

# The ocular protective effects of PLGA nanoparticles loaded with lutein, in fat diet Wistar rats, treated with systemic glucocorticoids

Adriana DINU, Bogdana VIRGOLICI, Daniela MIRICESCU, Alexandra TOTAN, Horia VIRGOLICI, Maria GREABU, Maria MOHORA  
"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

## ABSTRACT

**Introduction.** The carotene lutein is found in ocular layers and has powerful antioxidant, anti-inflammatory and anticarcinogenic properties. Posterior subcapsular cataract develops after prolonged glucocorticoid use.

Poly(lactic-co-glycolic acid) nanoparticles (PLGA-NPs) is one of the most successfully developed biodegradable polymer used as antioxidant delivery system.

The purpose of our study was to evaluate the ocular protective effects of PLGA -NPs loaded with lutein, in Wistar rats, on fat diet, treated with systemic glucocorticoids.

**Materials and methods.** Healthy Wistar male rats (n= 25, 12 months aged) were divided in 5 equal groups. the control group (S) with standard diet and no treatment and groups with Prednison treatment (1 mg/kg body weight), with and without PLGA-NPs loaded with lutein (1 mg/kg body weight), fed either with standard or fat diet. The duration of treatment was 6 weeks and it was done by gavage. The eye examination was done by two ophtalmologists as a blind test and photos of the eyes were made. Serum metabolic parameters were measured.

The rats treated with Prednison developed cataract and uveitis and the most severe injured eye was observed in the group with high caloric fat diet and no lutein as a supplement. Also, Prednison treatment produced dysglycaemia and dyslipidemia. The co-administration of lutein as PLGA-NPs with Prednison prevented the lens, retina and choroidal injuries, reduced the increased levels of glycaemia, triglyceridemia and increased the serum total antioxidant capacity.

**Conclusion.** The PLGA lutein nanoparticles assured global eye protection, reducing the ocular side effects of prednison.

**Keywords:** lutein PLGA-Ns, ocular protection, glucocorticoids, Wistar rats

## INTRODUCTION

Glucocorticoids are important immunosuppressive and anti-inflammatory drugs. They are

widely used in the treatment of autoimmune disorders, and other conditions such as asthma, skin disorders, allergic reactions. The anti-inflammatory and immunosuppressive effects are manifested

Corresponding author:

Assoc. Prof. Bogdana Virgolici, MD, PhD  
E-mail: hvirgolici@yahoo.com

Article History:

Received: 9 March 2020  
Accepted: 19 March 2020

by reducing the expression of pro-inflammatory cytokines, adhesion molecules, circulating monocytes, macrophages [1].

Long-term administration of glucocorticoids such as prednisone has side effects and is correlated with the occurrence of fractures, infections and cataracts [2].

Glucocorticoids have metabolic side effects, increase the risk of hyperglycemia, in a dose- and time-dependent manner in healthy and diabetic patients. Hyperglycemia may appear at a daily prednisone concentration of 2.5 mg/day [2].

Worldwide, cataracts are a major cause of blindness and vision impairment among the adult population. Cataracts usually develop over the age of 50, with a higher rate in sunny areas and higher incidence in women than in men [3].

Nowadays, surgery is the only option for cataract patients for vision recovery [4]. Lutein is a carotenoid with anti-inflammatory properties that present beneficial effects on eye health. Lutein administration improves or even prevents age-related macular degeneration and cataract. Food lutein is absorbed by small intestine mucosa, embedded in chylomicrons, and transported to the liver. From the liver to the peripheral tissues, such as the retina, lutein is loaded in chylomicrons structure. Lutein absorption can be affected by protein, Fe and Zn deficiency [5].

PLGA (polylactide-co-glycolic acid) nanoparticles loaded with lutein is an excellent alternative to remove these inconvenience. PLGA is a biodegradable polymer, whose hydrolytic degradation leads to lactic and glycolic acid, metabolized *via* the Krebs cycle, with minimal side effects. Pinocytosis and clathrin-mediated endocytosis are the mechanisms of PLGA internalization in cells. PLGA reaches the cytoplasm in less than 10 minutes after incubation, rapidly escaping endo-lysosomes [6].

