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Original Article

Trimetazidine prevents pirarubicin-induced myocardial damage: a possible antioxidant mechanism

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Abstract: In the present study, we investigated the effect and possible mechanism of action of trimetazidine in pirarubicin-induced myocardial damage. Thirty-six Wistar rats were randomly divided into control, model, and treatment groups. The rats in the model and treatment groups were injected through the vena caudalis with pirarubicin (2.5 mg/kg once a week) for six weeks. The control group rats were injected in the same manner with 0.9% NaCl for 6 weeks. The rats in the treatment group received an intragastric infusion of trimetazidine at 5.4 mg/kg/d for 8 weeks, while those in the control and model groups received infusions of 0.9% NaCl. At the end of the experiment, we measured the levels of superoxide dismutase, cardiac enzymes, and free radical mediators. Myocardial tissue was examined using light and electron microscopy. The levels of myoglobin, troponin, and alanine aminotransferase (ALT) were lower in the treatment group than in the model group ($P < 0.05$). Malondialdehyde (MDA) and nitric oxide (NO) levels were lower after treatment than in the model group ($P < 0.05$), and nonprotein sulfhydryl (NPSH) and superoxide dismutase (SOD) levels were higher in the treated animals than in the model group ($P < 0.05$). In the model group, the structure of myocardial cells was severely damaged, they were arranged in a disorderly manner, and myocardial myofilament dissolution and fracturing were observed. In the treatment group, the structure of myocardial cells was orderly, and the structure of the myocardium was essentially preserved. Treatment with trimetazidine reduced mitochondrial damage and relieved myocardial injury, indicating that trimetazidine exerted a protective effect on cardiomyocytes that were exposed to pirarubicin. The mechanism underlying this effect may be related to its antioxidative activities.

Keywords: Trimetazidine, pirarubicin, myocardial damage, oxidative stress

Introduction

Pirarubicin (THP) is a newer generation anthracycline antitumor antibiotic [1, 2]. THP and THP-based combination chemotherapies have demonstrated effectiveness against a variety of tumors [3, 4]. However, the toxic side effects of THP seriously restrict its clinical applications. The progressive, dose-dependent development of cardiomyopathy results in irreversible congestive heart failure [5] mainly as a result of damage to the structure of mitochondria. Because mitochondria affect the metabolism of the heart, THP induces serious toxic effects in the heart [6].

Trimetazidine (1-(2,3,4-trimethoxybenzyl) piperazine; TMZ) is, like ranolazine and L-carnitine, a cardioprotective drug [7, 8]. It blocks fatty acid oxidation and increases glucose utilization, eventually leading to a reduction in intracellular acidosis [9]. Numerous studies have suggested that TMZ inhibits the production of free radicals and preserves myocyte structure and function [10, 11]. However, few studies have evaluated the cardioprotective effect of using TMZ after chemotherapy, and the effects of clinical intravenous chemotherapy and long-term trimetazidine intervention have not been reported. Therefore, in the present study, we evaluated the protective effect of TMZ and the mechanism underlying its protection against THP-induced myocardial injury in rats.

Materials and methods

Animal treatment

A total of 36 healthy male Wistar rats (280 ± 20 g, license: SCXK-(Ji) 2007-0003) were obtained

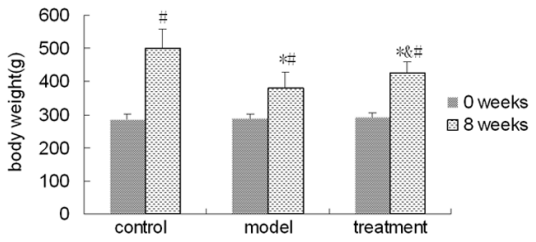

Figure 1. Weights of rats in various groups at 8 weeks. *P < 0.05 compared to the control group; & P < 0.05 compared to the model group; and # P < 0.05 compared to 0 weeks.

