Current Medical Science
DOI https://doi.org/10.1007/s11596-019-2124-1
39(6):920-928,2019

Protective Effects of α-Lipoic Acid on Vascular Oxidative Stress in Rats with Hyperuricemia

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Summary: The aim of the present study was to observe the protective effects of α-lipoic acid (ALA) on vascular injury in rats with hyperuricemia (HUA). The ALA treatment groups (10, 30 and 90 mg/kg, respectively) were administered with ALA via gavage for 2 weeks. Subsequently, the levels of blood urea nitrogen (BUN), creatinine (CREA), uric acid (UA), total cholesterol (TC), high density lipoprotein-C (HDL-C) and low density lipoprotein-C (LDL-C) were measured; the activities of glutathione peroxidase (GSH-Px), catalase (CAT), malonaldehyde (MDA), superoxide dismutase (SOD) and xanthine oxidase (XOD) were also determined. The thoracic aorta of rats in each experimental group was observed under a light microscope; ultrastructural analysis was performed. SOD and CAT protein contents were investigated by Western blotting. The results revealed that: i) Compared with the model group, the levels of UA were decreased in the ALA groups and the levels of BUN, CREA,TC, and LDL-C decreased in the 30 and 90 mg/kg ALA groups (P<0.05); ii) compared with the model group, the activities of GSH-Px, SOD and XOD were increased and the levels of MDA were reduced in the 90 mg/kg ALA group (P<0.05); and iii) in the model and 10 mg/kg ALA groups, edema and shedding were observed in endothelial cells. Compared with the model and 10 mg/kg ALA groups, the 30 and 90 mg/kg ALA groups exhibited fewer swollen endothelial cells. In summary, the results of the present study indicated that HUA resulted in vascular oxidative stress injury and decreased the activity of antioxidative enzymes, which leads to endothelial cell damage and vascular lesions. ALA may serve as a therapeutic agent for the treatment of HUA-induced endothelial dysfunction.
Key words: α-lipoic acid; hyperuricemia; oxidative stress; uric acid; animal model

Hyperuricemia (HUA) refers to the increased serum uric acid (SUA) levels caused by a dysfunction in the metabolism of purines and/or decreased UA excretion[1], which results in the accumulation of UA in the blood and elevated blood UA levels[1, 2]. In recent years, increasing evidence has indicated that HUA is not only critical for the development of gout, but has also been reported as a potential pathogenic factor in the onset of some common disorders[3], including diabetes, hypertension, cardiovascular disease and chronic kidney disease[4].

UA is the final enzymatic product of purine metabolism. It was demonstrated that treatment with 5 and 10 mg/dL UA induced a relaxing effect against oxidative stress (OS) in our steatosis cell model; however, 30 mg/dL UA may aggravate OS[5]. At normal physiological levels in the plasma, UA is a potent antioxidant and protects the erythrocyte membrane from lipid oxidation by scavenging oxygen radicals[6]. UA is an effective antioxidant in the extracellular environment; however, when UA enters cells via specific organic anion transporters, it induces an oxidative burst reaction, which has been detected in vascular smooth muscle cells, endothelial cells, adipocytes, islet cells, renal tubular cells and hepatocytes[7–9]. In addition, HUA has been reported to increase OS in numerous cell types[8, 10–12] and induce endothelial damage, renal injury, insulin resistance and inflammation.

α-Lipoic acid (ALA) is a type of auxiliary factor of mitochondrial enzyme complexes. It inhibits lipid peroxidation, removes oxygen free radicals, and recovers and increases the levels of other antioxidants

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in the body[13]. Therefore, ALA can reduce oxidative stress[14], decrease the expression levels of inflammatory markers and improve endothelial cell function. ALA is widely applied for the treatment of a variety of diseases, including heart disease[15], tumors, hepatitis, and diabetes, and their associated complications; however, the effects of ALA on HUA-induced endothelial dysfunction have not yet been investigated. As OS alters numerous functions of the endothelium, the present study hypothesizes that ALA may protect rats with HUA from endothelial dysfunction.

