

Original Article

Artesunate protects against sepsis-induced cardiomyopathy by reducing Akt/FoxO3-mediated apoptosis and inflammation

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Abstract

Sepsis-induced cardiomyopathy (SICM) is a life-threatening complication of sepsis characterized by acute and reversible myocardial dysfunction, for which effective targeted therapies remain limited. Artesunate (ART), a well-established first-line antimalarial agent, has attracted increasing attention for its anti-inflammatory, antioxidant, and cytoprotective properties. However, its role in SICM has not been fully elucidated. In this study, a murine SICM model is established using lipopolysaccharide (LPS) to evaluate the effects of ART on animal mortality, cardiac function, histopathology, and biomarkers of myocardial injury. In parallel, the *in vitro* study is performed using neonatal rat cardiomyocytes and H9c2 cells exposed to LPS in the presence or absence of ART. Transcriptomic and metabolomic analyses are used to elucidate the molecular mechanisms underlying ART-mediated cardioprotection. ART administration significantly improves cardiac function, attenuates myocardial injury, alleviates the inflammatory response, and reduces cardiomyocyte apoptosis in LPS-challenged mice. Consistent results are observed *in vitro*. Integrated multi-omics analyses identify the Akt/FoxO3 signaling pathway as a critical target of ART. ART treatment contributes to increased Akt phosphorylation and subsequent suppression of FoxO3 nuclear translocation and its transcriptional activity. Pharmacological inhibition of Akt using MK2206 or genetic knockdown of *FoxO3* by siRNA abolishes the cardioprotective effects of ART. In addition, molecular docking analysis suggests a potential interaction between ART and Akt isoforms. Collectively, these findings demonstrate that ART possesses significant cardioprotective effects in experimental SICM through modulation of the Akt/FoxO3 signaling axis, leading to reduced apoptosis and inflammation. ART may serve as a therapeutic candidate for the management of SICM.

Key words artesunate, sepsis-induced cardiomyopathy, FoxO3 transcription factor, Akt, apoptosis, inflammation

Introduction

Sepsis remains a major global health challenge and is associated with substantial morbidity and mortality, affecting over 30 million people worldwide annually [1]. Clinically, sepsis is characterized by a severe systemic inflammatory response that precipitates multi-organ dysfunction, including injury to the heart, lungs, kidneys, and other vital organs, due to dysregulated immune responses to bacterial or viral infections [2]. Among the various etiological pathogens, Gram-negative bacteria are responsible for the majority of sepsis cases. Lipopolysaccharide (LPS), a structural component of the bacterial outer membrane, plays a central role in the development of endotoxemia, the initiation of a cytokine storm, and

the progression to septic shock [3].

Sepsis-induced cardiomyopathy (SICM) is a particularly severe and life-threatening complication of sepsis characterized by acute but potentially reversible myocardial dysfunction, including impaired systolic contractility, diastolic abnormalities, and reduced ejection fraction. Epidemiological studies indicate that SICM occurs in approximately 40% of septic patients and is associated with a markedly elevated mortality rate ranging from 70% to 90% [4]. Despite its clinical relevance, the pathogenesis of SICM is complex and multifactorial, involving sustained systemic inflammatory responses, cardiomyocyte apoptosis, mitochondrial dysfunction, oxidative stress injury, *etc.* [5,6]. Currently, therapeutic strategies

for SICM remain largely supportive, focusing on early fluid resuscitation and antibiotics [7]. However, there are no approved pharmacological therapies that specifically target the underlying mechanisms of SICM, underscoring the urgent need for novel and effective therapeutic approaches.

Artesunate (ART), a semi-synthetic derivative of artemisinin, has been widely employed as a first-line antimalarial agent for over five decades and has demonstrated favorable safety and pharmacokinetic profiles in clinical practice [8,9]. Beyond its well-established antimalarial efficacy, accumulating evidence suggests that ART possesses a broad spectrum of pharmacological properties, including but not limited to anti-inflammatory [10], anti-cancer [11], and potential cardioprotective effects [12]. Notably, emerging evidence has highlighted the promising role of ART in mitigating sepsis-related damage across multiple organs [13,14]. Nevertheless, whether ART confers direct protective effects against SICM and the mechanisms underlying such effects remain largely unexplored.

At the mechanistic level, ART has been reported to confer protective effects in various pathological models by modulating cell survival pathways, notably through the downregulation of pro-apoptotic factors and the upregulation of anti-apoptotic factors, thereby alleviating tissue injury [15,16]. In our preliminary investigation, we constructed the “SICM-related gene-ART candidate target” pharmacology network, with Gene Ontology (GO) enrichment analysis of the intersected gene identified apoptosis as a central biological process potentially involved in the therapeutic effects of ART on SICM. Based on these observations, we hypothesized that ART may alleviate SICM by suppressing cardiomyocyte apoptosis.

