THE ROLE OF ALPHA-LIPOIC ACID IN STREPTOZOTOCIN-INDUCED DIABETIC CATARACT

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Abstract. Alpha-lipoic acid (LA) is well known as a powerful antioxidant. The effect of LA against streptozotocin (STZ)-induced diabetic cataract in rabbits was investigated. Twenty New Zealand albino rabbits were divided into 4 groups. Group I served as the control one. The other three groups (II, III, and IV) received a single intravenous injection of STZ (50 mg/kg body weight). After the onset of diabetes, cataract was developed within 12 to 13 weeks in group II. Groups III and VI received a daily dose of 150 mg/day of LA for 8 weeks and 16 weeks after induction of diabetes, respectively. The activity of Na^+-K^+ATPase was measured in lens membrane. Soluble lens proteins were extracted and the following techniques were carried out: estimation of total soluble protein, gel filtration chromatography and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The results indicated a decrease in total soluble lens protein and Na^+-K^+ATPase activity, increase in molecular weight of all protein fractions and changes in the electrophoretic mobility of lens proteins in diabetic cataract group. These changes were reduced in rabbits treated with LA particularly the long period (16 weeks). It is concluded that LA treatment was associated with delayed development and progression of streptozotocin-induced diabetic cataract in rabbits.

Key words: lens proteins, alpha-lipoic acid, electrophoresis, streptozotocin.

INTRODUCTION

Chronic hyperglycemia is a major determinant in the development of secondary complication of diabetes, including diabetic cataract. Studies indicate that hyperglycemia and the duration of diabetes increase the risk of development of cataract [12, 27, 35]. Cataracts, characterized by cloudiness or opacification of the crystalline eye lens, are the leading cause of blindness all over the world-more so in developing countries [8]. Apart from aging, other risk factors such as nutritional deficiencies or inadequacies, trace metals, sunlight, smoking and certain drugs are known to increase the risk of cataract. Although the etiology of cataract is not fully understood, oxidative damage to the constituents of the eye lens is considered to be a major mechanism in the initiation and progression of various types of cataracts,

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including diabetic cataract [31]. Diabetes causes increased oxidative stress in various tissues, as evidenced by increased levels of oxidized DNA, proteins, and lipids, which are thought to play an important role in the pathogenesis of various diabetic complications [6]. Several studies have suggested that the intake of antioxidant-rich food may slow the progression of cataract [20, 25, 33, 37]. Experimental data have revealed some beneficial effect of alpha-lipoic acid (LA, also known as thioctic acid), a potent lipophilic free radical scavenger [26], on glucose metabolism by enhancing glucose uptake in muscle cells [14, 17]. It has been reported that the diabetes induced impairment of lens antioxidative defense, glucose intermediary metabolism via glycolysis, energy status and redox changes were partially prevented by alpha-lipoic acid [28]. Borenshtein *et al*. [3] stated that injection of alpha-lipoic acid significantly inhibited cataract development and reduced blood glucose levels in rats fed the high-energy diet. In another study, the protective effect of alpha-lipoic acid against oxidative damage in the rabbit conjunctiva and cornea exposed to ultraviolet radiation was found [10]. Lin *et al.* [23] concluded that alpha-lipoic acid prevents microvascular damage through downstream of mitochondrial overproduction of reactive oxygen species, and preserves pericyte coverage of retinal capillaries, which may provide additional endothelial protection. Johnsen *et al.* [15] tested the effect of lipoic acid treatment on the retina after a short diabetic insult and found that lipoic acid restored electroretinogram b-wave amplitude of diabetic animals to its control values.

In the present work the protective effect(s) of alpha-lipoic acid (LA) against streptozotocin (STZ)-induced diabetic cataract in rabbits was investigated.

