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Alpha lipoic acid protects lens from H_2O_2-induced cataract by inhibiting apoptosis of lens epithelial cells and inducing activation of anti-oxidative enzymes

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ABSTRACT

Objective: To determine whether alpha lipoic acid (LA) can effectively protect lenses from hydrogen peroxide (H_2O_2)-induced cataract. **Methods:** Lens from adult Sprague-Dawley rats were cultured in 24-well plates and treated without or with 0.2 mM of H_2O_2, 0.2 mM of H_2O_2 plus 0.5 mM, 1.0 mM, or 2.0 mM of LA for 24 h. Cataract was assessed using cross line grey scale measurement. Superoxide dismutase (SOD), glutathione (GSH-Px), lactate dehydrogenase (LDH), and malondialdehyde (MDA) activity or level in lens homogenates was measured. Apoptosis of lens epithelial cells in each group were detected by Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) Assay. **Results:** A total of 0.2 mM of H_2O_2 induced obvious cataract formation and apoptosis in lens' epithelial cells, but 0.5-2.0 mM of LA could block the effect of 0.2 mM H_2O_2 in inducing cataract and apoptosis. Furthermore, 0.2 mM of H_2O_2 significantly decreased SOD, GSH-Px, and LDH activity and significant increased MDA level in the lens, but 0.5-2.0 mM of LA blocked the effect of 0.2 mM H_2O_2. One mM of LA was found to be the most effective. **Conclusions:** LA can protect lens from H_2O_2-induced cataract. LA exerts protective effects through inhibition of lens' epithelial cell apoptosis and activation of anti-oxidative enzymes.

1. Introduction

Cataract is the leading cause of blindness worldwide, and it prevalence rises in tropical area because of increased UV light exposure and temperature[1]. It causes enormous socio-economic burden[2]. Although cataract surgery has become one of the most successful therapies for cataract, it still comes with many risks and complications[3]. It is estimated that if the onset of clinical cataract can be delayed for 10 years, half of the cataract surgeries will not be necessary[4]. Oxidative stress is believed to play a pivotal role in cataract formation and progression[5]. Recent studies have found that reactive oxygen species (ROS) produced by H_2O_2 cause epithelial cell damage and protein degradation followed by subsequent cataract formation in the lens[6-10]. At the same time, the human lens employs multiple anti-oxidative mechanisms to prevent or repair oxidative stress, including enzyme-dependent systems such as superoxide dismutases (SOD) and glutathione peroxidase (GSH-Px), or enzyme-independent systems, such as Vit C, Vit E, and CoQ. Among these anti-oxidative systems, glutathione (GSH) deficiency is very common in the lens[11].

Despite the definite role of ROS in cataractogenesis, recent studies suggest that traditional antioxidants, such as Vit C, Vit E and Beta-carotene, exhibited no evidence in preventing or slowing the progression of cataract[12]. New antioxidant treatments should be explored. Recently, α-lipoic acid (LA) has gain considerable attention as a powerful antioxidant[13]. LA is a disulfide compound found

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naturally in the mitochondria as an essential coenzyme of mitochondrial pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. LA can scavenge ROS directly or stimulate the synthesis of other antioxidants, such as glutathione[14-16]. Recent studies showed that LA treatment delayed the development and progression of diabetic cataracts in rat models[17,18]. Accordingly, LA was suggested to be a good candidate for protection against oxidative stress. We hypothesize that LA can protect lens from oxidative damage-induced cataract by regulating anti-oxidative enzymes to restore the balance of redox status in the lens.

In the present study, we investigated whether LA could reduce H_2O_2-induced lens opacity in cultured lenses and then investigated whether the protective effect is conducted by inhibiting lens' epithelial cell apoptosis and inducing activation of anti-oxidative enzymes in the lenses.

2. Materials and methods

2.1. Animals

Eight-week-old Sprague-Dawley rats (average weight 190 g) were purchased from the Chinese Scientific Academy. All experimental procedures were compliant to the ARVO statement for the Use of Animals in Ophthalmic and Vision research.

