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Trimetazidine Protects Against Atherosclerosis by Changing Energy Charge and Oxidative Stress

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Background: This study investigated the effect and the possible mechanism of trimetazidine in atherosclerosis.

Material/Methods: We established an atherosclerotic rat model by high-fat diet and vitamin D injection. Rats were separated into 3 different groups: control, atherosclerosis, and trimetazidine (n=10). The aortic artery was isolated and its morphological features were examined by hematoxylin and eosin (HE) staining. Serum low-density lipoprotein cholesterol (LDL-c), total cholesterol (TC), and triglycerides (TG) were analyzed using an automatic biochemical analyzer. Human aortic smooth muscle cells (HASMCs) were cultured and divided into 5 groups: no treatment, H₂O₂ treatment only, trimetazidine preincubation before H₂O₂ treatment, oxidized low-density lipoprotein (oxLDL) treatment only, and trimetazidine preincubation before oxLDL treatment. HASMCs proliferation was tested using the Cell Counting Kit-8. Reactive oxygen species (ROS) and malondialdehyde (MDA) levels, superoxide dismutase (SOD) activity of the aortic artery, and HASMCs were measured using commercially available kits.





Results: HE staining assay showed that trimetazidine suppressed the progression of atherosclerosis and reduced foam cell formation in the aortic artery without affecting serum lipid levels. HASMCs proliferation assay revealed that trimetazidine alleviated the inhibitory effect of H₂O₂ on HASMCs proliferation and inhibited oxLDL-induced proliferation of HASMCs. Moreover, trimetazidine ameliorated ROS up-regulation elicited by H₂O₂ or oxLDL in HASMCs. Additionally, trimetazidine restored SOD activity and reduced MDA content of HASMCs.

Conclusions: Trimetazidine suppressed the progression of atherosclerosis by enhancing energy value, decreasing ROS level of aortic artery, modulating HASMCs proliferation, and reducing oxidative stress in HASMCs.

MeSH Keywords: **Atherosclerosis • Hydrogen Peroxide • Muscle Cells • Oxidative Stress**

Abbreviations: **oxLDL** – oxidized low-density lipoprotein; **ROS** – reactive oxygen species; **HASMCs** – human aortic smooth muscle cells; **LDL-c** – low-density lipoprotein cholesterol; **TC** – total cholesterol; **TG** – triglycerides; **MDA** – malondialdehyde; **SOD** – superoxide dismutase

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Background

Atherosclerosis, the leading cause of cardiovascular diseases, is a metabolic disease caused by lipid metabolic disorder [1]. Atherosclerosis is also a chronic inflammatory condition that is responsible for acute cardiovascular events caused by plaque rupture and thrombosis [2]. The accumulation of cholesterol in the endothelial layer of arteries by low-density lipoprotein (LDL) and chronic inflammation leads to a high ratio of pro-oxidants to antioxidants, which are 2 prominent hallmarks of pathogenesis [1,3]. Subendothelial deposition of LDL and its oxidative modification represent the initial event in atherogenesis, which is followed by monocyte-derived macrophages infiltration, foam cell aggregation, and atheromatous plaque formation [1]. Oxidative stress induced by generation of excess reactive oxygen species (ROS) has been shown to play a critical role in atherosclerosis. Key molecular events in atherogenesis, such as oxidative modification of lipoproteins and phospholipids, macrophage infiltration, and foam cell formation, are facilitated by vascular oxidative stress [3]. Currently, there is no treatment in the guidelines for primary prevention of cardiovascular (CV) events, and the risk factors of CV disease emphasized the need for new pharmaceutical targets [4,5]. Many studies have demonstrated that antioxidants possess therapeutic benefits to fight against CV disease progression [6]. However, clinical trials evaluating antioxidants supplements failed to show the beneficial effects on CV outcomes [7].

Energy metabolism disorder is the common pathological basis of many chronic diseases [8]. Recent studies suggested that the development of atherosclerosis is also accompanied by energy depletion [9,10]. The dysfunctions of mitochondria, the cellular powerhouse, can directly promote cell death, inflammation, and oxidative stress, and ultimately alter the metabolism [11]. These are key processes in atherosclerosis. Human atherosclerotic aortas have increased mitochondrial DNA (mtDNA) oxidative lesions compared with normal controls [12]. MtDNA damage is seen early in atherogenesis in mice deficient for apolipoprotein E (ApoE^{-/-}) [13]. Reducing mtDNA damage and increasing mitochondrial respiration can decrease necrotic core and increase fibrous cap areas independently of changes in reactive oxygen species [13]. Therefore, targeting energy metabolic disorder in atherosclerosis is a promising new approach.

