Mechanism in bradycardia induced by Trimethyltin chloride: Inhibition activity and expression of Na+/K+-ATPase and apoptosis in myocardia

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ABSTRACT — Trimethyltin chloride (TMT) is a stabilizer by-product in the process of manufacturing plastic, which is a kind of very strong toxic substance, and has acute, cumulative and chronic toxicity. TMT may cause bradycardia in patients with occupational poisoning, the mechanism of which has not been reported. This study explored the mechanism of TMT resulting in bradycardia of C57BL/6 mice. TMT was administered to mice to measure heart rate, serum succinate dehydrogenase (SDH) level, and myocardial Na+/K+-ATPase activity and expression. The effects of TMT on myocardial apoptosis were observed by changing the expressions of caspase-3, Bax and Bcl-2 in myocardium. It was found that the heart rate and SDH activity in serum of mice gradually decreased with the increase of TMT dose compared with the control group. The activity and the expression of Na+/K+-ATPase in the heart tissue of mice exposed to TMT was measured and gradually decreased with the increase of dose and time. We measured the expression of Bcl-2, Bax, caspase-3 and cleaved caspase-3 in the heart tissues of TMT exposed mice and found that the expressions of Bax, caspase-3 and cleaved caspase-3 increased and the expressions of Bcl-2 decreased in the heart tissues of the TMT-exposed mice at different doses. With the extension of TMT exposure time, the expression of Bax and caspase-3 increased and the expression of Bcl-2 decreased in the heart tissues of TMT exposed mice. Our findings suggest the mechanisms of TMT resulting in bradycardia may be associated with the inhibited activity and decreased content of Na+/K+-ATPase, thus further leading to the changes of Bcl-2, Bax, caspase-3 and cleaved caspase-3 in the mice’s ventricular tissues.

Key words: Trimethyltin chloride (TMT), Apoptosis, Bcl-2, Bax, caspase-3, Na+/K+-ATPase

INTRODUCTION

Trimethyltin chloride (TMT), a colorless and highly toxic substance, is an impurity in the process of synthesizing organotin stabilizer. Organotin as a heat stabilizer in plastic products, especially polyvinyl chloride (PVC), can lead to serious occupational poisoning accidents (Tang et al., 2013a). TMT has high acute and cumulative toxicity, and long-term chronic TMT exposure can lead to hypokalemia, neurotoxicity symptoms and kidney stones (Tang et al., 2010, 2013b; Ren et al., 2015). However, the cardiotoxicity induced by TMT exposure has not been explored thoroughly. Only in recent clinic cases, we notice some cardiotoxic cases caused by acute TMT exposure, the main symptoms of cardiotoxicity include bradycardia, arrhythmia (Long et al., 2000; Yang et al., 2006; Tang et al., 2008; Sun et al., 2007) and, in a few cases, atrioventricular block, sinus tachycardia, T wave abnormality, repolarization abnormality and so forth (Yang et al., 2018).

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Aptosis plays an important role in tumor therapy, brain disease, aging and heart disease. Caspase 9, as an activating factor among the apoptosis-related caspases, further activates caspase-3 after it produces apoptotic bodies, thus causing apoptosis (Kim et al., 2015). Many studies have confirmed that Na+/K+-ATPase is associated with apoptosis, and because of this association, Na+/K+-ATPase can be used as an antitumor treatment target. Cerella et al. (2013) believed that the main target of cardiac glycosides was Na+/K+-ATPase, which could inhibit the proliferation of tumor cells by regulating the expression of the anti-apoptotic protein Bcl-2. Bufadienolides inhibit the proliferation of tumor cells and promote their apoptosis. Bufadienolides are cardiotonic steroids that bind to Na+/K+-ATPase and inhibit Na+/K+-ATPase activity. After bufadienolides were applied to breast and ovarian cancer cells, they showed significant cytotoxicity to the tumor cells, causing decreased Na+/K+-ATPase activity in the tumor cells and increased caspase3 and caspase 9 activation, which led to apoptosis (Garcia et al., 2019). Other results suggested that hydrogen sulfide may cause bradycardia. The mechanism may be that hydrogen sulfide causes oxidative stress in cardiomyocytes, damages mitochondria, increases the expression of pro-apoptotic proteins and decreases the expression of anti-apoptotic proteins. Hydrogen sulfide exposure reduced Na+/K+-ATPase activity in the myocardium (Wang et al., 2019). Bartlett et al. (2018) believed that heart diseases that are caused by aging are related to cell apoptosis, which may be caused by the Na+/K+-ATPase-mediated production of large amounts of reactive oxygen species (ROS) and, thus, the generation of oxidative stress.

