Original Article

Asian Pacific Journal of Tropical Biomedicine

apjtb.org

doi: 10.4103/apjtb.apjtb_346_24

Thrombopoietin ameliorates doxorubicin-induced toxicities in H9c2 myocardiocytes by inhibiting oxidative stress through the SIRT1/p38 MAPK signaling pathway

Xu-Han Zuo[1*], Yu Huang[1*], Bo-Cen Chen[2*], Ming-Yue Zhu[1], Cai-Cai Zhang[1], Han-Yi Jiao[1], Li-Fang Lu[1], Man Xiao[1✉], Han Wang[1✉]

[1]*Key Laboratory of Tropical Translational Medicine of Ministry of Education, School of Basic Medicine and Life Sciences, Hainan Medical University, Haikou, Hainan, China*

[2]*Experiment and Teaching Center for Clinical Skills, Hainan Medical University, Haikou, Hainan, China*

ABSTRACT

Objective: To explore whether thrombopoietin can exert a protective effect against doxorubicin-induced cardiotoxicity by modulating the sirtuin 1 (SIRT1) signaling pathway.

Methods: H9c2 cell viability was determined by CCK-8 and cardiomyocyte apoptosis was detected by TUNEL assay. The protein expressions of SIRT1 and p38 MAPK were measured by Western blot. RT-qPCR was also used to determine *SIRT1* mRNA expression. In addition, intracellular reactive oxygen species levels and antioxidant enzyme activities were evaluated.

Results: Thrombopoietin treatment reversed doxorubicin-induced decline in H9c2 cell viability. It also increased SIRT1 and decreased p-p38 MAPK protein expressions. In addition, thrombopoietin significantly attenuated doxorubicin-induced apoptosis and oxidative stress, and enhanced antioxidant enzyme activities. However, silencing *SIRT1* abrogated the protective effects of thrombopoietin, as evidenced by reduced cell viability and increased oxidative stress and reactive oxygen species levels.

Conclusions: Thrombopoietin alleviates doxorubicin-induced cardiomyocyte injury by reducing oxidative stress and apoptosis *via* the SIRT1/p38 MAPK pathway. However, its protective effects need to be further verified in animal tests.

KEYWORDS: Doxorubicin; Thrombopoietin; Oxidative stress; Sirtuin 1; Cardiotoxicity

1. Introduction

Doxorubicin (DOX) is effective as a broad-spectrum anthracycline. It plays significant roles in leukemia[1], solid tumors, soft tissue sarcomas, and breast cancer. However, the adverse effects of DOX limit its clinical use in the heart. DOX-induced cardiotoxicity causes myofiber deterioration through cardiomyocyte apoptosis[2], endothelial cell apoptosis[3], and intracellular calcium dysregulation[4] and is regulated by several different mechanisms. The main mechanisms studied include oxidative stress[5], mitochondrial dysfunction, DNA damage[6], iron toxicity[7], and autophagy. The main mechanisms of DOX-induced cardiomyopathy are related to oxidative damage and mitochondrial dysfunction. Oxidative stress is caused by an imbalance between reactive oxygen

Significance

Doxorubicin can produce side effects, such as cardiotoxicity. Thrombopoietin exerts a protective effect on the blood system and against drug- and ischemia-induced myocardial injury. In the present study, thrombopoietin protects against doxorubicin-induced myocardial injury by suppressing oxidative stress and apoptosis *via* the the SIRT1/p38MAPK pathway. This offers new insights into the mechanism by which thrombopoietin exerts its cardioprotective effects.

✉To whom correspondence may be addressed. E-mail: xiaohanzi2801@163.com (H.Wang); xiaoman_shen2004@163.com (M. Xiao).
*These authors contributed equally to this work.

How to cite this article: Zuo XH, Huang Y, Chen BC, Zhu MY, Zhang CC, Jiao HY, et al. Thrombopoietin ameliorates doxorubicin-induced toxicities in H9c2 myocardiocytes by inhibiting oxidative stress through the SIRT1/p38 MAPK signaling pathway. Asian Pac J Trop Biomed 2024; 14(9): 410-416.

Article history: Received 13 June 2024; Revision 24 June 2024; Accepted 18 September 2024; Available online 27 September 2024

species (ROS) and endogenous antioxidants[8–11]. Accumulating evidence shows that ROS overproduction is one of the major factors in DOX-induced myocardial toxicity, which leads to the development of oxidative damage and apoptosis in cardiomyocytes and ultimately to heart failure[9,12].

