STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, LXII, 2, 2017 (p. 41-52)
doi:10.24193/subbbiol.2017.2.04

Has the Fluocinolon-acetonid N ointment any effect on the kidneys and the thyroid gland structure and function?

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SUMMARY. Besides the naturally occurring glucocorticoids, there are many other synthetically produced glucocorticoids: dexamethasone, prednisolone, triamcinolone, triamcinolone acetonide, flumetazon, methyl prednisone and methylprednisolone. Corticosteroids are administered intravenously, orally, through inhalation directly onto the inflamed organ, eye drops and by applying skin ointments. Although long term use has its undesirable effects, e.g. high blood pressure, heart failure, diabetes and renal failure. Fluocinolon-acetonid N ointment is a synthetic derivate of the adrenocortical hormone, which is used for medical treatment purposes in dermatology. We also use it in our homes, mostly due to its anti-inflammatory effect, in the treatment of itching, and also in the acute keratosis. It is highly effective in serious, non contagious, dry skin inflammations, such as atopic eczema, seborrheic dermatitis, psoriasis, dermatitis or even in allergic reactions. In prolonged usage due to its liposoluble properties, it is easily absorbed into the bloodstream, which increases the chances of having side effects. The main objective of this study is to analyze the side effects of glucocorticoid excess when treatment is done with Fluocinolon- acetonid N ointment, to see if it has any effect on organs which have an important role in maintaining basal metabolism such as kidneys and thyroid gland. Our results demonstrate that fluocinolon treatment affects the structure and the function of kidneys and thyroid gland.

Keywords: glucocorticoid excess, kidney, thyroid gland

Introduction

Glucocorticoid, which is produced in the adrenal cortex, has the two most important representatives in the mammals: cortisol and corticosterone. Glucocorticoid hormones take part in inflammation, development and metabolic processes (Cain

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and Cidlowski, 2017). They regulate carbohydrate, protein, fat, calcium and bone metabolism and energy balance. Cortisol activates virtually all energy sources, so it can be quickly used by our organism (Stojanoski *et al.,* 2012). It heightens the blood sugar level, it starts the biolysis, and it even starts to break muscle proteins into free amino acids, if the energy level is not high enough in the body. The cortisol level increases in stress situations, but this hormone has a very important role at all levels which helps metabolism function correctly, as high blood pressure, it also regulates the body's use of protein, carbohydrate and fat. In adults, glucocorticoids are stress hormones with a wide range of physiological effects, which aid survival in environmental conditions that challenge homeostasis. They maintain blood flow and a supply of nutrients and oxygen to tissues when these resources are either scarce or in increased demand.

On the long term, high level of cortisol can cause serious problems in humans, reduces the pituitary ACTH secretion, this way areas such as adrenocortical will atrophy, the intellectual performance weakens, the immune system weakens also and the inflammatory processes in the body will become more common than in healthy organisms.

The mostly studied effect of glucocorticoid application was the impact on blood sugar level. The excess of glucocorticoids increases in the liver the storage of glycogen obstructs the movement of glycogen in the blood and it stimulates the gluconeogenesis (Nyirenda *et al.,* 2000; Hans *et al*., 2006; Waldron *et al.,* 2013; Ivy *et al.,* 2016). Glucocorticoids inhibit the uptake and usage of sugar in the muscle and adipose tissues (Grigoriadis *et al.,* 1988; Burén *et al.,* 2002; Lundgren *et al.,* 2004; Gounarides *et al.,* 2008). In a long term glucocorticoid application, initially insulin resistance appears and at the final stage diabetes mellitus can occur (Madar *et al.,* 1995).

Glucocorticoid excess has different effects on the cardiovascular system and kidneys, it raises the blood pressure (Grunfeld and Eloy, 1987; Singh *et al.,* 2012; Hunter *et al.,* 2014; Jeje and Raji, 2015b). In the vascular smooth muscle tissue glucocorticoids excess increases muscle fiber sensitivity to catecholamines. Glucocorticoids have an impact on many components of noradrenergic innervations: they increase the number of receptors, the receptors affect the connectivity to the G protein and the cAMP synthesis, induced catecholamine eliberation, furthermore it prolongs the effects of catecholamines on the smooth muscle, thus it rises the vascular and cardiac contractility (Fonyó, 2011). In addition to the direct effects of glucocorticoids, they can indirectly modify the vascular response, too. Glucocorticoids inhibit prostanoid synthesis, and this mechanism also prevents prostanoid induced vasodilatation (O'Sullivan *et al.,* 2013). The decreased level of glucocorticoids, in Addison-disease or after the removal of the adrenals, caused chronic low blood pressure in many patients (Fonyó, 2011). NaCl co transporter (NCC) is an important determinant of daily blood pressure variation. NCC activity in cells is regulated by

the circadian transcription factor per1. In vivo, circadian genes are entrained via the hypothalamic–pituitary–adrenal axis. Chronic corticosterone infusion increased bmal1, per1, sgk1, and tsc22d3 genes expression during the inactive phase. In the inactive phase pNCC was also elevated by corticosterone (Ivy *et al.,* 2016).