In the current study, we employed both an *in vivo* LPS-induced SICM mouse model and an *in vitro* LPS-challenged cardiomyocyte model to systematically investigate the protective role of ART in SICM and to elucidate the underlying mechanisms.

Material and Methods

Animal model of SICM and drug administration

C57BL/6 male mice (8–12 weeks old), weighing approximately 25 g, were obtained from Jiangsu Huachuang Sino Pharmatech (Taizhou, China). All mice were housed under standard laboratory conditions with a 12/12-h light/dark cycle at a controlled temperature of 25°C. Animals were randomly assigned to the following groups ($n = 9$ per group): normal control (Con), 50 mg/kg ART-treated control (ART), LPS-induced SICM model (LPS), SICM model treated with 12.5 mg/kg ART (LPS + ART12.5), SICM model treated with 25 mg/kg ART (LPS + ART25), and SICM model treated with 50 mg/kg ART (LPS + ART50). Sepsis was induced by intraperitoneal injection of LPS (CL2630; Sigma, St Louis, USA). ART (12.5, 25, or 50 mg/kg body weight; HY-N0193; MedChemExpress, Monmouth Junction, USA) was administered intraperitoneally 10 min prior to LPS (10 mg/kg body weight) injection and again 4 h post-injection. The control groups received an equal volume of saline. Twenty-four hours after LPS administration, cardiac function was assessed, and myocardial injury markers were measured to determine the severity of septic cardiomyopathy. Before sacrifice, the mice were anesthetized with 50 mg/kg of 1% sodium pentobarbital. Blood samples were collected in EDTA tubes (1.8 mg/mL) and centrifuged at 400 g for 20 min at 4°C to obtain plasma. Heart tissues were rapidly excised, either snap-frozen at -80°C for subsequent analysis or fixed in 4% paraformaldehyde (PFA) and embedded in paraffin. All

procedures were conducted in compliance with the Guidelines for the Care and Use of Laboratory Animals and approved by the Experimental Animal Ethics Committee of Chongqing Medical University (approval No. IACUC-SAHQCMU-2024-00153).

Cardiac function measurements

After anesthesia with 2% isoflurane, the mice were positioned supine on a heating pad to maintain body temperature. Cardiac function was assessed using transthoracic echocardiography with a linear transducer (VINNO 6 LAB; VINNO Technology, Suzhou, China). Echocardiographic images were acquired, and left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), left ventricular internal diameter at end-diastole (LVIDd), left ventricular internal diameter at end-systole (LVIDs), left ventricular posterior wall thickness at end-diastole (LVPWd), and at end-systole (LVPWs) were calculated using the system's dedicated software.

Assessment of myocardial injury markers

Plasma samples collected at the end of the experimental period were used to assess serum myocardial injury markers. The concentrations of brain natriuretic peptide (BNP; MM-0060M1) and cardiac troponin (cTn; MM-45262M1) were measured using ELISA kits (Jiangsu Medical Industrial Co., Ltd., Yancheng, China) according to the manufacturer's protocol. Absorbance values were determined via spectrophotometry using a Varioskan LUX microplate reader (Thermo Fisher Scientific, Waltham, USA). In addition, the levels of creatine kinase-MB (CK-MB; S03023; Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China) and lactate dehydrogenase 1 (LDH1; C058; Huili Life Sciences Co., Ltd., Changchun, China) were quantified using enzymatic assay kits. All biochemical measurements were conducted using an automated analyzer (Chemray 800; Rayto Life and Analytical Sciences Co., Ltd.).

Histological analysis

Heart tissues were fixed in 4% paraformaldehyde at 4°C for 24 h, dehydrated through a graded ethanol series, and embedded in paraffin. The paraffin-embedded tissues were sectioned into 5- μm -thick slices and mounted on glass slides for histological examination. To assess myocardial morphology, the sections were deparaffinized, rehydrated, and stained using hematoxylin and eosin (H&E) staining kits (G1005; Servicebio, Wuhan, China) according to the manufacturer's protocol. Light-field and fluorescence images were acquired using a Nikon Eclipse Ci-L microscope (Nikon, Tokyo, Japan).