1 MATERIALS AND METHODS

1.1 Animal Model

A total of 60 healthy adult male Sprague Dawley rats (7 weeks of age, 217.4±16.5 g), obtained from the Animal Center of Xinjiang Medical University [Urumqi, China; animal license: SYXK (Xin) 2003-0004] were housed in temperature-controlled cages under a 12 h light-dark cycle, and were allowed free access to water and standard chow. After 1 week of breeding for adaptation, the rats were randomly divided into 5 groups with 12 in each group: normal control, model and the 3 ALA groups (10, 30 and 90 mg/kg). The normal control group was provided with standard feed, and the other groups were generated via 10% yeast (YE) feed and a single intraperitoneal injection of 10 mg/kg/day potassium oxonate (OA). To confirm the successful establishment of HUA model, the UA levels in the blood were examined after 3 weeks. Following the analysis of UA levels, of the four groups administered with YE and OA, three ALA groups were generated via the administration of 10, 30 and 90 mg/kg ALA[16, 17], respectively, by gavage once a day for 2 weeks. The body weight of the rats was recorded; the dosage was adjusted according to alterations in body weight. Throughout the experimental process, the animals were provided free access to water.

1.2 Sample Collection and Test Methods

The general state of rats was observed every day, which included alterations in eating and behavioral changes. In weeks 3 and 5, the blood UA content was detected. On day 36, the rats were fasted for 12 h and then anesthetized with urethane (1000 mg/kg). Two sections of thoracic aorta about 1-cm-length each were taken for analysis. One section was fixed in a small volume of formaldehyde. Slices of the samples were then stained with hematoxylin and eosin for pathological analysis. The second section was fixed in a small volume of 5% glutaraldehyde. Analysis of the section ultrastructure was conducted under an electron microscope. The remaining samples were stored at –80°C for spare protein determination.

1.3 Blood Specimen Assay

The levels of blood urea nitrogen (BUN), creatinine (CREA), UA, triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were detected using a Beckman LX20 automatic biochemical analyzer (Beckman Coulter, Inc., Brea, USA). The expression of glutathione peroxidase (GSH-Px, A005), catalase (CAT, A007-1-1), malonaldehyde (MDA, A003-1), superoxide dismutase (SOD, A001-3) and xanthine oxidase (XOD, A002-1) was detected using kits (Nanjing Jiancheng Bioengineering Institute, China).

1.4 Arterial Specimen Assay

The thoracic aorta of rats was cut into sections, in which some were prepared as pathological sections for ultrastructural analysis via electron microscopy. The remaining sections underwent protein extraction for the detection of SOD and CAT protein expression via Western blotting. Total protein was extracted using RIPA cracking liquid. The gel used for SDS PAGE was produced using an SDS-PAGE kit. A pre-stained protein ladder (10 μL, cat. no. 26616, Thermo Fisher Scientific, Inc., USA) was added into the first lane of the gel; 30 μg protein sample was added into other lanes. The voltage of electrophoresis was 110 V and the electric current applied for electrotransfer was 200 mA for 1 h. The membranes were blocked with blocking buffer for 2 h. The primary antibodies against SOD (SC-101523, Santa Cruz Biotechnology, Inc., USA) and CAT (2363-1, Epitomics; Abcam, UK) were diluted with 5% skim milk. Subsequently, the membranes were then incubated with the primary antibody diluent [1:600 for SOD, 1:800 for CAT, 1:400 for β-actin (SC-47778, Santa, Cruz Biotechnology, Inc., USA)] overnight at 4°C. The secondary antibodies for the detection of SOD and β-actin (SC-2443, Santa Cruz Biotechnology, Inc., USA) and CAT (ZB-2301, OriGene Technologies, Inc., China) were diluted with 5% skim milk (1:8000); the membranes were incubated with the secondary antibody diluent for 90 min. Membranes were washed and incubated with a 3,3'-diaminobenzidine substrate kit for 10 min. Subsequently, images of the gel were obtained using the GelDoc XR System (Bio-Rad Laboratories, Inc., USA). The grayscale of the images were analyzed with Quantity One software (Bio-Rad Laboratories, Inc., USA).

1.5 Statistical Analysis

The data were expressed as mean±standard deviation and analyzed with SPSS 18.0 software (SPSS, Inc., USA). The homogeneity of variance of the data was determined via Levene's test. When the variance was homogeneous, one-way analysis of variance (ANOVA) was applied to test the differences within these groups, followed by a Student-Newman-Keuls test. Data that did not have homogeneity of variance and were analyzed with one-way ANOVA were adjusted via the Brown-Forsythe test or the

Games-Howell method for multiple comparisons.