Thrombopoietin (TPO) is an essential regulator of platelet production. It promotes megakaryocytic/platelet lineage and angiogenesis and inhibits apoptosis[13]. TPO is a key hematopoietic growth factor involved in regulating hematopoietic stem cell self-renewal, maintaining multidirectional differentiation, and genomic stability. Additionally, it is a specific physiological regulator of megakaryocyte proliferation, differentiation, maturation, and platelet production[14,15]. TPO signals are mediated through the MPL receptor[16]. Since TPO has been purified, its structure, function, and clinical use have been elaborated[17].

Sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide (NAD)-dependent deacetylase, enhances mitochondrial function and tolerance to oxidative stress in cardiomyocytes by regulating the activity of transcriptional regulators associated with metabolic and antioxidant pathways[18,19]. DOX causes a marked decrease in the antioxidant enzyme activity in cardiac myocytes[20]. The level of SIRT1 protein expression is decreased after DOX addition[21]. The activation of SIRT1 protects the heart from oxidative stress[22]. When SIRT1 expression is upregulated, oxidative stress is inhibited, leading to a decrease in ROS production and an increase in superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) activities.

In addition, a large number of studies have demonstrated that SIRT1 activation can reverse oxidative damage and control apoptosis in cardiomyocytes under DOX[23]. Some studies have shown that SIRT1 and SIRT1 agonists inhibit oxidative stress and apoptosis, and alleviate DOX-induced cardiotoxicity by modulating the p38 MAPK pathway[24,25].

However, whether TPO regulates oxidative stress *via* the SIRT1/p38 MAPK pathway has not yet been reported. Therefore, elucidating the role of SIRT1 in TPO regulation of oxidative stress is imperative. The present study aimed to investigate whether TPO inhibits DOX-induced myocardial injury through the upregulation of SIRT1 expression.

2. Materials and methods

2.1. Cell culture

H9c2 cells (Wuhan Procell Company) were cultured in Dulbecco's Modified Eagle Medium (Wuhan Procell Company) with 10% fetal bovine serum (Yisheng Biotechnology, Shanghai), 100 units/mL penicillin, and 100 mg/mL streptomycin and incubated at 37℃ in a 5% CO_2 incubator. Then H9c2 cells were treated with DOX (Yuanye, Shanghai) (2.5 μg/mL, 24 h) and TPO (3SBio Inc., Shenyang) (10 ng/mL, 24 h)[13].

2.2. Cell viability assay

The medium containing 10% CCK-8 (Biosharp) reagent was added as a replacement for the culture medium, avoiding air bubbles during the process. Then, the plates with CCK-8 were incubated in a 5% CO_2 incubator at 37℃ for 1-2 h, and the OD 450 nm value was controlled to between 0.8 and 1.2.

2.3. Small interfering RNA (siRNA) culture

Cells were cultured in 6-well plates and the medium in the 6-well plates was replaced with 2 mL of complete medium before transfection. A solution mixed with basal medium, siRNA, and Lipo8000 was added to the 6-well plate at 125 μL per well, left to stand for 20 min, and incubated for 24 h. The sequences of siRNAs are as follows: *SIRT1–1319* sense: 5'-GCACUAAUUCCAAGUUCUAUA-3', antisense: 5'-UAGAACUUGGAAUUAGUGCUA-3'; *SIRT1–2143* sense: 5'-GGUUGUUAAUGAAGCUAUAGC-3', antisense: 5'-UAUAGCUUCAUUAACAACCUC-3'; *SIRT1–1712* sense: 5'-CAAGAAGUACAGACUAGUAGG-3', antisense: 5'-UACUAGUCUGUACUUCUUGUG-3'. The sequence of the scrambled siRNA is sense: 5'-UUCUCCGAACGUGUCACGUTT-3', antisense: 5'-ACGUGACACGUUCGGAGAATT-3'.

2.4. Real–time quantitative polymerase chain reaction (RT–qPCR) assay

RNA was extracted with a total RNA extraction kit (Promag Bioproducts, Shanghai) and reverse transcribed into cDNA. Amplification was done by a two-step PCR method: pre-denaturation at 95℃ for 300 s, one cycle of denaturation at 95℃ for 10 s, and 40 cycles of annealing extension at 60℃ for 30 s. The following primers were designed for RT-qPCR: *SIRT1* forward 5'-TGATTGGCACCGATCCTCG-3', reverse 5'-CCACAGCGTCATATCATCCAG-3'; *GAPDH* forward 5'-GCCTGGAGAAACCTGCCAAG-3', reverse 5'-ATTGAGAGCAATGCCAGCCC-3'.

