Int J Ophthalmol, Vol. 1, No. 4, Dec.18, 2008 www. IJO. cn
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·Basic Research·

Carnosine inhibits cataract formation and inactivation of Na^+-K^+ATPase induced by a glucocorticoid

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Foundation item: Excellent Youths Research Award (No.04J011)
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Received: 2008-04-19 Accepted: 2008-09-06

Abstract

• AIM: To investigate whether carnosine can inhibit cataract formation and protect Na^+-K^+ATPase against inactivation induced by a glucocorticoid.

• METHODS: Two hundred and twenty clear lenses cultured *in vitro* were randomly divided into five groups: control group (DMEM), steroid group (DMEM+Dexamethason 10μmol/L), lower concentration carnosine-treated group (DMEM+Dexamethason 10μmol/L+Carnosine 2mmol/L), higher concentration carnosine-treated group (DMEM+Dexamethason 10μmol /L+ Carnosine 5mmol/L) and carnosine group (DMEM + Carnosine 5mmol/L). Progression of cataract formation was evaluated daily using a dissecting microscope. On 1, 3, 5 and 7 days, 10 lenses of every group were homogenized and the activity of Na^+-K^+ATPase was measured by using spectrophotometer.

• RESULTS: During the incubation, mistlike opacity was observed in the lenses of the control group and carnosine group, but in the steroid group appeared dense nuclear opacity, while both two carnosine-treated groups came out visible demarcation between nuclear and cortical regions on 7 days. A decrease in the activity of Na^+-K^+ATPase was found in the lens of the steroid group. On 3, 5, 7 days, Na^+-K^+ATPase activity decreased 22.34% (P=0.002), 47.98% (P<0.001), 75.37%(P<0.001) compared with that at 1 day, respectively. In the carnosine group, the activity of Na^+-K^+ATPase remained at the level of the control throughout the 7-day incubation, indicating that carnosine itself did not interfere with the original lens enzyme activity. In the lower concentration carnosine-treated group, on 3, 5, 7 days, the activity of Na^+-K^+ATPase increased 10.8% (P<0.05), 44.6% (P<0.01), 57.4% (P<0.01) of control activity, respectively. In the higher concentration carnosine-treated group, the activity of Na^+-K^+ATPase increased 11.3% (P<0.05), 45.7% (P<0.01), 57.6% (P<0.01), respectively on 3, 5, 7 days. The activity of Na^+-K^+ATPase in both two carnosine-treated groups were only 6.7% and 6.5% lower than that of the control group after 7-day incubation. After the 7-day incubation, the Na^+-K^+ATPase activity of the lenses in the steroid group decreased significantly compared with carnosine-treated groups(P< 0.01).

• CONCLUSION: Carnosine prevents the cataract formation induced by a glucocorticoid, and significantly inhibits the inactivation of Na^+-K^+ATPase induced by a glucocorticoid.

• KEYWORDS: carnosine; glucocorticoid; cataract; Na^+-K^+ATPase

Sun Y, Yan H. Carnosine inhibits cataract formation and inactivation of Na^+-K^+ATPase induced by a glucocorticoid. *Int J Ophthalmol* 2008;1(4):307-310

INTRODUCTION

Cataract, or loss of lens transparency, is a leading cause of blindness worldwide. A side effect associated with long-term treatment of various diseases with glucocorticoids is a high incidence of posterior subcapsular cataracts (PSC)[1,2]. The morbidity of steroid-induced cataract is positive correlated with the doses and using time of glucocorticoids. Despite various studies of steroid-induced cataracts, the precise molecular mechanism involved in the formation of steroid-induced cataract is unclear, because it is difficult to procure human samples. Equilibrium of ions is quite important to maintain lens transparence. Na^+-K^+ATPase is one of the key enzymes regulating the ionic balance in lens. Inhibition of Na^+-K^+ATPase by corticosteroid increases intracellular sodium concentrations and decreases potassium levels, which leads to the accumulation of water within lens fiber cells and the formation of cataract [3]. At present, the only effective treatment is surgery. Although the visual acuity could be improved by surgery, the operation risk, serve postoperative complications and the concomitant economic problems could not be ignored. Thus, it is quite important to find out effective anti-cataract drugs.