2 RESULTS

2.1 Alterations in Blood UA Levels

At week 3, rats of the model group exhibited mental depression and dull fur. Following the end of the intervention, no rats in each group had succumbed to death. The effects of OA and YE on the weight of rats was presented in fig. 1A. The results revealed that there was no difference in weight between the normal control group and model group prior to and following model induction. As presented in fig. 1B, after 3 weeks (prior to treatment), the levels of UA increased significantly within model group and ALA groups as compared with the normal control group, indicating that the models were successfully generated (P<0.05). Following model induction and ALA treatment, compared with the normal control group, the level of UA in the model group increased significantly; however, that of the 90 mg/kg ALA group was significantly decreased (P<0.05). The UA levels in the 10, 30 and 90 mg/kg ALA groups were significantly decreased as compared with that in the model group (P<0.05). The results suggested that with increasing of concentrations of ALA, the levels of UA were reduced accordingly. But, the differences between ALA treatment groups were not significant (P>0.05).

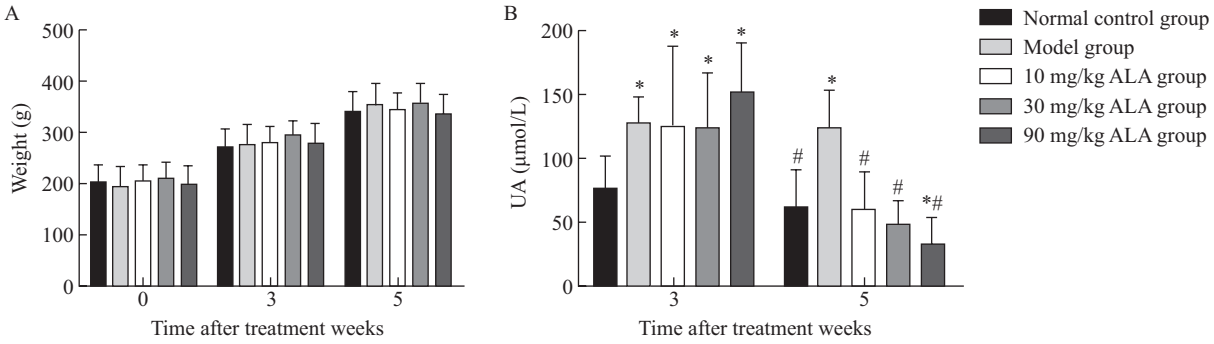

Fig. 1 A: Alterations in weight within the various groups. B: Alterations in blood uric acid levels (n=12, μmol/L). $^{*}P$<0.05 *vs.* the normal control group, $^{\#}P$<0.05 *vs.* the model group

2.2 Analysis of Blood Specimens

2.2.1 Alterations in the Kidney Index Five weeks after the treatment, the levels of BUN in the model and 10 mg/kg ALA groups were significantly increased compared with that in the normal control group; the levels of CREA in the model and ALA groups were also significantly increased (P<0.05) (fig. 2). Compared with the model group, the BUN and CREA levels in the 30 and 90 mg/kg ALA groups were significantly decreased (P<0.05). The BUN and CREA levels in the 10 mg/kg group did not exhibit notable differences as compared with those of the model group (P>0.05).

2.2.2 Alterations in Blood TC, TG, HDL-C and LDL-C levels As presented in table 1, 5 weeks after the treatment, compared with the normal control group, the levels of LDL-C in the model and 10 mg/kg ALA groups were significantly increased, whereas TG and HDL-C in the model, 10 and 30 mg/kg ALA groups decreased significantly (P<0.05). The levels of TC in the 30 and 90 mg/kg ALA groups also decreased significantly (P<0.05). Compared with the model group, the levels of TC and LDL-C in the 30 and 90 mg/kg ALA groups decreased significantly (P<0.05). HDL-C only in the 90 mg/kg ALA group decreased

