Article

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Unlocking the Protective Power of Sinomenine: How It Alleviates LPS-Induced Cardiomyocyte Inflammatory Injury Partially Through an Nrf2-Dependent Pathway

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Background: Myocarditis is an inflammatory cardiomyopathy characterized by cardiomyocyte injury, oxidative stress, and immune dysregulation. Sinomenine, a naturally occurring alkaloid with established anti-inflammatory properties, has been reported to have cardioprotective benefits. However, its underlying molecular mechanisms in myocarditis remain unclear. This study examined the effects and mechanisms of sinomenine in lipopolysaccharide (LPS)-induced cardiomyocyte injury.

Methods: An *in vitro* model of LPS-induced cardiomyocyte inflammatory injury was established using LPS treatment, with or without sinomenine pre-treatment. We evaluated the effects of sinomenine on cell viability, apoptosis, reactive oxygen species (ROS), malondialdehyde (MDA), superoxide dismutase (SOD), and the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) pathway in this cellular model using cell counting kit-8 (CCK-8), flow cytometry, Western blotting, enzyme-linked immunosorbent assay (ELISA), and immunofluorescence. In addition, we investigated the role of the Nrf2 pathway in the protective effects of sinomenine against myocardial cell injury through Nrf2 silencing.

Results: Sinomenine significantly alleviated the LPS-induced reduction in cell viability while suppressing apoptosis, oxidative stress, and inflammatory responses ($p < 0.05$). In particular, sinomenine reduced the p-p65/p65 ratio and the expression of Bax and cleaved caspase-3 ($p < 0.05$), promoted Nrf2 nuclear translocation without altering total Nrf2 expression, and increased the expression of the downstream antioxidant proteins NAD(P)H quinone dehydrogenase 1 (NQO1) and HO-1 ($p < 0.001$). Nrf2 silencing partially reversed the effects of sinomenine on cell viability, apoptosis, oxidative stress, inflammation, and Nrf2 and HO-1 expression ($p < 0.05$).

Conclusion: Sinomenine attenuates LPS-induced myocardial cell injury by reducing oxidative stress, apoptosis, and inflammation, and these effects are associated with activation of Nrf2/HO-1 signaling. These findings provide experimental evidence, rather than direct clinical evidence, supporting the potential therapeutic application of sinomenine in inflammatory myocardial injury.

Keywords: myocarditis; sinomenine; lipopolysaccharide; Nrf2; HO-1

Introduction

Myocarditis is an inflammatory cardiomyopathy caused by various reasons [1]. Oxidative stress and inflammatory infiltration are thought to be the main causes of cardiomyopathy [2]. The clinical manifestations of myocarditis include general fatigue, chest pain, arrhythmia, and even cardiac arrest [3]. Although severe myocarditis can be effectively treated with current medical technologies, its consequences remain challenging to manage, and there are currently very few effective ways to prevent the development of myocarditis [4]. Thus, a better understanding of myocarditis will help to develop more effective strategies for its treatment.

The cell walls of Gram-negative bacteria are largely composed of lipopolysaccharide (LPS), commonly referred to as endotoxin [5]. LPS is widely used to establish animal or cellular inflammation models for many diseases, including myocarditis [6,7,8]. The systemic immune response induced by LPS is a complicated pathological process, but the physiological and pathological changes involved are closely related to changes in heart function [9]. Improving cardiac function provides a new treatment strategy for LPS-induced myocardial injury.

Plant-derived compounds have been widely explored for their cardioprotective effects [10,11,12]. For example, Wang et al. [13] reported that leonurine may inhibit the activation of the nuclear factor kappa B (NF-κB) pathway to counteract oxidative stress and inflammation induced

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by myocarditis. Sinomenine is an active ingredient extracted from *Sinomenium acutum* (Caulis Sinomenii), a traditional Chinese medicinal plant [14]. Although the chemical structure of sinomenine is similar to that of morphine, it does not exhibit addictive properties [15]. Several studies have demonstrated that sinomenine may effectively alleviate inflammatory damage and reduce inflammation [16,17]. Sinomenine can inhibit the expression of downstream inflammatory genes by acting on NF-κB and nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) pathway [18]. Recent research has shown that Nrf2 activation can protect against experimental autoimmune myocarditis-induced injury, indicating that the Nrf2/HO-1 pathway may play a role in the onset and development of myocarditis [19].

At present, the therapeutic effects and underlying mechanisms of sinomenine in myocarditis remain to be investigated. Thus, this study aimed to investigate the therapeutic effects of sinomenine on LPS-induced myocarditis and explore potential downstream pathways to further elucidate the underlying molecular mechanisms.

Materials and Methods

Cell Culture and Treatment

Mouse HL-1 cardiomyocytes (CL-0605, Procell, Wuhan, China) were cultured in HL-1 cell-specific medium (CM-0605, Procell, Wuhan, China) at 37 °C with 5% CO_2. HL-1 cells were authenticated by short tandem repeat analysis and tested negative for mycoplasma contamination. To establish the myocarditis model, cardiomyocytes were treated with LPS (10 μg/mL; ST1470, Beyotime, Shanghai, China) for 12 h [13]. For sinomenine treatment, sinomenine (HY-15122, MedChemExpress, Princeton, NJ, USA) was dissolved in dimethyl sulfoxide (DMSO; ST038, Beyotime, Shanghai, China) at a final DMSO concentration of 0.1%. The treatment sequence was as follows: HL-1 cells were pre-treated with varying concentrations of sinomenine (0.1, 0.5, 1, 2, or 4 μmol/L) for 24 h [20]. The cells were then washed with PBS and subsequently stimulated with LPS (10 μg/mL) for 12 h. All endpoint measurements, including cell viability, oxidative stress markers, and inflammatory cytokines, were performed after 12 h of LPS stimulation.

