The investigation of menopausal hormone therapy on the histological transition of endometrial tissue and the demonstration of selected steroid hormones antioxidant effect

Ph. D. theses

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I. INTRODUCTION

Menarche and menopause as the entry and the exit of the reproductive ages are particular changes in women's life. With the onset of reproduction women become able to give rise for a new life enabled by the cyclic function of the hypothalamic-pituitary-ovary axis. The menopause is characterized by the cessation of the ovarian function resulting in the decrease of estrogens/progesterone production, the cessation of ovulatory cycles and menstruation. The term menopause by definition is the time when a menstruation is not followed by another one within 6 months regulated by the own hormone production of the ovaries. The change in the estrogen-progesterone(-androgen) production and ratio during menopause can lead to severe burden of everyday life. Such might be vegetative symptoms, psychical complaints, signs of urogenital atrophy and the alterations of the libido and the skin.

Considering that women live the quarter-third of their life in the menopause the importance of menopausal care became highlighted. The aim of current medicine is a preventive-curative health care which can be performed at the highest level in a well-organized cooperation of several medical specialities in case of menopausal syndrome as well. As the consequence of menopausal hormone therapy the menstrual period returns during menopausal care however it can be a sign of distinct histopathological changes of the endometrium either.
Around the millenary large-scale clinical trials including postmenopausal women pointed out detrimental cardiovascular and cerebrovascular effects of the replacement of estrogen and progesterone steroid hormones. Current studies showed beneficial effects in menopausal age women if appropriate risk assessment was performed prior to the onset of the smallest, relieving, necessary dose.

II. AIMS

Beyond in vitro data the best practice of menopausal hormone therapy can be performed safely based on in vivo experimental results and human clinical trials. Based on this my aims were the following:

1. Do selected steroid hormones have a beneficial effect on the antioxidant homeostasis in human neutrophil granulocytes?
2. Do selected steroid hormones have a beneficial effect on the antioxidant homeostasis in rats especially on the liver tissue itself?
3. In postmenopausal women what is the effect of hormone therapy (estrogen-only, sequential and continous combined estrogen and progesterone therapy) on endometrial bleeding, on the histological transition of the endometrium and on the incidence of endometrial neoplasia?
4. Finally I present a practice method in menopausal hormone therapy for the risk assessment, the therapeutical and follow-
up strategy. This was used as the first guideline for the Hungarian Menopause Outpatient Units which was in connection with one of my articles published more than a decade ago.

Briefly the aims of the development of the Menopause Outpatient Unit were the following:
- treatment of the vasomotor, psychical and other symptoms related to the estrogen deficiency in menopausal syndrome
- prevention and treatment of postmenopausal osteoporosis and arthropathy
- prevention of ischaemic heart disease
- treatment of the urogenital atrophy symptoms
- contribute to the continuous medical care of people from perimenopause until senescence
- in general the improvement of quality of life and life expectancy

III. PATIENTS AND METHODS

Experiments with human neutrophil granulocytes

For the demonstration that estrogen therapy has any influence on the intracellular granulocyte myeloperoxidase (MPO) activity and the secreted amount of the neutrophil cells eleven non-smoking, menopausal women (age: 48-64 years, mean: 56 years) started the following oral hormone replacement therapy protocol: estrogen-only (1 mg estriol + 2 mg estradiol) for 12 days, followed by estrogen +
gestagene combination (1 mg norethisterone-acetate + 1 mg estriol + 2 mg estradiol) for 10 days and subsequently by half-dose estrogen-only (0.5 mg estriol + 1 mg estradiol) for 6 days. Peripheral blood was drowned before the onset (Day 0), and on the 13th and 40th day of therapy. Due to non-compliance the third blood draw could be performed only in 6 of the 11 patients. After preparation of granulocytes secreted MPO quantity was measured with ELISA technique, while the intracellular MPO activity with spectrophotometric assay respectively. The results were expressed as mean peroxidase index (MPXI) which shows the deviation of the mean MPO activity of the proband's neutrophil population in comparison with the population average. (The reference range is between +10 and -10.) The measurements of the first two time-points were analyzed with two-sample, paired t-test, and the measurements of the third time-point were assessed with two-sample, equal and non-equal squares of the mean t-test.