TMT is an inhibitor of oxidative phosphorylation that can inhibit the activities of all kinds of ATPases, including Na+/K+-ATPase and Na+/K+-ATPase, as well as the production of cell energy (Besser et al., 1987; Tang et al., 2010; Ren et al., 2015). The weakened activity of Na+/K+-ATPase can lead to Q-T prolongation and arrhythmia (Pott et al., 2018). Bcl-2 can prevent apoptosis, and the inhibition of Na+/K+-ATPase may be related to apoptosis (Gilbert et al., 1996). TMT can release caspase 3 in the granulosa cells in the olfactory bulb (OB) and anterior olfactory nucleus (AON) of mice, causing abnormal function of the nerve cells in the OB and AON (Kawada et al., 2008).

Presently, the specific mechanism by which TMT causes bradycardia is not clear. This study explored the mechanisms and provided new evidence for preventing and curing the bradycardia caused by TMT.

MATERIALS AND METHODS

Drug treatment

TMT (Shanghai Civi Chemical Technology Co., Ltd, Shanghai, China) was diluted with 0.9% normal saline. Specific pathogen-free C57BL/6 mice (6-8 w, 18-25 g, male) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China, were housed in metallic breeding cages in a room with a light-dark cycle of 12 hr/12 hr, and were given free access to food and water. According to the previous TMT acute toxicity test and related research (Liu et al., 2008; Tang et al., 2013a), the mice were divided into four groups: NS control group, low-dose group (1.00 mg/kg TMT, ip), medium-dose group (2.15 mg/kg TMT, ip) and high-dose group (4.64 mg/kg TMT, ip). Additional mice were divided into four other groups: control group, 1 d group, 3 d group and 6 d group (2.15 mg/kg TMT, ip, in the treatment groups).

Twenty-four hours after the last exposure to TMT, 20% ethyl carbamate was used to anesthetize the mice (10 mL/kg, ip). The limb lead II of the mice was measured by electrocardiography. The calibration voltage was 2 mV, and the paper speed was 50 mm/sec. The heart rates were measured on 5 consecutive R waves with a stable electrocardiograph (ECG) baseline. The mean R-R intervals were measured, and the heart rates were calculated (heart rate= 60/R-R interval/2). After measuring the heart rates, blood was taken from the angular vein, the mice were sacrificed by cervical dislocation, and the myocardial tissues were stored at -80°C. The animal experiments were approved by the North Sichuan Medical College Ethics Committee.

Measurement of Succinate dehydrogenase activity in serum

The blood was not treated with anticoagulants and was centrifuged for 10 min at 3500 r/min. Then, the serum was obtained. The working fluid was prepared according to the instructions of the Succinate Dehydrogenase (SDH) Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The working fluid was added to the serum (serum:working fluid=100 μL:2.6 mL). After mixing these fluids, the mixture was immersed in a water bath at 37°C for 5 min. A spectrophotometer was used to perform colorimetric analysis at 600 nm, and the absorbance values were measured at 5 sec and 65 sec. The difference between the two absorbance values was calculated, which indicated the SDH activity in the serum.
Measurement of Na+/K+-ATPase activity
The hearts of the mice were collected, and the same part of the ventricular tissues was homogenized by a cold glass homogenizer. The homogenate of the hearts was centrifuged by freezing centrifugation (Eppendorf, Hamburg, Germany) at 3500 r/min and 4°C for 10 min, and then 200 μL supernatant fractions were extracted. The reagents were prepared according to the instructions of the ATP enzyme test kit (Nanjing Institute of Bioengineering, Nanjing, China), and the supernatant fractions were mixed with phosphorus fixative in a water bath at 37°C for 30 min. After cooling to room temperature, the absorbance values were measured at 660 nm by a multifunctional Enzyme Marker (M2 Molecular Devices, Shanghai, China), and then, the Na+/K+-ATPase activity was calculated.

