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Early lipoic acid intake protects retina of diabetic mice

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Abstract
The aim of this study was to test the effect of lipoic acid treatment on the retina after a short diabetic insult. Diabetes was induced by alloxan and mice were divided into sub-groups; control, diabetic, diabetic + insulin and all groups received ± lipoic acid (100 mg/kg body weight) for 3 weeks. GSH content, MDA concentration, GPx activity were measured and electroretinograms (ERG) were recorded. Early administration of lipoic acid to diabetic mice prevented the statistically significant decreases of GSH content and GPx activity and normalized MDA concentration. Moreover, lipoic acid restored electroretinogram b-wave amplitude of diabetic animals to control values. Lipoic acid has a protective effect on the diabetic retina.

Keywords: *Diabetes, oxidative stress, lipoic acid, antioxidant, retina, electroretinogram*

Introduction

Diabetic eye disease is a group of eye problems that may occur as a complication of diabetes. Diabetic eye disease includes Diabetic retinopathy, Cataract and Glaucoma. All can cause severe vision loss or even blindness. Extensive research has been carried out in order to find better ways to detect, treat and prevent vision loss in people with diabetes. In spite of all this research, diabetic retinopathy remains difficult to prevent and treat. Therapeutic approaches in patients with or at risk for diabetic retinopathy include drug therapy to reduce modifiable risk factors, laser photocoagulation and intraocular surgery [1], but none of these therapeutic options are fully satisfactory. Thus, the maintenance of a tight control of blood sugar levels [1,2] and elevated blood pressure [3,4] remains one of the most effective options to slow the onset and progression of retinopathy.

The retina has high content of polyunsaturated fatty acids and the highest oxygen uptake and glucose oxidation rate of all tissues. This phenomenon makes the retina highly susceptible to oxidative stress [5]. Correlations between hyperglycemia, changes in the redox homeostasis and oxidative stress have been suggested to be key events in the pathogenesis of diabetic retinopathy [6]. Experimental [7,8] as well as clinical studies [9,10] have demonstrated that oxidative stress contributes to the development of diabetic retinopathy. Oxidative stress has also been involved in the resistance of retinopathy to reverse, once good glycemic control is achieved, which is known as the

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'metabolic memory phenomenon' [11]. This phenomenon is attributed to an accumulation of damaged molecules and reactive oxygen species (ROS) that are not easily removed, even after good glycemic control is re-established. Therefore it would be an interesting therapeutic option to protect the diabetic patients at risk for developing retinopathy, from oxidative damage in a preventive manner. Thus, in this report we studied the effect of the antioxidant lipoic acid on early signs of diabetic retinopathy related to oxidative stress such as diminished glutathione (GSH) levels, decreased antioxidant defence enzymes as glutathione peroxidase (GPx activity) and increased membrane lipid peroxidation.

Lipoic acid is able to scavenge ROS and to interact with metabolites such as GSH to maintain a healthy cellular redox state [12]. It distributes to the mitochondria and serves as a critical cofactor for mitochondrial enzyme complexes and is regenerated via glycolytic flux. It is known that lipoic acid supplementation prevents diabetes-induced activation of NF-κB and also decreases the number of apoptotic capillary cells in experimental models of diabetic retinopathy [13,14]. Lipoic acid has been used for over 30 years in Germany for treatment of diabetes-induced neuropathy [15].

In addition to its antioxidant properties, lipoic acid has earlier been reported to increase tissue sensitivity to insulin and lower blood glucose levels [16,17], probably by increasing glucose uptake in muscle and fat cells through the insulin-signalling cascade [18,19]. Effects of lipoic acid on GSH metabolism have also been reported, where its administration increased *in vitro* the *de novo* synthesis of cellular GSH [20]. However, the effects of lipoic acid on early changes of oxidative stress markers in diabetic retina have not yet been studied. Since protecting the tissue from oxidative stress has been showed to hinder apoptosis and thereby ameliorating diabetic retinopathy [13], we decided to study the effect of this antioxidant in a preventive manner. Therefore, our aim was to assess the possible protective effect of lipoic acid on the early signs of diabetic-induced oxidative damage in mouse retina.