Immunohistochemistry (IHC) staining

Tissue sections were deparaffinized, rehydrated through a graded series of ethanol with decreasing concentrations, and subjected to heat-induced antigen retrieval in citrate buffer (pH 6.0) at 95°C for 20 min. Endogenous peroxidase activity was subsequently blocked using 3% hydrogen peroxide for 10 min. The sections were then incubated overnight at 4°C with primary antibodies against cleaved Caspase-3 (1:200, 25128-1-AP; Proteintech, Wuhan, China), CD68 (1:100, ab283654; Abcam, Cambridge, UK), and phospho-FoxO3 (1:200, R381118; Zenbio, Chengdu, China). After washing with phosphate-buffered saline (PBS), the sections were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (1:500, RGAR011; Proteintech) for 1 h at room

temperature. Immunoreactivity was visualized using 3,3'-diaminobenzidine (DAB; D5905; Sigma) as the chromogenic substrate, followed by counterstaining with hematoxylin. Finally, the sections were dehydrated and mounted with neutral balsam. Images were captured using a Nikon Eclipse Ci-L light microscope.

Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay

Cardiomyocyte apoptosis was evaluated using the TUNEL assay with a commercial kit (G1501; Servicebio) following the manufacturer's protocol. Nuclear counterstaining was performed with 4',6-diamidino-2-phenylindole (DAPI; 36308ES20; Yeasen, Shanghai, China). Images were captured using a Nikon Eclipse Ci-L light microscope. The proportion of TUNEL-positive nuclei relative to the total number of nuclei was calculated to quantify the extent of apoptosis.

Measurement of reactive oxygen species

To assess oxidative stress injury, intracellular reactive oxygen species (ROS) levels in frozen myocardial tissues and H9c2 cells were measured using dihydroethidium (DHE; G1746; Servicebio) and 2',7'-dichlorofluorescein diacetate (DCFH-DA; G1706; Servicebio), respectively, according to the manufacturer's instructions. Fluorescence images were captured using a Nikon Eclipse Ci-L light microscope. The fluorescence intensity of ROS in both myocardial tissues and cells was quantified using ImageJ software (NIH, Bethesda, USA).

Network pharmacological analysis

The possible targets among ART were predicted and integrated using Swiss Target Prediction (<http://www.swisstargetprediction.ch/>) with a probability greater than 0.1, and all the target names were verified using the UniProt database (<https://www.uniprot.org/>). In the GeneCards (<https://www.genecards.org/>), DisGeNET (<https://www.disgenet.org/>), and OMIM (<http://www.omim.org/>) databases, "septic cardiomyopathy" was used as the keyword to search, download, and integrate all related genes. Targets in OMIM with a score of at least 0.02 in DisGeNET and at least 1 in GeneCards were regarded as SICM-related genes, with duplicates removed. The intersection targets of ART against SICM were obtained using the jVenn online software mapping tool platform (<https://www.bioinformatics.com.cn/>). Therapeutic targets and protein-protein interaction (PPI) networks were constructed using the STRING database (<https://string-db.org/>). The "SICM-related genes and ART candidate targets" network was further mapped by Cytoscape 3.10.0 software using the MCC algorithm. GO enrichment analysis of the intersected genes was performed using the ClusterProfiler R package (version 4.12.6).

Transcriptome sequencing analysis

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, USA). RNA sequencing (RNA-seq) and subsequent data processing were conducted by Shanghai Applied Protein Technology Corporation (Shanghai, China). For quality control, genes were pre-filtered based on counts greater than 20 and reads per kilobase million (RPKM) exceeding 1 in at least one sample. Differentially expressed genes (DEGs) were identified using the DESeq2 package, applying a fold change threshold greater than 1 and an adjusted *P* value (false discovery rate, FDR) less than 0.05. The *P* value was

calculated using the Z test (Pearson's chi-square test) within the Agilent GeneSpring GX software suite. The statistical significance of gene expression was further represented by the negative logarithm ($-\log_{10}$) of the *P* value. GO enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were performed using the ClusterProfiler R package (version 4.12.6).

Metabolomics analysis

Non-targeted metabolomics analysis was conducted by Shanghai Applied Protein Technology Corporation. Metabolic profiling of the control (LPS; *n* = 5) and treatment (LPS + ART50; *n* = 5) groups was performed using ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF MS). The resulting data were subjected to multivariate statistical analyses, including principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), to evaluate intergroup metabolic differences. Hierarchical clustering analysis (HCA) and metabolite correlation analysis were employed to investigate the relationships among different metabolites. Metabolites with a variable importance in projection value > 1 were evaluated using Student's *t* test. *P* < 0.05 was considered to indicate statistically significant results. The identified metabolites were mapped to the KEGG Compound Database and subjected to pathway enrichment analysis using the KEGG Pathway Database.

