Corticosteroid Biosynthesis and Metabolism in Adrenal Tumors

Dissertation (PhD thesis)

Nikolette Szücs MD

Supervisor: Károly Rácz MD, DSc

Program Director: László Rosivall MD, DSc

2nd Department of Medicine, Faculty of Medicine,

Semmelweis University

2005

INTRODUCTION

The adrenal cortex is one of the earliest examined and fairly best studied endocrine organ, which plays important roles in the homeostasis of the organism. After discovery of the most important glucocorticoid (cortisol) and mineralocorticoid (aldosterone) hormones produced by the adrenals and the recognition of the most important physiological regulatory regulatory mechanisms, the knowledge about novel regulatory factors and mechanisms is still under progress. One of these new factors seems to be leptin, discovered only in the last decade, which plays a well-known role in energy homeostasis and in regulating the quantity of fat tissue. Based on these new data it seemed worthwhile to study the connection between leptin and corticosteroids, which have also a role in the same processes.

During the metabolism of cortisol, numerous active, partially active or inactive metabolites are produced. One of these compounds is 6β-hydroxycortisol, whose importance, despite numerous studies, is still debated. The possibility arises that the quantitative determination of this compound may help in the diagnosis of various clinical diseases.

Hypertension occurs approximately 30 percent of the adult population, and the majority of secondary forms are in close connection with aldosterone overproduction of the adrenals. The recognition of these forms of hypertesion is of great importance, because therapy differs substantially from that used in pateints with essential hypertension. These observations prompted us to analyze the data of our large number of patients with primary aldosteronism, examined at the 2nd. Department of Medicine, Semmeweis University.

AIMS OF THE STUDY

My aim in this work was to study those aspects of corticosteroid production and metabolism which may add some new aspects to the better understanding of the hormone secretion of adrenocortical adenomas, may help to establish a more accurate diagnosis, and may offer a chance for better therapy. The study was based in part on hormone determinations of blood and/or adenoma tissue samples of patients examined at the 2nd Department of Medicine, Semmelweis University. The other part of the study analyzed clinical and hormone laboratory findings of these patients.

In the study I tried to find answers to the followig five issues

1. I wanted to explore, whether the newly recognized neuroendocrine hormone, leptin, could have, in addition to its well-known role in energy homeostasis, a direct effect on the corticosteroid production of different types of human adrenocortical adenomas. Is it posible that leptine could produce different effects on cells isolated from various adrenocortical adenomas?
2. I wanted to study, whether commonly used dinamic tests for investigatons of the hypothalamic-pituitary-adrenal axis have any influence on plasma levels of the cortisol metabolite 6β-hydrocortisol, produced mainly in the liver. Does plasma concentrations of 6β-hydroxycortisol in healthy individuals, in patients with liver diseases or in those with Cushing's syndrome differ under basal condition, after 1 mg dexamethasone administration, and/or after exogenous ACTH stimulation? Do measurements of cortisol and/or 6β-hydroxycortisol concentration in saliva, a good marker of

biologically active free hormone fraction, offer a better means for the detection of an occult overproduxction of hormones present in the majority of patients with clinically non-hyperfunctioning adrenal adenomas,

3. Is it possible to prove the role of the adrenals, as a possible extrahepatic source of 6β-hydroxycortisol, with measurement of hormone secretion in isolated cell systems obtained from adrenocortical tissues? Does the 6β-hydroxycortisol production differs in different types of hormon producing adenocortical adenomas, as compared to cells isolated from adrenocortical tissues surrounding the adenoma? Do hormone measurements in samples obtained from selective catheterization of the adrenal veins confirm the results of these in vitro studies?

4. What is the efficacy of the most frequently used diagnostic tests in differentiating the two common subtypes of primary aldosteronism when analyzing retrospectively the data of 187 patients with primary aldosteronism, examined at the 2nd Department of Medicine, Semmelweis University between 1958 and 2004? Do these data confirm recent observation on the changing proportion of the two most common subtypes of primary aldosteronism, and/or on the increasing number of normokalemic patients?

5. Is it possible to elaborate the most optimal method in localizing unilateral aldosterone producing adenomas with a retrospective analysis of the data of the ”gold standard” method, which detects hormones in blood samples obtained with adrenal vein samplings?