from the Experimental Animal Center of Jilin University (Changchun, China). The protocol used in this study was approved by the ethics committee of the First Bethune Hospital of Jilin University. The rats were divided into the following groups: control group (n=10), model group (n=13), and treatment group (n=13). The rats in the model and treatment groups were injected with pirarubicin (2.5 mg/kg, once a week) through the vena caudalis for six weeks to establish a myocardial damage model. Pirarubicin (Cat. 1105c2) was purchased from Shenzhen Main Luck Pharmaceuticals Inc. (Shenzhen, China). In the treatment group, TMZ (5.4 mg/kg/d, once a week for 8 weeks) was administered via gavage one day before pirarubicin administration began. TMZ (Cat. 2000-958) was purchased from Servier.

Inclusion and exclusion criteria

Rats were included in our study if they (i) were male, (ii) weighed 280 ± 20 g, and (iii) survived to the end of the experiment. Rats that did not meet these criteria were excluded from the study.

Cardiac enzyme detection

Following the 8-week treatment period, oral feeding was withheld for 12 hours before anesthesia was administered. After the rats were anesthetized, blood was collected using a retro-orbital bleeding protocol. After the samples were rested for 30 min at room temperature, serum was separated using centrifugation at 12,000 rpm for 5 min at a low temperature. Troponin I and myoglobin levels were measured using chemiluminescence (Johnson 5600 Biochemical Analyzer; kit: 20120110). Alanine aminotransferase (ALT) levels were measured using a continuous monitoring procedure (Japan Hitachi Automatic Biochemical Analyzer1210; Kit: 20120218).

Measurement of free radical mediators

Myocardial tissue was washed with cold 0.9% NaCl, and the liquid was then completely aspirated. After the left ventricular myocardium was isolated and dissociated, a 10% tissue homogenate was prepared by adding cold 0.9% NaCl. The supernatant was separated using centrifugation at 3500 rpm for 15 min at 4°C. After the sample was centrifuged, the supernatant was stored at -20°C. The dithio-bis-nitrobenzoic acid (DTNB) method was used for the nonprotein sulfhydryl (NPSH) assay. Malondialdehyde (MDA) levels were measured using the thiobarbituric acid (TBA) method. Nitric oxide (NO) content was measured using the nitric reductase method. The kits used in this study were provided by the Nanjing Jiancheng Bioengineering Institute, and the procedures were performed according to the kit instructions. SOD activity was determined using the pyrogallol method (Johnson 5600 Biochemical Analyzer and Instrument; kit: 20120810).

Echocardiography detection

After 8 weeks of treatment, the rats were anesthetized via an intraperitoneal injection of 10% chloral hydrate. After the chests of the rats were shaved, the rats were immobilized in the left lateral decubitus position on a laboratory-manufactured fixed table. Ultrasounds were performed using an ALOKA-5500 ultrasonic diagnostic instrument (Japan). All ultrasound measurements were performed by the same experienced physician using the same ultrasound diagnostic apparatus.

Observation of myocardial structure

The rats were anesthetized using diethyl ether. Two pieces of myocardial tissue were obtained from the anterior wall of the left ventricular. One sample was fixed in 10% neutral-buffered formalin and then embedded in paraffin. Paraffin sections were cut and subjected to hematoxylin-eosin (HE) staining after routine deparaffinization and hydration. The following criteria were used for cardiac histopathology scoring: (0 points) the myocardial fibers were arranged

