

Metabolic Parameters in Wistar Rats Treated with Glucocorticosteroids and Vitamin E-Charged Poly Lactic-Co-Glycolic Acid (PLGA) Nanoparticles

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Glucocorticosteroids are the mainstay of treatment in many inflammatory and autoimmune disorders. Their administration can be associated with various side effects, similar to those within the metabolic syndrome. The beneficial effects of vitamin E in metabolic syndrome were demonstrated by many studies. Nowadays, vitamin E can be administered through a new and innovative system delivery, such as polylactic-co-glycolic acid nanoparticles (PLGA-NPs). The aim of this experimental study was to compare the metabolic and haematological parameters after Prednisone administration as a single drug or in combination with vitamin E delivered by PLGA-NPs. Wistar male rats (n=15, age 10-12 months), on standard diet were randomised in three groups, for 6 weeks as: group SC served as a control, group SP was treated with Prednisone (1 mg/kg/day) by oral gavage and group SN received Prednisone (1 mg/kg/day) and vitamin E-charged PLGA nanoparticles (1 mg/kg/day) by oral gavage. In the Prednisone treated group versus control, higher levels ($p < 0.05$) were demonstrated for visceral fat weight, glycosylated hemoglobin (HbA1c), cholesterolemia and triglyceridemia. The association of PLGA-NPs charged with vitamin E to Prednisone prevented ($p < 0.05$) hypercholesterolemia and visceral fat development induced by the cortisol intake. In conclusion, polylactic-co-glycolic acid nanoparticles (PLGA-NPs) charged with Vitamin E may demonstrate promising results in decreasing side effects induced by glucocorticosteroids, by reducing the amount of visceral fat and cholesterolemia.

Keywords: prednisone; vitamin E-charged PLGA nanoparticles; Wistar rats

Glucocorticosteroids (GC) are important drugs used for treating many chronic autoimmune and inflammatory disorders. The side effects of the drug depend on the dose and the duration of treatment [1]. Many side effects of GC are components or criteria found within the metabolic syndrome: insulin resistance, dyslipidemia, high blood pressure, visceral adiposity, hepatic steatosis [2-4]. Even though the pathophysiology of GC-induced insulin resistance is still unclear, one hypothesis is that 5-hydroxytryptamine formed in liver and adipose tissue, as a result of chronic GC administration is responsible for insulin resistance [5]. Moreover, it was demonstrated that insulin sensitivity was impaired in healthy patients receiving a low dose of glucocorticosteroids (7.5 mg/day) for 2 weeks [1].

Long-term glucocorticoid administration was responsible for the alteration of the circadian rhythm and had a negative effect on the lipid metabolism and gut microbiota, resulting in slow body growth and fat mass deposits [6].

Vitamin E, a lipophilic vitamin, known with anti-oxidant and anti-inflammatory effects [7], proved to have beneficial effects by preventing dysmetabolism [8-12]. Moreover, in high doses, glucocorticoid-treated rats, vitamin E and selenium administration improved the liver antioxidant defence mechanism [13].

Poly(lactic-co-glycolic acid) nanoparticles (PLGA-NPs) are FDA-approved biodegradable polymers with multiple applications in nanomedicine, serving as advanced drug delivery systems. They have a minimal risk of toxicity, considering the fact that they can be metabolised in lactic acid and glycolic acid, that can further enter in the Krebs cycle [14, 15].

The aim of this study was to assess if PLGA-vitamin E charged nanoparticles may have a potential beneficial role by counteracting Prednisone side effects in Wistar rats, focusing on the evaluation of some metabolic and haematological parameters.

Experimental part

Materials and methods

The animal protocol was approved by the Committee of the Ethics of Scientific Research of 'Carol Davila' University of Medicine and Pharmacy, Bucharest, Romania. The experiment was performed at the Biobase of the Faculty of Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, in accordance with the EU Directive 2010/63 approved in 2014, regarding the protection of animals used for experimental and other scientific purposes.

Male Wistar rats (n=15; age=10-12 months; weight=380-410 g), raised on standard diet, were randomly divided into three experimental groups (n=5 for each group). The rats were housed in cages at 20-22°C temperature, 12 h light and 12 h dark cycles, humidity 50 ± 5% and had access to standard rodent chow and water *ad libitum*.

For 6 weeks, group SC served as a control. Group SP received Prednisone (1 mg/kg/day) and group SN was treated both with Prednisone (1 mg/kg/day) and vitamin E-charged PLGA nanoparticles (1 mg/kg/day). Prednisone (Prednisone-Richter, 5 mg /tablet) was from Gedeon Richter Romania S.A) and PLGA-Vitamin E nanoparticles were obtained from the Department of Biological and Agricultural Engineering, Louisiana State University, 70803, Louisiana, United States. Both drugs were dissolved in

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distilled water and were administered by oral gavage. PLGA polymeric nanoparticles, 95 ± 2 nm were spherical in shape and had a negative zeta potential (-38 mV).

After 6 weeks of treatment, the animals were fasted for 12 h and the body weight was assessed. Then, the rats were sacrificed by cervical dislocation. Blood samples and the liver were collected from each animal. The liver and the visceral fat were weighed.

