

Protective role of α -lipoic acid in hyperuricemia-induced endothelial dysfunction

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Abstract. The aim of the current study was to determine the effects of α -lipoic acid (LA) on hyperuricemia and endothelial dysfunction, and to uncover the underlying mechanism of its action. A hyperuricemic rat model was established by administration of uric acid (UA) and the rats were orally fed with 2 g/kg/day LA or phosphate-buffered saline. Primary rat aortic endothelial cells were subsequently isolated, and a cell viability assay, apoptosis assay, enzyme nitric oxide synthase (eNOS) activity assay and mitochondrial function assay were all performed. For the *in vitro* study, human umbilical vein endothelial cells were used and western blotting was performed to assess Akt signaling activity. The results of the current study indicated that LA inhibited apoptosis, enhanced eNOS activity and production of nitric oxide (NO), and rescued mitochondrial mass and function in uric acid (UA)-treated endothelial cells. LA activated Akt signaling and inhibition of Akt signaling abolished the effects of LA on cell viability, NO production, ROS production and ATP levels in UA-treated endothelial cells. Therefore, the current study demonstrated that LA attenuated oxidant stress and inhibited apoptosis in UA-treated endothelial cells by activating Akt signaling. The results indicate that LA may serve as a therapeutic approach to treat hyperuricemia-induced endothelial dysfunction.

Introduction

Uric acid (UA) is the final enzymatic product of purine metabolism (1). UA is present in the blood as a result of endogenous production and food intake, and 70% of UA is excreted from the kidney, with the remainder being primarily eliminated by the intestine (1,2). At normal physiological levels in the plasma, UA is a major antioxidant and protects the erythrocyte membrane from lipid oxidation by scavenging oxygen radicals (3,4).

However, disruption of this balance, primarily due to the defective elimination of UA, may lead to hyperuricemia (5).

Hyperuricemia is a common metabolic syndrome and is characterized by high levels of UA in the blood (6). Generally, men with serum urate (ionized form of UA) levels of >7.0 mg/dl and women with urate levels of >5.7 mg/dl are considered to be hyperuricemic (7-9). An analysis conducted from 2007 to 2008 found that the overall prevalence of hyperuricemia among American adults was 21.4% (7). Although hyperuricemia has been identified for many years, the pathophysiology of hyperuricemia remains poorly understood. Previous studies have demonstrated that hyperuricemia is highly associated with a number of diseases including gout, cardiovascular disease and endothelial dysfunction (4,10,11).

Nitric oxide (NO) is an important protective molecule in the vasculature and is primarily synthesized from L-arginine in endothelial cells by the enzyme nitric oxide synthase (eNOS) (12). Endothelial dysfunction, such as impaired NO production, is often identified in patients with cardiovascular and renal diseases, indicating that a correlation exists between hyperuricemia and endothelial dysfunction (13,14). In addition, it has been demonstrated that administering an inhibitor of uricase in rats leads to hypertension and vascular disease, but both renal injury and hypertension were at least partially reversed by supplementation with L-arginine, an eNOS substrate (15). Furthermore, Khosla *et al* (13) have indicated that hyperuricemia may induce endothelial dysfunction by inhibiting NO production.

Lipoic acid (LA), also known as alpha lipoic acid, is an 8-carbon fatty acid in mitochondria and is an important prosthetic group of various enzymatic complexes (16,17). In addition to its role in mitochondrial dehydrogenase reactions, LA exhibits antioxidant activity and has been used as therapeutic agent in the treatment of different pathologies related to oxidative stress, including type 2 diabetes mellitus, alcoholic liver disease and Alzheimer's disease (18-20). However, the effects of LA on hyperuricemia-induced endothelial dysfunction have not yet been investigated. Since oxidant stress alters many functions of the endothelium, it was hypothesized that LA may protect rats with hyperuricemia from endothelial dysfunction.

Materials and methods

Animal study. A total of 30 male Wistar rats (250-300 g, 8-10 weeks old) obtained from the Experimental Animal

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Center of Harbin Medical University (Harbin, China) were used in the current study. The rats were housed individually in a room ($23\pm 1^{\circ}\text{C}$ and 55% humidity) and subjected to a 12-h light/dark cycle with *ad libitum* access to food and water. In the present study, animal modeling and drug intervention were initiated simultaneously following the division of the animals into three groups: Control (phosphate-buffered saline, PBS) group, model group and model+LA group ($n=10$ per group). Rats in the control group were intraperitoneally injected with PBS (0.5 ml/day). The rats in the other two groups were intraperitoneally injected with 250 mg/kg/day oxonic acid potassium salt (Sigma-Aldrich; Merck Millipore, Darmstadt, Germany) and 250 mg/kg/day UA (Shanghai Chemical Reagent Co., Ltd., Shanghai, China) to induce hyperuricemia. To confirm the successful establishment of hyperuricemia models, UA levels in the blood were examined after 14 days. Rats in model+LA group were orally fed with 2 g/kg/day α -lipoic acid (LA; Shanghai Chemical Reagent Co., Ltd.) prior to feeding for 6 weeks. The present study was approved by the Institutional Animal Care and Use Committee of Heilongjiang Provincial Hospital.

