THE EFFECTS OF VARIOUS DOSES OF PREDNISOLONE ADMINISTRATION ON SERUM VASPIN LEVELS IN RATS

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Abstract

Aim. The aim of this study was to investigate the dose-dependent effects of prednisolone administration on serum vaspin levels and correlate this with changes in the BMI and lipogenesis in rats.

Materials and Methods. Twenty-four albino Wistar male rats weighing between 190–240 g were divided into four groups, three experimental (5 mg/kg, 10 mg/kg, and 20 mg/kg prednisolone) and one control. The prednisolone groups were given once-daily doses for 30 days, orally. In addition, the rats were weighed, and their height and waist circumferences were measured once a week. At the end of 30 days, vaspin and glucose levels were measured from blood samples.

Results. In the prednisolone groups, the vaspin levels significantly increased when compared with the control group. The control group has a serum vaspin level of 155 ± 20.99 pg/mL and this level has been increased by prednisolone administration in a dose dependent manner. In the prednisolone groups, especially the 10 mg/kg and 20 mg/kg groups, the glucose levels increased in a dose dependent fashion.

Conclusion. Prednisolone administration significantly increased serum glucose and vaspin levels in a dose dependent manner, indicating that the increase in the serum vaspin levels could be related to the increase in the serum glucose concentration. Vaspin can be a molecule that is released in response to increased glucose and can be a rebound defense mechanism to modulate the blood glucose concentration. We suggest vaspin as a potential target for the treatment and diagnosis of diabetes mellitus and other metabolic disorders.

Key words: Adipose tissue, glucose, prednisolone, vaspin.

INTRODUCTION

Corticosteroids are hormones synthesized from cholesterol and released from the adrenal gland into

the blood under the control of the adrenocorticotrophic hormone (1-3). Glucocorticoids (GCs) play an important role in glucose metabolism. They increase glucose production in the liver and decrease use of insulin-dependent glucose in the peripheral tissues. They inhibit glucose entry into cells with peripheral insulin resistance and, as a result, cause the serum glucose level to increase (4-6). GCs also affects lipid metabolism in two ways. First, the changes in body fat accumulation that occur in hypercortisolism cause Cushing's syndrome. The second is by increasing the effects of other hormones in adipose tissues and increasing the levels of free fatty acids through lipolysis. At high doses, GCs increase lipolysis, antagonize the lipolytic effect of insulin, and increase appetite. GCs increase protein synthesis in the liver but inhibit it in other tissues (7-10).

Adipose tissue, which is dominated by fat cells, is a specialized connective tissue for lipid synthesis and storage, and it is the largest source of variable energy for an organism that can be adjusted depending on the organism's energy needs. In adult mammals, adipose tissues are formed by loosely adhering lipid-rich cells, called adipocytes, and may also contain some structural cells, such as fibroblasts, leukocytes, macrophages, and preadipocytes (yet unfilled fat cells) (11, 12). Precursor cells in adipose tissue are stimulated by adipogenic hormones like glucocorticoids, insulin, and triiodothyronine.

Fatty tissues can behave like an active endocrine gland, secreting many bioactive peptides and hormones. These secretory products communicate with other cells through the endocrine, paracrine, and autocrine pathways (13, 14). These proteins are called "adipokins" or "adipocytokines", and they play a role in homeostasis, immune response, the bloodstream, and steroid metabolism. Balancing the adipocytokines,

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which are produced from adipose tissue, provides homeostasis in the glucose and lipid metabolism pathways (12, 14).

Vaspin is a newly discovered adipocytokine, first described in 2005, that plays a regulatory role in the metabolism of glucose and lipids released from visceral fatty tissue, and it is a member of the serine protease inhibitor family (serpin). Vaspin is an adipocytokine that has been isolated from the visceral adipose tissue of Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model for type 2 diabetes. They are characterized by abdominal obesity, insulin resistance, hypertension, and dyslipidemia when obesity and insulin plasma concentrations reach peak levels (15, 16).

In the light of metabolic effects of vaspin, this study aimed to investigate serum vaspin levels in rats after chronic prednisolone administration at different doses. In addition, serum glucose levels, BMI, and regional lipogenesis were evaluated.

MATERIALS AND METHODS

Animals

A total of 24 albino Wistar male rats weighing 190–240 g were obtained from the Medical Experiment Practice and Research Center of the Ataturk University (Erzurum, Turkey). They were kept in standard laboratory conditions under a natural light and dark cycle and were fed a normal diet with free access to water. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by the local animal care committee (No. 18/20.02.2014) of Ataturk University.

Experimental Procedure

The rats were divided into four main groups. A total of 24 rats were used, with six in each of prednisolone-induced groups and six in the control group. At the beginning of the experiment, the weight, waist circumference, and length of the animals were measured. Prednisolone and normal saline were given orally by gavage.

Blood Samples

After 30 days of prednisolone administration, the animals were sacrificed using a high-dose anesthetic (thiopental sodium - 50 mg/kg). The rats were opened from the thoracic cortex at the level of the sternum, and 5 mL blood samples were taken intracardially from each rat after the pericardium was removed. The collected blood was kept for 10 minutes until completely clotted, then the serum was obtained by centrifuging at 1500 g for five minutes at 4°C, and stored at -80°C until analysis.

