The effects of systemic hormonal replacement therapy on the skin of postmenopausal women

A.V.D. Sauerbronn\textsuperscript{a*}, A.M. Fonseca\textsuperscript{a}, V.R. Bagnoli\textsuperscript{a}, P.H. Saldiva\textsuperscript{b}, J.A. Pinotti\textsuperscript{a}

\textsuperscript{a}Departments of Obstetric and Gynecology, Hospital das Clínicas of University of São Paulo Medical School, São Paulo, Brazil

\textsuperscript{b}Department of Pathology, University of São Paulo Medical College, São Paulo, Brazil

Received 28 May 1999; received in revised form 31 August 1999; accepted 1 September 1999

Abstract

Objective: The aim of this study was to determine the effects of hormonal replacement therapy on the skin of postmenopausal women. Method: Forty-one postmenopausal women were randomly allocated to receive either hormonal replacement (valerate estradiol — 2 mg/day for 21 days and cyproterone acetate — 1 mg/day for 10 days) or placebo, both in a cyclic scheme for 6 months. Neither patients nor investigators were aware of the group allocation. Histologic changes were evaluated by skin biopsy of the left upper arm at baseline and after 6 months of treatment, utilizing computerized image analysis to assess the ratio area of epidermis/basement membrane length (AE/BML), ratio area of keratin/basement membrane length (AK/BML) and collagen and elastic fibers content. Result: Collagen content of the left upper arm increased after 6 months of treatment only in the hormonal group (+6.49%; \textit{P} < 0.05). Other parameters did not present any significant alteration after treatment in both groups. Conclusion: Hormonal replacement for climacterics increases skin collagen content. © 2000 International Federation of Gynecology and Obstetrics.

Keywords: Hormonal replacement therapy; Menopause; Skin; Collagen

* Corresponding author.
E-mail address: q802834@zaz.com.br (A.V.D. Sauerbronn)
1. Introduction

Hypoestrogenism has been implicated in many diseases, signs and symptoms such as osteoporosis, cardiovascular disease, Alzheimer disease, uro vaginal hypotrophy and hot flushes. These factors cause the quality of life to deteriorate. Despite the small importance attributed to skin, mucous and their annexes, they are also likely to be influenced by hypoestrogenism, interfering with the well-being sensation and quality of life.

Human skin presents nine different types of collagen and 80% of them correspond to type I and 15% to type III [1]. Collagen type I is the main constituent of skin, corresponding to 70% of its dehydrated weight. Collagen fibers are the main factor to assure skin resistance.

Skin collagen behavior is comparable to collagen in other locations of the human body. When a correlation is done between skin collagen and bone density, for example, we can see many similarities such as timing of peak, which occurs between 30 and 35 years [2] and a faster rate of loss at the initial postmenopausal years than at the later ones, with approximately 30% being lost in the first 5 years [3]. The average rate of loss of skin collagen is 2.1% per postmenopausal year [4].

After menopause, the different layers of the skin are changed — keratin layer is reduced, epidermis is thinner, with flattening of interpapillary crests and dermoepidermal junction. A decrease of collagen type I in the skin leads to a decrease of skin thickness, making it more transparent and vulnerable to injuries [5].

Positive effects of estrogen on the skin collagen were demonstrated in different areas of the human body [4–7]. These results apply mainly to collagen type I, the most important constituent of dermis, nevertheless, collagen type III also responds positively to estrogen [8].

Under different schemes and doses, estrogen makes skin thicker, either in solar protected areas [3,9,10] or solar exposed areas [10–12]. On the other hand, there are also studies demonstrating that HRT does not promote positive effects on the skin [1,10,13,14].

Although there are many studies on the influence of sexual steroids on the skin, there are still many controversies, mainly with systemic treatment under regular doses.

2. Objective

The aim of this study was to evaluate the effects of systemic HRT on the skin of postmenopausal women, analyzing the total amount of collagen fibers, elastic fibers, area of keratin and epidermis.

3. Method

This was a randomized, double-blind, placebo-controlled study. We studied 41 postmenopausal women from the outpatient clinic of the Department of Obstetrics and Gynecology of ‘Hospital das Clínicas’ from São Paulo University Medical School, to be observed during 6 consecutive months, receiving either HRT (group H, n = 21) or placebo (group P, n = 20). Group H received medication consisting of 21 tablets, the first 11 with 2 mg of valerate estradiol and the last 10 with 2 mg of valerate estradiol associated with 1 mg of cyproterone acetate. Group P (control) received 21 tablets of placebo. Study medication was disposed in a calendar-blister, making it easier for patients to follow the correct sequence of ingestion. Neither patients nor investigators knew at any time, which drug was placebo or hormones.