Trimetazidine has been used as antianginal agent for decades. It can make ischemic myocardial cells shift fatty acid oxidation to glucose aerobic oxidation and enhance their anti-ischemic ability [14]. Compared with traditional anti-ischemic drugs, trimetazidine exerts direct effects on myocardial ischemia without inducing hemodynamic changes [15]. Patients with chronic angina and diabetes treated by trimetazidine show no significant change in the lipid file and glycemic status [16]. Besides its cardiac protection ability, recent evidence suggests that trimetazidine

may also play an important role in treatment of other diseases, such as contrast-induced nephropathy [17] and peripheral arterial disease [18]. Trimetazidine exerts CV protection against ischemia-reperfusion injury due to its effect on endothelial function improvement and vasodilatory effect on coronary arteries [19]. Moreover, trimetazidine has direct protective effects on the biological functions of endothelial progenitor cells against H₂O₂-induced injury [20]. Trimetazidine has also been shown to have antioxidative and anti-inflammatory effects in reducing carotid artery stenosis by decreasing proliferation of vascular smooth muscle cells (VSMCs) and apoptosis of human umbilical vessel endothelial cells (HUVECs) [21]. However, it is not clear whether trimetazidine has effects on atherosclerosis. Therefore, we hypothesized that early administration of trimetazidine may have a protective effect against atherogenesis by improving energy metabolism of the aorta and reducing oxidative stress. In this study, we observed the energy and ROS levels in aortic arteries of rats fed a high-fat diet, and we detected H₂O₂-induced or oxLDL-induced oxidative stress of human aortic smooth muscle cells (HASMCs) to show whether trimetazidine is beneficial for atherosclerosis, and we explored the preliminary mechanism.

Material and Methods

Animals

We purchased 30 male Wistar rats (120–140 g), 6 weeks old, from the Experimental Animal Center of Sichuan University (Sichuan, China). All rats were randomly assigned to the control group (n=10), atherosclerotic group (n=10), or trimetazidine group (n=10). The rats in the atherosclerotic group and trimetazidine group were fed a high-fat diet (HFD, 92.5% normal chow, 2% cholesterol, 0.5% sodium cholate, 5% lard) (Beijing HFK Bio-Technology, China) for 12 weeks and were injected with vitamin D (total dose 700 000 u/kg) intraperitoneally on the first 3 days [22]. The rats in the control group were fed a normal diet at the same time interval. In addition, the trimetazidine group rats received a dose (30 mg/kg/day) of trimetazidine (Wansheng Pharmaceuticals Co., Ltd., Beijing, China) by gavage for 12 weeks [23]. Then, all the rats were anesthetized by 100% diethylether and sacrificed. The aortic artery from the root to the abdominal aorta were isolated and washed briefly in 0°C phosphate-balanced solution. Each tissue sample was separated and put into 3 EP tubes for further testing. All animals received humane care and all experimental procedures were performed in accordance with animal protocols approved by the Sichuan University Animal Care Committee.

Blood lipid analysis

At the end of the 12th week, after rats were fasted overnight, blood samples were obtained from the tail vein. Serum

low-density lipoprotein cholesterol (LDL-c), total cholesterol (TC), and triglycerides (TG) were analyzed by an automatic biochemical analyzer (HITACHI 7600-020, Hitachi, Ltd., Tokyo, Japan).

Hematoxylin and eosin (HE) staining

Pathological section of aortic root tissue was HE stained according to the manufacturer's instructions. Morphological changes of endothelium, smooth muscle cells, and foam cells were observed by light microscopy.

Primary aortic smooth muscle cell culture

Human aortic smooth muscle cells (HASMCs, catalog no. 6110) and smooth muscle cell medium (catalog no. 1101) were purchased from ScienCell Company, USA. HASMCs were cultured in medium supplemented with 2% fetal bovine serum (GIBCO, Carlsbad, CA, USA), maintained in a 5% CO₂ atmosphere at 37°C, and passages 4–8 were used throughout the study. Trimetazidine used for HASMCs treatment was a gift from the Beijing Wansheng Pharmaceuticals Co., Ltd. Trimetazidine powder was weighed and dissolved in dimethyl sulfoxide to a stock concentration of 10 mmol/l. HASMCs were pretreated with 10 μM trimetazidine for 12 h and then exposed to oxidized low-density lipoprotein (oxLDL, 80 μg/ml, Guangzhou Yiyuan Biological Technology Co., Ltd, Guangzhou, China) for 24 h. HASMCs were also treated with 10 μM trimetazidine for 36 h and 100 μM H₂O₂ was added 2 h before termination.

HASMCs proliferation detected by CCK-8 assay

HASMCs proliferation was tested using the Cell Counting Kit-8 (CCK-8, Nanjing Jancheng Bioengineering Institute, Nanjing, China) assay. HASMCs were seeded in 96-well cell culture plates at 5±10³ cells per well and divided into 5 groups: no treatment, 100 μM H₂O₂ treatment only, 10 μM trimetazidine preincubation before H₂O₂ treatment, 80 μg/ml oxLDL treatment only, and 10 μM trimetazidine preincubation 12 h before oxLDL treatment. All cells were cultured at 37°C for 36 h. At each time, cells were washed. We added 10 μl CCK-8 solution to each well for incubation at 37°C for 1 h. Cell proliferation of each well was determined by measuring the absorbance at 490 nm. Each treatment was performed in triplicate.

ADP, ATP, and AMP measured by high-performance liquid chromatography

Aortic tissue homogenate was made and extracted on ice for 30 min, then centrifuged at 1500 g at 4°C for 10 min. The supernatant was collected and centrifuged again at 10 000 g 4°C for 10 min, sediment particles were saved, and precooled acidic liquid was added. pH value was adjusted to around 6.5. The mixture was centrifuged at 6000 g for 10 min. Afterwards, 30 ul

supernatant was taken and ADP, ATP, and AMP were measured by high-performance liquid chromatography. ATP, ADP, and AMP peaks were identified according to the corresponding retention times and confirmed by 'spiking' with added exogenous ATP, ADP, and AMP. Concentrations of ATP, ADP, and AMP were calculated from standard curves. The cellular energy charge (EC) was calculated as $[\text{ATP}+0.5\text{ADP}/(\text{ATP}+\text{ADP}+\text{AMP})]$ [24].