2.5. Western blot analysis

To extract the protein, the cultured cell precipitate was added to the protein lysate, and the protein concentration was determined

at OD 562 nm after incubation with a BCA Protein Assay Kit (Biosharp) for 30 min in a 37℃ incubator, the volume was made up with phosphate-buffered saline (PBS) and then added to the loading buffer boiled at 100℃. After preparing the gel, the samples were added under 90 V constant pressure for 50 min, the PVDF membrane which had been activated in methanol was immersed in the transmembrane solution, 280 A for 60 min and then the protein samples were transferred to the membrane and closed in milk powder for 1 h. The membrane was washed three times in Tris Buffered Saline with Tween 20 (TBST) and probed with the primary antibodies at 4℃ overnight. Then the membrane was washed three times with TBST for 5 min and incubated with the secondary antibody for 1 h. Subsequently, the exposure solution was dropped onto the membrane, and the immunoreactive bands were developed and analyzed on a gel imager (e-blot). The primary antibodies were as follows: rabbit anti-SITR1 (Abcam: ab189494, 1:1 000), anti-p38 MAPK (CST#9212, 1:1 000), anti-p-p38 MAPK (Abcam: ab178867, 1:1 000), anti-GAPDH (Abcam: ab9485, 1:2 500).

2.6. ROS assay

ROS kit (Beyotime Biotechnology) diluted with DCFH-DA with a basal medium at a ratio of 1:1 000. The original cell culture medium in a 6-well plate was removed, and 1 mL of the diluted DCFH-DA was added. The cells were then incubated in a 37℃ incubator for 20 min. After incubation, the cells were washed three times with a basal medium, and ROS was observed using a fluorescence microscope.

2.7. TUNEL assay

Using the One Step TUNEL Apoptosis Assay Kit (Beyotime Biotechnology), cells were prepared and washed once with PBS. They were then fixed with an immunostaining fixative for 30 min and incubated with an immunostaining permeabilizing solution for 5 min at room temperature. After washing twice with PBS, 50 μL of the TUNEL assay solution was added to the samples and incubated for 60 min at 37℃, protected from light. The samples were washed three times with PBS, sealed with an anti-fluorescence quenching sealing solution, and subsequently observed under a fluorescence microscope.

2.8. Determination of SOD, catalase, and GPx activity

SOD assay kit (Nanjing Institute of Bioengineering), CAT assay kit, and GPx assay kit from the Nanjing Institute of Bioengineering were utilized to collect the cell sediment. A specific volume of PBS was added to the cell sediment, ensuring that the ultrasound probe remained submerged below the liquid level. The ultrasound was applied at a power of 300 W, using an ice water bath, with intervals of 3-5 s for a total of four cycles. Protein concentration was determined using the BCA method.

2.9. Statistical analysis

All statistical analyses in this study were performed with GraphPad Prism 9.5.1. All the above experimental results were obtained from three independent experiments. Data were analyzed by one-way analysis of variance (ANOVA). $P<0.05$ indicates a statistically significant difference.

3. Results

3.1. TPO attenuates DOX-induced cardiac injury and increases SIRT1 expression in H9c2 cells

After treatment, cell viability was detected using the CCK-8 method. The results showed that there was no significant change in

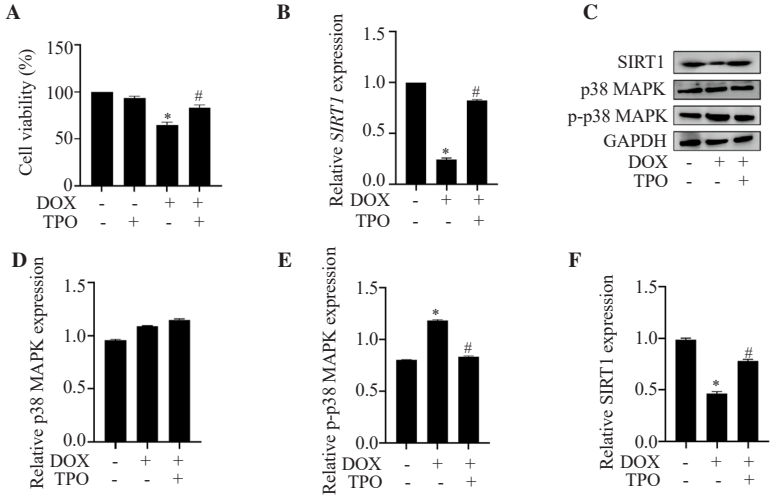

Figure 1. Thrombopoietin attenuates doxorubicin-induced cardiotoxicity. (A) Cell viability was evaluated by CCK8 assay. H9c2 cells were treated with doxorubicin (2.5 μg/mL) and thrombopoietin (10 ng/mL). (B) The mRNA expression of *SIRT1* in H9c2 cells was determined after different treatments. (C-F) Representative blots and quantitative data. Data are normalized to GAPDH. Results are expressed as mean ± SEM of three experiments and analyzed using one-way ANOVA. $^{*}P<0.05$ compared to the normal H9c2 group, $^{\#}P<0.05$ compared to the doxorubicin group. TPO: thrombopoietin, DOX: doxorubicin.

