A novel peptide HSP-17 ameliorates oxidative stress injury and apoptosis in H9c2 cardiomyocytes by activating the PI3K/Akt pathway

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Background: Oxidative stress and cell apoptosis play pivotal roles in the pathogenesis of doxorubicin (DOX)-induced myocardial injury. Heat shock protein-derived peptide (HSP-17) is a peptide which is low-expressed in DOX treated mouse heart tissue. It has high bioactivity and interspecies sequence consistency, and is predicted to have myocardial protective effect.

Methods: Firstly, we added 1 μM DOX to H9c2 cell culture medium for 24 hours to construct the myocardial cytotoxicity model. Then we detected the effect of HSP-17 on DOX induced H9c2 cardiomyocyte injury by measuring cell viability and lactate dehydrogenase (LDH) level. In addition, reactive oxygen species (ROS) and tetraethylbenzimidazolylcarbocyanine iodide kits are used to evaluate the effect of the HSP-17 peptide on DOX-induced oxidative stress injury to cardiomyocytes, and the detection of apoptosis related proteins and flow cytometry were applied to detect the level of apoptosis. Furthermore, the protein expression levels [phosphorylated Akt (p-Akt) and phosphorylated PI3K (p-PI3K)] of the PI3K/Akt pathway were also detected by western blotting.

Results: We found that the HSP-17 peptide can increase cell viability, protect mitochondrial potential, reduce LDH levels, and reduce ROS and cardiomyocyte apoptosis. In addition, we also observed that HSP-17 upregulated the expression level of p-Akt, and LY294002, a typical inhibitor of PI3K/Akt, was found to eliminate the protective roles of HSP-17.

Conclusions: In conclusion, this study demonstrated that the HSP-17 peptide protected H9c2 cells against oxidative stress and apoptosis via PI3K/Akt pathway activation, which provides a new idea for the treatment of DOX-induced myocardial injury.

Keywords: Doxorubicin (DOX); oxidative stress; apoptosis; HSP-17; PI3K/Akt

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Introduction

At present, tumors are becoming increasingly common in the clinic and are a major cause of human mortality, presenting a growing threat to human health. As an anthracycline antibiotic, doxorubicin (DOX) is one of the most commonly used broad-spectrum and highly effective antitumor drugs. However, DOX has an inevitable toxic effect on the heart while killing tumor cells (1); the cardiotoxicity caused by DOX seriously limits its widespread application in clinical antitumor therapy (2). Therefore, reducing the toxicity of DOX has become a challenge that needs to be urgently overcome. Due to its high affinity for cardiomyocytes, DOX can easily accumulate in cardiomyocytes, inducing cardiomyocyte injury (3). It has been reported that DOX can also produce excessive reactive oxygen species (ROS), cause mitochondrial oxidative stress damage, induce cardiomyocyte apoptosis, and eventually lead to heart failure (4). Therefore, the inhibition of DOX-induced oxidative stress injury and cardiomyocyte apoptosis may be an important therapeutic target for DOX-induced myocardial injury.

A peptide is a type of bioactive substance with a small molecular weight, simple structure, and wide tissue distribution (5). Peptides exert a unique biological activity; they are widely involved in biological events such as oxidative stress (6), cell proliferation (7), and apoptosis (8). Studies on peptides in cardiovascular diseases are increasing. For example, the α-calcitonin gene-related peptide has an anti-hypertensive effect, alleviates myocardial remodeling, and promotes angiogenesis and myocardial survival (9). The adiponectin agonist ADP355, an adiponectin-based active peptide, ameliorates DOX-induced cardiac injury by reducing oxidative stress and cardiomyocyte apoptosis (10). Also, osteocrin peptides can reduce oxidative stress injury, inflammation, cell apoptosis, and cardiac dysfunction in DOX-induced cardiac injury (11). In addition, peptides are highly clinically significant for drug transformation due to their small molecular weight, low toxicity, and good targeting abilities, as well as the ease with which they enter cells (5). Therefore, peptides may provide a novel approach for treating DOX-induced myocardial injury.

PI3K/Akt is a serine-threonine protein kinase that plays a vital role in regulating cardiomyocyte proliferation, apoptosis, and glucose uptake (12). Akt has an important protective effect on cardiomyocytes under oxidative stress. In addition, it is known that the PI3K/Akt signaling pathway exerts a key function in DOX-induced myocardial oxidative stress injury and apoptosis (13). It has been reported that DOX can significantly suppress the PI3K/Akt signaling pathway in cardiomyocytes, which is closely associated with its cardiotoxicity (14). Previous experiments have also indicated that a Shenmai injection can sustain mitochondrial homeostasis and improve cardiac function by activating the AMPK and PI3K/Akt pathways in DOX-induced cardiotoxicity (15). Therefore, targeting the PI3K/Akt signaling pathway could prove beneficial for the treatment of DOX-induced myocardial injury.