Measurement of reactive oxygen species (ROS) level

2',7'- dichlorodihydrofluorescein diacetate (DCHF-DA) is a dye that can be oxidized by ROS to fluorescent compound dichlorofluorescein (DCF), which is monitored by fluorescence microplate spectrophotometry. ROS level is quantified by the fluorescence intensity of DCF. ROS level of aortic tissue and HASMCs were assessed by a commercial ROS assay kit according to the manufacturer's protocol (Nanjing Jancheng Bioengineering Institute, Nanjing, China).

Superoxide dismutase (SOD) and malondialdehyde (MDA) levels detection

SOD activities and MDA contents in the media were measured by commercially available kits using colorimetric assay from Nanjing Jancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's protocols.

Statistical analysis

All data are presented as mean ± standard deviation (SD). SPSS 16.0 was used for statistical analysis. Differences between experimental groups were determined by one-way ANOVA or t test. P<0.05 was considered statistically significant.

Results

Trimetazidine inhibited the development of atherosclerotic lesion in rats fed HFD

To investigate potential therapeutic effect of trimetazidine in the development of atherosclerosis, we tested the effect of trimetazidine on atherosclerosis in HFD-fed rats. HE staining of aortic roots showed that foam cells developed in the arterial wall, with thick intima and endothelial cell deficiency in the atherosclerotic group (Figure 1A, 1B). Rats treated with trimetazidine had fewer foam cells and decreased aortic lesion area compared with the atherosclerotic group (Figure 1C). Trimetazidine had no significant effect on serum lipid profile, including TG, TC, and LDL-c (Figure 1D). Body weight was monitored every 2 weeks. As shown in Figure 1E, body weight of Wistar rats in 3 groups were all increased at the end of the experiment. Body weight of rats in the atherosclerosis group

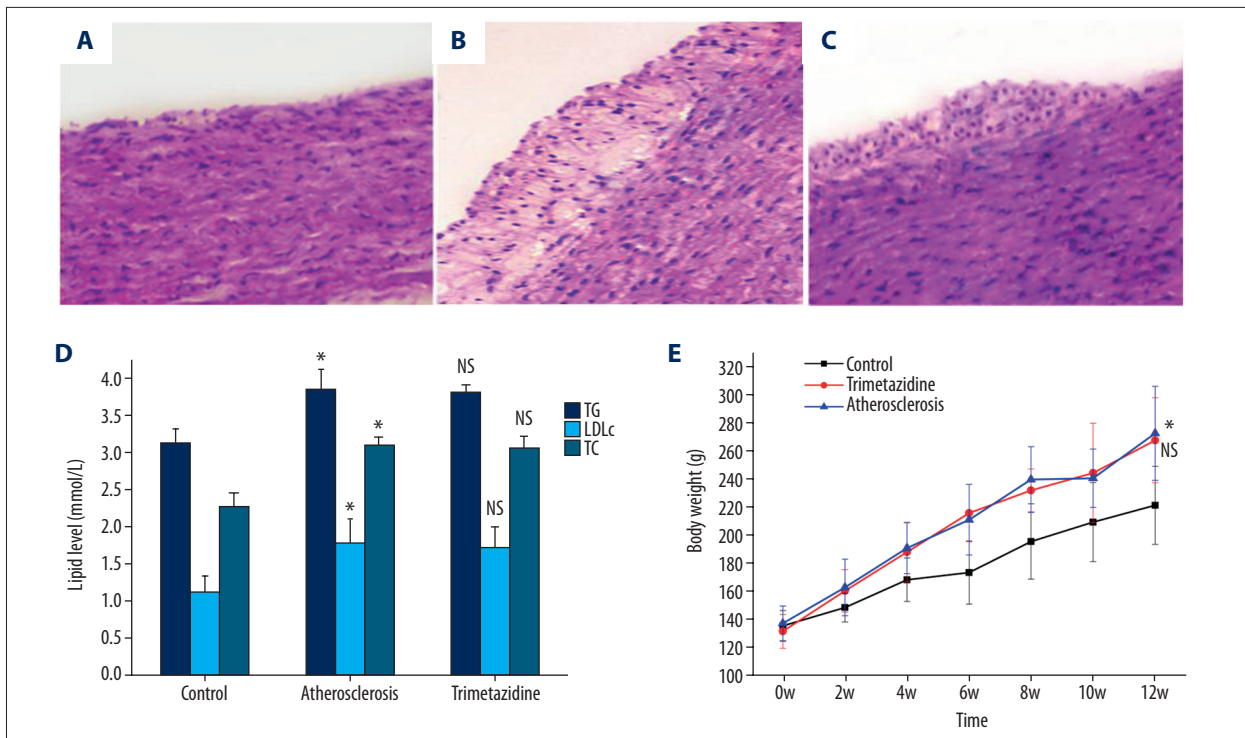


Figure 1. Effect of trimetazidine on the development of atherosclerosis in rats fed HFD. HE staining of cross-sections of rat aortic roots in each group (A: Control. B: Atherosclerosis. C: Trimetazidine. Scale bar: 200 μ m). Trimetazidine markedly inhibited the development of atherosclerosis in aortic arteries (n=10 per group). (D) Error bars show serum lipid level in each group. Trimetazidine had no significant effect on serum lipid profile. (E) Rat body weights in 3 groups were all increased at the end of the experiment. Body weights in the atherosclerosis group were significantly increased compared to the control group at each time point, while trimetazidine had no effect on the body weight changes compared with the atherosclerosis group. Data are mean \pm standard deviation. n=10, * P<0.05. NS indicates no significance vs. atherosclerosis group.

were significantly increased compared to the control group at each time point, while trimetazidine had no effect on the body weight changes compared with the atherosclerosis group. No change of feeding behavior was observed in our experiment.