the viability of H9c2 treated with TPO (10 ng/mL) alone compared to the normal group, proving that TPO did not cause damage to H9c2 cells. However, the viability of H9c2 cells was reduced significantly after DOX (2.5 μg/mL) treatment (P<0.05). In contrast, TPO alleviated DOX-induced toxicity in H9c2 cells by markedly increasing cell viability (P<0.05) (Figure 1A). In addition, RT-qPCR results (Figure 1B) exhibited that DOX reduced the mRNA expression of *SIRT1* in H9c2 cells, while TPO reversed this effect. Western blot analysis further corroborated the results of RT-qPCR assay. DOX notably downregulated the expression of SIRT1 protein (P<0.05), while TPO treatment markedly upregulated its protein expression (P<0.05). Moreover, p38 MAPK phosphorylation was activated by DOX in H9c2 cells (P<0.05), which was significantly inhibited by TPO treatment (P<0.05).

3.2. TPO attenuates siSIRT1–induced decline in H9c2 cell viability

According to RT-qPCR results (Figure 2A), the screening of siRNAs identified Mouse-SIRT1-2143 had the highest silencing efficiency, so it was used for subsequent experiments. H9c2 cell viability significantly decreased after silencing *SIRT1* (P<0.05) (Figure 2B). TPO treatment could abrogate siSIRT1-induced decline in cell viability (P<0.05). The PCR results (Figure 2D) showed that *SIRT1* mRNA expression was significantly reduced after silencing *SIRT1*, and TPO could reverse this change (P<0.05), which was further verified in Western blot results (Figure 2C and 2E).

3.3. TPO inhibits DOX–induced increase in ROS

The fluorescence intensity of ROS significantly increased in the DOX group. By contrast, TPO treatment significantly reduced DOX-induced ROS level in H9c2 cells. Furthermore, silencing *SIRT1* caused a marked rise in ROS level which was inhibited by TPO treatment (Figure 3).

3.4. TPO inhibits DOX–induced cardiomyocyte apoptosis

As shown in Figure 4, green fluorescence pronouncedly increased in the DOX group, but it decreased in the TPO group, indicating that TPO attenuated DOX-induced apoptosis. In addition, silencing *SIRT1* caused damage to H9c2 cells by increasing cell apoptosis, which was reversed by TPO treatment. These findings demonstrated that TPO attenuates H9c2 cell apoptosis caused by *SIRT1* silencing.

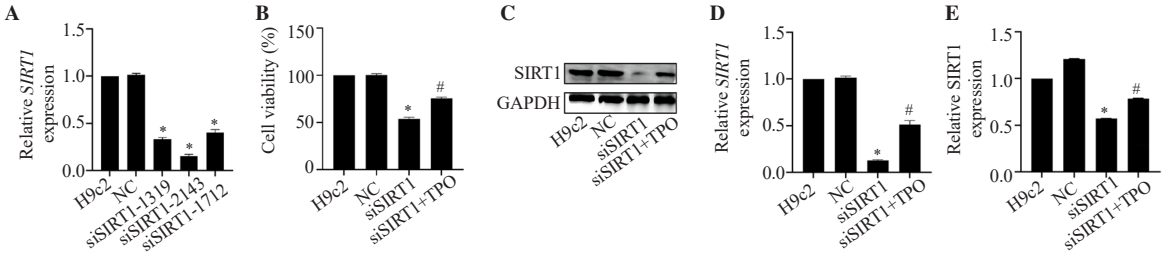

Figure 2. Thrombopoietin attenuates siSIRT1-induced cardiac injury in cells. (A) Expression of *SIRT1* after transfection with siRNA. siSIRT1-2143 shows the highest silencing efficiency, therefore, it is used for subsequent experiments. (B) The viability of H9c2 cells after *SIRT1* silencing. (C) Western blot bands. (D-E) The mRNA and protein expression of SIRT1 in H9c2 cells. Results are expressed as mean ± SEM of three experiments and analyzed using one-way ANOVA. $^{*}P$<0.05 compared to the normal H9c2 group, $^{\#}P$<0.05 compared to the siSIRT1 group. NC: negative control.