Carnosine has anti-glycation, anti-oxidant and free-radical scavenging roles and has been proposed to be a potential therapeutic agent for some conformational diseases including cataract and Alzheimer's disease [4]. Babizhayev *et al* [5] and Seidler *et al* [6] had proved the anti-cataract function of carnosine in their *in vivo* and *in vitro* studies. Furthermore, carnosine's protection against steroid-induced inactivation of

important enzymes in lens including esterase and catalase has been reported [7,8]. The aim of the study is to investigate whether carnosine can protect Na^+-K^+ATPase against inactivation induced by a glucocorticoid, which is pertinent to cataract formation.

MATERIALS AND METHODS

Materials One hundred and twenty one-month-old Sprague-Dawley rats were provided by Animal Laboratories of Fourth Military Medical University (Xi'an, China). Carnosine was purchased from Sigma Chemical Co. Ltd. (Beijing,China). The kit of Na^+-K^+ATPase was from Jiancheng Biology Company (Nanjing, China).

Methods

Isolation and culture of rat lenses Two hundred and twenty lenses were obtained by posterior approach from eyes of one-month-old Sprague-Dawley rats. They were incubated at 37℃, 50mL/L CO_2 atmosphere in Dulbecos MEM (Sigma) supplemented with 100mL/L fetal calf serum, 0.584g/L Glutamine, 5mg/L Transferrin, 100kU/L Penicillin and 0.1g/L Streptomycin. Lenses were immediately transferred into culture medium. Approximately 24 hours after the preparation of organ cultures, clear lenses were selected and randomly divided into five groups: control group (basic medium), steroid group (basic medium with 10μmol/L dexamethasone), lower concentration carnosine-treated group (basic medium with 10μmol/L dexamethasone and 2mmol/L carnosine), higher concentration carnosine-treated group (basic medium with 10μmol/L dexamethasone and 5mmol/L carnosine) and carnosine group (basic medium with 5mmol/L carnosine). Lenses were cultured for 7 days at 37℃ under 50mL/L CO_2. During the incubation, lenses were evaluated daily by using a dissecting microscope. The degrees of nuclear opacity were graded into five levels. Stage 0 = Clear lens, Stage 1 = lens with mistlike opacity, Stage 2 = lens with visible demarcation between nuclear and cortical regions, Stage 3 = lens with light nuclear opacity, Stage 4 = lens with dense nuclear opacity and Stage 5 = whole lens opacity[9].

Na^+–K^+ATPase activity assay On 1, 3, 5 and 7 days, ten lenses were used in each group, and each lens was immersed in cold saline (50mg of lenses/mL of saline) respectively. All further treatments were performed at 0-4℃. Lenses were shattered and centrifugated at 3 000r/min for 15 minutes. The supernatant was used to assay the Na^+-K^+ATPase activity. The enzyme activity was measured by 756-PC spectrophotometer(Shanghai, China). The hydrolytic activity of Na^+-K^+ATPase was assessed as ATP hydrolysis under maximal velocity conditions by measuring the release of inorganic phosphate (Pi) from ATP at 660nm. One unit of enzyme activity was defined as producing 1μmol Pi per milligram of protein per hour at 37℃.

Statistical Analysis Statistical significance of differences was examined with Kruskal-Wallis H test to ranked data and repeated measurement data variance analysis to different times and groups' comparison. Results of opacification are presented as median values and ranges. Results of Na^+-K^+ATPase activity are presented as the mean±SEM.

RESULTS

Isolated Whole Rat Lens System Whole lenses were cultured for 7 days without (control group, carnosine group) or with (steroid group, two carnosine-treated groups) dexamethasone. Along with the incubation, opacification of rat lenses gradually aggravated. In the steroid group, a part lens appeared hazy opacity during the second day of incubation. On 3 days, a part lens appeared visible demarcation between nuclear and cortical regions, most lenses appeared hazy opacity. On 4 days, lenses appeared light nuclear opacity. On 6 days, lenses appeared dense nuclear opacity. On 7 days, a part lenses were whole opacity. A time-dependent aggravation in opacification of lenses in the steroid group was observed up to 7days incubation. In the control group and carnosine group most lenses incubated for 4 days remained clear and appeared similar to fresh lenses and a little lenses appeared a hazy opacity, a little lenses with visible demarcation between nuclear and cortical regions on 7 days. In both two carnosine-treated groups, on 2 days, a part lens appeared a hazy opacity. On 4 days, a part lenses with visible demarcation between nuclear and cortical regions. On 6 days, a part lenses were light nuclear opacity, most lenses appeared visible demarcation between nuclear and cortical regions. Since day 3, significant statistical differences of lenses opacification appeared between the steroid group and other four groups (P<0.05). Carnosine exhibited inhibition of cataract formation induced by a glucocorticoid (Table 1).