Trimetazidine increased energy charge in atherosclerotic aortic arteries

EC, which indicates the content of high-energy phosphate bonds in cells, is an index used to measure the energy status of biological cells [24]. To test energy level change of aortic arteries in rats fed HFD and the positive function of trimetazidine on energy metabolism of atherosclerosis, ADP, ATP, and AMP of the aorta tissue were measured by high-performance liquid chromatography. The peak times of ATP, ADP, and AMP were 4.25, 4.88, and 7.09 min, respectively, in the chromatograms of standard samples. Energy charge decreased significantly in the atherosclerotic group compared to the control group. Trimetazidine increased energy charges of the rat's atherosclerotic aortic arteries (Figure 2A, 2B).

The effects of trimetazidine on HASMCs proliferation treated by oxLDL or H₂O₂

The effects of trimetazidine on HASMCs proliferation were determined by CCK-8 assay. Cell viability using different concentrations of trimetazidine (0, 0.1, 1, 10, 50, and 100 μ M) were firstly tested by CCK-8 and we found that 50 μ M trimetazidine affected cell survival (Figure 3A). We found that 80 μ g/ml oxLDL significantly promoted HASMCs proliferation, and trimetazidine with concentration 0.1–10 μ M inhibited oxLDL-stimulated HASMCs proliferation dose-dependently (Figure 3B). The effect of 10 μ M trimetazidine was most obvious. As shown in Figure 3C, the results showed that 100 μ M H₂O₂ reduced HASMCs proliferation, and pretreatment with 10 μ M trimetazidine alleviated the inhibitory effects of H₂O₂ on HASMCs proliferation.

Trimetazidine reduced ROS levels of atherosclerotic aortic arteries and HASMCs treated with H₂O₂ or oxLDL

ROS level was quantified by the fluorescence intensity of DCF and detected by fluorescence microplate spectrophotometer.