Isolation of rat aorta endothelial cells. After 6 weeks of feeding with LA, primary rat aortic endothelial cells were isolated as described previously (21). Briefly, the rats were sacrificed by continuous 5% isoflurane (RWD Life Science, Shenzhen, China) exposure until 1 min after the cessation of breathing. Endothelial cells were then collected from a freshly dissected thoracic aorta. The vessel was ligated at each end with a 5-0 silk suture and PBS solution (2.68 mmol/l KCl, 1.47 mmol/l KH_2PO_4 , 136.9 mmol/l NaCl, and 8.1 mmol/l Na_2HPO_4), containing 8 mg/ml collagenase B to destroy extracellular structure and 0.1% bovine serum albumin (BSA) to stop further digestion, was injected. Following a 40-min incubation at 37°C , the vessel was opened longitudinally and loosely adhered cells were dislodged by repeated flushing with a pipette. Cells were centrifuged ($1,000 \times g$) at -4°C for 5 min and collected for further assay.

Cell culture. Human umbilical vein endothelial cells (HUVECs) were purchased from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum, (Thermo Fisher Scientific, Inc.) 100 U/ml penicillin and 10 $\mu\text{g}/\text{ml}$ streptomycin (Beyotime Institute of Biotechnology, Haimen, China) and incubated at 37°C for at least 24 h in a humidified atmosphere containing 5% CO_2 . HUVECs were first treated with 0.1 mM UA with or without 0.2 mM LA for 24 h. The Akt inhibitor MK-2206 (10 μM ; Selleck Chemicals Co., Ltd., Shanghai, China) was used to inhibit Akt signaling.

Western blot analysis. Rat aorta endothelial cells or HUVECs were collected and homogenized in radioimmunoprecipitation assay buffer with protease inhibitor cocktail (BestBio, Shanghai, China). Equal quantities of protein (30 $\mu\text{g}/\text{lane}$) were separated by electrophoresis on 10% SDS-polyacrylamide gels and sequentially electrophoretically transferred to nitrocellulose membranes. Following blocking with 5% BSA for

1 h at room temperature, the membranes were subjected to immunoblotting with primary antibodies (overnight at 4°C). The following primary antibodies were used: Cleaved caspase-3 (1:1,000; sc-22171-R; Santa Cruz Biotechnology, Inc.), p-Akt (1:2,000; sc-135650; Santa Cruz Biotechnology, Inc.), Akt (1:1,000; sc-377457; Santa Cruz Biotechnology, Inc.) and β -actin (1:1,000; cat. no. 4970; Cell Signaling Technology, Inc., Danvers, MA, USA). β -actin was used as a loading control. Following incubation with appropriate goat anti-rabbit (1:10,000; sc-2030) and goat anti-mouse (1:10,000; sc-2031) secondary antibodies conjugated with horseradish peroxidase (Santa Cruz Biotechnology, Inc., Dallas, TX, USA), blot bands were visualized using PierceTM ECL Western Blotting Substrate kit (Pierce Protein Biology; Thermo Fisher Scientific, Inc.). Densitometric analysis of western blots was performed using Image J software (version 1.44; National Institutes of Health, Bethesda, MD, USA).

Cell viability assay. Cell viability was assessed using an MTT cell proliferation and cytotoxicity assay kit (C0009; Beyotime Institute of Biotechnology) according to the manufacturer's instructions. Briefly, the cells were seeded in a 96-well plate at a density of 2×10^3 cells/well and incubated for 24 h. Following treatment, 10 μl MTT solution was added to each well for 4 h followed by incubation with 10 μl formazan solution for 4 h at 37°C . The absorbance levels of the wells were measured using a microplate spectrophotometer at 570 nm. Each experiment was repeated 4 times and the data were expressed as a percentage of the control.

Measurement of reactive oxygen species (ROS) production. ROS generation was assessed in arbitrary units by the dichloro-dihydro-fluorescein diacetate (DCFH-DA; Beyotime Institute of Biotechnology) assay according to the manufacturer's introductions. The fluorogenic substrate DCFH-DA was oxidized to highly fluorescent 2',7'-dichlorofluorescein (DCF) by ROS and used to monitor ROS dose generation. For *in vivo* detection, rat aorta endothelial cells were incubated in DCFH-DA for 1 h. DCF was measured using excitation/emission wavelengths of 485/530 nm using a spectrophotometer (NanoDrop 8000; Thermo Fisher Scientific, Inc.). For *in vitro* measurement, the HUVECs were treated with DCFH-DA for 1 h in the dark at 37°C following the indicated treatments. Fluorescence intensity was measured by flow cytometry using an LSR II Flow Cytometer (BD Biosciences, Franklin Lakes, NJ, USA) and analyzed using FlowJo software version 9.5.2 (TreeStar, Inc., Ashland, OR, USA).

