PREVENTIVE EFFECTS OF ALPHA-LIPOIC ACID ON DIABETIC NEPHROPATHY IN A RAT MODEL

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ABSTRACT

Objective: Diabetic nephropathy is the major cause of chronic renal failure, and oxidative stress has an important role in its etiology. Aim of this study is to investigate the effects of the antioxidant alpha-lipoic acid (ALA) treatment on proteinuria, antioxidant enzyme and inflammatory cytokine levels in the renal tissue and renal histopathology in diabetes.

Design: Thirty male Wistar rats were divided into three groups: normal control group (n=10), diabetic control group (n=10), and ALA-treated diabetic group (n=10). Diabetes was induced by a single intravenous injection of streptozotocin. Rats which completed 4 weeks of the study period were sacrificed after 24-hour urine collection for albuminuria detection. Histopathological examination was performed. Antioxidant enzyme and inflammatory marker levels in renal tissue were measured.

Results: The 24-hour urinary albumin levels were significantly reduced in the ALA-treated diabetic group compared with the diabetic control group. In ALA administered rats, histopathological findings such as tubular dilation, tubular epithelium necrosis, glomerular focal necrosis, and interstitial inflammation were observed at a lesser extent than the diabetic control group. Any difference was not detected between the diabetic control group, and the ALA group with respect to the levels of GSH, GSH-r, MDA, TNF-α and IL-6.

Conclusion: ALA administration led to regression of histopathologic and morphologic lesions in extracted rat kidney. Also, positive effect of ALA on albuminuria was detected. However, no positive effect of ALA was found for antioxidant parameters at the tissue level.

Key words: Alpha-lipoic acid, diabetes mellitus, diabetic nephropathy, oxidative stress, antioxidants.

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Introduction

Diabetic nephropathy (DN) is a major complication associated with type 2 diabetes and is a leading cause of end-stage renal disease⁴. It is characterized functionally by proteinuria and pathologically by glomerular hypertrophy, mesangial expansion and tubulointerstitial fibrosis leading to the loss of renal function⁵. In spite of the intensive control of blood glucose and blood pressure, DN remains an important clinical problem. Therefore, new therapeutic drugs for controlling DN are needed.

The underlying mechanisms of the evolution of DN are extremely complex, and several growth factors or metabolic products have been identified as contributing factors⁶,⁷. Among these factors, reactive oxygen species are thought to play an important role in the development of DN⁸. It has been hypothesized that an increase in oxidative stress as a result of chronic hyperglycemia activates several signaling pathways that alter gene expression⁹. Recent studies have suggested that inflammation plays a role in the progression of DN⁹. Accordingly, many studies have focused on slowing down the progress of DN by reducing oxidative stress as well as controlling blood glucose and blood pressure levels.

Alpha lipoic acid, a naturally occurring dithiol compound which plays an essential role in mitochondrial bioenergetic reactions, has gained considerable attention as an antioxidant for use in managing diabetic complications⁴. Lipoic acid quenches
reactive oxygen species, chelates metal ions, and reduces the oxidized forms of other antioxidants such as vitamin C, vitamin E, and glutathione\(^9\).

The aim of the present study was to investigate the therapeutic effects of ALA on proteinuria, oxidative stress and inflammation by characterizing biochemical and histopathological changes in an experimental diabetic rat model.

**Material and methods**

**Experimental Design**

All animal studies were performed in accordance with the Adnan Menderes University Animal Experiments Institutionale Ethics Committee guidelines for animal care and use. Animals were housed at constant temperature (20-22°C) and humidity (50-60%) with a 12-h light and 12-h dark cycle. They were allowed free access to water and standard rat chow. Thirty male Wistar rats (weight 200-250 g) were divided into three groups as diabetic control group (n=10), ALA-treated diabetic group (n=10), and normal control group (n=10). Diabetes was induced by a single i.v. injection of streptozotocin (45 mg/kg in 100 mmol/l sodium citrate buffer, pH 4.5). Non-diabetic animals were sham-injected with buffer only. After 3 days from streptozotocin injection, rats with plasma glucose levels >300 mg/dL were included in the study. After induction of diabetes, rats were divided randomly into an untreated diabetic group and a treated diabetic group, receiving ALA (MEDA Pharma GmbH & Co. KG, Bad Homburg, Germany) 2ml/day (100 mg/kg, intraperitoneal administration). In order to limit hyperglycaemia and ensure that animals maintained body weight, diabetic rats received 2 IU NPH insulin every other day.

