

Original Article

Kaempferol attenuated cisplatin-induced cardiac injury via inhibiting STING/NF- κ B-mediated inflammation

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Abstract: Cardiovascular complications have been well documented as the downside to conventional cancer chemotherapy. As a notable side effect of cisplatin, cardiotoxicity represents a major obstacle to the successful treatment of cancer. It has been reported that kaempferol (KPF) possesses cardioprotective and anti-inflammatory qualities. However, the effect of KPF on cardiac damage caused by conventional cancer chemotherapy remains unclear. In this study, we clarified the protective effect of KPF on cisplatin-induced heart injury, and conducted in-depth research on the molecular mechanism underlying this effect. The results showed that KPF protected against cardiac dysfunction and injury induced by cisplatin *in vivo*. In H9c2 cells, KPF dramatically reduced cisplatin-induced apoptosis and inflammatory response by modulating STING/NF- κ B pathway. In conclusion, these results showed that KPF had great potential in attenuating cisplatin-induced cardiac injury. Besides, greater emphasis should be placed in the future on natural active compounds containing KPF with anti-inflammatory effects for the treatment of these diseases.

Keywords: Kaempferol, cisplatin, inflammation, STING

Introduction

Cardiovascular complications caused by cancer chemotherapy drugs have become a common problem in cancer treatment. Cisplatin (cis-diamminedichloroplatinum [II], CDDP), a platinum anti-tumor drug, is applied to the treatment of many solid tumors involving the endometrium, neck, head, bladder, testis, ovary, kidney and lung [1]. Although platinum-like chemotherapy drugs have been reported with reliability and effectiveness in the treatment of cancer, the side effects of cisplatin, such as neurotoxicity and cardiotoxicity, represent major obstacles for the successful treatment of cancer [1-3]. A raft of studies have revealed that cisplatin could increase the levels of plasma troponin I (TPI), creatine kinase (CK), and creatine kinase isoenzyme MB (CK-MB), which may be caused by the damage of myocardial membrane structure and function [4]. In a word,

cardiac injury induced by cisplatin eventually leads to congestive heart failure and abrupt cardiac death. Therefore, revealing the mechanism of cisplatin-induced toxicity will be helpful in the quest for new drugs to treat cisplatin-related toxicity.

Apoptosis of cardiomyocytes has been demonstrated in cisplatin-induced cardiotoxicity models *in vivo* and *in vitro* [5-7]. The molecular mechanisms of cisplatin-induced toxicity are complicated and unclear. Results from a large number of studies showed that multiple pathways were involved in cisplatin-induced toxicity, including mitochondrial damage, toxic metabolites, inflammatory response, and so on [6, 8-10]. Inflammatory response is an inevitable occurrence that usually leads to secondary damage to cells or tissues [11]. There is increasing evidence that cisplatin could induce a large number of inflammatory cytokines and chemo-

kines, including transcription activation of the nuclear factor- κ B (NF- κ B), which regulates the expression of a variety of inflammatory factors including tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6), monocyte chemotactic protein 1 (MCP-1), and so on [12]. It has been reported that cardiac dysfunction induced by cisplatin is related to mitochondrial membrane depolarization [2, 13]. Accumulating evidence has illustrated that cisplatin could directly interact with mitochondria and rapidly induce the release of cytochrome C and NAD(P)H decay [14, 15]. And the cell damage induced by cisplatin can be reversed by the afore-mentioned cisplatin permeability transition pore inhibitor [15]. In addition, cisplatin inhibited respiration, including ADP phosphorylation rate, respiration control rate, and ADP/O rate, and increased the production of ROS [16, 17]. Furthermore, mtDNA leaks into the cytosol during cisplatin-induced mitochondria damage and mitochondrial permeability transition [18]. As a key regulator, mtDNA also activated the cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING) signal pathway [19]. mtDNA-activated cGAS induces the production of 2'3'-cGAMP, which binds to STING and activates downstream signal pathway [20]. Studies have also reported that STING-mediated inflammation could promote myocardial damage and kidney damage caused by cisplatin-induced mitochondrial stress [21, 22]. In addition, in *in vivo* animal models, cisplatin can induce a significant increase in myocardial TNF- α and myocardial peroxidase activity [12, 23]. To sum up, STING-mediated inflammation activated by mtDNA leakage may be involved in cisplatin-induced apoptosis.

Kaempferol (KPF), a kind of flavonoids, widely exists in all kinds of vegetables and fruits [24, 25]. A large number of studies have reported that KPF has anti-inflammatory and antioxidant activities [26, 27]. A recent study has shown that the decrease of serum inflammatory cytokines was closely related to the daily consumption of high levels of flavonol, especially KPF [28]. KPF has shown great anti-tumor potential by inhibiting metastasis, inflammation and angiogenesis [25]. With a focus on the patented medical potential of natural products, we clarified the protective effect of KPF on cisplatin-induced heart damage and conducted in-depth research on the molecular mechanism underlying this effect. Our results showed that

KPF could reduce myocardial injury and diastolic dysfunction induced by cisplatin, and reduce the inflammation and apoptosis of cardiomyocytes. The cardioprotective effect of KPF was closely related to its inhibition of STING-mediated NF- κ B activation.

Methods

Chemicals

KPF and cisplatin were procured from Selleck (Houston, Texas, USA). For the *in vivo* experiments, KPF was dissolved in 1% sodium carboxyl methyl cellulose (CMC-Na), and for the *in vitro* experiments, it was dissolved in DMSO.

Animals

All the experimental procedures were in compliance with "The Detailed Rules and Regulations of Medical Animal Experiments Administration and Implementation". The animal research protocols were approved by the institutional review boards of Zhejiang University School of Medicine, Hangzhou, China. C57BL/6 mice (male, 18-22 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The animals were fed with a normal animal diet and kept at a constant temperature in room with a 12:12 light/dark cycle. Cisplatin-induced cardiac injury animal model was established by subcutaneous injections of cisplatin at 3 mg.kg⁻¹.2 day⁻¹ for 1 week in saline buffer. KPF was orally administrated at 10 mg.kg⁻¹.day⁻¹ for 2 weeks before injection of cisplatin, while the control group was orally administrated with 1% CMC-Na. Under ether anesthesia, the mice underwent euthunization with sodium pentobarbital anesthesia. Afterwards, their blood and heart tissues were collected.

