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ORIGINAL ARTICLE

THE EFFECT OF TRAUMATIC EXPOSURE ON THYMUS WEIGHT LEVEL AFTER DEXAMETHASONE APPLICATION IN RATS

EFEKAT TRAUME NA MASU TIMUSA NAKON PRIMENE DEKSAMETAZONA KOD PACOVA

Milica Knežević[1], Milica Ivanišević[1], Natalija Kojović[1], Ana Starčević[1,2]

[1] Univerzitet u Beogradu, Medicinski fakultet, Beograd, Srbija
[2] Institut za anatomiju "Niko Miljanić", Medicinski fakultet, Beograd, Srbija

Correspondence: knezmima@gmail.com

Abstract

Introduction: Thymus is the central lymphoid organ responsible for proper immune cell maturation, hence ensuring functional T cell repertoire. Stress induces elevated levels of hormones that profoundly alter immune response. Susceptibility to physiologically synthesised and exogenously applied glucocorticoids make thymus an ideal substrate for anatomical and morphological analysis.

Aim: Our research aimed to investigate the impact of endogenous and exogenous glucocorticoids on thymus weight level.

Material and methods: Experimental procedure was conducted on male Wistar rats, 12 in total, divided into 2 groups - control and experimental. Latter was exposed to two kinds of stressors. Acute stress included immobilization with exposure to the predator's odor. Chronic social stress included rotation of the animals held in pairs. On the 11th day of the experimental procedure, half of the experimental group received dexamethasone treatment (impact of endogenous + exogenous glucocorticoids) while the other half did not (impact of endogenous glucocorticoids). After the experiment, animals were sacrificed and their thymuses were obtained and measured. For statistical analysis, ANOVA was used to test differences between groups and LSD test for each group testing.

Results: Results showed statistically significant differences between the thymus mass of different groups ($F=4.336$, $p=0.048$). The part of the experimental group that received dexamethasone had a smaller thymus weight level compared to the part of the experimental group that received no treatment ($p=0.024$). No statistically relevant results were obtained after comparing thymus masses from impact of endogenous glucocorticoids and control group ($p>0.05$).

Conclusion: Exogenous glucocorticoids induce morphological changes in thymus which are observed in decreased weight level. Stress induced thymus apoptosis, but it was not sufficient to lead to decrease in thymic mass. Our further experiments will put emphasis on understanding of morphological and anatomical changes caused by stress.

Keywords:
thymus,
stress,
dexamethasone,
glucocorticoids

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Sažetak

Uvod: Timus je organ u kome dolazi do sazrevanja prekursora imunskih ćelija kako bi se obezbedio funkcionalan T-ćelijski repertoar. Neadekvatan imunski odgovor često je posledica povećanog lučenja hormona povezanih sa stresom. Kako na timus deluju endogeno sintetisani i egzogeno aplikovani glukokortikoidi, ovaj organ predstavlja idealan supstrat za analizu morfoloških i funkcionalnih promena koje nastaju kao posledica stresa.
Cilj: Cilj istraživanja je ispitivanje uticaja endogenih i egzogenih glukokortikoida na promene mase timusa.
Materijal i metode: Kao eksperimentalne životinje korišćeni su odrasli Vistar pacovi, 12 jedinki podeljenih u dve grupe - kontrolnu i eksperimentalnu. Stres paradigmu su činili hronični i akutni stres kojima je bila izložena samo eksperimentalna grupa. Imobilizacija uz izloženost mirisu predatora bila je deo akutnog stresa, dok je hronični socijalni stres predstavljala svakodnevna rotacija životinja koje su držane u parovima. Jedanaestog dana eksperimenta polovina eksperimentalne grupe je tretirana deksametazonom (uticaj endogenih + egzogenih glukokortikoida), dok druga polovina nije dobila nikakav tretman (uticaj endogenih glukokortikoida). Po završetku eksperimentale procedure životinje su žrtvovane, a njihovi timusi izolovani i izmereni. Za statističku obradu podataka korišćeni su ANOVA test i LSD test.
Rezultati: Dokazano je postojanje značajne razlike u masi timusa među grupama ($F = 4{,}336$, $p = 0{,}048$). Deo eksperimentalne grupe koji je primio deksametazon imao je značajno manji timus u odnosu na grupu koja je izložena samo stresu ($p = 0{,}024$). Nije otkrivena značajna razlika u masi timusa između grupe kojoj nije dat deksametazon i kontrolne grupe ($p > 0{,}05$).
Zaključak: Egzogeni glukokortikoidi (deksametazon) indukuju smanjenje mase timusa. Međutim, samo izlaganje stresu nije bilo dovoljno da dovede do smanjenja masetimusa. Naša istraživanja koja su u toku upotpuniće ove rezultate i dovesti do boljeg razumevanja morfoloških i anatomskih promena koje izaziva stres.