The small interfering RNA (siRNA) negative control (siNC) (C03002, 5′-TTCTCCGAACGTGTCACGTTTC-3′) and siRNA targeting Nrf2 (siNrf2; C02001, 5′-AGGCAGCCATGACTGATTTAA-3′) were purchased from GenePharma (Shanghai, China). HL-1 cardiomyocytes were seeded into 6-well cell culture plates (84011ES03, Yeasen, Shanghai, China) at a density of 2×10^5 cells per well. Transfection was performed when cells reached 70%–80% confluence. Briefly, 1 μg of siRNA was transfected into the cells in each well using Lipofectamine 3000 reagent (L3000015, Invitrogen, Carlsbad, CA, USA). The cells were incubated at 37 °C for 48 h after transfection. To verify the effect of Nrf2 silencing on the protective effects of sinomenine against myocardial inflammation *in vitro*, the cells were first transfected with siRNA, then pretreated with sinomenine (0.5 or 1 μmol/L) for 24 h, and finally stimulated with LPS (10 μg/mL) for 12 h. Notably, the control group and the LPS-only group were treated with an equal volume of DMSO without sinomenine to eliminate solvent effects and ensure consistent experimental conditions across all groups.

Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from HL-1 cardiomyocytes using RNAiso Plus (9108, Takara, Beijing, China), followed by quantification using a NanoDrop One/Onec (ND-ONEC-W, Thermo Fisher Scientific, Waltham, MA, USA). After cDNA synthesis using a PrimeScript RT reagent kit (RR036A, Takara, China), the cDNA was amplified and analyzed using an ABI 7500 real-time PCR system (Applied Biosystems, Foster, CA, USA) with qPCR Mix (RR430A, Takara, China). The qPCR reaction system (20 μL) contained 10 μL of 2× qPCR Mix, 0.8 μL of each primer (10 μM), 0.4 μL of ROX Reference Dye II, 2 μL of cDNA template, and 6 μL of RNase-free water. The amplification protocol was as follows: initial denaturation at 95 °C for 30 s; 40 cycles of 95 °C for 5 s and 60 °C for 15 s; and final melting curve analysis from 65 °C to 95 °C. The $2^{-\Delta\Delta Ct}$ method, normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was used to analyze the data [21]. All primer (Tsingke, Beijing, China) sequences were as follows. Nrf2: 5′-TAGATGACCATGAGTCGCTTGC-3′ (Forward), 5′-GCCAAACTTGCTCCATGTCC-3′ (reverse); GAPDH: 5′-TGTGTCCGTCGTGGATCTGA-3′ (Forward), 5′-CCTGCTTCACCACCTTCTTG-3′ (reverse). The amplification efficiency of each primer pair was determined using a standard curve, and the efficiencies ranged from 95–105% for both Nrf2 and GAPDH.

Detection of Oxidative Stress

The levels of malondialdehyde (MDA) (BC0025, Solarbio, Beijing, China) and superoxide dismutase (SOD) (S0101, Beyotime, China) in cell supernatants were measured and analyzed using a microplate reader (Multiskan FC, Thermo Fisher Scientific, USA). For MDA quantification, the thiobarbituric acid (TBA) reaction was employed, and absorbance was measured at 532 nm and 600 nm. SOD activity was determined by measuring absorbance at 450 nm according to the kit instructions.

Western Blot

HL-1 cardiomyocytes were lysed using a total protein extraction kit (BC3711, Solarbio, China) or Nuclear Protein Extraction Kit (EX1470, Solarbio, China) to obtain total or nuclear proteins, respectively. Thereafter, pro-

tein concentrations were quantified using a bicinchoninic acid kit (P0011, Beyotime, China). The proteins were then separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (P0690, Beyotime, China), electrotransferred onto polyvinylidene fluoride (PVDF) membranes (BS-PVDF-45, Biosharp, Beijing, China) and blocked with 5% skim milk for 2 h. Following overnight incubation with specific primary antibodies, the membranes were incubated with secondary antibodies for one hour. Subsequently, BeyoECL Plus (P0018M, Beyotime, China) was added for signal detection. Protein signals were analyzed using ImageJ software (v1.52, NIH, Bethesda, MD, USA). Information on antibodies from Abcam (Cambridge, UK) was as follows: GAPDH: ab181602, 1/10,000, 36 kDa; cleaved caspase-3: ab214430, 1/500, 17 kDa; Nrf2: ab313825, 1/3000, 68 kDa; NAD(P)H quinone dehydrogenase 1 (NQO1): ab80588, 1/10,000, 30 kDa; Bax: ab32503, 1/1000, 21 kDa; HO-1: ab13243, 1/2000, 34 kDa; Lamin B: ab16048, 0.1 µg/mL, 66 kDa; Goat anti-rabbit IgG H&L (HRP) (ab205718, 1/5000). Information on antibodies from Cell Signaling Technology (CST) (Danvers, MA, USA) was as follows. p65: #8242, 1/1000, 65 kDa; p-p65: #3039, 1/1000, 65 kDa.

Cell Viability Assay

The cell counting kit-8 (CCK-8) (C0037, Beyotime, China) was used to assess the effects of sinomenine on cell viability. HL-1 cardiomyocytes (approximately 1×10^4 cells/well) were seeded into 96-well plates and incubated with sinomenine (0.1, 0.5, 1, 2, or 4 µmol/L) for 24 h, followed by LPS treatment for 12 h as required. After 12 h of LPS treatment, CCK-8 reagent (10 µL per well) was added, and the cells were incubated for 2 hours. A GENios Pro plate reader (450 nm, Tecan, Männedorf, Switzerland) was then used to determine the relative cell viability. Relative cell viability was calculated using the following formula: Relative cell viability = (optical density (OD) value of the experimental group – OD value of the blank group) / (OD value of the control group – OD value of the blank group) × 100%.

