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Effects of α-lipoic acid on biomarkers of oxidative stress in streptozotocin-induced diabetic rats

A. C. Maritim[a], R. A. Sanders[b], J. B. Watkins, III[b,*]

[a]*Moi University, Faculty of Health Sciences, Eldoret, Kenya*
[b]*Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN 47405, USA*

Abstract

Increased oxidative stress and impaired antioxidant defense mechanisms are important factors in the pathogenesis and progression of diabetes mellitus and other oxidant-related diseases. This study was designed to determine whether α-lipoic acid, which has been shown to have substantial antioxidant properties, when administered (10 mg/kg ip) once daily for 14 days to normal and diabetic female Sprague-Dawley rats would prevent diabetes-induced changes in biomarkers of oxidative stress in liver, kidney and heart. Serum glucose concentrations, aspartate aminotransferase activity, and glycated hemoglobin levels, which were increased in diabetes, were not significantly altered by α-lipoic acid treatment. Normal rats treated with a high dose of α-lipoic acid (50 mg/kg) survived but diabetic rats on similar treatment died during the course of the experiment. The activity of glutathione peroxidase was increased in livers of normal rats treated with α-lipoic acid, but decreased in diabetic rats after α-lipoic acid treatment. Hepatic catalase activity was decreased in both normal and diabetic rats after α-lipoic acid treatment. Concentrations of reduced glutathione and glutathione disulfide in liver were increased after α-lipoic acid treatment of normal rats, but were not altered in diabetics. In kidney, glutathione peroxidase activity was elevated in diabetic rats, and in both normal and diabetic animals after α-lipoic acid treatment. Superoxide dismutase activity in heart was decreased in diabetic rats but normalized after treatment with α-lipoic acid; other cardiac enzyme activities were not influenced by either diabetes or antioxidant treatment. These results suggest that after 14 days of treatment with an appropriate pharmacological dose, α-lipoic acid may reduce oxidative stress in STZ-induced diabetic rats, perhaps by modulating the thiol status of the cells.

Keywords: Streptozotocin; Diabetes; Alpha-lipoic acid; Antioxidant; Oxidative Stress; Rat

1. Introduction

α-Lipoic acid is a disulfide compound that functions as a coenzyme in pyruvate dehydrogenase and α-ketoglutarate dehydrogenase mitochondrial reactions, leading to the production of cellular energy (ATP). α-Lipoic acid and its reduced form, dihydrolipoic acid, reduce oxidative stress by scavenging a number of free radicals in both membrane and aqueous domains, by chelating transition metals in biological systems, by preventing membrane lipid peroxidation and protein damage through the redox regeneration of other antioxidants such as vitamins C and E, and by increasing intracellular glutathione [1-3]. α-Lipoic acid may also be effective in both prevention and treatment of oxidative stress in a number of models or clinical conditions, including ischemia-reperfusion injury [4-6], diabetes [7-11], HIV infection [4,12], and neurodegenerative diseases [13].

Oxidative stress, the prevalence of oxidant factors over antioxidant mechanisms, plays a central role in the pathogenesis and progression of diabetes and its complications. Hence, it is likely that a substance known to reduce oxidative stress in vivo would reduce progression of cell damage in clinical diabetes. α-Lipoic acid has been reported to have a number of potentially beneficial effects in both prevention and treatment of oxygen-related diseases: for example, the enhancement of glucose utilization in type II diabetes [1], and the reduction of the development of diabetic complications [10,11,14-17]. Research on this compound is now lively in view of these potential benefits in diabetes. Clinical studies using doses of 600 mg α-lipoic acid 1x, 2x, or 3x per day showed reduction in plasma lipid peroxides [18], reduction in neuropathy symptoms [19], and an increase in insulin sensitivity in type-2 diabetes [9]. A dose of 600 mg/day in a 60-70 kg person is roughly 10 mg/kg body weight, which is similar to the dose used in rats. Although

* Corresponding author. Tel.: +1-812-855-3201; fax: +1-812-855-4436.