Cardiac function

7 days after cisplatin treatment, transthoracic echocardiography was conducted on the mice to examine their cardiac function and structure. Transthoracic echocardiography (VisualSonics, Toronto, Canada) was performed to measure cardiac function in a non-invasive manner. The LV internal dimension in diastole (LVIDs), LV end-systolic volume (LVESV), LV internal dimension in systole (LVIDd), and LV end-diastolic volume (LVEDV) were assessed from M-mode images. The equations of Fractional shortening

Table 1. Primers used for real-time qPCR assay

Gene	Species	Primers (FW)	Primers (RW)
TNF- α	Mouse	TGATCCGCGACGTGGAA	ACCGCCTGGAGTTCTGGAA
IL-6	Mouse	CCAAGAGGTGAGTGCTTCCC	CTGTTGTTCACTCTCTCCCT
MCP-1	Mouse	TCACCTGCTGCTACTCATTACCA	TACAGCTTCTTTGGGACACCTGCT
HMGB-1	Mouse	GGCGAGCATCCTGGCTTATC	GGCTGCTTGTCTATCTGCTG
β -actin	Mouse	CCGTGAAAAGATGACCCAGA	TACGACCAGAGGCATACAG
TNF- α	Rat	TACTCCCAGGTCTCTTCAAGG	GGAGGCTGACTTTCTCCTGGTA
IL-6	Rat	GAGTTGTGCAATGGCAATTC	ACTCCAGAAGACCAGAGCAG
MCP-1	Rat	GTCACCAAGCTCAAGAGAGAGA	GAGTGGATGCATTAGCTTCAGA
HMGB-1	Rat	GCATCCTGGCTTATCCATTGG	GGCTGCTTGTCTATCTGCTG
β -actin	Rat	AAGTCCCTCACCTCCCAAAG	AAGCAATGCTGTACCTTCCC

(FS) = $[(LVIDd - LVIDs)/LVIDd] \times 100\%$ and Ejection fraction (EF) = $(LVEDV - LVESV)/LVEDV \times 100\%$ were respectively used to calculate FS and EF.

Heart histology

After the cisplatin treatment, the heart tissues were obtained and fixed in 4% formaldehyde buffered with PBS (pH 7.2). The second step was meant to prepare 5- μ m-thick paraffin-embedded sections. Thereafter, the sections were submitted to hematoxylin and eosin (H&E) treatment.

Myocardial TUNEL staining

Sections were deparaffinized and TUNEL staining was conducted following the manufacturer's operating procedures (Yeasen, Shanghai, China). Images were taken with the microscope (NIKON A1R/A1, Nikon, Japan).

Cell culture and treatment

After they were cultured in DMEM medium (Gibco, Eggenstein, Germany) that contained 4.5 g/L glucose, H9c2 cells were maintained at 37°C in an atmosphere of 5% CO₂ and 95% humidity. The cells were pretreated with KPF (1, 5, or 10 μ M) for 1 h, and then incubated with cisplatin (10 μ M) or PBS (1 μ L).

MTT assay

After the afore-mentioned treatment, the cells were rinsed three times, and the medium was converted into 1 mg/mL MTT solution (100 μ L/well, Sigma). Cells were incubated at 37°C for 4 h. A microplate reader was used to measure

the absorbance at 570 nm in order to determine cell viability.

Western blot analysis (WB)

Protocol of WB was represented in detail in the published paper [7]. Antibodies for α -Tubulin and GAPDH were obtained from Proteintech group (Chicago, Illinois, USA). Antibodies for p-STING, p-TBK1, TBK1, BAX, BCL2, NF- κ B p65, and p-NF- κ B p65 were procured from Cell Signaling Technology (Boston, Massachusetts, USA).

Real-time quantitative PCR (RT-qPCR)

Protocol of RT-qPCR was described in a previous paper [29]. The primer sequences of TNF- α , IL-6, HMGB1, MCP-1 and β -actin genes were synthesized with Invitrogen (Invitrogen, Shanghai, China) (Table 1). The quantitative analysis of genes was normalized to β -actin.

Determination of TNF- α and IL-6 by enzyme-linked immunosorbent assay (ELISA)

Following the manufacturer's instructions, the protein levels of TNF- α and IL-6 in heart tissue or medium supernatant were determined by ELISA kits (Bioscience, San Diego, CA).

Silencing STING by siRNA

In accordance with the manufacturer's operating procedures, specific siRNA (sense sequence: 5'-CCAACCUGCAUCCAU CCAUTT-3'; anti-sense sequence: 5'-AUGGA UGGAUGCAGGUU-GGTT-3') for STING obtained from Genepharma Co., LTD. (Shanghai, China) was transfected into H9c2 cells using siRNA-Mate (Genepharma, Shanghai, China).