Flow Cytometry

Reactive oxygen species (ROS) and apoptosis were assessed by flow cytometry. The Annexin V-fluorescein isothiocyanate (FITC) Apoptosis Detection Kit (CA1020, Solarbio, China) was used for apoptosis analysis. Briefly, HL-1 cardiomyocytes were stained with Annexin V-FITC (5 µL) and propidium iodide (PI) (5 µL) staining solution for 15 min in the dark. For ROS analysis, a ROS assay kit (S0033S) was purchased from Beyotime (China). Briefly, HL-1 cardiomyocytes were collected, suspended in diluted 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), and incubated for 20 minutes. To ensure complete contact between the probe and the cells, the suspension was inverted and mixed every three to five minutes. The cell apoptosis rate and relative fluorescence intensity were then measured by flow cytometry (CytoFLEX; Beckman Coulter, Brea, CA, USA).

Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA technique was used to measure the levels of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α). The tests were performed according to the instructions provided with the TNF-α ELISA kit (PT512) and IL-6 ELISA kit (PI326), both purchased from Beyotime (China). Absorbance at 450 nm was measured using a microplate reader (Model 680; Bio-Rad, Hercules, CA, USA).

Immunofluorescence Staining

Immunofluorescence staining was used to detect Nrf2 nuclear translocation. The cardiomyocytes were fixed and permeabilized using the Image-iT fixation/permeabilization kit (R37602, Invitrogen, USA), followed by blocking with BlockAid Blocking Solution (B10710, Invitrogen, USA). Subsequently, the cardiomyocytes were incubated with an Nrf2 antibody (#14596, 1/200, CST, USA), followed by incubation with Alexa Fluor 488 conjugated anti-rat IgG (#4416, 1/1000, CST, USA) for 30 min. 4′,6-diamidino-2-phenylindole, dilactate (DAPI; D3571, Invitrogen, USA) was used to stain the nucleus. Afterward, images of Nrf2 nuclear translocation were captured using EVOS Imaging Systems (4471136, Thermo Fisher Scientific, USA) at 400× magnification. The nuclear translocation of Nrf2 was quantified using ImageJ software (v1.52, NIH, Bethesda, MD, USA) by analyzing the co-localization of Nrf2 (green fluorescence) and DAPI (blue fluorescence). Briefly, three randomly selected fields per group were analyzed. The nuclear regions were defined by DAPI staining, and the Nrf2 fluorescence intensity within these regions was measured. The nuclear Nrf2 fluorescence intensity was normalized to the corresponding nuclear area and expressed relative to the Control group. All measurements were performed by two independent investigators blinded to group allocation, and the average value was used for statistical analysis.

Statistical Analysis

The experiment was conducted independently at least three times. All quantitative data were tested for normality using the Shapiro-Wilk test. Data conforming to a normal distribution were expressed as the mean ± standard deviation. All statistical analyses were performed using Graphpad Prism software (version 8.0; Graphpad software, Boston, MA, USA). A p value < 0.05 was considered statistically significant. One-way analysis of variance (ANOVA) was used to compare multiple groups, followed by the Bonferroni post-hoc test.