2.2. Chemicals and reagents

Alpha lipoic acid was purchased from Sigma (St. Louis, MI, USA). H_2O_2 was purchased from Shanghai Chemical Tech (Shanghai, China). MEM medium was purchased from Gibco (Grand Island, NY, USA). 10% chloral hydrate was from the Pharmacy of 2nd Xiangya Hospital, Central South University. TUNEL assay kit was from KeyGEN Biotech Company Ltd (Shanghai, China). SOD, GSH-Px, LDH, and MDA colorimetric kits were purchased from Nanjing Jiancheng Bioengneering Institute (Nanjing, China). All other agents were of analytical purity from the Central Laboratory of 2nd Xiangya Hospital, Central South University.

2.3. Lens culture and treatments

Rats were sacrificed with 10% chloral hydrate overdose. The eyeballs were surgically removed immediately and washed with pre-warmed, sterile PBS with 80 IU/mL penicillin and 100 μg/mL streptomycin. The eyeballs were dissected under the microscope. The extracted lenses were placed in 24-well plates containing 2 mL of MEM medium in each well and cultured at 37 ℃, 5% CO_2. The MEM medium was refreshed every 12 h. After 24 h of culture, lenses damaged during dissection were discarded.

Transparent lens were divided into 5 groups with 10 lenses in each group: lenses were cultured with MEM medium only (control group), 0.2 mM of H_2O_2 (H_2O_2 treatment), or 0.2 mM of H_2O_2 plus 0.5 mM, 1.0 mM, and 2.0 mM of LA (H_2O_2+LA), respectively, for 24 h.

2.4. Lens opacity measurement

Image of each lens was acquired with a digital camera under the same lighting, distance, focus and angle. During imaging, lenses were positioned on top of a black and white crossline of 0.5 mm in width. Adobe photoshop 10.0 was used to analyze the gray scale value of the crossing point for each lens.

2.5. TUNEL assay

The lens capsule was dissected from the equator using Vannas scissors. Poly-L-Lysine slides were used to pick up the anterior capsule with lens epithelial cells facing up. Flat mount of the anterior capsule was fixed in 4% paraformaldehyde/0.01M PBS for 30 min at room temperature followed by washing with 0.01 M of PBS for 3 times. Apoptosis in cultured lens' epithelial cells was detected by TUNEL staining according to the manufacturer's instructions. The manufacturer's positive sample slides were used as positive control.

2.6. Tissue homogenization

After 24 h of culture, lenses from each group were washed with pre-chilled 0.9% saline, dried with filter paper, weighed and collected in a glass vial on ice. Tissues were promptly cut with Vannas scissor and then homogenized in a glass homogenizer containing PBS. Tissues were fully homogenized for 6 min and spin down in a refrigerated centrifuge at 1 000 r/min for 5 min. The supernatant was harvested. Protein concentration was measured using a spectrophotometer with Coomassie brilliant blue at 595 nm.

2.7. Measurement of enzyme activities

Total SOD activity was determined spectrophotometrically at 550 nm as described previously[19]. The values of SOD were expressed as U/mg protein. The activity of LDH was measured by the 2,4-dinitrophenylhydrazine method as previously described[20]. The values were expressed as U/mg protein. The activity of GSH-Px was determined as previously described[21] with few modifications. Briefly, the rate of glutathione oxidation by H_2O_2, as catalyzed by the Gpx present in the supernatant, was determined. MDA is formed from the breakdown of polyunsaturated fatty acids and serves as a convenient parameter for determining the extent of the peroxidation reaction. MDA was measured by the thiobarbituric acid method[22]. The results were expressed as nmol/mL.

2.8. Statistical analysis

Data were presented as mean±SD. The comparison between each group (n=10) was carried out with SPSS 16.0

software as follow: lens opacity (cataractous) scale: ANOVA; TUNEL: ANOVA; SOD, GSH-Px, LDH and MDA level: LSD-*t* test. A $P< 0.05$ was considered statistically significant.