MATERIALS AND METHODS

New Zealand albino rabbits with an average body weight of 2.5±0.5 kg were selected from the animal house facility at the Research Institute of Ophthalmology, Giza, Egypt. The research protocol was approved by the local ethical committee that applies the ARVO (The Association for Research in Vision and Ophthalmology) statements for using animals in ophthalmic and vision research. Group I (n = 5) is the control group whereas the other experimental rabbits received a single intravenous injection of STZ (50 mg/kg) [16, 18]. After 72 hours, fasting blood glucose level was monitored. Animals having blood glucose level higher than 145mg/dl were considered diabetic while others which did not satisfy this condition were excluded from the experiment. The diabetic animals were randomly distributed into three groups, 5 rabbits each, namely II, III and VI. Cataracts developed in group II in about 12 to 13 weeks while the two groups III and IV received an oral daily dose of 150 mg alpha-lipoic acid [9] for 8 weeks and 16 weeks, respectively after induction of diabetes (i.e., after 72 hours of STZ injection).

Na^+-K^+ATPase ACTIVITY

After animals' decapitation, eyes were enucleated from the eye globe, then the lenses were freed from the eye and their capsules were removed carefully. Each lens capsule was weighed in a separate container then homogenized in extraction medium (0.32M sucrose, 1 μM EDTA and 0.15% deoxycholic acid). Na^+-K^+ ATPase measurement was carried out on the lens membrane by the method of Bowler and Tirri [4]

SOLUBLE LENS PROTEIN STUDIES

The lenses, without their capsules, were weighed then homogenized separately in de-ionized water and centrifuged at 16.000 rpm to extract soluble lens proteins then stored at –20 ºC for the following measurements.

ESTIMATION OF TOTAL SOLUBLE PROTEIN

Total proteins in the soluble part of crystalline lens were determined by the method of Lowry *et al.* [24].

GEL FILTRATION CHROMATOGRAPHY

Sephacryl G 200 purchased from Pharmacia fine chemicals (Uppsala, Sweden) was used for the chromatographic separation. The column (1.6×100cm) was eluted with phosphate buffer (pH 7.4) and fractions of 7.5 mL/20 min were collected using the fraction collector (type Haake Buchler instruments, Inc. Saddle Brooke, made in U.S.A.). The optical density of the collected fractions was measured using a spectrophotometer (type UV-visible Recording 240 Graphical, Shimadzu, Japan) at 280 nm. The elution pattern was drawn by plotting the fraction number against the optical density of each fraction for all groups. Determination of void volume and calibration of the column were done to calculate the molecular weights for all protein fractions according to Andrews [1].

SDS POLYACRYLAMIDE GEL ELECTROPHORESIS

Soluble lens proteins were separated according to their molecular weights by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli [21] using 5% stacking gel and 12% separating gel. The data were represented graphically with an automatic scanner (model R-112, manufactured by Beckman).

STATISTICAL ANALYSIS

Data were expressed as the mean ± SD. Comparison between multiple groups was performed using analysis of variance (ANOVA). Commercially available statistical software package (SPSS-11, for Windows) was used where the significance level was set at $p < 0.05$. All the spectral analysis was performed with OriginPro 7.5 software (Origin Lab Corporation, Northampton, MA, USA).

RESULTS

Table 1 illustrated the total soluble lens proteins of control rabbits (group I), diabetic cataract rabbits (group II) and rabbits received a daily dose of 150 mg/day of LA for 8 weeks and 16 weeks (group III and group IV). The diabetic cataract group was characterized by a significantly decreased content of soluble protein relative to the control value (303.6±3 mg/g wet wt). After treatment with LA, total soluble lens protein was increased compared to the diabetic cataract group but it is still less than control even after treatment with LA for 16 weeks. On the other hand, Na^+-K^+ATPase activity (Table 1) shows a similar behavior as the total proteins. For normal lens membrane, the enzyme activity was 50.12±4 µMpi /hour/g wet wt. After induction of diabetes, it decreased to 32.6±2.8 µMpi /hour/g wet wt. Treatment with LA resulted in increased Na^+-K^+ATPase activity compared to the diabetic cataract group and this increase is directly proportional with the time of treatment with LA.