In the kidneys, it increases sodium retention in the renal tubules and potassium excretion. Long term treatment with synthetic glucocortikoids causes hypertension and low potassium levels. Dexamethasone treatment of female rats during pregnancy reduced renal mass and the number of nephrons in offspring rats (Martins *et al.,* 2003).

Long-term glucocorticoid treatment is associated with central obesity in humans, which is also typically observed in most patients with Cushing's syndrome. The mechanisms by which exogenous glucocorticoids alter metabolism and induce weight gain are poorly understood (Paggioli *et al.,* 2013).

Side effects of gGlucocorticoid-based drugs on adult rat kidneys and thyroid gland were less studied. This article describes the side effects of treatment with Fluocinolon- acetonide N ointment, on the level of kidney and thyroid gland structure and function in adult Wistar rats. In addition we analyzed and measured t body mass and thyroid gland mass variation during the short term of treatment.

Materials and methods

Experiments were carried out in adult (60-day-old) male Wistar rats. The animals were kept under standardized bioclimatic conditions and fed on common rat chow, with water *ad libitum*.

Commercial Fluocinolon-N ointment containing 25 mg Fluocinolon-acetonid-N/100 g excipient, was applied topically to the skin at 2 cm^2, for four consecutive days, by smearing 50 mg ointment/100 g b.w on the inguinal region, the daily dose of Fluocinolon-acetonid-N being equals to 12.5 μg/100 g b.w. The animals were divided into the following groups: C-control group and FC –fluocinolon treated group.

After 16 hours of fasting and 24 hours following the cessation of treatments, the treated animals together with controls were sacrificed by exsanguinations. The body weight and the thyroid glands weights were measured with an accuracy of 0.00001 g immediately after excision. The significance levels were determined by parametric t-test. A $p<0.05$ was considered statistically significant.

For structural analysis the organs of slaughtered animals were fixed in Bouin liquid and afterwards processed in view of being embedded in paraffin. The fragments were sectioned at the Reichert-Austria type microtome with a thickness of 7 μ. The staining of kidney was made by means of hematoxilin-eosin method and for rendering

evident of the thyroid secretion, the staining of the sections was made by means of Hurduc and co-workers (Muresan *et al.,* 1974). The histological preparations obtained were examined on the Olympus microscope with digital camera.

Results and discussion

Body and thyroid gland weights change during treatment

The treated and control animals' body weight measured in our experiment are summarized in Table 1. Based on the table values of the control group during the treatment their body weight slightly increased, while in the body weight of treated animals with Fluocinolon-acetonid N a significant decrease can be observed.

Table 1.

Changes in body weight during treatment with Fluocinolon-acetonid N. ± the mean value illustrated. The % quantitative variation compared to control animals in body weight. P – significant

Animal group	1th day treat.	2th day treat.	3th day treat.	4th day treat.
C	181.7 ± 3.8	187.5 ± 3.9 +3.17%	188.6 ± 3.5 +3.82%	183.3 ± 3.9 +0.90% P> 0.5
FC	148.3 ± 4.5	145.1 ± 4.6 -2.15%	137. 8 ± 4.1 -7.09%	126.7 ±4.9 -14.56% P<0.01

There are a lot of data about the effect of glucocorticoid excess upon the fetal development. The hypothalamic–pituitary–adrenal (HPA) axis fulfills important functions during fetal development and the transition to extrauterine life. However, alterations to the HPA axis during fetal life, for example following pharmacological treatment with corticosteroids, may affect development and future health of the individual (Li *et al.,* 2013). Prenatal dexamethasone leads to low birth weight and compromises organogenesis (Martins *et al.,* 2003).

During the experiment in the treated animals parallel with the decrease in body weight we observed a slight decrease in the thyroid gland mass (Fig. 1). The magnitude of the reduction in thyroid gland mass was not significant.