Measurement of NO production. To evaluate the UA-mediated reduction of NO production, the rat serum and HUVEC medium was collected and stored at -80°C until use. Finally, medium nitrite concentration was measured as an indicator of NO production by the Griess reaction method (22). The value of different levels of NO production was expressed relative to the control (PBS) animal group.

eNOS activity assay. eNOS activity was measured using an NOS activity assay kit (NOVA Biologics, Oceanside, CA, USA), according to the manufacturer's protocol. Briefly,

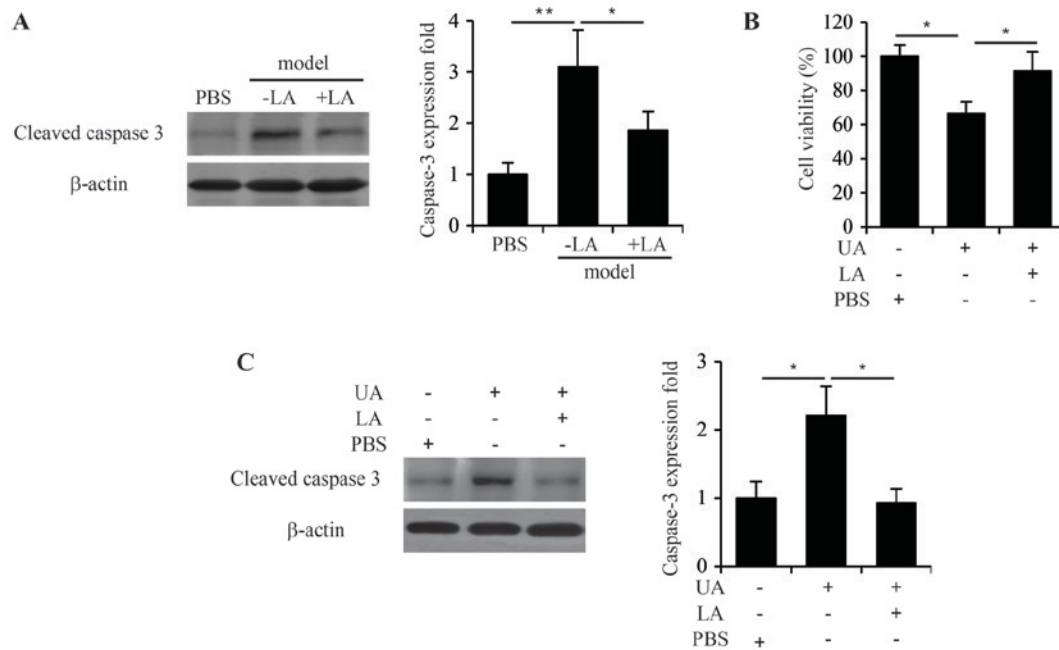


Figure 1. LA inhibits uric acid-mediated vascular endothelial cell apoptosis *in vivo* and *in vitro*. (A) Representative western blots indicate the levels of cleaved caspase-3 in isolated aorta from control and hyperuricemic model rats with or without LA treatment. * $P < 0.05$, ** $P < 0.01$ vs. hyperuricemic mice without LA treatment. (B) Cell viability was evaluated by MTT assay in HUVECs from control (PBS), UA-LA or UA+LA groups. * $P < 0.05$ vs. cells treated with UA alone. (C) Representative western blots reveal levels of cleaved caspase-3 in HUVECs from Ctrl (PBS), UA-LA or UA+LA groups. * $P < 0.05$ vs. cells treated with UA alone. PBS, phosphate-buffered saline; LA, α -lipoic acid; UA, uric acid; HUVECs, human umbilical vein endothelial cells.

rat aorta endothelial cells or HUVECs were homogenized in lysis buffer with protease inhibitor cocktail (BestBio). The homogenate was centrifuged (12,000 \times g) at 4°C for 15 min and collected. The supernatant was then added into the NOS reaction buffer containing L-arginine, NADPH and calcium. The reaction was stopped by adding a stop buffer (50 mM HEPES, pH 5.5 and 5 mM EDTA) from the kit. Finally, the reaction product with NO was quantified at 530 nm using a spectrophotometer (NanoDrop 8000). The results were normalized against the mean value of control and expressed as fold changes.