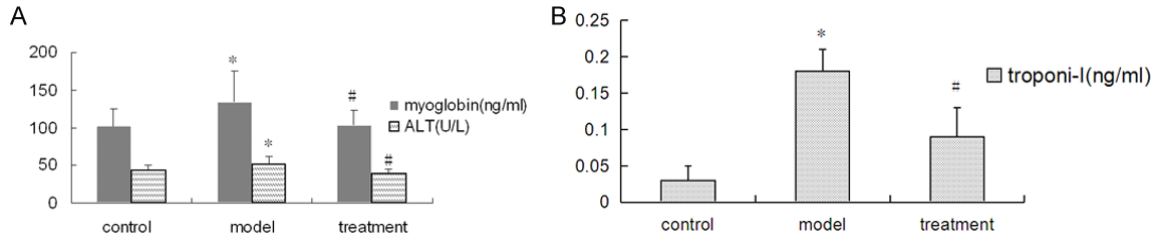

Figure 2. Myocardial enzymes (A. Myoglobin and ALT, B. Troponi-I) in rats in various groups at 8 weeks. *P < 0.05 compared to the control group; and #P < 0.05 compared to the model group.

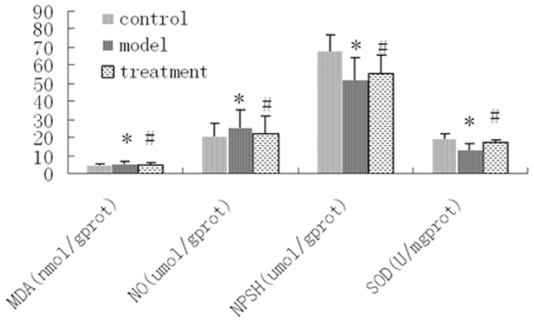

Figure 3. Levels of MDA, NO, NPSH and SOD activity in the myocardium of rats in the three groups at 8 weeks after THP administration. *P < 0.05 compared to the control group; and #P < 0.05 compared to the model group.

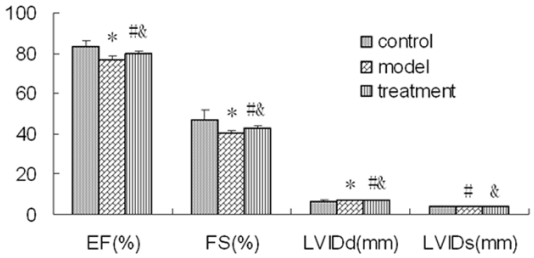

Figure 4. Echocardiography results in the three groups at 8 weeks after THP administration. *P < 0.01, #P < 0.05 compared to the control group; and &P < 0.05 compared to the model group.

orderly, the Z and M lines were clear, and the nucleus was obvious; (1 point) the lesion range was less than 5%, and the lesion showed cell swelling, mild interstitial edema, and infiltration by a small number of inflammatory cells; (2 points) the lesion range was 5-20%, and the lesion showed mild interstitial edema, inflammatory cell infiltration, and scattered punctate necrosis; (3 points) the lesion range was 20-35%, and the lesion showed moderate interstitial edema, inflammatory cell infiltration, and multifocal necrosis; (4 points) the lesion range was 35-50%, the lesion showed serious interstitial edema, inflammatory cell infiltration, and multifocal necrosis, and there were contacts between the necrotic areas of the lesion; and (5 points) the lesion range was more than 50%, and the lesion showed serious interstitial edema, inflammatory cell infiltration, and focal necrosis.

Eight weeks after the experiment began, the other sample was fixed in 4% glutaraldehyde at 4°C. Pieces of the heart (1×1×1 mm) were processed successively in 1% osmic acid fixations for 3 hours, dehydrated in an ethanol series, embedded in Epon 812 epoxy resin, and sectioned. Uranyl acetate and lead citrate double-staining and transmission electron microscopy (JEM-1200EX) were used to analyze the sections, and photographic images were captured. This procedure was performed on the remaining heart samples of eight animals that were randomly chosen in each group. Four sections were randomly chosen from each heart. One-hundred cells were examined, and the positive rate (%) was calculated.

Statistical analysis

All statistical analyses were performed using SPSS 11.5 software (IBM-SPSS, Inc., Armonk, New York, USA). All data are presented as the means ± standard deviations. One-way analysis of variance (ANOVA) was used for multiple comparisons, and *P* values < 0.05 were considered to be statistically significant.