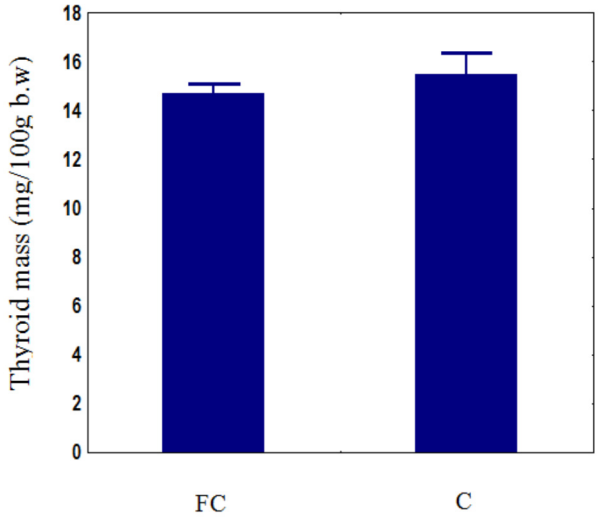

Figure. 1. Weight change in thyroid gland in treated (FC) and controlled (C) animals

Weight decrease of the thyroid gland can be associated with the decrease of the colloidal follicle diameter. Weight decrease of the thyroid gland is in harmony with our structural results.

Histological studies of the kidney in the control and treated group

Kidney sections from control animals displayed normal histological structure (Fig. 2), the outer part is the cortex, and the medulla is inside within the renal pelvis side portion.

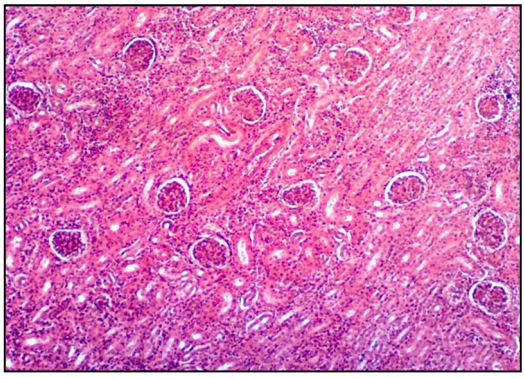

Figure 2. Renal histological section of the C-groups, Hematoxilin-eozin staining 10x

In animals treated with Fluocinolon-acetonid N, kidney sections are majorly different from the control animals, dystrophy of the renal tissues is clearly visible. The number of glomeruli is far less than in the control animal kidney sections. The few existing Bowman capsules shrunk, they blend into the environment of tubules, and they are hardly recognizable. The hialinoid is present in some places in the Bowman capsule, specifically in the vascular basement membrane which has become thicker (Fig.3 a, b).

In some parts of the renal tissues, on the wall of the renal tubules, necrotic lesions can be seen, where the epithelial cell nucleus has pyknotic, the cell membrane has also been destroyed. Among the renal tubules hyperemia is visible.

The bleeding and also the atrophic glomerulonephritis suggest that the Fluocinolon-acetonid N based treatment causes glucocorticoid excess due to its vasoconstrictor effect, glomerulus atrophy, and while in the rest of the kidney tissue hyperemia occurs, which leads to necrosis in the walls of the renal tubules. These findings are compatible with the data from other studies according to which glucocorticoid excess can produce very severe rat nephropathy (Kamphuis *et. al* 2007; de Vries *et al.,* 2010). From this we can speculated that glucocorticoids have vasoconstrictor effect on renal tissues. This is also proven by the fact that beta-adrenoreceptor stimulation significantly participates in glucocorticoid excess-induced metabolic and gravimetric alterations (Kis *et al.,* 2001; Kis and Crăciun, 2003 a, b).

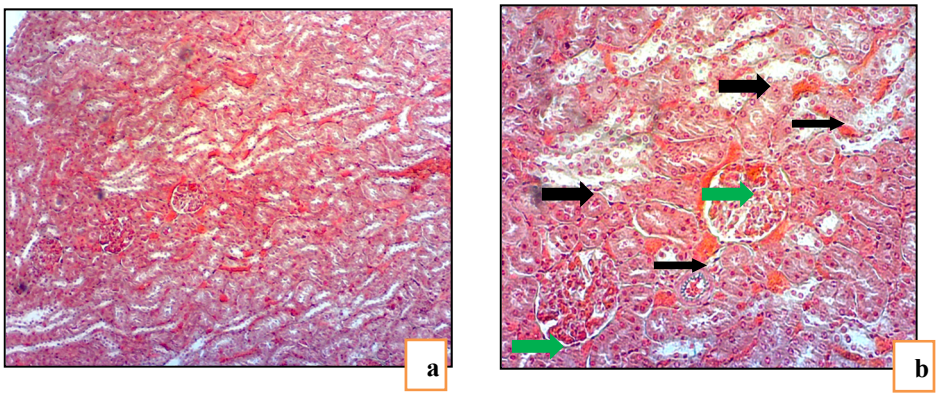

Figure 3. Renal histological section in treated group a) The renal section in FC-groups, the Bowman capsule shrunk, it is difficult to recognize, and also the numbers of normal glomeruli are less 10x. b) Nephrons dystrophy can be seen on the kidney section. The thin arrow points to the epithelial cell necrosis in the tubules, the thick points to the hemorrhage in the renal tissues and the green arrow to the hialinoid in the Bowman capsule, Hematoxilin-eozin staining, 20x