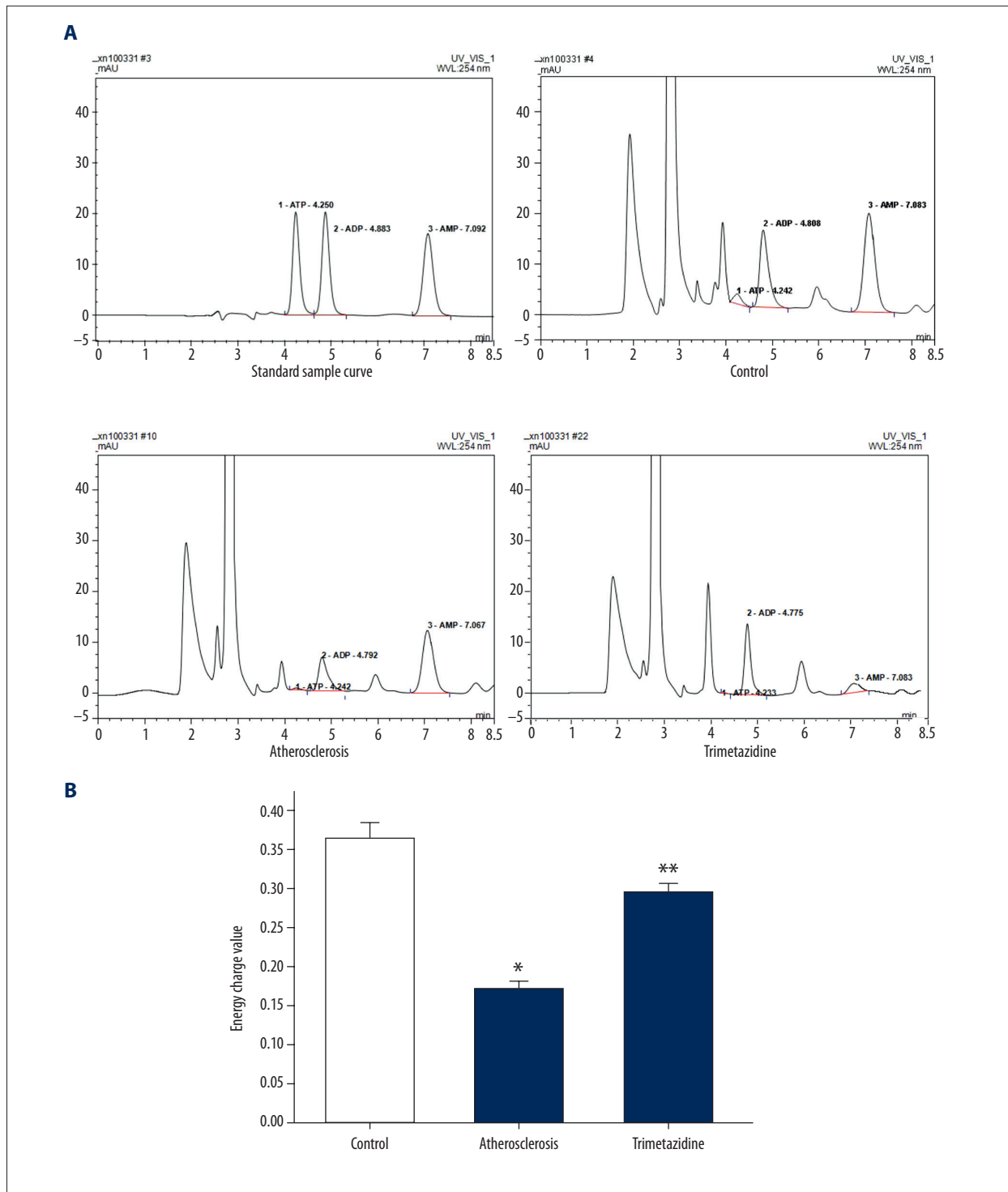


Figure 2. Changes in energy charge in atherosclerotic aortas after trimetazidine treatment. **(A)** The chromatograms of ATP, ADP, and AMP. The peak times of ATP, ADP, and AMP were 4.25, 4.88, and 7.09 min, respectively, in the chromatogram of a standard sample. **(B)** Energy charge value of rat aortic artery measured by high-performance liquid chromatography. The formula $[EC=ATP+0.5ADP/(ATP+ADP+AMP)]$ was used to measure the energy charge level. Energy charge values in the atherosclerotic group decreased remarkably compared to the control group, * $P<0.05$. Pretreatment with trimetazidine produced much more energy charge value than in the atherosclerotic group, ** $P<0.05$. $n=10$, error bars indicate that data are expressed as the mean \pm standard deviation.