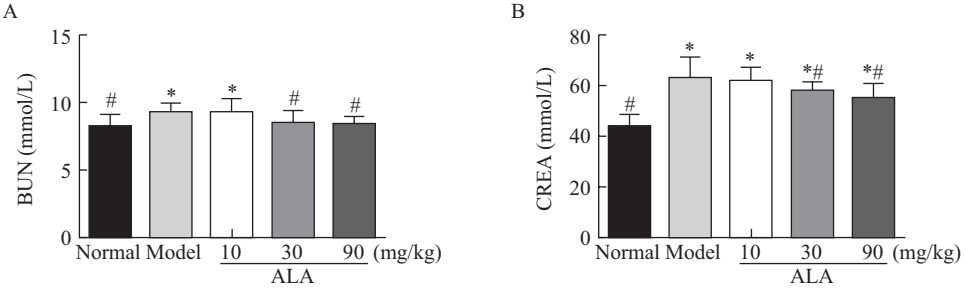

Fig. 2 Alterations in the kidney indexes BUN (A) and CREA (B) (n=12, mmol/L)
$^{*}P$<0.05 *vs.* the normal control group, $^{\#}P$<0.05 *vs.* the model group. ALA, α-lipoic acid; BUN, blood urea nitrogen; CREA, creatinine

significantly (P<0.05). In addition, the levels of TC, TG, HDL-C and LDL-C in the 10 mg/kg ALA group exhibited no significant differences as compared with the model group (P>0.05).

2.2.3 Changes in OS Indicators As presented in table 2, GSH-Px activity was lower in the model, 10 and 30 mg/kg ALA groups than in the normal control group; however, GSH-Px activity was significantly higher in the 90 mg/kg ALA group than in the model group (P<0.05). SOD activity in the serum was lower in the model and 10 mg/kg groups than in the normal control group, and it was significantly higher in the 30 and 90 mg/kg ALA groups than in the model group (P<0.05). CAT activity in the serum was significantly lower in the 90 mg/kg ALA group than in the normal control group (P<0.05). MDA activity in the serum was higher in the model group than in the normal control group, and it was significantly lower in the 10, 30 and 90 mg/kg ALA groups than in the model group (P<0.05). In addition, XOD activity in the serum was lower in the model group than in the normal control group; XOD activity in the serum was significantly higher in the 10, 30 and 90 mg/kg ALA groups than in the model group (P<0.05).

2.3 Detection of Thoracic Aorta Indexes

2.3.1 Western Blotting for the Analysis of SOD and CAT Protein Expression within Rat Thoracic Aortic Tissue The optical density (OD) values computed via Quantity One software was presented in fig. 3. The grayscale value for SOD was significantly lower in the model, 10, 30 and 90 mg/kg ALA groups than in the normal control group (P<0.05; fig. 3A). The OD values for SOD in the 30 and 90 mg/kg ALA groups were significantly higher than that of the model group (P<0.05); the OD value for CAT in the model and 10 mg/kg ALA groups were significantly lower than that in the normal control group (P<0.05; fig. 3B). The 30 and 90 mg/kg ALA groups exhibited significantly higher

Table 1 Alterations in blood TC, TG, HDL-C and LDL-C levels (n=12, mmol/L)

Group	TC	TG	HDL-C	LDL-C
Normal control	1.33±0.09	1.34±0.60$^{\#}$	0.60±0.04$^{\#}$	0.26±0.03$^{\#}$
Model	1.27±0.12	0.90±0.10*	0.47±0.06*	0.29±0.05*
10 mg/kg ALA	1.31±0.17	0.89±0.13*	0.44±0.04*	0.31±0.05*
30 mg/kg ALA	1.14±0.12$^{*\#}$	1.03±0.30*	0.48±0.05*	0.25±0.03$^{\#}$
90 mg/kg ALA	1.05±0.15$^{*\#}$	1.10±0.29	0.58±0.05$^{\#}$	0.23±0.02$^{\#}$
P	<0.001	0.011	<0.001	<0.001

$^{*}P$<0.05 *vs.* the normal control group, $^{\#}P$<0.05 *vs.* the model group. ALA, α-lipoic acid; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride

Table 2 Effects of ALA on SOD, GSH-Px, CAT, MDA and XOD activities (n=12)

Group	GSH-Px (U/L)	SOD (U/mL)	CAT (U/mL)	MDA (nmol/mL)	XOD (U/L)
Normal control	2271.43±210.26$^{\#}$	519.90±60.52$^{\#}$	6.82±3.88	3.65±0.55$^{\#}$	29.55±3.27$^{\#}$
Model	1644.71±198.54*	410.48±48.83*	4.61±4.48	6.35±1.73*	26.16±4.57*
10 mg/kg ALA	1754.62±253.84*	433.19±56.71*	4.23±4.09	4.05±1.51$^{\#}$	31.98±4.69$^{\#}$
30 mg/kg ALA	1893.75±312.96*	485.43±80.13$^{\#}$	4.96±3.73	3.85±0.90$^{\#}$	29.92±4.36$^{\#}$
90 mg/kg ALA	2151.83±214.44$^{\#}$	494.70±34.50$^{\#}$	2.65±1.94*	2.85±0.64$^{\#}$	29.62±3.20$^{\#}$
P	<0.001	<0.001	0.117	<0.001	0.021