Western blotting
RIPA pyrolysis solution containing 1% PMSF (P0013B, Beyotime, Shanghai, China) was added to the homogenates of the hearts, and then, they were processed by an Ultrasonic Cell Disruptor (SCIENTZ-IID SCIENTZ Xinzhi Technology, Zhejiang, China). The mixtures were centrifuged for 10 min at 4°C and 12000 r/min, and then, the supernatant fractions were extracted. A BCA protein quantitative kit was used to determine the protein concentrations of the samples. The protein samples were loaded in a volume of 20 μg/well, and SDS-PAGE (80 V and 110 V for 1 hr each) was carried out with a 10% separation gel and 5% concentration gel. After electrophoresis, the proteins were transferred to membranes at 250 V and 0.28 A for 80 min. The PVDF membranes were placed in a closed container with 5% skimmed milk and shaken for 2 hr at room temperature. The membranes were incubated in the primary antibodies (rabbit monoclonal antibody to Sodium Potassium ATPase alpha subunit (Abcam, Cambridge, MA, USA); rabbit monoclonal antibody to Bax (Abcam); rabbit monoclonal antibody to Bcl-2 (Abcam); rabbit monoclonal antibody to caspase-3 (Abcam); rabbit polyclonal antibody to cleaved caspase-3 (Abcam) and beta-Actin (bs-0061RB, BioSS, Beijing, China)) at 4°C overnight. After washing with TBST, the membranes were incubated with the secondary antibody (horseradish peroxidase-labeled goat anti-rabbit IgG A0208, Beyotime) at room temperature for 1 hr. According to the BeyoECL Star hypersensitive ECL chemiluminescence kit (Beyotime), the luminescent solution was prepared, the gel imaging system was used for imaging, and ImageJ software was used to analyze the bands.

Statistical analysis
The experimental data were analyzed by statistical software SPSS 20.0, and the results were expressed as Mean ± SEM. The differences among groups were analyzed by one way-ANOVA, and the differences between control groups and experimental groups were analyzed by LSD test, a = 0.05.

RESULTS

Analysis of arrhythmia caused by TMT
According to the collected literature reports (http://www.cnki.net, http://www.Wanfangdata.com.cn), A total of 601 cases of trimethyltin chloride poisoning with complete case reports were collected, including 240 cases of arrhythmia, accounting for 39.9% of the total cases. The incidence of arrhythmia was as high as 39.9%. Data statistics are shown in Table 1.

Table 1. A summary of arrhythmia caused by trimethyltin chloride poisoning in China from 1998 to 2017.

| Province    | Cases | Number of TMT poisonings | Proportion of arrhythmia n (%) | Accident Time | Reference                  |
|-------------|-------|--------------------------|--------------------------------|---------------|---------------------------|
| Jiangxi     | 4     | 225                      | 75(33.3)                       | 1998-2017     | Long et al., 2000; Yang et al., 2018; Li et al., 2019 |
| Zhejiang    | 4     | 114                      | 54(47.3)                       | 1997-2008     | Zhu, 2006; Luo et al., 2008; Ding et al., 2003; Zhang et al., 2006 Yang et al., 2006; Fang et al., 2004; Xia et al., 2007; Bai et al., 2013; Luo et al., 2005; Xiao and Wen, 2000; Zhang, 2001; He et al., 2002; Xia et al., 2002 |
| Guangdong   | 9     | 215                      | 91(42.3)                       | 1998-2013     |                         |
| Shanghai    | 1     | 4                        | 1(25.0)                        | 2010          | Fu and He, 2010          |
| Jiangsu     | 1     | 3                        | 1(33.3)                        | 2008          | Zhu et al., 2008         |
| Liaoning    | 1     | 40                       | 18(45)                         | 2011          | Liu, 2011                |
| Total       | 20    | 601                      | 240(39.9)                      |               |                           |

