URINARY STEROID PROFILES IN ENDOCRINE AND PSYCHIATRIC DISEASES

PhD Thesis

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INTRODUCTION

Nearly 60 years ago, in 1950 the Nobel Prize was awarded to three American researchers (P. S. Hench, E. C. Kendall, and T. Reichstein) for their discoveries relating to the hormones of the adrenal cortex, their structure and biological effects. Thence steroids have been basal monitoring and therapeutical requirements in the medical routine.

Several experimental and clinical data show that physiological and pathological changes in steroid metabolism play an important role in the development of some diseases. The qualitative and quantitative determination of steroid hormones is important in medical diagnostics as well as in the investigation into the pathophysiology of various diseases, and can also help therapy.

Among the various methods that may be applied to measure steroid hormones and their metabolites, the use of chromatographic methods and immunoessays are the most widespread currently. Individual steroid measurements (generally immunoessays) from serum, plasma, saliva and urine can be performed rapidly and easily and can mainly be automatized. Their disadvantage is that they provide information about the steroids present in the circulation only at the moment of sampling, so they do not inform us about the circadian rhythm of the hormonal secretion.

The method known as urinary steroid profile is a multicomponent analysis, measuring several steroid groups simultaneously. The results of urinary measurements provide information relating to steroid metabolism in the glands and the periphery as well. By eliminating the daily fluctuations that are characteristic for certain steroids, measurements from urine collected during a period of 24 hours provide an overall picture concerning one-day steroid metabolism, the metabolic pathways of steroids and also the functioning of the enzymes taking part in the synthesis. The other advantage is that the procedure is non-invasive, and it is important in the case of examination of stress-related diseases.

The urinary steroid profiling has outstanding importance in the differential diagnostics and research of several endocrine diseases. In Hungary, with medical diagnostic aims, urinary steroid profile tests are allowed to be performed only at the Institute of Bioanalysis, Faculty of Medicine, University of Pécs, being a member of an international organization that coordinates laboratories using this same method.

We have examined urinary steroid profiles in some endocrine and psychiatric diseases with a complex endocrine background connected to the modification of steroid metabolism.

AIMS OF STUDY

These days eating disorders and infertility problems are very common. Several risk factors have been identified in their development; however, their pathophysiology has not been completely cleared and can be associated with modified steroid metabolism. We wanted to study whether a specific urinary steroid profile of these diseases existed or whether the detected changes could be interpreted in the framework of diagnostics and therapy.

1. Our goal was to recognise the changes of steroid metabolism in women with eating disorders.

2. We wanted to investigate the urinary steroid profile of patients with poor and normal response to controlled ovarian stimulation.

3. We aimed to recognize the changes of steroid metabolism prior to oocyte pick-up and three weeks after embryo transfer of women who underwent controlled ovarian stimulation.

4. Our aim was to investigate the urinary steroid metabolite profiles for patients who failed to achieve pregnancy and for patients with ongoing pregnancy after in vitro fertilization

5. We intended to compare the urinary steroid metabolite levels of women at the day of oocyte pickup with treated endometriosis and control women.

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 according to severe criteria.

The patients had not received medication influencing steroid metabolism, had not had endocrine or other serious diseases before the collection of urine samples, collected the urine samples precisely and agreed to take part in the research.

The controls were age-matched healthy women from the Department of Obstetrics and Gynecology and from the Institute of Bioanalysis of the Faculty of Medicine, University of Pécs.

2. DETERMINATION OF STEROID-PROFILE

Before the gas chromatographic analysis we performed sample preparation consisting of the following steps: extraction of steroids from urine, releasing of metabolites secreted as conjugates by enzymatic hydrolysis, derivatisation of the polar groups (hydroxyl and keto groups) influencing heat degradation and volatility. We removed the silylating reagent excess by column chromatography.

The steroid derivates were separated by gas chromatography. The separations were carried out in a capillary column with non-polar stationary phase. A mass spectrometer was used as detector. The quantitation of the metabolites was carried out in the selected ion monitoring mode. The Target Ion was used for the quantitative analysis of the steroid metabolites. The steroid identification in each sample was based on the ratio of Qualifier Ions to Target Ion and the difference between the retention times of each steroid. The quantification was obtained by relating the peak areas of the individual components to the area of the internal standard.

RESULTS AND DISCUSSION

We studied the 24-hour urinary steroid metabolite profile of patients suffering from endocrine or neural pathographies affecting steroid metabolism. The 24-hour urinary steroid volumes measured in patient samples were compared to those of healthy control subjects of similar ages by way of statistical tests in compliance with the distribution of samples.

1. Neuroendocrine studies have engaged in changes of cortisol (F) metabolism in eating disorders. It is known that high cortisol excretion is accompanied with eating disorders, but normal plasma level has been reported, too. The high cortisol level can be the effect of the hyperactivity of hypothalamic-pituitary-adrenal (HPA) axis. We could not detect significant differences in the urinary cortisol level and in the sum of cortisol metabolites in patients. However, in five patients the mean values of the total cortisol metabolites were very high, supporting others' findings and pointing out the important role of cortisol and its metabolites in eating disorders. Further investigations are required to clarify the reason of the different results.