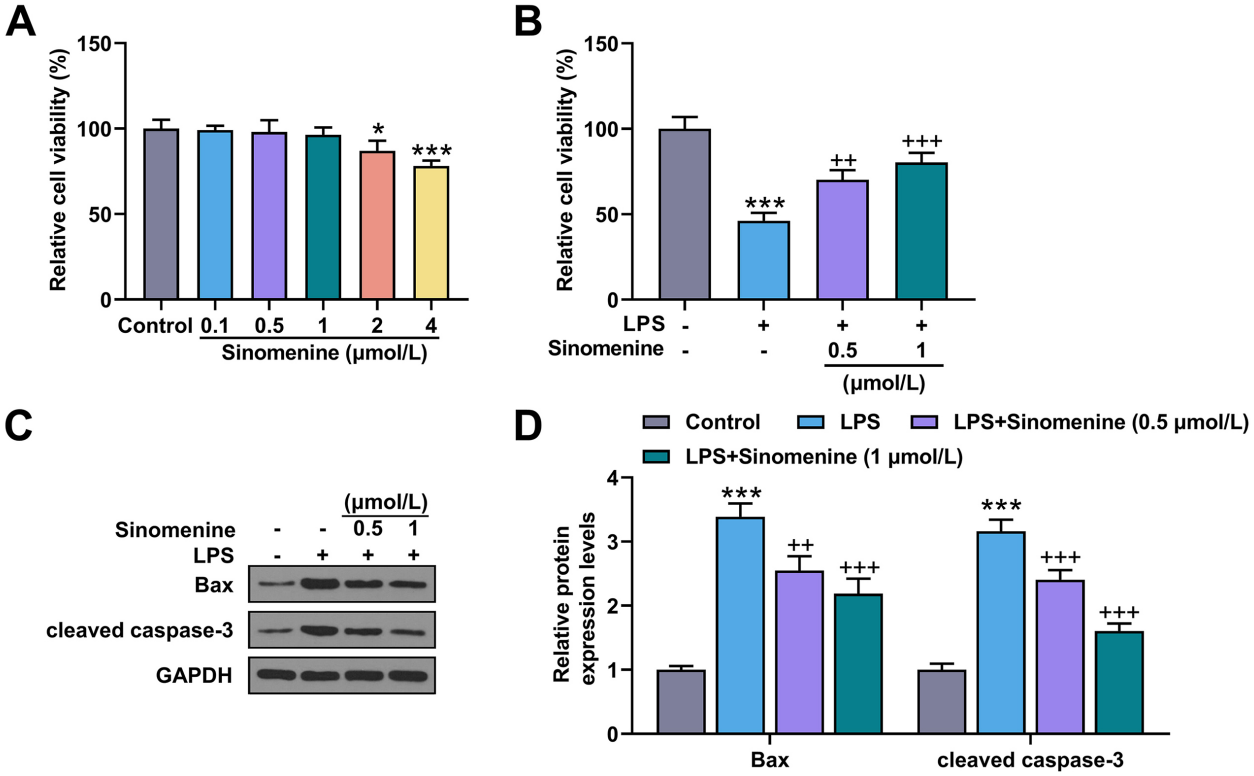

Fig. 1. The effects of sinomenine on the viability and apoptosis-related protein expression of LPS-induced HL-1 cells were investigated *in vitro*. (A) Cardiomyocytes were treated with different concentrations of sinomenine for 24 hours, and cell viability was assessed using the cell counting kit-8 (CCK-8) assay. (B) Cardiomyocytes were treated with sinomenine and LPS, and cell viability was assessed using the CCK-8 assay. (C,D) The expression of Bax and cleaved caspase-3 in cells from the Control, LPS, LPS+sinomenine-0.5, and LPS+sinomenine-1 groups was evaluated by Western blotting. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal control. $^{*}p < 0.05$, $^{***}p < 0.001$ vs Control; $^{++}p < 0.01$, $^{+++}p < 0.001$ vs LPS. Data are expressed as the mean ± standard deviation (n = 3 independent biological replicates). LPS, lipopolysaccharide.

Results

Effect of Sinomenine on LPS-Induced HL-1 Cardiomyocyte Injury In Vitro

We established a LPS-induced HL-1 cardiomyocyte injury model *in vitro* to further confirm the effect of sinomenine on LPS-induced myocardial damage. Sinomenine at concentrations below 2 μmol/L had no significant effect on cardiomyocyte viability, whereas 2 and 4 μmol/L sinomenine significantly decreased cell viability ($p < 0.05$, Fig. 1A). Therefore, we selected 0.5 and 1 μmol/L sinomenine for subsequent experiments.

Subsequently, we observed that sinomenine treatment improved cardiomyocyte viability, which was reduced by LPS ($p < 0.01$, Fig. 1B). In addition, sinomenine reversed the LPS-induced increases in Bax and cleaved caspase-3 expression ($p < 0.01$, Fig. 1C,D). Flow cytometry results showed that sinomenine reduced LPS-induced cardiomyocyte apoptosis and ROS levels ($p < 0.001$, Fig. 2A–D). Furthermore, we found that sinomenine reduced LPS-induced increases in MDA, TNF-α, and IL-6 while simultaneously restoring SOD activity reduced by LPS ($p < 0.05$, Fig. 2E–H). Additionally, LPS increased nuclear Nrf2 (green) levels, whereas sinomenine significantly enhanced LPS-induced Nrf2 nuclear translocation ($p < 0.05$), without affecting total Nrf2 expression (Fig. 3A–E). Mechanistically, sinomenine up-regulated the expression of NQO1 and HO-1 and inhibited p65 activation in LPS-treated cells ($p < 0.05$, Fig. 3F–H). Among these results, the effects of 1 μmol/L sinomenine were more pronounced. Therefore, 1 μmol/L sinomenine was used in subsequent mechanistic experiments.

The Impact of Nrf2 Silencing on the Amelioration of Myocardial Injury by Sinomenine In Vitro

Through *in vitro* experiments, we found that the mechanism by which sinomenine reduces myocardial injury may be associated with the Nrf2/HO-1 pathway. Therefore, we performed an *in vitro* rescue experiment using Nrf2 silencing. As shown in Fig. 3I, compared with the siNC group, transfection with siNrf2 significantly reduced Nrf2 mRNA expression in HL-1 cells ($p < 0.001$), confirming successful Nrf2 knockdown.