Cell culture and treatment

Neonatal rat cardiomyocytes (NRCMs) were isolated from 1- to 3-day-old male and female Sprague-Dawley rats (Jiangsu Huachuang Sino Pharmatech) in a manner described previously [17]. Briefly, excised hearts were dissected into small pieces and subjected to sequential cycles of digestion for 7 min per cycle in 7 mL of HEPES-buffered saline solution (0.08% trypsin, 0.06% collagenase type IV, 136 mM NaCl, 4.2 mM NaHCO₃, 5.5 mM glucose, 4 mM KCl, and 8.3 mM HEPES). The cells were incubated at 37°C for 1 h. Non-adherent cells were primary cardiomyocytes, which were collected and cultivated for the experiment. NRCMs and H9c2 cells, which were obtained from Haixing Technology (Shenzhen, China), were cultured in Dulbecco's Modified Eagle Medium (DMEM; 11995065; Gibco, Carlsbad, USA) supplemented with 10% fetal bovine serum (FBS; A5670701; Gibco) at 37°C in a humidified incubator with 5% CO₂. To mimic *in vivo* conditions, cardiomyocytes were treated with LPS (10 µg/mL) in the presence or absence of ART (5 µM). The cells were divided into four groups: control (untreated), LPS-treated (LPS), ART-treated (ART), and LPS combined with ART-treated (LPS + ART). ART or PBS was administered 4 h prior to LPS treatment. Cells were harvested for subsequent analyses 24 h after LPS exposure.

Cell viability

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8; CA1210; Solarbio, Beijing, China) according to the manufacturer's instructions. Briefly, H9c2 cells were seeded in 96-well plates and treated with various concentrations of ART, with or without LPS, to determine the optimal ART concentration. After 24 h of incubation, 10 µL of CCK-8 solution was added to each well, followed by incubation for 1 h. Cell viability was determined by measuring absorbance at 450 nm using a Varioskan LUX spectrophotometer (Thermo Fisher Scientific).

Immunofluorescence (IF) staining

For cultured NRCM and H9c2 cell preparation, cells were rinsed with sterile PBS and fixed with PFA for 20 min at room temperature. Next, the cells were permeabilized with 0.1% Triton X-100 for 15 min at room temperature, followed by a blocking step with 5% (w/v) BSA for 1 h at room temperature. Then, the cells were incubated at 4°C overnight with primary antibodies against phospho-nuclear factor- κ B p65 (p-P65; 1:200, 82335-1-RR; Proteintech) or FoxO3 (1:200, 66428-1-Ig; Proteintech). After overnight incubation, DyLight 594-conjugated goat anti-rabbit IgG (1:500, A23420; Abbkine, Wuhan, China) or DyLight 488-conjugated goat anti-rabbit IgG (1:500, A23220; Abbkine) served as the secondary antibody, followed by DAPI staining. Images were captured using a Nikon Eclipse Ci-L microscope and further processed with ImageJ software.

Protein extraction and western blot analysis

Heart tissues and cells were homogenized using RIPA lysis buffer (P0013B; Beyotime, Shanghai, China). After centrifugation, the protein concentration of the samples was determined using the bicinchoninic acid (BCA) protein assay (P0010; Beyotime). Proteins were then separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene fluoride (PVDF) membrane (IPVH00010; Merck Millipore, Burlington, USA). The membranes were blocked at room temperature in rapid blocking buffer (PS108P; Epizyme Biotech, Shanghai, China), followed by overnight incubation at 4°C with the following primary antibodies against Bax (1:1000, 50599-2-Ig; Proteintech), Bcl-2 (1:1000, 26593-1-AP; Proteintech), phospho-FoxO3 (1:2000, R381118; Zenbio), FoxO3 (1:1000, 66428-1-Ig; Proteintech), Akt (1:1000, 9272; Cell Signaling Technology, Beverly, USA), phospho-Akt (1:1000, 9271; Cell Signaling Technology), SIRT1 (1:2000, R25721; Zenbio), JNK1/2/3 (1:1000, R24780; Zenbio), phospho-JNK1/2/3 (1:1000, R381100; Zenbio), AMPK (1:2000, 2532; Cell Signaling Technology), phospho-AMPK (1:1000, 2531; Cell Signaling Technology), and GAPDH (1:2000, 5174; Cell Signaling Technology). After washing three times with TBST, the membranes were incubated with either rabbit anti-goat or mouse anti-goat secondary antibodies (1:8000, HA1001 or HA1006; HUABIO, Hangzhou, China) for 1 h at room temperature. The bands were visualized using enhanced chemiluminescence reagents and detected with a ChemiDoc XRS+ Imaging System (Bio-Rad, Hercules, USA). Band intensity was quantified using ImageJ software, and protein expression levels were normalized to GAPDH. Data are expressed as fold changes relative to the control group.

siRNA and transfection

Oligonucleotides for siRNA were obtained from Sangon Biotech (Shanghai, China). NRCMs and H9c2 cells were transfected with 100 nM of control siRNA (NC) (5'-AGGUAGUGUAAUCGCCUUG-3') or rat *FoxO3* siRNA (5'-CCUCAUGGAUGACCUGCUATT-3') by Lipofectamine 3000 Reagent (Invitrogen). At 24 h after transfection, the cells in each group were correspondingly treated.