E-mail address: watkins@indiana.edu (J.B. Watkins III)

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the lack of regulation of companies selling OTC nutriceuticals means that the actual dose contained may or may not match that advertised, α-lipoic acid is readily available in concentrations from 50 to 500 mg per tablet [20]. Additional investigators have assessed different doses of α-lipoic acid, ranging from 10 to 100 mg/kg body weight for their experimental animal studies on diabetes with no reported toxic effects [21]. Therefore, two doses of α-lipoic acid (10 and 50 mg/kg body weight) were chosen for our study and their effects in normal and streptozotocin (STZ)-induced diabetic rats were compared.

The present study was designed to extend the current information on antioxidant effects of α-lipoic acid, as well as to determine its effects on rats having 30 days of uncontrolled type I diabetes. The markers of oxidative stress evaluated in normal and STZ-induced diabetic rats after subacute treatment with α-lipoic acid included the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, and the concentrations of glutathione and glutathione disulfide in livers, kidneys and hearts.

2. Materials and methods

All chemicals used in the study were purchased from Sigma Chemicals (St. Louis, MO).

Female Sprague-Dawley rats were purchased from Harlan Sprague Dawley (Indianapolis, IN). Purina Rat Chow (No. 5012, St. Louis, Mo) and water were provided ad libitum. Animal husbandry and experimentation were consistent with United States Public Health Service guidelines [22].

Rats were randomly divided into four groups at the start of the experiment. In two groups, diabetes was induced by a single ip injection of streptozotocin (90 mg/kg body weight) in freshly prepared 10 mM sodium citrate, pH 4.5. The diabetic rats included in the study had blood glucose concentrations >350 mg/dl (Sigma Infinity glucose kit). Thirty days after streptozotocin, the experimental groups comprised the normal control group (n = 8); normal rats treated with DL-α-lipoic acid (either 10 or 50 mg/kg/day, ip, for 14 days, n = 8 for each dose); the untreated diabetic group (n = 8); and the remaining diabetic group treated with DL-α-lipoic acid (either 10 or 50 mg/kg/day, ip, for 14 days, n = 8 for each dose group). Rats in both normal and diabetic control groups received 0.25 ml of vehicle (water), ip, each per day. Each day, α-lipoic acid powder was weighed and completely dissolved in sterile water by the drop-wise addition of 5N sodium hydroxide as described by Cameron et al [23].

2.1. Tissue collection

After 14 days of treatment with α-lipoic acid or vehicle, the rats were sacrificed under 2% (ih) isoflurane-induced anesthesia. Blood was drawn by cardiac puncture into heparinized syringes; part was used to measure total glycated hemoglobin and glycated hemoglobin (HbA_{1c}) levels (Sigma diagnostic kits 442B and 441B respectively), and the remaining portion was centrifuged (3000 x g, 4°C, for 10 min). Glucose concentration and aspartate aminotransferase activity (Sigma kits, St. Louis, MO) were determined in all plasma samples. Livers, kidneys and hearts were removed, weighed, rinsed in ice-cold 1.15% potassium chloride, quick frozen in liquid nitrogen and kept at -80°C for subsequent biochemical analyses.

2.2. Assays

For each tissue, an appropriate portion was homogenized in ice-cold Tris buffer, pH 7.4. The homogenates were centrifuged at 105,000 x g for 1 hr, and the supernatants (cytosols) were used for measuring the activities of superoxide dismutase [24], catalase [25], glutathione peroxidase [26], and glutathione reductase [27]. Another 0.25 g aliquot of each tissue was homogenized in 3.75 ml ice-cold 0.1M sodium phosphate-5 mM EDTA (pH 8.0), then 1 ml 25% phosphoric acid was added. After vortexing for 10 sec, the samples were centrifuged at 100,000 x g for 30 min. The resulting supernatants were assayed for concentrations of both reduced glutathione (GSH) and glutathione disulfide (GSSG) concentrations [28]. All assays were performed in duplicate. Protein concentrations of cytosols and homogenates were determined according to Lowry et al [29].