Cholesterol, triglycerides, glycosylated hemoglobin, ALT, albumin were measured by spectrophotometry, by using kits from SC Biosystems Diagnostic SRL.

The values were expressed as the mean \pm standard deviation (SD). In order to compare the differences between two groups, a Student's t-test (two tailed, unpaired) was used and $p < 0.05$ results were considered statistically significant.

Results and discussions

It is known that cortisol can cause growth failure during childhood and apparently the results on the body weight, in this study, are a paradox because the observed rats were adults, one year old and not pups. We demonstrated that both administration of Prednisone and vitamin E charged-PLGA-NPs determined a statistically significant reduction in the body weight ($360.60 \text{ g} \pm 18.27$, $p < 0.05$) when compared to the control group.

In the Prednisone-treated group ($379.60 \text{ g} \pm 21.38$) the average rat weight was lower compared to that in the control group ($392.00 \text{ g} \pm 14.14$), but without statistical significance (table 1).

Martinez et al. showed that Wistar rats treated with intraperitoneal (i.p.) Dexamethasone (1 mg/kg/day), for ten days were thinner than the animals in the control group [16]. Similar results were obtained by other authors with the same drug, at different doses as: 0.4 mg/kg i.p. for 4 weeks [17], 8 mg/kg, i.p., for few days [2] and 0.1 mg/kg, 3 times/week for 4 weeks injected subcutaneously [18].

Sato et al showed that Sprague Dawley rats that received topical ocular Dexamethasone had a reduced body weight. The authors considered that the drug produced a stomach irritation and a change in the taste that inhibited the appetite and promoted weight loss [19]. Another study performed on Wistar rats vs. a control group, concluded that the administration by gavage of low-doses of Dexamethasone solution (0.01 mg/kg/day and 0.05 mg/kg/day) for 7 weeks determined weight loss [6]. The researchers considered that an alteration of bone density may contribute to the weight loss [6, 20].

The peripheral administration of glucocorticoids seems to have different effects compared to the central administration. In this regard, Wistar rats treated for a short period of time with Dexamethasone through intracerebroventricular infusion had an increased appetite and body weight gain [21].

In our study the co-administration of vitamin E-charged PLGA nanoparticles and Prednisone accentuated weight loss in Wistar rats. But, if we observe the ratio values calculated as visceral fat weight/body weight, will notice the highest result in the SP group (SP vs. SN, $p < 0.05$ and SP versus SN group $p < 0.001$). In other words, the

	SC (n=5)	SP (n=5)	SN (n=5)
Body weight (g)	392.00 \pm 14.14	379.60 \pm 21.38	360.60 \pm 18.27 a
Liver weight (g)	14.79 \pm 0.23	12.60 \pm 1.56 b	11.22 \pm 0.88 a
Visceral fat weight (g)	5.63 \pm 1.63	8.98 \pm 2.56 b	3.09 \pm 0.33 a. c
ALT (GPT) (U/L)	94.3 \pm 11.31	97.64 \pm 12.62	71.74 \pm 7.16 a. c
Albumin (g/dl)	2.65 \pm 0.21	2.55 \pm 0.51	2.72 \pm 0.48
Glycosylated Hb %	3.9 \pm 0.3	5.1 \pm 0.36 b	4.7 \pm 0.55
Triglycerides (mg/dl)	96.5 \pm 4.95	134.80 \pm 21.86 b	116.80 \pm 4.09 a
Cholesterol (mg/dl)	88.50 \pm 12.02	118.80 \pm 13.55 b	80.00 \pm 8.51 c
WBC ($\times 10^9/l$)	6.78 \pm 0.01	8.45 \pm 2.63	7.58 \pm 1.58
RBC ($\times 10^{12}/l$)	8.86 \pm 1.18	9.81 \pm 0.44	8.79 \pm 0.72 c
HBG (g/dl)	14.95 \pm 0.91	15.86 \pm 0.32	15.70 \pm 0.90
PLT ($\times 10^9/l$)	909.50 \pm 106.77	887.20 \pm 215.23	917.75 \pm 54.94

a $p < 0.05$ SC vs. SN

b $p < 0.05$ SC vs SP

c $p < 0.05$ SP vs SN

Legend: ALT-alanin aminotransferase, WBC-white blood count, RBC-red blood count, HBG-hemoglobin, PLT-plateletes number.

Table 1
THE RESULTS OF THE EXPERIMENTAL STUDY

administration of Prednisone increases the visceral fat development. The co-administration of Vitamin E as NPs to Prednisone reversed the high value of the ratio visceral fat weight/ body weight.

In our study, by measuring the weight of the visceral fat we obtained arguments that emphasize that the treatment with Prednisone increases the amount of the visceral fat and Vitamin E as NPs co-administration prevents this effect. These results are as following: the visceral fat weight measured in SP group versus SC group ($8.98 \text{ g} \pm 2.56$ vs. $5.63 \text{ g} \pm 1.63$, $p < 0.05$) and visceral fat weight in the SN group vs SP group ($3.09 \text{ g} \pm 0.33$ vs. $8.98 \text{ g} \pm 2.56$, $p < 0.01$).