Enzymatic Assays On 1 day, the activity of Na^+-K^+ATPase had no differences between the five groups. During the incubation, the activity showed a time-dependent loss. In the steroid group, the enzyme activity decreased significantly, compared with day 1, the activity decreased 22.34% (P= 0.002) on 3 days, 47.98% (P<0.001) on 5 days and 75.37% (P<0.001) on 7 days, respectively. In the carnosine group, the activity of Na^+-K^+ATPase remained at the level of the control throughout the 7-day incubation, indicating that carnosine did not interfere with the original lens enzyme activity. In the lower concentration carnosine-treated group, on 3, 5, 7 days, the activity of Na^+-K^+ATPase increased 10.8% (P<0.05), 44.6% (P<0.01), 57.4% (P<0.01) of control activity, respectively. In the higher concentration carnosine-treated group, the activity of Na^+-K^+ATPase increased 11.3% (P<0.05), 45.7% (P<0.01), 57.6% (P<0.01), respectively on 3, 5, 7 days (Figure 1). The

Table 1 Protective effect of carnosine on steroid-induced opacification of rat lenses analyzed by ocular staging (n=10)

	Ocular staging, stages 0-5						
Group	1d	2d	3d	4d	5d	6d	7d
DMEM	0(0-0)	0(0-0)	0(0-1)	0(0-1)	1(0-1)	1(0-2)	1(0-2)
DMEM+DEX	0(0-1)	1(0-2)	2(0-3)[a]	2(1-3)[a]	3(2-3)[b]	4(2-4)[b]	4(2-5)[b]
DMEM+DEX+2mM C	0(0-1)	0(0-1)	0(0-1)[c]	1(0-2)[c]	1(0-2)[d]	1(0-3)[d]	2(0-3)[d]
DMEM+DEX+5mM C	0(0-1)	0(0-1)	0(0-1)[e]	1(0-2)[e]	1(0-2)[f]	1(0-2)[f]	2(0-3)[f]
DMEM +5 mM C	0(0-0)	0(0-0)	0(0-1)	0(0-1)	1(0-1)	1(0-2)	1(0-2)

DMEM: Dulbeccos MEM; Dex: 10μmol/L dexamethasone; C: carnosine. The numbers outside parenthesis present median value of opacification stages and inside parenthesis present the range of the ocular staging.

[a]P<0.05,DMEM+DEX *vs* DMEM;[b]P<0.01,DMEM+DEX *vs* DMEM;[c]P<0.05,DMEM+DEX *vs* DMEM+DEX+2mmol/L C;[d]P<0.01, DMEM+DEX *vs* DMEM+DEX+2mmol/L C; [e]P<0.05, DMEM+DEX *vs* DMEM+DEX+5mmol/L C; [f]P<0.01, DMEM+DEX *vs* DMEM+DEX+5mmol/L C (Kruskal-Wallis H test)

activity of Na^+-K^+ATPase in two carnosine-treated groups was only 6.7% and 6.5% lower than that of the control group after the 7-day incubation, respectively. After 5-day incubation, the Na^+-K^+ATPase activity of the lenses in the steroid group decreased significantly compared with carnosine-treated groups (P<0.01). The extent of inactivation and protection was significantly different after 7-day incubation (P< 0.01).

DISCUSSION

Several mechanisms have been proposed for the changes leading to lens opacification induced by steroid including osmotic failure. It is known that the maintenance of ionic balance has been shown to be crucial for lens transparency[10]. Na^+-K^+ATPase protein is critical for maintaining cellular ion gradients and volume and for transepithelial ion transport. It could not only keep intra-cellular high concentration of K^+ and low concentration of Na^+, and also maintain the Na^+ dependent transport mechanism to transport the amino acids and solute. However, the activity of Na^+-K^+ATPase is inhibited in steroid-induced cataract. Mayman reported an 80% inhibition of calf lens Na^+-K^+ATPase by 10-4mol/L dexamethasone. The inhibition showed a dose-dependent effect and resulted in a reduction in light transmission by the lens [11]. Since then, many studies proved that the steroid-induced inactivation of Na^+-K^+ATPase and increase of ionic flow induce damage of cellular membrane, which leads to sodium and water accumulation and cataract.