$^{*}P$<0.05 *vs.* the normal control group, $^{\#}P$<0.05 *vs.* the model group. CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malonaldehyde; SOD, superoxide dismutase; XOD, xanthine oxidase

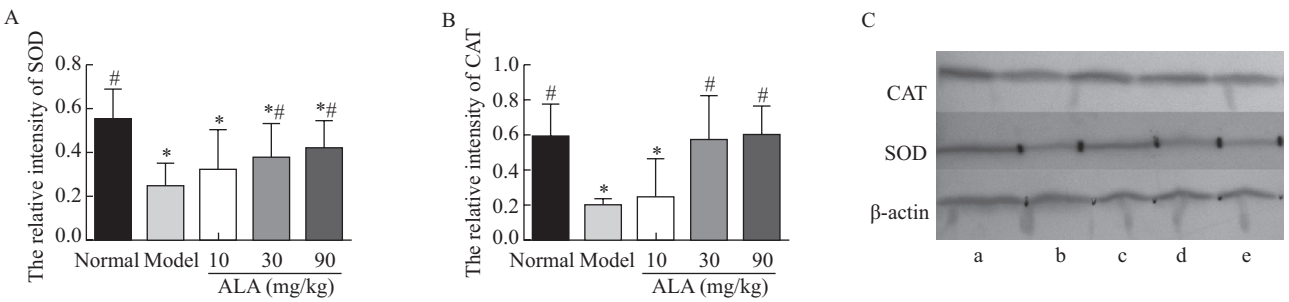

Fig. 3 Comparison of relative expression presented by grayscale intensity

Expression of (A) SOD and (B) CAT (β-actin served as the internal reference, n=12). $^{*}P$<0.05 *vs.* the normal control group, $^{\#}P$<0.05 *vs.* the model group. C: protein expression levels of CAT and SOD in the thoracic aorta from rats within the various groups (Western blotting). (C-a) Normal control, (C-b) model, (C-c) 90 mg/kg ALA, (C-d) 30 mg/kg ALA and (C-e) 10 mg/kg ALA groups. ALA, α-lipoic acid

OD values for CAT than the model group (P<0.05).

2.3.2 Pathological and Ultrastructural Alterations of the Thoracic Aorta In the normal control group, the intima surface of the aorta was smooth and intact, and each structural layer was normal (fig. 4A). The smooth muscle of the tunica media was neatly arranged and of uniform thickness; endothelial cells were closely arranged and did not fall off. In addition, endothelial cell adhesion and inflammatory cell infiltration were rarely observed. In the model group (fig. 4B) and 90 mg/kg ALA group (fig. 4C), edematous and deciduous endothelial cells in the rats were observed, as well as intimal bulge. In the 30 and 90 mg/kg ALA groups, the number of swollen endothelial cells was decreased as compared with the model and 10 mg/kg ALA groups.

In the normal control group (fig. 5A), the surface of the intima was observed to be smooth and the endothelial cells appeared to be flattened. Beneath the endothelial cells, thin loose connective tissue containing a little collagen and a small number of elastic fibers are observed. The internal elastic membrane was continuous, intact, smooth and wavy; the elastic membrane of the tunica media was continuous with parallel arrangement and layers of cells. Additionally, the smooth muscle cells were multinucleated and as a monolayer.

In the model group (fig. 5B), on the surface of intima, a region of endothelial cells bulged toward the vascular lumen. This swelling or shedding of cells was visible in the endotheliocytes; the density of the nuclear matrix also increased. Additionally, the internal elastic lamina was thicker, some of which was fractured and missing. The smooth muscle cell layer was uneven and increased branching of smooth muscle cells was observed. Furthermore, smooth muscle cells were detected within the lumen. Mitochondria were swollen and denser.