Quantification of mitochondrial DNA (mtDNA). To determine the mtDNA copy number in rat aorta endothelial cells or HUVECs, reverse transcription quantitative-polymerase chain reaction (RT-qPCR) was performed. Genomic DNA and RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, Inc.), following the manufacturer's instructions. cDNA was synthesized using SuperScript® III reverse transcriptase (Thermo Fisher Scientific, Inc.). In the reverse transcription step, 20 ng sample RNA, 40 units SuperScript III reverse transcriptase and 5 pmol reverse primers were mixed in 5 μ l reverse transcription buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂ and 10 mM DTT), the mixture was then incubated at 50°C for 1 h. The cDNA amplification was performed in 25 μ l mixture containing 12.5 μ l 2X Power SYBR-Green PCR Master mix (Applied Biosystems; Thermo Fisher Scientific, Inc.), 0.5 μ M specific forward and reverse primers, and 5 μ l template DNA with thermal cycling conditions of 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec. The amplification of mtDNA content was obtained as the ratio between the copy number of the mitochondrial

tRNA gene and that of the internal reference gene β -actin. qPCR was performed on the 7500HT Fast Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The values were determined relative to the control sample following normalization to β -actin and calculated by the $-2^{\Delta\Delta C_q}$ method (23). The primers (Taihe Biotechnology Co., Ltd., Guangzhou, China) used were as follows: Rat *mtDNA*, forward, 5'-ATCCTCCCAGGATTTGGAAT-3' and reverse 5'-GGGGAAGCGAGGTTGACCTG-3'; rat β -actin, forward, 5'-TAAAGACCTCTATGCCAACAC-3' and reverse, 5'-TAAAGCCATGCCAAATGTCTC-3'; human, *mtDNA* forward, 5'-AAAATATTAAACACAACTACCACCTACCTCCCTCACCAT-3' and reverse, 5'-ACCGGTAGGAATTGC GATAA-3'; human β -actin, forward, 5'-TCACCCACACTG TGCCCATCTACGA-3' and reverse, 5'-TCGGTGAGGATC TTCATGAGGTA-3'.

Measurement of intracellular adenosine triphosphate (ATP) content. ATP concentration was assessed using an ATP Assay kit (S0026; Beyotime Institute of Biotechnology) according to manufacturer's protocol. Briefly, rat aorta endothelial cells or HUVECs was collected and lysed respectively. Cell lysate was centrifuged at 12,000 \times g at 4°C for 15 min and the supernatant was collected for ATP analysis. ATP samples were diluted at the following concentrations to construct a standard curve of ATP: 3.90, 625, 7.8, 125, 15.625, 31.25, 62.5, 125, 250 and 500 μ M. ATP testing solution (100 μ l) was added to each well of an enzyme-coated plate for 5 min. Subsequently, supernatant (100 μ l) was added to each well. The results were revealed by measuring the luminescence generated in an ATP-dependent luciferin-luciferase and intracellular ATP levels were expressed relative to control.