Ključne reči:
timus,
stres,
deksametazon,
glukokortikoidi

Introduction

In order to respond to stress induced situations, organisms have developed different coping strategies and compensatory mechanisms which are able to maintain homeostasis. The core of these mechanisms are positive and negative feedback loops that engage the central nervous system and endocrine glands. The main stress response is activation of the hypothalamic-pituitary-adrenal axis (*HPA*), followed by activation of the sympathetic nervous system (1). Cortisol, which is considered to be primary stress hormone, along with other hormones produced by adrenal gland, glucocorticoids, mineralocorticoids and catecholamine have pivotal roles in regulation of every physiological process involved in our well-being (1). These mechanisms are designed to be an acute response, hence when stress is prolonged, secreted hormones induce physiological changes in the neuroendocrine system, as well as in the immune system (1). Stress hormone receptors are found in immune cells, consequently impairing their function directly by binding to specific sites and indirectly, with increased production of inflammatory mediators (2). Due to the complexity of immune system regulation, which includes everything from gene expression to hormone and neuronal adjustment, it was initially hard to separate impacts of different hormones. At first, glucocorticoids by suppressing lymphocyte activity should prevent autoimmune reactions which is highly beneficial for organism, but in cases of prolonged stress, this down-regulation turns into immunosuppression which increases susceptibility to myriad of infective agents and diseases (3). As a consequence of altered gene expression, secretion of inflammatory mediators called cytokines (IL-2, IL-4, IL-5, IL-13, IL-15, TNFα) is inhibited (4). Since they have a major role in remodeling of damaged tissue in wound healing and immune cell proliferation, these processes are impaired (5). Higher incidence of autoimmune disease and allergic reaction during stress response are connected with altered cytokine levels (6). Elevated levels of IL-6, which are a direct consequence of catecholamines secretion, along with other proinflammatory cytokines and acute phase reactants are connected with insulin resistance, atherosclerosis and coronary heart diseases (7). This mediator, in turn, induces elevated cortisol levels through activation of HPA axis hormone secretion which demonstrates the complexity and intertwined regulation of two main stress response mechanisms (8).

Due to the diversity of cytokine effects, it comes as no surprise that they have a huge impact in thymus, an organ responsible for T cell maturation. Contrary to their role in the periphery, in thymus they induce thymocyte proliferation and migration, thus regulating different T cell selection (9). Glucocorticoids induce apoptosis of CD4+ and CD8+ T cell precursors and participate in forming functional T cell repertoire (3). Fine adjustment of T cell maturation is of great significance because any mistake can lead to development of autoimmune disease

or inappropriate response to antigens. Histological structure of this organ includes separate parts with closely regarded barriers and receptors, so macromolecules from periphery have little to no effect on cells. Although it is separated, the thymus is not spared of glucocorticoids and catecholamines influence, consequently stress will have an impact on this tissue as well (9). It is known that stress induces thymus atrophy along with morphological changes in noradrenergic innervation which all leads to depletion of T cell repertoire and immune system malfunction with higher incidence of allergic and autoimmune reactions. Conclusively, activation of the HPA axis stimulates secretion of glucocorticoids and cytokines which together induce depletion of immune cells in the thymus, regarded in decrease of thymic mass (10). Sympathetic activation in stressful situations acts in the same manner through stimulation of β-adrenergic receptors (11).