Materials and methods

Experimental design

Male albino mice weighing 25–30 g (Harlan Iberica SL, Barcelona, Spain) were housed in a temperature- and humidity-controlled room with a 12-h light/dark cycle (less than 100 cds/m^2 luminance during the light phase) and were provided with food and water *ad libitum*. All animal manipulations were done according to international regulations of European Economic Community (directive 86/608/CEE) and ARVO (Association for Research in Vision and Ophthalmology). Mice were randomly assigned to diabetic and control groups. Baseline random blood glucose concentrations were measured with a glucometer (Precision PCx; Medisence, Cambridge, UK). Diabetes was induced by a single subcutaneous injection of 200 mg alloxan/kg body weight (66 mg/ml) in 0.1 m citrate buffer, pH 4.5 and the control group received a subcutaneous injection of citrate buffer alone. Mice were considered diabetic with a blood glucose level higher than 16 mm, 4 days after alloxan treatment. Then, animals were divided into sub-groups (control, control + lipoic acid, diabetic, diabetic + lipoic acid, diabetic + insulin, diabetic + insulin + lipoic acid) and maintained with their respective treatment for 3 weeks. At least 20 animals were used in each group. Lipoic acid (Sigma, St. Louis, MO) was administered ip daily at a dose of 100 mg/kg body weight and insulin at 500 mU/g body weight. Blood samples were taken daily from the tail vein to assay blood glucose levels and glycated hemoglobin (HbA1c) was determined in blood samples obtained by heart puncture immediately before the animals were killed.

Electroretinogram (ERG)

After 3 weeks of treatment, mice adapted to darkness overnight were anaesthetized with ketamine (100 mg/kg body weight) and azepromazine (2.5 mg/kg body weight). Anaaesthetic and midriatic colirium were administered. An active wire loop electrode was used to record responses and needle electrodes were placed in the neck and tail of the mice, that served as the reference and ground, respectively. The stimuli were flashes with a maximum duration of 5 ms (mean 4; range 100; intensity 1 (14 cds/m^2)). In front of the white standard flash a 2.5 logarithmic unit optical density filter was placed. A 2-s interval was set between the flash shots. The filter bandpass of the amplifier and pre-amplifier was set to 350 Hz. Results were recorded on a MacLab computer equipment (Castle Hill, Australia).

Sacrifice and tissue sampling

Anaesthetized mice were decapitated and eyes were immediately enucleated. Lenses were removed from enucleated eyes due to their high GSH concentration. Lenseless eyes were homogenized in pre-chilled 0.2 m potassium phosphate buffer, pH 7.0. The homogenate was used to assay GPx activity, GSH, malondialdehyde (MDA) and protein concentrations. Samples were kept frozen (-80°C) until biochemical assays were performed.

Biochemical assays

GPx activity was assayed as reported by Lawrence et al. [21] towards hydrogen peroxide. MDA concentration was measured by liquid chromatography

(HPLC) according to a modification of the method of Richard et al. [22], as previously described [23]; the GSH content was quantified by the method of Reed et al. [24] on HPLC; and protein concentrations by Lowry et al.'s [25] method.

Statistical analysis

The results are presented as mean values ± SEM. Statistical significances were assessed by the Student's *t*-test. The level of significance was set at $p \leq 0.05$.

Results

Confirmation of experimental diabetes

Before treatment, there was no significant difference between any group in weight or random glucose levels. Three weeks after induction of diabetes by injection of alloxan, random blood glucose concentration in the diabetic and control groups were 29.5 ± 1.3 and 5.3 ± 0.4 mm, respectively ($p \leq 0.05$) as shown in Table I. No statistical differences in glycated haemoglobin (HbA1c) were found between the diabetic group and the diabetic group receiving lipoic acid. As expected, weight in the diabetic animals was somewhat lower than in the control group (Table I).