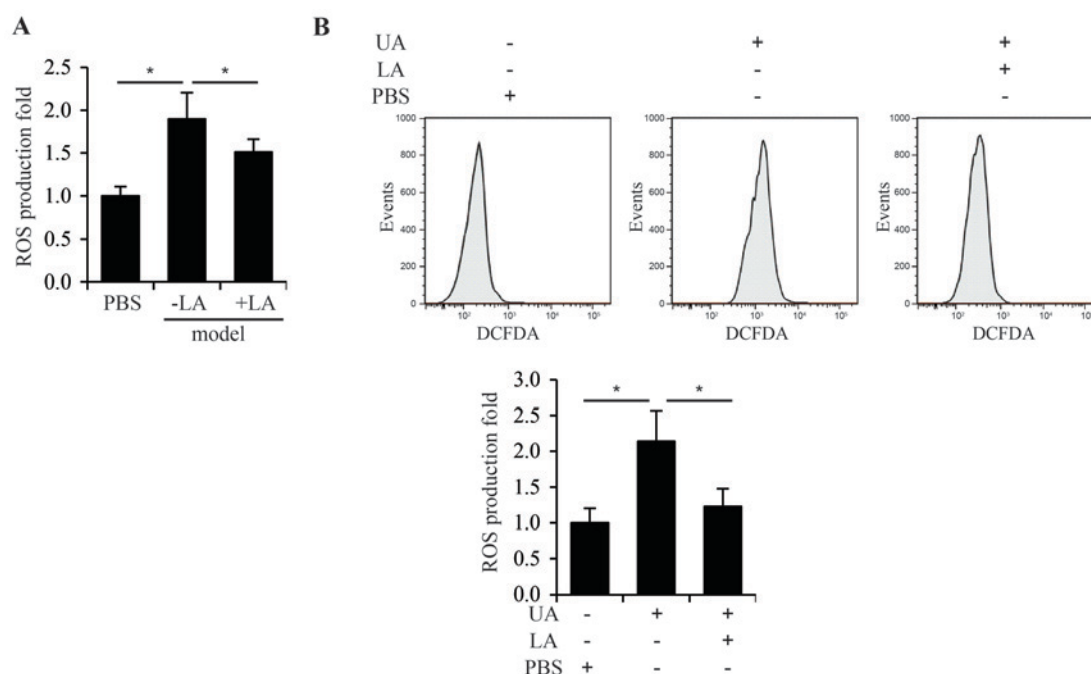


Figure 2. LA reduces vascular endothelial cell ROS levels mediated by UA *in vivo* and *in vitro*. (A) ROS production in isolated aorta from control and hyperuricemic model rats without or with LA treatment. * $P < 0.05$ vs. hyperuricemic mice without LA treatment. (B) ROS detection by flow cytometry in human umbilical vein endothelial cells from control (PBS), UA-LA or UA+LA groups. * $P < 0.05$ vs. cells treated with UA alone. ROS, reactive oxygen species; UA, uric acid; LA, α -lipoic acid; PBS, phosphate-buffered saline; DCFDA, dichloro-dihydro-fluorescein diacetate.

Statistical analysis. All data are presented as the mean \pm standard error of the mean. Data graphics and statistical analysis were performed using SPSS software, version 13.0 (SPSS, Inc., Chicago, IL, USA). Comparisons between groups were conducted by Student's t-test. The majority of experiments were performed at least 3 times with independent replicates. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

LA inhibits UA-induced vascular endothelial cell apoptosis *in vivo* and *in vitro*. To investigate whether LA regulates UA-induced apoptosis, hyperuricemic rats were fed with or without LA, and aorta endothelial cells were then surgically isolated to assess the expression of cleaved caspase-3. As shown in Fig. 1A, hyperuricemia markedly induced cleaved caspase-3 expression ($P < 0.01$) in rat aortic endothelial cells and this was significantly inhibited by LA treatment ($P < 0.05$), suggesting that LA protects vascular endothelial cells from hyperuricemia-induced apoptosis *in vivo*. Subsequently, *in vitro* assays were performed to test if LA increases the viability of HUVECs in presence of UA. The results revealed that 1 mM LA significantly rescued the reduction in cell viability induced by treatment 300 μ M UA ($P < 0.05$; Fig. 1B). Consistently, LA suppressed cleaved caspase-3 expression induced by UA in HUVECs ($P < 0.05$; Fig. 1C).

LA reduces vascular endothelial cell ROS levels stimulated by UA *in vivo* and *in vitro*. Subsequently, the effect of LA on ROS production in vascular endothelial cells of hyperuricemic rats was investigated. Production of ROS was high in aortic endothelial cells of hyperuricemic rats, whereas ROS levels significantly decreased following LA treatment

($P < 0.05$; Fig. 2A). Furthermore, there was a significant elevation of ROS production in UA treated HUVECs, however, a significant decrease in ROS levels was observed in the UA+LA group ($P < 0.05$; Fig. 2B). This result further confirmed that LA reduces vascular endothelial cell ROS levels increased by UA.

LA increases NO production and eNOS activity of vascular endothelial cells *in vivo* and *in vitro*. The effect of LA treatment on UA regulated NO production and eNOS activity was studied. High serum UA concentration resulted in a 38.2% reduction in NO production and 31.5% reduction of eNOS activity in hyperuricemic rats compared with the control group. The inhibition of NO production and eNOS activity by hyperuricemia was reversed by LA treatment ($P < 0.05$; Fig. 3A and B). These results were supported by the results of *in vitro* assays. LA abrogated the UA-mediated reduction of NO production and eNOS activity (Fig. 3C and D).

LA rescues vascular endothelial cell mitochondrial mass and function *in vivo* and *in vitro*. It has been reported that UA-induced endothelial dysfunction is associated with decreased intracellular ATP concentrations and mitochondrial alterations (24). Therefore, the present study evaluated whether LA treatment affects mitochondrial mass and function in the process of hyperuricemia. Mitochondria were isolated from endothelial cells in rats from the control, model+PBS and model+LA groups, and ATP concentration and mitochondrial DNA (mtDNA) levels were examined. ATP concentration and mtDNA levels were significantly down-regulated in the model+PBS group ($P < 0.05$) and, as expected, LA diminished the UA-mediated decrease in ATP concentration and mtDNA levels ($P < 0.05$; Fig. 4A and B). In addition,

