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Original scientific paper

DEXAMETHASONE EFFECTS ON SERUM GLUCOSE AND POTASSIUM CONCENTRATIONS AND PLATELET COUNT OF WISTAR RAT

Muhamed Fočak, Edhem Hasković, Damir Suljević, Erna Islamagić

Department of Biology, Faculty of Science,
Zmaja od Bosne 33–35, Sarajevo, 71000 Bosnia and Herzegovina
mfocak10@gmail.com

Dexamethasone is synthetic corticosteroide which influences the metabolism of glucose, ion transport in organisms and values of certain hematological parameters. In this research, dexamethasone was applied in musculus gluteus maximus of Wistar rat species in form of dexamethasone phosphate sodium solution in 1 mL ampoules. Each ampoule contains 4 mg of dexamethasone, administered each 24 hours in 4 days period. Glucose and potassium concentrations in serum, and platelet count in whole blood were observed. After the experiment and statistical processing of collected data, it was determined that concentrations of serum glucose and platelet count were significantly increased, while values of serum potassium were significantly decreased, compared with control group.

Key words: corticosteroids; thrombocytopoiesis; hypokalemia; rats; glucose

ВЛИЈАНИЕ НА ДЕКСАМЕТАЗОНОТ ВРЗ СЕРУМСКАТА ГЛУКОЗА, КОНЦЕНТРАЦИЈАТА НА КАЛИУМ И БРОЈОТ НА ТРОМБОЦИТИТЕ КАЈ СТАОРЦИ ОД СОЈОТ WISTAR

Дексаметазонот е синтетички кортикостероид кој влијае на метаболизмот на глукозата, транспортот на јони во организмот и на вредноста на одредени хематолошки параметри. Во оваа испитување се аплицираше дексаметазон на лабораториски стаорци од сојот Wistar, во musculus gluteus maximus во форма на раствор на дексаметазон – натриум фосфат во ампули од 1ml. Секоја ампула содржи 4 mg дексаметазон, и се применуваше на секои 24 часа, во период од четири дена. Мерени се вредностите на серумската глукоза, калиумот и бројот на тромбоцити во полна крв. После експериментот и статистичката обработка на добиените податоци, утврдено е дека има значително зголемување на серумската глукоза и бројот на тромбоцитите, додека вредностите на калиумот во серумот, беа значително намалени во споредба со контролната група.

Клучни зборови: кортикостероиди, тромбоцитопоеза, хипокалиемија, стаорци, глукоза

INTRODUCTION

Dexamethasone (DX) is a fluoridated glucocorticosteroid with the strongest anti-inflammatory and immunosuppressive activity among drugs. After its introduction into organism, its bioavailability is 78%. Its maximum serum concentration is reached after 10–30 minutes, and after 60 minutes it binds to albumins (68%) Zhou & Cidlowski [1]. At the cellular level, DX, similarly to natural glucocorticosteroids, binds to glucocorticosteroid receptor (GR), with molecular weight of 94 kDa, Duma et al. [2].

DX impacts metabolism of carbohydrates, proteins (increased catabolism of glucose, urea and uric acid in blood), activates lipolysis and changes body fat tissue redistribution. Parenteral intake of dexamethasone highly suppresses the central stress response system (hypothalamic – pituitary – adrenal axis), as well as secretion of cortisol, Levin & Maibach [3]. Inhibition of cortisol releasing leads to carbohydrate metabolism change by increasing mobilization of gluconeogenic substrates that takes place in the liver, as well as decreased glucose utilization McMahon et al.; De Feo et al. [4, 5]. Synthetic glucocorticosteroids activate glucose metabolism in human liver, Pagano et al.; Rooney

et al. [6, 7] while other authors demonstrated that glucocorticosteroids did not have such effect Malerbi et al.; Wajngot et al. [8, 9]. DX completely inhibits stimulatory effects of insulin, probably because of insulin's inability to maintain the level of fructose-2,6-phosphate, Rooney et al. [7]. Inhibition of insulin secretion leads to inactivation of enzyme pyruvate kinase, Klein et al. [10]. Application of synthetic steroids increases releasing of glucose from the cell, and its blood concentration. Considering that potassium transport is in relation to glucose transport, potassium ions also leave the cell, Malerbi et al.; Wajngot et al. [8, 9]. Also, DX decreases potassium ions absorption in gut and increases their excretion. Long term usage of DX may cause diseases as cataract or glaucoma, Bent et al. [11].

Dexamethasone acts as an antiemetic drug, inhibits the processes such as phagocytosis, lysosomal digestion, synthesis and releasing of cytokines and reduces the number of lymphocytes, eosinophils and monocytes. Synthetic corticosteroids, applied in a large dose have a high impact on occurrence of thrombocytopenia. In application, seven days after achieving the maximum value, platelets number was reduced, Kühne et al. [12]. Application of DX in concentration of 40 mg a day was sufficient for an initial increase in the platelet count in adult rats, Andersen; Stasi et al. [13, 14].