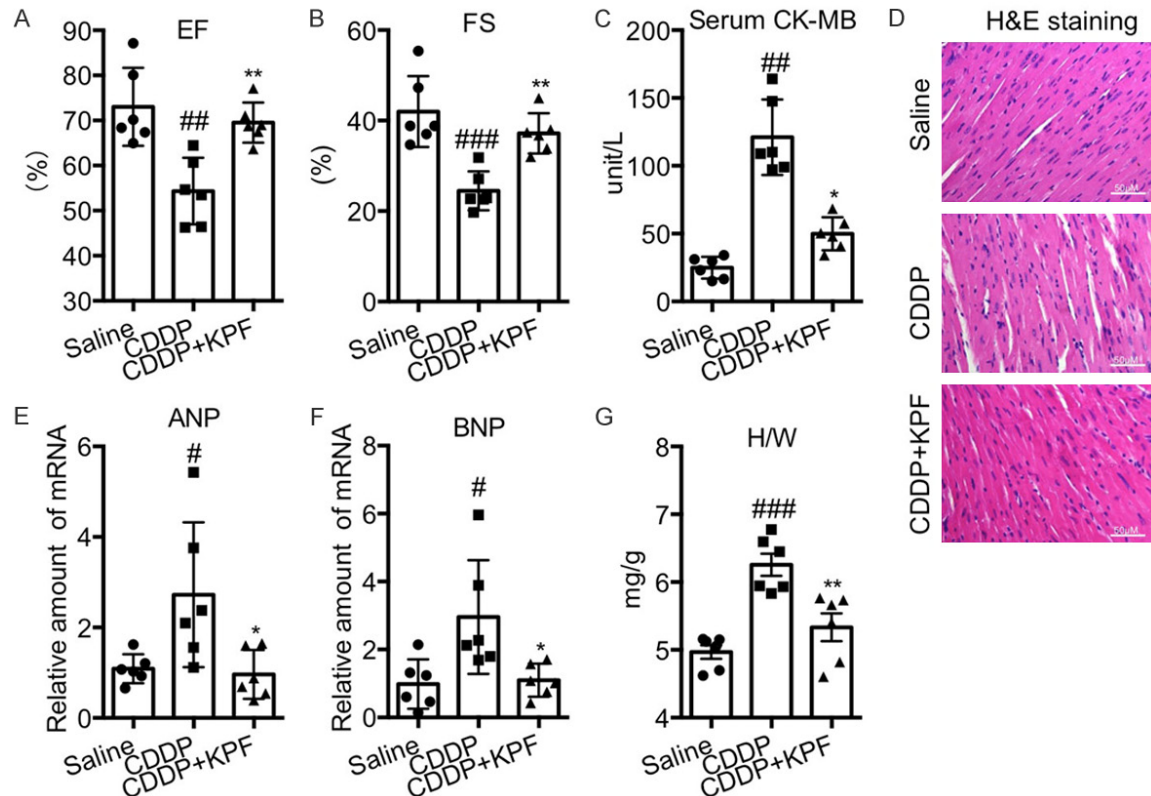


Figure 1. KPF attenuated cisplatin-induced hypertrophy and cardiac dysfunction. (A, B) Eject fraction (A) and Fractional shortening (B) were measured by echocardiography. (C) The level of serum CK-MB was detected by the ELISA Kit. (D) The represent images were observed for myocardial fiber disorder. Heart tissues were subjected to (H&E). (E, F) RT-qPCR was used to detect the mRNA levels of ANP and BNP. (G) The ratio of heart to body weight (HW/BW) is assumed to reflect cardiac hypertrophy. (n=6 in each group; #, vs. Saline group, #P<0.05, ##P<0.01, ###P<0.001; *, vs. CDDP group, *P<0.05, **P<0.01).

Statistical analysis

The statistics reported are presented as means + SEMs (standard errors of the mean) in the present study. Analysis of variance (ANOVA) was used to evaluate the statistical significance of differences. A statistically significant result was obtained at P<0.05.

Results

KPF mitigated hypertrophy and cardiac dysfunction induced by cisplatin

To explore the cardioprotective effect of KPF on cisplatin-induced hypertrophy and cardiac dysfunction, mice treated with cisplatin were pre-treated with KPF. As shown in **Figure 1A, 1B**, cisplatin induced the decrease of EF and FS, while treatment with KPF significantly improved cisplatin-induced cardiac dysfunction. Cisplatin induced an increase in serum CK-MB levels, but it was improved in KPF-treated mice (**Figure 1C**). Histological analysis of heart tissues sh-

owed increased myocardial fiber disorder in cisplatin-induced mice, but the change was reversed in KPF-treated mice (**Figure 1D**). We also measured the related indexes of cardiac hypertrophy, ANP and BNP. Cisplatin enhanced mRNA levels of ANP and BNP, but these changes were reversed by treatment with KPF (**Figure 1E, 1F**). The increase in the heart to body weight (HW/BW) ratio was assumed to be caused by cardiac hypertrophy. **Figure 1G** showed that KPF reduced the cisplatin-induced increase of the ratio of HW/BW. The above data indicated that KPF had a protective effect on hypertrophy and cardiac dysfunction caused by cisplatin.

KPF reduced cisplatin-induced cardiomyocyte apoptosis in myocardial tissue

To further investigate the protective effect of KPF, we analyzed cardiomyocyte apoptosis in the myocardial tissues. As shown in **Figure 2A**, pretreatment with KPF significantly attenuated cisplatin-induced cardiomyocyte apoptosis