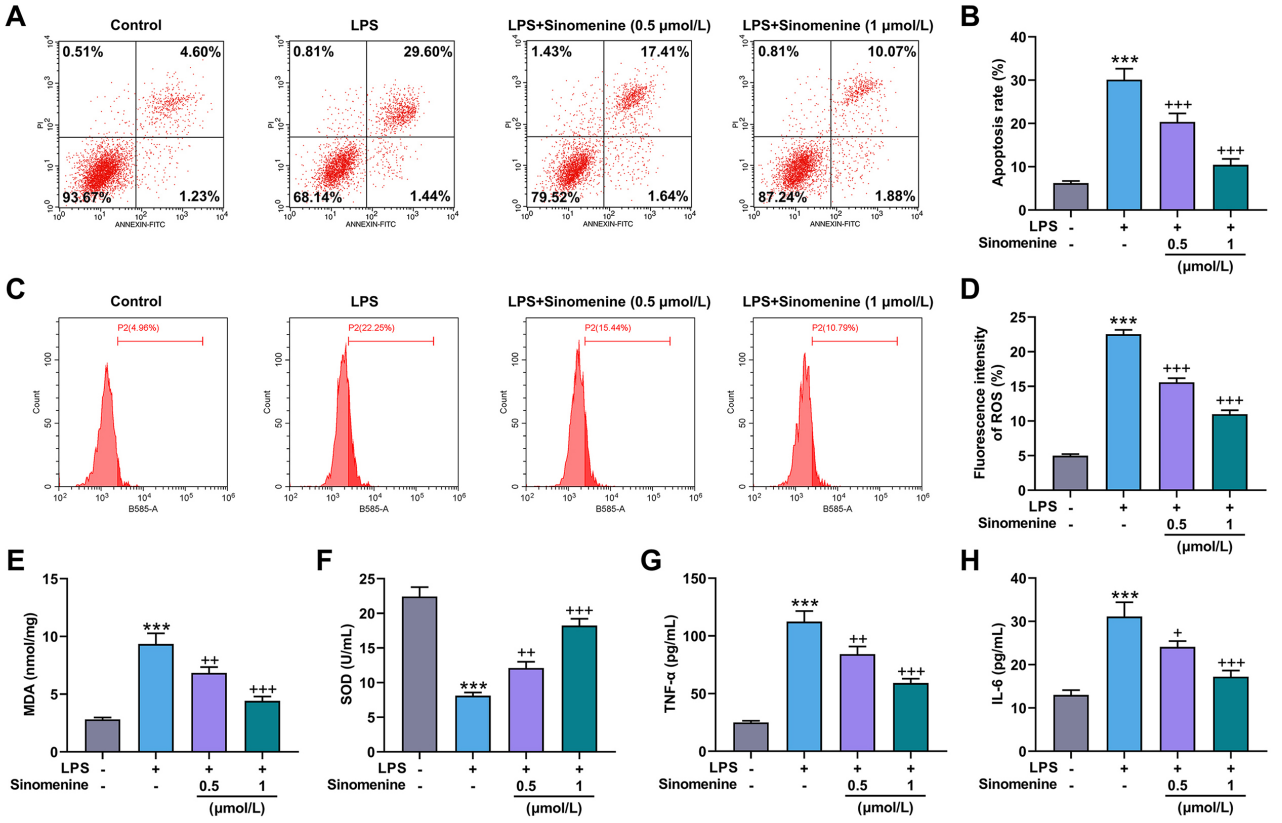

Fig. 2. The effects of sinomenine on apoptosis, oxidative stress, and inflammatory factors in LPS-induced HL-1 cells. (A–D) Apoptosis and ROS levels in the Control, LPS, LPS+sinomenine-0.5, and LPS+sinomenine-1 groups were evaluated by flow cytometry. (E,F) The level of malondialdehyde (MDA) and the activity of superoxide dismutase (SOD) in the cell supernatant were determined using the corresponding kits. (G,H) Enzyme-linked immunosorbent assay (ELISA) was used to detect the levels of the inflammatory factors tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) in the cell supernatant of each group. $^{***}p < 0.001$ vs Control; $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ vs LPS. Data are expressed as the mean ± standard deviation (n = 3 independent biological replicates). ROS, reactive oxygen species.

Neither LPS nor sinomenine significantly affected total Nrf2 levels, whereas Nrf2 silencing significantly reduced total Nrf2 expression ($p < 0.05$, Fig. 4A,B). Compared with the LPS group, the LPS+sinomenine-1 group exhibited a significant increase in nuclear Nrf2 levels, which was partially reversed by Nrf2 silencing ($p < 0.05$, Fig. 4A,C). Similarly, the protective effects of sinomenine on cardiomyocyte viability and its inhibitory effects on apoptosis and ROS production were partially reversed by Nrf2 silencing ($p < 0.001$, Fig. 4D–H). Furthermore, the effects of sinomenine on reducing MDA, TNF-α, and IL-6 levels and increasing SOD activity were partially reversed by Nrf2 silencing in LPS-induced cardiomyocytes ($p < 0.001$, Fig. 4I–L). In addition, compared with the control group, LPS treatment significantly increased NQO1, HO-1 levels and the p-p65/p65 ratio ($p < 0.05$, Fig. 5A–C). Compared with the LPS group, the LPS+Sinomenine-1 group exhibited a decreased p-p65/p65 ratio and increased NQO1 and HO-1 levels, whereas Nrf2 silencing partially reversed these changes ($p < 0.05$, Fig. 5A–C).

Discussion

Myocarditis is a disease characterized by edema, degeneration, and necrosis of cardiomyocytes [1,22]. LPS induces an acute inflammatory response that leads to myocarditis and is widely used to establish models of myocarditis [1]. Sinomenine is an extract of the Chinese herbal medicine Caulis Sinomenii and is currently mainly used to treat chronic inflammatory conditions, such as rheumatoid arthritis and chronic nephritis [23,24]. However, the protective effect of sinomenine against myocarditis has been less extensively studied. Therefore, this study used a LPS-induced HL-1 cardiomyocyte injury model to explore the therapeutic effect of sinomenine on myocarditis. Importantly, we found that sinomenine significantly attenuated LPS-induced cardiomyocyte injury, and this effect was at least partly dependent on the activation of the Nrf2 signaling pathway.

Reducing LPS-induced myocardial damage may provide valuable evidence for the clinical treatment of my-