Effects of lipoic acid administration on antioxidants defence enzymes

Oxidative stress, as assessed by the concentration of GSH and GPx activity, was present in the retina of diabetic mice after only 3 weeks of diabetic insult. Thus, GSH concentration and GPx activity showed significant decreases in the diabetic group with respect to controls ($p \leq 0.05$ vs control, for both parameters, Figure 1). Lipoic acid administration had a protective effect on the retina of diabetic mice, since it prevented the decrease of GSH concentration ($p \leq 0.05$ with respect to all other groups, Figure 1A), as well as of GPx activity ($p \leq 0.05$ with respect to all other groups, Figure 1B). No differences were observed in insulin-treated animals with respect to controls in any of the oxidative stress markers analysed.

Effect of lipoic acid administration on lipid peroxidation

After 3 weeks of alloxan injection, mice retina had MDA contents significantly elevated when compared to control animals ($p \leq 0.05$, Figure 2). Lipoic acid administration reduced MDA content in the retina of diabetic mice so that it was not significantly different from the control group ($p \leq 0.05$). No differences were observed between the insulin-treated animals and the control ones.

Effects of lipoic acid administration on electroretinogram recordings

The retinal function was tested by means of electroretinogram recordings. ERG b-wave amplitude was significantly decreased in diabetic animals with respect to all other groups ($p \leq 0.05$, Figure 3), suggesting an early impairment of the retinal function due to the diabetic insult. Administration of lipoic acid restored the b-wave amplitude to 77% of control values and was significantly different from the diabetic group. No differences were observed in insulin-treated diabetic mice with respect to controls.

Discussion

Herein we report the effect of lipoic acid treatment on the early changes of the redox status in diabetic retina. Retina is markedly sensitive to oxygen free radical damage, due to its high levels of polyunsaturated lipids. The results show that after 3 weeks of diabetes, MDA levels in the retina were increased, when compared to controls, and that lipoic acid was able to prevent this effect. MDA is a well accepted oxidative stress marker for pathological processes [26]. Since oxidative stress represents an imbalance between excess formation and/or impaired removal of ROS, the antioxidant defence system of the cell is a crucial part of the overall oxidative stress experienced by cells. Our data showed that diabetes induces a decrease of antioxidant defence systems (GSH content and GPx activity) and that lipoic acid prevented these effects in mice retina.

Table I. General characteristics of the experimental groups.

	Plasma glucose (mm)†	HbA1c (%)‡	Body weight (g)‡
Control ($n=15$)	5.3 ± 0.4	3.33 ± 0.25	33.54 ± 0.82
Control+LP ($n=12$)	5.4 ± 0.5	2.92 ± 0.26	32.11 ± 1.39
Diabetic ($n=14$)	29.5 ± 1.3*	6.80 ± 0.27*	23.80 ± 1.39*
Diabetic+LP ($n=12$)	24.8 ± 1.3*	5.87 ± 0.26 *	29.29 ± 0.85*
Diabetic+Ins ($n=18$)	12.9 ± 1.2*	4.88 ± 0.24*	33.16 ± 0.89
Diabetic+Ins+LP ($n=14$)	13.5 ± 0.8*	4.62 ± 0.24*	32.91 ± 0.40

LP: Lipoic acid. Ins: insulin. *$p \leq 0.05$ vs Control.
HbA1c: Glycated haemoglobin.
Data are means ± SEM or range of means. †throughout study; ‡at the end of the study.