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The main electrocardiographic abnormalities in patients with trimethyltin chloride poisoning were sinus bradycardia, sinus arrhythmia, atrioventricular block, sinus tachycardia, ST segment depression, prolonged QT interval, U wave, PR interval abnormality, P, R, T wave amplitude abnormality and ST segment deviation, etc. The incidence of arrhythmia was higher in moderate and severe poisoning patients, and even cardiac arrest crisis and death occurred.

Changes in heart rates and SDH activities induced by TMT in mice

After treating mice with 1.00 mg/kg, 2.15 mg/kg and 4.64 mg/kg TMT, the heart rates of these mice were significantly lower than those of the control-treated mice (Fig. 1A), and the heart rates of the mice after TMT exposure for 1 d, 3 d and 6 d were significantly lower than those of the control mice (Fig. 1B).

To observe the effect of trimethyltin chloride on energy metabolism in mice, the succinate dehydrogenase (SDH) activity in the serum was measured. The results showed that the SDH activities of mice exposed to 1.00 mg/kg and 2.15 mg/kg TMT were significantly lower than those of the control mice, and the decrease in the serum SDH activities in the mice exposed to 4.64 mg/kg TMT was the most obvious (Fig. 1C). The SDH activities of the mice treated with 2.15 mg/kg TMT for 1 d, 3 d and 6 d decreased significantly compared with those of the control mice, and the SDH activities were always inhibited with increasing exposure time (Fig. 1D).

Effects of TMT on Na⁺/K⁺-ATPase activities in mice’s hearts in vivo and in vitro

Normal mouse ventricular homogenates were treated with TMT at different doses. After incubating in a water bath at 37°C for 2 hr, the activity of Na⁺/K⁺-ATPase in the TMT-exposed myocardial homogenates gradually decreased as the TMT dose increased (Fig. 2A). In addi-
tion, normal mouse myocardial tissue homogenates were treated with 2.15 mg/kg TMT or with normal saline as the control, and the homogenates were incubated for 0 hr, 2 hr, 4 hr, and 6 hr in a water bath at 37°C. The activity of Na+/K+-ATPase continuously decreased with increasing exposure time. At 0 hr and 2 hr, the activities of Na+/K+-ATPase in the mice in the TMT-treated group exhibited no significant difference compared with those in the mice in the control group; at 4 hr and 6 hr, the activities of Na+/K+-ATPase in the mice in the TMT-exposed groups were significantly lower than those in the mice in the control group (Fig. 2B). These results suggest that TMT exposure in vitro can significantly decrease the activity of Na+/K+-ATPase in the myocardial tissues of mice, and the activity of Na+/K+-ATPase gradually decreases with increasing TMT exposure dose and time. The changes in the Na+/K+-ATPase activities in the myocardium of mice were further verified in vivo. After 24 hr of TMT exposure, the activities of Na+/K+-ATPase in the myocardium of the mice in the 2.15 mg/kg and 4.64 mg/kg TMT groups were significantly lower than those of the mice in the control group (Fig. 2C). After 1 d, 3 d and 6 d of TMT exposure, the Na+/K+-ATPase activities of the heart tissues in the 2.15 mg/kg TMT groups were significantly lower than those of the heart tissues in the control group (Fig. 2D).

The expression of Na+/K+-ATPase in hearts of mice exposed to TMT
The above study has confirmed that TMT exposure can lead to the decrease of Na+/K+-ATPase activities in mice’s cardiac tissue. We further verified the effect of TMT on the expressions of Na+/K+-ATPase proteins cardiac tissues.

