ORIGINAL RESEARCH

Effects of Cadmium Exposure on Oxidative Stress in Atherosclerotic Rats by Downregulating TopBP1 Expression to Induce Mitochondrial DNA Damage

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ABSTRACT

Objective • To explore the mechanism of the effect of cadmium exposure on TopBP1-induced mitochondrial DNA damage in atherosclerotic rats to affect oxidative stress.

Methods • 50 rats were established atherosclerotic model, and they were divided into model control group (MC group), low-dose cadmium exposure group (LD group), medium-dose cadmium exposure group (MD group), high-dose cadmium exposure group (HD group), and positive control group, with 10 rats in each group. Rats in the LD group, MD group, and HD group were intraperitoneally injected with different doses of cadmium acetate solution for intervention, rats in the PC group were intraperitoneally injected with oxidized banking solution, and those in the MC group were injected with normal saline. 10 rats were taken as the normal control group (NC group). Human umbilical vein endothelial cells were taken for cell experiments, normal saline was added as the blank control group (group A), cadmium acetate solution was added (group B), oxidized bankning solution was added (Group C), and oxidized bankning solution and cadmium acetate solution were added (Group D). Western blot and fluorescence quantitative PCR were used to detect the protein and mRNA expressions respectively. ROS, MDA, and SOD were detected by ELISA, apoptosis of endothelial cells was detected by flow cytometry, and arterial plaque damage was observed by oil red O staining.

Results • The relative expressions of TopBP, Bax, and Bcl-2 proteins in rat aortic tissues in each group were significantly different (all $P < .05$). The relative expressions of TopBP1 and Bcl-2 proteins in the aortic tissues of rats in NC group, MC group, LD group, MD group, HD group, and PC group decreased (all $P < .05$), while the relative expressions of Bax protein in those groups were increased (all $P < .05$). Similarly, the relative expression levels of Topbp1mRNA, BaxmRNA, and Bcl-2mRNA in the aortic tissues of rats in each group were significantly different (all $P < .05$). There were statistically significant differences in the expression levels of ROS, MDA, SOD, and mtDNA expression levels in the aortic tissues of rats in each group. There were statistically significant differences in TopBP1, Topbp1mRNA, and mtDNA among groups (all $P < .05$); while the relative expression of TopBP1 and Topbp1mRNA in groups A, B, C, and D decreased (all $P < .05$), the expression levels of mtDNA in those group increased (all $P < .05$), and the apoptosis rates of endothelial cells were also increased (all $P < .05$).

Conclusion • Cadmium exposure can down-regulate the expression of TopBP1 in atherosclerotic rats, aggravate mitochondrial DNA damage, promote oxidative stress response, and then induce the development of atherosclerosis. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Cadmium has a long half-life after entering the body,[1] and cadmium exposure can lead to a variety of adverse reactions, which can cause kidney and liver dysfunction, pulmonary edema, testicular injury, and hematopoietic system damage.[2-5] Atherosclerosis is a chronic inflammatory disease characterized by apoptosis and local inflammation.[6] At present, the mechanism of the effect of cadmium exposure on the development of atherosclerosis has not been fully understood, but studies have confirmed that cadmium exposure can cause mitochondrial dysfunction,[7] which in turn promotes atherosclerosis.[8,9] In this process, the

downregulation of human DNA topoisomerase IIβ binding protein 1 has a critical effect on mitochondrial DNA damage.[10] To further understand the mechanism of cadmium-linked mitochondrial DNA damage induced by human DNA topoisomerase II β binding protein 1 on the functioning of endothelial cells participating in atherosclerosis, this study was conducted. In this paper, the effects of cadmium exposure on the development of atherosclerosis were preliminarily discussed through the establishment of an atherosclerosis model and cell experiment verification, to provide a theoretical basis for future research.

MATERIALS AND METHODS

Experimental materials

Experimental animals: There were sixty 4-month-old Specific Pathogen Free (SPF grade) SD rats, weighing 165-175 g. Feeding conditions: (1) separate cage feeding; (2) room temperature 25°C; and (3) humidity 50%. This study met ethical requirements and was approved by the animal ethics committee.