After checking the inclusion/exclusion criteria for admission (Table 1), patients were oriented about nature, procedures and objectives of the study. Each patient received a randomization number, in accordance with her order of inclusion.

All criteria described in Table 1 were observed, taking into consideration familiar and personal history (amnnessis), as well as physical and gynecologic examination. The following subsidiary exams were carried on in order to check other parameters: transvaginal ultrasonography; pap smear; and bilateral mammography.
Table 1
Inclusion and exclusion criteria

**Inclusion criteria**
1. Postmenopausal women (natural or artificial menopause for at least 1 year).
2. Intact uterus.
3. Indication to receive HRT.

**Exclusion criteria**
1. Any kind of diseases with cutaneous implications.
2. Presence or suspicion of pregnancy.
3. Presence or suspicion of chronic or acute hepatic disease.
4. History of idiopathic jaundice or severe pruritus of pregnancy.
6. Abnormal pap smear.
7. History or presence of thromboembolic disease.
8. Sickle cell anemia.
9. History, presence or suspicion of uterine or mammary neoplasm.
10. History of pituitary or hepatic benign tumors.
11. History of familial hiperlipidemy or hiperlipoproteinemy.
13. Diabetes mellitus or other endocrine diseases.
15. History or presence of otosclerosis.
16. Multiple sclerosis, epilepsy, porphyria, tetany.
17. Smokers more than 10 cigarettes per day or women who drink more than 40 ml of alcohol per day.
18. Radiotherapic treatment or use of cytostatics.
20. History of use of estrogen implants in the last 2 years.
21. Use of drugs that interfere with sexual steroids metabolism (i.e. barbiturics, benzodiazepinics and rifampicin).

Patients were oriented to take one tablet per day with a small amount of water, always at the same time, until the end of the calendar-blistter, followed by a free interval of 7 days. On the 8th day a new calendar blister was initiated.

3.1. Procedures during visits of evaluation

During the study, patients received cards to note complaints and vaginal bleedings. When this was not possible, they were oriented to note just the days with bleeding, as well as days of tablets intake or any other relevant information to the treatment in a blank page. If necessary, they were oriented to ask their friends or relatives for help.

This study comprised a total of four visits, including anamnesis, detailed physical and gynecological examination. At each visit, cycle calendars were checked and filed in the respective report forms. Blisters were dispensed to the patients every visit, 1 month earlier than necessary until next visit, due to safety reasons. Each visit took place between days 20 and 28 of the cycle.

3.2. Skin biopsy

Skin biopsies were carried out twice: at inclusion visit and on the fourth visit (6-month treatment), between days 20 and 28 of the cycle. We used a dermatological punch of 3 mm (Stiefel®) to extract a fragment from keratin to hypoderms, at the medial part of the left arm, approximately 4 cm above pleat of elbow. The second biopsy was performed 1.0 cm from the scar of the first biopsy and equidistant from pleat of elbow. This area is sun protected with the advantage of being very aesthetic. Suture was not necessary.

After collection, all fragments were fixed in a formalin (buffer) solution 10% and sent to the histology laboratory where they were included in
paraffin blocks and kept until the end of the study.

3.3. Histological analysis of fragments of the skin

3.3.1. Optic microscopy

The fragments of the skin were cut in three slices, each 3–4 μm thick. The slices were submitted to different methods of coloration, in accordance with the parameters to be analyzed, by the following coloring processes: hematoxin-eosin (HE) (to evaluate epidermis area, keratin area and length of basal membrane), oxidized resorcin-fuscin (ORF) (quantitative evaluation of elastic fibers), and picrosirius (PS) (evaluation of collagen fibers of dermis) [15].

Quantitative histology evaluation was carried out through computerized digitized image analyzer (software Optimas®). Computerized image analysis has shown its value in quantifying microscopic structures as elastic [16], and collagen fibers [17].

Each slice had five microscopic fields analyzed under vision of 400×. All data were entered in a chart and randomization codes were broken only after the last slice was analyzed. To compare epidermis data, we calculated the rates ‘area of epidermis/basement membrane length’ (AEBML) and ‘area of keratin/basement membrane length’ (AK/BML) in each microscopic field.