The aim of this research is to examine how stress induces changes in thymus weight and emphasize any possible difference between the effect of endogenous and exogenous glucocorticoids on the same trait. The hypothesis is that decrease in weight level will be the consequence of both HPA activation and dexamethasone administration. Animal stress models enabled a close insight into mechanisms and connections between any threat to homeostasis and tendency towards disease. Decreased weight level of thymus as a sequel of atrophy after stress exposure is a proof of the profound impact that it exerts on many levels. These studies are an important part of psychoneuroimmunology and behavioral studies within translational medical research.

Material and methods

For this experiment twelve 4-week-old male Wistar albino rats were used, weighed from 220 to 350g. The rats were raised in the vivarium of Galenika a.d. under conditions of alternating 12-hour light and dark intervals. The experimental animals were kept in two in properly marked macrolon cages with steel wire covers. The ambient temperature ranged from 18°C to 20°C with relative humidity of 55-65%. The animals were fed with a full feed mixture for rats that contained 20% of protein (Veterinary Institute Subotica) and water was obtained from the Belgrade plumbing. The experimental animals had food at their disposal and their care was conducted in compliance with procedures of Galenika a.d.

After acclimatization that lasted for 7 days, the animals were divided in 2 groups: an experimental group (8 animals) that was exposed to stress and a control group (4 animals) that was not exposed to stress. According to an experimental procedure described by Zoladz and Zohar, stressors included acute stress along with predator odor exposure and daily social stress (12,13). Material with odor was collected from the cat's toilet box after the 48h period when it was available for urination and defecation. The treatment lasted 20 minutes and was performed twice during the experimental period, on the first day during daylight and 10 days later in the dark. Since animals were held in pairs, social stress included their rotation on a daily basis, starting from the second day to the 31st, last day of the experiment. It was carefully monitored that original rats aren't re-matched within 48 hours. Pharmacological treatment consisted of subcutaneous administration of dexamethasone (dexasone) (50 mcg/kg b. m.) since low doses are better for investigating chronic effects. It was performed on the 11th day, after the second acute stress imposition. These injections were given only to half of the experimental group, and the other half received no treatment. After the experiment, animals were sacrificed by decapitation (**Figure 1**).

Thymuses were isolated in order to evaluate their weight levels. After obtaining weight results, statistical analysis was performed. One way ANOVA test was used for statistical analysis and as a post hoc test we used LSD test for each group testing.

Results

In total, 12 thymuses were weighed, 4 from the control group, 4 from the impact of endogenous glucocorticoids and 4 from the impact of endogenous + exogenous glucocorticoids. After using ANOVA in EZR software, a statistically significant difference between these groups was found and results of the F test (F=4.336, p=0.048) showed that there is a difference in the weight of thymus (**Table 1**).