Our previous study analyzed the peptide profiles of the heart tissue of normal mice and DOX-induced cardiotoxic mice, and identified 236 differentially expressed peptides (fold change ≥2; $P<0.05$). Among them, a 17-amino acid peptide is derived from heat shock protein HSPB6, ASAPLPGFSAPGRLFDQ (HSP-17), with a peptide ranker score of 0.82 (http://bioware.ucd.ie/~compass/biowareweb/; a ranker score >0.5 indicates possible high bioactivity) (16) attracted our attention and was predicted to have cardioprotective effects. Recent studies have shown that HSPB6, a small heat shock protein, plays an important protective role in stress-induced injury in cardiovascular disease (17,18). It has been reported that icariin can protect H9c2 cells from ischemia-reperfusion injury by upregulating the expression of HSPB6 (19). This study also reported that HSPB6 competes with Bcl-2 to bind Beclin 1 (BECN1), which may alleviate the inhibition of Bcl-2 on BECN1 and autophagy, thereby protecting the myocardium and alleviating heart failure (20). In addition, small heat shock proteins also play an important role in oxidative stress (21). A study has shown that some heat shock proteins, including

Highlight box

Key findings

- HSP-17 peptide is the first time to be identified and validated its function.

What is known and what is new?

- Oxidative stress and apoptosis play pivotal roles in the pathogenesis of doxorubicin (DOX)-induced cardiotoxicity.
- HSP-17 peptide can alleviate DOX induced oxidative stress injury and apoptosis in H9c2 cardiomyocytes by activating PI3K/Akt signaling pathway.

What is the implication, and what should change now?

- Peptides are highly clinically significant for drug transformation due to their small molecular weight, low toxicity, and good targeting abilities, as well as the ease with which they enter cells. Our study can provide a new idea for the treatment of DOX-induced myocardial injury.

Hspb6, can limit cell death by inhibiting oxidative stress damage (22). Some researchers found that the transplantation of Hsp20 transgenic rat mesenchymal stem cells (Hsp20-MSCs) to the myocardial infarction area can improve cardiac function by enhancing the activation of Akt and increasing the secretion of growth factors to resist oxidative stress injury (23).

In the present study, H9c2 cells were used to construct a stable DOX-induced cardiomyocyte injury model. We found that the HSP-17 peptide can increase cell viability, and reduce cardiomyocyte apoptosis as well as the levels of LDH and ROS. It was also shown that HSP-17 upregulated the expression of phosphorylated Akt (p-Akt) in the DOX-induced cardiomyocyte injury model, and LY294002 (LY) (24), a typical inhibitor of PI3K/Akt, was shown to eliminate the protective roles of HSP-17. In summary, this study elucidated the effect of the HSP-17 peptide in DOX-induced cardiomyocyte injury, providing novel insights into the treatment of DOX-induced myocardial injury. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6007/rc).

Methods

Peptide synthesis

HSP-17 and scramble peptide (Scr) were chemically synthesized by Shanghai Science Peptide Biological Technology Co., Ltd. (China) with >95% purity. A cell-penetrating peptide RKKRRQRRR (derived from human immunodeficiency virus type 1 transactivator of transcription) was conjunct to HSP-17 and Scr to facilitate their penetrating cytomembrane. The amino acid sequences of the peptides were as follows: Peptide HSP-17, RKKRRQRRR-ASAPLPGFSAPGRLFDQ; Scr, RKKRRQRRR-PAFGLPSADPSLAGFQR. Scr was used as the control.

Cell culture and intervention

The H9c2 cells were purchased from the Chinese Academy of Science (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% streptomycin and penicillin in an incubator (5% CO_2 and 95% air at 37 ℃). The passage culture of H9c2 cells was usually performed every 2 days, and cells that were in good condition were used in the experiment.

Treatment with 1 μM of DOX was performed for 24 h to establish a DOX-induced myocardial injury *in vitro* model, as previously described (25). The peptide was then added to the culture medium 2 h before DOX administration. The survival status of H9c2 cells was observed under the microscope, and the effects of DOX and peptides on cells were recorded by taking pictures under the microscope.

Cell counting kit-8 (CCK-8) assay

The H9c2 cells (1×10^4/well) suspended in 100 μL of DMEM were seeded in a 96-well plate and treated (as described in section 2.2) following attachment. Next, 10 μL of CCK-8 reagent (Dojindo Molecular Technologies, Inc. Japan) was added to the culture medium and incubated in the dark at 37 ℃ for 2 h. The optical density (OD) was then tested at 450 nm using a microplate reader (Molecular Devices, LLC, America). Cell viability was calculated using the OD values of the experimental and control wells.

Lactate dehydrogenase (LDH) release

The H9c2 cells (1×10^4/well) were cultured in a 96-well plate and then treated as described in section 2.2. Next, all of the culture supernatant fluid was transferred to another 96-well plate, and the LDH level was assessed using an LDH assay kit (Beyotime Institute of Biotechnology, China), according to the manufacturer's instructions. The OD was then detected at 490 nm.

Flow cytometry

Flow cytometry is a common experimental method for detecting apoptosis. This experiment aimed to detect the effect of the HSP-17 peptide on the DOX-induced apoptosis of H9c2 cells using flow cytometry. The H9c2 cells were seeded in a six-well plate (5×10^5/well) and treated as described in section 2.2. The cells were then washed with cold PBS and trypsinized. Subsequently, the cells were suspended in a 500 μL binding buffer, and 5 μL Annexin V fluorescein isothiocyanate (AnnexinV-FITC) and 5 μL of propidium iodide (PI) were added. Subsequently, the cells were cultured in the dark for 15 min. Finally, flow cytometry was performed (BD Biosciences, America).

Detection of intracellular ROS

As described in section 2.2, the cells were seeded in a six-well plate (5×10^5/well) and then dyed with 10 μM of 2',7'-Di

chlorodihydrofluorescein diacetate (DCFH-DA) (Beyotime Institute of Biotechnology) in the dark for 20 min at 37 ℃. After washing twice with DMEM, the images were captured using a fluorescence microscope (Carl Zeiss AG, Germany).