PATIENTS AND METODS

Patients, blood- and tissue samples

The study included the results of clinical data and hormonal findings obtained from blood, salivary, and/or adrenal adenoma tissue samples of patients examined at 2nd Department of Medicine, Semmelweis University. Blood an salivary samples were used for hormone measurements and/or molecular biologic analysis. Adrenocortical adenoma tissue samples were obtained from patients treated with surgical intervention, and were used for in vitro analysis of hormone production of isolated adrenocortical cell systems.

RESULTS AND CONCLUSION

1. Using *in vitro* testings of isolated adenoma cells obtained from patients with aldosterone producing adenomas, adrenocortical adenomas causing Cushing’s syndrome and non-hyperfunctioning adrenocortical adenomas, I found that human recombinant leptin significantly inhibited basal and ACTH-stimulated cortisol and corticosterone secretion in all types of adenoma cells. Based on these results and previous observations from the literature one may hypothesize, that in patients with adrenal adenomas causing Cushing’s syndrome (and also in at least some patients with non-

hyperfunctioning adrenal adenomas), glucocorticoid oversecretion causes elevated plasma leptin concentration, and this increased plasma leptin concentration may inhibit glucocorticoid production in the adrenal adenoma cells. Therefore, it is possible, that the inhibitory effect of leptin on glucocorticoid secretion is part of a counterregulatory mechanism, which limits further increases of glucocorticoid production in glucocorticoid-producing adenoma cells. In addition, I showed that leptin, which exerts an inhibiting effect on cortisol and corticosterone secretion, does not influence basal and ACTH-stimulated aldosterone secretion in those adenoma cell types which exhibited measurable aldosterone production. One may postulate that the lack of inhibiting effect of leptin on aldosterone production may be associated with an increased ACTH sensitivity of aldosterone production.

2. I showed that in patients with chronic alcoholic liver disease after at least two weeks of alcohol abstinence, the plasma 6β-hydroxycortisol concentration after ACTH administration was significantly higher than in control subjects, while plasma 6β-hydroxycortisol concentration during midnight, in the morning and after administration of 1 mg dexamethason did not differ in the two groups. These findings raise the possibility that after a two-week abstinence in patients with chronic alcoholic liver disease, plasma 6β-hydroxycortisol may be a sensitive indicator of hepatic 6β-hydoxylase (and perhaps of other drug metabolizing enzymes).
3. Using measurements of plasma and salivary cortisol and 6β-hydroxycortisol concentrations in the morning, after 1 mg dexamethasone administration, and after ACTH stimulation in

patients with adrenocortical adenomas causing Cushing's syndrome, as well as in patients with non-hyperfunctioning adrenocortical adenomas, I showed that plasma and salivary 6β-hydroxycortisol concentrations are far more sensitive indicators of both occult and overt cortisol oversecretion compared to plasma and salivary cortisol concentrations. Based on these findings plasma and salivary 6β-hydroxycortisol values may become useful biochemical markers of occult or overt cortisol overproduction in patients with adrenocortical adenomas.

4. With the use of human adrenal and human adrenocortical adenoma cells, as well as venous blood samples obtained from selective adrenal vein catheterisation I showed that 6β-hydroxycortisol is not only a metabolite exclusively produced by the liver, but it is also a secretory product of the normal and adenoamtous adrenocortical cells. It seems likely that 6β-hydroxycortisol is under the regulatory influence of cortisol (and ACTH, which regulates cortisol secretion), and that in adrenocortical adenomas causing cortisol overproduction, the increased secretion of 6β-hydroxycortisol of adrenocortical cells may contribute to the increase of plasma and salivary 6β-hydroxycortisol levels.
5. Analyzing the results of clinical and laboratory findings of 187 patients with primary aldosteronism examined at the 2nd Department of Medicine, Semmelweis University, between 1958 and 2004, I showed that the frequency of idiopathic hyperaldosteronism as compared to aldosterone-producing adenomas and the number of normokalemic patients considerably differed from the results of the published international clinical

studies. The reason for this difference may be the conseqence of the fact, that the plasma aldosterone/plasma renin ratio suitable for screening of primary aldosteronism is rarely used in Hungarian hypertensive patients, Moreover, I showed that among the diagnostic methods of primary aldosteronism, the postural test combined with furosemide administration has a specificity of 92% and a sensitivity of 69% when patients who show an increase of plasma cortisol level during the test are excluded. For the first time in Humgary, a DNA-based screening method was introduced for the detection of the chimeric aldosterone-synthase/11β-hydroxylase gene present in patients with familial hyperaldosteronism type I, but no sach a case was found among the 30 patients examined.

