J Mal Vasc 2003; 28 : 190-193)

The cardiovascular effects of Hormone Replacement Therapy (HRT) have been extensively studied at the macrocirculatory level, by examining the interactions of this therapy with hemostatic variables, serum lipids and the lipoprotein profile, carbohydrate metabolism and nitric oxide regulation (1, 4) and the results of these studies suggest that estradiol provides protection against cardiovascular disorders. However, two recent studies based on the occurrence of clinical events, the Heart and Estrogen/Progestin Replacement Study (HERS) and the Coumadin Aspirin Reinfection Study (CARS) made this issue controversial, by suggesting that HRT may not afford such protection (5, 6). The role of the microcirculation in the onset of symptoms of coronary heart disease has been shown and the prevalence of these symptoms is decreased by HRT (1). The microcirculation may therefore be a target for HRT as well as the macrocirculation.

The microcirculation is easily accessible for evaluation in the skin, where measurement of blood flow and microvascular reactivity allow non invasive evaluation of the functional reserve of the cutaneous microcirculation.

To gain insight into the impact of HRT on the cutaneous microcirculation, we investigated the effect of HRT on laser-Doppler cutaneous microcirculatory blood flow and reactivity in healthy postmenopausal women, in a controlled, randomized, double-blind study.

METHODS

STUDY POPULATION

This prospective randomized double-blind placebo controlled study was approved by the local Ethics Committee and all subjects included gave written informed consent to participate. The supplier of the study was Aventis Laboratory.

All these subjects were healthy non smoker (for at least 12 months), normal weight (BMI between 18 and 27) 54 to 67 year old postmenopausal women. All subjects had reported at least 12 months of amenorrhea and were willing to use HRT. All patients had normal blood pressure, blood lipids and blood glucose levels. Before inclusion, hormonal status was evaluated (estradiol level) and all participants underwent mammograms and had pap smears taken.

TREATMENT DESIGN

Sixteen women were randomly assigned to either the treatment or placebo group. Those in the treatment group were given 1 mg/day of oral micronized 17 beta estradiol (E) for 14 days, and then E + 0.25 mg/day of progesterone (P), for the next 14 days. The other group was given similar placebo treatment. The same 28-day sequence was completed for 6 cycles in both groups. The treatment therefore lasted for 6 cycles and was scheduled as follows : D1-14 : E, D15-28 : E+P, for each cycle.

MICROCIRCULATORY EVALUATION

The cutaneous microcirculation was assessed by a laser-Doppler velocimeter (Model PF4, Perimed, Stockholm, Sweden).

With this technique, a fiber-optic probe delivers a laser light to the skin and this light is diffusely scattered. A fraction of the light is also scattered by moving erythrocytes within the microcirculation, thus forming a beam of detectable Doppler shifts. The intensity of this beam, expressed in arbitrary units, is linearly related to the product of the erythrocyte concentration and velocity, giving an index of the microcirculatory flux. The results of laser-Doppler velocimetry correlate with those of other methods used to assess peripheral blood flow. Repeated measurements of this flow from the same area of skin under carefully controlled conditions produce coefficients of variation of 9-14% (12).

Here, patients were studied in a quiet temperature-controlled laboratory (22.5 ± 0.3 °C), after an acclimatization period of 10 minutes, to allow them to reach thermal equilibrium. Subjects were asked to refrain from caffeine and alcohol absorption before the laser-Doppler test.

The laser-Doppler probe was placed on the dorsum of the foot, an area containing few arteriovenous anastomoses.

In order to study both fluxes and reactivity, we recorded fluxes in the supine and then dependent positions and after post ischemic hyperemia. The dependent position elicits a type of vasoconstriction called the venoarteriolar reflex, which constitutes around 79 % of the supine flux in normal subjects (12), and concerns a vasoconstrictive part of vasoreactivity. Ischemia elicits a type of vasodilation called hyperemia, which constitutes around 400 % of the supine flux in normal subjects (13), and concerns a vasodilative part of vasoreactivity.

The protocol comprised the following 4 parts (fig. 1) : 1) flux recording in the supine position (a), 2) flux recording in the dependent position (b), 3) residual flux recording with a tourniquet placed for 4 minutes around the ankle and inflated above the systolic pressure (c), (this parameter is called the biological zero and its value is subtracted from the value of each parameters measured), and 4) the peak flux recording after the release of the tourniquet (d).

![Fig. 1. Scheme of the laser-Doppler velocimetry protocol. a = flux recording in the supine position, b = flux recording in the dependent position, c = residual flux recording with an ankle tourniquet (biological zero), d = flux at the peak of hyperemia, H= hyperemia, VAR = Venoarteriolar reflex.](image-url)

This set of microcirculation measurements was repeated 5 times: at inclusion (baseline), and on first cycle at days 12-14 (E period) and days 26-28 (E+P period), and on sixth cycle at days 12-14 (E period) and days 26-28 (E+P period).

The venoarteriolar reflex (VAR) was calculated as the supine minus dependent position flux, \( \text{VAR} = a - b \), and hyperemia (H), as \( d \) (peak flux recording after the release of the tourniquet).