Western blotting

The H9c2 cells were seeded in six-well plates (5×10^5/well) and treated as described in section 2.2. Western blotting was then performed as previously described (26). Briefly, a total of 30 μg of protein was transferred to a polyvinylidene fluoride (PVDF) membrane following SDS-PAGE. The membrane was blocked with 5% non-fat milk buffer and cultured overnight at 4 ℃, followed by the addition of primary antibodies purchased from Cell Signaling Technology, Inc., USA: Anti-poly (ADP-ribose) polymerase (PARP; cat No. 9542, 1:1,000), anti-cleaved-casepase3 (cat No. 9661, 1:1,000), anti-β-actin (cat No. 4970, 1:5,000), anti-Akt (cat No. 9272, 1:1,000), and anti-phospho-Akt (cat No. 4060, 1:1,000). After washing with Tris-Buffered Saline and Tween 20 (TBST) and culturing with secondary antibodies, the membrane was visualized using an enhanced chemiluminescence (ECL) reagent (Millipore, America). Protein expression was quantified according to its grey value, which was analyzed using ImageJ 1.8.0 (National Institutes of Health, America).

Detection of MMP

The H9c2 cells (5×10^5/well) were seeded in a 6-well plate and treated as described in section 2.2. They were washed using PBS and stained with 1 ml of tetraethylbe nzimidazolylcarbocyanine iodide (JC-1) working buffer (Beyotime Institute of Biotechnology, China) for 20 min at 37 ℃. After washing twice with dilution buffer, the cells were photographed using a fluorescence microscope (Nikon,Janpan).

Statistical analysis

Statistical analysis was performed using GraphPad Prism software (v8.0.1; GraphPad Software, Inc. America). All results are presented as the mean ± SD. An unpaired two-sided Student's *t*-test or one-way analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons was used to measure the statistical differences. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Preliminary functional exploration of the HSP-17 peptide

First, the bioactivity and interspecific sequence conservatism of the HSP-17 peptide was analyzed using Peptide Ranker: (http://bioware.ucd.ie/~compass/biowareweb/) and National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi). It was found that the predicted bioactivity score of the HSP-17 peptide was 0.82, indicating that it had high bioactivity (Figure S1), and the sequence consistency of HSP-17 among various species was as high as 94.1% (Figure S2). To study the effect of HSP-17, a model of cytotoxicity was established in H9c2 cells following treatment with 1 μM of DOX for 24 h. Different concentrations of HSP-17 were shown to enhance cell viability in a concentration-dependent manner following DOX treatment (*Figure 1A*). As a control peptide, there was no statistical difference in the effect of Scr on cell viability. Similarly, the HSP-17 peptide decreased LDH release in a concentration-dependent manner (*Figure 1B*). Moreover, there was no significant difference in the effect of HSP-17 between 10 μM and 20 μM. Therefore, we speculated that 10 μM may be the most suitable concentration of the HSP-17 peptide for myocardial protection. The cell death rate was also evaluated and was found to be lowered by 10 μM HSP-17 following DOX treatment (*Figure 1C*,*1D*).

The HSP-17 peptide attenuated DOX-induced oxidative stress injury in H9c2 cells

Oxidative stress injury is a vital biological event in DOX-induced myocardial injury. To study the effect of HSP-17 on DOX-induced oxidative stress injury in H9c2 cardiomyocytes, ROS production was detected using DCFH-DA staining, and the result indicated that 10 μM HSP-17 peptide significantly reduced the ROS level (*Figure 2A*). In addition, JC-1 fluorescence detection illustrated that HSP-17 protected the mitochondrial potential (*Figure 2B*).

The HSP-17 peptide attenuated the apoptosis of H9c2 cardiomyocytes following DOX treatment

Cardiomyocyte apoptosis is the main cause of DOX-induced myocardial injury. Therefore, flow cytometry and western blotting were used to further explore the effect of the HSP-17 peptide on DOX-induced H9c2 cardiomyocyte apoptosis. The experimental results indicated that the