This experimental result is consistent with other similar research results, according to which the high amount of glucocorticoid is harmful, resulting in increased blood pressure (Hunter *et al.,* 2016; Ivy *et al.,* 2016; Jeje and Raji, 2017). In long-term treatment with glucocorticoids, increased blood pressure in the heart and vascular

system disease can occur which change kidney function. A number of experimental results demonstrate that dexamethasone treatment causes glomerulosclerosis, which significantly reduces the number of glomeruli (Singh *et al.,* 2012). Similar results are shown in the treatment with dexamethasone in mice, where the glomerulus number significantly reduced (Woods and Week, 2005). Wade and coworkers (1979) observed the thickening of renal tubule basic membrane after a treatment with cortisone.

Moritz and co-workers (2011), Woods and Weeks (2005), Ortiz and co-workers (2003) studied how exogenous glucocorticoids reduces the number of nephrons in fetal sheep kidneys. In dexamethasone treated pregnant sheep kidneys, the number of glomeruli decreases by 25%. Treated pregnant sheep's newborn lambs had a reduced number of nephrons in their adulthood, the arterial blood pressure was higher than in the non-treated pregnant sheep's offspring. The authors are assuming that the reduced nephron numbers have contributed in the hypertension formation.

After glucocorticoid treatment similar changes can be observed in other mammals such as rats, rabbits and mice (Ortiz *et al.,* 2003; Dickinson *et al.,* 2007; O'Sullivan *et al.,* 2013). Maternal administration of dexamethasone for 48 h early in rat kidney development results in offspring with a reduced nephron endowment. The authors hypothesized that dexamethasone may indirectly inhibit nephrogenesis by inhibiting ureteric branching morphogenesis (Shingh *et al.,* 2007).

Damage of the kidney tissues may explain the hematological effect of dexamethasone treatment. Prenatal cortisol excess inhibits erythropoietin production in fetal sheep and disturbed the maturation of erythrocytes (Jeje and Raji, 2015b).

Findings from this study suggest that Fluocinolon-acetonid N treatment could retard growth and subsequent development of nephropathy.

Histological studies of the thyroid gland in control and treated groups

The control animals showed normal structures of follicular thyroid, they were in different secretory phases. The follicle wall is made up of cubical epithelium (Fig 4 a, b).

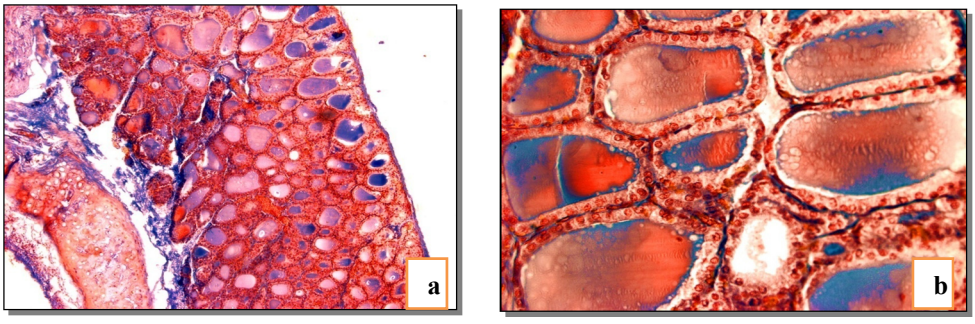

Figure 4 a) Thyroid histological section of the control animals, 1x. **b**) The thyroid follicle walls are covered with cubical epithelium, Hurduc staining, 40x

In the treated animals the thyroid show structural and functional changes in comparison to the thyroid tissue of the control animals (Fig. 5 a). Follicle diameters are much lower as compared to control animals, larger diameter edge of the gland follicles can be observed.

The number of smaller follicles is much higher than the ones in the controlled thyroid tissue. Towards in the central part of glandular tissues, the follicle diameter decreases, this occurs in empty follicles as well (Fig. 5 b).