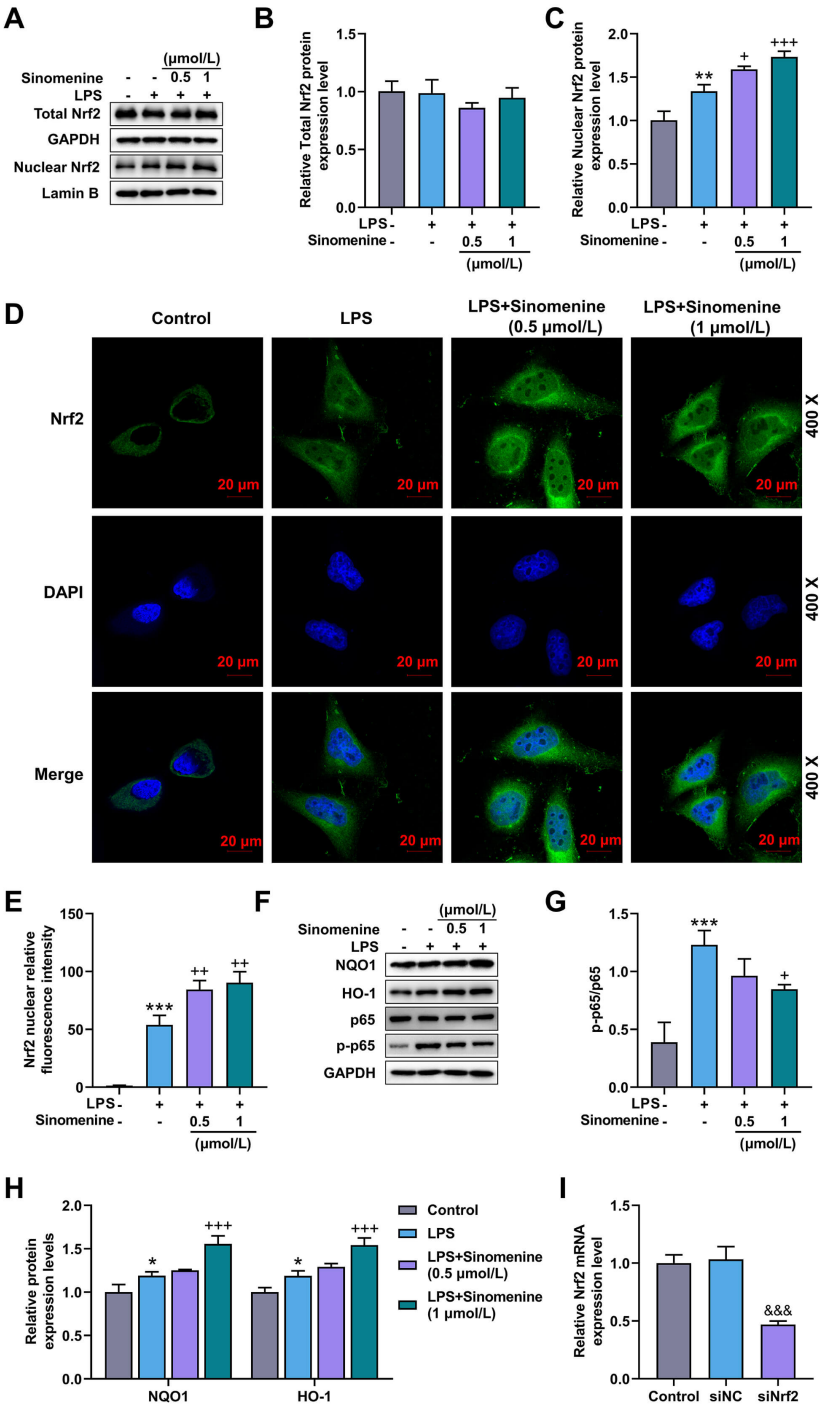

Fig. 3. Regulatory effects of sinomenine on the LPS-induced Nrf2 signaling pathway and p65 activation. (A–C) The expression of total and nuclear Nrf2 in the Control, LPS, LPS+sinomenine-0.5, and LPS+sinomenine-1 groups was detected by Western blotting. GAPDH or Lamin B served as the internal control. (D,E) Immunofluorescence staining was used to detect Nrf2 nuclear translocation in the Control, LPS, LPS+sinomenine-0.5, and LPS+sinomenine-1 groups. Magnification ×400; scale bar, 20 μm. Nrf2-positive signals were visualized as green fluorescence, while nuclei were stained blue. (F–H) Western blotting was performed to detect the expression levels of NQO1, HO-1, p65, and p-p65 in the Control, LPS, LPS+sinomenine-0.5, and LPS+sinomenine-1 groups. GAPDH served as the internal control. (I) Nrf2 expression in the Control, siNC and siNrf2 groups was detected by qRT-PCR. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs Control; $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ vs LPS; $^{\&\&\&}p < 0.001$ vs siNC. Data are expressed as the mean ± standard deviation (n = 3 independent biological replicates). Nrf2, nuclear factor erythroid 2-related factor 2; NQO1, NAD(P)H quinone dehydrogenase 1; HO-1, heme oxygenase-1; siNC, small interfering RNA (siRNA) negative control.