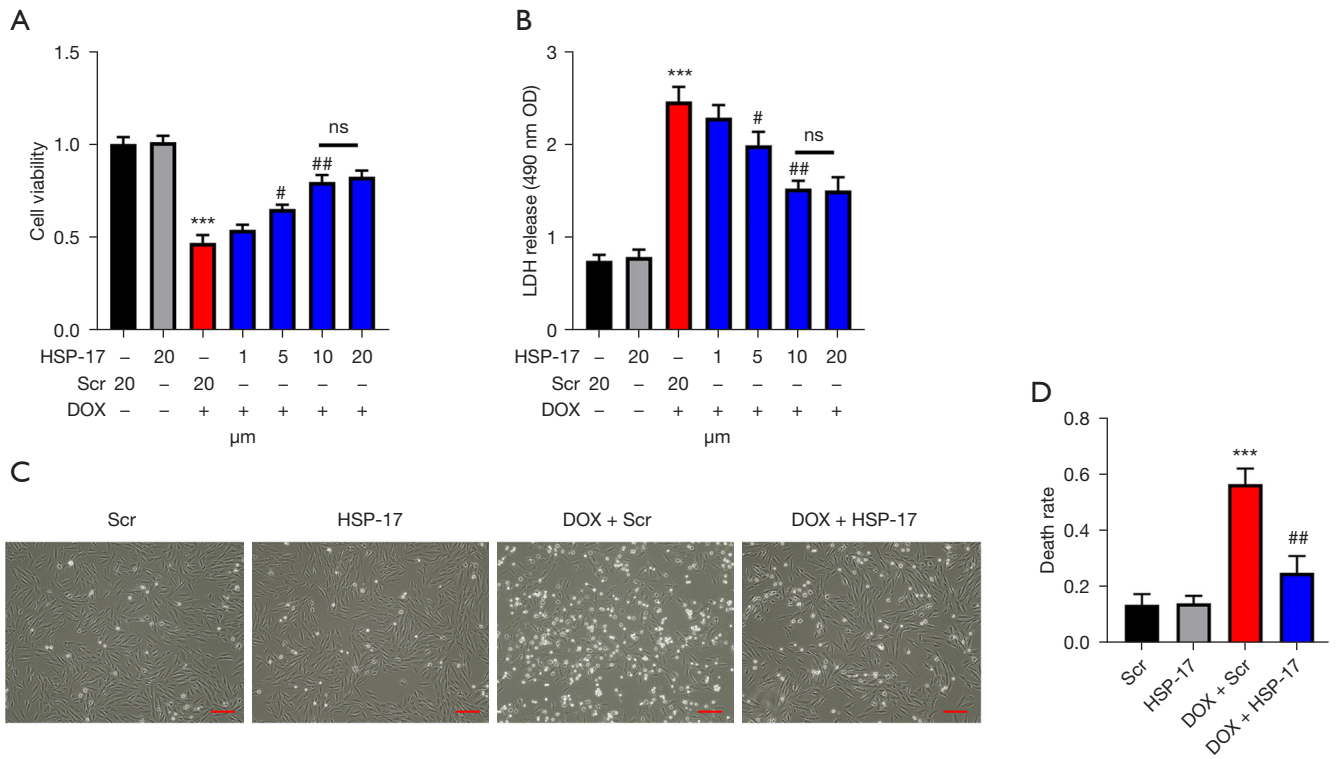

Figure 1 Preliminary functional exploration of the HSP-17 peptide. (A) Viability of H9c2 cells pretreated with different concentrations of HSP-17 for 2 h and then cultured with 1 μM of DOX for 24 h. (B) LDH release level of H9c2 cells pretreated with different concentrations of HSP-17 for 2 h and then cultured with 1 μM of DOX for 24 h. (C) The cell death rate was measured, and the concentration of HSP-17 was 10 μM (magnification, ×100; scale bar =50 μm). (D) Quantitative analysis of the cell death rate. The data are presented as the mean ± SD. ***P<0.001 *vs.* the Scr group; #P<0.05 *vs.* the DOX + Scr group; ##P<0.01 *vs.* the DOX + Scr group. ns, no statistical significance; DOX, doxorubicin; Scr, scramble peptide; HSP-17, a 17-amino acid peptide is derived from heat shock protein; LDH, lactate dehydrogenase; OD, optical density.

number of apoptotic cells was decreased (*Figure 3A*,*3B*), and cleaved PARP (c-PARP) activation and cleaved caspase-3 (c-caspase-3) activation were also lower following HSP-17 peptide treatment (*Figure 3C-3E*).

The HSP-17 peptide activated PI3K/Akt signaling in DOX-treated H9c2 cells

The PI3K/Akt signaling pathway plays a key role in DOX-induced myocardial oxidative stress injury and apoptosis. The key proteins involved in the PI3K/Akt signaling pathway were analyzed by western blotting. The results indicated that 10 μM of the HSP-17 peptide activated the PI3K/Akt pathway in DOX-induced cardiomyocyte injury, as indicated by the increase in p-Akt and phosphorylated PI3K (p-PI3K; *Figure 4A-4C*). The above results confirmed our hypothesis that the HSP-17 peptide acts against DOX-induced cardiomyocyte injury by activating the PI3K/Akt signaling pathway.

LY eliminates the effect of HSP-17 against DOX-induced cardiomyocyte injury

To further confirm whether HSP-17 protected the H9c2 cardiomyocytes against DOX-induced injury by activating the PI3K/Akt signaling pathway, LY, a typical inhibitor of the PI3K/Akt pathway, was used to perform rescue experiments. The western blot analysis results demonstrated that the expression of p-Akt was inhibited when LY was added to the cells following co-treatment with HSP-17 and DOX (*Figure 5A-5C*).

Next, phenotypic rescue experiments were performed to confirm our hypothesis. LY was found to eliminate the protective effects of HSP-17 on cell viability following DOX treatment (*Figure 5D*). Finally, LY also eliminated the inhibition of HSP-17 on the expression of apoptosis