Fig. 2. The changes of Na+/K+-ATPase activities in mice’s hearts induced by TMT in vivo and in vitro. A: The activity of Na+/K+-ATPase was measured after the homogenate of normal mice was exposed to 0, 0.100, 0.215 and 0.464 mg/mL TMT for 2 hr; B: The activity of Na+/K+-ATPase was measured after the tissue homogenate of normal mice was exposed to 0.215 mg/mL TMT for 0, 2, 4, 6 hr. The control group was given normal saline followed by the same treatment. n = 3, compared with the control group, *: \( P < 0.05 \), **: \( P < 0.01 \); C: 32 mice were infected with 0, 1.00, 2.15 and 4.64 mg/kg TMT, and the activity of Na+/K+-ATPase in heart tissue was measured after 24 hr; D: 48 Mice were exposed to 2.15 mg/kg TMT and the activity of Na+/K+-ATPase in heart tissue was measured after 1 d, 3 d and 6 d, respectively. In the control group, the activity of Na+/K+-ATPase in heart tissue was measured after normal saline was given. n = 8, compared with the control group, **: \( P < 0.05 \), ***: \( P < 0.01 \).
of mice. We found that the expression of Na⁺/K⁺-ATPase in the myocardiums of mice decreased gradually with the increase dosage of TMT after TMT exposure for 24 hr (Fig. 3A and B). The Na⁺/K⁺-ATPase contents in the myocardiums of mice decreased significantly after 2.15 mg/kg TMT exposure for 3 d and 6 d, compared with the control group (Fig. 3C and D).

**Changes of Bcl-2, Bax, caspase-3 and cleaved caspase-3 in cardiomyocytes of mice induced by TMT**

The expressions of Bcl-2, Bax, caspase-3 and cleaved caspase-3 proteins in myocardial tissues of mice exposed to TMT were detected to further verify the effect of TMT on apoptosis of myocardial tissues of mice. The expressions of Bcl-2 proteins in the myocardiums of mice decreased gradually after exposing to different doses of TMT for 24 hr, compared with the control group (Fig. 4A and B). The expressions of Bax proteins in 2.15 mg/kg and 4.64 mg/kg TMT groups increased significantly compared with the control group (Fig. 4A and C). At the same time, the expressions of caspase-3 and cleaved caspase-3 proteins in the myocardia of TMT-exposed mice in all three dose groups were higher than that of the mice in control group, and the cleaved caspase-3 expression were more significant in the 2.15 mg/kg TMT group (Fig. 4A, D and E). The expressions of Bcl-2 proteins in myocardial cells of mice exposing to 2.15 mg/kg TMT for 1 d, 3 d and 6 d were lower than that of the mice in control group (Fig. 4F and G). Meanwhile, with the prolongation of exposure time, we found that the expressions of Bax, caspase-3 and cleaved caspase-3 proteins increased gradually in the myocardia of mice with 2.15 mg/kg TMT exposure (Fig. 4F, H, I and J).

**DISCUSSION**

In the early stage, patients exposed to TMT have some ordinary symptoms, such as headache, dizziness, sleep disorders, limb weakness, memory loss, nausea and vomiting, chest tightness, palpitation, tinnitus, dry cough and poor acceptance and other nonspecific symptoms, which are easily ignored by patients themselves or misdiagnosed by professional doctors (Xia et al., 2007). With the accumulation of TMT in the body, patients’ conditions can rapidly progress, and patients develop a series of abnormal symptoms of the respiratory, digestive, and nervous systems and abnormal mental behavior; these symptoms includes dyspnea, abdominal pain, loss of appetite, weight loss, persistent headache, neck prickling, mental...