Cytoplasmic and nuclear protein extraction

Nuclear and cytoplasmic fractionation was conducted using a commercial kit (P0028; Beyotime) following the manufacturer's instructions. NRCMs and H9c2 cells with the indicated treatment were harvested and suspended in an equal volume of solution A containing 1% PMSF, and cell lysates were mixed with 10 μ L of

solution B by vortexing and placed on ice for 1 min. Tissue sample fragments were homogenized with a mixture of solutions A and B by a glass homogenizer. The supernatant (cytoplasmic protein) was obtained after centrifugation at 13,684 g for 5 min; 50 μ L of solution C was used to suspend the sediment. Samples were mixed by vortexing for 30 s every 2 min 15 times, and the supernatant (nuclear protein) was collected after centrifugation. Nuclear proteins and cytoplasmic proteins were analyzed separately by western blot analysis.

Molecular docking

As an active ingredient, ART was selected to dock with three proteins in potential therapeutic targets. The 2D structures of small-molecule ligands were obtained from the PubChem database (<http://pubchem.ncbi.nlm.nih.gov/>). These structures were then imported into ChemOffice software to generate their corresponding 3D conformations, which were subsequently saved in MOL2 file format. High-resolution crystal structures of protein targets were retrieved from the RCSB Protein Data Bank (<http://www.rcsb.org/>) to serve as molecular docking receptors. Prior to docking, the protein structures were preprocessed using PyMOL software, including the removal of water molecules and phosphate groups, and saved in PDB format. Molecular docking was performed using AutoDock Vina 1.5.6 to investigate the potential interactions between proteins and ligands. Both the protein and ligand structures were pretreated with AutoDock, including hydrogenation of the protein, removal of water molecules, and assignment of torsional flexibility for ligands. The docking grid box was then defined, and the optimal binding conformation was selected based on the docking score. Visualization and interaction analysis between the test compounds and key amino acid residues were carried out using PyMOL and Discovery Studio 2019, generating both 2D interaction diagrams and 3D structural representations.

Statistical analysis

Data are presented as the mean $\pm$ standard error of the mean (SEM) and analyzed using GraphPad Prism 10.0 software (GraphPad Software, San Diego, USA). Comparisons between two groups were performed using an unpaired Student's *t* test. Differences among multiple groups were assessed by one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test. A *P* value < 0.05 was considered statistically significant.

Results

ART alleviates sepsis-induced cardiac dysfunction and myocardial injury

The experimental design for the animal study is illustrated in Figure 1A. The general condition of septic mice was evaluated by survival rate, murine sepsis score (MSS), and body weight. To determine whether ART could improve survival in SICM, we first assessed the 24-h survival rate. While we observed a trend toward increased survival in ART-treated mice compared to the LPS control group, this difference did not reach statistical significance, likely due to the limited sample size (Figure 1B). The mean MSS was significantly elevated in the LPS group compared to the Con group, while ART administration at dosages of 25 mg/kg and 50 mg/kg markedly decreased the MSS (Supplementary Figure S1A). Additionally, mice in the LPS group exhibited significant weight loss by the end of the experiment, whereas ART treatment showed a trend toward weight