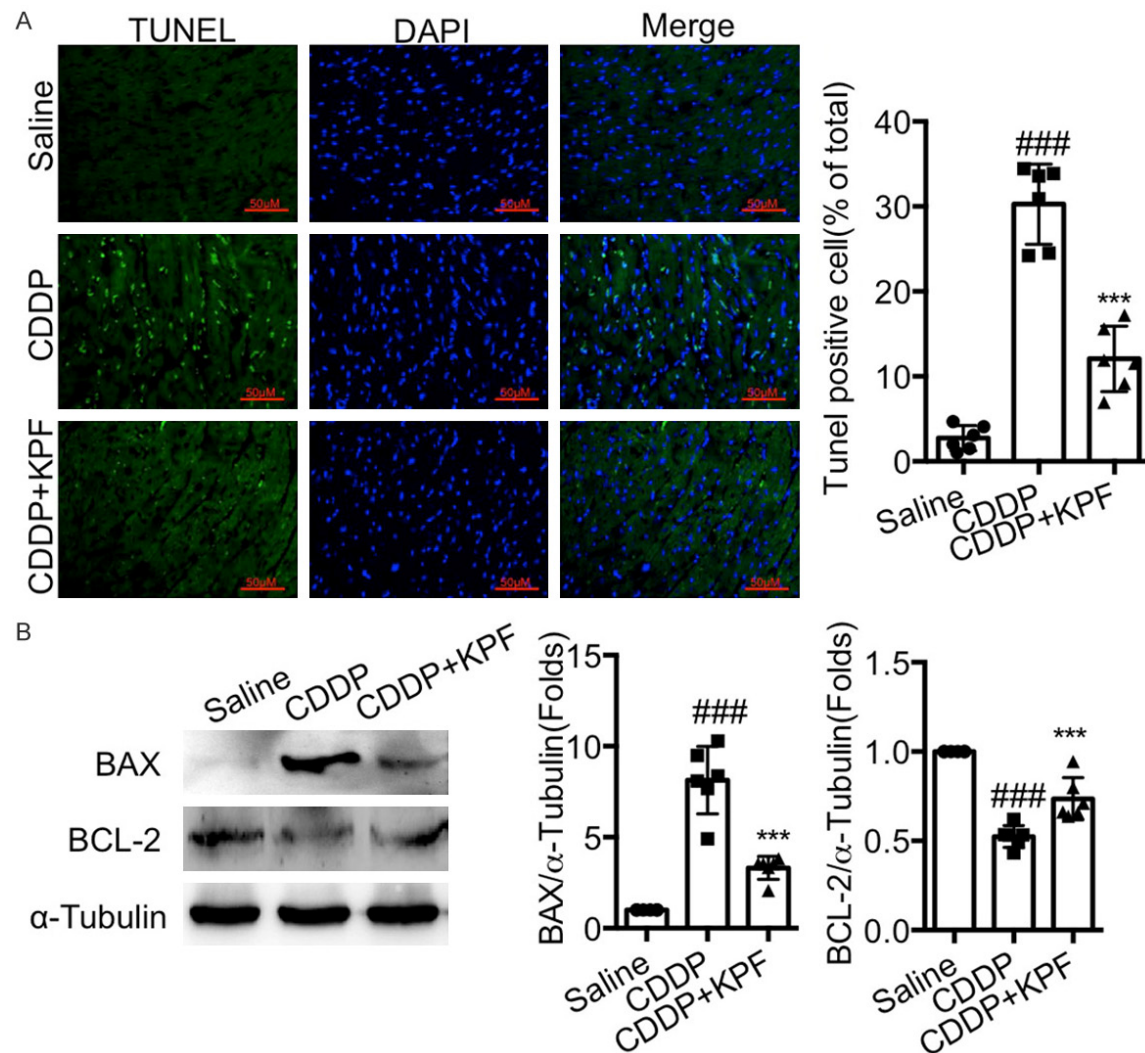


Figure 2. KPF reduced cisplatin-induced cardiomyocytes apoptosis in myocardial tissue. A. The representative images of TUNEL staining and quantitative analysis were used to demonstrate cardiomyocyte apoptosis. B. Western blot analysis and quantitative analysis were used to detect the expression of apoptosis-related protein BAX and anti-apoptosis-related protein BCL-2. (n=6 in each group; #, vs. Saline group, ###P<0.001; *, vs. CDDP group, ***P<0.001).

measured by TUNEL staining. On the other hand, cisplatin increased the expression of apoptosis-related proteins (BAX) and decreased the expression of anti-apoptosis-related protein (BCL-2), but these changes induced by cisplatin were reversed by treatment of KPF (Figure 2B). These data indicated that KPF had the protective effect of cisplatin-induced cardiomyocytes apoptosis.

KPF significantly suppressed cardiac inflammation induced by cisplatin in myocardial tissue

Immunohistochemical staining showed that the expression of CD68 in myocardial tissue was increased by cisplatin, indicating that increa-

sed macrophages infiltrated into myocardial tissue. The increase was reversed by pretreatment with KPF (Figure 3A). Real-time qPCR analysis (Figure 3B-E) was performed to further validate the mRNA levels of inflammatory factors in cardiac tissue and the protein levels of IL-6 and TNF- α in cardiac tissue were measured by ELISA (Figure 3F, 3G). The results revealed that cisplatin could induce the mRNA levels of IL-6, HMGB1, TNF- α and MCP-1 and the protein contents of IL-6 and TNF- α in cardiac tissue, but these changes were reversed by treatment of KPF. Since the NF- κ B signal pathway is considered critical for inflammation, we tested it in the cardiac tissue. As Figure 3H suggested, cisplatin induced the phosphoryla-

