Laboratory Research

Protective effects of trimetazidine against vascular endothelial cell injury induced by oxidation

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Objective To explore the protective effects of trimetazidine on vascular endothelial cells injury induced by hydrogen peroxide (H$_2$O$_2$) and its pharmacological mechanisms of anti-oxidation. Methods Human umbilical vein endothelial cells (HUVECs) were injured by H$_2$O$_2$. Next, the cells were treated with three different concentrations of trimetazidine (1μmol/L, 10μmol/L, 100μmol/L, respectively). The viability of cells was detected by methyl thiazolyl tetrazolium (MTT) assay. In addition, malondialdehyde (MDA) contents, superoxide dismutase (SOD) and secretion of NO were measured. Results Trimetazidine could enhance the viability of the injured HUVECs induced by oxidation, decrease the level of MDA, enhance the SOD activity, and increase the secretion of nitrogen oxide. These effects were in a certain dose-dependent manner and the difference was significant among the three concentrations (P<0.05). Conclusions Our results suggest that trimetazidine may protect lipid peroxidation and prevent oxidation-induced cellular dysfunction of HUVECs (J Geriatr Cardiol 2008; 5:248-251)

Key words Trimetazidine; endothelial cells; oxidative damage

Atherosclerosis (AS) is a direct factor that contributes to the incidence of cardiovascular and cerebrovascular disease’s and one of the leading causes that threaten the health of mankind. A number of studies have shown that the oxidative damage of vascular endothelial cell is directly related to the development of AS. One of the earliest events in the atherosclerotic process is oxidation of low density lipoprotein (LDL). Oxidized LDL (oxLDL) is shown to play an important role in the oxidative injury of vascular endothelial cells. Trimetazidine is a long chain 3-ketoyl coenzyme A thiolase (3-KAT) inhibitor, as well as a new metabolic drug, which can protect against myocardial ischemia and improve activities of endothelial cells. In this study, we established an oxidative injury model that human umbilical vein endothelial cells (HUVEC) were oxidized by hydrogen peroxide (H$_2$O$_2$) in vitro. Then the protective effects of trimetazidine on the injured HUVEC were observed, and their possible mechanisms of anti-oxidation mediated by this drug were analyzed.

Materials and methods

Materials Human umbilical vein endothelial cells (HUVEC) were purchased from Cell Culture Conservation Center of Wuhan University. Medium 199 was from Invitrogen (USA). Calf serum was from Sijiqing Biomaterial Engineering Research Institute of Hangzhou. Malondialdehyde (MDA), superoxide dismutase (SOD) and nitric oxide (NO) determination kits were all from Jiancheng Bioengineering Research Institute of Nanjing. Trimetazidine was a kind gift from Servier Pharmaceutical Company.

Cell culture The endothelial cells were cultured by medium 199 supplemented with 10% fetal bovine serum, then placed into the incubator with 37°C, 5% CO$_2$, 95% atmosphere and saturation humidity. After a confluent monolayer formed, cells passaged, which were supported by 0.25% trypsin and 0.2% ethylene diamine tetraacetic acid (EDTA), then cells were seeded in the 24-well or 96-well plates.

Preparation of trimetazidine and hydrogen peroxide Trimetazidine was dissolved in fluid of dimethyl sulfoxide (DMSO) and diluted to different concentrations by cell culture medium according to the requirement of experiments (the final concentration of DMSO did not exceed 0.5%). H$_2$O$_2$ (30%) was diluted into different concentrations by cell culture medium if necessary.

Treatments of endothelial cells The cultured endothelial cells were divided into five groups when the cells were in log phase and coalesced into monolayer. Group A was the normal control group, cells of group B were only injured by hydrogen peroxide (the final concentration of H$_2$O$_2$ was 2mmol/L). In groups C, D, and E, endothelial cells were pre-incubated with trimetazidine un-
under their respective concentrations (1μmol/L, 10μmol/L and 100μmol/L). Twenty-four hours later, cells were challenged by H_2O_2 for 4 h. Finally, the changes in cell appearance were observed to calculate the index of morphology.

**Cell proliferation assay**

After endothelial cells being injured by H_2O_2 for 4 h, the cell cultures were added with 20μl MTT (5g/L) per well for another 4 h in 37℃. Next, 150l DMSO were added into cell wells and oscillated for 10 minutes, and the absorbance at 490nm was read in each well.

**Malondialdehyde assay**

Cells were seeded in 24-well plates, grouped and treated as described previously. The supernatant fluid was collected from every group and the contents of MDA were detected according to the kit.

**Superoxide dismutase assay**

Cells were seeded in 24-well plates, grouped and treated as described previously. Supernatant fluid of 100μl was collected from every group, then the activity of SOD was evaluated according to the kit.

**Nitric oxide assay**

Cells were seeded in 24-well plates, grouped and treated as described previously. Supernatant fluid of 100μl was collected from every group, then the quantity of NO was detected by the method of nitrate reductase according to the kit.

**Statistical analysis**

Data were shown as means±SD. Differences between groups were analyzed by grouped t-test using SPSS10.0. A probability (P) value<0.05 was considered statistically significant.

**Results**

**Morphological changes with treatment of trimetazidine**

The cells in normal control group were in a good condition as showed under the inverted phase contrast microscope (Fig.1a). Cell was shown as a polygon or short spindle shape and the boundary was distinct. All cells appeared uniform and arranged as monolayer cobblestones. Cells adhered strongly, and conjunctions between cells were tight. In the injured control group, cells shrank into a small round; the inter-space between cells widened; cell boundary was obscure; cell membrane was not integrated and even damaged to break (Fig.1b). There were some large lost zones because many cells fell off. Cell appearance of trimetazidine intervened groups was different from that of normal control group but better than the injured control group. Intercellular space was diminished and conjunctions between cells were increased, and the cell boundary was comparatively clear.