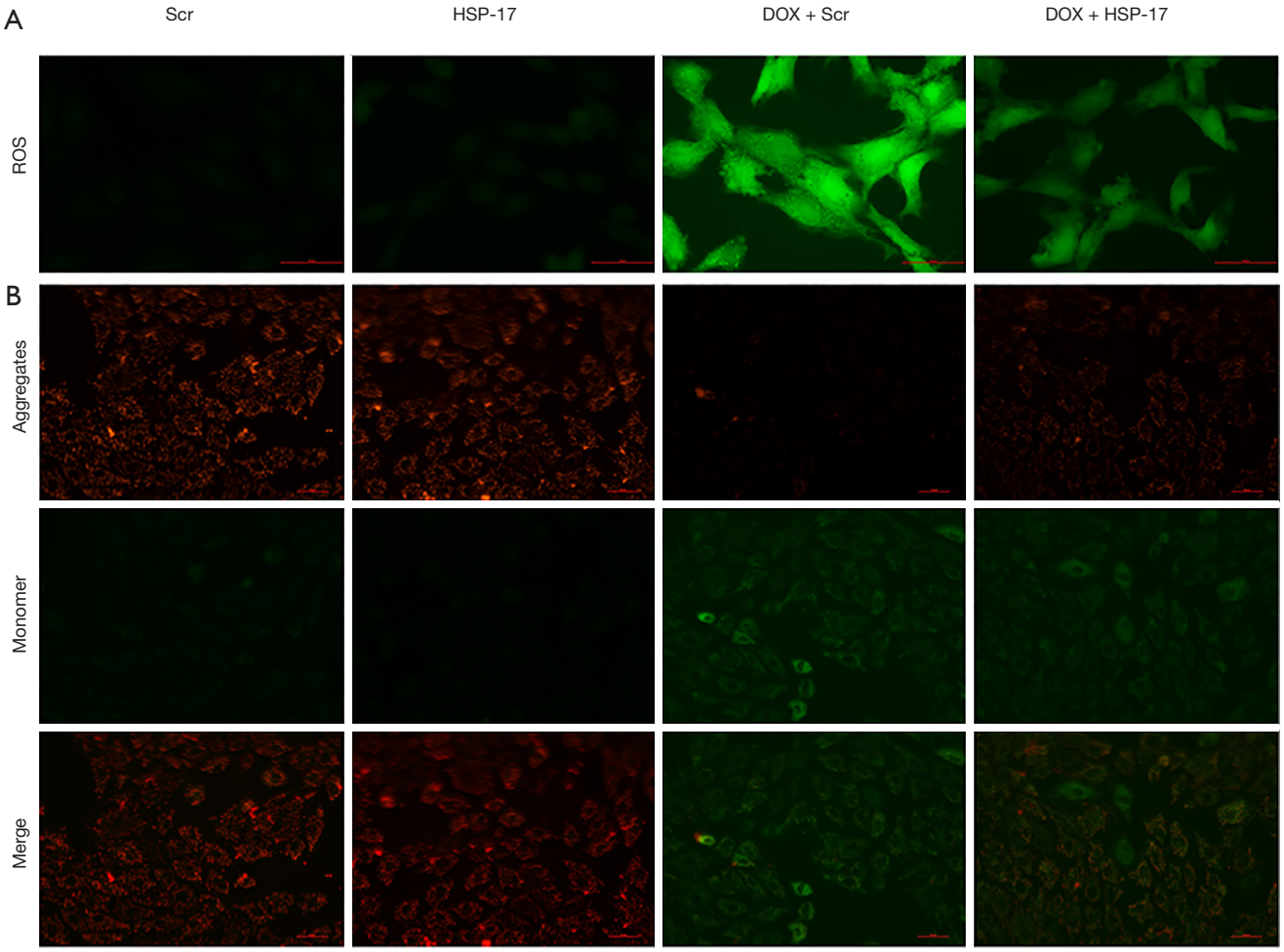

Figure 2 The HSP-17 peptide attenuates DOX-induced oxidative stress injury in H9c2 cells. The peptide concentration used was 10 μM. (A) Intracellular ROS was detected using DCFH-DA staining. Magnification, ×400. Green, ROS. Scale bar =50 μm. (B) Representative images of the mitochondrial membrane potential in H9c2 cells. The H9c2 cells were stained with 1 mL of JC-1 working buffer for 20 min at 37 ℃. Magnification, ×200. Green, Monomer; Red, Aggregates. Scale bar =50 μm. DOX, doxorubicin; Scr, scramble peptide; HSP-17, a 17-amino acid peptide is derived from heat shock protein; ROS, reactive oxygen species; DCFH-DA, dichlorodihydrofluorescein diacetate.

proteins (PARP and c-caspase-3, *Figure 5E-5G*). These findings indicated that LY could rescue the protective effect of HSP-17, and further confirmed the above hypothesis that the HSP-17 peptide protected cardiomyocytes against DOX-induced injury by activating the PI3K/Akt signaling pathway.

Discussion

It is well known that DOX can cause dose-dependent cardiotoxicity, which can lead to cardiomyopathy (2); however, no effective prevention and treatment strategies have been identified thus far. Therefore, our team has long been committed to researching therapeutic approaches for DOX-induced cardiotoxicity. In the present study, the HSP-17 peptide was shown to alleviate DOX-induced oxidative stress injury and apoptosis in H9c2 cardiomyocytes. In addition, through western blotting combined with rescue experiments, it was found that the HSP-17 peptide exerts a cardioprotective effect by activating the PI3K/Akt signaling pathway. This study provides evidence that HSP-17 may become a novel candidate molecule to treat DOX-induced myocardial injury.