The 10 mg/kg ALA group (fig. 5C) demonstrated few cytoplasmic protrusions of endothelial cells on the surface of intima into the vessel. The connective tissue beneath the endotheliocyte layer exhibited an irregular arrangement; endothelial cells presented notable swelling. As the endothelial cells were arranged in a nonuniform manner compared with the subendothelial cells, a vacuole formed. Compared with the model group, the density of the endothelial matrix decreased. In addition, part of the internal elastic membrane was not connected tightly. Pyknosis was detected in

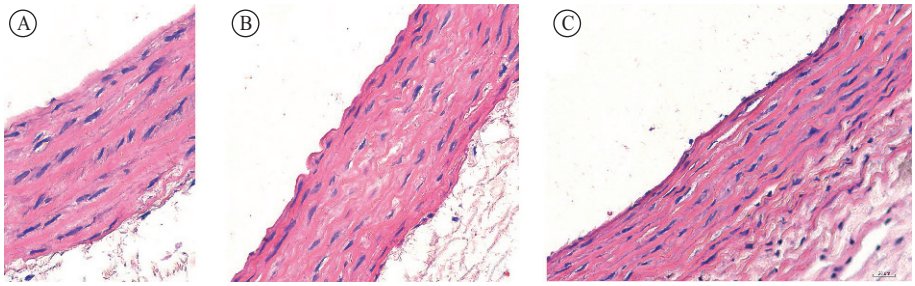

Fig. 4 Pathological alterations of the thoracic aorta of rats under a microscope (hematoxylin and eosin staining, ×400)
(A) Normal control, (B) model and (C) 90 mg/kg ALA groups. ALA, α-lipoic acid

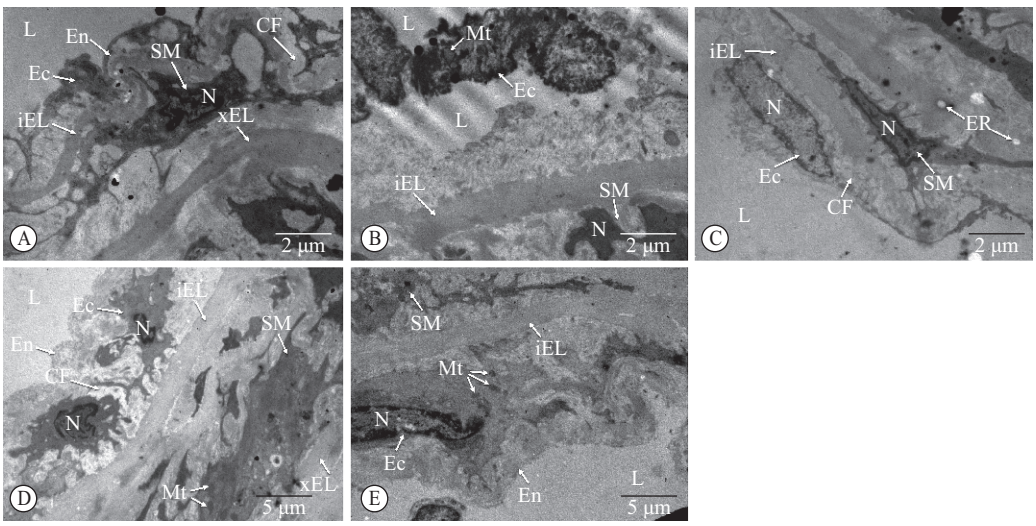

Fig. 5 Ultrastructure of the thoracic aorta of rats in different groups as analyzed via an electron microscope (8000×)
(A) Normal control, (B) model, (C) 10 mg/kg ALA, (D) 30 mg/kg ALA and (E) 90 mg/kg ALA groups. ALA, α-lipoic acid; CF, collagenous fiber; Ec, endothelial cells; En, endothelium; ER, endoplasmic reticulum; L, lumen; N, nucleus; iEL, internal elastic lamina; Mt, mitochondria; SM, smooth muscle cell; xEL, external elastic laminae

smooth muscle cells and the endoplasmic reticulum. The amount of heterochromatin and the number of mitochondria were increased.

In the 30 mg/kg ALA group (fig. 5D), more heterochromatin in the nucleus was detected than in the normal group; the thickness of subendothelial layer decreased as compared with that of the model group. Additionally, the extent of edema of the endothelial cells decreased compared with the low dose group (10 mg/kg ALA). Furthermore, an increased number of swelled mitochondria in the external elastic membrane was reported. Analysis also revealed that the smooth muscle cell layer was discontinuous.