In contrast to the visceral fat, in this study, the liver weight was decreased by Prednisone treatment. In the SP group the average liver weight, $12.60 \text{ g} \pm 1.56$ was lower ($p < 0.05$) compared to the control group, $14.79 \text{ g} \pm 0.23$. The rats that received vitamin E as NPs associated to Prednisone had also a smaller liver ($11.22 \text{ g} \pm 0.88$, $p < 0.05$) than those in the control group.

Many researchers demonstrated a smaller weight of the liver in the rats treated with Dexamethasone either by gavage (0.01 mg/kg/day and 0.05 mg/kg/day for 7 weeks) [6] or subcutaneously injected (0.1 mg/kg , 3 times/week for 4 weeks) [18] versus control. In the literature, some results are contrary and increased liver weight was noticed in Wistar rats that received Dexamethasone intraperitoneally in doses either of 8 mg/kg for few days [2] or 0.4 mg/kg for 4 weeks [17].

In our study, rats that received glucocorticoids, by gavage had a smaller liver compared to the control group. Moreover, vitamin E PLGA-NPs associated with Prednisone had the strongest effect in reducing liver weight.

The liver can be one of the most affected organs by the glucocorticoid therapy and transaminases activity can reflect the function of the liver. It was demonstrated that Prednisolone therapy (0.5 mg per animal, for 30 days) can also induce significant liver damage [22]. Experimental studies done on rodents with Dexamethasone treatment gave different results: either elevated ALT activity was shown after topical ocular drug [19] and i.p. administration [17] or lower ALT activity after subcutaneous administration of low doses [18]. In our study, the treatment with Prednisone by gavage (1 mg/Kg) did not influence the ALT activity.

Kim et al. showed that vitamin E reduced the abnormal level of transaminases in patients with non-alcoholic steatohepatitis, with no effect on the metabolic profile [23]. Moreover, a similar effect of lowering the transaminases was observed in patients with fat liver disease [24].

In our study, the rats that received, by gavage, vitamin E PLGA-NPs associated to Prednisone had a lower level of ALT, in comparison to ALT values from the other two groups. Our team demonstrated that the beneficial hepatic effect of the association of vitamin E PLGA-NPs to Prednisone is more obvious in the obese rats, on a high caloric diet (unpublished yet, data). In our data, the above normal level of ALT, in the obese rats treated with Prednisone (120 IU/L) was reversed ($p < 0.05$) to normal level (84 IU/L) by the association of Vitamin E PLGA-NPs.

Albumin reflects the synthesis function of the liver and it is a marker of liver injury. In our study there weren't any differences between the studied groups. There is a dual relation between albumin and glucocorticoids. On the one hand, in a study conducted by Hasona et al., a lower level of serum albumin was demonstrated in Wistar rats receiving glucocorticosteroids (Dexamethasone 0.1 mg/kg , 3 times/week for 4 weeks injected subcutaneously) when compared to rats in the normal group [18]. On the

other hand, albumin transports cortisol and when the protein is in a low concentration, the free cortisol increases and its metabolic effects becomes stronger [25].

Although the precise mechanisms through which changes in cortisol secretion impairs glucose metabolism is not known, there is a strong relation between diurnal cortisol patterns and type 2 diabetes mellitus development [26]. In an experimental study, Vitamin E in combination with flaxseeds significantly decreased glycemia in the diabetic groups compared to the unsupplemented diabetic group [27]. In our study, the glycosylated hemoglobin was significantly increased in SP group versus SC group, and Vitamin E intake as PLGA-NPs couldn't reverse the values.

The effects of cortisol on lipid profile were uniformly described, in many studies, as increasing triglyceridemia and cholesterolemia. High doses of Dexamethasone 8 mg/kg for 6 days or moderate doses 0.4 mg/kg for 4 weeks increase both cholesterolemia and triglyceridemia [2, 17].

Vitamin E administration was efficient in reducing the elevated level of triglycerides in mice treated with a high-fat diet (C57BL/6J mouse, a model of insulin resistance) [9, 10]. In clinical studies, patients with non-alcoholic steatohepatitis who received vitamin E had a lower level of plasma cholesterol and triglycerides [28].

In our study the administration of Prednisone 1 mg/kg , for 6 weeks, increased both serum triglycerides and cholesterol, while the PLGA-NPs charged with vitamin E associated to Prednisone prevented the increase of cholesterolemia.

The values of the haematologic parameters were in the normal range in all the three studied groups. In a clinical study, vitamin E supplementation intake, for 3 months, in performance athletes had positive effects, by preventing the oxidative stress induced modification of the haematological parameters [29].

Conclusions

In conclusion, polylactic-co-glycolic acid nanoparticles (PLGA-NPs) charged with vitamin E may demonstrate promising results in decreasing side effects induced by glucocorticosteroids, by reducing the amount of visceral fat and cholesterolemia.

Further experimental studies are needed to determine the appropriate dose of vitamin E PLGA-NPs that can decrease the side effects induced by glucocorticosteroids intake, in humans.

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