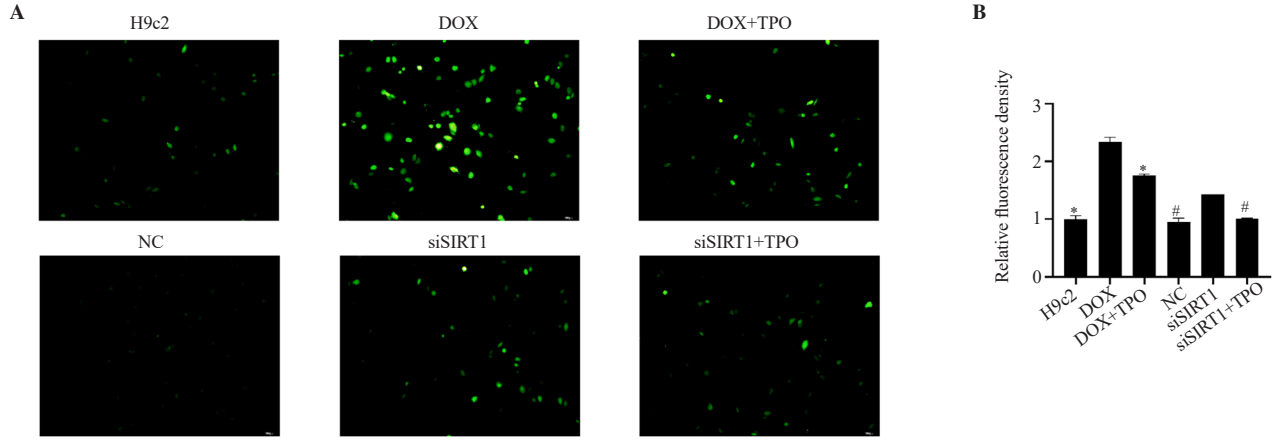

Figure 3. ROS detection in H9c2 cardiomyocytes (scale bar = 100 μm). Results are expressed as mean ± SEM of three experiments and analyzed using one-way ANOVA. $^{*}P$<0.05 compared to the doxorubicin group, $^{\#}P$<0.05 compared to the siSIRT1 group.

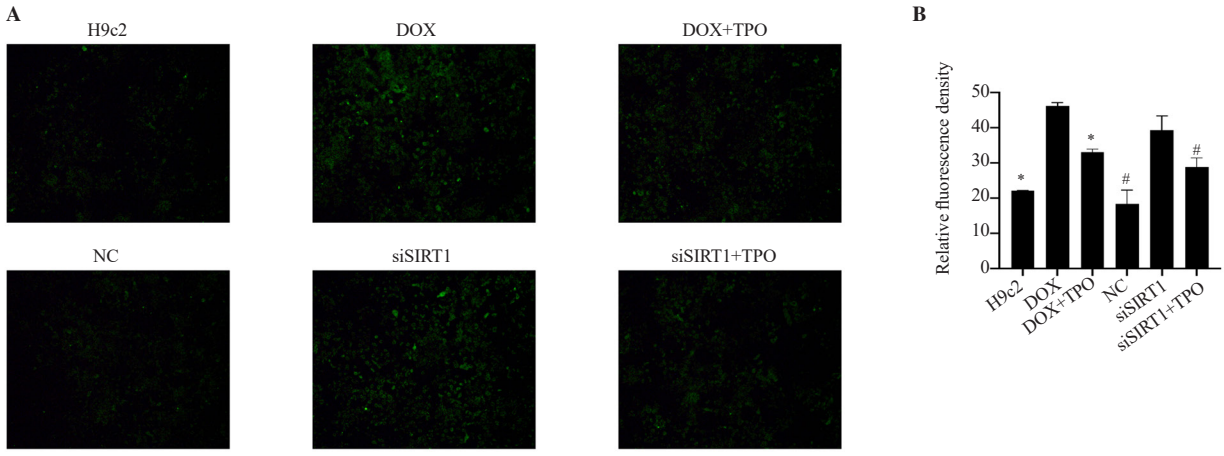

Figure 4. Detection of apoptosis after thrombopoietin treatment by TUNEL assay (scale bar = 100 μm). Results are expressed as mean ± SEM of three experiments and analyzed using one-way ANOVA. $^{*}P<0.05$ compared to the doxorubicin group, $^{\#}P<0.05$ compared to the siSIRT1 group.