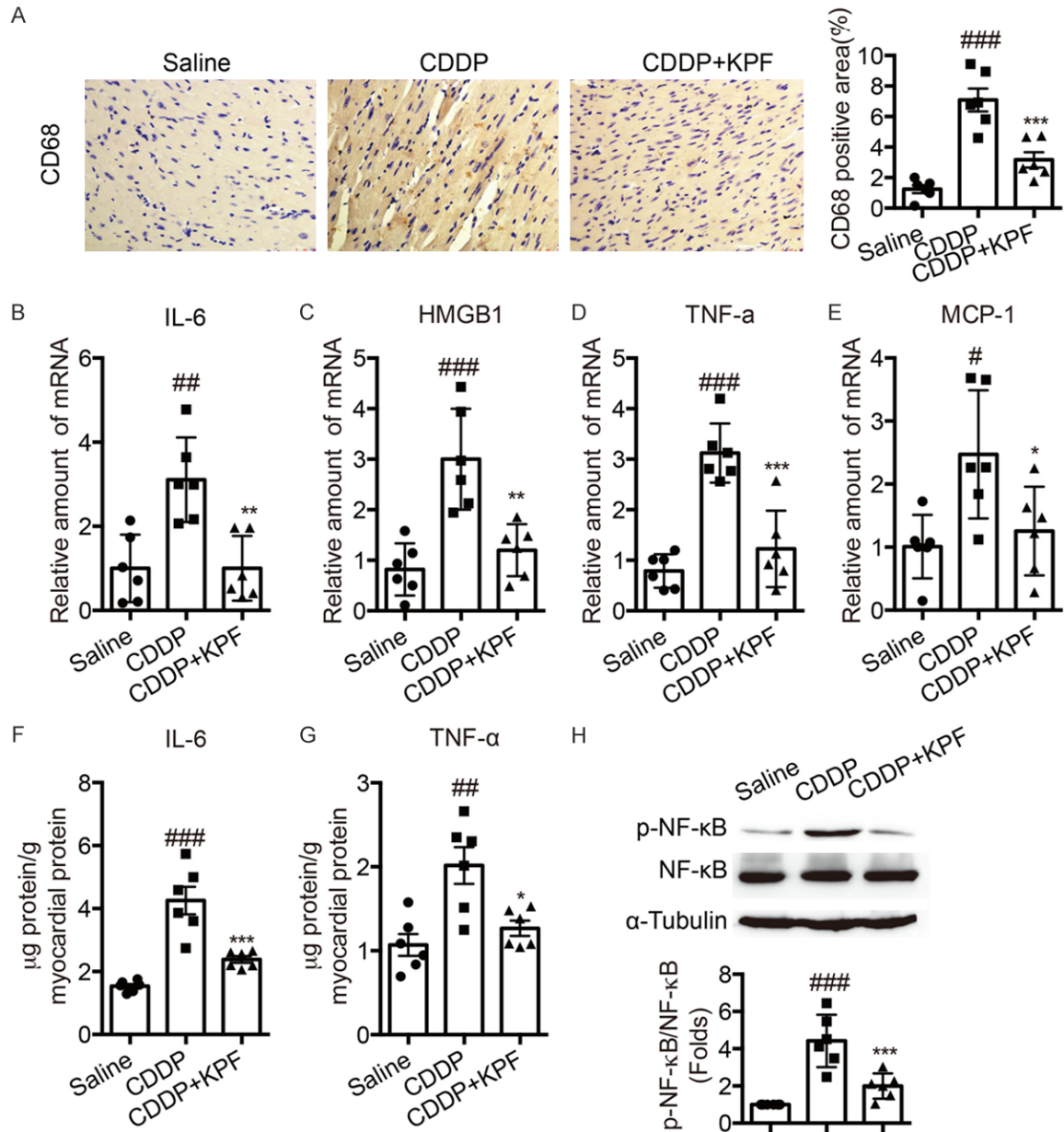


Figure 3. KPF significantly suppressed cardiac inflammation induced by cisplatin in myocardial tissue. (A) The representative images of immunohistochemistry staining of CD68 and quantitative analysis were used to demonstrate macrophage infiltration. (B-E) RT-qPCR was used to detect the mRNA levels of IL-6 (B), HMGB1 (C), TNF- α (D), and MCP-1 (E). (F, G) The protein contents of IL-6 (F) and TNF- α (G) were detected with the corresponding ELISA Kit. (H) Western blot analysis and quantitative analysis were used to detect the phosphorylation and expression of NF- κ B. (n=6 in each group; #, vs. Saline group, #P<0.05, ##P<0.01, ###P<0.001; *, vs. CDDP group, *P<0.05, **P<0.01, ***P<0.001).

tion of NF- κ B, while KPF effectively inhibited phosphorylation of NF- κ B induced by cisplatin in cardiac tissue. These results indicated that KPF could significantly inhibit the cardiac inflammation caused by cisplatin, which might contribute to the cardioprotective effects of KPF.

KPF inhibited cisplatin-induced cell death in H9c2 cells

First, we established a cell model in which cisplatin inhibits the viability of H9c2 cells. As **Figure 4A** showed, cisplatin at concentrations of 5 and 10 μ M markedly inhibited the viability