The increased level of the stress marker allo-tetrahydrocorticosterone (aTHB) in the urine of young women with eating disorders shows the stress involvement in these diseases, which may derive from their continuous dissatisfaction with their body shape.

An increasing interest in the role of dehydroepiandrosterone and dehydroepiandrosterone sulfate in different diseases is observable among researchers. Their effect has been studied in breast cancer, osteoporosis, depression etc. Our findings on the significantly changed urinary dehydroepiandrosterone level suggest the possibility of the therapeutical use of this component in human eating disorders.

2. The success of *in vitro* fertilization and other assisted reproductive technologies is critically dependent on optimizing ovarian stimulation protocols that try to provide good quality oocytes and embryos. Poor responder patients characteristically have low oocyte yield, high cancellation rate, and few available embryos for transfer. The cause of poor ovarian response is not cleared yet. In some cases it correlates with advanced reproductive age (>40 years). Other factors, such as advanced endometriosis, as well as previous ovarian surgery can lead to a poor ovarian response.

Certain studies notified altered ovarian steroidogenesis, as assessed by follicular steroid levels in women undergoing controlled ovarian hyperstimulation using Gonadotropin-releasing hormone (GnRH) agonists. Likewise differences in the role of granulosa-luteal cells were found in patients undergoing IVF with GnRH agonists and antagonists. It was confirmed that the follicular level of estradiol was decreased in poor responders, and granulosa cells of poor responders produced significantly less progesterone than those of normoresponders. It is consistent with our results, but we found just one progesterone metabolite (Δ5-PD) that was significantly decreased in poor responders. The urinary level of another progesterone metabolite (pregnanediol) was also lower, but not to a level of statistical significance. The rest of progesterone metabolites did not differ between poor responder and normoresponder groups. Furthermore we have shown decreased levels of urinary dehydroepiandrosterone (DHEA) in poor responders. In terms of the other androgen metabolites and all of the corticoids the two groups did not vary. The adrenal cortex is the site of massive formation of very weak androgenic steroids, DHEA and DHEA-sulphate, which are partially converted to more potent androstenedione and testosterone in the periphery. Our findings on the significantly decreased level of the DHEA point to the role of testosterone, androstenedione and DHEA in poor ovarian response. In addition some studies have shown decreased levels of follicular fluid testosterone in poor responders. Others have found that DHEA improved response to ovarian stimulation even after controlling for gonadotrophin dose.

Based on the above results, we suppose that ovarian granulosa cell steroid production is adversely altered in poor responders.

It is needful to point out that our study was executed in vivo, whereby we compared the actual urinary concentrations of steroid metabolites. We did not execute *in vitro* experiments to determine whether the groups differed in granulosa cell function following gonadotrophin stimulation.

3. Successful embryo transfer

The hormonal events surrounding implantation and early pregnancy are complex and still poorly understood. The concentrations of androsterone (An), etiocholanolone (Et), pregnanediol (PD), tetrahydro-11-dehydrocorticosterone (THA) and tetrahydro-corticosterone (THB) were significantly higher three weeks after embryo transfer compared to the values obtained before the oocyte retrieval in patients with a successful pregnancy. The An and Et are androgen metabolites, the PD is a progesterone derivative, THA and THB are corticosterones.

The increased levels of two androgens, androsterone and etiocholanolone, in patients with an ongoing IVF pregnancy may originate from the increased production by the ovaries or adrenal glands, from increased peripheral aromatase activity, or from decreased clearance from the body. The significantly increased androgen metabolite levels are very interesting findings. Previous studies have described that the elevation of the androgen concentration is one of the important factors that might cause miscarriage, which is contradictory with our results. Some studies have highlighted an association between high testosterone concentration, abnormal endometrial development and adverse pregnancy outcome.

The increased level of pregnanediol is obviously connected to the progesterone production. The most likely reason for the stable high progesterone levels is that IVF induction methods result in multiple luteinized follicles. Some studies measured high concentrations of progesterone during the first trimester. Csapo et al. proved the importance of progesterone during the first weeks of pregnancy. They found that the removal of the corpus luteum prior to the seventh week of gestation led to pregnancy loss, but pregnancy could be maintained even after removal of the corpus luteum by external administration of progesterone. Progesterone also helps with uterine smooth muscle quiescence and local vasodilatation, by inducing nitric oxide synthesis in the decidua.

The function of the hypothalamic-pituitary-adrenal axis is altered during pregnancy. The increased levels of tetrahydro-11-dehydrocorticosterone and tetrahydro-corticosterone point to the role of corticosterones in early pregnancy and fetal development. Previous studies have found that plasma corticosterone concentrations increased with the progress of the pregnancy.

Unsuccessful embryo transfer

In the group of patients who failed to achieve pregnancy, the concentrations of tetrahydrocortisone (THE), tetrahydrocortisol (THF), allo-tetrahydrocortisol (aTHF) and α-cortolone (α-CL) were significantly higher three weeks after the embryo transfer compared to the values obtained prior to oocyte retrieval. THE and α-CL are cortisone metabolites, THF and aTHF originate from cortisol.