**Urinary analysis**

Renal albumin excretion was used as a marker for early diabetic nephropathy. On the 4th week, 24-hour urinary albumin was measured. For urinary measurements, rats were housed in metabolic cages for 24 hour, and several drops of toluene dye were added to the urine collection beaker to inhibit microbial growth. Urinary albumin was quantified by a competitive ELISA method. On the following day, rats were sacrificed by decapitation. Both kidneys were washed out with saline and immediately stored at -80°C until being processed for biochemical and pathological investigations.

**Biochemical analysis**

The tissue levels of GSH, GSH-r, MDA, TNF-\(\alpha\), and IL-6 were measured by standard enzymatic methods.

Tissue homogenizing was performed by a homogenizer (B. Braun, Melsungen, Germany) using a 1/10 homogenizing buffer (1 mM, pH 7,4). The buffer, containing the protease inhibitors, 0.2 \(\mu\)M phenyl-methylsulfonyl fluoride (PMSF, Sigma, Cat. no: P-7626), and 1mM ethylene- daimine tetraacetic acid (EDTA, Sigma, Cat. No: E-9884), was used to homogenize samples at +4°C. Tissue GSH levels were determined by the method of Ellman et al., tissue GSH-r levels were determined by the method of Beutler et al., and tissue MDA levels were measured by the method of Draper and Hadley spectrophotometrically (UV-160 Shimadzu spectrophotometer, Shimadzu Corp., Kyoto, Japan) using thiobarbituric acid as a reactive substance\(^{10,11,12}\).

**Histopathological analysis**

Paraffin embedded tissues were cut into 4 \(\mu\)m thick sections and stained with hematoxylin & eosin, Masson trichrome, Gomoramine methenamine silver, periodic acid-Schiff. We examined these sections with a light microscope. In each kidney section, the degree of focal necrosis for each glomerulus, expansion of Bowman's capsule, degeneration and necrosis for tubular epithelium, tubular dilatation, interstitial inflammation, vascular congestion and thickening were graded from 0 to 3 as follows: grade 0: normal (-); grade 1: mild (+); grade 2: moderate (++); grade 3: severe (+++).

**Statistical analysis**

All results are presented as means ±SD. Data was analyzed using SPSS version 14.0. Statistical significance was evaluated using Mann-Whitney U tests (corrected for ties), chi-square tests and Spearman's rank correlation coefficients (corrected for ties, 1-tailed probabilities). P values of less than 0.05 were considered statistically significant.

**Results**

Six rats (four from the diabetic and two from normal control group) died during the study period as a result of potential diabetic complications. Changes in blood glucose levels at the beginning and at four weeks after induction of diabetes in each experimental group were shown in Table 1.
Plasma glucose level was significantly increased in the diabetic groups compared to the control group (p=0.0001). No significant changes between diabetic control group and ALA-treated diabetic group was noted (p=0.374), although glucose levels in the ALA-treated diabetic group decreased compared to the diabetic control group.

Albumin excretion was elevated significantly in rats in both diabetic groups at four weeks after induction of diabetes when compared to the normal control group (p=0.025). Renal albumin excretion was significantly reduced in the ALA-treated diabetic group compared to the diabetic control group (p=0.009). There was no statistically significant difference in the levels of GSH, GSH-r, MDA, TNF-α and IL-6 among the study groups (Table 2).

In renal histopathology, diabetic group showed significant increase in focal necrosis for each glomerulus (p=0.02), expansion of Bowman's capsule (p=0.018), necrosis for tubular epithelium (p=0.02), tubular dilatation (p=0.017), and interstitial inflammation (p=0.016) compared with normal control group (Figures 1, 2). ALA-treated diabetic group showed decreased focal necrosis for each glomerulus (p=0.008), expansion of Bowman's capsule (p=0.006), necrosis for tubular epithelium (p=0.008), tubular dilatation (p=0.018), and interstitial inflammation (p=0.046) compared with diabetic control group (Figures 2, 3). There was no statistically significant difference in degeneration for tubular epithelium, vascular congestion and thickening among the study groups.