Means and standard errors were calculated, the data were analyzed by analysis of variance (ANOVA) and Dunnett's test, and significance was set at $p < 0.05$.

3. Results

In this study, the STZ diabetic rats had uncontrolled, Type 1, insulin-dependent diabetes mellitus for 30 days prior to antioxidant treatment. Although normal rats survived treatment with both 10 and 50 mg/kg of α-lipoic acid ip for 14 days, all of the diabetic rats (n = 8) treated with the higher dose of α-lipoic acid (50 mg/kg body weight, ip) died 3-6 days after onset of treatment. All diabetic rats treated with the lower dose of lipoic acid showed no signs of morbidity.

Glucose concentration in blood plasma of STZ-induced diabetic rats was significantly higher (approximately 3-fold) than in the normal control group and was not changed by α-lipoic acid treatment of diabetic rats (Table 1). Aspartate aminotransferase activity in plasma was elevated about 2-fold in diabetic rats compared to the controls, and was not altered by 14 days of α-lipoic acid treatment. Both total glycated hemoglobin and glycated HbA_{1c} concentrations in diabetic rats were significantly increased compared to the values in normal rats, and were not normalized by α-lipoic acid treatment (Table 1). The final body weights of diabetic

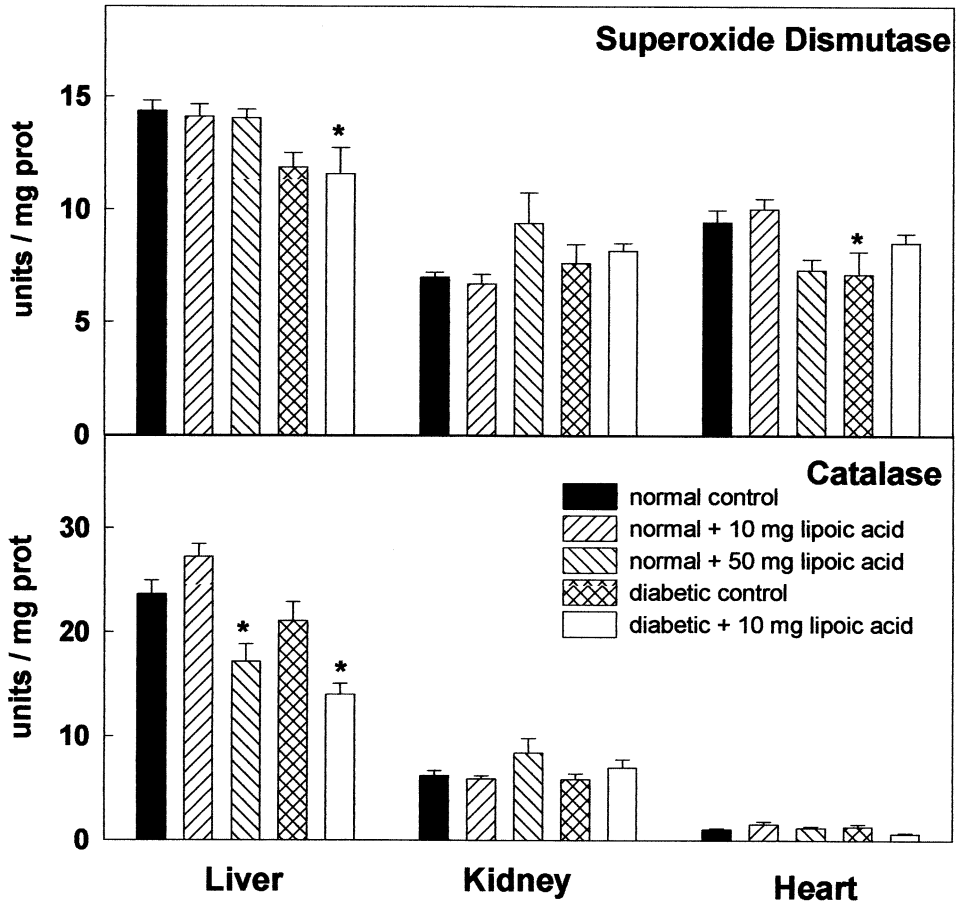

Fig. 1. Effects of treatment with α-lipoic acid on activities of superoxide dismutase and catalase in the liver, kidney, and heart from normal and streptozotocin-induced diabetic rats. One unit of superoxide dismutase activity is that which produces 50% inhibition of the reduction of cytochrome C in the presence of superoxide radical. One unit of catalase activity liberates half of the peroxide oxygen from solution in 100 s at 25°C. * represents significantly different from normal control at $p < 0.05$.

animals were lower than normal controls. The liver weights were increased and liver weight/body weight ratio almost doubled in diabetic rats as compared to normals. There was no significant difference found between body weights, liver weights and liver weight/body weight ratios in diabetic rats treated with α-lipoic acid and the corresponding untreated diabetic rats (Table 1).