Results

Trimetazidine ameliorates pirarubicin-induced changes in behavior and body weight

Symptoms were observed in the model and treatment groups at 1 week after pirarubicin was administered. These included poorer men-

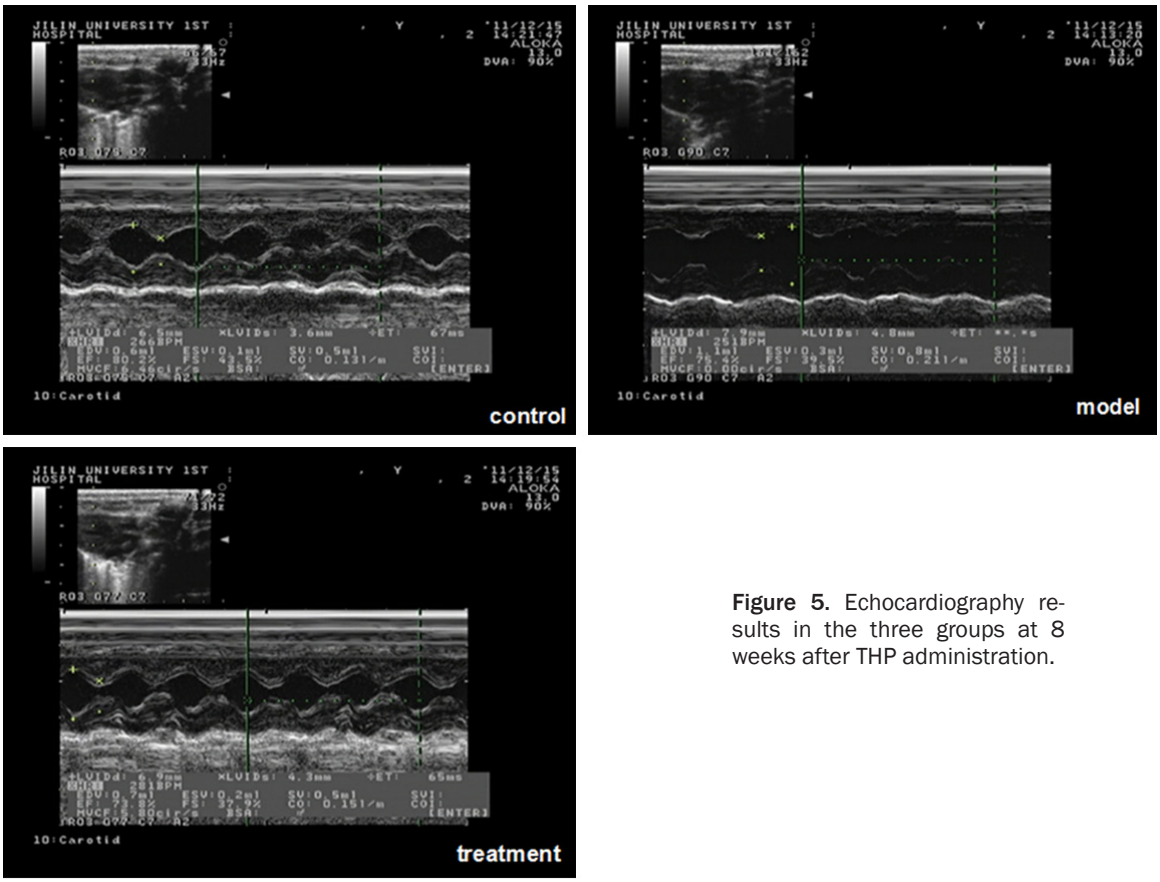

Figure 5. Echocardiography results in the three groups at 8 weeks after THP administration.

tal functions, diminished activity, and decreased diet. Some rats exhibited depilation, diarrhea, and weight loss. The symptoms observed in the treatment group were less severe than those observed in the model group. During the 8-week experimental period, 3 rats in the model group and 1 rat in the treatment group died. The body weight of the rats increased with time. The weight in the model group was lower than the weight in the control group (379.23 ± 47.81 vs. 499.11 ± 59.98, $P < 0.05$), and the weight in the treatment group was higher than the weight in the model group (424.80 ± 35.73 vs. 379.23 ± 47.81; $P < 0.05$), as shown in **Figure 1**.