The aim of our study was to test the antioxidant effects of PLGA-loaded with lutein in Wistar rats with cataracts induced by prednisone administration and hypercaloric diet.

## MATERIALS & METHODS

Old Wistar male rats (12 months old,  $n = 25$ , weight = 380-410 g) raised on standard diet (3.5 kcal/g), were randomly divided into five experimental groups ( $n=5$  for each group). Then for 6 weeks, the experimental animals were fed with different diet. Group S (control) and Group SP (standard-prednisone) continued this diet without or with Prednisone (1 mg/kg/day). Group F (fat) and FP (fat- prednisone) were fed with a high ca-

loric/high fat diet (5.15 kcal/g) without or with Prednisone. FPL group (fat- prednisone- lutein) received high fat diet and treatment with Prednisone and Lutein PLGA-NPs (1 mg/kg body) in the same time. The treatment was done by oral gavage. The rats were held in cages at 20-22°C temperature, 12 h light and 12 h dark cycles, humidity  $50 \pm 5\%$  and had access to standard rodent meal and water *ad libitum*.

Lutein PLGA-NPs were obtained from the Department of Biological and Agricultural Engineering, Louisiana State University, 70803, Louisiana, United States and Prednisone (Prednisone-Richter, 5 mg /tablet) was from Gedeon Richter Romania S.A).

PLGA polymeric nanoparticles,  $95 \pm 2$  nm were spherical in shape and had a negative zeta potential (-38 mV). Before being sacrificed the animals were fasted for 12 h and sedated with Ketamine (25 mg/kg weight). Blood samples and the eyes were collected from each animal.

## Biochemical analysis

Blood samples were centrifuged at 3,000 rpm for 5 min and the serum was used for biological measurements. Kits had been provided from Biosystems (Spain). It was respected manufacturer's indications.

Total antioxidant capacity assay (TAC) *sau* (Total Antioxidant Status-TAS, Randox, Crumlin, UK activity was performed using an analysis kit), on a semiautomatic biochemistry analyzer. The method is based on the ability of antioxidant molecules to quench the long-lived ABTS $\cdot^+$ , a blue-green chromophore with characteristic absorption at 734 nm, in comparison to that of Trolox, a water-soluble vitamin E analogue.

**Statistical analysis** was performed using a Student's t-test (two tailed, unpaired). Mean  $\pm$  standard deviation (SD) values was calculated. Statistical significance was set at a p-value of  $< 0.05$ .

The EU Directive 2010/63 approved in 2014, was respected regarding the protection of animals used for experimental and other scientific purposes. The experiment was accomplished at the Biobase of the Faculty of Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

## RESULTS

The serum biological results and clinical examination results are depicted in the following tables and figures.

## DISCUSSION

The main cause of blindness in the world is cataract and 50% of the populations over 80 have loss of vision because of cataract. Light and oxygen are environmental agents that produce free oxygen species and eye is a sensitive organ to oxidative stress. Cataract, glaucoma and inflammation are common diseases of the human eye and antioxidant supplements have beneficial effects [7-9].

In animal models, for cataract screening, researchers have been used in vivo and in vitro (incubated lenses) experiments with triggers like: sel-

enite, naphthalene, H<sub>2</sub>O<sub>2</sub>, etc [10]. In the present experimental study, Prednison was given by gavage, for 6 weeks, in Wistar rats with two types of diet, standard and obesogenic.

Many types of cataract: nuclear, cortical and posterior subcapsular cataract have been described [11]. Corticosteroids increase the risk of posterior subcapsular type of cataract. It seems that steroids do not directly act on the lens but rather produce an imbalance of ocular cytokines and growth factors [12]. Recently, it was demonstrated that distinct metabolic compartments exist in the lens and these explains different etiologies of nuclear and cortical cataract, even the one induced by Prednison [13].

Dysglycaemia and oxidative stress are the principal culprits in almost all types of cataract [14]. Lower levels of systemic and lens antioxidants including lutein and reduced glutathione (GSH) are associated with cataract [15].