Experimental cells: Human umbilical vein endothelial cells (HUVECs, ZQ00446) were purchased from Shanghai Zhongqiao Biotechnology Company.

Experimental reagents and instruments: Oil red O staining kit was purchased from Nanjing Jiancheng Bioengineering Institute. Human DNA topoisomerase IIβ Binding protein 1, B-cell lymphoma factor 2 (Bcl-2), and Bax antibody were purchased from Proteintech (China); ELISA kits for reactive oxygen species (ROS), nitric oxide (NO), malondialdehyde (MDA), and superoxide dismutase (SOD) were purchased from Nanjing Camillo Bioengineering Co., Ltd; Fluorescence microscope was purchased from Guangzhou Dezhen Technology Co., Ltd. and chemiluminescence imaging system was purchased from Guangzhou Yuwei biotechnology Instrument Co., Ltd.

Establishment and administration of the atherosclerosis model

Fifty rats were selected to establish the atherosclerosis model and divided into model control group (MC group), low-dose cadmium exposure group (LD group), medium-dose cadmium exposure group (MD group), high-dose cadmium exposure group (HD group), and positive control group (PC group), with 10 rats in each group. At 8 weeks after modeling, rats in the LD group, MD group, and HD group were intraperitoneally injected with different doses of cadmium acetate solution, rats in the PC group were intraperitoneally injected with oxidized bankning solution, and rats in the MC group were intraperitoneally injected with an equal volume of normal saline. Another 10 rats in the same period as the modeling were taken as the normal control (NC) group. When the modeling was started for the rats in the other groups, the rats in the NC group were injected with an equal volume of normal saline through the tail vein on the first day and were fed with ordinary feed. The dosage, frequency, and feeding days of the NC rats were the same as those of the modeling rats. After 8 weeks of modeling, the rats in the NC group were injected with an equal volume of normal saline intraperitoneally.

Detection of TopBP1mRNA, BaxmRNA, Bcl-2mRNA, and mtDNA in rat aortic tissues

The expressions of TopBP1mRNA, BaxmRNA, Bcl-2mRNA, and mtDNA in rat aortic tissues were detected by real-time quantitative PCR method (qRT-PCR). The specific operation was as follows: Rat aortic tissues were taken in a grinder, and then liquid nitrogen was added and ground evenly. After the lysate was used to organize the tissues, TRIzol solution was used to extract total RNA from the tissues, and then cDNA was synthesized by reverse transcription. The relative expressions of TopBP1mRNA, BaxmRNA, and Bcl-2mRNA were analyzed by $2^{-\triangle\triangle Ct}$ calculation method with glyceraldehyde phosphate deoxygenase (GAPDH) as an internal reference. Cytochrome B (Cytb) was a marker gene of rat mtDNA, and a standard curve was prepared and converted to copy number (copies/ μL).

Detection of TopBP1, Bax, and Bcl-2 proteins in rat aorta

Western blotting (WB) was used to detect the expressions of TopBP1, Bax, and Bcl-2 proteins in rat aortic tissues. The specific operation was as follows: rat aortic tissues were taken in a grinder, liquid nitrogen was added and ground evenly, and then lysate was used to extract the total tissue protein. Antibodies TopBP1, Bax, Bcl-2, and GAPDH were added first, and Goat anti-rabbit secondary antibody was added after overnight incubation. Image J software was used to quantify the gray values of protein bands.

Detection of ROS, MDA, and SOD levels in rat aortic tissues

2 g of rat aortic tissues were weighed and added into normal saline to make homogenate. Then it was centrifuged for 10 min (3000 r/min), and then the supernatant was sucked. The levels of ROS, MDA, and SOD in rat aortic tissues were detected by enzyme-linked immunosorbent assay (ELISA).