3.4. Ethical aspects

This protocol was approved by the ‘Hospital das Clínicas’ from University of São Paulo Medical School IRB and every patient was included only after giving her free written consent for participation.

3.5. Statistical analysis

Classifiable variables were presented in tables with absolute and relative frequencies. The rate of these factors were compared with the chi-square test or Fischer’s exact test in both hormone and placebo groups.

Continuous variables were presented in tables with mean and S.D. values. The mean related to each group was compared with the Student t-test, when distribution was normal, or Wilcoxon’s test of sum of posts.

The variable evaluated in more than one condition (visits) were studied through the mean profile analysis, where the following hypothesis were tested:

- $H_0$: mean profiles are parallel, both groups present the same behavior along the time.
- $H_1$: both mean profiles are coincident, there is no difference among the means of both groups along the visits.
- $H_2$: there is no effect of time, means are constant along the visits.

The level of significance adopted in this study was 0.05.

4. Results

During the study there were three patients who discontinued study medication — one due to the suspicion of thrombophlebitis and two others due to protocol violation; one of them was substituted and therefore, this study included a total of 41 patients of whom 38 completed the study, 19 belonging to the group H and 19 group P.

There was no difference, between the groups at baseline, concerning skin color ($P = 0.408$), chronological age ($P = 0.730$), time of menopause ($P = 0.386$), body mass index (BMI) ($P = 0.554$), number of pregnancies ($P = 0.843$), parity ($P = 0.436$) and Kupperman’s Index (KI) ($P = 0.734$).

No patient discontinued treatment while this study was going on, nevertheless there were patients that forgot to take tablets, in both groups. The highest rate of forgotten tablets in the same cycle in group P was 14 tablets cycle 4, and four tablets in group H cycle 4. The remaining patients forgot to take from one to four tablets in the same cycle and there were no missed tablets in more than two cycles for each patient in both groups.

Although groups P and H had 19 women each, we were not able to have data of microscopic evaluation from all of them; this was due to the
fact that not all the final material provided confident analysis. In most of these cases we could not proceed with histological analysis, and the main cause was technical problems with inclusion and fixation of the fragment, which presented altered persisting architecture even after several slices of the block. Therefore, we had 16 cases in group P and 18 in group H for epidermis analysis, 14 cases in group P and 17 in group H for collagen analysis, and 16 cases in group P and 18 in group H for elastic fibers analysis.

4.1. Collagen fibers

There was no difference at baseline in both groups concerning collagen fibers content (group H = 21897.40 ± 1635.33; group P = 22014.63 ± 1858.10; \( P > 0.05 \)). After 6 months of treatment, there was a statistical significant increase of collagen content in group H, compared to baseline (23318.20 ± 2027.56; 21897.40 ± 1635.33; \( P < 0.05 \)). There were no changes in group P (Table 2).

4.2. Other skin parameters

In other skin parameters such as thickness of epidermis (AE/BML), thickness of keratin (AK/BML) and elastic fibers content, there were no significant differences between baseline and 6-month treatment in both groups.

5. Discussion

The comparative use of placebo and a cyclic estroprogestogenic preparation may be considered inappropriate for a double-blind study, once hormones can induce withdrawal bleeding. On the other hand, postmenopausal endometrium is hypotrophic in most women and once we begin HRT, it may take some time until deprivation bleeding occurs. This phenomenon may take approximately 2–3 months in certain women and there are others that do not bleed, or bleed too little.

In the placebo group only one patient bled during the 6-month treatment, while in the hormone group there was one woman that never bled. Five other patients did not present bleedings from two to four consecutive cycles in the hormone group. Therefore, it was not possible to assure which medication each patient was taking, placebo or hormone, what endorses the double-blind pattern.