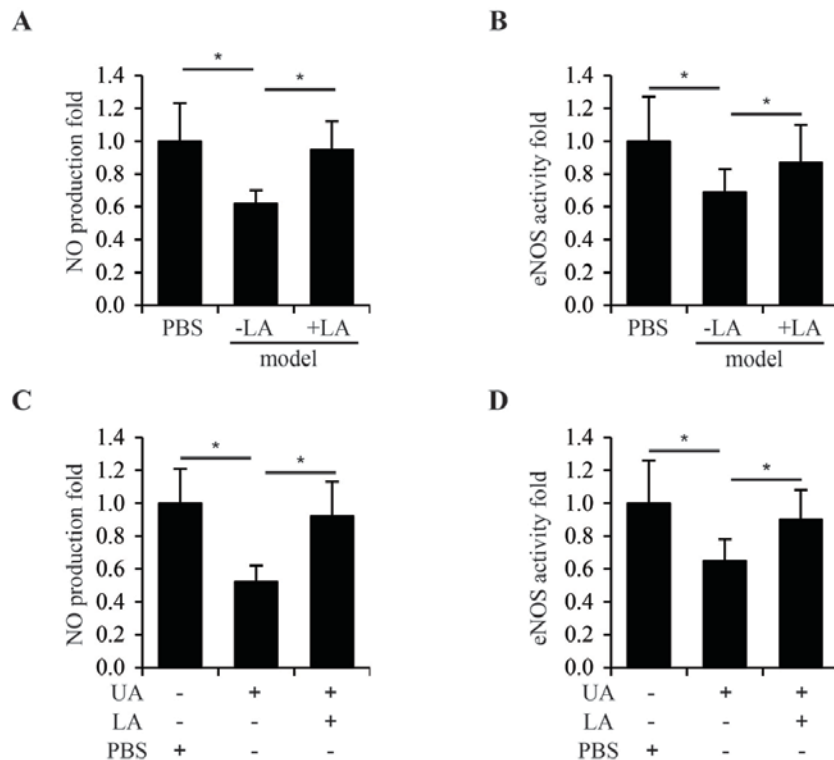


Figure 3. LA increases NO production and eNOS activity of vascular endothelial cells *in vivo* and *in vitro*. Histograms represent (A) NO production of rat serum and (B) eNOS activity of rat isolated aorta from control, hyperuricemic model rats without or with LA treatment. *P<0.05 vs. hyperuricemia without LA treatment. Histograms represent (C) NO production and (D) eNOS activity in human umbilical vein endothelial cells from control (PBS), UA-LA or UA+LA groups. *P<0.05 vs. cells treated with UA alone. NO, nitric oxide; eNOS, enzyme nitric oxide synthase; UA, uric acid; LA, α -lipoic acid; PBS, phosphate-buffered saline.

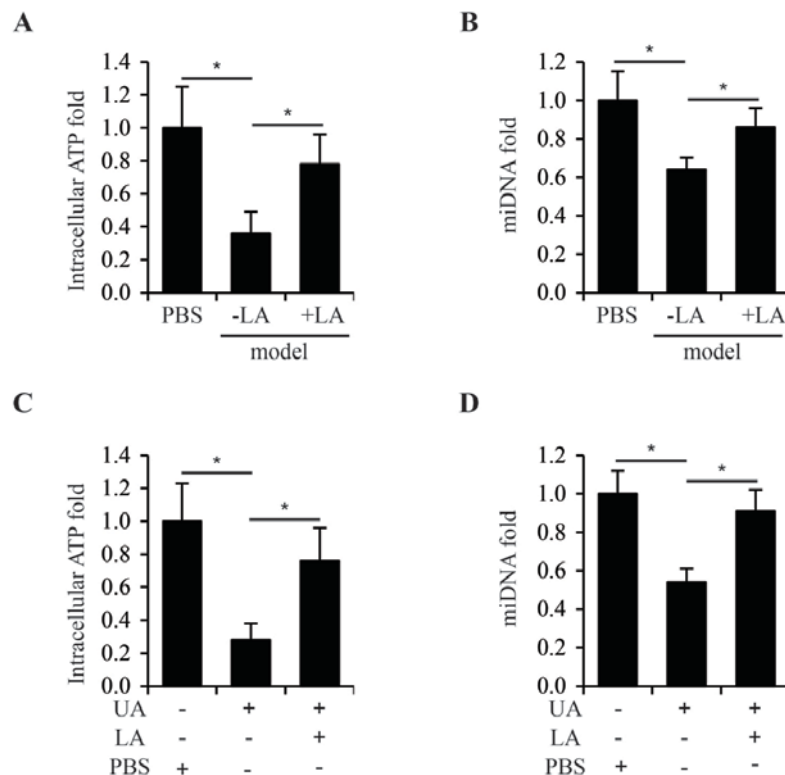


Figure 4. LA rescues mitochondrial mass and function of vascular endothelial cells *in vivo* and *in vitro*. Histograms represent intracellular (A) ATP and (B) miDNA levels in mitochondria isolated from the rat aorta tissues of control and hyperuricemic model rats without or with LA treatment. *P<0.05 vs. hyperuricemia without LA treatment. Histograms represent (C) intracellular ATP and (D) miDNA levels in mitochondria of human umbilical vein endothelial cells in control (PBS), UA-LA or UA+LA groups. *P<0.05 vs. cells treated with UA alone. LA, α -lipoic acid; PBS, phosphate-buffered saline; UA, uric acid; ATP, adenosine triphosphate; miDNA, mitochondrial DNA.