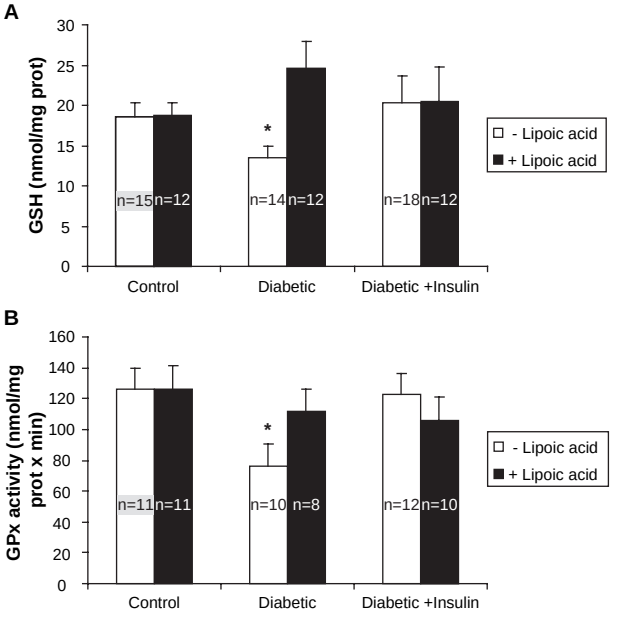

Figure 1. GSH content (A) and GPx activity (B) of mouse retina in the different groups studied. * $p \leq 0.05$ vs all other groups ($n \geq 8$).

Increased oxidative stress in diabetes is considered a contributing factor in the development of diabetic complications, including retinopathy [7,27,28], and reactive oxygen species have been reported to act as a causal link between the elevated glucose levels and the other relevant metabolic changes involved in the development of diabetic complications [29]. Herein we report that the alterations of oxidative stress markers in the retina, that are already present after just 3 weeks of diabetic condition, were completely prevented when lipoic acid was administrated. Other studies reported that administration of lipoic acid prevented impairment of ion demand [30], as well as retinal capillary cell death and microvascular damage [13,14], however in both of these studies the lipoic acid was administered from the first day of the experiment and througout 11 months or 30 weeks, respectively. Moreover, these studies postulated that apoptosis of retinal capillary cells is mediated through sequential events involving caspases and Nuclear Factor kappa B (NFκB) and that inhibition of

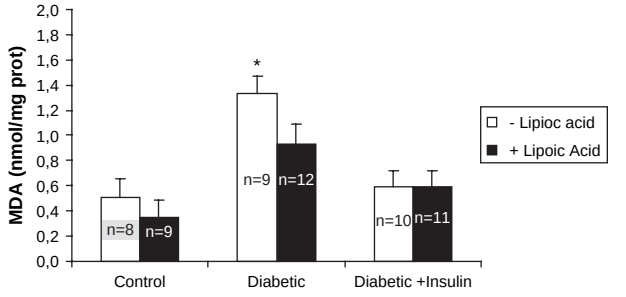

Figure 2. MDA concentration of mouse retina in the different groups studied. * $p \leq 0.05$ vs control group ($n \geq 8$).

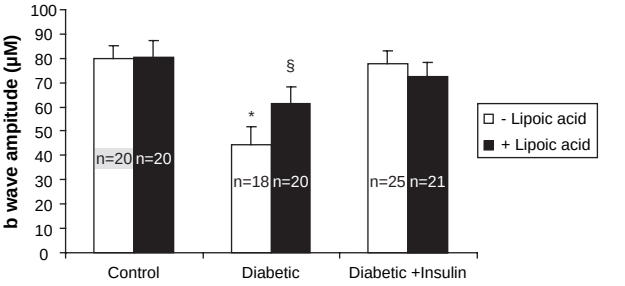

Figure 3. Lipoic acid restores the b-wave amplitude in diabetic animals to control values. * $p \leq 0.05$ vs all other groups. § $p \leq 0.05$ vs control+lipoic acid group ($n \geq 18$).

superoxide accumulation prevents apoptosis. We postulate that lipoic acid prevents the development of diabetic retinopathy due to the prevention of superoxide accumulation and thereby hindering the following sequential events leading to apoptosis. In support of this theory is the reported resistance of diabetic retinopathy to reverse attributed to accumulation of damaged molecules and reactive oxygen species (ROS) [11]. The results of the present study suggest that in order to protect the retina from potential apoptosis and microvascular damage, lipoic acid must be administrated as early as possible.

Changes in the redox status of neuronal tissue can lead to various functional impairments, as we described previously [8,31]. Impaired electroretinogram recordings were observed after only 3 weeks of diabetes, suggesting a fast acting deterioration of retinal function possibly due to oxidative stress damage. The administration of lipoic acid prevents the decrease of the b-wave amplitude in electroretinogram recordings of diabetic mice. Lipoic acid thereby provides protection to the retina as a whole and to ganglion cells in particular. Thus, our data show that *in vivo* administration of lipoic acid provides a functional protection to the retina most probably by protecting it from oxidative damage induced by the diabetic condition.