Growing evidence shows that oxidative stress and apoptosis play important roles in the pathogenesis of DOX-induced cardiotoxicity (27-29). It has been confirmed

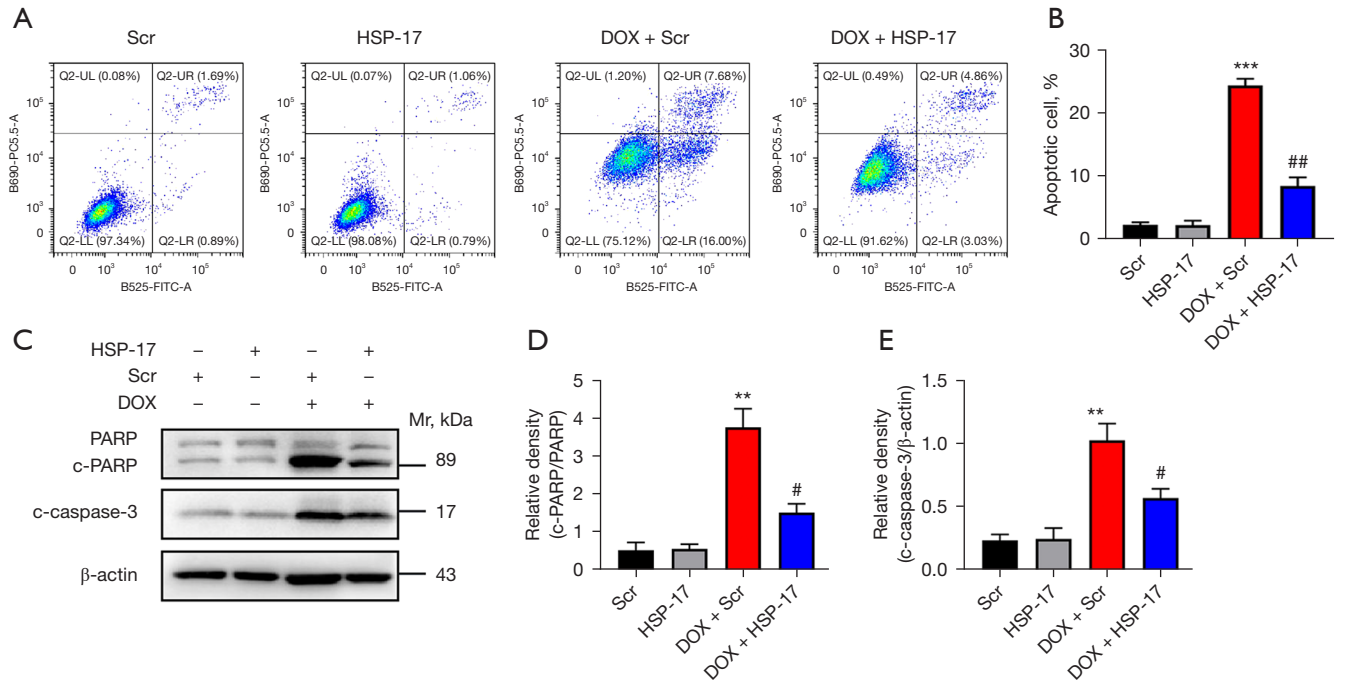

Figure 3 The HSP-17 peptide attenuated H9c2 cardiomyocyte apoptosis following DOX treatment. The peptide concentration used was 10 μM. (A) Representative photographs of flow cytometry. (B) Quantitative analysis of apoptotic cells. (C) Apoptotic proteins (PARP and c-caspase-3) were analyzed using western blotting. (D) Quantitative data of the protein expression of PARP. (E) Quantitative data of the protein expression of c-caspase-3. Data are presented as the mean ± SD. **P<0.01 *vs.* the Scr group; ***P<0.001 *vs.* the Scr group; #P<0.05 *vs.* the DOX + Scr group; ##P<0.01 *vs.* the DOX + Scr group. DOX, doxorubicin; Scr, scramble peptide; HSP-17, a 17-amino acid peptide is derived from heat shock protein; c-PARP, cleaved PARP; c-caspase-3, cleaved caspase-3; PARP, poly (ADP-ribose) polymerase.