Trimetazidine reduces the levels of cardiac enzymes

Myoglobin, troponin, and alanine aminotransferase (ALT) levels were higher in the model group than in the control group (myoglobin: 134.50 ± 40.87 vs. 101.69 ± 22.8, troponin-I: 0.18 ± 0.03 vs. 0.03 ± 0.02, ALT: 51.19 ± 9.81 vs. 43.54 ± 6.81; $P < 0.05$) and lower in the rats that were administered trimetazidine than in the model rats (myoglobin: 102.85 ± 19.57 vs. 134.50 ± 40.87, troponin-I: 0.09 ± 0.04 vs. 0.18 ± 0.03, ALT: 39.51 ± 5.26 vs. 51.19 ± 9.81; $P < 0.05$), as shown in **Figure 2**.

Trimetazidine suppresses oxidative stress

The levels of MDA and NO were higher and the levels of NPSH and SOD were lower in the model group than in the control group. MDA and NO levels were lower (MDA: 5.01 ± 1.44 vs. 5.41 ± 1.32, NO: 22.31 ± 9.61 vs. 25.73 ± 9.58; $P < 0.05$), and NPSH and SOD levels were higher (NPSH: 55.53 ± 9.96 vs. 51.99 ± 12.35, SOD: 17.58 ± 0.97 vs. 13.34 ± 3.21; $P < 0.05$) in the rats administered trimetazidine than in the model group, as shown in **Figure 3**.

Trimetazidine improves echocardiography parameters

The left ventricular ejection fraction (EF) and fractional shortening (FS) were lower, but the left ventricular internal diastolic diameter (LVIDd) and left ventricular internal systolic diameter (LVIDs) were higher, in the model group

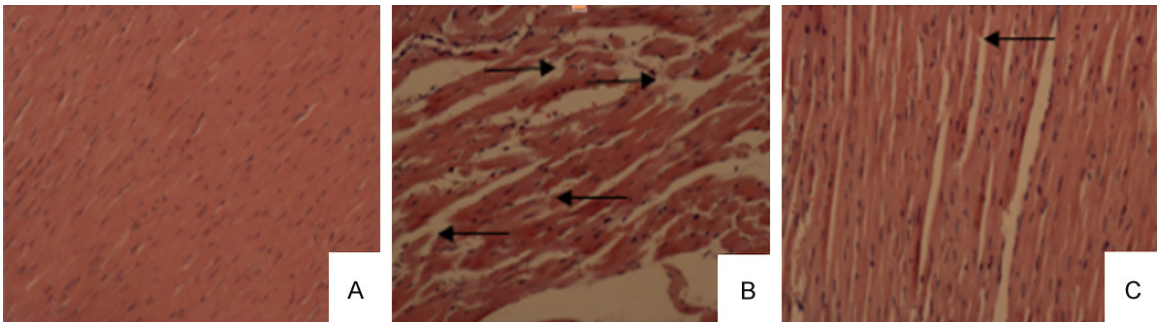

Figure 6. Observation of myocardial tissue obtained from rats in various groups and examined using an optical microscope (×200). A. Control group; B. Model group: multiple visible myocardial cells, myofilament dissolved (→), fractured (←); C: Treatment group: partial dissolution, fracture (←).