**Proliferation effects of endothelial cells**

The proliferation of endothelial cells treated by trimetazidine was detected by the MTT assay (Fig.2). With statistical analysis, the optical density (OD) of H_2O_2 injured control group was obviously lower than that of other groups. There were some statistical differences between normal control group and the trimetazidine treatment groups. In the trimetazidine interfered groups, the optical density was increased corresponding with the increase in concentrations of trimetazidine, and the differences between groups had statistical significance (P<0.01 or P<0.05).
MDA contents

MDA contents from endothelial cells treated by trimetazidine were also analyzed (Fig 3). Through statistical analysis, the MDA contents of cells in \( \text{H}_2\text{O}_2 \) injured control group were obviously lower than those in other groups, and the differences had statistical significance \((P<0.01)\) or \((P<0.05)\).

![Figure 3](image1)

**Figure 3** Effects of trimetazidine on endothelial cells about MDA contents

SOD activity of endothelial cells

The SOD activity of endothelial cells with different trimetazidine treatments was detected (Fig.4). Through statistical analysis, the SOD activity of cells in \( \text{H}_2\text{O}_2 \) injured control group was obviously lower than that in normal control group and the differences were significant \((P<0.01)\). The SOD activity of cells in all trimetazidine groups were higher than in the \( \text{H}_2\text{O}_2 \) injured control group \((P<0.01)\).

![Figure 4](image2)

**Figure 4** Effects of trimetazidine on endothelial cells about SOD contents

Secretion of NO

The NO secretion of endothelial cells treated by trimetazidine was also detected (Fig.5). Through statistical analysis, the NO concentration in \( \text{H}_2\text{O}_2 \) injured control group was obviously lower than that in normal control group and the differences were significant \((P<0.01)\). The NO level in all trimetazidine groups were higher than that in \( \text{H}_2\text{O}_2 \) injured control group \((P<0.01\) or \(P<0.05)\).

![Figure 5](image3)

**Figure 5** Effects of trimetazidine on endothelial cells about NO contents

Discussion

Trimetazidine is a new metabolic drug as well as a 3-KAT inhibitor. It can directly irritate myocardium and indirectly promote glucose metabolism. It can also reduce the requirement of oxygen in the process of forming hepatic high energy phosphate, cut down the consumption of oxygen while generating adenosine triphosphate and increase the efficacy about utilize oxygen in myocardial anoxia. Trimetazidine can remove oxygen free radical and limit the cytolysis and endomembrance damage which were caused by free radicals. Besides, trimetazidine can activate nitric oxide synthase and mediate the production of vasodilatation factor. Experiment and clinic study indicated that trimetazidine can not only protect cardiocyte and improve heart function, but also improve the function of endothelial cells.

The injury of vascular endothelial cells is a common pathological change in the development of many cardiovascular diseases especially in the progression of atherosclerosis and it is also a start link of atherosclerosis proved by document. In ordinary condition, the rate of growth and apoptosis is low and the balance between growth and apoptosis can stabilize the number of endothelial cells and maintain the vascular function in normal state. Apoptosis is an active death process that initiated by a cell itself. Oxidative stress is one of the factors which result in apoptosis.

\( \text{H}_2\text{O}_2 \) can modify LDL into ox-LDL that will promote the development of atherosclerosis. In this study, we investigated that oxidative damage could alter the morphology and function of endothelial cells by construction of an injured cell model caused by hydrogen peroxide.

NO released from endothelial cells mediates many functions such as inhibiting platelet and mononuclear adhesion to endothelial cells, regulating angiogenesis and the growth of vascular endothelial cells and maintaining endothelial layer integrity.\(^5\) The decreased level of NO is usually regarded as a marker of endothelial dysfunction. The concentration of secreted NO was different in each group in this study, with the lowest in the injured group \((62.72\pm3.62)\), and the highest in the 100\(\mu\)mol/L trimetazidine treated group \((87.60\pm4.99)\). The difference was statistically significant. Our results indicated that trimetazidine could enhance endothelial cells to secrete NO, and promote the function recovery of endothelial cell injured with oxidation.

\( \text{H}_2\text{O}_2 \) can lead to cell damage via protein polymerization by MDA produced from lipid peroxidation. In this study,
the concentration of MDA was 0.13±0.00 in the normal group, 3.22±1.3 in the injured control group, with the lowest (0.65±0.10) in the 100μmol/L trimetazidine treated group. Our research confirmed that H$_2$O$_2$ could increase MDA through lipid peroxidation. Because trimetazidine can obviously degrade MDA derived from impaired cells, it can protect cells by anti-peroxidation.

In normal state, human body has a consummation antioxidation system such as SOD and glutathione peroxidase (GSH-PX), which have considerable defensive effect inside cytoplasm and chondosome. H$_2$O$_2$ can decrease the activity of antioxidase and eliminate oxygen free radicals which will aggravate the cell damage. This study demonstrated that trimetazidine could promote the activity of SOD inside the oxidative injury endothelial cells, thus protect cells against free radicals. MTT is an assay that detects cell proliferative activity, which may indirectly reflect the viable count of cells. In the study, the optical density of trimetazidine groups was higher than that in injured control group, which indicated that trimetazidine may maintain or even increase cell activity. Thereby, it could protect cells through alleviating the oxidative damage in endothelial cells caused by H$_2$O$_2$.

In conclusion, our study demonstrated that trimetazidine had protective effects on injured endothelial cells caused by oxidation in vitro. The molecular mechanism was probably related to elevating activity of anti-oxidase and protecting chondosome inside the cells.

References

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