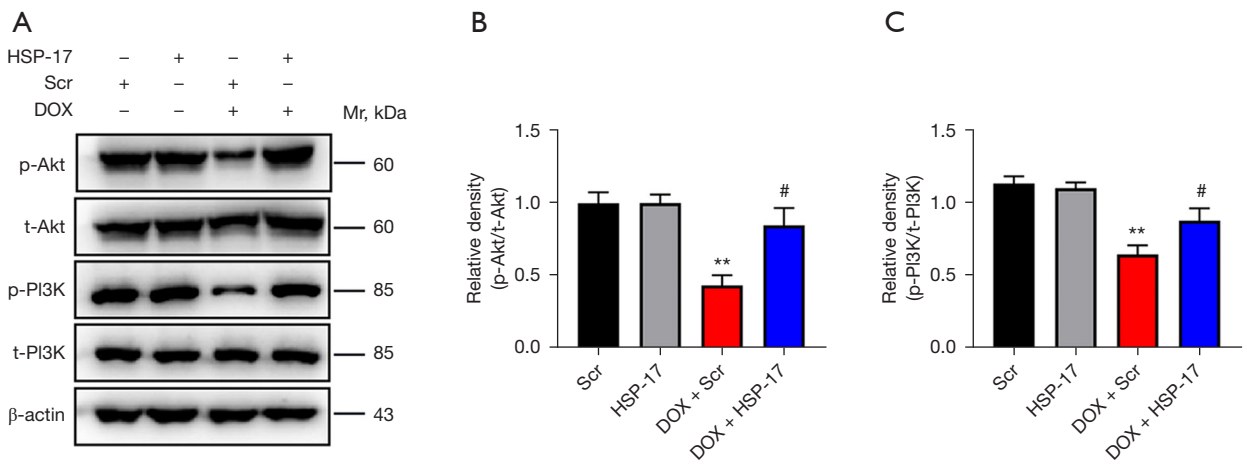

Figure 4 The HSP-17 peptide activates the PI3K/Akt pathway in DOX-treated H9c2 cardiomyocytes. The peptide concentration used was 10 μM. (A) The main proteins involved in the PI3K/Akt signaling pathway were analyzed by western blotting. (B) Quantitative data of the relative expressions of p-Akt and t-Akt. (C) Quantitative data of the relative expressions of p-PI3K and t-PI3K. Data are presented as the mean ± SD. **P<0.01 *vs.* the Scr group; #P<0.05 *vs.* the DOX + Scr group. DOX, doxorubicin; Scr, scramble peptide; HSP-17, a 17-amino acid peptide is derived from heat shock protein; p-Akt, phosphorylated Akt; t-Akt, total Akt; p-PI3K, phosphorylated PI3K; t-PI3K, total PI3K.

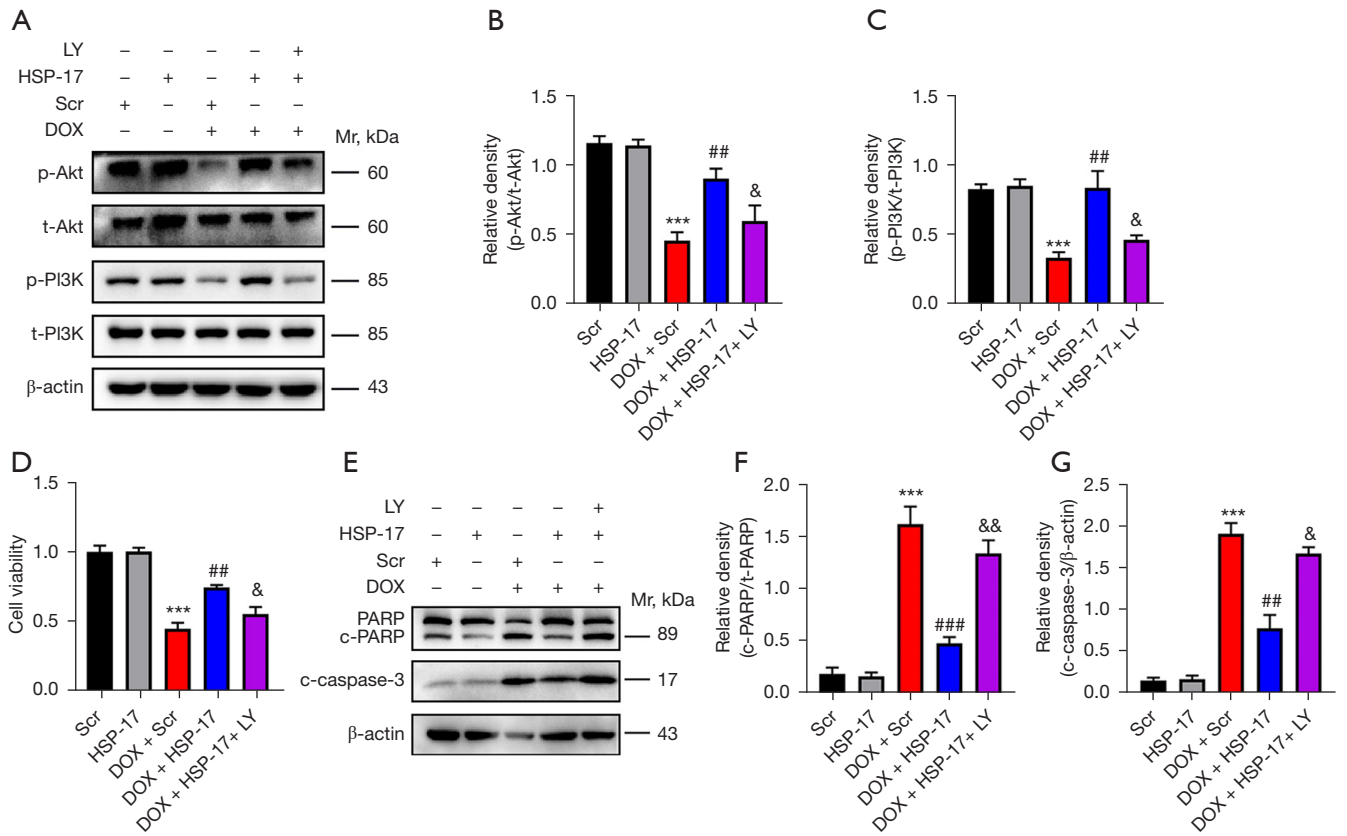

Figure 5 LY eliminates the protective effect of HSP-17. (A) The protein expression of p-Akt was inhibited when LY was added to the cells following co-treatment with HSP-17 and DOX. (B) Quantitative data of the relative expressions of p-Akt and t-Akt. (C) Quantitative data of the relative expressions of p-PI3K and t-PI3K. (D) Cell viability was measured following LY treatment. (E) The apoptotic proteins were detected by western blot analysis. (F) Quantitative data of PARP protein expression. (G) Quantitative data of c-caspase-3 protein expression. Data are represented as the mean ± SD. ***P<0.001 *vs.* the Scr group; ##P<0.01 *vs.* the DOX + Scr group; ###P<0.001 *vs.* the DOX + Scr group; &P<0.05 *vs.* the DOX + HSP-17 group; &&P<0.01 *vs.* the DOX + HSP-17 group. LY, LY294002; DOX, doxorubicin; Scr, scramble peptide; HSP-17, a 17-amino acid peptide is derived from heat shock protein; p-Akt, phosphorylated Akt; t-Akt, total Akt; p-PI3K, phosphorylated PI3K; t-PI3K, PARP, poly (ADP-ribose) polymerase; total PI3K; c-PARP, cleaved PARP; c-caspase-3, cleaved caspase-3.