In order to restore the depleted antioxidant level in the lens, it is important to enhance the delivery and accumulation of antioxidants in the lens. In Sprague-Dawley rats with Dexamethasone (5 µM) induced cataract, N-acetylcysteine amide elevated the GSH/GSSG ratio in the lenses, limiting the lipid peroxidation [16]. In other experimental studies, the benefic effect of carotenoids was explained by increasing the antioxidant defence in the eyes, but, interesting, only in H<sub>2</sub>O<sub>2</sub> induced cataract, lutein didn't mitigate the low level of GSH [10].

Lutein is a beta-carotene which accumulates preferentially in the macula lutea. It defends oxidative damage from ultraviolet light and it is known as a protective factor in age macular degeneration [5]. But, lutein, also delays the cataract onset and progression. For these reason it can be recommended as a supplement for eye diseases [17,18].

**TABLE 1.** Comparison of the average values of serum parameters for groups: S and F

Parameters	S group	F group	F vs S
Cortisol µg/dl	7.25±1.06	9.45±2.33	Ns
FBG mg/dl	86±2.62	109.5±4.94	p<0.0001
Hb A1c%	3.8±0.02	4.9±0.14	p<0.0005
Ionic Na mmol/l	124.56±2.85	195.23±8.26	Ns
Triglycerides mg/dl	96.5±4.94	241.5±12.79	p<0.0035
HDL cholesterol mg/dl	24±4.24	23±2.82	Ns
Cholesterol mg/dl	88.5±12.02	76.2±13.43	Ns
TAC mmol/l	5.25±0.49	4.25±1.9	Ns

FBG fasting blood glucose, Hb A1c glycated hemoglobin, Na serum sodium  
HDL high density lipoprotein TAC total antioxidant capacity

**TABLE 2.** Comparison of the average values of serum parameters for groups: S and SP

Parameters	S group	SP group	S vs SP
Cortisol µg/dl	7.25±1.06	9.2±3.02	p<0.001
FBG mg/dl	86±2.62	106.75±3.59	p<0.0001
Hb A1C %	3.8±0.02	5.05±0.91	p<0.02
Ionic Na mmol/l	124.56±2.85	132.7±6.08	p<0.026
Triglycerides mg/dl	96.5±4.94	134.8±21.85	p<0.005
HDL cholesterol mg/dl	24±4.24	20.04±3.4	p<0.05
Cholesterol mg/dl	88.5±12.02	118.8±13.55	p<0.0006
TAC mmol/l	5.25±0.49	4.775±0.63	Ns





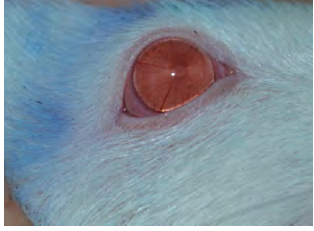





FBG fasting blood glucose, Hb A1c glycated hemoglobin, Na serum sodium  
HDL high density lipoprotein TAC total antioxidant capacity

**TABLE 3.** Comparison of the average values of serum parameters for groups: F and PF and also for PFL and PF

Parameters	F group	PF group	F vs PF	PFL group	PF vs PFL
Cortisol µg/dl	9.45±2.33	6.1±1.13	p<0.02	7.92±3.22	Ns
FBG mg/dl	109.5±4.94	121±7.07	p<0.01	96±18.16	p'<0.02
Hb A1C %	4.9±0.14	5.9±0.42	p<0.001	4.775±0.74	p'<0.01
Ionic Na mmol/l	195.23±8.26	136±22.7	Ns	132.09±15.8	Ns
Triglycerides mg/dl	241.5±12.79	403.5±30.4	p<0.001	342±49.5	p'<0.02
HDL cholesterol mg/dl	23±2.82	17.7±2.5	p<0.05	19.8±2.8	Ns
Cholesterol mg/dL	76.2±13.43	91.5±12.02	p<0.026	66.5±12.81	p'<0.013
TAC mmol/l	4.25±1.9	3.1±1.31	Ns	5.1±0.99	p'<0.017

FBG fasting blood glucose, Hb A1c glycated hemoglobin, Na serum sodium  
HDL high density lipoprotein TAC total antioxidant capacity

**TABLE 4.** Clinical eye examination results

Wistar group	Clinical examination 1	Clinical examination 2
S group-transparent lens		
SP group-cataract, uveitis and rubeosis iridis		
F group-cataract and uveitis		
PF group-glaucoma, cataract rubeosis iridis		
PFL group almost normal aspect		

Metabolic disorders (obesity, diabetes mellitus), life style (long use of steroids, smoking), aging and poor diet are important risk factors for cataract.