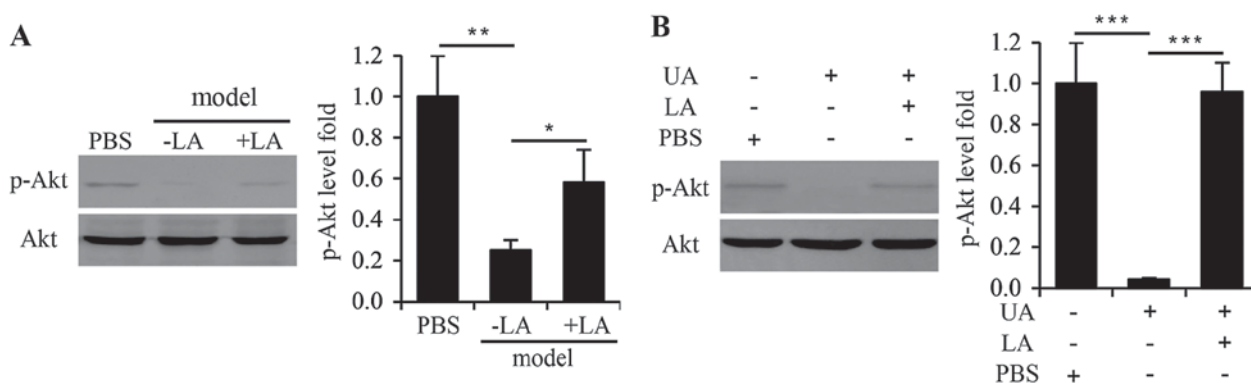


Figure 5. LA regulates vascular endothelial cell Akt signaling pathway inhibited by UA in vivo and in vitro. (A) Representative western blots indicate the levels of p-Akt in isolated aorta from control and hyperuricemic model rats without or with LA treatment. * $P < 0.05$, ** $P < 0.01$ vs. hyperuricemia without LA treatment. (B) Representative western blots show the levels of p-Akt in human umbilical vein endothelial cells from control (PBS), UA-LA or UA+LA groups. *** $P < 0.001$ vs. cells treated with UA alone. LA, α -lipoic acid; UA, uric acid; PBS, phosphate-buffered saline.

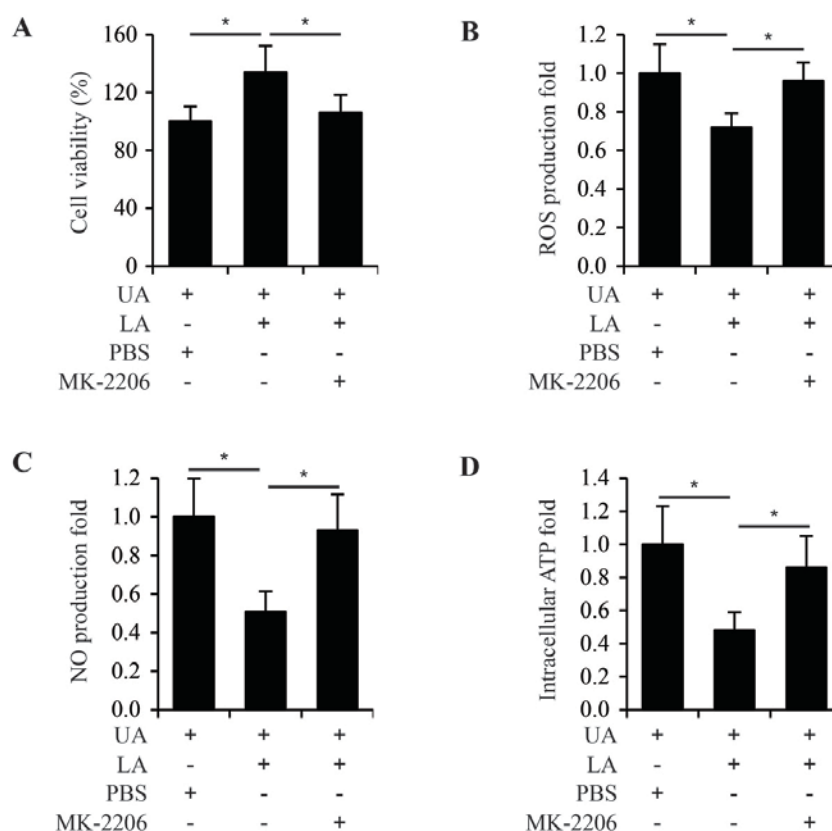


Figure 6. LA rescues uric acid-mediated vascular endothelial cell dysfunction via the Akt signaling pathway. Histograms represent (A) cell viability, (B) ROS production, (C) NO levels and (D) intracellular ATP concentrations in UA treated human umbilical vein endothelial cells from PBS, LA or LA+MK-2206. * $P < 0.05$ vs. cells treated with LA alone. LA, α -lipoic acid; ROS, reactive oxygen species; NO, nitric oxide; PBS, phosphate-buffered saline; UA, uric acid; ATP, adenosine triphosphate.

the reduction of ATP concentration and miDNA levels by UA was reversed following LA treatment in HUVEC cells ($P < 0.05$; Fig. 4C and D).