In vivo experiments with rats
Six separated, randomly selected groups of adult male Sprague-Dawley rats (n = 5 for each group), weighing 500-600 g, were studied. Three groups (control, Group 1 and 3) were fed lipid-rich food (cholesterol: 1%, olive oil: 10 %) and three groups (Untreated, Group 2 and 4) were fed normal, conventional food for 28 days. Drinking fluid was provided in automated watering devices. In Group 1 and 2 of experimental animals DHEA (400 µg/ml), in Group
3 and 4 DHEA-S (150 µg/ml) was added to the drinking water. The untreated and the control group received unaffected water. All groups had free access to food and water. The rats were housed on a controlled 12-h/12-h light/dark schedule and an ambient temperature. Blood samples were collected at day zero (Day 0) and day 28 (Day 28) by dorsal tail venipuncture. Measurement of total scavenger capacity (TSC) was carried out using a commercially available chemiluminometric assay kit. This method is applicable for the measurement of total free radical production irrespectively of its origin. The relative luminescence of every plasma sample was determined by comparing it to a blank control (sample/blank quotients). Decrease in sample/blank quotient means better antioxidant status (increased TSC value).

Fat content in the liver was determined by Sudan-staining from the fresh frozen tissue samples which were stored at -70 °C. A relative score of 0 to 3 was used for the evaluation of the liver fat content. Every group was evaluated with the sum of scores of individual animals.

Liver superoxide dismutase (SOD) catalase and glutathione S transferase (GST) activity was measured from the homogenized slices of liver tissue which was stored on -70 C by the Ewing-Janero, the Goth spectrophotometric and the modified Mauch-Dudler methods respectively. Protein contents were determined by the biuret reaction.
The matched sample/blank quotients of individual samples were analyzed. The TSC data from day zero (Day 0) and day 28 (Day 28) were compared with paired sample two-tailed t-test corresponding to the normal distribution. Significance level was set to 0.05. Statistical analysis was not performed with the liver histology and enzyme activity values of Day 28.

Effect of postmenopausal hormone therapy on endometrial bleeding and the histological transition of the endometrial tissue

In the six-year period between January 1, 2000 and December 31, 2005, 5893 patients were given care at the Menopause Outpatient Unit of the 1st Department of Obstetrics and Gynaecology at Semmelweis University. Among these women, 2122 patients (36.00%) have received hormone replacement therapy while 3771 of them (64.00%) did not rely on such treatment. Among the 5893 patients, 707 (12.00%) reported irregular bleeding. Of those bleeding 707 women, 577 had not received hormone replacement treatment earlier (15.3% with bleeding in the non-treated group) however in 130 patients one or another form of hormonal treatment was applied (6.13% with bleeding in the treated group), as part of their postmenopausal care. The 3771 patients without hormone therapy had the mean age of 52.3 years (range: 38-79 years). The 707 women with irregular bleeding had the mean age of 54.7 years (range 37-81 years). In my sample, of the 130 hormonally treated patients who had presented at our department for irregular bleeding, only 5 women
(3.85%) were given estrogen only, 85 patients (65.38%) received sequential combined preparations, while 40 women (30.77%) took continuous combined preparations. (The estrogen-only treatments were not started at our department.) The difference between the mean ages of the treated and non-treated groups was analyzed with two-sample t-test. The patients suffering from menstrual irregularity underwent fractioned curettage in which cervical and corporal scrapes were obtained for histological investigations.

Introduction to the methods of the Menopause Outpatient Unit Model
In my full PhD-theses the Menopause Outpatient Unit Model - published in 1996 - was described in details which is a complex, multi-level care system and its abstract would abundantly exceed the space of this short theses.

