STEROID PROFILES IN ENDOCRINE AND PSYCHIATRIC DISEASES

PhD Thesis

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INTRODUCTION

Breast cancer, osteoporosis and depression are common health problems among women. Multiple factors may play role in the etiology of the diseases including environmental, genetic and endocrine factors. It is known that estrogens are particularly important in the process of breast cancer and osteoporosis. High estrogen level has been identified as a major risk factor for breast cancer, in contrary the estrogen deficiency may inducate osteoporosis. Changes in the sex steroids are connected to the development of depression during pregnancy, after child birth and menopause. Recent studies suggest that androgens have significant role in the pathophysiology of these diseases, too.

Several methods are known for the measurement of steroid hormones and their metabolites of significance under healthy and pathophysiological conditions, such as colorimetric, fluorimetric, chromatographic methods and immunoassay. Single steroid measurements (usually immunoassays) from serum, plasma, saliva and urine allow rapid, simple analysis, which can be automated, but give only momentary information about the circulating steroids.

The gas chromatographic (GC) method known as urinary steroid profile or spectrum is a multicomponent analysis, measuring several steroid groups simultaneously. Methods developed by C.H.L. Shackleton – a prominent researcher in steroid profile measurements – are worldwide accepted and serve as the base of steroid analysis. The GC-measurements of the 24-hour urinary steroid metabolites provide a comprehensive overview of the metabolism and function of the enzymes involved overcoming the problem of timing of blood samples in relation to the circadian level of certain steroids. The other advantage, with particular importance in pediatrics and in the case of examination of stress-related diseases, is that procedure is non-invasive and the analytical sample can easily be obtained. The urinary steroid profiling technique possesses a significant importance in the differential diagnostics and research of several endocrine diseases. The Institute of Bioanalysis, Faculty of Medicine, University of Pécs as member of an international network is the only one Hungarian team applying the steroid profile method.

We have examined urinary steroid profiles in different diseases (breast cancer, osteoporosis, depression) affecting mainly women with a complex endocrine background connected to the modification of steroid metabolism.

AIMS OF STUDY

In the past few years we have examined urinary steroid profiles in different pathographies. The following questions raised.

-are specific changes in the steroid profiles of diseases?

-are there characteristic changes in the functions of certain enzymes?

-could the detected changes be applied for diagnostics or therapy?

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1. Since our laboratory has been working from the mid-90's to analyze urinary steroid-profile of breast cancer in postmenopausal women we continued this project in case of patients with breast cancer, who developed the disease before and around menopause. The goal was to understand the changes of metabolism in different states of menopause.

2. We have examined the role of androgens and the 11β-hydroxysteroid dehydrogenase (11β-HSD) enzyme in osteoporosis in postmenopausal women.

3. Another aim was to obtain information on the level of cortisol and dehydroepiandrosterone (DHEA) in depressed patients, considering sex and age. In addition we examined the ratio of certain metabolites, which can be possible markers of depression.

MATERIALS AND METHODS

1. SUBJECTS

24-hour collection of urine samples was applied. Patients from the clinics of the Faculty of Medicine, University of Pécs were chosen, with the help of clinicians.

The patients did not receive medication influencing steroid metabolism before the collection of urine samples and agreed to participate in the research.

The controls were age and sex-matched healthy people, except the controls for breast cancer, were the control women were patients of the surgical department undergoing operation from other reasons.

2. DETERMINATION OF STEROID-PROFILE

Before the GC measurements we performed sample pre-treatment consisting of the following steps: extraction of steroids from urine, releasing of metabolites secreted as conjugates by enzymatic hydrolysis and derivatization of the polar groups (hydroxyl and keto groups) influencing heat degradation and volatility. The derivatizating reagent would destroy the capillary column used for GC measurements; therefore we removed the reagent excess by column chromatography.

The steroid derivates were injected into the GC in liquid phase. The separations were carried out in a capillary column with nonpolar stationary phase, using temperature program and flame ionization detection. The metabolites were identified by their relative retention times. The quantification was obtained by relating the peak areas of the individual components to the area of the internal standard.

RESULTS AND DISCUSSION

We analyzed the 24-hour urinary steroid profile of patients suffering from three endocrine or neural pathographies affecting steroid metabolism. The 24 hour quantity of steroid metabolites measured in samples from patients (μmol/24h), the main metabolite amounts characteristic to steroid production and the metabolite ratios characteristic to enzyme function were compared to the steroid values of the controls using appropriate statistical tests corresponding to the sample distribution.

1. According to our results we obtained different changes in women with breast cancer in different menopausal status. The significantly decreased values of androsterone (An) ($p<0,05$) in premenopausal women and the decreased values of 5α-androstanediol (5α-Ad) and androstenediol (Δ5-Ad) ($p<0,05$) in perimenopausal women point out the importance of androgens in pre and perimenopausal breast cancer. The decreased level of androgen metabolite observed in premenopausal women with breast cancer shows the changes of testosterone and androstendion metabolism, while in women with perimenopausal breast cancer it shows the change in the metabolism of the main adrenal androgen, DHEA.

According to other's experiments carried out on certain tissue cultures the androgens can express antiestrogenic effect under high estrogen level conditions. Is it possible that the lack of androgens at premenopausal women with relatively high estrogen level can increase the risk of cancer?