Treatment of normal rats with either dose of α-lipoic acid did not affect the activity of superoxide dismutase in any tissue (Fig. 1, top panel). Superoxide dismutase activity in heart of diabetic animals was decreased to 76% of normal controls, whereas activity in liver and kidney was not significantly changed by diabetes. α-Lipoic acid treatment of diabetic rats decreased hepatic superoxide dismutase activity but normalized the enzyme's activity in the heart. The bottom panel of Figure 1 illustrates that catalase activity was not significantly altered by diabetes in liver, heart, or kidney. Treatment of diabetic rats with α-lipoic acid decreased hepatic catalase activity by 41% compared to normal controls. Although catalase activity was lower by 27% in liver, there was no change in renal or cardiac catalase activity of normal rats treated with 50 mg/kg α-lipoic acid.

The top panel of Fig. 2 shows that there were no significant changes in hepatic, renal or cardiac glutathione reductase activity in STZ-induced diabetic rats as compared with normal controls. Treatment with α-lipoic acid had no influence on glutathione reductase activity. Normal rats treated with α-lipoic acid (10 mg/kg) showed no significant changes in glutathione peroxidase activity levels in all three tissues compared with control group. In contrast, at 50 mg/kg α-lipoic acid, glutathione peroxidase activity was increased in both livers (163%) and kidneys (179%) as compared to untreated normal rats (Fig. 2, bottom panel). In diabetic rats, renal glutathione peroxidase activity was increased by 214% compared with normal control rats. Treatment of diabetic animals with α-lipoic acid did not alter glutathione peroxidase activity in any tissues as compared to diabetic controls.

As shown in Fig. 3 (top panel), GSH concentrations were normal in liver, kidney, and heart of STZ-induced diabetic rats. α-Lipoic acid treatment of diabetic rats had no effect on reduced GSH levels in any tissue. In normal rats, α-lipoic acid treatment at 50 mg/kg resulted in a 60% increase in hepatic GSH concentration, with no significant change in