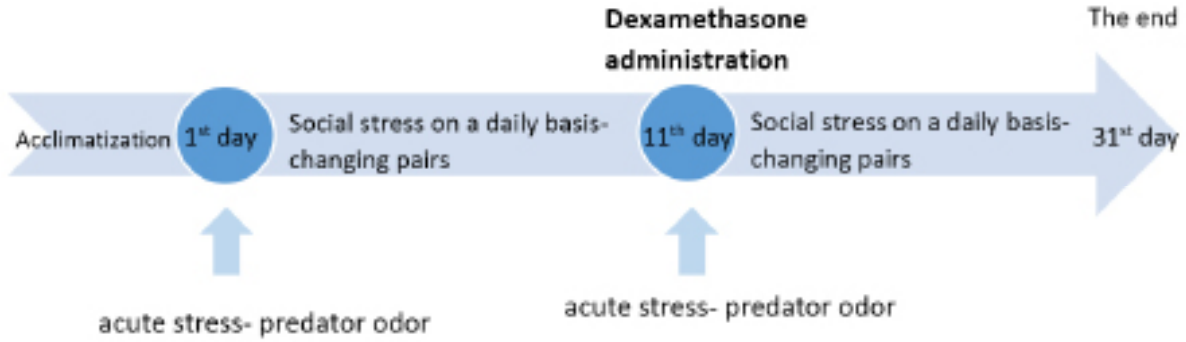

Figure 1. Timeline

Table 1. ANOVA analysis

Group	N	Mean	SEM	F test
Impact of endogenous glucocorticoids	4	0.475	0.039	
Impact of endogenous+ exogenous glucocorticoids	4	0.362	0.018	F=4.336 p=0.048
Control	4	0.445	0.028	
Total	12	0.427	0.021	

Statistically significant difference in thymus mass was observed in the LSD test between the impact of the endogenous glucocorticoids group and impact of the endogenous + exogenous glucocorticoids group (p=0.024). No statistically relevant results were obtained after comparing masses of impact of endogenous glucocorticoids and control group (p=0.82) (**Figure 2**).

Discussion

Activation of the HPA axis and consequential rise in glucocorticoids and mineralocorticoids, increased sympathetic activity along with high levels of circulating cytokines are hallmarks of stress response. In previous research it was noticed that exposure to chronic stress has a noteworthy effect on the immune system. It is known to cause atrophy of the thymus and dexamethasone application intensifies this effect (14). Our group investigated stress' influence on thymus and whether exogenous glucocorticoid treatment modifies these outcomes. It is important to emphasize that glucocorticoid can modify immune response, hence leading to immunosuppression.

Obtained results showed significant reduction in thymus weight after exogenous glucocorticoid treatment which is supported by previous reports (14, 15). The underlying mechanism of noticed change in mass is apoptosis of thymic cells (16). In addition, apoptotic changes are not equally distributed - cortex showed higher susceptibility than medulla (17). Another study showed severe depletion of tolerance-inducing MHC medullary epithelial cells which is contrary to previous statement, yet again, it supports the fact of decrease in weight and confirms the thymus sensitivity (18). Flow cytometry experiments confirmed dexamethasone induced apoptosis of thymocytes, natural killer cells and cytotoxic T cells respectively (19, 20). High vulnerability of the thymus to the dexamethasone treatment is due to the inability of the T cells to effectively metabolize corticosteroids (21). Another explanation lies in the fact that there are two types of glucocorticoid receptors - type I and type II adrenal steroid receptors. Type II has higher affinity for dexamethasone and it is the only one detected in the thymus (22). Moreover, it is important to stress that immature thymocytes are more susceptible to apoptosis after drug administration than mature cells, hence most obvious differences in weight will be observed in the thymus itself (20, 23). Dexamethasone mediated thymus atrophy can be reversed with full recovery after a certain period of time (24). Full recovery of thymic structure is possible after pregnancy and infection, not only after exogenous glucocorticoid administration (25) (**Figure 3**).