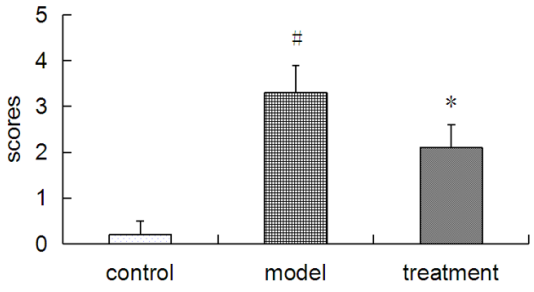

Figure 7. Pathological changes were observed in the myocardial tissues of various groups using an optical microscope. # P < 0.05 compared to the control group, and *P < 0.05 compared to the model group.

than in the control group (EF: 76.98 ± 1.52 vs. 83.04 ± 3.55, FS: 40.46 ± 1.40 vs. 47.24 ± 4.55, LVIDd: 7.16 ± 0.25 vs. 6.68 ± 0.28, P < 0.01; LVIDs: 4.20 ± 0.17 vs. 3.92 ± 0.39, P < 0.05). EF and FS were higher than in the rats treated with trimetazidine than in the model group (EF: 79.47 ± 1.54 vs. 76.98 ± 1.52, FS: 43.09 ± 0.68 vs. 40.46 ± 1.40; P < 0.05), and LVIDd and LVIDs were lower in the rats treated with trimetazidine than in the model group (LVIDd: 6.88 ± 0.32 vs. 7.16 ± 0.25, LVIDs: 3.91 ± 0.25 vs. 4.20 ± 0.17, P < 0.05), as shown in **Figures 4**, **5**.

Trimetazidine prevents myocardial damage observed under optical microscopy

After 8 weeks, the myocardial cells of the rats in the control group were neatly arranged, and their cellular structures were intact (**Figure 6A**). In the model group, myocardial cells were arranged in a disorderly manner, their structure was severely damaged, and myocardial myofilament dissolution and fracturing were visible (**Figure 6B**). In the treatment group, the structure of the myocardial cells was orderly, the structure of the myocardium was essentially preserved, and partial dissolution and fracturing were comparatively absent (**Figures 6**, **7**).

Trimetazidine prevents myocardial damage observed under electron microscopy

After 8 weeks, in the control group, cardiomyocyte sarcomeres were arranged in an orderly fashion, the Z and M lines were clear, and there were many long, oval, and longitudinally arranged mitochondria (**Figure 8A**). In the model group, myocardial myofilaments were dissolved, fractured, or absent, the number of mitochondria was lower, and cytoplasmic matrices were cavitated (**Figure 8B**). In the animals treated with trimetazidine, the cardiomyocyte sarcomeres were neatly arranged, the number of local myofilaments was slightly lower, and the surrounding mitochondria were oval and arranged parallel to each other between myofilament bundles (**Figure 8C**). Hence, the application of trimetazidine reduced mitochondrial damage and relieved myocardial injury (**Figures 8**, **9**).

Discussion

In the present study, we evaluated the effect of trimetazidine and suggest a potential mechanism underlying its activity in pirarubicin-induced myocardial damage. The main findings of this study are the following: 1. injecting THP caused myocardial damage, including the dissolution and fracturing of myofilaments; and 2. TMZ protected against cardiotoxicity via an antioxidative pathway and by upregulating NP-SH and SOD and downregulating MDA and NO.

THP is a derivative of anthracycline that exhibits strong antiproliferative activity. However, its clinical uses are severely limited by its cardio-