IV. RESULTS

Results of the studies with human neutrophil granulocytes
The MPXI was increased parallel to the estrogen treatment. The means of those with 12 day long treatment (-0.2) was significantly higher compared to the controls (-5.5). The white blood cell count (6.1 vs. 6.56 Giga/liter) and the neutrophil percentage (60.2 vs. 62.0) had no significant change during the study. A similar trend can be observed in the comparison of the MPXI values at the first and third blood draws (p<0.05).
No further relationship was seen between the second and the third sampling time-points which also means there was no further increase in the index with the continuation of the estrogen administration, however additional progesterone was used and a 50% estrogen dose reduction was carried out in the next 6 days. Thereafter 12 days further, initial dose of estrogen was used before the third blood draw.

The release of the MPO in comparison with the first and the second moreover the first and the third blood-draws was increased as the result of a shorter and a longer period of estrogen administration (p<0.05). The means of the MPO release values were: 52.8±7.7 before the treatment, 251.4±70.0 after 12 days and 554.3±142.0 mU/ml after 40 days. The amount of the released enzyme was still significantly increasing during the long term estrogen therapy in contrast with the MPXI (p<0.05).

*Results of the in vivo experiments with rats*

TSC changes (Day 0 vs. Day 28) are expressed as the average sample/blank ratios in every five-member group. The decrease of the ratio marks the increase of TSC. There was no significant change in the untreated group. The TSC was numerically reduced in the control group on the high-fat diet; however, this change was not significant. In the groups on the normal diet (Group 2 and 4) with DHEA or DHEAS treatment, there was a trend toward reduced TSC values but the differences did not reach statistical significance (Group 2: 0.315
± 0.108 vs. 0.890 ± 1.199 (p=0.165) and Group 4: 0.152 ± 0.059 vs. 0.205 ± 0.352 [p=0.369]). The groups on the high-fat diet with DHEA or DHEAS treatment (Group 1 and 3) showed significantly increased TSC (Group 1: 0.198 ± 0.065 vs. 0.096 ± 0.048 [p=0.020] and Group 3: 0.210 ± 0.028 vs. 0.093 ± 0.101 [p=0.004]). The p-value was lower than 0.01 in Group 3 (high-fat diet + DHEAS treatment). Neither group had significant change in average body weight during the study period.

Liver samples showed no Sudan-positivity in the untreated group and in the groups on normal diet with DHEA or DHEAS treatment (Group 2 and 4). The groups on the high-fat diet, control and with DHEA or DHEAS treatment (Control group, Group 1 and 3) showed different levels of positivity with Sudan-staining and the total score of liver fat content was eight, two and one respectively.

The groups on a high-fat diet have lower SOD activity, while all groups on a normal diet have elevated SOD activity. The prooxidant effect of the lipid load couldn't be diminished by our antioxidant steroids, however these seemed to increase the SOD activity in normal diet in concordance with their antioxidant manner. Catalase and GST activities changed synchronously with each other. In their case the lipid load had rather a stimulating effect on their activity. The groups on a high-fat diet with DHEA or DHEAS treatment (Group 1 and 3) showed preferably decreased catalase and GST activities. In case of normal diet the investigated weak androgens
increased the activity of these antioxidant enzymes as well like we have seen with the SOD.

Results of the effects of postmenopausal hormone therapy on endometrial bleeding and the histological transition of the endometrial tissue

The patients who had not received hormone treatment turned out to be significantly younger (p<0.0001). The menopausal patients given care at our outpatient unit between 2000 and 2005 had estrogen monotherapy in 669 cases, sequential combined in 731 while continuous combined in 722 cases.

In the group not receiving hormonal treatment, the incidence of bleeding was more than two-folds higher than in the treated group (15.30% vs. 6.13%, p<0.001).

In the histology results of the patients with sequential combined treatment in the vast majority they had - like the non-treated - proliferative phase (36.47% and 34.66%). In contrast to this with continuous combined therapy patients had proliferative phase only in 12.50% of the cases. Although this is influenced by the timing of the sampling and the phase of the sequential treatment the difference is remarkable however not significant (p=0.6208). In the 30.00% of the continuous combined group the endometrium was atrophic and moreover 40.00% was not evaluated which also can be due to the result of atrophy. In the sequential combined therapy the atrophy was present only in 20.00% and by adding the non evaluated results it
was altogether 25.88%. In comparison with the 71.18% value of the continuous combined group the difference is highly significant (p<0.002). In the non treated group the proliferative phase (34.66%) and the atrophy (23.92 [17.16+6.76]%) incidence was approximately the same like in the sequential combined group.