Table 1

Total soluble protein content of rabbit lens and activity of Na^+-K^+ ATPase of rabbit lens membrane for the different studied groups

	Total soluble protein (mg/g wet wt)	Na^+-K^+ ATPase (µ Mpi/h/g wet wt)
Control	303.6 ± 3	50.1 ± 4
Diabetic cataract	†232 ± 3.3	†32.6 ± 2.8
α-Lipoic acid (8 weeks)	†248 ± 2.8	†40.5 ± 2.4
α-Lipoic acid (16 weeks)	†260 ± 4.2	†42.7.± 2.5

†Statistically significant

Figure 1 shows the chromatographic elution pattern for control animals, diabetic cataract group and those treated with LA for 8 weeks and 16 weeks. The chromatographic elution pattern for control rabbits was eluted into four fractions, namely α, β_H (high molecular weight), β_L (low molecular weight), and γ-crystalline. The first peak (α-crystalline) eluted at molecular weight range 461.7– 473.7 kDa (kilodalton). This was followed by β_H and β_L crystalline with molecular weight range 259–267 kDa for β_H and 99.7–109.7 kDa for β_L. The fourth peak (γ-crystalline) was eluted at molecular weight range 35.8–43.8 kDa. These four peaks were shifted towards high molecular weight in diabetic cataract group. On the other hand, the chromatographic elution pattern of rabbits treated with LA for 8 weeks and 16 weeks was shifted to lower molecular weight compared to the diabetic cataract group. These changes were given in Table 2 in which the molecular weights of α, β_H, β_L, and γ-crystalline for all studied groups were calculated, and the intensities of all peaks were also shown. It is clear from this table that the molecular weight of β_H for LA group (16 weeks) is mimicking the normal value. Moreover, the peak intensity of α, β_H and β_L crystalline for diabetic cataract group was significantly decreased while the intensities of all peaks for those two groups treated with LA were unchanged.

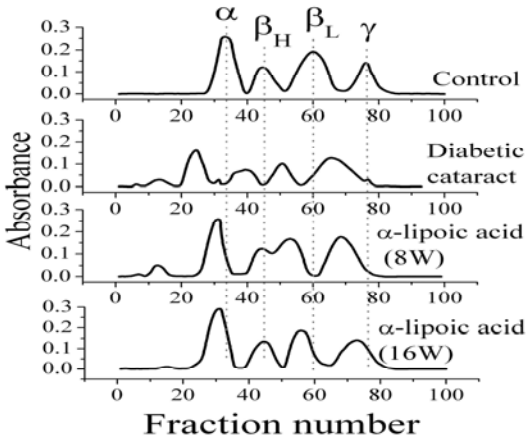

Fig. 1. Chromatographic elution pattern of soluble lens proteins for control, diabetic cataract group and groups of rabbits treated with alpha-lipoic acid for 8 and 16 weeks.

The left panel of Figure 2 shows the electrophoretic patterns of lens proteins for control rabbits, diabetic cataract group and those treated with LA for 8 weeks and 16 weeks, while the right panel shows the SDS polyacrylamide gel electrophoresis image of all the studied groups as well as the molecular weight marker that includes carbonic anhydrase (29 KDa), ovalbumin (45 KDa), albumin (66 KDa) phosphorylase b (97.4 KDa), β-galactosidase (116 KDa) and myosin (205 KDa).

Table 2

Molecular weights and intensities of α, β_H, β_L, γ-crystalline of rabbit lens for the different studied groups

	α-crystalline	β_H-crystalline	β_L-crystalline	γ-crystalline
Control	467.7±6 0.26±0.07	263±4 0.12±0.03	104.71±5 0.19±0.06	39.81±4 **0.15**±0.06
Diabetic cataract	†831.8±7 †0.17±0.04	†346.7±6 †0.07±0.01	†181.9±3 †0.11±0.02	†75.9±8 **0.15**±0.04
α-Lipoic acid (8 weeks)	†540.2±10 0.26±0.03	†275.4±3 0.12±0.02	†158.5±7 0.17±0.05	†64.6±11 0.18±0.05
α-Lipoic acid (16 weeks)	†560.6±13 0.3±0.04	263.8±9 0.14±0.03	†138.1±4 0.18±0.02	†53 .7±7 0.14±0.02

†Statistically significant. First line in each cell indicates the molecular weight ± SD. Second line in each cell indicates the intensity of the peak ±SD.

The control pattern was characterized by the presence of 10 peaks, which reflect the different soluble protein fractions with specific intensities and broadening that covered the molecular weight range 39–180 kDa. The diabetic cataract profile revealed the reduction of the soluble lens protein peaks to 8 fractions which covered the molecular weight range 73–186 kDa and the disappearance of the last two fractions (high mobile fractions). Although the molecular weight range for both groups treated with LA was 35–177 kDa, treatment with LA for 8 weeks was associated with broad high mobile region. With continuous treatment with LA for 16 weeks, the high mobile fractions have appeared with low molecular weight as compared to the diabetic cataract.