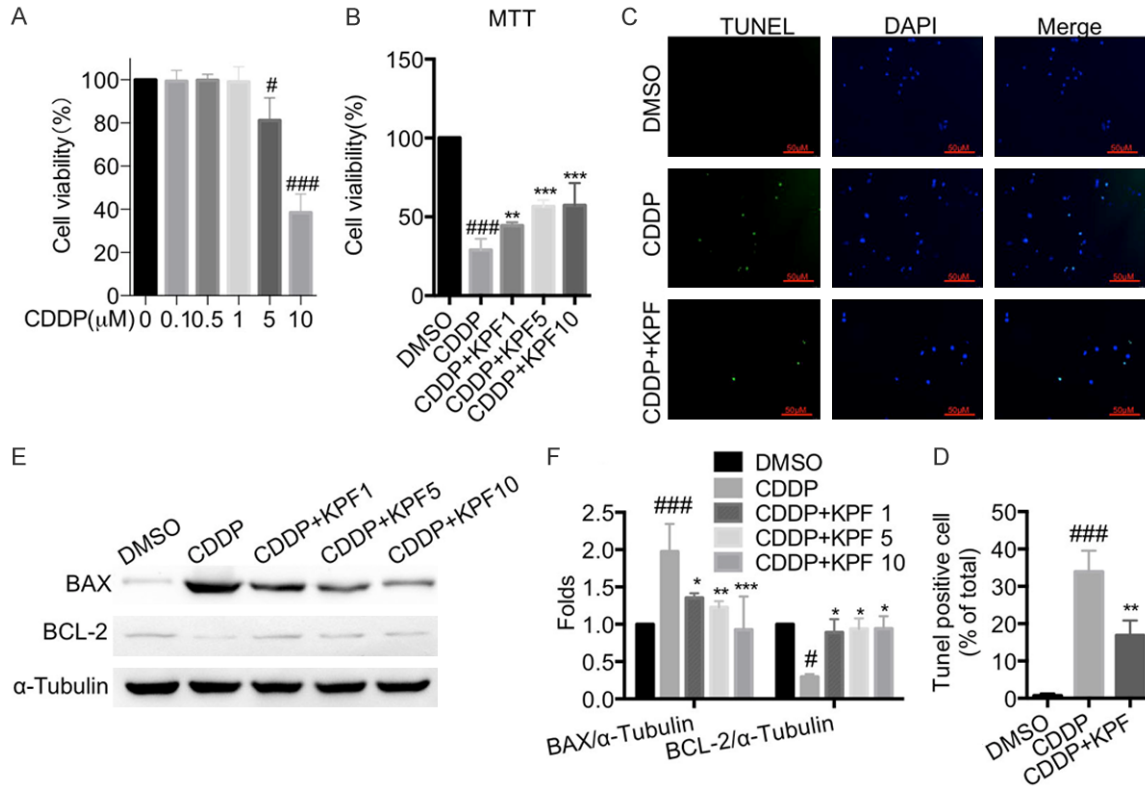


Figure 4. KPF inhibited cell death induced by cisplatin in H9c2 cells. (A) After incubation with cisplatin (0.1, 0.5, 1, 5, or 10 μ M) for 24 h, MTT assay was performed to detected cell viability in H9c2 cells. (B) Following 1 h pretreatment with KPF (1, 5, or 10 μ M), H9c2 cells were incubated with cisplatin (10 μ M) for 24 h and then cell viability was measured by MTT assay. (C, D) Following 1 h pretreatment with KPF (1, 5, or 10 μ M), H9c2 cells were incubated with cisplatin (10 μ M) for 24 h and then cell apoptosis was measured by TUNEL staining. The representative images of TUNEL staining (C) and quantitative analysis (D) were used to perform apoptosis in H9c2 cells. (E, F) Following 1 h pretreatment with KPF (1, 5, or 10 μ M), H9c2 cells were incubated with cisplatin (10 μ M) for 24 h and then total protein were collected. Western blot analysis and quantitative analysis were used to detect the expression of BAX and BCL2. (n=3 independent experiments; #, vs. Ctr or DMSO group, #P<0.05, ###P<0.001; *, vs. CDDP group, *P<0.05, **P<0.01, ***P<0.001).

of H9c2 cells. Subsequently, we evaluated the viability of H9c2 cells induced by cisplatin (10 μ M) after they had been pretreated with KPF. H9c2 cells were incubated with 1, 5, or 10 μ M KPF for 1 h, and then exposed to cisplatin (10 μ M) for 24 h. As shown in **Figure 4B**, cisplatin significantly inhibited the proliferation of H9c2 cells, but pretreatment with KPF had a dose-dependent protective effect on H9c2 cells. Moreover, TUNEL staining showed that KPF reduced cisplatin-induced cardiomyocytes apoptosis (**Figure 4C, 4D**). On the other hand, cisplatin increased the expressions of apoptosis-related proteins (BAX) and decreased the expression of anti-apoptosis-related protein (BCL-2), but these changes induced by cisplatin were reversed by treatment with KPF in H9c2 cells (**Figure 4E, 4F**). These results showed that

KPF dose-dependently protected against cisplatin-induced cell death in H9c2 cell.

KPF inhibited the expression of cytokine induced by cisplatin

The inflammatory reaction of myocardial tissue is widespread, which aggravates the injury of myocardial cells and leads to apoptosis. Therefore, we examined whether KPF inhibited the release of inflammatory cytokines induced by cisplatin. RT-qPCR showed that cisplatin could increase the mRNA levels of inflammatory cytokines, including HMGB1, MCP-1, TNF- α , and IL-6 (**Figure 5A**), and the secretion of IL-6 and TNF- α in cell culture medium (**Figure 5B**). Treatment with KPF reduced both the mRNA levels of HMGB1, MCP-1, TNF- α , and IL-6 and

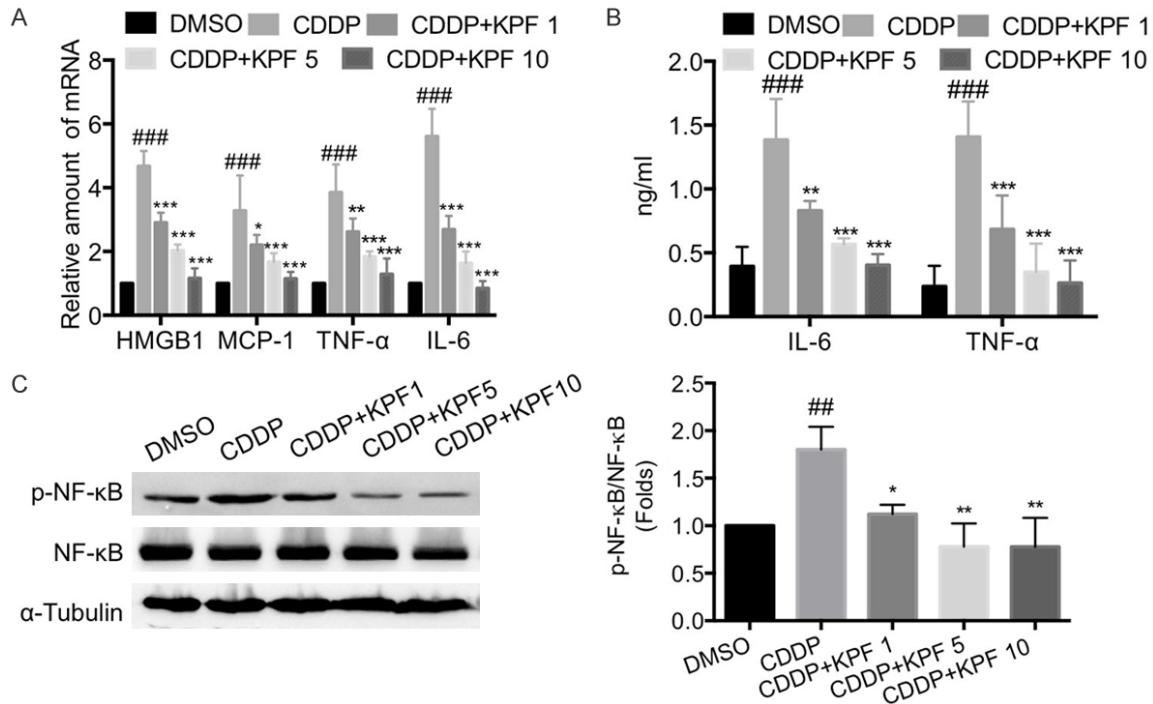


Figure 5. KPF inhibited the expression of cytokine induced by cisplatin. A. After being pretreated with KPF (1, 5, or 10 μ M) for 1 h, H9c2 cells were incubated with cisplatin (10 μ M) for 6 h and then total RNA was collected. RT-qPCR was used to detect the mRNA levels of HMGB1, MCP-1, TNF- α and IL-6. B. Following 1 h pretreatment with KPF (1, 5, or 10 μ M), H9c2 cells were incubated with cisplatin (10 μ M) for 24 h and then culture medium were collected. The contents of IL-6 and TNF- α were detected by the corresponding ELISA Kit. C. Following 1 h pretreatment with KPF (1, 5, or 10 μ M), H9c2 cells were incubated with cisplatin (10 μ M) for 1 h and then total protein was collected. Western blot analysis and quantitative analysis were used to detect the phosphorylation and expression of NF- κ B. (n=3 independent experiments; #, vs. DMSO group, ##P<0.01, ###P<0.001; *, vs. CDDP group, *P<0.05, **P<0.01, ***P<0.001).