3. Results

Transparent lenses were cultured in MEM medium and treated with 0.2 mM of H_2O_2, or 0.2 mM of H_2O_2 plus 0.5 mM, 1.0 mM, or 2.0 mM of LA for 24 h. Lens opacity measurement showed that 0.2 mM of H_2O_2 induced obvious cataract formation in lenses, but 0.5-2.0 mM LA could block the effect of 0.2 mM of H_2O_2 (Figure 1). We further investigated whether H_2O_2 induces apoptosis in epithelial cells of the lens. TUNEL staining showed that 0.2 mM of H_2O_2 induced obvious apoptosis in lens's epithelial cells. One mM of LA significantly inhibited the apoptotic role of 0.2 mM of H_2O_2 (Figure 2). The biological activity assay showed that 0.2 mM of H_2O_2 significantly decreased SOD (Figure 3a), GSH-Px (Figure 3b), and LDH (Figure 3c) activity in lenses, but 0.5-2.0 mM of LA blocked the effect of 0.2 mM of H_2O_2. However, 1 mM of LA was most effective. In contrast, 0.2 mM of H_2O_2 induced significant increase in MDA level in Lens, but LA blocked the effect of H_2O_2 in MDA activity (Figure 3d). Also, 1 mM LA was most effective in blocking the effect of H_2O_2 in MDA activity.

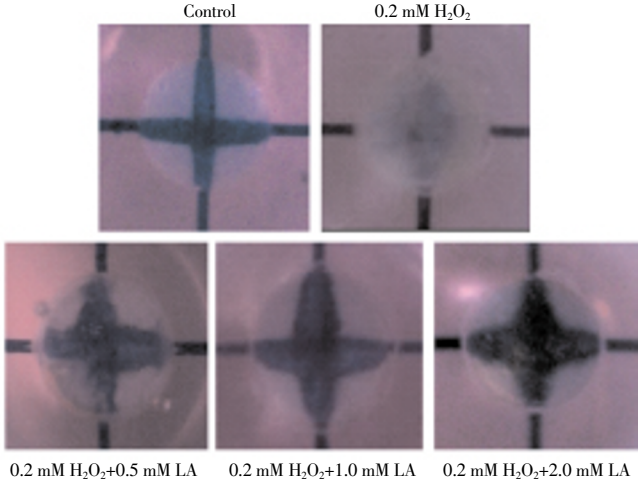

Figure 1. Alpha lipoic acid protected lens against H_2O_2-induced cataract formation.

A representative image from each group (*n*=10).

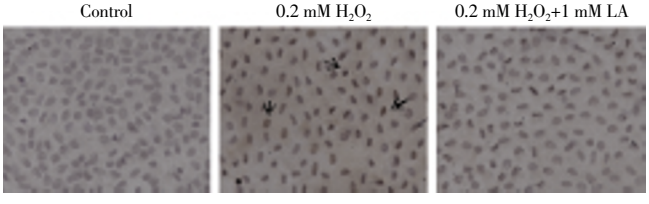

Figure 2. Alpha Lipoic acid inhibited H_2O_2-induced apoptosis in lens epithelial cells.

Representative TUNEL staining of lens' epithelial cells (*n*=10). Arrows indicate positive staining.

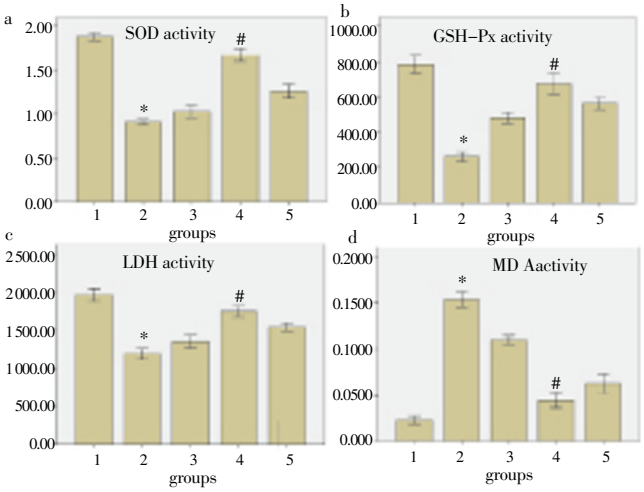

Figure 3. The protective role of LA.