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**Fig. 3.** Expression of Na⁺/K⁺-ATPase in Myocardiums of Normal Mice Exposing to TMT at Different Doses and Times. A: Na⁺/K⁺-ATPase expression bands under different doses of TMT exposure; B: Na⁺/K⁺-ATPase gray value expression bands under different doses of TMT exposure; C: Na⁺/K⁺-ATPase expression bands under different times of TMT exposure; D: Na⁺/K⁺-ATPase gray value expression bands under different times of TMT exposure. n= 3, compared with the control group, *: P < 0.05; **: P < 0.01; ***: P < 0.001.
disorders and coma (Saary and House, 2002; Pluta and Ostrowska, 1987; Fortemps et al., 1978; Morita et al., 2006). Additionally, TMT poisoning affects heme metabolism and cardiovascular function (Cameron et al., 1991). The main electrocardiogram manifestations of TMT poisoning patients are sinus bradycardia and sinus arrhythmia, and a few patients may have atrioventricular block, sinus tachycardia, T wave abnormality and repolarization abnormality (Wang et al., 2017). With the increasing use of plastics in daily life, the cardiotoxicity caused by TMT occupational exposure requires urgent attention. SDH is one of the most basic enzymes in cell metabolism and is a marker of mitochondrial function. When succinate dehydrogenase decreases, it not only inhibits its own function but also interrupts the fourth dehydrogenation reaction of the tricarboxylic acid cycle, which leads to the degradation of phospholipids in the mitochondrial membrane, the lack of calcium carriers, the decrease in active calcium uptake by mitochondria, the decrease in ATP synthesis, the insufficient contractile energy of the myocardium, the apoptosis of myocardial cells and so on (Philipson et al., 1980; Qiao et al., 2005). With the increase in TMT dosage and the increase in TMT exposure time, the heart rates of the mice decreased, and the activities of SDH in the serum continued to decrease, which showed that the damage to the myocardial cells induced by TMT might be related to disrupted mitochondrial function in the mice. The inhibition of Na+/K+-ATPase could lead to the imbalance of Na⁺ and K⁺ distribution inside and outside the cell membrane, the prolongation of QT interval, of the reduction in cardiac conduction velocity and bradycardia, which is consistent with the experimental results in this study (Pott et al., 2018). The main function of Na⁺/K⁺-ATPase is to maintain the concentration gradient of Na⁺ and K⁺. The decrease in the Na⁺/K⁺-ATPase activity prevents intracellular Na⁺ efflux and then causes intracellular Na⁺ overload, which activates Na⁺-Ca²⁺ exchange proteins on the cell membrane and promotes Ca²⁺ entry into cells, resulting in intracellular Ca²⁺ overload (Shattuck et al., 2015). Studies on embryonic death in zebrafish have found that *schneckentempo* (*ste*) is associated with decreased resting heart rate and that the absence of ste is related to *dihydrolipoyl succinyltransferase* (DLST); moreover, DLST plays a key role in the citric acid cycle in mitochondria, thus affecting the production of ATP. There-

![Fig. 4. Effects of TMT exposure on expressions of Bcl-2, Bax, caspase-3 and cleaved caspase-3 proteins in Mice’s Cardiac Myocytes. A: The expression bands of Bcl-2, Bax, caspase-3 and cleaved caspase-3 proteins under different doses of TMT exposure; B: The gray value expression bands of Bcl-2 proteins under different doses of TMT exposure; C: The gray value expression bands of Bax proteins under different doses of TMT exposure; D: The gray value expression bands of caspase-3 proteins under different doses of TMT exposure. E: The gray value expression bands of cleaved caspase-3 proteins under different doses of TMT exposure. F: The expression bands of Bcl-2, Bax, caspase-3 and cleaved caspase-3 proteins under different times of TMT exposure; G: The gray value expression bands of Bcl-2 proteins under different times of TMT exposure; H: The gray value expression bands of Bax proteins under different times of TMT exposure; I: The gray value expression bands of caspase-3 proteins under different times of TMT exposure; J: The gray value expression bands of cleaved caspase-3 proteins under different times of TMT exposure. n= 3, compared with the control group, *: P < 0.05; **: P < 0.01; ***: P < 0.001. Vol. 45 No. 9
fore, it is believed that DLST protein is related to the production of ATP in heart tissue, which further affects the energy metabolism of the heart, leading to bradycardia (Keßler et al., 2015). A study showed that *Lycium barbarum* polysaccharide has a protective effect on the myocardial injury caused by ischemia-reperfusion, and the mechanism is that *Lycium barbarum* polysaccharide enhances the activity of the Na⁺/K⁺-ATPase and inhibits the apoptosis of myocardial cells (Hou et al., 2017).