the secretion of IL-6 and TNF- α in H9c2 cells (Figure 5A, 5B). In addition, KPF effectively reduced the phosphorylation level of NF- κ B in cisplatin-induced H9c2 cells. These results suggested that KPF might alleviate cisplatin-induced injury in H9c2 cells via inhibition of inflammatory response.

STING was involved in cisplatin-induced inflammation

Research has shown that the cGAS/STING signal pathway is capable of detecting cytosolic DNA and inducing innate immunity [20]. Cao et al. reported that mice lacking cGAS showed significantly ameliorated early survival after MI, reduced pathological remodeling, abolished the induction of inflammatory programs, and promoted the conversion of macrophages to a repair phenotype [30]. Figure 6A showed that cisplatin increased the phosphorylation of STING and TBK1 in cardiac tissues as quanti-

fied from immunoblotting assay, while KPF treatment reversed these changes in cisplatin-induced mice. On the other hand, cisplatin induced a time-dependent activation of STING and TBK1 (Figure 6B). However, pretreatment with KPF inhibited cisplatin-stimulated phosphorylation of STING and TBK1 in H9c2 cells in a dose-dependent manner (Figure 6C). Next, we investigated whether the anti-apoptotic effect of KPF is STING-dependent in cisplatin-challenged H9c2 cells. The mRNA level of STING was knocked down by siRNA (Figure 6D). As shown in Figure 6E, inhibiting the expression of STING by siRNA prior to cisplatin exposure induced an increase in the expression of BAX and elevated the expression of BCL-2 in cisplatin-challenged H9c2 cells. In addition, KPF failed to improve the cell viability in cisplatin-challenged H9c2 under the condition of knockdown STING (Figure 6F). In order to further confirm that the anti-inflammatory effect of KPF depends on its inhibition of STING, we