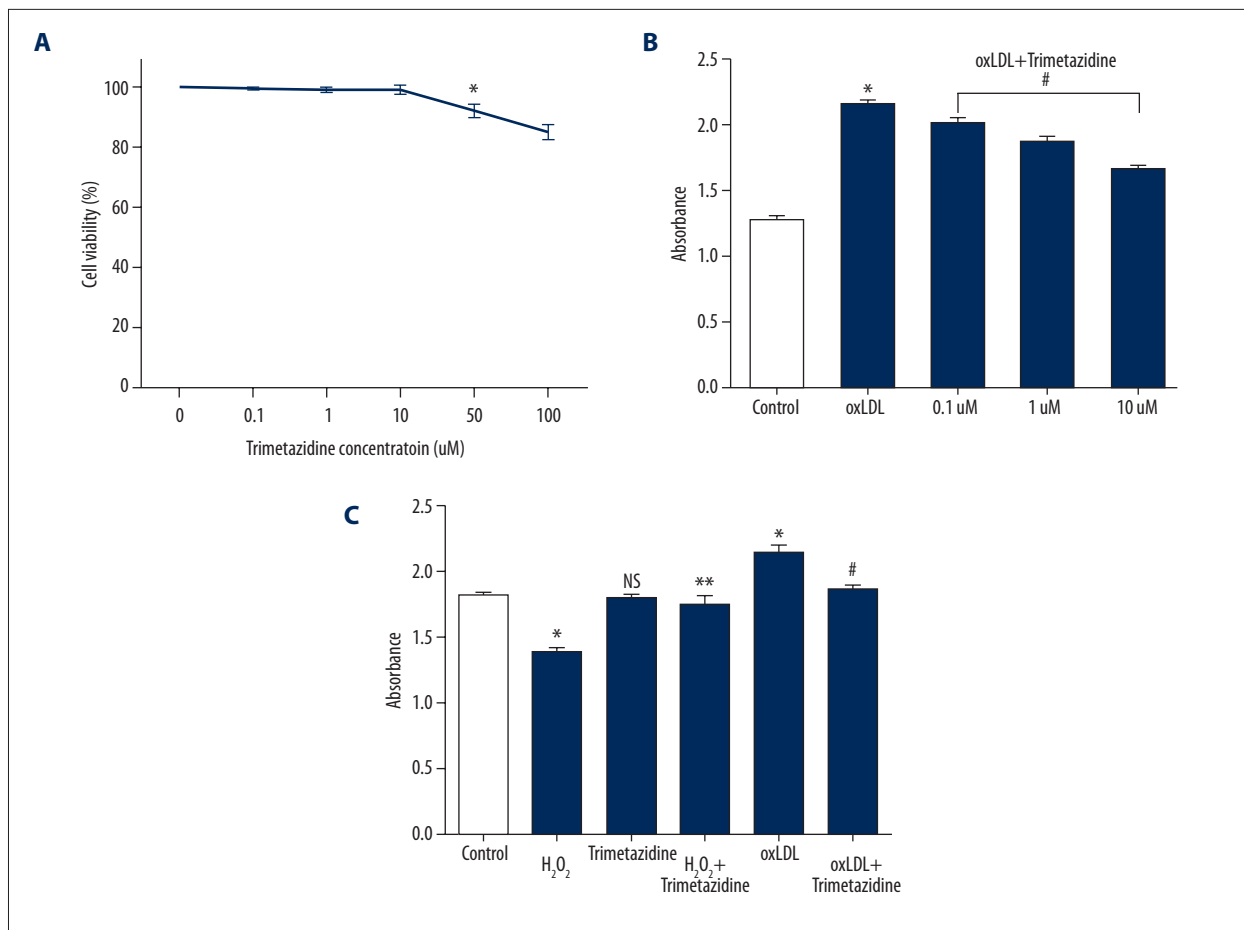


Figure 3. Effects of trimetazidine on HASMCs proliferation treated by oxLDL or H₂O₂. **(A)** Cell viability detected by CCK-8 assay. HASMCs were treated by different concentrations of trimetazidine (0, 0.1, 1, 10, 50, and 100 μM). Trimetazidine at 50 μM significantly affected cell survival. **(B)** HASMCs were treated with different concentrations of trimetazidine (0.1, 1, and 10 μM) and 80 μg/ml oxLDL. **(C)** Cells were pretreated for 12 h with or without trimetazidine, then treated with H₂O₂ (100 μM) for 2 h or oxLDL (80 μg/ml) for 24 h. H₂O₂ (100 μM) inhibited HASMCs proliferation. Trimetazidine pretreatment alleviated the inhibitory effect of H₂O₂ on HASMCs proliferation. Trimetazidine had an anti-proliferation effect on HASMCs against oxLDL. n=3. * P<0.05 vs. control. # P<0.05 vs. oxLDL. ** P<0.05 vs. H₂O₂.

Atherosclerosis accompanied by large ROS production and trimetazidine pretreatment reduced ROS levels markedly. The mean fluorescence intensity of ROS increased significantly in the atherosclerotic group compared to the control group. In the trimetazidine group, the mean fluorescence intensity of ROS decreased compared to the atherosclerotic group (Figure 4A). Both H₂O₂ and oxLDL induced ROS production in HASMCs. Cells pretreatment with trimetazidine before H₂O₂ or oxLDL stimulation produced much less ROS than cells incubated with only H₂O₂ or oxLDL, respectively (Figure 4B).

Trimetazidine modulated MDA levels and SOD activities on HASMCs against H₂O₂ and oxLDL

As shown in Figure 5A and 5B, oxLDL and H₂O₂ treatment separately significantly increased MDA levels and decreased SOD

activities of HASMCs. Trimetazidine pretreatment reduced MDA levels and increased SOD activities in HASMCs. The oxLDL- and H₂O₂-induced oxidative stress injury were notably attenuated by trimetazidine pretreatment.

Discussion

Our experiment showed an energy charge decrease in the process of atherosclerosis and shows that metabolic modulator trimetazidine can protect against atherogenesis. We showed that the protective effect of trimetazidine was associated with enhanced energy charge and reduced ROS level in rat atherosclerotic aortic arteries. Trimetazidine inhibited oxLDL-induced HASMCs proliferation and alleviated the inhibitory effect of H₂O₂ on HASMCs proliferation. Trimetazidine reduced ROS production