Cortisol may influence the production of luteal progesterone. Some studies have reported a negative association between cortisol and progesterone around the time of implantation. The discovery of corticotrophin-releasing factor receptors on the ovary is also consistent with the possible existence of a down-regulatory effect of stress on steroidogenesis. Other studies have shown that hormones of the hypothalamic-pituitary-adrenal axis are able to suppress gonadal function by inhibiting GnRH release.

4. The hormonal events in early pregnancy are very complex processes, and still poorly understood. We found that the concentrations of pregnanediol (PD) and pregnanetriol (PT) (progesterone metabolites) are significantly higher, and the concentration of tetrahydrocortisol (THF) (a cortisol metabolite) is significantly lower in pregnancy.

The endocrine system and the immune system interact closely during the maintenance of the pregnancy. At the decidua, under the influence of sex steroids, there is a dramatic increase of a unique population of lymphocytes, the uterine natural killer (uNK) cells in early pregnancy. The role of these cells in human pregnancy is still not definitively established. However, they are believed to promote placental and trophoblast growth and provide immunomodulation at the maternal-fetal interface. Uterine natural killer cells are hormonally regulated by progesterone, estrogen and prolactin.

In the pregnant group the lower level of THF (compared to the group failing to achieve pregnancy) is probably a maternal adaptation to the pregnancy. In the non-pregnant group the higher THF level suggests the chance of possible miscarriages. The increased cortisol level may decrease the production of progesterone around the time of implantation.

Furthermore, immune challenges in mice appear to promote a shift in the Th1/Th2 cytokine ratio, which has been associated with low progesterone levels and early spontaneous abortion. It was found in rabbit cell-culture studies that glucocorticoids can cause degeneration and premature aging of the trophoblast.

5. We compared the urinary steroid metabolite levels at the day of oocyte pickup of women treated with endometriosis and control women. We did not find significant differences in the androgen, progesterone or corticoid metabolites.

Studies have reported increased progesterone levels in the follicular fluid at the day of oocyte pick-up in patients who underwent controlled ovarian stimulation. In these patients there was a connection between the progesterone level and the severity of endometriosis, while the testosterone level in the follicular fluid was reciprocally proportional to the severity of the disease.

Our results do not reflect this; further investigations are needed to clear the question.

CONCLUSIONS

I studied the gas-chromatographical steroid-profile method that can determine urinary steroid metabolites.

This method can be applied in endocrinology, gynecology, pediatrics, dermatology, neurology and medicine, since the profiles have important diagnostical values in adrenal and gonadal diseases having characteristic steroid profile.

The investigated psychiatric and gynecological diseases, statuses have complex endocrine background, steroid hormones play multiple and half-known roles in their pathophysiology. We observed changes in the steroid profile in several cases in the examined diseases compared to the same age and same sex control or patient groups.

Main results:

1. Our results confirm the role of dehydroepiandrosterone in eating disorders, therefore we have raised the possibility of its therapeutical use. The increased level of the stress marker allo-tetrahydrocorticosterone refers to the involvement of stress in these diseases, but the relevance of hormone alteration to the pathophysiology of eating disorders remains to be elucidated.

2. We pointed out that ovarian granulosa cell steroid production is adversely altered in poor responders, therefore significantly lower progesterone production can be observed. Our findings on the significantly decreased level of the dehydroepiandrosterone point to the role of testosterone, androstenedione and dehydroepiandrosterone in poor ovarian response. The decreased level of dehydroepiandrosterone in poor responders suggests the possibility of the therapeutical use of the component.

3. The increased level of pregnanediol in patients with ongoing pregnancy highlights the key role of the progesterone during the first trimester. We pointed out the importance of corticosterones in early pregnancy and embryonal development with the altered level of tetrahydro-11-dehydrocorticosterone and tetrahydro-corticosterone. The significantly increased androsterone and etiocholanolone levels seem to contradict the results of previous studies that described the elevation of the androgen concentration as one of the important factors that might cause miscarriage. Further researches are required to clarify this outcome.

   We confirmed the possible existence of a down-regulatory effect of stress on steroidogenesis, with the high cortisone and cortisol metabolite levels in patients who failed to achieve pregnancy. We found a negative association between cortisol and progesterone around the time of implantation, which can be important in maintaining pregnancy.

   We found that the concentrations of pregnanediol and pregnanetriol were significantly higher, and the concentration of tetrahydrocortisol was significantly lower in pregnant patients than in women who failed to achieve pregnancy. The study concludes that the production of pregnanediol, pregnanetriol and tetrahydrocortisol is altered in early pregnancy. In the pregnant group the lower level of tetrahydrocortisol is a maternal adaptation to the pregnancy.

4. We did not find significant differences in the androgen, progesterone or corticoid metabolites between women with treated endometriosis and control women. Some previous studies observed higher levels of progesterone, other studies show lower levels of testosterone in the follicular fluid. Further investigations are needed to clear this question.

Similar changes found in the level of certain metabolites are accompanied by various symptoms in different diseases. Therefore the urinary steroid metabolite determination is suitable for detecting the changes; however, to explain the exact role of different components further tissue-specific examinations are necessary.

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