There are many studies about the effects of HRT on the skin, nevertheless only a few of them were double-blind, placebo-controlled, and all of

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Visit</th>
<th>n</th>
<th>Mean</th>
<th>S.D.</th>
<th>Median</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone</td>
<td>1</td>
<td>17</td>
<td>21897.4</td>
<td>1635.3</td>
<td>22224.7</td>
<td>17941.3</td>
<td>24511.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>17</td>
<td>23318.2</td>
<td>2027.6</td>
<td>22964.9</td>
<td>19778.0</td>
<td>27438.5</td>
</tr>
<tr>
<td>Placebo</td>
<td>1</td>
<td>14</td>
<td>22014.6</td>
<td>1858.1</td>
<td>22338.6</td>
<td>18187.8</td>
<td>25079.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>14</td>
<td>22057.2</td>
<td>2405.7</td>
<td>22367.9</td>
<td>16706.7</td>
<td>25370.9</td>
</tr>
</tbody>
</table>

Hypothesis \( H_{0j} \) \( P \) 0.034

Contrast Visit 1 Visit 4
Hormone \( \times \) placebo \( > 0.05 \) \( < 0.05 \)
Contrast Visit 1 \( \times \) visit 4 Hormone Placebo
\( < 0.05 \) \( > 0.05 \)

*Abbreviations.* Min, minimum; Max, maximum; S.D., standard deviation.
them employed different estrogen, different ways of administration, different schemes of treatment, and evaluated different aspects of the skin.

The first double-blind study was from Bologna et al. [19], they demonstrated that there were no significant differences between transdermal estradiol plus medroxiprogestosterone acetate (MPA) and placebo plus MPA groups, concerning many cutaneous signs and symptoms related to the aging process, such as dryness of sun exposed skin and pruritus. On the other hand, they demonstrated that earlier menopause is related to degenerative changes in elastic fibers.

Another double-blind study was from Maheux et al. [9]. They evaluated the effects of conjugated equine estrogens (CEE) alone, compared to placebo on the thickness of the skin measured by both ultrasound and biopsy. Skin thickness was found significantly increased in the hormone group with both methods.

Unfortunately, the double-blind studies above have not measured skin collagen. This is the first double-blind, placebo-controlled study, which evaluated the effects of the association valerate estradiol and cyproterone acetate on the skin. We have studied collagen and elastic fibers, as well as epidermis and keratin thickness. We have found a significant increase in the amount of collagen fibers through computerized image analysis in the hormone group after a 6-month treatment period (+6.49%; \(P < 0.05\)). Our results are in accordance with many other studies [2,6,7,20]. Considering that skin collagen loss rate is 30% during the first 5 years of postmenopause [3], 6.49% of increase in skin collagen rates after 6-month treatment period may be clinically relevant.

There are published studies that evaluated the effects of valerate estradiol (E\(_2\)V) on postmenopausal women's skin. Punnonen et al. [11] studied the effects of E\(_2\)V alone and associated to levonorgestrel. The authors verified that at the end of the treatment period (from 3 to 6 years), every group presented increased skin thickness. Despite the fact we have not evaluated skin thickness, we found no alteration in epidermis (AE/CMB) and keratin (AQ/CMB) thickness.

There are few references to the effects of sexual steroids on elastic fibers. It is mentioned that they appear to be less fragmented and thicker after estrogen therapy [19]. Our study did not find any correlation between estrogen treatment and elastic fibers. Perhaps the low turnover rate of elastic fibers (estimated in years) may explain our results.

Skin collagen increase with HRT is limited, and after a certain time of treatment it will not change [3,7]. Quantitative alterations of collagen reach the highest level after 1.5–2 years of treatment. The effects of sexual steroids on the skin may vary depending on the kind of estrogen, as well as the dose and time of administration. There is a range for optimal dosage, which, if not observed, will not produce favorable results [3,7]. Despite this, weaker estrogens seem to present better results than stronger estrogens as estradiol, at least when the topic route is used [18].

Extrapolation of data from scientific studies to clinical practice must be careful. It is always important to remember that there are many aspects which may influence the results. In the past, cutaneous collagen was considered to increase with aging, based on rat experiments [21]. Interpretation of the results of scientific studies must always be carried out on a critical basis. We must not forget that we always prescribe medication expecting to achieve a specific effect, however, not every patient will present the same answer [22].

Although most results of several clinical studies indicate a positive influence of estrogen on the skin, more studies are necessary, in order to clarify the effects of systemic HRT on the skin of postmenopausal women.

6. Conclusion

The effects of valerate estradiol plus cyproterone acetate association on the skin of postmenopausal women evaluated after a 6-month treatment period were:

1. an increase of collagen fibers content of the dermis (+6.49%);
2. it does not interfere in the thickness of the epidermis;
3. it does not interfere in the thickness of the keratin; and
4. it does not change the elastic fibers content of the dermis.

References