### Table 1: Changes in plasma glucose (mg/dl) in each experimental group at the beginning and at the end of the study.

<table>
<thead>
<tr>
<th></th>
<th>Normal control (n=8)</th>
<th>Diabetic control (n=6)</th>
<th>ALA-treated diabetic (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean±SD (range)</td>
<td>mean±SD (range)</td>
<td>mean±SD (range)</td>
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<tr>
<td>Plasma glucose</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>First week</td>
<td>114±13.8 (100-136)</td>
<td>528±85.4 (384-600)</td>
<td>544±95.4 (309-600)</td>
</tr>
<tr>
<td>Four weeks</td>
<td>159±50.6 (75-241)</td>
<td>600± (600-600)</td>
<td>512±163 (235-600)</td>
</tr>
</tbody>
</table>

### Table 2: Biochemical characteristics of experimental animals.

<table>
<thead>
<tr>
<th></th>
<th>Normal control (n=8)</th>
<th>Diabetic control (n=6)</th>
<th>ALA-treated diabetic (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean±SD (range)</td>
<td>mean±SD (range)</td>
<td>mean±SD (range)</td>
</tr>
<tr>
<td>GSH</td>
<td>107.83±19.46</td>
<td>128.28±11.33</td>
<td>118.81±23.98</td>
</tr>
<tr>
<td>GSH-r</td>
<td>0.41±0.07</td>
<td>0.47±0.14</td>
<td>0.50±0.06</td>
</tr>
<tr>
<td>MDA</td>
<td>1.24±0.42</td>
<td>1.17±0.30</td>
<td>1.28±0.46</td>
</tr>
<tr>
<td>TNF-α</td>
<td>12.19±7.33</td>
<td>12.87±2.40</td>
<td>11.01±3.90</td>
</tr>
<tr>
<td>IL-6</td>
<td>375.04±69.50</td>
<td>411.63±55.96</td>
<td>395.67±69.42</td>
</tr>
<tr>
<td>24-h albuminuria</td>
<td>0.059±0.078</td>
<td>0.177±0.139</td>
<td>0.033±0.022</td>
</tr>
<tr>
<td>(mg/day)*</td>
<td>(0.010-0.215)</td>
<td>(0.050-0.429)</td>
<td>(0.015-0.078)</td>
</tr>
</tbody>
</table>

*GSH= Glutathione; GSH-r= Reduced glutathione; GSH-Px= Glutathione peroxidase; MDA= Malondialdehyde; TNF= Tumour necrosis factor; IL= Interleukin

* p<0.05

In renal histopathology, diabetic group showed significant increase in focal necrosis for each glomerulus (p=0.02), expansion of Bowman's capsule (p=0.018), necrosis for tubular epithelium (p=0.02), tubular dilatation (p=0.017), and interstitial inflammation (p=0.016) compared with normal control group (Figures 1, 2). ALA-treated diabetic group showed decreased focal necrosis for each glomerulus (p=0.008), expansion of Bowman's capsule (p=0.006), necrosis for tubular epithelium (p=0.008), tubular dilatation (p=0.018), and interstitial inflammation (p=0.046) compared with diabetic control group (Figures 2, 3). There was no statistically significant difference in degeneration for tubular epithelium, vascular congestion and thickening among the study groups.

Figure 1: Renal tissue demonstrating mild degrees of congestion in the control group (HE, x200).

Figure 2: Renal tissue demonstrating tubular dilatation, and degeneration (short arrows) and focal glomerular necrosis (long arrows), interstitial inflammation (asterix), marked dilatation in the space of Bowman’s capsule (arrow head) in the diabetic control group (HE, x200).

Figure 3: Renal tissue demonstrating congestion, dilatation within the space of Bowman’s capsule, and mild tubular dilatation in the ALA-treated diabetic group (HE, x200).
Discussion

For the complexity of the mechanisms of DN, it is necessary to develop new drugs to deal with more than one pharmacological target. In our study, we were able to demonstrate significant protection against early nephropathy (albuminuria) and regression in histopathological lesions in extracted kidneys from rats. However, no positive effect of ALA was found on antioxidant parameters at the tissue level.