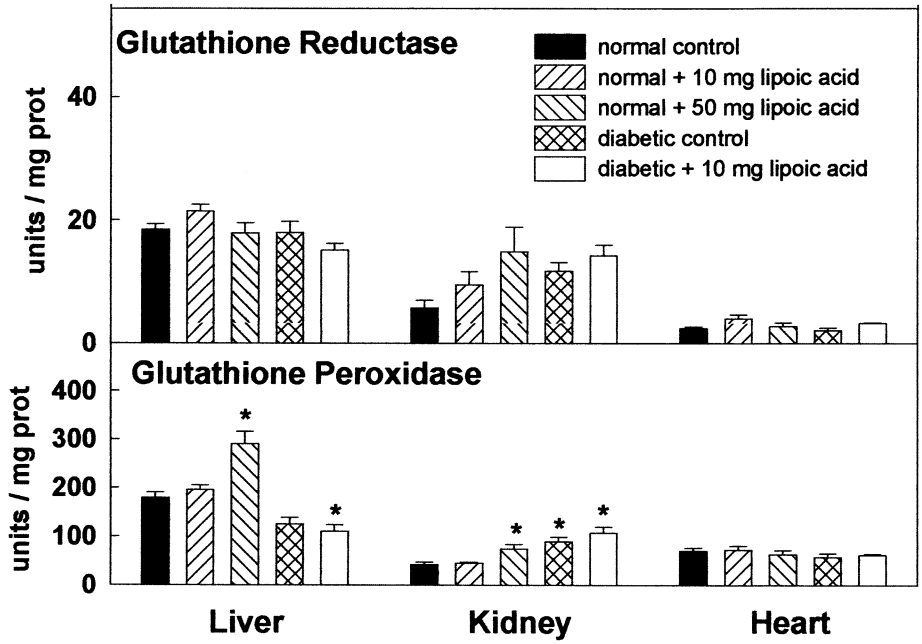

Fig. 2. Effects of treatment with α-lipoic acid on activities of glutathione reductase and glutathione peroxidase in the liver, kidney, and heart from normal and streptozotocin-induced diabetic rats. One unit of glutathione peroxidase or glutathione reductase activity oxidizes 1 nmol of NADPH/min at 30°C. * represents significantly different from normal control at $p < 0.05$. n/a indicates not assayed.

renal or cardiac GSH levels. After both doses of α-lipoic acid, glutathione disulfide concentrations (Fig. 3, bottom panel) were increased in liver of normal rats. There was no change in renal or cardiac GSSG concentrations after either dose in normal rats. When compared to normal controls, levels of GSSG were increased in hearts (155% of normal) of diabetic rats and were normalized after α-lipoic acid treatment.

4. Discussion

Experimental and clinical studies have indicated the potential usefulness of exogenous α-lipoic acid as an antioxidant therapeutic agent for the prevention and treatment of diabetes mellitus. α-Lipoic acid treatment has been reported to reduce the development of diabetic complications such as retinopathy [15], cataract formation [14,30,31], neuropathy

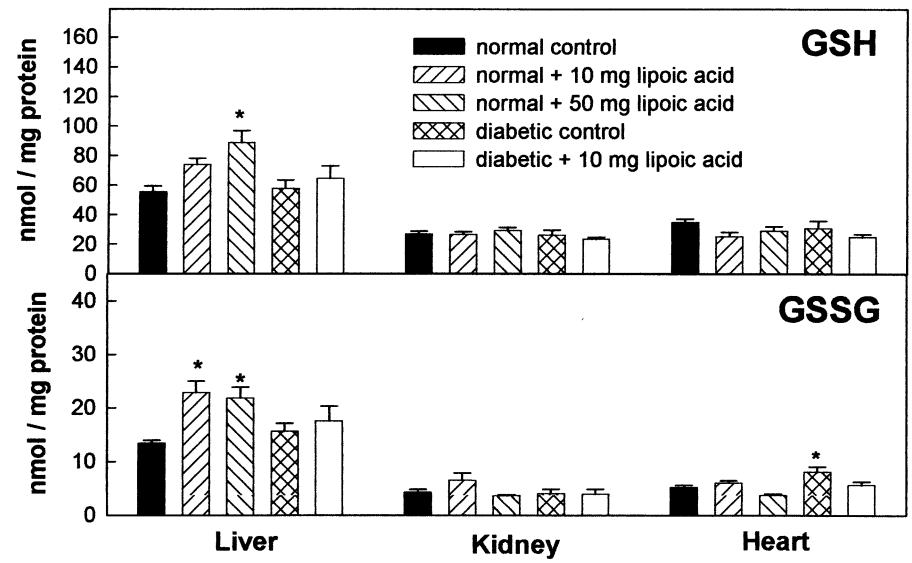

Fig. 3. Effects of treatment with α-lipoic acid on concentrations of reduced glutathione (GSH) and glutathione disulfide (GSSG) in the liver, kidney, and heart from normal and streptozotocin-induced diabetic rats. * represents significantly different from normal control at $p < 0.05$.