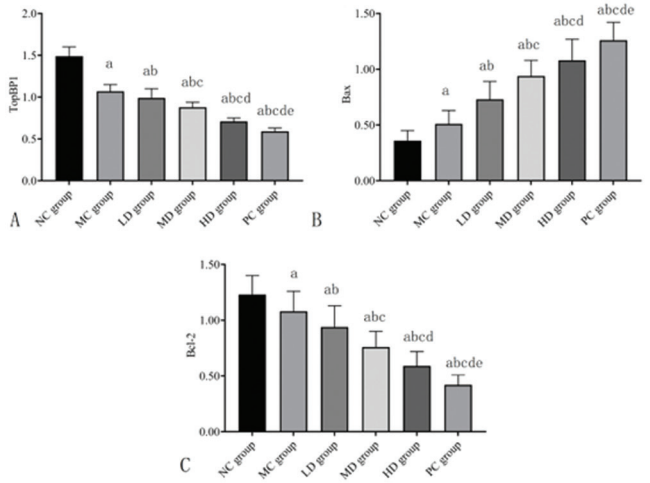
Detection of aortic plaque injury in rats

The aortic tissues of rats were taken and made into sections, and then 3:2 saturated oil red O stock solution was added for staining. The atherosclerotic plaques of the aorta of atherosclerotic rats were observed under a microscope.

Cell research

HUEVCs were cultured after resuscitation, and logarithmically growing cells were inoculated into 96 well plates harvested at a density of 1×10^6/mL and divided into normal control group (group A), cadmium intervention group (group B), TopBP1 inhibitor intervention group (Group C), and cadmium + TopBP1 inhibitor mixed intervention group (Group D). 30 μmol/l cadmium acetate was added to group B for intervention, 250 μmol/L oxidized bankning solution was added to group C for intervention,

Figure 1. The Expression Levels of TopBP1 and Apoptosis Related Proteins in Rat Aortic Tissues



[a]$P < .05$ Compared with NC Group
[b]$P < .05$ Compared with MC Group
[c]$P < .05$ Compared with LD Group
[d]$P < .05$ Compared with MD Group
[e]$P < .05$ Compared with HD Group

30 μmol/L cadmium acetate and 250 μmol/L oxidized bankning solution was added to group D for intervention, and same amount of normal saline was added to group A.

Statistical analysis

SPSS22.0 software was used to analyze and process the data. The measurement data in line with normal distribution was expressed in the form of the mean ± standard deviation ($\bar{x} \pm s$). One-way analysis of variance was used to compare the mean of multiple groups, and the LSD test was used for pairwise comparison. When $P < .05$ indicated that the difference was statistically significant.

RESULTS

Expression levels of TopBP1 and apoptosis-related proteins in rat aorta tissues

The WB test results (Figure 1 and Figure 2) showed that the relative expressions of TopBP, Bax, and Bcl-2 proteins in aortic tissues of rats in each group were significantly different (all $P < .05$), and the relative expressions of TopBP1 and Bcl-2 proteins in aortic tissues of rats in NC group, MC group, LD group, MD group, HD group, and PC group were decreased in turn (all $P < .05$), while the relative expressions of Bax protein in those group were increased in turn (all $P < .05$).

Gene expression levels of TopBP1 and apoptosis-related protein in rat aorta tissues

The RT-PCR results (Figure 3) showed that the relative expression levels of TopBP1mRNA, BaxmRNA, and Bcl-2mRNA in aortic tissues of rats in each group were significantly different ($P < .05$), and the relative expression levels of TopBP1mRNA and Bcl-2mRNA in aortic tissues of rats in NC group, MC group, LD group, MD group, HD group, and PC group were decreased in turn (all $P < .05$), while the relative expression levels of BaxmRNA protein in those group were increased in turn (all $P < .05$).