In our study, the rats on obesogenic diet, associated with Prednison had the “worst picture” of metabolic parameters and eye aspect. The high caloric/high fat diet increased the levels of fasting plasma glucose, of glycated hemoglobin and of serum triglycerides and didn't influence significantly the levels of total antioxidant capacity and cholesterolemia (Table 1). Each time, when rats were treated with Prednison, regardless the diet (obesogenic or standard), dysglycaemia and dyslipidemia were present (Table 2). Our results are in accordance with clinical studies which

demonstrated that there is a negative correlation between cortisol and cholesterol/HDLc ratio and a positive correlation between saliva cortisol and fasting glycaemia and between Dexamethasone treatment and high glycaemia [19-21].

By MRI imaging, it was demonstrated that the lens has a unique internal microcirculation system to deliver antioxidants in different regions, and sodium is important in this mechanism [13]. In both groups treated only with Prednison, the serum level of ionic sodium was significantly increase (Tables 2 and 3). Maybe serum sodium can influence the ocular level of this metal.

In the high fat diet Wistar rats, the association of lutein loaded PLGA-NPs to Prednison, decreased

the increased values for glycaemia, triglyceridemia and improved the systemic antioxidant defence of the body. Bodoki et al., by comparison free lutein or lutein-loaded NPs administered orally or topically in selenite cataract, demonstrated that the severity of lens injury was significantly reduced in rats treated with topical applications of lutein-loaded NPs [22]. Recently, it was demonstrated that in diabetic cataract, autoregenerative redox nanoparticles can act as an antioxidant and glycation inhibitor [23].

Experimental studies demonstrated that lutein has also hepatoprotective effects, „fighting“ against xenobiotics and reversing the histopathological abnormalities. This antioxidant reduced the elevated levels of ALT and AST enzymes in ethanol treated rats [24]. Also, it was demonstrated that PLGA-NPs loaded with vitamin E had visceral tissue protective effects and lowered cholesterolemia [25].

In accordance with the dysmetabolic modifications, oral treatment with glucocorticoids produced eye injuries: cataract, uveitis, glaucoma and rubeosis iridis. While cataract is the clouding of the lens, the rubeosis iridis is associated with impaired ciliary body perfusion, with a subsequent decrease in aqueous production [26].

The eyes from S group, control group, healthy group, on standard diet had transparent cornea and crystallin, while the eyes from SP Group, with

prednisolone treatment (1mg/kg body weight), standard diet had uveitis and rubeosis iridis (Table 4).

In the eyes from F group with fat diet, cataract and uveitis were observed.

In group PF, with prednisolone treatment (1 mg/kg body weight) and fat diet, glaucoma, cataract and rubeosis iridis were present (Table 4).

Group PFL with Prednisolone treatment (1 mg/kg body weight), fat diet and PLGA NPs loaded with lutein (1 mg/kg body weight), normal aspect is observed. The association of lutein to the glucocorticoids prevented the inflammatory and degenerative modifications in the eye (Table 4).

## CONCLUSION

PLGA-NPs loaded with lutein (1 mg/kg body) had ocular protective effects in high fat diet Wistar rats treated with Prednisolone (1 mg/kg body) and improved sugar and lipid metabolism.

## Acknowledgments

Authors thank to Assoc. Prof. Iuliana Ionascu, MD, PhD, University of Agronomic Sciences and Veterinary Medicine, 59 Mărăști Blvd., 011464, Bucharest, Romania) and to lecturer Ramona Barac, MD, PhD, Department of Ophthalmology, Carol Davila University of Medicine and Pharmacy, 8 Eroilor Sanitari, 050474, Bucharest, Romania.

All the authors had equal contribution.

*Conflict of interest:* none declared  
*Financial support:* none declared

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