**STATISTICAL ANALYSIS**

Continuous data are expressed as means ± standard error of the mean (SEM). For comparisons within and between the placebo and treatment groups, we used an analysis of variance.

**RESULTS**

**POPULATION CHARACTERISTICS**

Eight of the 16 women aged from 54 to 67 years who fulfilled the inclusion criteria and participated in the study were randomly assigned to the treatment group, and 8 to the placebo group.

*Table I* shows their baseline characteristics. No significant difference was found between the two groups for any of the baseline parameters (*table I*).

**EFFECTS OF TREATMENTS**

Intragroup analysis of variance showed no significant change in either of the two groups for any of the hemodynamic parameters measured during the 6 successive phases of the study.

Intergroup analysis of variance only showed a significant difference for venoarteriolar reflex at 6th cycle during EP period, when it was 1.2 ± 2 arbitrary units in the treatment group versus 7 ± 1.7 in the placebo group (\( F = 5, p = 0.041 \)).

*Table II* shows the laser-Doppler velocimetry measurements on the first day of the study (day 0) and after 6 months of treatment (days 12-14 : E period of cycle 6 ; days 26-28 : EP period of cycle 6) for the supine cutaneous flux, the postural venoarterial reflex, and hyperemia.

**DISCUSSION**

The results of the present study show that postmenopausal hormone therapy did not perturb either resting supine cutaneous microcirculatory blood flow or postischemic hyperemia.

To our knowledge, only three non-randomized studies have dealt with cutaneous laser-Doppler flow and reactivity among post-menopausal women on HRT (7, 8, 10).

Brooks-Asplund and Kenney (8), using a methodology based on laser-Doppler velocimetry, are expressed in arbitrary units.

**TABLE I** – Characteristics of the study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>Total Group</th>
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<tr>
<td>Number of patients</td>
<td>8</td>
<td>8</td>
<td>16</td>
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<tr>
<td>Age (years)</td>
<td>58.4 ± 0.6</td>
<td>57 ± 0.7</td>
<td>57.7 ± 0.5</td>
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<tr>
<td>a</td>
<td>11.8 ± 1.8</td>
<td>13.2 ± 3.9</td>
<td>12.49 ± 2.1</td>
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<tr>
<td>VAR</td>
<td>5.6 ± 1.2</td>
<td>6 ± 3.3</td>
<td>5.8 ± 1.7</td>
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<tr>
<td>H</td>
<td>35.2 ± 3.9</td>
<td>48.3 ± 11</td>
<td>42.8 ± 6.4</td>
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<th>Days 26-28 (cycle 6)</th>
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<tr>
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Résultats des données laser-Doppler dans les 2 groupes placebo et traitement substitutif au début de l’étude (jour 0) et au sixième cycle (jour 12-14 et jour 26-28 de ce 6e cycle)

a, VAR et H correspondent au schéma de la figure 1 où a= flux du dos du pied en position allongée, VAR= réflexe veino-arteriolaire et H= hyperémie. Les résultats du laser-Doppler sont exprimés en unités arbitraires.
similar to ours, reported similar results, i.e., that HRT did not affect either baseline or maximal cutaneous blood flow, as measured by laser-Doppler velocimetry. In the two other studies (7, 10), the authors, reported that HRT increased both endothelium-dependent and endothelium-independent vasodilation, but the methodology used in these studies was very different, as it consisted of laser-Doppler imaging and iontophoresis.

Recently Vuilleumier and coll. (14) have shown in a large study that postischemic forearm skin hyperemia is inversely correlated to cardiovascular risk in healthy women using the Framingham risk score. In this study risk scores were low as well as in ours, and HRT had no significant impact on postischemic forearm skin hyperemia.

In the present study, the only significant change in laser-Doppler hemodynamics was a diminution of the venoarteriolar reflex, which was found at the end of the study after 6 months of HRT treatment, but was not found in the placebo group. This result might be placed alongside that reported by Hassan and coworkers, who found that VAR diminished during the luteal phase of the normal menstrual cycle (15) and may be related to the progesterone induced vasodilatation.

Our results for the cutaneous microcirculation are similar to those found for other organs such as the heart, where, for instance, HRT did not affect endothelium-independent myocardial perfusion (16).

Note, however, that the present study had several limitations: first, the microrcirculatory blood flow varied considerably as a consequence of the physiologic rhythmical vasomotion, a usual limitation of laser-Doppler velocimetry. As against this, the possible resulting bias was limited by the carefully controlled conditions of the study, including the methodology used for the laser-Doppler measurements. Secondly, only a few subjects were studied, and the methodology using 5 repeat measures throughout the 6 phases of the study reduced its statistical power. Thus, the statistical tests used in the study were based on a difference methodology and not an equivalence methodology. Therefore, it is possible that there are differences between HRT and placebo which were not found in our study.

In conclusion, hormone replacement therapy did not perturb either resting supine cutaneous microcirculatory blood flow or post ischemic hyperemia.

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RÉFÉRENCES