Table 1
General characteristics of normal, diabetic and α-lipoic acid treated rats[a].

Groups	Glucose (mg/dl)	Serum AST (U/ml)	Total glycated Hb (%)	Glycated HbA_{1c} (%)	Body weight (g)	Liver weight (g)	Liver wt/body wt (%)
Normal	156 ± 12	88 ± 9	3.5 ± 0.2	2.2 ± 0.1	248 ± 5	8.6 ± 0.4	3.4 ± 0.1
Normal + lipoic acid 50 mg/kg	131 ± 9	68 ± 7	n/a	n/a	221 ± 4	8.4 ± 0.3	3.8 ± 0.1
Normal + lipoic acid 10mg/kg	143 ± 6	97 ± 7	3.3 ± 0.2	2.0 ± 0.1	230 ± 5	7.9 ± 0.4	3.4 ± 0.1
Diabetic	481 ± 21[b]	183 ± 20[b]	11.9 ± 0.5[b]	4.4 ± 0.1[b]	191 ± 6[b]	12.6 ± 0.9[b]	6.6 ± 0.4[b]
Diabetic + lipoic acid 10mg/kg	431 ± 43[b]	181 ± 22[b]	10.3 ± 1.2[b]	4.2 ± 0.4[b]	181 ± 13[b]	10.0 ± 0.6[b]	5.6 ± 0.3[b]

[a] Values are means ± SE for 8 rats
[b] Indicates significantly different from Normal at $p < 0.05$
n/a indicates not assayed

[10,32,33], vascular complications [16,34] and nephropathy [17,35,36]. In addition to its antioxidant properties, there is evidence that α-lipoic acid can prevent β-cell destruction leading to type I diabetes [1], enhance glucose uptake in type II diabetes [2,8,9,18,37,38] and prevent glycation of some proteins like albumin [4,39].

α-Lipoic acid is both water- and fat-soluble, making it highly effective at reducing free radicals, including lipid peroxides, in cellular membranes, as well as scavenging free radicals at their mitochondrial source [40]. Inside cells and tissues, α-lipoic acid is reduced to dihydrolipoic acid, which is even more potent as an antioxidant [1-3].

In the present study (Table 1), it was apparent that α-lipoic acid treatment had no effect on reduced body weights, hyperglycemia, and increased liver weights or liver weight/body weight ratio of diabetic rats. α-Lipoic acid treatment did not have a substantial effect on blood glucose levels. These observations are consistent with previous findings [14,41].

Protein glycation and glucose oxidation have been implicated in the pathogenesis of chronic diabetic complications [42,43]. α-Lipoic acid has been tested in a number of model systems with conflicting results about its protection against glycation [3]. In our study, both total glycated hemoglobin and glycated $HbA_{1c,}$ which were elevated in diabetic rats, were not significantly reduced after 14 days of α-lipoic acid treatment.

Treatment of diabetic rats with α-lipoic acid had divergent effects on superoxide dismutase activity, decreasing activity in liver and yet restoring cardiac levels to normal. With diminished superoxide dismutase activity in diabetic rats, superoxide anion radicals, which are continuously generated as a consequence of hyperglycemia, are converted to hydrogen peroxide. In the presence of inadequate catalase or glutathione peroxidase activity levels to degrade hydrogen peroxide, more hydrogen peroxide could be converted to toxic hydroxyl radicals [44] that contribute to the oxidative stress of diabetes.