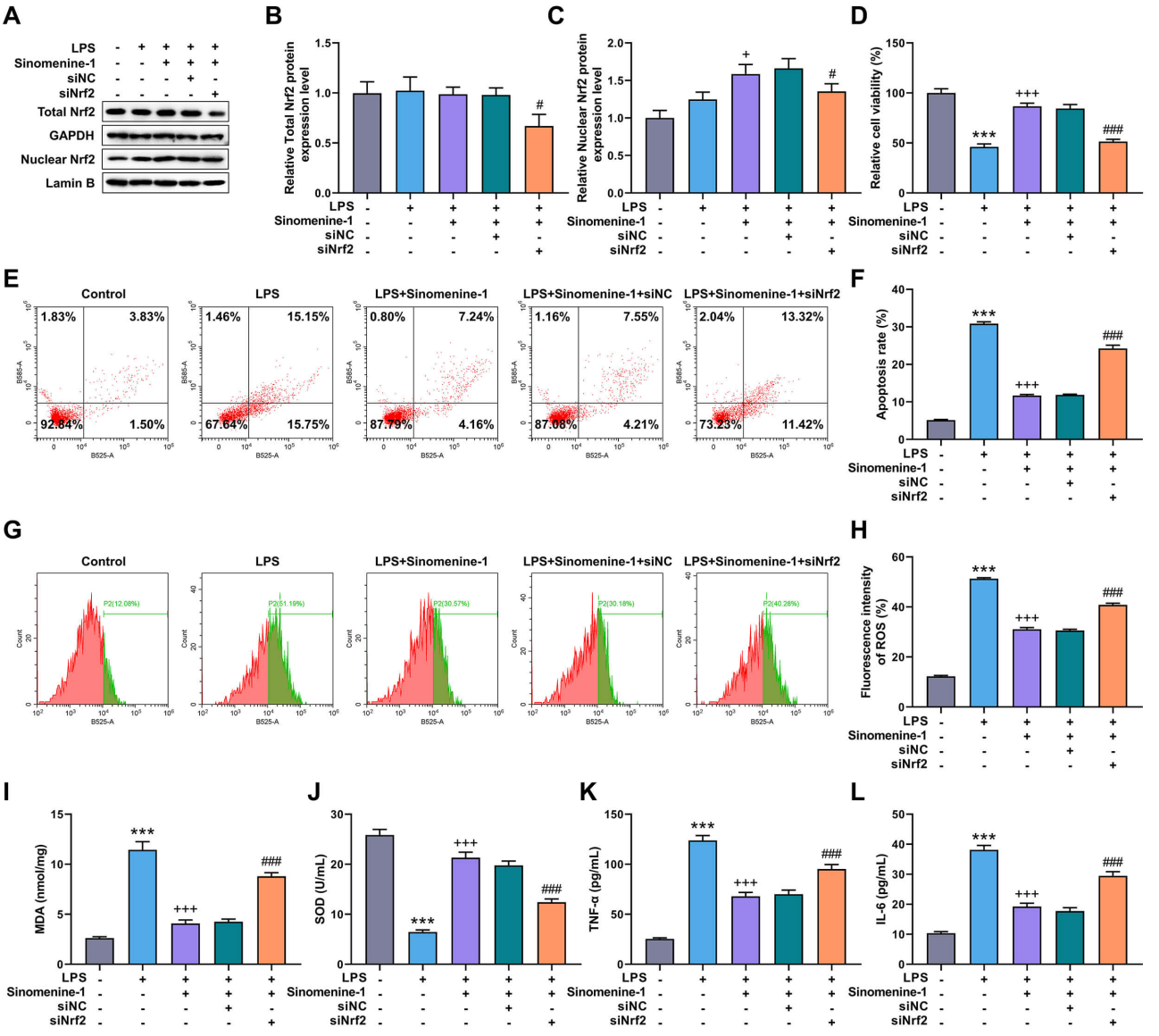

Fig. 4. Nrf2 silencing attenuates the protective effects of sinomenine on LPS-induced oxidative stress, apoptosis, and inflammation. (A–C) Nrf2 expression in cells from the Control, LPS, LPS+Sinomenine-1, LPS+Sinomenine-1+siRNA negative control (siNC), LPS+Sinomenine-1+siNrf2 group cells was detected by Western blotting. (D) The viability of myocardial cells in each group was assessed using the CCK-8 assay. (E–H) Apoptosis and ROS levels in each group were assessed by flow cytometry. (I,J) The level of MDA and the activity of SOD in the cell supernatant of each group were determined using the corresponding kits. (K,L) ELISA was used to detect the levels of the inflammatory factors TNF-α and IL-6 in the cell supernatant of each group. $^{***}p < 0.001$ vs Control; $^{+}p < 0.05$, $^{+++}p < 0.001$ vs LPS; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs LPS+Sinomenine-1+siNC. Data are expressed as the mean ± standard deviation (n = 3 independent biological replicates).

ocarditis. For example, Su et al. [25] found that Geniposide can also reduce LPS-induced damage and inhibit inflammatory responses. In this study, we found that sinomenine protected cardiomyocytes against LPS-induced oxidative stress and inflammatory responses. These findings suggest that sinomenine may represent a potential therapeutic candidate for inflammatory myocardial injury; nevertheless, its clinical efficacy and safety require further validation in animal models and clinical studies.

Recent research has demonstrated that sinomenine provides a variety of benefits, including anti-inflammatory, anti-depressant, and anti-cancer properties [26]. For instance, sinomenine treats rheumatoid arthritis by regulating gut microbiota, which in turn affects tryptophan metabolism and activates aryl hydrocarbon receptors [27]. Li et al. [28] showed that sinomenine targets guanylate-binding protein 5 (GBP5) and regulates the P2X purinoceptor 7 (P2X7) receptor to reduce collagen-induced arthritis