Many anti-cataract drugs have been investigated including antioxidants and/or free radical scavengers like vitamin E, N-(2-mercaptopropionyl)glycine (MPG) [12,13], glucocortico-steroid receptor RU486 [14,15] and aspirin [16]. Carnosine is responsible for a variety of activities related to the protection

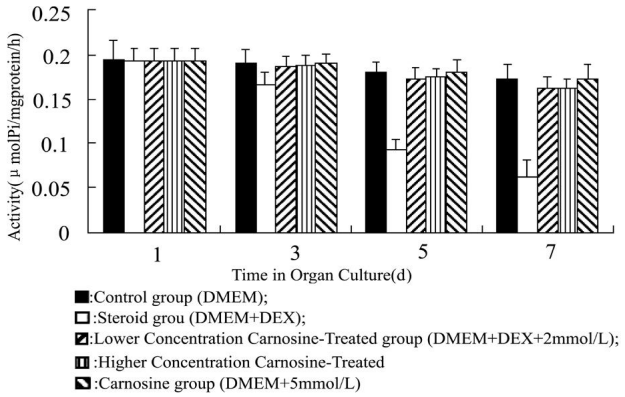

Figure 1 Protective effect of carnosine on inactivation of Na^+–K^+ATPase induced by a glucocorticoid. DMEM: Dulbeccos MEM; Dex: 10μmol/L dexamethasone; C:carnosine. Data are expressed as mean±SEM

of the protein from oxidation and glycation, but recent studies have shown that this small molecule also has anti-cataract function *in vivo* and *in vitro* [5,6]. The anti-cataract mechanisms of carnosine including carnosine inhibits glycosylation and formation of advance glycation end product[17]; carnosine raises the metabolic rate of protein and hydrolyze the cross-linking protein and scavenge free-radical to protect α-crystallin against oxidative stress, protects Na^+-K^+ATPase to maintain the fluid and electrolyte balance, inhibits the cross-linking of protein and damage of DNA [4]; carnosine protects against the inactivation of some key metabolic enzymes and protects α-crystallin modification and decreased chaperone induced by glycation and steroid[7,18,19].

Our data showed that a high concentration (10mmol/L) of dexamethason induced nuclear opacity in organ-cultured rat lens. In the steroid group, a time-dependent aggravation in opacification of lenses was observed up to 7-day incubation.

However, in both two carnosine-treated groups, most lenses appeared visible demarcation between nuclear and cortical regions on 6 days. After 3-day incubation, significant statistical differences of lenses opacification appeared between the steroid group and carnosine-treated groups ($P<0.05$). Carnosine exhibited inhibition of cataract formation induced by a glucocorticoid.

It was reported in an *in vitro* study which rats lenses occurred cortex opacity induced by Solu-Medrone, the activity of Na^+-K^+ATPase decreased 20% in the initial stage, while in advanced stage the rate was 27%[20]. Our previous results had shown the enzyme activity of Na^+-K^+ATPase decreased 23.5% and 75.8% on 3 and 7 days compared to that on 1 day [21]. The present data showed a time-dependent decrease of Na^+-K^+ATPase activity in the steroid group was observed up to the 7-day incubation. With the inactivation of Na^+-K^+ATPase, the cellular membrane was damaged and ionic transport disordered, lens opacity occurred. However, in the carnosine-treated groups, the activity was only 6.7% and 6.5% lower than the control group after 7-day incubation, respectively. After 5-day incubation, the extent of inactivation and protection was significantly different ($P<0.01$). Our data suggests that the inactivation of Na^+-K^+ATPase contribute to the development of steroid-induced cataract and carnosine protect the inactivation of Na^+-K^+ATPase induced by a glucocorticoid, which may contribute to maintain the ionic transport balance and transparency of lens.

However, no significant difference was shown in protective effect between two concentrations of carnosine. Our previous results showed that carnosine in concentrations of 50mmol/L and 100mmol/L could protect steroid-induced (-crystallin modification and decreased chaperone by directly reacting with a glucocorticoid [18]. We chose lower concentrations (2mmol/L and 5mmol/L) in order to avoid this reaction. The results showed that carnosine protected the inactivation of Na^+-K^+ATPase induced by a glucocorticoid, but no significant difference in protective effect was shown between two concentrations, the reason may be that concentration difference was not sufficient to cause significant difference in protective effect.

In conclusion, carnosine was used as an inhibitor on steroid-induced lens opacity in this study, we found that carnosine effectively inhibited the opacity of rat lenses and protected the inactivation of Na^+-K^+ATPase induced by a glucocorticoid *in vitro*. These results suggest that carnosine may have a potential role in treatment of for steroid-induced cataracts.

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