The sum of the main cortisol metabolites ($p<0,01$) and decrease in the level of several main cortisol metabolites (tetrahydrocortisone-THE, tetrahydrocortisol-THF, allotetrahydrocortisol-aTHF, β-cortolone-β-Cl, $p<0,05$) were obtained in the cases of women with premenopausal breast cancer.

The observed subnormal cortisol metabolit level in premenopause, and the subnormal level of the progesterone metabolite, the pregnanediol (PD) in postmenopause support that glucocorticoids and progesterone play different roles in menopause.

2. According to literature the androgens have a positive effect on the bone-density. We expected that the level of androgen metabolites would be lower in postmenopausal women with osteoporosis compared to the healthy women.

We observed a significant decrease of some androgen metabolites (etiocholanolone-Et, 11-keto-androsterone- 11-O-An, $p<0,05$, and DHEA, $p<0,01$) and in the sum of certain characteristic metabolites (sum of An and Et, sum of 11-deoxi-17-ketosteroids, $p<0.05$) which supports the possibility that besides the female sex hormones the androgens can play role in the process of osteoporosis after menopause. In certain assumptions the effect of androgens against osteoporosis can have particular role in the estrogen-deficient state after menopause.

There are several possibilities for the treatment of osteoporosis after menopause. To achieve optimal results a lot of factors have to be taken into consideration. According to literature the most effective treatments are the hormone-substitutions in the prevention and treatment of the disease. The subnormal DHEA level observed in our experiments indicates that this component can be applied therapeutically in postmenopausal osteoporosis. The detection of DHEA-level and its effect on bone-density can help the prevention in certain cases and can make the treatment more efficient.

The decrease of cortisol metabolites (THE, $p<0.01$ and β-CL, α-cortolone- α-CL, $p<0,05$) seems to be contradictory with the known osteoporosis-inducing effect of glucocorticoids. Is it possible, that this decrease is the compensation of the organism that led to the decrease of the osteoporosis-inducing hormone?

In the index of 11β-HSD activity we did not find differences between the two groups. 11β-HSD catalyzes the introversions of active glucocorticoids and their inert 11-keto derivatives and it has two izozyme types. The type-1 isozyme, probably an 11β-reductase in vivo, may amplify glucocorticoid action during the diurnal nadir. It is assumed that the ratio of 11-oxo/11β-hydroxy metabolites of cortisol reflects primary 11β–HSD I activity, i.e. peripheral conversion of inert glucocorticoids to active forms.

3. Based on literature we expected the changes in the DHEA and cortisol levels and in the ratio of cortisol/DHEA metabolites in depressed patients. It is difficult to compare the contradictory data in the literature due to the differences in the applied methods and differences in the age and sex of the patients.

In our work we found marked gender differences in depressed patients. The increase of cortisol level ($p<0,01$) in depressed women and the decrease of DHEA level ($p<0,01$) in depressed men may suggest that the function of the hypothalamic-pituitary-adrenal axis

changes differently in man and women and the effect of the two steroids prevails differently. Despite of this there was no difference in the ratio of cortisol and DHEA in any of the cases.

Based on the urinary steroid metabolite ratios (the ratio of 11-oxo and 11-OH cortisol metabolites, $p<0,01$) it was proven that the function of 11β-HSD enzyme system changed in both sexes compared to age- and gender-matched controls. Our results support that independently from gender, depressed individuals convert inactive cortisone to active cortisol more readily than the controls do, but the degree of decreases were greater in men.

We concluded that the separated examination of men and women would be useful in clarifying the biochemical changes in of depression. It can be very interesting to examine the connection between the rate of changes and the severity of depression symptoms. The decreased DHEA level suggests a new possibility in the applied medication.

CONCLUSIONS

Summarizing our experiences with employing the urinary steroid determination we observed changes in the steroid profile in several cases in the examined diseases compared to the same age and same sex control groups.

Profiling urinary steroids has the advantage to give a comprehensive overview about the synthesis of daily steroids the including glandular and peripheral steroid metabolism. The changes in the levels of single metabolites and in the ratio of certain metabolites point out the importance of the role of certain enzymes and steroid groups, thus providing help in the recognition and treatment of diseased states.

Main results:

1. Various hormonal changes can be observed in women with breast cancer, developing the disease in different status of the menopause. In the cases of pre- and perimenopause we observed subnormal level of certain androgen metabolites. Further important changes are the differences of glucocorticoids and the progesterone metabolism in certain menopausal status.

2. In postmenopausal osteoporosis we observed a significant decrease in the level of several androgen metabolites which can indicate that after menopause the effect of sexual steroids on the bone loss is not only connected to estrogens. The unexpected decrease of cortisol and its metabolites seems to be contradictory with the fact that the increased level of glucocorticoids can induce osteoporosis.

3. With our work we draw attention to the fact that there are sex differences in the steroid metabolism of depressed patients. A possible explanation can be that the function of hypothalamic-pituitary-adrenal axis changes differently in the two sexes, but its confirmation requires further research. We confirmed that the function of 11β-HSD enzyme controlling the cortison and cortisol transformation shifted into the direction of cortison→cotisol in both sexes with depression.

4. The decreased level of DHEA in depressed men and women with osteoporosis suggests the possibility of therapeutically use of the component.

It is obvious that the similar changes in the level of certain metabolites are accompanied by various symptoms in different diseases. Thus this method is applicable for the detection of changes, but the discovery and specification of the role of certain components requires further tissue-specific examinations.

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