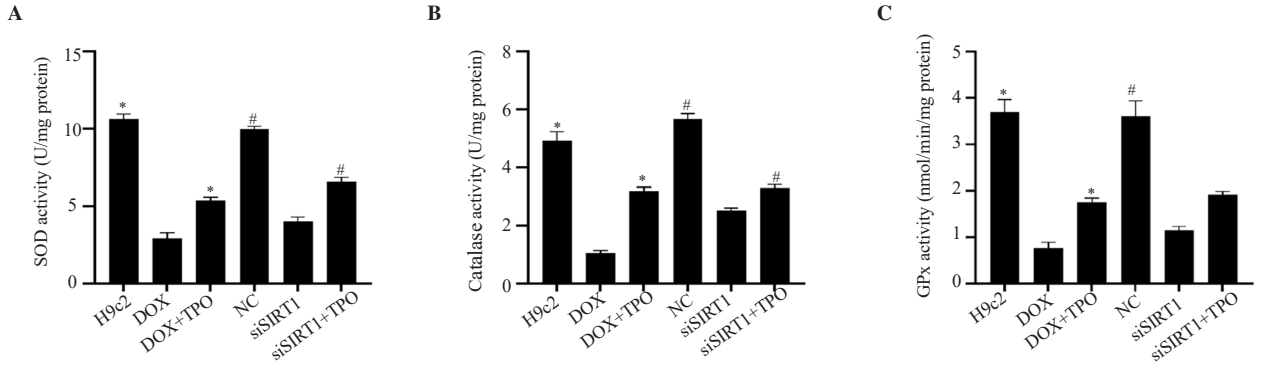

Figure 5. SOD, catalase, and GPx activities after thrombopoietin treatment. Results are expressed as mean ± SEM of three experiments and analyzed using one-way ANOVA. $^{*}P<0.05$ compared to the doxorubicin group, $^{\#}P<0.05$ compared to the siSIRT1 group. SOD: superoxide dismutase, GPx: glutathione peroxidase.

3.5. TPO reverses DOX-induced decrease in antioxidant enzyme activity

SOD, catalase, and GPx activities were significantly reduced in the DOX group compared to the normal H9c2 group ($P<0.05$). TPO treatment notably enhanced antioxidant enzyme activities ($P<0.05$) (Figure 5). Moreover, silencing *SIRT1* induced a significant decline in SOD, catalase, and GPx activities ($P<0.05$). TPO abrogated its reducing effects in these enzyme activities.

4. Discussion

DOX is a class of anthracycline anticancer drugs widely used for the treatment of a wide range of solid and hematological malignancies. However, its clinical application is severely limited due to DOX-induced cardiotoxicity[26]. The current findings are consistent with those observed in earlier studies. DOX caused cardiomyocyte damage[27,28] and elevated ROS leading to apoptosis and eventually myocardial toxicity[29,30]. Previous studies found that TPO alleviated DOX-induced myocardial toxicity by inhibiting autophagy and apoptosis[13]. In the present study, TPO alleviated DOX-induced myocardial toxicity by inhibiting ROS and apoptosis. Our findings corroborated the results of previous studies, indicating that TPO protects cardiac cells against DOX-induced cellular damage.

SIRT1 belongs to the Sirtuin family of NAD-dependent enzymes that play multiple roles in metabolism and disease biology. SIRT1 has a key function in the regulation of multiple physiological processes such as oxidative stress, inflammation, apoptosis and autophagy[25]. In the current study, we found that DOX inhibits the level of SIRT1 in cardiomyocytes, while TPO can increase its level.

Oxidative stress plays a critical role in DOX-induced myocardial injury, and ROS levels and antioxidant enzyme activity are major indicators of oxidative stress in cells. The level of ROS was significantly increased, and the activity of antioxidant enzymes was decreased in DOX-treated cells, indicating that DOX causes oxidative stress in cardiomyocytes[31]. DOX-induced upregulation

of ROS levels and a decrease in antioxidant enzyme activity were reversed by TPO treatment.

p38 MAPK is known as a downstream effector of SIRT1[25]. Moreover, SIRT1 acts as an inhibitor of p38 MAPK, inhibiting its phosphorylation and biological function, as well as oxidative stress by suppressing the expression of p38 MAPK and inhibiting ROS production[32]. The results of Western blot analysis showed that SIRT1 expression was downregulated and p-p38 MAPK expression was upregulated after the addition of DOX. Silencing *SIRT1* caused no changes in ROS levels of DOX-treated cells, indicating that DOX increased oxidative stress levels in cardiomyocyte through the SIRT1/p38 MAPK pathway. Conversely, the downregulation of SIRT1 expression was reversed by TPO treatment, and the phosphorylation of p38 MAPK was alleviated, thus reducing the level of ROS. These findings suggested that TPO may attenuate DOX-induced cardiotoxicity in H9c2 cells by inhibiting oxidative stress through the SIRT1/p38MAPK pathway.

However, this study has some limitations. Firstly, animal experiments are not conducted for further validation. Secondly, in terms of cellular experiments, there is no further knockdown validation of the p38 MAPK gene. Therefore, further studies are warranted to verify the protective effects of TPO.

In summary, TPO alleviates DOX-induced cardiomyocyte injury by reducing oxidative stress and apoptosis *via* the SIRT1/p38 MAPK pathway. It may be used as a protective agent in the treatment of cardiomyocyte injury, which needs further investigation.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

This study was supported by the Natural Science Foundation of Hainan Province High-level Talent Project (grant number 820RC644) and Innovative Research Projects for Postgraduate Students at Hainan Medical University (grant number HYYS2022B08).

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

XHZ and YH contributed to data curation, investigation, and visualization. XHZ wrote the original draft. BCC planned the experimental protocols, ensuring that each step effectively supported the validation of our research hypotheses. MYZ, CCZ, HYJ, and LFL contributed to the final version of the manuscript. HW and MX supervised the project.