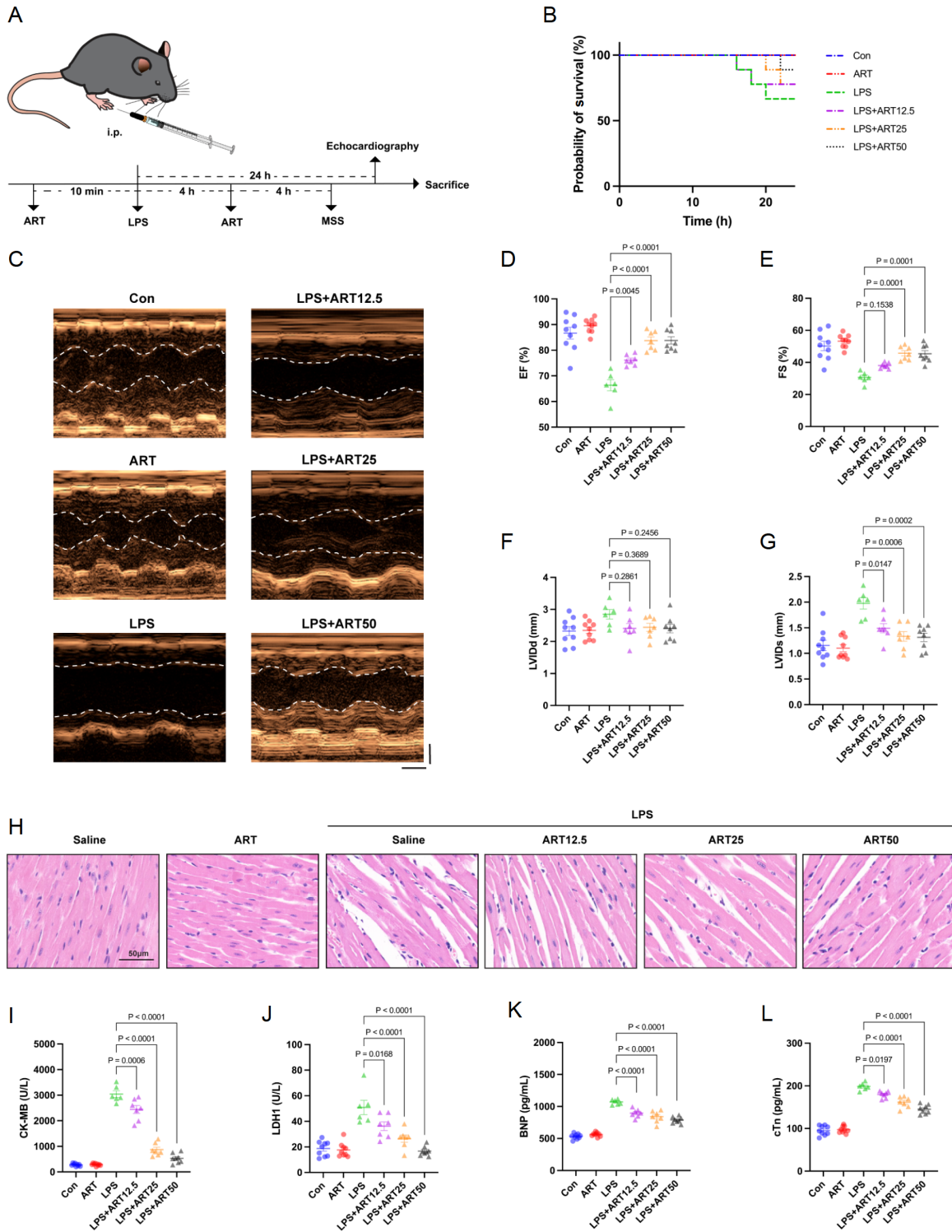


Figure 1. ART alleviates sepsis-induced cardiac dysfunction and myocardial injury (A) Schematic illustration of the study. (B) Survival curve among NC, ART, LPS, LPS + ART12.5, LPS + ART25, and LPS + ART50 groups. $n = 9$. (C) Representative M-model echocardiographic images from mice. Scale bar (horizontal), 0.05 s; scale bar (vertical), 0.1 cm. (D–G) Echocardiographic assessment of LVEF, LVFS, LVIDs, and LVIDd in the indicated groups. $n = 9$ in Con and ART groups, $n = 6$ in the LPS + ART50 group, $n = 7$ in LPS + ART12.5 and LPS + ART25 groups, $n = 8$ in the LPS + ART50 group. (H) Representative H&E staining images of experimental groups. Scale bar, 50 μm . (I–L) Levels of myocardial injury biomarkers in the indicated groups, including CK-MB (I), LDH1 (J), BNP (K), and cTn (L). $n = 9$ in Con and ART groups, $n = 6$ in the LPS + ART50 group, $n = 7$ in LPS + ART12.5 and LPS + ART25 groups, $n = 8$ in the LPS + ART50 group.

recovery compared to the LPS group (Supplementary Figure S1B).

Cardiac injuries were further evaluated by echocardiography, serum biomarkers, and histological examination. Echocardiographic analysis revealed that LVEF, LVFS, LVIDd, and LVIDs were significantly impaired in the LPS group compared to the Con group. ART treatment notably improved these parameters, suggesting a protective effect against LPS-induced cardiac dysfunction (Figure 1C–G). No significant differences were observed in LVPWd and LVPWs among the groups (Supplementary Figure S1C,D), which may be attributed to the short experimental duration. Histological analysis showed severe pathological damage in the LPS group, characterized by myocardial fiber degeneration and necrosis, nuclear pyknosis and disintegration, cytoplasmic dissolution, and inflammatory cell infiltration dominated by lymphocytes with fibrous tissue proliferation. These pathological alterations were substantially attenuated following ART treatment at higher dosages (Figure 1H). Furthermore, serum levels of myocardial injury biomarkers—CK-MB, cTn, LDH1, and BNP—were significantly elevated in the LPS group compared to the Con group. ART administration significantly reduced the concentrations of these biomarkers, indicating amelioration of myocardial injury (Figure 1I–L).