As mentioned in the introduction, good glycemic control remains one of the most effective options to prevent or delay the worsening of diabetic retinopathy. However, good glycemic control is difficult to achieve and maintain for long periods of time for a number of patients. Lipoic acid is an interesting therapeutic option for diabetic patients due to its ability to increase tissue sensitivity to insulin and lower glycemia level [16,17] and thereby help to maintain glycemic control. Interactions of lipoic acid with glucose metabolism have been previously described to explain this lowering effect of glucose and eventually lactate levels, as is the inhibition of the pyruvate dehydrogenase kinase, thus increasing the activity of the pyruvate dehydrogenase complex [32]. However, the mechanism of how lipoic acid prevents diabetic retinopathy needs to be further clarified.

In summary, we can conclude that lipoic acid has a protective effect delaying the onset and eventually slowing the progression of retinopathy and propose that lipoic acid should be administered as early as possible to the diabetic patients in order to reduce and thus, at least partly, protect the diabetic retina.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Bloomgarden ZT. Screening for and managing diabetic retinopathy: current approaches. Am J Health Syst Pharm 2007;64:S8–S14.

[2] Kawasaki S, Hasegawa O, Satoh S, Saito T, Ishio H, Fukushima H, Kato S, Yamashita H, Terauchi Y, Sekihara H. Development and progression of retinopathy after inpatient management of diabetes. Intern Med 2006;45:1267–1271.

[3] Tyrberg M, Melander A, Lovestam-Adrian M, Lindblad U. Retinopathy in subjects with impaired fasting glucose: the NANSY-Eye baseline report. Diab Obes Metab 2007;epub ahead of print.

[4] Klein R, Klein BE, Moss SE, Wong TY. The relationship of retinopathy in persons without diabetes to the 15-year incidence of diabetes and hypertension: Beaver Dam eye study. Trans Am Ophthalmol Soc 2006;104:98–107.

[5] Anderson RE, Rapp LM, Wiegand RD. Lipid peroxidation and retinal degeneration. Curr Eye Res 1984;3:223–227.

[6] Kowluru RA, Chan PS. Oxidative stress and diabetic retinopathy. Exp Diabetes Res 2007;2007:43603.

[7] Miranda M, Muriach M, Johnsen S, Bosch-Morell F, Araiz J, Romá J, Romero FJ. Oxidative stress in a model for experimental diabetic retinopathy: treatment with antioxidants. Arch Soc Esp Oftalmol 2004;79:289–294.

[8] Muriach M, Bosch-Morell F, Alexander G, Blomhoff R, Barcia J, Arnal E, Almansa I, Romero FJ, Miranda M. Lutein effect on retina and hippocampus of diabetic mice. Free Radic Biol Med 2006;41:979–984.

[9] Matteucci E, Giampietro O. Oxidative stress in families of type 1 diabetic patients. Diabetes Care 2000;23:1182–1186.

[10] Turk HM, Sevinc A, Camci C, Cigli A, Buyukberber S, Savli H, Bayraktar N. Plasma lipid peroxidation products and antioxidant enzyme activities in patients with type 2 diabetes mellitus. Acta Diabetol 2002;39:117–122.

[11] Kowluru RA. Effect of reinstitution of good glycemic control on retinal oxidative stress and nitrative stress in diabetic rats. Diabetes 2003;52:818–823.

[12] Packer L, Tritschler HJ. Alpha-lipoic acid: the metabolic antioxidant. Free Radic Biol Med 1996;20:625–626.

[13] Lin J, Bierhaus A, Bugert P, Dietrich N, Feng Y, VomHagen F, Nawroth P, Brownlee M, Hammes HP. Effect of R-(+)-alpha-lipoic acid on experimental diabetic retinopathy. Diabetologia 2006;49:1089–1096.