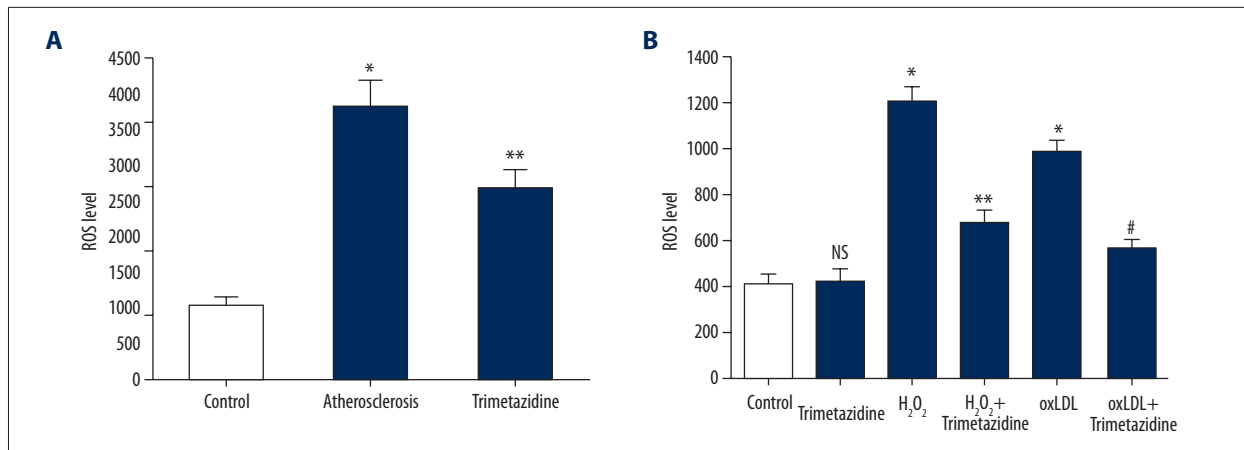


Figure 4. Effect of trimetazidine on ROS level in aorta of rats and HASMCs. **(A)** ROS level of rat aortic artery in atherosclerosis group increased remarkably compared to the control group, * $P < 0.05$. Trimetazidine treatment reduced ROS production compared to the atherosclerosis group, $n = 10$ per group, ** $P < 0.05$. **(B)** Ox-LDL and H₂O₂ induced ROS generation in HASMCs and the effect of trimetazidine. Cells were pretreated for 12 h with or without trimetazidine (10 μ M), then treated with H₂O₂ (100 μ M) for 2 h or ox-LDL (80 μ g/ml) for 24 h. Both H₂O₂ and oxLDL induced ROS production. Cells pretreated with trimetazidine before H₂O₂ or oxLDL stimulation produced much lower ROS levels than cells incubated with only H₂O₂ or oxLDL, respectively. Treatment with trimetazidine only had no effect on the ROS level. DCHF-DA is a dye that can be oxidized by ROS to the fluorescent compound DCF, as monitored by fluorescence microplate spectrophotometry. ROS level is quantified by the fluorescence intensity of DCF. Data are shown as mean \pm SD and are expressed as DCF fluorescence intensities compared with unstimulated cells. $n = 3$. * $P < 0.05$ vs. control. ** $P < 0.05$ vs. H₂O₂. # $P < 0.05$ vs. ox-LDL. NS indicates no significance vs. control.

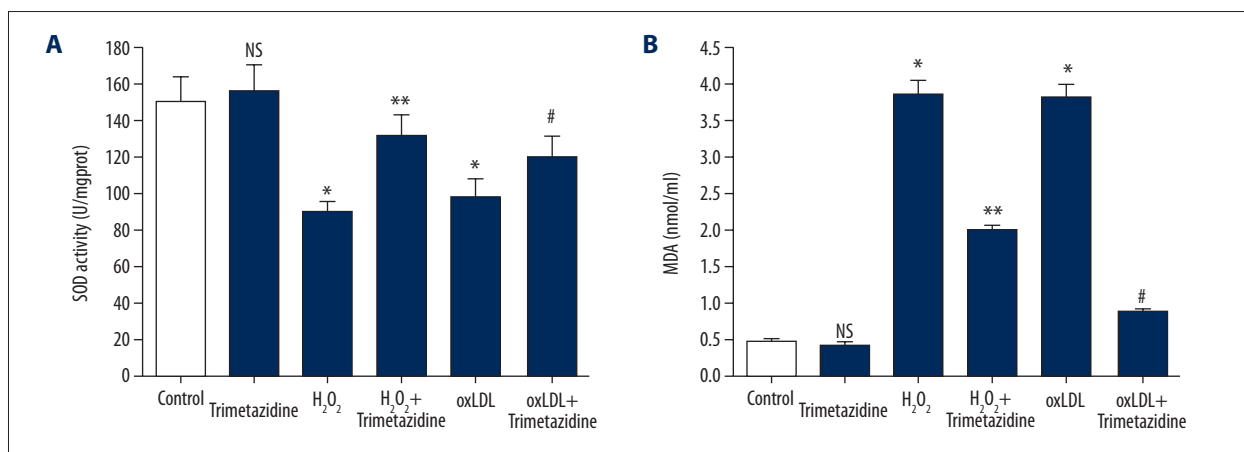


Figure 5. Effect of trimetazidine on superoxide dismutase (SOD) activity and malondialdehyde (MDA) level. **(A)** Trimetazidine restored SOD activity of HASMCs, which inhibited by H₂O₂ and oxLDL. Both H₂O₂ and oxLDL decreased SOD activity. Cells pretreated with trimetazidine before H₂O₂ or oxLDL stimulation had increased SOD activity than cells incubated with only H₂O₂ or oxLDL, respectively. Trimetazidine alone had no effect on SOD activity. $n = 3$. * $P < 0.05$ vs. control. ** $P < 0.05$ vs. H₂O₂. # $P < 0.05$ vs. ox-LDL. NS indicates no significance vs. control. **(B)** Trimetazidine reduced MDA content of HASMCs stimulated by H₂O₂ and oxLDL. Both H₂O₂ and oxLDL increased MDA content. Trimetazidine pretreatment reduced MDA level stimulated by H₂O₂ or oxLDL. Trimetazidine alone had no effect on the MDA level. $n = 3$. * $P < 0.05$ vs. control. ** $P < 0.05$ vs. H₂O₂. # $P < 0.05$ vs. ox-LDL. NS indicates no significant difference vs. control.

of HASMCs stimulated by oxLDL or H₂O₂, and trimetazidine-mediated antioxidative effects of HASMCs were associated with increased SOD activity and decreased MDA content.

Trimetazidine, with the chemical name 1-(2,3,4 preparation benzyl) piperazine hydrochloride, selectively inhibits long-chain 3-ketone acyl coenzyme A sulfur solution enzyme (3-KAT), makes cellular metabolism preferable to glucose aerobic oxidation, prevents intracellular acidosis and calcium overload, helps maintain