It was observed that the dosage of 50 mg/kg with ART made the most significant improvement in both general condition and cardiac function. Therefore, heart tissues of mice in the LPS + ART50 group (LPS + ART in the following text) were selected for further analysis.

Collectively, these results suggest that ART treatment can alleviate both systemic symptoms and cardiac dysfunction in a murine model of SICM.

ART attenuates myocardial inflammation and apoptosis in SICM mice

Based on the pharmacology network analysis of “SICM-related genes and ART candidate targets”, a total of 27 intersecting genes were identified (Figure 2A). The PPI network showed a complex interaction map among the predicted targets of ART, with dense connections indicating strong functional associations (Figure 2B). The hub gene network highlights key targets, including CASP8, CASP9, CASP3, STAT3, EGFR, PARP1, SRC, PPARG, GSK3B, and MMP9, suggesting their potential central roles in ART-mediated biological effects (Figure 2C). GO enrichment analysis highlighted apoptosis as a prominently associated biological process (Figure 2D). To experimentally validate this prediction, apoptosis in cardiac tissue was assessed via TUNEL staining and by examining the expressions of apoptosis-related proteins, Bax and Bcl-2, through western blot analysis. As shown in Figure 3A,B, LPS exposure significantly increased the percentage of TUNEL-positive nuclei in mouse heart tissues, indicating enhanced cardiomyocyte apoptosis. ART treatment markedly reduced this apoptotic response. Correspondingly, western blot analysis revealed that LPS induced upregulation of the pro-apoptotic protein Bax and downregulation of the anti-apoptotic protein Bcl-2. ART administration effectively reversed these alterations (Figure 3C–E). In addition, IHC staining demonstrated elevated expression of cleaved caspase-3 in the LPS group, which was substantially reduced by ART treatment (Figure 3F).

Given that sepsis is characterized by a robust systemic inflammatory response, we also evaluated inflammatory markers in cardiac tissue. LPS significantly increased the accumulation of

CD68-positive inflammatory cells, whereas ART treatment clearly mitigated this effect (Figure 3G). These findings suggest that ART exerts cardioprotective effects in septic mice by reducing both myocardial apoptosis and inflammation.

Furthermore, as oxidative stress is a key contributor to sepsis-induced myocardial injury, we assessed ROS levels in heart tissue. DHE staining revealed that ROS production was significantly elevated in the LPS group, while ART treatment notably decreased ROS accumulation (Supplementary Figure S2A,B).

Taken together, these results indicate that ART attenuates myocardial inflammation, apoptosis, and oxidative stress in a murine model of SICM.

ART alleviates inflammation and apoptosis *in vitro*

To validate the protective role of ART in SICM, *in vitro* assays were then conducted using NRCMs and H9c2 cardiomyocytes exposed to LPS. As illustrated in Supplementary Figure S3A, ART demonstrated no significant cytotoxicity up to a concentration of 40 μ M, confirming its safety profile. When H9c2 cells were subjected to LPS stimulation, ART treatment significantly improved cell viability, with the most pronounced protective effect observed at a concentration of 5 μ M (Supplementary Figure S3B). This concentration was therefore selected for subsequent experiments.

Western blot analysis revealed that LPS exposure markedly increased the expression of the pro-apoptotic protein Bax while decreasing the expression of the anti-apoptotic protein Bcl-2. ART administration effectively reversed these LPS-induced alterations in apoptotic protein expression (Figure 4A–C and Supplementary Figure S3C–E). Consistent with these findings, TUNEL staining showed a significant increase in the number of apoptotic (TUNEL-

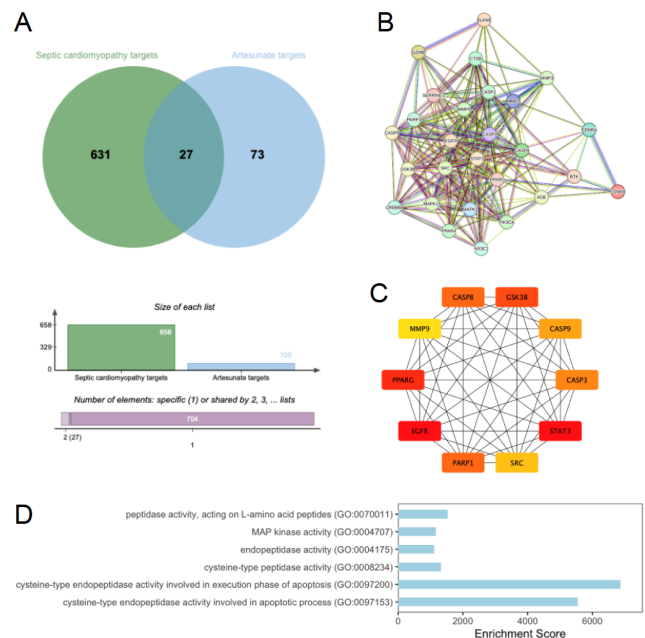


Figure 2. Network pharmacological analysis of ART in the sepsis-induced cardiomyopathy (A) Venn plot of targets among ART and SICM targets. (B) PPI networks constructed using the STRING database. (C) “SICM-related genes and ART candidate targets” network mapped by Cytoscape using MCC algorithm. (D) GO enrichment analysis of the intersected genes.