References

[1] Zhang M, Ge Y, Xu S, Fang X, Meng J, Yu L, et al. Nanomicelles co-loading CXCR4 antagonist and doxorubicin combat the refractory acute myeloid leukemia. *Pharmacol Res* 2022; **185**. doi: 10.1016/j.phrs.2022.106503.

[2] Nitobe J, Yamaguchi S, Okuyama M, Nozaki N, Sata M, Miyamoto T, et al. Reactive oxygen species regulate FLICE inhibitory protein (FLIP) and susceptibility to Fas-mediated apoptosis in cardiac myocytes. *Cardiovasc Res* 2003; **57**(1): 119-128.

[3] Grethe S, Coltella N, Di Renzo MF, Pörn-Ares MI. p38MAPK downregulates phosphorylation of Bad in doxorubicin-induced endothelial apoptosis. *Biochem Biophys Res Commun* 2006; **347**(3): 781-790.

[4] Arai M, Yoguchi A, Takizawa T, Yokoyama T, Kanda T, Kurabayashi M, et al. Mechanism of doxorubicin-induced inhibition of sarcoplasmic reticulum $Ca^{(2+)}$-ATPase gene transcription. *Circ Res* 2000; **86**(1): 8-14.

[5] Kong CY, Guo Z, Song P, Zhang X, Yuan YP, Teng T, et al. Underlying the mechanisms of doxorubicin-induced acute cardiotoxicity: Oxidative stress and cell death. *Int J Biol Sci* 2022; **18**(2): 760-770.

[6] Kawano I, Adamcova M. MicroRNAs in doxorubicin-induced cardiotoxicity: The DNA damage response. *Front Pharmacol* 2022; **13**. doi: 10.3389/fphar.2022.1055911.

[7] Ta N, Qu C, Wu H, Zhang D, Sun T, Li Y, et al. Mitochondrial outer membrane protein FUNDC2 promotes ferroptosis and contributes to doxorubicin-induced cardiomyopathy. *Proc Natl Acad Sci U S A* 2022; **119**(36). doi: 10.1073/pnas.2117396119.

[8] Songbo M, Lang H, Xinyong C, Bin X, Ping Z, Liang S. Oxidative stress injury in doxorubicin-induced cardiotoxicity. *Toxicol Lett* 2019; **307**: 41-48.

[9] Wu YZ, Zhang L, Wu ZX, Shan TT, Xiong C. Berberine ameliorates doxorubicin-induced cardiotoxicity *via* a SIRT1/p66Shc-mediated pathway. *Oxid Med Cell Longev* 2019; **2019**. doi: 10.1155/2019/2150394.

[10]Cheng X, Liu D, Song H, Tian X, Yan C, Han Y. Overexpression of Kininogen-1 aggravates oxidative stress and mitochondrial dysfunction in DOX-induced cardiotoxicity. *Biochem Biophys Res Commun* 2021; **550**: 142-150.

[11]Liu D, Ma Z, Xu L, Zhang X, Qiao S, Yuan J. PGC1α activation by pterostilbene ameliorates acute doxorubicin cardiotoxicity by reducing

oxidative stress *via* enhancing AMPK and SIRT1 cascades. *Aging (Albany NY)* 2019; **11**(22): 10061-10073.

[12]Hu C, Zhang X, Wei W, Zhang N, Wu H, Ma Z, et al. Matrine attenuates oxidative stress and cardiomyocyte apoptosis in doxorubicin-induced cardiotoxicity *via* maintaining AMPKα/UCP2 pathway. *Acta Pharm Sin B* 2019; **9**(4): 690-701.

[13]Wang H, Wang H, Liang EY, Zhou LX, Dong ZL, Liang P, et al. Thrombopoietin protects H9C2 cells from excessive autophagy and apoptosis in doxorubicin-induced cardiotoxicity. *Oncol Lett* 2018; **15**(1): 839-848.

[14]Baker JE, Su J, Hsu A, Shi Y, Zhao M, Strande JL, et al. Human thrombopoietin reduces myocardial infarct size, apoptosis, and stunning following ischaemia/reperfusion in rats. *Cardiovasc Res* 2008; **77**(1): 44-53.

[15]Tong W, Lodish HF. Lnk inhibits Tpo-mpl signaling and Tpo-mediated megakaryocytopoiesis. *J Exp Med* 2004; **200**(5): 569-580.

[16]Kuter DJ, Begley CG. Recombinant human thrombopoietin: basic biology and evaluation of clinical studies. *Blood* 2002; **100**(10): 3457-3469.