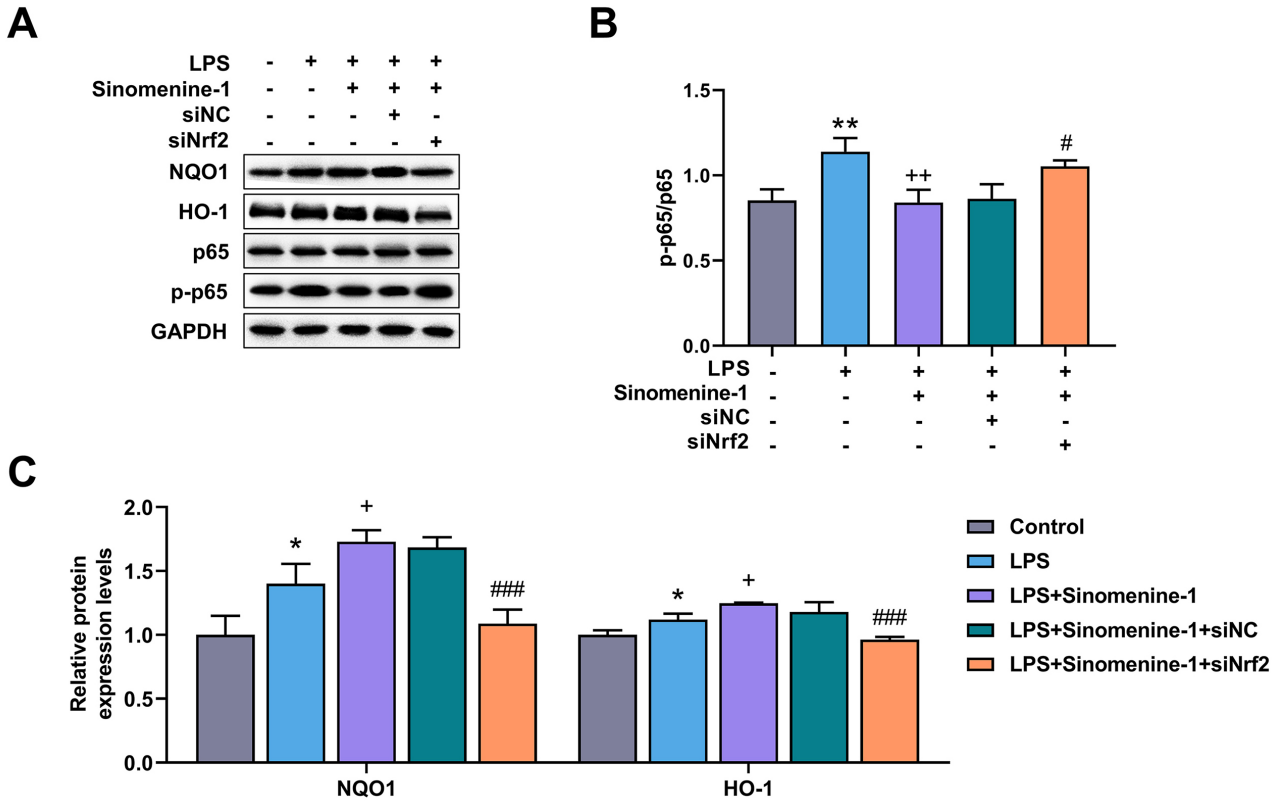

Fig. 5. The influence of Nrf2 silencing on the NF-κB pathway in sinomenine-treated, LPS-induced HL-1 cells. (A–C) The expression levels of NQO1, HO-1, p65, and p-p65 in cardiomyocytes from the Control, LPS, LPS+Sinomenine-1, LPS+Sinomenine-1+siNC, and LPS+Sinomenine-1+siNrf2 groups were determined by Western blotting. GAPDH served as the internal control. $^{*}p < 0.05$, $^{**}p < 0.01$ vs Control; $^{+}p < 0.05$, $^{++}p < 0.01$ vs LPS; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs LPS+Sinomenine-1+siNC. Data are expressed as the mean ± standard deviation (n = 3 independent biological replicates). NF-κB, nuclear factor kappa B.

in mice. Zhang et al. [29] demonstrated that sinomenine attenuates traumatic spinal cord injury and regulates oxidative stress and inflammation through the Nrf2 pathway. These findings suggest that sinomenine exerts its effects on various diseases by regulating specific signaling pathways; however, its underlying mechanism in myocarditis remains unclear. This study provides evidence that sinomenine attenuates LPS-induced cardiomyocyte injury in association with the activation of Nrf2 and its downstream antioxidant response, as well as the inhibition of NF-κB p65 activation. Notably, Nrf2 silencing partially reversed the protective effects of sinomenine, indicating that Nrf2-dependent signaling is an important contributor, rather than the sole mediator, of its cardioprotective effects. Nevertheless, these findings do not establish a direct upstream-downstream relationship between Nrf2 and NF-κB, and further studies are required to elucidate the potential crosstalk between these pathways.

Nrf2 is a key regulator of the body's tolerance to oxidative stress and is involved in several pathophysiological processes [30,31]. Nrf2 is located in the cytoplasm, interacts with cytoplasmic chaperone proteins, and remains inactive under normal physiological conditions [32]. When cells are damaged, external stimuli activate Nrf2, which then translocates to the nucleus, binds to antioxidant response elements, induces the expression of various antioxidant proteins (such as SOD) and antioxidant defense enzymes (such as HO-1), and reduces the production of ROS and the peroxide end product MDA, ultimately contributing to the antioxidant response [33,34]. Our findings demonstrate that Nrf2 silencing can counteract the beneficial effects of sinomenine on oxidative stress-induced damage and LPS-induced inflammation. These findings suggest that Nrf2 activation is involved in the protective effects of sinomenine against LPS-induced cardiomyocyte injury in this cellular model. However, whether Nrf2 is a direct molecular target of sinomenine cannot be determined from the present knockdown experiments, and further studies are needed to clarify this relationship.

This study has several limitations. First, this study was conducted exclusively using an LPS-induced HL-1 cardiomyocyte injury model *in vitro*, which mainly reflects inflammatory and oxidative stress-related cellular damage and cannot fully represent the complex pathophysiology of clinical myocarditis (e.g., inflammatory cell infiltration, edema, and cardiac dysfunction). No *in vivo* experiments

were performed. Therefore, the current findings cannot be directly extrapolated to the complex pathophysiology of clinical myocarditis, and future studies using animal models of myocarditis are needed to validate these observations. Second, the long-term protective effects of sinomenine on cardiomyocytes, as well as its potential toxicity and side effects, remain unclear and require further comprehensive *in vivo* and clinical evaluation.

Conclusion

In summary, this study demonstrates that sinomenine effectively alleviates LPS-induced cardiomyocyte inflammatory injury by suppressing oxidative stress, apoptosis, and inflammatory responses. Nrf2 knockdown experiments revealed that the cardioprotective effects of sinomenine are associated with the activation of Nrf2/HO-1 signaling. These results suggest that strategies targeting the Nrf2 pathway may represent a potential therapeutic approach for inflammatory myocardial injury and provide additional experimental evidence supporting the protective role of sinomenine in cardiomyocytes under inflammatory conditions. However, these conclusions are based on an *in vitro* LPS-induced HL-1 cell model, and further *in vivo* and clinical studies are warranted to confirm the translational potential of these findings.

Availability of Data and Materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Author Contributions

XY, HFL and FZ designed the research study; BX and KY performed the research; HT and BSY collected and analyzed the data. BSY has been involved in drafting the manuscript and all authors have been involved in revising it critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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