that DOX-induced cardiotoxicity is associated with the ROS level and the activation of the apoptotic proteins, caspase-3 and PARP (30,31). In addition, Zhang *et al.* found that fibronectin type III domain containing 5 (FNDC5) can reduce DOX-induced myocardial oxidative stress injury and cardiomyocyte apoptosis by activating the Akt signaling pathway (32). In the present study, we verified the crucial role of the HSP-17 peptide in DOX-induced cardiomyocyte injury, primarily manifesting as oxidative stress and apoptosis. Consistent with the above studies, we observed that DOX treatment caused an increase in the ROS level in H9c2 cells. In addition, 10 μM of the HSP-17 peptide was proven to reduce the ROS level. Our study further demonstrates that HSP-17 could inhibit the activation of PARP and caspase-3 and reduce the number of apoptotic cells. Therefore, oxidative stress and apoptosis are the key factors of DOX-induced myocardial injury, and an effective treatment for DOX-induced cardiac damage can be developed using anti-oxidative stress and anti-apoptosis agents as a starting point.

It is known that the PI3K/Akt signaling pathway plays a key role in DOX-induced myocardial oxidative stress injury and apoptosis (32,33). The present study demonstrated that the HSP-17 peptide could activate the PI3K/Akt signaling pathway in DOX-induced cardiomyocyte injury, thereby alleviating cardiomyocyte apoptosis. In addition, LY, as an inhibitor of the PI3K/Akt signaling pathway, abolished the cardioprotective effect of HSP-17. Similarly, brain-

derived neurotrophic factor has been shown to reduce DOX-induced cardiac dysfunction in rats by activating the Akt signaling pathway (34). In addition, hydrogen sulfide has been reported to protect H9c2 cardiomyocytes from DOX-induced cytotoxicity via the PI3K/Akt/forkhead box protein O3 signaling pathway (35). The present results are consistent with those of the aforementioned studies and further substantiate the important effect of the PI3K/Akt pathway in DOX-induced myocardial injury.

Despite confirming that the HSP-17 peptide could protect cardiomyocytes from DOX-induced oxidative stress injury and apoptosis by activating the PI3K/Akt signaling pathway, this study had certain limitations that should be noted. Firstly, the effect (if any) of different peptide modification methods on the function of HSP-17 remains to be verified. Furthermore, the function of the HSP-17 peptide on DOX-induced cardiotoxicity *in vivo* needs to be further elucidated. Therefore, in future work, we intend to evaluate the function of HSP-17 *in vivo* and modify it to clarify its function.

Conclusions

In conclusion, to the best of our knowledge, the present study was the first to show that the HSP-17 peptide can alleviate DOX-induced oxidative stress injury and apoptosis by activating the PI3K/Akt pathway in H9c2 cardiomyocytes. Our results also suggested that HSP-17 is an attractive new candidate molecule for the prevention and treatment of DOX-induced cardiotoxicity.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6007/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6007/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6007/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Figure S1 Bioactivity analysis of the HSP-17 peptide. HSP-17 peptide bioactivity was analyzed using the peptide ranker score (http://bioware.ucd.ie/~compass/biowareweb/).

Species	Peptide sequences																	Identity
Mus musculus	A	S	A	P	L	P	G	F	S	A	P	G	R	L	F	D	Q	100%
Neotoma lepida	A	S	A	P	L	P	G	F	S	A	P	G	R	L	F	D	Q	100%
Rattus norvegicus	A	S	A	P	L	P	G	F	S	T	P	G	R	L	F	D	Q	94.10%
Dipodomys ordii	A	S	A	P	L	P	G	F	S	T	P	G	R	L	F	D	Q	94.10%
Oryctolagus cuniculus	A	S	A	P	V	P	G	F	S	A	P	G	R	L	F	D	Q	94.10%
Homo sapiens	A	S	A	P	L	P	G	L	S	A	P	G	R	L	F	D	Q	94.10%
Bos taurus	A	S	A	P	L	P	G	L	S	A	P	G	R	L	F	D	Q	94.10%
Gulo	A	S	A	P	L	P	G	L	S	A	P	G	R	L	F	D	Q	94.10%
Sheep	A	S	A	P	L	P	G	L	S	A	P	G	R	L	F	D	Q	94.10%
Gorilla	A	S	A	P	L	P	G	L	S	A	P	G	R	L	F	D	Q	94.10%
Goat	A	S	A	P	L	P	G	L	S	A	P	G	R	L	F	D	Q	94.10%
Sousa chinensis	A	S	A	P	L	P	G	L	S	A	P	G	R	L	F	D	Q	94.10%

Figure S2 Sequence consistency of the HSP-17 peptide. Analysis of the HSP-17 peptide sequence consistency in different species (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

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