Biochemical analysis

In Atatürk University Research Hospital Medical Biochemistry Laboratory, a Beckman Coulter AU5800 model (USA) and Beckman Coulter kits were used to measure the glucose levels photometrically. The vaspin levels were studied by ELISA (enzyme-linked immunosorbent assays) in the Molecular Pharmacology Research Laboratory of the Atatürk University Faculty of Medicine Department of Pharmacology (RayBio ELISA kits; USA; reference range: 1–10,000 pg/mL; lowest measurable value: 26.2 pg/mL). The results are expressed as pg/mL.

Statistical Analysis

The statistical evaluation was performed using the SPSS 17.0 (Statistical Package for Social Sciences) program. The results obtained from the experiments were given as mean $\pm$ standard deviation (SD). The significance level difference between the groups was analyzed using a one-way analysis of variance by the Turkey technique from the ONE-WAY ANOVA test and the post-hoc test. The results were evaluated as a 95% confidence interval and $p < 0.05$ was considered to be statistically significant.

RESULTS

This study evaluated blood vaspin and glucose levels in the rats which received chronic prednisolone treatment. Our results demonstrated that vaspin levels were dose dependently increased in prednisolone given rats when compared to control group. In line with vaspin levels blood glucose levels were significantly increased in high dose prednisolone

Table 1. Experimental plan

Groups	Number of Animals	Treatment
I	6	Control (2 mL normal saline)
II	6	Prednisolone (5 mg/kg)
III	6	Prednisolone (10 mg/kg)
IV	6	Prednisolone (20 mg/kg)

Table 2. Blood vaspin and glucose levels and 30-day weight, BMI and waist circumference measurements

Experimental Group	Vaspin (pg/mL)	Glucose (mg/dL)	30 Day Weight Change	30 Day BMI Change	30 Day Waist Circumference
Control	155 ± 20.99	132 ± 5.98	57.48 ± 5.95	0.058 ± 0.005	0.857 ± 0.048
5 mg/kg Prednisolone	203.12 ± 18.56*	135 ± 12.59	6.57 ± 0.51**	0.004 ± 0.000	-0.714 ± 0.014 *
10 mg/kg Prednisolone	225.16 ± 35.77**	148 ± 11.67 *	1.57 ± 0.06 **	-0.017 ± 0.001	-0.857 ± 0.070 *
20 mg/kg Prednisolone	244.77 ± 35.06**	158 ± 10.97**	-7.14 ± 0.81 **	-0.036 ± 0.002 *	-2 ± 0.236 **

*p < 0.05 and **p < 0.001 significance when compared to the control group.

groups. We also evaluated 30 day weight change, BMI change and waist circumference values in our study. Prednisolone administration caused significant dose-dependent weight loss, decreased BMI change and waist circumference when compared to control group (Table 2).

DISCUSSION

Both GCs and vaspin are effective modulators of glucose and fat metabolism. In this study, in order to study these effects we investigated the serum vaspin levels in rats who were given different doses of GCs. Vaspin is an adipokine that is released from visceral fat tissue and has antiprotease effects (11,15). Studies have shown that the level of vaspin released from the visceral adipose tissue is related to the body fat percentage and plasma glucose levels, and it has been suggested that there is a compensatory mechanism related to obesity (17-19).

In this study, the serum vaspin and glucose levels in rats were shown to increase according to the prednisolone dose. There may be several reasons for this increase. One of them is that prednisolone, as well as vaspin, may accelerate lipolysis and may also increase the output of free fatty acid and glycerol from the adipocytes. As described above, in studies conducted in humans, plasma vaspin levels were found to be related to body fat percentage and plasma glucose level, and serum vaspin levels were found to be higher in obese subjects with higher fat ratios than in lean subjects (11, 18, 20). Another reason for the increase in serum vaspin levels due to the dosage may be because of a reaction of the rat metabolism to prednisolone because of the subsequent increased serum glucose level. In this study, serum glucose levels were increased with increasing prednisolone, which was an expected finding that was in accordance with the literature (20). Because GCs affect glucose metabolism, they increase the plasma glucose level and cause hyperglycemia. We can think of the increase in serum vaspin levels as an opposition response by the organism to bring the increased glucose back to normal levels.

Studies have shown that the administration of recombinant vaspin to obese rats reverses insulin sensitivity and the gene expression that initiates insulin resistance in dietary-induced obese mice (20). In cases where diabetes cannot be controlled and diabetic weight loss occurs, it has been shown that vaspin levels decrease and can be normalized by insulin and pioglitazone therapy (21). Heiker J *et al.* found that vaspin inhibited insulin-degrading kallikrein 7, and they suggested that the glucose-lowering effect of vaspin was due to the prolongation of the insulin half-life through the inhibition of kallikrein 7 rather than through an increased insulin sensitivity (22).

All of these studies suggest that the increasing level of vaspin in prednisolone-treated rats may be a compensatory mechanism developed by the organism to normalize the blood glucose level, and that increased plasma vaspin levels occurring with increased blood glucose levels could indicate that vaspin improves glucose tolerance.

In this study, the administration of prednisolone for 30 days resulted in an increase in serum vaspin and glucose levels according to the dosage. It was concluded that the increasing vaspin level may be linked to the effects of GCs on lipid metabolism or to the increasing blood glucose level. Our work and literature review indicate that vaspin may have the potential to reduce hyperglycemia back to normal limits, and that it could act as a biomarker for obesity and diabetes.

Conflict of interest

The authors declare that they have no conflict of interest.

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