Lenses were cultured with MEM media (group 1), 0.2 mM of H_2O_2 (group 2), or 0.2 mM of H_2O_2 plus 0.5 mM (group 3), 1.0 mM (group 4), or 2.0 mM (group 5) of LA for 24 h.

a) SOD activity in Lenses. H_2O_2 decreased SOD activity in lenses, but LA blocked the effect of H_2O_2. 1 mM LA was most effective. b) GSH-Px activity in lenses. H_2O_2 decreased GSH-Px activity in lenses, but LA blocked the effect of H_2O_2 1 mM of LA was most effective. c) LDH activity in Lenses. H_2O_2 decreased LDH activity in lenses, but LA blocked the effect of H_2O_2. 1 mM LA was most effective. d) MDA level increase in Lenses. H_2O_2 increased MDA activity in lenses, but LA blocked it.

The effect of H_2O_2 in MDA activity. 1 mM LA was most effective. *$P<$ 0.001 *vs.* control (group 1); #$P<$ 0.001 *vs.* H_2O_2 treatment group (group 2).

4. Discussion

H_2O_2 is the most common source of ROS in the lenses of cataract patients. In this study, we demonstrated that LA protected cultured lenses from H_2O_2-induced cataract through inhibiting H_2O_2-induced epithelial cell apoptosis. The H_2O_2-induced cataract was associated with decreases in SOD, GSH-Px, and LDH activities and an increase in MDA activity in the lens. Li *et al*[23] found that lens' epithelial cell apoptosis is a common mechanism of all noncongenital cataracts, suggesting that lens epithelial cells are essential for maintenance of metabolic homeostasis and transparency of the lens. Previous studies demonstrated that lens epithelial cells can produce high local oxygen concentrations when chronically exposed to light[24]. The oxidative stress induced by H_2O_2 has been recognized as an important mediator of apoptosis in lens' epithelial cells, which had been identified as an important molecular basis for both the initiation and the progression of cataract[25]. Therefore, H_2O_2 is a classical model that induces cataract formation.

LDH is an indicator of cell integrity and has been reported to diminish markedly during cataract genesis[26]. In this study, the level of LDH decreased in H_2O_2-treated lenses compared to the control lenses. The decrease was blocked in LA treated lenses. During oxidative stress, there is an increase in lipid peroxidation, which is considered to be the basic mechanism of cellular damage caused by free radicals, characterized by a rise in the level of MDA[27]. We observed that the level of MDA was significantly higher in H_2O_2-treated lenses than in the control lenses, which is consistent with previous

reports[28]. However, our study showed that LA significantly inhibited H_2O_2-induced increase in MDA levels in lenses.

In addition to quenching reactive oxygen species directly, LA may prevent protein, lipid or DNA from oxidative damage by regulating other cellular antioxidant systems. Glutathione is one of the major intracellular antioxidants in the lens and plays an important role in protecting cells from oxidative damage[29-34]. LA induces three enzymes which are essential in oxidative stress protection and normal lens metabolism (SOD, LDH, and GSH-Px). LA can effectively recover their activity in H_2O_2-treated lenses, which parallel the role of LA in inhibiting lens epithelial cells from H_2O_2-induced apoptosis. Our study suggests that LA has a potential therapeutic role in the prevention of cataract.

In conclusion, the present study demonstrated that LA could significantly reduce H_2O_2-induced cataract formation and protect lens epithelial cells against H_2O_2-induced apoptosis. We also showed that LA upregulates the activities of SOD, GSH-Px, LDH and reduces MDA production in H_2O_2-induced oxidative stress. Our findings suggest that LA is a powerful antioxidant, which might be a promising candidate for cataract prevention.

Conflict of interest statement

We declare that we have no conflict of interest.

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