[17]Li L, Yi C, Xia W, Huang B, Chen S, Zhong J, et al. c-Mpl and TPO expression in the human central nervous system neurons inhibits neuronal apoptosis. *Aging (Albany NY)* 2020; **12**(8): 7397-7410.

[18]Chan SH, Hung CH, Shih JY, Chu PM, Cheng YH, Lin HC, et al. SIRT1 inhibition causes oxidative stress and inflammation in patients with coronary artery disease. *Redox Biol* 2017; **13**: 301-309.

[19]Alves-Fernandes DK, Jasiulionis MG. The role of SIRT1 on DNA damage response and epigenetic alterations in cancer. *Int J Mol Sci* 2019; **20**(13). doi: 10.3390/ijms20133153.

[20]Liao W, Rao Z, Wu L, Chen Y, Li C. Cariporide attenuates doxorubicin-induced cardiotoxicity in rats by inhibiting oxidative stress, inflammation and apoptosis partly through regulation of akt/gsk-3β and Sirt1 signaling pathway. *Front Pharmacol* 2022; **13**. doi: 10.3389/fphar.2022.850053.

[21]Sun Z, Lu W, Lin N, Lin H, Zhang J, Ni T, et al. Dihydromyricetin alleviates doxorubicin-induced cardiotoxicity by inhibiting NLRP3 inflammasome through activation of SIRT1. *Biochem Pharmacol* 2020; **175**. doi: 10.1016/j.bcp.2020.113888.

[22]Abd El-Aziz TA, Mohamed RH, Pasha HF, Abdel-Aziz HR. Catechin protects against oxidative stress and inflammatory-mediated cardiotoxicity in adriamycin-treated rats. *Clin Exp Med* 2012; **12**(4): 233-240.

[23]Xu F, Xu J, Xiong X, Deng Y. Salidroside inhibits MAPK, NF-κB, and STAT3 pathways in psoriasis-associated oxidative stress *via* SIRT1 activation. *Redox Rep* 2019; **24**(1): 70-74.

[24]Wang AJ, Tang Y, Zhang J, Wang BJ, Xiao MJ, Lu G, et al. Cardiac SIRT1 ameliorates doxorubicin-induced cardiotoxicity by targeting sestrin 2. *Redox Biol* 2022; **52**. doi: 10.1016/j.redox.2022.102310.

[25]Ruan Y, Dong C, Patel J, Duan C, Wang X, Wu X, et al. SIRT1 suppresses doxorubicin-induced cardiotoxicity by regulating the oxidative stress and p38MAPK pathways. *Cell Physiol Biochem* 2015; **35**(3): 1116-1124.

[26]Tai P, Chen X, Jia G, Chen G, Gong L, Cheng Y, et al. WGX50 mitigates doxorubicin-induced cardiotoxicity through inhibition of mitochondrial ROS and ferroptosis. *J Transl Med* 2023; **21**(1): 823.

[27]Chen L, Byer SH, Holder R, Wu L, Burkey K, Shah Z. Wnt10b protects cardiomyocytes against doxorubicin-induced cell death *via* MAPK modulation. *PLoS One* 2023; **18**(10). doi: 10.1371/journal.pone.0277747.

[28]Anwar MJ, Alenezi SK, Azam F, Mahmood D, Imam F, Alharbi KS. *Nigella sativa* oil alleviates doxorubicin-induced cardiomyopathy and neurobehavioral changes in mice: *In vivo* and *in-silico* study. *Asian Pac J Trop Biomed* 2022; **12**(7): 312-322.

[29]Jeong Y, Lee SH, Lee J, Kim MS, Lee YG, Hwang JT, et al. Water extract of *Capsella bursa-pastoris* mitigates doxorubicin-induced cardiotoxicity by upregulating antioxidant enzymes. *Int J Mol Sci* 2023; **24**(21). doi: 10.3390/ijms242115912.

[30]Wagh SS, Patil KR, Mahajan UB, Bagal PD, Wadkar AR, Bommanhalli B, et al. Phloretin-induced suppression of oxidative and nitrosative stress attenuates doxorubicin-induced cardiotoxicity in rats. *Asian Pac J Trop Biomed* 2022; **12**(3): 124-131.

[31]Barangi S, Hayes AW, Karimi G. The role of lncRNAs/miRNAs/Sirt1 axis in myocardial and cerebral injury. *Cell Cycle* 2023; **22**(9): 1062-1073.

[32]Li S, He Q, Chen B, Zeng J, Dou X, Pan Z, et al. Cardamonin protects against iron overload-induced arthritis by attenuating ROS production and NLRP3 inflammasome activation *via* the SIRT1/p38MAPK signaling pathway. *Sci Rep* 2023; **13**(1): 13744. doi: 10.1038/s41598-023-40930-y.

Publisher's note

Edited by Liang Q, Tan BJ