In cases of adrenal insufficiency and Addison's disease, DX is prescribed for patients who do not tolerate prednisone or methylprednisolone, Charmandari et al. [15]. It is used in oncology in order to avoid side effects of chemotherapy treatment in cancer patients. DX is administered in cases of patients with spinal compression caused by tumour, Harousseau et al. [16].

In this research we examined the effects of dexamethasone on glucose and potassium ion serum concentrations, as well as its impact on platelet count in Wistar rats. It was important to determine side effects of dexamethasone in a dose used in the treatment of a wide variety of conditions in children and adolescents.

EXPERIMENTAL SECTION

Rattus norvegicus (Wistar species) lab rats used for this research were bred in the laboratory at the Department of Biochemistry and Physiology (Faculty of Science, University of Sarajevo). The acquisition, care, accommodation, use, and disposition of rats in this research is in compliance with the international convention of American Psychological Association [17]. Serum potassium ions and glucose level were analysed, as well as platelet count in the control (n = 30) and experimental (n = 30) group of animals. Both groups were of the same age (60 – 65 days) and had equal distribution of genders (14 males and 16 females).

Experimental design. Before the experiment, body mass was measured in order to determine adequate DX doses. Animals were anaesthetized with ethoxyethane before cardiopuncture. DX sodium phosphate solution in ampoules of 1 ml (Dexason® Galenika a.d. Beograd) was used. One ml of this solution contains 4 mg of DX sodium phosphate. Proper dose of dexamethasone was calculated by Clark's formula

$$\left(\frac{adult\ dose \cdot weight\ (Lbs)}{150} = child\ dose\right).$$

DX was applied in musculus gluteus maximus every 24 hours for four days. Blood samples were collected in test tubes without anticoagulant for serum analysis, and for haematological analysis test tubes with heparin were used. Further samples were centrifuged on 3000 rpm for 15 minutes in Heraeus – sepatech centrifuge.

Potassium concentration was determined by turbidimetric method in alkaline environment with tetraphenylborate (Potassium liquarapid, Human) and serum glucose by enzyme test with hexokinase (Glucose liquUV mono, Human). Spectrophotometer Spectronic 20 Genesys^{IM} was used for quantification. Measuring of platelet count was performed by Fonio's method, Adams [18].

Using IBM SPSS v.21 (IBM Corp., Armonk, NY, USA), statistical differences between the control and experimental group of rats treated with dexametasone were calculated by the t test, ANOVA and Shapiro-Wilk W.

RESULTS AND DISCUSSION

Males and females in the control group had a higher average body weight than the experimental group. Males in both groups had higher values of weight than female rats. T-test and ANOVA showed statistically significant values between the compared groups ($p < 0.05$). Shapiro-Wilk W test showed the presence of normal distribution of data (Table 1).

Table 1

Estimated average values and statistical analysis of rats weight in both genders (males and females) and groups (control and experimental)

Body weight (g)		♂♂		♀♀	
		Cont.	Exp.	Cont.	Exp.
		231.80 ± 25.22	216.85 ± 25.53	223.50 ± 21.87	201.70 ± 24.72
t test	p	0.01		0.01	
	Sig.	P < 0.05		P < 0.05	
ANOVA	p	0.03		0.04	
	Sig.	P < 0.05		P < 0.05	
Shapiro-Wilk	p	0.98**		0.62**	

Results of analysed biochemical and haematological parameters in the control and experimental group are presented in Table 2. Among samples in the control group, small variations in potassium ion concentration were detected; however, a difference between two groups in platelet count was noticed.

High values of serum glucose concentration and increased platelet count were determined in the experimental group, while concentration of potassium was decreased. Low coefficient of variation showed significant difference in individual values of the analysed parameters. This was interpreted as an individual response to corticosteroid treatment (Table 2).

ANOVA showed that the obtained values of glucose and potassium concentration and platelets count in the experimental group are statistically significant in regard to the control group ($p < 0.01$).

Table 2

The values of hematological and biochemical parameters in the control and experimental groups and statistical evaluation of analysed parameters

Statistical parameters		Glucose (mmol/L)		Potassium (mmol/L)		Platelets (10^9/L)	
		Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
Mean value ± SD		3.94± 0.80	11.44 ± 2.75	5.73 ± 1.97	2.71 ± 0.73	898.20 ± 62.35	1197.15 ± 96.10
Minimum value		2.26	6.40	2.40	1.57	789.00	1070.00
Maximum value		4.86	19.70	8.16	4.09	1005.00	1366.00
LCL		3.47	10.15	4.63	2.36	863.66	1152.17
UCL		4.41	12.72	6.83	3.05	932.73	1242.12
Skewness		– 0.94	0.96	– 0.35	0.02	0.02	0.58
Kurtosis		2.79	5.35	1.66	2.20	2.33	1.96
Coefficient of variation		21.97	24.05	34.38	27.03	6.94	8.03
t-test	P	0.000		0.000		0.000	
	Sig.	p<0.01		p<0.01		p<0.01	
ANOVA	P	0.001		0.001		0.000	
	Sig.	p<0.01		p<0.01		p<0.01	
Shapiro-Wilk W	P	0.42*		0.06*		0.03**	