Figure 2. The Strip Chart of WB

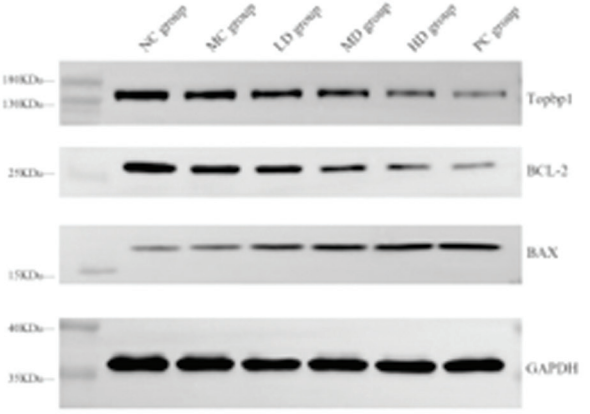
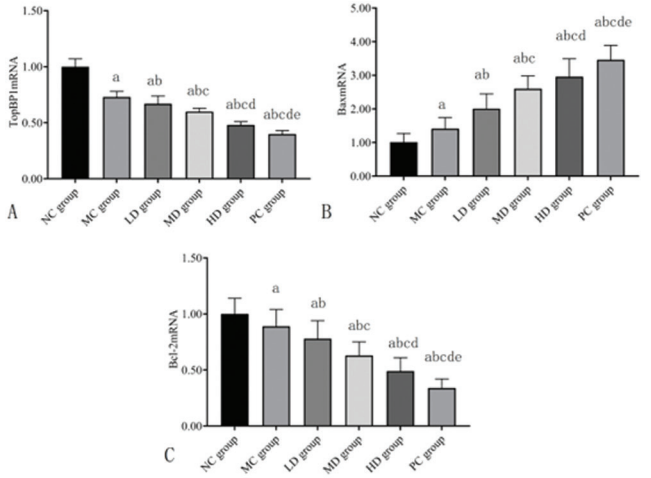


Figure 3. The Expression Levels of TopBP1 and Apoptosis Related Proteins Gene in Rat Aortic Tissue



[a]$P < .05$ Compared with NC Group
[b]$P < .05$ Compared with MC Group
[c]$P < .05$ Compared with LD Group
[d]$P < .05$ Compared with MD Group
[e]$P < .05$ Compared with HD Group

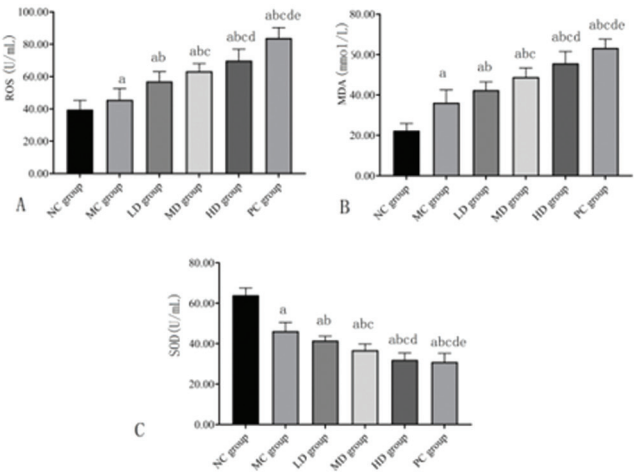
Expression levels of ROS, MDA, and SOD in rat aorta tissues

The results of ELISA showed that the expression levels of ROS, MDA, and SOD in aortic tissues of rats in each group were significantly different ($P < .05$), and the expression levels of ROS and MDA in aortic tissues of rats in NC group, MC group, LD group, MD group, HD group, and PC group were increased (all $P < .05$), while the expression levels of SOD in those group were decreased (all $P < .05$), as shown in Figure 4.

Expression level of mtDNA in rat aorta tissues

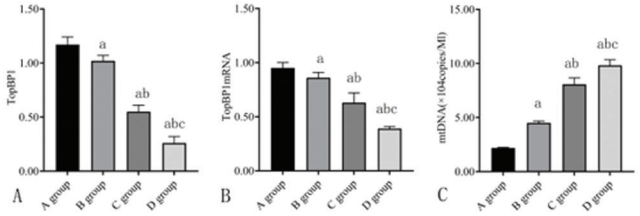
The difference in mtDNA expression levels in the aortic tissues of rats in each group was statistically significant ($P <$

Figure 4. The Expression Levels of ROS, MDA, and SOD in Rat Aortic Tissues



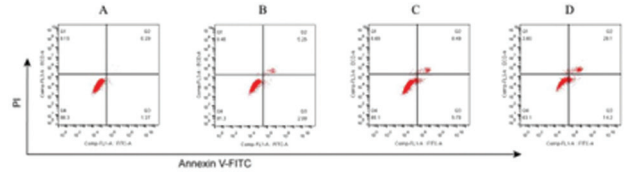
[a]$P < .05$ Compared with NC Group
[b]$P < .05$ Compared with MC Group
[c]$P < .05$ Compared with LD Group
[d]$P < .05$ Compared with MD Group
[e]$P < .05$ Compared with HD Group