Table 3 summarizes the intensity, percentage change and width estimated from the electrophoretic patterns of Figure 2. The peak intensities of diabetic cataract group were decreased while the pronounced decrease was noticed in peak 4 (–55.5%). The LA-8 weeks treated group was characterized by decreased percentage change of intensity of all peaks, but not peak (6), as compared to the control ones. This decrease in the peak intensities was reduced relative to diabetic group (but not for peaks 5 and 7). For the long term LA treated-group (16 weeks), the peak intensities fluctuated relative to the normal ones; decreased in all peaks except for peaks 2 and 8. The peak width of all studied groups fluctuated relative to the control; the changes (increase) were dominant in both diabetic cataract group and 8 weeks-LA treated one.

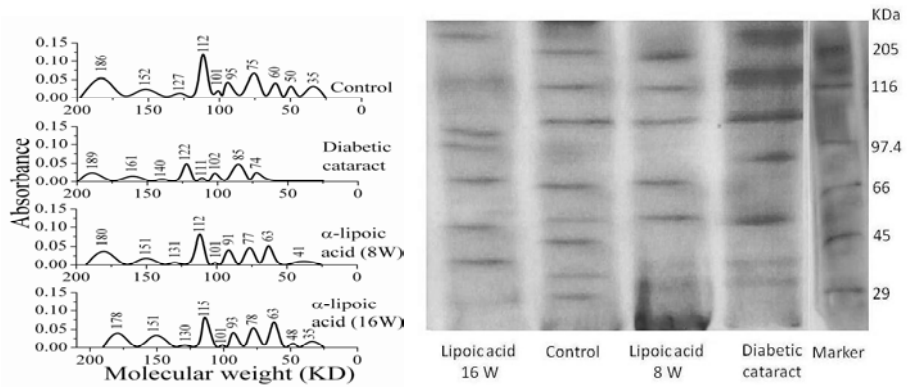

Fig. 2. The left panel represents the electrophoretic profile of rabbits' lens protein for control group, diabetic cataract group and those treated with alpha-lipoic acid for 8 and 16 weeks; the right panel shows the stained gels image.

DISCUSSION

At present, the only treatment for cataract is surgery. It has been estimated that a delay in cataract onset by 10 years could reduce the need for cataract surgery by as much as half [19]. Any antioxidant that prevents or slows the progression of cataract has a significant health impact. Oxygen radicals play an important role in the origin of a wide range of diseases [5, 30]. Diabetes mellitus in both experimental animal models and humans is associated with marked reductions in the levels of plasma antioxidants such as α-tocopherol, ascorbic acid, uric acid, and glutathione causing lowered plasma antioxidative capacity. Various biochemical imbalances contribute to the development of diabetic micro-and macrovascular complication, including atherosclerosis. Among these complications, hyperglycemia-induced cataract formation is thought to be caused by the accumulation of sorbitol [7, 22]. Many studies have suggested that oxidative stress has also a crucial role in diabetes-induced cataract formation [11, 36]. This gives the impetus to investigate the effect of alpha lipoic acid on streptozotocin-induced diabetic cataract in rabbits. Streptozotocin (STZ) was used to induce experimental diabetes because it is a simple, inexpensive and available method [13, 34].

After injection of STZ, the content of the soluble lens proteins was decreased relative to the control (Table 1), and this decrease is partly due to protein insolubilization. LA treatment was found to exert a positive effect represented by preventing the decrease in the soluble lens proteins as compared to the STZ group (although it still decreased as compared to normal). This may be attributed to the less cross-linking/aggregation of soluble proteins.