6. Analyzing the results of hormone determinations in selective selective adrenal vein samples I showed that the adrenal vein aldosterone/cortisol ratio, frequently used in the international literature, produced a false negative result in the adenomatous (dominant) side in 5 of the 43 surgically treated patients with aldosterone-producing adenomas. I found that these the false negative results can be avoided by calculating the aldosterone/cortisol ratio in suprarenal vein followed by a correction with the same ratios in vena cava inferior. This new method of calculation proved to be useful for the localization of unilateral adenomas when adrenal vein sampling was succesful only in the nonadenomatous side.

ACKNOWLEDGEMENTS - A THANK YOU NOTE

I would like to thank for all help in connection with my thesis to:

•Professor dr. Zsolt Tulassay, the head of 2nd Department of Medicine, Faculty of Medicine, Semmelweis University, who made it possible for me to carry out my work at his Clinic.

•Professor dr. Edit Gláz whose invaluable advice and encouragement to strive for high standards was of great help to me.

•Professor dr. Károly Rácz, my Subject Supervisor, who provided me with every possible help and advice.

•Professor dr. László Rosivall, my Program Director, who made it possible that as a PhD student I could carry out my research work.

•dr. Miklós Tóth, Senior Staff Member, for his invaluable assistance in the processing of patients'clinical and laboratory test results.

•dr. Ibolya Varga, Scientific Researcher and all the assistants at the Endocrine and Isotope Laboratory of 2nd Department of Medicine, Faculty of Medicine, Semmelweis University

•All PhD students working at the laboratory

My husband, my children and my parents for all their patience.

LIST OF PUBLICATIONS

PUBLICATIONS INCLUDED IN THE THESIS

Szücs N., Varga I., Jakab Cs., Patócs A., Gláz E., Tóth M., Kiss R., Rácz K.: Leptin inhibits cortisol and corticosterone secretion in pathologic human adrenocortical cells. Pituitary 4: 71-77, 2001.

Szücs N., Varga I., Patócs A., Tóth M., Gláz E., Rácz K.: Secretion of 6β-hydroxycortisol by normal human adrenals and adrenocortical adenomas. Steroids 68:477-482, 2003. **IF: 2,44**

Szücs N., Varga I., Patócs A., Tóth M., Jakab Cs., Gláz E, Rácz K.: Plasma 6β-hydroxycortisol measurment for assessing altered hepatic drug metabolizing enzym activity. Acta Physiologica Hungarica 90:217-223, 2003.

Varga I., Jakab Cs., **Szücs N.**, Patócs A., Tóth M., Kiss R., Gláz E., Rácz K.: Plasma and salivary 6β-hydroxycortisol measurements for assessing adrenocortical activity in patients with adrenocortical adenomas. Horm Metab Res 35:421-426, 2003. **IF: 1,669**

Gláz E., **Szücs N.**, Varga I.: Az aldoszteronról a XXI. század elején. LAM 15 (2):96-107, 2005.

Szücs N., Gláz E., Varga I., Tóth M., Kiss R., Patócs A., Jakab Cs., Perner F., Járay J., Horányi J., Dabasi G., Molnár F., Major L., Fütő L., Rácz K., Tulassay Zs.: A primer aldosteronismus diagnosztikája és a kezelés eredményei 187 beteg adatainak retrospektív elemzése alapján. Orvosi Hetilap (közlésre elfogadva)

OTHER PUBLICATIONS

Somogyi A., Prechl J., Pusztai P., Kocsis I., **Szücs N.**: Vörösvértestek scavanger kapacitásának vizsgálata diabetes mellitusban. Diabetologica Hungarica 3 :109-114, 1995.

Tóth M., **Szücs N.**, Rácz K., Varga I., Hüttl K., Perner F., Gláz E. Neurofibromatosis I. típusának endocrin szövődményei. Magyar Orvosi Hetilap 137:1683-1688, 1996.

Hagymássy L., Rigó J., Tóth M., **Szücs N.**, Kékes K., Hüttl K.: Nerofibromatosissal szövődött HELLP szindroma. Magyar Nőorvosok Hetilapja 6:1161-164, 1998.