DN is the most common cause of end-stage renal disease and the characteristics of this diabetic complication include macrovascular and microvascular damage. However, there are few effective agents that prevent the development of diabetic nephropathy, although strict control of hyperglycemia is possible with the use of several diabetic treatments. Oxidative stress has been known to play an important role in the development and progression of DN, and the formation of reactive oxygen species is a direct consequence of hyperglycemia. Therefore, antioxidant treatment is a potential antifibrotic therapy for DN[4, 5].

Prolonged supplementation of the diet of STZ-induced diabetic rats with ALA was associated with attenuation of both hyperglycemia and diabetic renal injury. Amelioration of hyperglycemia has been previously documented in both human and experimental diabetes treated with ALA. Earlier studies demonstrating a hypoglycemic effect of ALA in the STZ-induced diabetic rat have been acute (hours to days) and used high parenteral doses of this agent. No increases in plasma insulin levels were observed in the diabetic rats that were given ALA compared with untreated diabetic rats, suggesting that ALA does not act as an insulin secretagogue, reduce insulin clearance, or protect pancreatic-cells from injury in this model[13, 14]. In the present study, a detectable hypoglycemic effect of ALA was not observed. A delay in expression of the hypoglycemic effect of ALA was previously reported in the literature[15]. It is possible that delayed expression of the hypoglycemic action of ALA is linked to progressive cellular accumulation of ALA, including possibly in insulin-responsive tissues such as muscle and fat[16]. Whether the hypoglycemic effect of ALA in diabetes involves its antioxidant activity or other mechanisms is uncertain[17]. However, recent studies indicated that ALA protects against oxidative stress-induced insulin resistance in muscle cells[18].

Proteinuria in DM is considered to have both a hemodynamic (glomerular capillary hypertension and hyperfiltration) and a structural/cellular basis (alterations in basement membrane, mesangial cell matrix, and podocyte function)[19]. The progression of nephropathy is correlated with the degree of albuminuria. As in a previous study, diabetic rats developed modest proteinuria by 4 weeks, but this was prevented by ALA[20]. The mechanism of the antiproteinuric effect of ALA is not clear. Three months treatment with oral ALA has been reported to attenuate proteinuria in patients with either type 1 or 2 diabetes and overt nephropathy[21]. As previously proposed, increased intraglomerular pressure rather than hyperfiltration may be the key hemodynamic determinant of diabetic renal injury[22].

Attenuation of renal injury by ALA may be linked to its antioxidant activity. The antioxidant properties of both ALA and its reduced form dihydroliopic acid, which is rapidly generated from ALA in many tissues, are previously reported. ALA has also conferred protection against ischemia-reperfusion injury in a number of in vitro and in vivo experimental models[23, 24, 25]. As noted above, there is considerable evidence that increased oxidative stress may participate in the pathogenesis of diabetic complications, including nephropathy[21, 26]. In this regard, administration of ALA has been reported to attenuate neuropathy in experimental and human diabetes in association with reduced markers of oxidative stress[22]. However, in the present study, favourable antioxidant or anti-inflammatory effect on tissue level has not been found.

This study demonstrates that whatever the precise mechanism of its actions, ALA supplementation prevents or delays the development of advanced diabetic renal injury.

Conclusions

The precise mechanisms by which ALA and other antioxidants alter diabetic renal injury are not known. In the present study, the protective effect of ALA therapy on renal structural damage, and albuminuria was observed in diabetic nephropathy. Positive effects in glomerular, tubular, and vascular system have been detected. However, favourable antioxidant or anti-inflammatory effect on tissue level has not been found. According to these results, it can be said that ALA therapy can slow down the development, and progression of diabetic nephropathy, just like some other complications of diabetes.
References


2) Lee EY, Lee MY, Hong WY, Chung CH, Hong SY. Blockade of oxidative stress by vitamin c ameliorates albuminuria and renal sclerosis in experimental diabeti-


5) Chiu J, Khan ZA, Farhangkhoee H, Chakrabarti S. Curcumin prevents diabetes-associated abnormalities in the kidneys by inhibiting p300 and nuclear factor-


11) Draper HH, Hadley M. A review of recent studies on the metabolism of exogenous and endogenous malondi-


17) Jacob S, Streeper RS, Fogt D. The antioxidant alpha-


26) Craven P, DeRubertis R, Kagan VE, Melhem MF, Studer RK. Effects of dietary supplementation with vit-


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