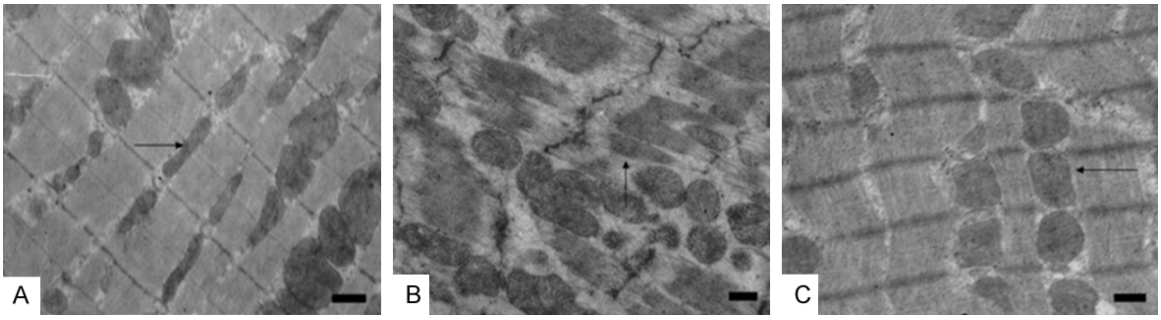

Figure 8. The myocardial tissues of rats in various groups were observed under an electron microscope (×7500), bar =500 nm. A: Control group: cardiomyocyte sarcomeres were arranged in parallel, the Z and M lines were clear, and the mitochondria were oval and arranged in an orderly fashion (→); B: Model group: myocardial muscle bundles were dissolved (↑), fractured, or absent; the number of mitochondria was lower; and the cytoplasmic matrix was cavitated; C: Treatment group: cardiomyocyte sarcomeres were arranged in line (←), the number of local myofilaments was slightly lower, and the surrounding mitochondria were oval and arranged in parallel between muscle bundles.

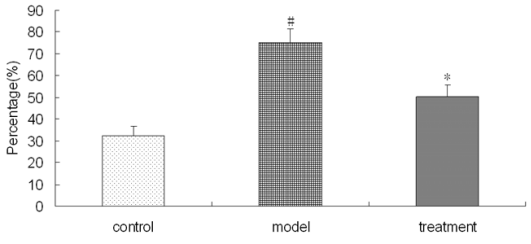

Figure 9. Percentage of muscle bundles exhibiting dissolution in various groups when observed under an electron microscope. #P < 0.05 compared to the control group; *P < 0.05 compared to the model group.

toxic side-effects [5, 12]. THP can intercalate itself into mitochondrial membranes, affecting these organelles and causing the generation of damaging reactive oxygen species (ROS). THP increases ROS concentrations beyond physiological levels [5]. ROS directly or indirectly activate several signaling pathways that lead to cardiomyocyte apoptosis and ultimately to heart failure. Many studies have suggested that the mechanism underlying this protection against cardiotoxicity involves antioxidative activities [13-16]. Sun et al. [14] showed that myricitrin effectively reduced doxorubicin (DOX)-induced cell toxicity by counteracting oxidative stress and increasing the activity of antioxidant enzymes. Das et al. suggested that beet root juice protected against DOX toxicity in cardiomyocytes by reducing the DOX-induced generation of ROS [15].

TMZ is an anti-ischemic agent that is widely used to treat coronary artery disease and does not affect the hemodynamic determinants of myocardial oxygen consumption [17, 18]. The anti-ischemic effects of TMZ have been experimentally assessed in a variety of models [19, 20]. TMZ reportedly protects against smoking-induced left ventricular remodeling by attenuating oxidative stress, apoptosis, and inflammation [10]. It has also been shown that TMZ can shift fatty acid oxidation toward glucose oxidation and attenuate myocardial ischemia/reperfusion injury by activating AMPK and ERK signaling [8].

Study limitations

Our sample size was relatively small, and this may have resulted in unreliable outcomes. Moreover, only one dose of TMZ (5.4 mg/kg/d) was administered in our study. We were therefore unable to determine the effect of using different doses of TMZ on myocardial damage.

In conclusion, in the present study, we provide the first evidence showing that TMZ exerts protective effects in cardiomyocytes that were damaged by pirarubicin, and these effects were most likely related to its antioxidant activity. Hence, the results of the present study may promote the development of a novel drug that can be used to treat pirarubicin-induced cardiotoxicity.

Disclosure of conflict of interest

None.

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