LA regulates UA-mediated dysfunction of vascular endothelial cell via Akt signaling pathway. Lastly, the mechanisms underlying the protective roles of LA on hyperuricemic vascular endothelial cells were investigated. Western blotting was performed to analyze Akt signaling

pathway activity. The expression of phosphorylated Akt was decreased in hyperuricemia model group and UA treated HUVECs, which indicated that Akt signaling pathway was inhibited by UA *in vivo* and *in vitro*. LA partially reversed the reduction of Akt phosphorylation in the model+PBS group and UA treated HUVECs (Fig. 5A and B). Furthermore, using the Akt signaling inhibitor MK-2206, Akt signaling in UA treated HUVECs was blocked. The results of cell ability assay, ROS production, NO production and ATP concentration detection

revealed that LA failed to regulate UA-mediated HUVECs dysfunction in the presence of MK-2206 (Fig. 6). Collectively, these findings suggest that LA regulates UA-mediated dysfunction of vascular endothelial cells via the Akt signaling pathway.

Discussion

It has been demonstrated that hyperuricemia is an independent risk factor for cardiovascular disease (25). High levels of UA induce endothelial dysfunction by generating ROS (13). Allopurinol is the most commonly used therapeutic strategy to treat chronic hyperuricemia by decreasing purine synthesis (26). However, allopurinol therapy may produce serious reactions, such as Stevens-Johnson syndrome (27). Thus, there has been increasing interest in developing new approaches to control serum UA levels. LA is an antioxidant that protects tissues against oxidative damage (19). In the current study, the effects of LA on apoptosis, ROS levels, NO production and mitochondrial function in endothelial cells were investigated, both *in vitro* and *in vivo*. The results of the present study indicate that LA treatment reverses endothelial dysfunction by regulating Akt signaling in hyperuricemic rats.

Firstly, it was demonstrated that LA attenuated oxidative stress in endothelial cells stimulated by UA. Oxidative stress has been long implicated in endothelial dysfunction in cardiovascular disease. A number of enzyme systems are involved in the elevated vascular production of ROS, including xanthine oxidase, NADPH oxidase, cyclooxygenase and the mitochondrial electron transport chain (28). Although UA is an antioxidant oxypurine, hyperuricemia was found to induce endothelial dysfunction by inhibiting the production of NO, which is a key endothelium-derived relaxing factor generated by eNOS (12). Upregulated ROS levels were also found to decrease NO bioavailability by converting NO to ONOO⁻ (12). In the current study, it was revealed that LA treatment suppressed ROS accumulation, enhanced NO production, and rescued eNOS activity in hyperuricemic rats and UA-treated HUVECs. Notably, eNOS may become uncoupled, such that the enzymatic production of O₂⁻ occurs instead of NO, resulting in a significant increase in oxidative stress (29). Further studies are necessary to investigate if uncoupling of eNOS is involved in hyperuricemia-induced endothelial dysfunction and to determine the effects of LA on eNOS uncoupling.

Secondly, it was demonstrated that LA inhibited UA-induced apoptosis of endothelial cells. Mitochondria serve a crucial role in apoptosis and excessive ROS production may induce oxidative damage in mitochondrial proteins and membranes (30). In the present study, mitochondrial mass and function, and expression of cleaved caspase-3 were measured in the LA-treated endothelial cells of hyperuricemic rats. The results indicated that LA suppressed UA-induced loss of mitochondria function and apoptosis. It has been demonstrated that long-term administration of LA inhibits the apoptosis of retinal capillary cells and development of retinopathy in diabetic rats (31). Furthermore, LA was found to induce apoptosis in human colon cancer cells by increasing mitochondrial respiration (32). These findings revealed a potential clinical application of LA for other diseases. However, it has been reported that LA induces insulin autoimmune syndrome (33).

Increased attention should be given to the side effects of LA in patients with hyperuricemia.

Finally, the results of the current study demonstrated that LA suppressed UA-stimulated endothelial dysfunction by activating Akt signaling. It has previously been observed that LA activates eNOS by activating Akt signaling (34). By contrast, inhibition of Akt signaling by high levels of UA may be associated with UA-induced insulin resistance, indicating that Akt signaling serves an essential role in endothelial cell function (35). Collectively, the results of the present study demonstrate that LA exerts protective effects in hyperuricemic rats by activating Akt signaling.

In conclusion, the results of the current study demonstrated that LA attenuated oxidant stress and apoptosis stimulated by UA in endothelial cells, and that activation of Akt signaling is required for the protective role of LA in UA-treated endothelial cells. The results indicate that LA may be used as a therapeutic strategy to treat hyperuricemia-induced endothelial dysfunction.

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