myocardial cell energy metabolism in ischemic state, increases cardiac systolic function, and protects myocardial cells [25]. Metabolic reconstruction, first proposed by Van BilSen [26], means that materials such as sugar and fat metabolic disorder in cells can cause energy metabolic pathways changes, which result in abnormal structure and function of organs. Cell energy metabolism disorder decreases membrane potential, cell membrane permeability of ions increases, metabolic enzymes activity decreases, lysosome are damaged, and cytoskeletons become unstable [27]. Our experiment showed that as atherosclerosis developed after 12 weeks of high-fat diet, energy charge values decreased, indicating an energy disorder exists in the atherosclerotic aortic artery. The energy metabolism of atherosclerosis has not been explicitly defined. It is reported that the unstable plaque with macrophages show signs of severe metabolic stress, including ATP depletion, low glucose concentrations, and lactate accumulation [28]. ATP depletion can directly lead to macrophage death and plaque instability. Moreover, macrophages in the atherosclerotic plaque have a high demand for cellular energy because of increased rates of cholesterol efflux under the condition of inflammation and require the efficient use of either glycolysis and/or fatty acid metabolism to maintain necessary levels of ATP to meet these demands [29]. The efficient removal of cholesterol in macrophage is ATP-dependent [30]. In terms of the efficiency of aerobic capacity, the oxidation of glucose metabolism is superior to FFA oxidative metabolism [31]. Based on the above studies, we hypothesized that trimetazidine had beneficial effects on atherosclerosis. Our experiment showed that the trimetazidine reduced foam cell formation, alleviated the progress of rat aortic artery atherosclerosis, and increased the energy value without affecting the lipid level. This means that trimetazidine did not influence the absorption of lipids, possibly because trimetazidine improves energy metabolism by directly acting on mitochondria. A previous study showed that trimetazidine protects cardiomyocytes from palmitate-induced mitochondrial fission and dysfunction [32].

Oxidative stress has been shown to be involved in the occurrence and development of atherosclerosis, mainly by oxidation, inducing endothelial dysfunction, promoting local inflammation and smooth muscle cell proliferation, and by causing mitochondrial damage [33]. It refers to the imbalance between ROS and antioxidant capacity in tissues. Exogenous antioxidant vitamin E is reported to inhibit progression of atherosclerosis by decreasing lipid peroxidation, monocyte proatherogenicity, and platelet aggregation [34]. Some natural antioxidants like flavonoids also help reduce oxidative stress in lowering the risk of atherosclerosis and CVD [35,36]. Beyond the metabolic effects, trimetazidine has been shown to inhibit cardiac fibrosis, lower nicotinamide adenine dinucleotide phosphate-oxidase levels, and reduce reactive oxygen species production [37], as well as protecting against IR injury by increasing endogenous antioxidants and regulating oxidative stress in the whole

body [38,39]. Our experiment showed that trimetazidine effectively reduced the ROS level of aortic arteries in rat fed a high-fat diet. To further explore the exact effects of trimetazidine on oxidative stress of HASMCs, the main component of the aortic wall, which plays a key role in atherogenesis and restenosis, we used oxLDL and H₂O₂ as stimulators. H₂O₂ is a strong pro-oxidative factor, and the role of H₂O₂ in modulating VSMCs proliferation and viability is controversial. Previous studies have reported that exogenous ROS was associated with VSMC proliferation [40,41], and they were also shown to affect viability of VSMC and cause cell death dose-dependently [42]. The positive and negative effects of H₂O₂ on modulating VSMC growth may be concentration-dependent [43]. Our results showed that 100 µM H₂O₂ inhibited HASMCs proliferation, and pretreatment with trimetazidine alleviated the inhibitory function of H₂O₂ on HASMCs. OxLDL is the major pathogenic factor of foam cell formation and can alter the fragile balance between survival and death of foam cells and VSMCs, thereby leading to plaque instability and, finally, to atherothrombotic events. OxLDL has been shown to affect VSMC growth by inducing proliferation or apoptosis, and it has proinflammatory effects that promote expression of proinflammatory molecules in VSMCs [44,45]. In addition, oxLDL decreases SOD mRNA and protein levels by binding to lectin-like oxidized LDL receptor-1 (LOX-1) [46]. Our study showed that 80 µg/ml oxLDL stimulated HASMCs proliferation and pretreatment with trimetazidine reduced HASMCs proliferation against oxLDL. We also detected the direct production of oxidative stress and ROS production of HASMCs, and we tested ROS and MDA content and SOD activity as parameters of oxidative stress, since MDA content is an indicator of lipid peroxidation and oxidative damage, while the main antioxidant enzyme, SOD, which breaks down superoxide into oxygen and hydrogen peroxide, is widely distributed throughout all aerobic cells and in extracellular fluids [47]. Our study showed that both H₂O₂ and oxLDL induced ROS production, decreased SOD activity, and elevated MDA content of HASMCs. Treatment with trimetazidine markedly reduced ROS levels and MDA content against oxLDL and H₂O₂. The antioxidative enzyme SOD activity was restored, suggesting enhanced HASMCs ability to remove oxygen free radicals to avoid cell biological function damages. Trimetazidine provided HASMCs with a more efficient antioxidative defense system compared with controls.

Our study has certain limitations. Firstly, we only observed the morphologic changes of rat aortic arteries. However, there was no quantified evaluation of atherosclerosis by quantification. Secondly, we didn't assess how trimetazidine affects ROS and MDA levels or SOD activity. Thirdly, we did not consider sex differences and only used male rats. Thus, our results may not be applicable to atherosclerosis in female rats. Fourthly, the combined effect of vitamin D plus trimetazidine against trimetazidine only was not evaluated. Further studies are warranted.

Conclusions

Trimetazidine plays a protective role in atherogenesis by increasing energy charge and reducing ROS production. Trimetazidine decreases oxLDL- and H₂O₂-induced oxidative stress in HASMCs with involvement of antioxidation. Our findings may provide new evidence for expanding application of

trimetazidine in protecting against atherogenesis. A follow-up quantitative study will be carried out to demonstrate the effect and underlying mechanism of trimetazidine on atherosclerosis.

Conflict of interest

None.

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