Ascleposide, as a type of cardiac glycoside, inhibits cell proliferation and promotes apoptosis. The mechanism is that ascleposide leads to the inhibition of Na⁺/K⁺-ATPase, down-regulates the expression of α1 subunit of Na⁺/K⁺-ATPase, causes cell cycle abnormality, activates caspase, reduces the expression of Bel-2 and Mcl-1 protein, and increases the expression of bak, thus causing damage to the mitochondrial membrane, in turn it causes apoptosis (Ganesan et al., 2016). Through the study of the toxic effect of copper oxide nanoparticles (CuO-NPs) on zebrafish embryos, it was found that CuO-NPs could lead to decreased Na⁺/K⁺-ATPase activity and oxidative stress injury in zebrafish embryos, which further led to the apoptosis of zebrafish embryo cells, as well as decreased heart rate and hatchability of zebrafish embryos (Ganesan et al., 2016). Studies have shown that cadmium can reduce the activity of LDH, GSH and SOD in primary tubular cells of duck kidney and promote the generation of ROS and MDA. The activity of Na⁺/K⁺-ATPase was inhibited, the expression of Bax and caspase-3 was increased, and that of Bcl-2 was decreased. In conclusion, cadmium can promote cell apoptosis and damage renal tubular epithelial cells through the oxidative stress of renal tubular epithelial cells and the effect of Na⁺/K⁺-ATPase activity (Zhuang et al., 2019).

The increase of intracellular Ca²⁺ concentration is the initiating factor of apoptosis. Previous studies have shown that the concentration of intracellular free calcium increases continuously before apoptosis induced by various pathways. At the same time, the increase of extracellular K⁺ concentration decreases the absolute value of cell resting potential, increases excitability and causes arrhythmia (Galang et al., 2000; Sanguinetti et al., 1996). The inhibition of Na⁺/K⁺-ATPase activity is also related to the process of apoptosis induced by unbalanced Bel-2/Bax ratio (Huang et al., 2015). When the expression level of Bel-2 is high, the heterodimer of Bel-2/Bax can be formed, and the apoptosis is inhibited. When the expression level of Bax is high, the homodimer of Bax/Bax can be formed, which promotes the apoptosis (McClintock et al., 2002). Caspase is the initiator and executor of mammalian cell apoptosis. Caspase-3 is the most critical apoptotic protease in the downstream of caspase cascade. Bcl-2 blocks the activation of upstream caspase protease by interfering with the release of cytochrome C and then inhibits cell apoptosis (Kim et al., 2014). Bax, a component of ion channels on mitochondrial membrane, enables cytochrome C to pass through mitochondrial membrane and form a polymer complex with apoptotic protease activator-1(Apaf-1) to activate precursors, leading to caspase-3-mediated activation of downstream effectors (Liu et al., 2016). In this study, the activities and contents of Na⁺-K⁺-ATPase in mice’s heart tissues decreased with the increase of TMT dosage and the prolongation of exposure time, which was consistent with the inhibition of SDH and mitochondrial damage. The expression of Bax protein increased with the increase of TMT dosage and the prolongation of exposure time, while the expression of Bcl-2 protein decreased in that condition.

In conclusion, the bradycardia induced by trimethyltin chloride in mice may be related to the decrease of activity and content of Na⁺-K⁺-ATPase, and the expression of Bax, Bcl-2, caspase-3 and cleaved caspase-3 in mice’s cardiomyocytes may be altered by TMT exposure, suggesting that TMT exposure can induce apoptosis of cardiomyocytes.

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**Conflict of interest**—The authors declare that there is no conflict of interest.

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