[14] Kowluru RA, Odenbach S. Effect of long-term administration of alpha-lipoic acid on retinal capillary cell death and the development of retinopathy in diabetic rats. Diabetes 2004;53:3233–3238.

[15] Ziegler D. Treatment of diabetic polyneuropathy: update 2006. Ann NY Acad Sci 2006;1084:250–266.

[16] Jacob S, Henriksen EJ, Tritschler HJ, Augustin HJ, Dietze GJ. Improvement of insulin-stimulated glucose-disposal in type 2 diabetes after repeated parenteral administration of thioctic acid. Exp Clin Endocrinol Diabetes 1996;104:284–288.

[17] Kamenova P. Improvement of insulin sensitivity in patients with type 2 diabetes mellitus after oral administration of alpha-lipoic acid. Hormones (Athens) 2006;5:251–258.

[18] Estrada DE, Ewart HS, Tsakiridis T, Volchuk A, Ramlal T, Tritschler H, Klip A. Stimulation of glucose uptake by the natural coenzyme alpha-lipoic acid/thioctic acid: participation of elements of the insulin signaling pathway. Diabetes 1996;45:1798–1804.

[19] Yaworsky K, Somwar R, Ramlal T, Tritschler HJ, Klip A. Engagement of the insulin-sensitive pathway in the stimulation of glucose transport by alpha-lipoic acid in 3T3-L1 adipocytes. Diabetologia 2000;43:294–303.

[20] Han D, Handelman G, Marcocci L, Sen CK, Roy S, Kobuchi H, Tritschler HJ, Flohe L, Packer L. Lipoic acid increases de novo synthesis of cellular glutathione by improving cysteine utilization. Biofactors 1997;6:321–338.

[21] Lawrence RA, Parkhill LK, Burk RF. Hepatic cytosolic non-selenium dependent glutathione peroxidase activity: its nature and the effect of selenium deficiency. J Nutr 1978;108:981–987.

[22] Richard MJ, Guiraud P, Meo J, Favier A. High performance liquid chromatography separation of malondialdehyde thiobarbituric acid adduct in biological materials (plasma and human cell) using a commercially available reagent. J Chromatogr 1992;577:9–18.

[23] Romero MJ, Bosch-Morell F, Romero B, Rodrigo JM, Serra MA, Romero FJ. Serum malondialdehyde: possible use for the clinical management of chronic hepatitis C patients. Free Radic Biol Med 1998;25:993–997.

[24] Reed DJ, Babson JR, Beatty PW, Brodie AE, Ellis WW, Potter DW. High-performance liquid chromatography analysis of nanomole levels of glutathione, glutathione disulfide, and related disulfides. Anal Biochem 1980;106:55–62.

[25] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–275.

[26] Halliwell B. Oxidative stress markers in human disease: application to diabetes and to evaluation of the effects of antioxidants. In: Packer L, Rösen P, Tritschler HJ, King GL, Azzi A, editors. Antioxidants in diabetes management. New York: Marcel Dekker; 2000. p 33–52.

[27] Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 1999;48:1–9.

[28] Kowluru RA, Tang J, Kern TS. Abnormalities of retinal metabolism in diabetes and experimental galactosemia. VII. Effect of long-term administration of antioxidants on the development of retinopathy. Diabetes 2001;50:1938–1942.

[29] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813–820.

[30] Berkowitz BA, Roberts R, Stemmler A, Luan H, Gradianu M. Impaired apparent ion demand in experimental diabetic retinopathy: correction by lipoic acid. Invest Ophthalmol Vis Sci 2007;48:4753–4758.

[31] Johnsen-Soriano S, Bosch-Morell F, Miranda M, Asensio S, Barcia J, Roma J, Monfort P, Felipo V, Romero FJ. Ebselen prevents chronic alcohol-induced rat hippocampal stress and functional impairement. Alcohol Clin Exp Res 2007;31:486–492.

[32] Korotchkina LG, Sidhu S, Patel MS. R-lipoic acid inhibits mammalian pyruvate dehydrogenase kinase. Free Radic Res 2004;38:1038–1092.

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