The 90 mg/kg ALA group (fig. 5E) exhibited few cytoplasmic protrusions of endothelial cells on the surface of intima into the vessel. The internal elastic lamina was continuous and edema of the mitochondria in the smooth muscle layer appeared to be ameliorated. A few samples demonstrated similarities to the normal control group. In addition, the edema of endothelial cells was alleviated and the number of mitochondria decreased.

3 DISCUSSION

HUA has been associated with traditional metabolic diseases. HUA was an independent risk factor for cardiovascular disease, including high blood pressure, diabetes, obesity and others[18, 19]. High levels of UA have been reported to induce endothelial dysfunction by generating reactive oxygen species (ROS)[20]. Allopurinol is the most commonly used therapeutic agent to treat chronic HUA by inhibiting XOD[21]; however, treatment with allopurinol may result in serious side effects, such as Stevens-Johnson syndrome[22]. Thus, there is increasing interest focused on developing a novel method to control SUA levels. ALA and dihydrolipoic acid are mutually transformed via metabolism and are regenerated in the body to eliminate numerous accelerated aging-associated free radicals[23]. Unlike other antioxidants, ALA in the oxidized and reduced states has been reported to exhibit antioxidant effects, and dihydrolipoic acid in particular was reported to possess the highest antioxidative potency[24]. ALA by eliminating ROS such as MDA and hydrogen peroxide, reducing and recycling SOD and other antioxidants, becomes the ideal antioxidant.

As an organic metabolite with dual effects of antioxidation and pro-oxidation, UA has been investigated in numerous studies. An early study conducted by our research group using L-02 cells revealed that 5 and 10 mg/dL UA may have a protective effect on the steatosis cells, and 30 mg/dL UA was proposed to promote cellular oxidative stress[5]. In the present study, 10% YE feed combined with 100 mg/kg OA, which was administered intraperitoneally to rats, was applied to generate the HUA rat model. At week 3, the SUA levels were significantly higher within rats of the model group than in the normal control group. There are numerous methods to build hyperuricemia model in rats, such as that reported by Renuka *et al*[25], which involves an injection with ammonium chloride to rats for 8 weeks to generate the HUA model; however, the results demonstrated that this method can cause liver damage. In the studies of Li *et al*[26], Zhu *et al*[27] and Nguyen *et al*[28], models of HUA were generated via an intraperitoneal injection of OA for only 1 h, and were employed to investigate the effects of drugs on reducing blood UA levels. In addition, Zhang *et al*[29] conducted oral gavage on rats with 10% fructose feed to generate a model of HUA. On the 14th day, the SUA levels were significantly higher in the model group than in the control group; however, obesity was simultaneously observed. Ma *et al*[30] conducted oral gavage on rats with 0.5% purine and 10% YE feed for 18 days; however, notable kidney injury was detected. Generally, the establishment of the HUA model mainly occurs via the high intake of purines and/or inhibited excretion of UA. In the present study, by establishing the HUA model with YE and OA, increases in the levels of UA and CREA were observed in the model group compared with the control group at 3 weeks. Increased CREA levels suggested that renal function had been impaired in the rat model. The aim of the present study was to investigate the effects of ALA on a basic model of HUA. Simple rat models of HUA were desired for analysis as the existing methods for generating HUA models were not suitable for investigation in the present study. In numerous animal studies, elevated levels of UA have been reported to induce the OS reaction in a variety of cells[31, 32]. Epidemiological studies have demonstrated that a variety of chronic diseases are accompanied with increased UA levels[33]; however, the association between diseases and UA levels is unclear. Therefore, whether the animal model of HUA in the present study can be established without damaging other tissues and organs requires further investigation. In contrary, the method of generating a model of HUA employed in the present study was aimed at minimizing unnecessary injury to animals, thereby avoiding potential confounding factors.

HUA in the population is usually accompanied with disorders in lipid metabolism, including elevated LDL-C, TC, TG and decreased HDL-C levels[34–36]. As HUA is usually associated with several other chronic diseases[37], it is difficult to determine whether alterations in the levels of these indicators are directly caused by HUA; however, recent studies have demonstrated a significant correlation between UA and lipid metabolism abnormalities after adjusting for dietary habits, hypertension and diabetes[37]. The results of the study that investigated HUA in a population was

not completely consistent[38, 39]; high concentrations of blood UA were reported to promote OS, which can induce disorders in lipid metabolism. In the present study, HUA rat models did not present with serious symptoms of lipid metabolic disorders. Compared with the normal control group, TC levels were not statistically significantly different in the model group; however, the levels of TG were decreased in the model group. Following intervention with ALA, the various indexes analyzed were similar to those of the normal control group. It was demonstrated that ALA may affect blood lipid metabolic alterations in the HUA rat model in the present study. The underlying mechanism of ALA in HUA remains unknown and requires further investigation; however, the effects of ALA on HUA may be associated with its antioxidative capacity.