The majority of follicles filled with colloid with a vesicular structure (Fig. 5 c), which suggests an increase in endocytosis of the thyroid hormones from the colloid.

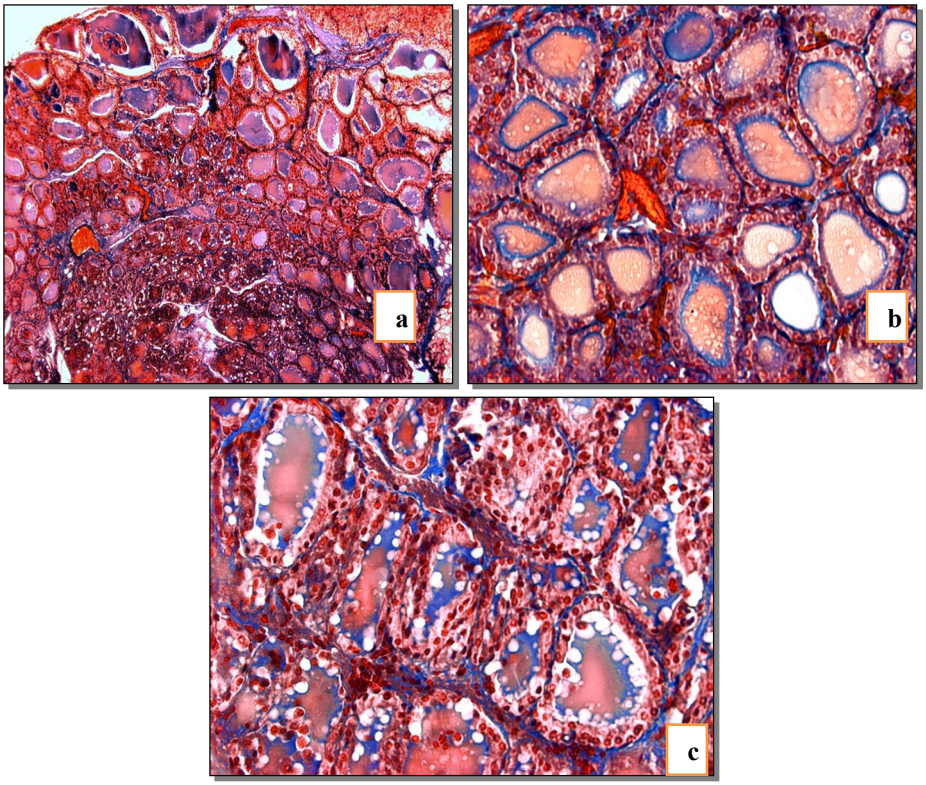

Figure 5 a) The thyroid of FC treated animals, 6x. **b**) Hormone generating follicles, 20x **c**) Vesicular structured follicles, Hurduc staining, 40x

As a result of the histological studies, we can conclude, that glucocorticoid excess induced increases of thyroid hormone releasing operation, which is reflected in the histological structure. This observation is consistent with bibliographic data,

according to which glucocorticoid excess increases the release of thyroid hormones. Literature data demonstrate that the thyroid gland is sensitive to glucocorticoid excess (Martino *et al* 2001; Menconi *et al* 2007; Nadolnik 2012).

Some results demonstrate that fluocinolon treatment effects hormone-producing pituitary cells, especially somatotrop, kortikotrop, gonadotrop and tireotrop cells. Fluocinolone treatment in Wistar rats caused ultrastructural changes in the tireotrop cells and reduction in secretory granules number, which leads to a reduction in the release of tireotrop hormone from pituitary gland (Kis and Crăciun, 2005). Fluocinolon treatment causes an excess of glucocorticoid-induced changes in hormone production in the thyroid regulation, this upsets the negative feed-back mechanisms and empties the thyroid which reduces the hormone production. The decreased hormone production is demonstrated by the finding that glucocorticoid excess causes significant weight loss in young and mature animals. The growth of various ages of Wistar rats were studied after treatment with Fluocinolon-acetonid N, and there has been a significant growth rhythm disruption in young animals which were born with reduced body mass. Their weight loss can be explained with the balance disruption of somatotropic and thyroxine / triiodothyronine hormone release (Kis and Crăciun, 2005).

Conclusions

1. The dose of Fluocinolon-acetonid N ointment used in the present study induced low body weight in adult male Wistar rats.
2. The glucocorticoid excess induced by Fluocinolon treatment increases the release of thyroid hormones.
3. In our experimental protocol the more sensitive organ to exogenous glucocorticoids is the kidney.
4. In our study the rat's kidney exhibited a discrete oligonephronia.

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