Figure 5. The Expressions of TopBP1 and mtDNA in Vascular Endothelial Cells



[a] $P < .05$ Compared with A Group
[b]$P < .05$ Compared with B Group
[c]$P < .05$ Compared with C Group

Figure 6. Detection of Endothelial Cell Apoptosis by Flow Cytometry



.05), and the mtDNA expression levels in the aortic tissues of rats in the NC group, MC group, LD group, MD group, HD group, and PC group were increased in turn (all $P < .05$).

Aortic plaque injury in rats

The results of oil red O staining showed that the vascular structure of rats in the NC group was normal without atherosclerotic plaque. The inner walls of blood vessels in the LD group, MD group, and HD group were thickened, and the lumens were narrowed to varying degrees, showing the formation of atherosclerotic plaque, which was most obvious in the HD group and PC group.

Effects of cadmium acetate and TopBP1 inhibitor on mitochondrial DNA of endothelial cells

There were statistically significant differences in TopBP1, TopBP1mRNA, and mtDNA of cells in each group (all $P < .05$). The relative expressions of TopBP1, TopBP1mRNA of cells in groups A, B, C, and D were decreased (all $P < .05$), while the expression levels of mtDNA in those groups were increased (all $P < .05$), as shown in Figure 5.

Effects of cadmium acetate and TopBP1 inhibitor on endothelial cell apoptosis

There was a statistically significant difference in the apoptosis rate among the groups ($F = 55.001$, $P < .05$). The apoptosis rates of group A, group B, group C, and group D were (6.09 ± 2.65)%, (15.16 ± 1.08)%, (19.71 ± 1.53)%, and (24.67 ± 2.01)% respectively, and showed an increasing trend (all $P < .05$), as shown in Figure 6.

DISCUSSION

Atherosclerosis is a multifactorial disease of the cardiovascular system, which is related to aging, inflammation, and oxidative stress.[11-14] Some external factors such as smoking, diabetes, and infection may also lead to the development of the disease. With more and more studies, an in-depth mechanistic understanding of the effect of cadmium exposure, and cadmium exposure-linked cardiovascular disease has also received attention. Mice and rabbits are animals commonly used to prepare atherosclerosis.[11,15] Due to the advantages of rapid reproduction, easy genetic operation, and the ability to monitor the occurrence of atherosclerosis within a reasonable time range, mice have become the main species for the study of experimental atherosclerosis. TopBP1 is a multi-BRCT domain scaffold protein, which plays an important role in the replication, repair, and signal transmission of DNA damage.[16] Studies have shown that the downregulation of TopBP1 can cause DNA to replicate stress, leading to the accumulation of DNA damage.[17] Mitochondria are semi-autonomous organelles that provide cellular energy through the respiratory chain and play a key role in cell survival and death. In addition, mitochondria are involved in regulating cellular metabolism and ion balance and play important signaling functions. Mitochondria contain unique DNA (mtDNA) in the form of circular chromosomes, which are involved in the coding of proteins required for most respiratory chain functions,[18] so mtDNA expression level can be used as an important indicator to evaluate the degree of mitochondrial damage. In addition to generating energy, the mitochondrial respiratory chain also produces a large number of free radicals, ROS, and other by-products, of which the level of mitochondrial ROS depends on the respiratory activity, metabolic level, and the normal functioning of mitochondria. Although low levels of ROS have important signaling functions, a large amount of ROS production will harm the surrounding cell structure, thereby changing DNA, proteins, and other molecules. mtDNA is located near the mitochondrial membrane that reacts. The abnormal functioning of mtDNA can lead to dysfunction, reduced energy, and increased ROS generation in the respiratory chain. In addition, the deletion of mtDNA can

trigger its compensatory hyperproliferation, a process that may lead to a further increase of ROS, forming a vicious cycle, ultimately leading to cell death and damage to surrounding tissues, thereby causing local pro-inflammatory conditions. It is well known that vascular endothelial cell apoptosis is an important step in promoting the development of atherosclerotic plaque,[19] and mtDNA damage can promote the transformation of atherosclerotic plaques to vulnerable plaques by selectively inducing the apoptosis of vascular endothelial cells.[20,21]