LCL – lower control limit, UCL – upper control limit, *data are normally distributed, ** rejected null hypothesis

When biochemical and haematological parameters of both examined groups were compared, it was noticeable that after dexamethasone application there was a large increase of glucose concentration and decrease of potassium concentration, while platelet count was very high in the experimental group ($p<0.001$). Shapiro-Wilk W test showed the presence of normal distribution of analysed parameters for individual cases, while platelet count values showed larger variations from normal distribution (Table 2).

Pearson's coefficient indicated signifycantly negative correlation between glucose and potassium concentration, meaning that increase in glucose concentration was followed by decrease in potassium concentration.

Application of DX for a period of four days resulted in clearly observable changes in biochemical and haematological parameters in rat blood. Intramuscular injection of DX had a high impact on glucose metabolism and its homeostasis. In general, in Wistar rats, low serum glucose values were determined Xu et al. [19]. This could be explained by high muscle metabolism, and therefore high rate of glycollisis which correlates with decreased level of serum glucose. Application of dexamethasone results in increase of serum glucose values from 3.94 mmol/l to 11.4 mmol/l, because of the influence on gluconeogenetic pathway in the liver, wherefore glucose utilisation in cells is reduced, McMahon et al.; De Feo et al. [4, 5]. Other studies show antagonistic effect in regard to corticosteroids and insulin, where peripheral uptake of glucose is inhibited and gluconeogenesis is triggered, Wang et al. [20]. Similar results were established by authors Olefsky; Hans et al. [21, 22].

Glucose and potassium ions share the same metabolic pathways. During an increased glucose concentration, potassium-dependent channels are activated and potassium ions are secreted into blood. This results in higher overall potassium concentration in blood and thereafter its secretion in renal tubule with ketone bodies, Olsson & Kallner [23]. When taking synthetic corticosteroids, glucose blood concentration increases because glucose leaves the cells. Considering that potassium transport is dependent on glucose transport, potassium also leaves the cells.

Potassium concentration levels (5.73 mmol/l) in the control group of rats were higher compared to results of author Woldow [24], yet similar to the research of authors Zorbas et al. [25] in rats with hypokinesia, and results of authors Bia et al. [26]. Because of related metabolic pathways of glucose and potassium, decrease in concentration of serum potassium ions in the experimental group of rats was observed (2.71 mmol/l). Klein et al. [10] also show that after intraperitoneal application of dexamethasone, excretion of potassium ions by kidneys was excessive with similar values in statistical significance.

Glucocorticoids induce a rapid natriuresis and kaliuresis in rodents Marissal-Arvy & Mormede; Muller et al.; Campen et al. [27, 28, 29]. The effect of DX on Na^+/K^+ pump subunit expression and muscle exchange of K^+ during exercise in humans was investigated, Nordsborg et al. [30]. The results indicate that an increased Na^+/K^+ pump expression per se is of importance for thigh K^+ reuptake at the onset of low and moderate intensity exercise, but less important during high intensity exercise.

DX inhibits synthesis and excretion of a large number of interleukins (among them IL-3 has important role in thrombocytopoiesis). Otherwise, it has a high impact on thrombopoietin production and secretion, and this glycoprotein has a positive effect on thrombocytopoiesis. Platelet number in the control group of rats ($898.2 \cdot 109$/l) is similar to the results observed in the research by Bourchier & Weston [31]. DX application leads to statistically significant increase in platelet count. In the research of authors Andersen; Stasi et al. [13, 14], it is reported that the application of dexamethasone in concentration of 40 mg/day is sufficient only for initial increase of platelet count in adult rats. DX positively affects gene activation responsible for thrombopoietin synthesis.

To determine the mechanism by which platelet counts increase after corticosteroid therapy for human immune thrombocytopenic purpura (ITP), Mizutani et al. [32] studied the platelet kinetics using prednisolone – treated ITP-prone mice, (NZW x BXSB) F1. These results suggest that corticosteroids improve platelet counts not only by suppressing systemic reticulo-endothelial phagocytic function, but also by reducing antibody production.

Based on the statistically processed data and analysis of the results of biochemical and haematological parameters, it can be concluded that DX significantly affects the concentration of biochemical (glucose and potassium) and haematological (platelet count) parameters after four days of application in the dose calculated by Clark's formula.

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