By the advance of the age the occurrence of the proliferative phase was significantly decreased and the occurrence of atrophic endometrium was significantly increased in the continuous combined group. The occurrence of secretion phase was approximately the same between the non treated and the sequential combined groups (16.63% vs. 16.47%). There was no secretion phase in the continuous combined group.

There was altogether 8 cases with hyperplasia simplex in almost the same ratio in the sequential and the continuous combined groups (7.06% ND 5.00%). In the non treated group there was 52 cases (9.01%) who had hyperplasia simplex. Hyperplasia complex with atypia and endometrial carcinoma (1.39% and 0.35% respectively) was seen in the non treated group exclusively but not in any treated group. Hyperplasia was present mainly in the menopause and earlier while in the treated group after the menopause. The two endometrial carcinomas were also seen after the menopause.

Endometrial polyp was more frequently present in the treated groups but due to the low number of cases statistical analysis could not be performed. In other categories of the endometrial histology I have
not found any significant difference between the treated and the non treated groups.

Based on the comparison of the cervical scrape histology results cervical polyp was more frequent in the treated groups (20.00%, 15.30% and 15.00% respectively) than in the non treated group (6.07%). The occurrence of chronic cervicitis was very low in the continuous combined group (2.50%) compared to the 16.30 % of the non treated and the 11.76% of the sequential combined groups. Microglandular hyperplasia was present equally in both main groups but endometrial carcinoma was present only in the non treated group (in 2 cases).

V. CONCLUSIONS
The administration of estrogens in postmenopausal age women increases the secreted amount of the myeloperoxidase enzyme from the neutrophil granulocytes and its intracellular activity as well. The increased myeloperoxidase enzyme amount and activity might lead to the dramatic decrease of the prooxidant hydrogen-peroxide and the superoxide-anion finally resulting in an antioxidant effect. This may be responsible for the antioxidant effect of estrogens on several pathological processes with the generation of free radicals like carcinogenesis, atherosclerosis or aging.

In vivo rat experimental results showed that the weak androgen dehydroepiandrosterone and its sulphate produce a significant increase in total scavenger capacity only on high-fat diet. In these
groups these steroids were able to compensate the increase of liver fat content to a certain extent as well. In the local antioxidant system of the liver the superoxide-dismutase activity was lower in high-fat diet and higher in normal diet groups while the changes in the catalase and glutathione-S-transferase activities (decreases) were remarkable in those groups which had significant total scavenger capacity changes (increases). These enzymatic changes have relevance only locally in the liver and they contribute less to the systematic total scavenger capacity changes. As a main outcome this effect might be favourable for the whole body redox-homeostasis.

Menopausal hormone therapy has a key role not only in the treatment of the unbecoming vasomotor, urogenital, bone metabolism and psychical changes of the climacterics but also for the control of the endometrial function. With an appropriate professional background and under the auspices of a Menopause Outpatient Unit the individualized prevention, treatment and follow-up strategy is essential for the prevention and early diagnosis of side-effects as breast cancer as well.

During menopausal hormone therapy the continuous combined treatment resulted in atrophic endometrium which subsequently showed a decreased in the occurrence of endometrial hyperplasia and the chance of uterine bleeding. The group without treatment had two and a half-time more cases with bleeding. Proliferation phase and endometrial hyperplasia was mainly seen in non treated cases. Those who received hormone replacement therapy had no complex
hyperplasia with atypia but only its simplex form. Cervical polyp was more frequently present in patients with hormone replacement therapy. Based on my results the postmenopausal hormone replacement therapy decreases the occurrence of postmenopausal bleeding and might prevent the development of endometrial carcinoma. This beneficial effect can be related to a change in the antioxidant state.
PUBLICATIONS RELATED TO THE THESSES


Book chapters related to the theses:


PUBLIATIONS NOT CLOSELY RELATED TO THE

THESES