Hagymássy L., Rigó J., Tóth M., **Szücs N.**, Kékes K., Hüttl K.: Neurofibromatosis type 1 with pregnancy associated renovascular hypertension and HELLP syndrome.: Am. J. Obstet. Gynecol 179: 272-274, 1998. **IF: 2,634**

Hubina E., Kovács L., Szabolcs I., Rimanóczky É., Ferencz A., Czirják S., Tóth M., **Szücs N.**, Rácz K., Góth M.: Növekedési hormonnal történő

hormonpótló kezelés hatásai súlyos növekedési hormonhiányos felnőttekben. Magyar Orvosi Hetilap 141.(44) 2375-2379, 2000.

Cserepes É., **Szücs N.,** Patkós P., Csapó Zs., Molnár F., Tóth M., Dabasi G., Ésik O., Rácz K..: Ovarian steroid cell tumor and a contralateral ovarian thecoma in a postmenopausal woman with severe hyperandrogenism. Gynecological Endocrinology 16:213-216, 2002. **IF: 0,899**

Szücs N., Mészáros J., Czirják S., Mondok Á., Varga I., Gláz E.: Acromegáliás betegek tartós hatású octreotid kezelésével szerzett tapasztalatok. Magyar Orvosi Hetilap 143. (19) Suppl.2. 1066-1070, 2002.

Tóth M., Tőke J., Kiss E., Bernád I., Miheller P., **Szücs N.**, Rácz K.: Acromegalia McCune-Albright szindromában. Magyar Orvosi Hetilap 143. (19) Suppl.2. 1070-1073, 2002.

Patócs A., Tóth M., Barta Cs., Sasvári-Székely M., Varga I., **Szücs N**, Jakab Cs., Gláz E., Rácz K.: Hormonal evaluation and mutaion screening for steroid 21-hydroxilase deficiency in patients with unilateral and bilateral adrenal incidentalomas. European Journal of Endocrinology 147: 349-355, 2002. **IF: 2,56**

Hubina E, Kovács L., Szabolcs I., **Szücs N.**, Tóth M., Rácz K., Czirják S., Góth M.: The effect of gender on growth hormone replacement in growth hormone deficient patients. Horm Metab Res 36:247-253, 2004, **IF: 1,946**

Majnik J.,**Szücs N.**, Patócs A., Tóth M., Balogh K., Varga I., Gláz E., Rácz K.: Effect of single doses of dexamethasone and adrenocorticotrop hormone

on serum bone markers in healthy subjects and in patients with adrenal incidentalomas and is Cushing syndrome. J Endocrinol Invest 27:747-753, 2004. **IF: 1,525**

Patócs A., Karádi É., Tóth M., Varga I., **Szücs N.**, Balogh K., Majnik J., Gláz E., Rácz K.: Clinical and biochemical features of sporadic and hereditary pheochromocytomas: an analisis of 41 cases in a single endocrine center. Eur J Cancer Prev 13:403-409, 2004. **IF: 1,785**

Hubina E., Lakatos P.,Kovács L., Szabolcs I., Rácz K., Tóth M., **Szücs N.**, Góth M.: Effects of 24 monts of growth hormone (GH) treatment on serum carboxylated and undercarboxylated osteocalcin levels in GH-deficient adults. Calcif Tissue Internet. 74: 55-59, 2004. **IF: 2,258**

Patócs A., Likó I., Varga I., Boros A., Fütő L., Kun I., Bertalan R., Tóth S., Pázmány T., Tóth M., **Szücs N.,** Horányi J., Gláz E., Rácz K.: Novel mutation of the CYP17 gene in two unrelated patients with combined 17α-hydroxylase/17,20-lyase deficiency: demonstration of absent enzyme activity by expressing the mutant CYP17 gene and three-dimensional modeling. J.Steroid Biochem. 97: 257-265, 2005. **IF: 2,715**

Balogh K., Hunyady L., Patócs A., Valkusz Zs., Bertalan R., Gergics P., Majnik J., Tőke J., Tóth M., **Szücs N.**, Gláz E., Fütő L., Horányi J., Rácz K., Tulassay Zs.: Az 1-es típusú multiplex endokrin neoplasia klinikai tünetei, diagnózisa és kezelése. A genetikai vizsgálatok hazai tapasztalatai. Orvosi Hetilap 146 (43) 2191-2197, 2005.