Both catalase and glutathione peroxidase catalyze the transformation of hydrogen peroxide within the cell to harmless products, thereby curtailing the quantity of cellular destruction inflicted by lipid peroxidation byproducts [45-48]. The ability of α-lipoic acid or its reduced form, dihydrolipoic acid, to scavenge hydroxyl radicals and chelate transition metals in Fenton reactions restricts the molecular damage of hydroxyl radicals and so reduces cellular need for catalase. Diminution of hepatic catalase activity in normal and diabetic rats is consistent with previous studies showing that α-lipoic acid or dihydrolipoic acid scavenge various reactive oxygen species such as superoxide, hydroxyl radicals and singlet oxygen [3].

Glutathione peroxidase plays a much greater role in detoxification of hydrogen peroxide than does catalase [49,50]. Glutathione peroxidase activity was increased in kidneys of diabetic rats and was still elevated after α-lipoic acid treatment, reflecting a response to an increase in the rate of hydrogen peroxide production. The decrease in hepatic glutathione peroxidase activity levels may be linked to increased oxidative stress in diabetes accompanied by hyperglycemia, as is the case observed with catalase activity in the liver.

Reduced glutathione and its oxidized counterpart glutathione disulfide constitute a major redox buffer system of the cell. After treatment with 10 mg/kg α-lipoic acid, hepatic GSH concentration was not significantly altered in normal or diabetic rats, though treatment with the high dose increased hepatic GSH concentration in normal rats. α-Lipoic acid has the ability to correct deficient thiol status of cells [51], increase de novo synthesis of GSH [37,52], and enhance GSH levels in diabetic [14] and normal animals [53]. Additionally, α-lipoic acid can maintain high levels of vitamin C and participate in vitamin E recycling, thus complementing some of the functions of GSH [30]. Glutathione disulfide levels were increased in heart of diabetic rats and after treatment with α-lipoic acid in liver of normal rats, but remained normal in diabetic kidneys. Treatment with α-lipoic acid restored GSSG content to normal levels in heart, reflecting the association between glutathione and α-lipoic acid.

Selection of the appropriate dose of α-lipoic acid for use

in chemically-induced diabetes is critical. In this study, all of the diabetic rats died after only a few days of treatment with 50 mg/kg of α-lipoic acid, whereas normal rats treated with same dose all survived, though with significant effects to hepatic catalase activity, glutathione peroxidase activity, and reduced and oxidized glutathione concentrations, as well as renal glutathione peroxidase activity. There are no reported deleterious effects with α-lipoic acid treatment, which has an LD50 of approximately 400-500 mg/kg in rats. Fatal complications have, however, been reported in thiamine-deficient rats receiving a relatively low dose of lipoic acid (20 mg/kg) intraperitoneally, in comparison to thiamine sufficient rats [3]. One study began treatment with 100 mg/kg lipoic acid at the same time that streptozotocin was injected to induce diabetes [15]. These rats did not have diabetes at the time antioxidant treatment began, so that the animals were healthier when they encountered lipoic acid. Thus, Obrosova et al [15] examined the effects of the antioxidant on the development of the disease, whereas our present study examined the effect of lipoic acid on ameliorating preexisting disease and its complications. After examining these two studies, it would appear that the severity of the disease has an impact on the dose of lipoic acid tolerated by the diabetic rats. Although the mechanisms by which α-lipoic acid caused deaths in the diabetic rats in the present study were not established, factors in toxicity may include persistent hyperglycemia of 30 days duration, increase in ketone bodies, or blood lactate levels in combination with the concentration of α-lipoic acid. It is also possible that the elevated pH of the vehicle necessary to dissolve the α-lipoic acid was not tolerated well by animals that were already ill.

From these observations, it is possible to conclude that α-lipoic acid may reduce some oxidative stress in diabetes by alleviating lipid peroxidation through scavenging of free radicals, or by increasing the activities of antioxidant enzymes which then detoxify free radicals. The role of α-lipoic acid in the modulation of glutathione may be significant in the restoration of cell redox status and minimization of cell damage due to reactive oxygen species. Selection of appropriate pharmacological doses of α-lipoic acid for use in diabetes is critical. Further investigations into the role and mechanism of antioxidant action of this readily available dietary supplement are warranted.

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