As aforementioned, ALA has high antioxidant capacity. The disulfide bond of ALA can directly react with ROS[40]. In addition, ALA can chelate transition metals and inhibit the production of ROS[41]. Higher than normal levels of UA may damage tissue and organs, which may cause harmful effects via OS. In the present study, the model group exhibited decreased activities of SOD, GSH-px and CAT; however, the levels of MDA were increased compared with those in the normal control group; OS was observed following the generation of the model group. Recent studies have reported similar results and proposed the possible mechanism in detail[8, 42]. With the intervention of ALA, the activities of SOD and GSH-px were significantly increased, and the content of MDA was significantly decreased, which was consistent with the results of several recent studies[40, 43]. In addition, CAT activity decreased in response to ALA treatment. In a study by Khan *et al*[44], ALA was applied to alleviate rat nerve toxicity induced by bisphenol A. It was reported that intervention with ALA significantly ameliorated OS within rats and enhanced the activity of CAT. Liu *et al*[45] used ALA to ameliorate high-fat diet-induced cerebral injury within mice. Following intervention with ALA, the expression levels of CAT were notably increased compared with the mice fed with a pure high-fat diet. The present study did not report any similar results. The decreased activity of CAT following treatment with ALA was not expected in the present study and requires further study. In the present study, an interesting phenomenon was reported, in which successful modeling was accompanied with decreased XOD activity. XOD is the key enzyme of UA synthesis in mammals; increased XOD activity is conducive to the generation of UA with the production of ROS. Therefore, decreased XOD activity may help inhibit the development of OS. Similar results were reported in a study of renal injury in a rat model of HUA by Asakawa *et al*[46]; however, further investigation is required. We speculated that OS induced by high UA levels may impair XOD activity. However, the long duration of high-purine feeding reversed the effect that the decrease of XOD activity should exert, and finally increased the levels of UA. Following intervention with ALA, the activity of XOD had recovered, while UA levels were decreased. The possible cause of this observation remains unknown. The present study suggested that this may not be accounted for by the antioxidative ability of ALA; however, further investigation is required.

Elevated levels of UA have been reported to induce vascular endothelial dysfunction *in vivo* and *in vitro* in numerous studies. One of the main mechanisms proposed has suggested that UA may promote the occurrence and development of endothelial cell OS[47, 48]. Endothelial disorders can cause increased blood pressure, which leads to a variety of chronic cardiovascular diseases. In the present study, based on the analysis of the rat thoracic aorta tissue, ALA was reported to significantly reduce the degree of OS within rat models of HUA; however, the morphological changes of endothelial cells in the thoracic aorta of the model group were not significant. In a study regarding vascular endothelial dysfunction, Yang *et al*[49] used 750 mg/kg of OA via gavage for 12 weeks to induce a model of HUA for analysis. The results revealed changes of vascular endothelial morphologies under an optical microscope. The method described in the present study to induce HUA may not be effective to result in notable changes in the morphology of rat vascular endothelial cells. Higher doses and increased durations for inducing the HUA model may be key factors associated with alterations in animal endothelial morphology. The results of electron microscopy demonstrated that the changes in endothelial cell morphology in the model group were alleviated following intervention with ALA. Numerous studies have revealed that ALA can alleviate the dysfunction of endothelial cells, in which the antioxidative capacity of ALA may play a major role[50–52].

An issue remains unresolved in this field of research. Following the intervention of ALA, the levels of UA reduced by varying degrees as the intervention dose increased. The association between the decrease in UA levels and ALA intervention requires further investigation; whether the aforementioned results were due to increased UA excretion or reduced UA production remains unknown. In conclusion, ALA significantly alleviates OS within a rat model of HUA, improves the dysfunction of thoracic aortic endothelial cells and regulates the metabolism of lipids *in vivo*.

Acknowledgments

This study was supported by Southwest Medical University and Xinjiang Medical University and we thank them for their funding and technical assistance.

Conflict of Interest Statement

The authors declare that they have no competing interests.

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(Received Jan. 20, 2019; revised Oct. 6, 2019)