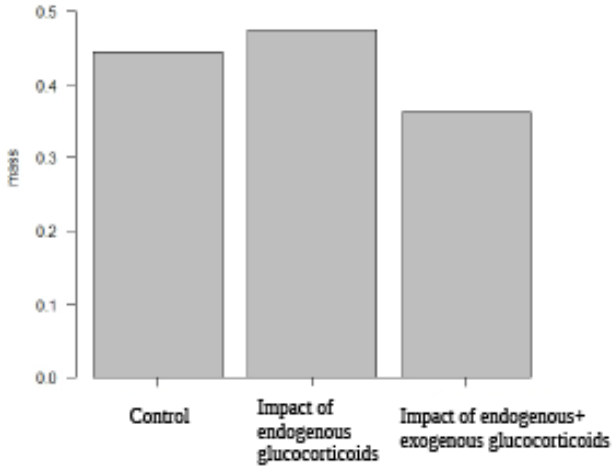

Figure 2. Comparison of mean values between examined groups

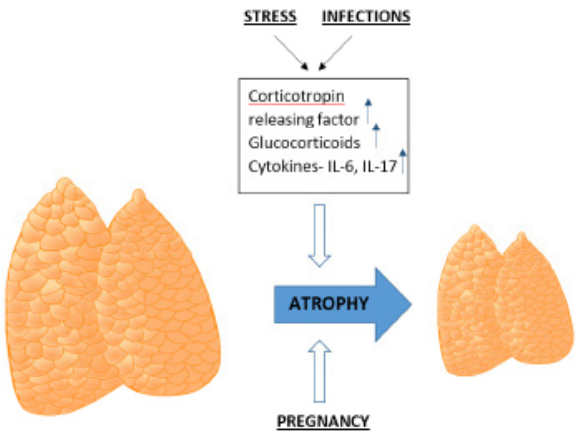

Figure 3. Inducers of thymic atrophy

Additionally, gradual decrease in thymus weight level was observed after application of different doses of dexamethasone (14). All of the above mentioned supports our results and undoubtedly confirms correlation between thymus weight and dexamethasone application.

Several studies showed that endogenous glucocorticoids affect thymus in the same manner as dexamethasone, which leads to organ involution followed by decrease in mass (10, 26–29). Again, weight loss might be due to the apoptosis triggered by glucocorticoids and elevated gene expression of corticotropin-releasing factor, the most important regulator of the HPA axis (30–32). Locally secreted cytokines, along with those secreted because of HPA and sympathetic activation may contribute to this degeneration. It is proven that IL-6 and IL-17 are crucial for this process (33). Treatment with cytokines followed by the stress exposure results in decreased weight level because they regulate migration, induce apoptosis of the immune cells and ensure appropriate microenvironment (9, 34). Our results do not support these observations since no significant reduction in thymus weight level was found after the exposure to stress and, therefore, high endogenous glucocorticoid levels. Various factors can induce observed discrepancy. Endogenous glucocorticoids are bound to corticosteroid-binding globulin (*CBG*) in order to be distributed throughout the body (35). Dexamethasone is not attached to CGB, hence its effects are facilitated effortlessly (22). Type II glucocorticoid receptor is prominent in the thymus and it has higher affinity to exogenous glucocorticoids. Glucocorticoids might exert their effects via mineralocorticoid receptors. In order to avoid overstimulation, an enzyme called 11β-hydroxysteroid dehydrogenase metabolizes hormones and decreases their affinity (36). On a cellular level, heat-shock proteins modify the function of the receptor, either by stimulation or suppression (37). Furthermore, the foundation of thymic structure are closely regarded barriers that ensure immune cell development and they are responsible for limited hormone permeability (38). These statements suggest that the effects of endogenous glucocorticoids might be altered on numerous levels, thus their activity cannot always be interpreted as a decrease in weight level.

Initially, the hypothesis was that stress induced hormones, endogenous glucocorticoids as well as dexamethasone will notably change thymus mass. After the experiment, the conclusion was made that only application of dexamethasone results in significant weight reduction. The experiment was conducted on a small experimental group which is the limitation of this research. Our results are encouraging and should be validated by a larger sample size.

Conclusion

Stress poses a threat to homeostasis, hence stimulating compensatory mechanisms to minimize the damage and ensure appropriate response. Stress induced hormones and cytokines are known to disrupt morphology of thymus. The outcome is efficiently observed in reduction of its weight level. Our study puts an emphasis on the correlation of dexamethasone administration with reduction of thymus mass and shows that exogenous and endogenous glucocorticoids have major effects.

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