Table 3

Summary of the different parameters that resulted from the differentiation of the electrophoresis curves

	Peak (1)	Peak (2)	Peak (3)	Peak (4)	Peak (5)	Peak (6)	Peak (7)	Peak (8)	Peak (9)	Peak (10)
Control	0.055±0.01 27.5±3	0.024±0.001 23.75±0.9	0.014±0.001 16.25±0.4	0.11±0.05 11.25±1.3	0.02±0.003 3.75±0.8	0.042±0.006 10±1.5	0.067±0.004 15±1.7	0.042±0.006 10±0.7	0.033±0.007 8.75±2	0.033±0.002 15±1.8
Diabetic cataract	†0.026±0.004 –52.7% †22.4±0.9	†0.016±0.005 –33.3% †25±1.2	†0.007±0.002 –50% †15±0.7	†0.049±0.01 –55.5% †15±1.6	†0.011±0.009 –45% †3.25±0.3	†0.024±0.003 –42.9% †8.75±1	†0.049±0.007 –26.9% 15±1.4	†0.027±0.004 –35.7% †8.75±1	–	–
Lipoic acid (8w)	†0.038±0.007 –30.9% †25±2.4	†0.02±0.004 –16.7% †25±1.8	† 0.008±0.02 –42.9% †11.25±2	†0.082±0.008 –25.5% †15.5±1.4	†0.008±0.006 –60% 3.75±1	†0.042±0.03 0 11.25±2.1	†0.048±0.02 –28.4 15±1.6	†0.053±0.009 +26.2% †11.25±1.3	†0.012±0.04 –63.6% †23.75±3	–
Lipoic acid (16w)	†0.041±0.01 –25.5% 25.7±2.5	†0.034±0.008 +41.7% 24±2	†15.6±3.5 –42.9% †11±0.7	†0.082±0.005 –25.5% 12.75±3	†0.009±0.005 –55% 4±0.9	†0.042±0.04 0 11.25±2	†0.055±0.01 –17.9 †12.5±2.5	†0.068±0.05 +61.9 †13.5±0.8	†0.013±0.02 –60.6 9±0.5	†0.019±0.009 –42.4 †18±0.7

† Statistically significant. The first line in each cell reflects the peak intensity ± S.D., while the second line in each cell reflects the percentage change of the intensity of the peak, and the third line in each cell reflects the width of the peak ±SD.

Na^+-K^+ATPase activity was inhibited after STZ injection and this was due to the oxidative capacity of STZ. Also Na^+-K^+ATPase activity changes play a major role on the ionic transport through the cell membrane. The results indicated that Na^+-K^+ATPase activity of lens membrane for rabbits treated with LA is increased compared to diabetic cataract group and this reflects that LA reduce the incidence of the induced-diabetic cataract especially after 16 weeks of treatment. This is in agreement with Kojima *et al.* [18] who concluded that LA treatment delayed development and progression of cataract in rats with STZ-induced diabetes due to its oxidative stress.

The normal rabbit soluble lens crystallins, separated by column chromatography, were eluted in four fractions α, β_H, β_L and γ-crystallins in the range previously reported by Bloemendal [2]. Also the aggregation of lens proteins and the shift of all crystallins towards higher molecular weight after STZ injection are due to cataract formation. The changes in lens crystallins were evidenced by SDS-PAGE in diabetic cataract group that appeared in the electrophoretic mobility and these changes are characteristics of cataract. Takemoto and coworkers [32] concluded that the formation of covalently linked, high molecular weight protein aggregated has been thought to play an important role in opacification of lens. The chromatographic elution patterns for soluble lens proteins of rabbits treated with LA indicate the enhancement in the molecular weight for all crystallins especially after 16 weeks treatment. These enhancements explain the repair which clearly appeared in the electrophoretic mobility for rabbits treated with LA.

The mechanism by which LA may prevent cataract in diabetes was proposed to be due to its potent antioxidant effects in both its oxidized form (lipoic acid) and reduced form (dihydrolipoic acid, DHLA). This property is undoubtedly responsible for much of its protective effect in diabetic cataract. Packer [29] hypothesizes that LA enters the lens (via a fatty acid carrier) and is converted to DHLA. DHLA has the potential to regenerate ascorbic acid from ascorbyl radicals. The ascorbic acid can then regenerate vitamin E from tocopheryl radicals. Alternately lipoic acid could directly spare vitamins C and E. The increases in vitamins C and E would result in decreased utilization of Glutathione (GSH) and a relative increase in its levels in the lens. In conclusion, alpha-lipoic acid treatment may delay the development and/or progression of cataract in rabbits with streptozotocin-induced diabetes.

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