In this study, rats were used to establish an atherosclerosis model, which was intervened by a high-fat diet, immune stimulation, and emotional restraint. Oil red O staining showed the formation of plaques, which could be used for further experimental research. Compared with normal rats, the expression levels of TopBP1 and TopBP1mRNA in the aorta of atherosclerotic rats were decreased; compared with non-intervened atherosclerotic rats, the expression levels of TopBP1 and TopBP1mRNA in atherosclerotic rats intervened with different doses of cadmium acetate and oxidized bankning (TopBP1 inhibitor) were decreased to varying degrees, among which oxidized bankning intervention was the most obvious, and cadmium acetate intervention had a dose-dependent relationship. The results showed that the downregulation of TopBP1 was associated with atherosclerosis, and cadmium acetate could downregulate the expression of TopBP1 in the aorta of atherosclerotic rats. The results of cellular experiments showed that cadmium acetate and oxidized bankning could also reduce the expression levels of TopBP1 and TopBP1mRNA in endothelial cells, and the combination of the two had a synergistic effect. The downregulation of TopBP1 and TopBP1mRNA was more obvious, which further confirmed that cadmium exposure had a regulatory effect on TopBP1 expression in endothelial cells.[18,19] From the results of this study, the expression levels of mtDNA and ROS in the aortic tissues of atherosclerotic rats were found to be higher than those of normal rats. The expression levels of mtDNA and ROS in atherosclerotic rats intervened by different doses of cadmium acetate were all increased in a dose-dependent manner. As a positive control compound, oxidized bankning had the highest effect on the expression levels of mtDNA and ROS in atherosclerotic rats. On the other hand, the study found that the expression of mtDNA was increased in the endothelial cells pretreated with cadmium acetate and (or) oxidized bankning. The above results suggest that mitochondrial DNA damage occurs in atherosclerotic rats, and cadmium acetate and oxidized bankning can aggravate this effect, which has an important connection with the downregulation of TopBP1 expression. Oxidative stress can produce a large number of oxygen free radicals, produce oxidative reactions, and cause endothelial damage; and ROS can reflect the total amount of collective oxygen free radicals. MDA can reflect the degree of lipid peroxidation in the body and SOD and GSH-Px can scavenge the oxygen free radicals. If there is too much ROS and MDA in the body and too little SOD and GSH-Px, it can lead to oxidative damage of vascular endothelium and promote atherosclerosis.[19,20] The results of this study found that MDA in the arterial tissues of atherosclerotic rats was increased and SOD was decreased, while the degree of change was more obvious in the atherosclerotic rats treated with cadmium acetate and oxidized bankning, indicating that cadmium exposure can aggravate vascular oxidative stress in atherosclerotic rats. In addition, cadmium acetate intervention can reduce the expression of anti-apoptotic protein Bcl-2 and promote the expression of apoptotic protein Bax in the aortic tissues of atherosclerotic rats. The increase in the apoptosis rate of endothelial cells suggests that cadmium exposure may affect the apoptosis of vascular endothelial cells.

In conclusion, cadmium exposure can downregulate the expression of TopBP1 in atherosclerotic rats, aggravate mitochondrial DNA damage, promote oxidative stress response, and then induce the development of atherosclerosis.

FUNDING
There was no funding.

ETHICS
This investigation was approved by Suzhou Medical College of Soochow University. All patients participating in this study have signed a formal consent form.

DATA AVAILABILITY STATEMENT
All data obtained in this study can be found in published articles.

AUTHOR DISCLOSURE STATEMENT
This study has no conflicts of interest.

ACKNOWLEDGEMENT
Junqi Xiao and Xiangtai Zeng designed the study; AJunqi Xiao ,Xiangtai Zeng,Yang xe and Fengen Liu carried out the experiment; Tao Liu and Leiying Zhang collected tissue samples; Junqi Xiao and Huilin Luo analyzed these data; Junqi Xiao and Xiangtai Zeng wrote and revised the paper; All authors read the final version of the article and agreed to submit.

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