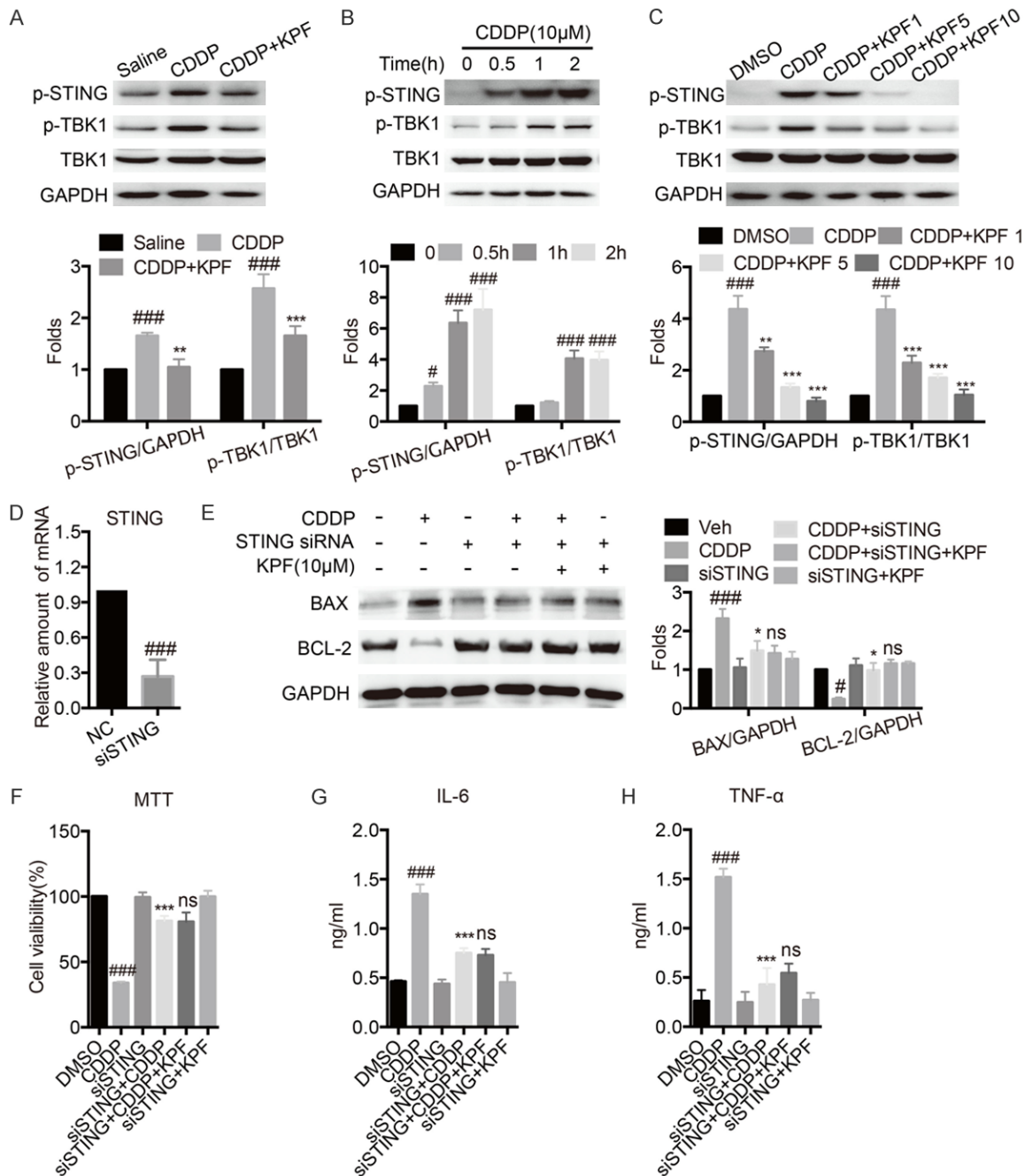


Figure 6. STING was involved in cisplatin-induced inflammation. (A) Western blot analysis and quantitative analysis were used to detect the phosphorylation of STING and TBK1 in cardiac tissue. (n=6 in each group; #, vs. Saline group, ###P<0.001; *, vs. CDDP group, **P<0.01, ***P<0.001). (B) H9c2 cells were incubated with cisplatin (10 μM) for 0.5, 1 h, or 2 h, and then total protein was collected. Western blot analysis and quantitative analysis were used to detect the phosphorylation of STING and TBK1. (C) Following 1 h pretreatment with KPF (1, 5, or 10 μM), H9c2 cells were incubated with cisplatin (10 μM) for 1 h and then total protein was collected. Western blot analysis and quantitative analysis were used to detect the phosphorylation of STING and TBK1. (D) Specific siRNA for STING were transfected into H9c2 cells for 24 h and then total RNA were collected. RT-qPCR was used to detect the mRNA level of STING. (E-H) Transfecting specific siRNA of STING into H9c2 for 24 h, H9c2 cells were pretreated with KPF (10 μM) for 1 h and then incubated with cisplatin (10 μM) for 24 h. Western blot analysis and quantitative analysis were used to detect the expression of BAX and BCL-2 (E) and cell viability (F) was measured by MTT assay. The contents of IL-6 (G) and TNF-α (H) in the culture medium were detected by the corresponding ELISA Kit. (n=3 independent experiments; #, vs. Ctrl or DMSO group, ###P<0.01, ###P<0.001; *, vs. CDDP group, *P<0.05, **P<0.01, ***P<0.001).

observed the effect of KPF on cisplatin-induced inflammatory response by knocking down the expression of STING. As shown in **Figure 6G, 6H**, cisplatin failed to increase the inflammatory factors in STING-knockdown H9c2 cells. These results suggested that STING was involved in cisplatin-induced inflammation and apoptosis, and that KPF suppressed cisplatin-induced inflammation and apoptosis, which is mediated by blocking the STING/NF- κ B signal pathway.

Discussion

Cisplatin, as an antineoplastic drug, is widely used in the clinic, accompanied by such side effects as cardiotoxicity, nephrotoxicity, hepatotoxicity, etc. It has been reported that the cardiotoxicity caused by cisplatin is mainly manifested in changes of ECG, arrhythmia, acute myocardial infarction and autonomic cardiovascular dysfunction (including atrial fibrillation, occasional sinus bradycardia, supraventricular tachycardia, ventricular arrhythmia, and occasional complete atrioventricular node) [1, 31-34]. At its final stage, cardiotoxic events can result in congestive heart failure and abrupt cardiac death. Therefore, it is necessary to find an effective strategy to treat cardiotoxicity and understanding the mechanism leading to cardiotoxicity will help to develop new methods and means to prevent cisplatin-related toxic effects.

KPF is a kind of flavonol that has nonnegligible potential in preventing inflammation and oxidative stress. It is reported that KPF could relieve Ang II-induced cardiac remodeling and heart failure [35]. In this study, we found that KPF could attenuate cisplatin-induced cardiac dysfunction and hypertrophy *in vivo* (**Figure 1**). Furthermore, KPF could reduce cisplatin-induced cardiomyocyte apoptosis (**Figure 2**). These findings indicated that KPF had a protective effect on myocardial damage caused by cisplatin. Furthermore, we found that cisplatin induced an increase in the expression of inflammatory factor and macrophage infiltration *in vivo*, but KPF treatment reversed these changes (**Figure 3**). These results indicated that KPF might attenuate cisplatin-induced cardiac injury via inhibition of NF- κ B-mediated inflammation.

Cisplatin induced an increase in caspase-3 activity and the expression of BAX and BAK

(pro-apoptotic proteins of BCL-2 family) in the cardiac tissue of the mouse model, indicating that the apoptosis of cardiomyocyte was increased [13, 36, 37]. As an intracellular DNA sensor, cGAS can detect self and nonself DNA [20]. After binding to cytosolic double-stranded DNA (dsDNA), cGAS induces synthesis of cyclic GMP-AMP (cGAMP) by consuming adenosine 50-triphosphate (ATP) and guanosine 50-triphosphate (GTP) [20, 38]. The second messenger, cGAMP, induced STING set to be a polymer, which increased the production of type 1 IFN through transcription activation of interferon regulatory factor 3 (IRF3) [39]. Interestingly, we found that the phosphorylation of STING and TBK1 was increased (**Figure 6A**) and NF- κ B was activated (**Figure 3H**) by cisplatin in cardiac tissue. KPF had the ability to reduce the phosphorylation of STING and TBK1 (**Figure 6A**) and inhibited the activation of NF- κ B in cisplatin-induced mice (**Figure 3H**). Similar results were observed in cisplatin-stimulated H9c2 cells. To determine the role of STING in the cardioprotective effect of KPF, STING siRNA was transfected into H9c2 cells (**Figure 6D**). KPF was not able to inhibit the expressions of inflammatory factors and reduce cardiomyocytes apoptosis induced by cisplatin in H9c2 cells (**Figure 6E-H**). These suggested that KPF could inhibit cardiac inflammatory response induced by cisplatin through blocking STING/NF- κ B signal pathway.

KPF has shown great anti-cancer potential through the regulation of signal transduction pathways related to oxidative stress, inflammation, angiogenesis, and apoptosis [14]. In the present project, we found that KPF relieved cardiac injury induced by cisplatin via inhibiting STING/NF- κ B-mediated inflammatory response. Moreover, our results clearly indicated that targeting STING might be a new strategy for treating heart failure induced by cisplatin. In the future, more attention should be paid to the natural active compounds containing KPF with anti-inflammatory effects for treating these diseases.

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Disclosure of conflict of interest

None.

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