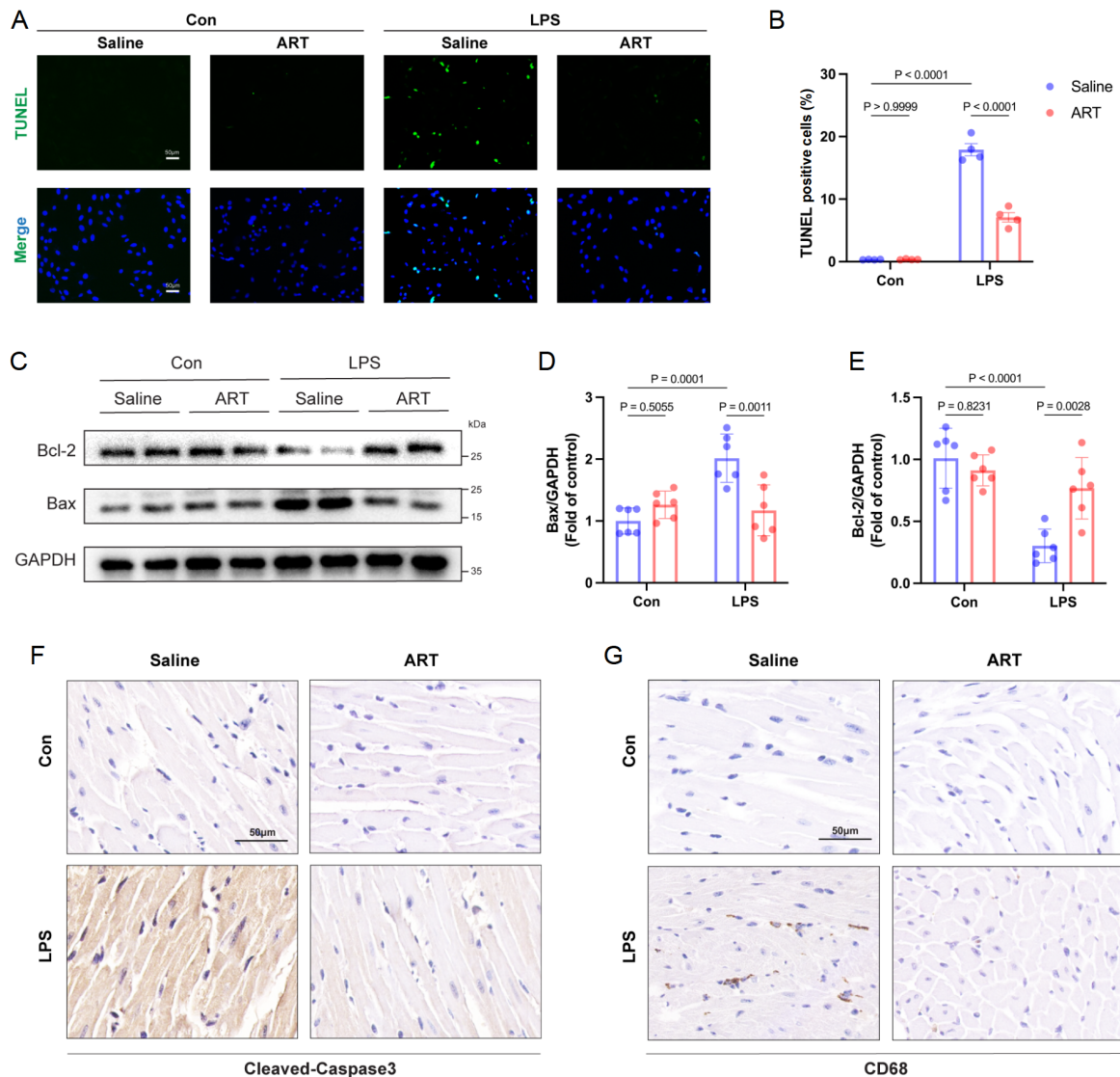


Figure 3. ART alleviates myocardial inflammation and apoptosis in SICM mice (A,B) Representative images of TUNEL staining and qualification of positive cells in the experimental groups. $n = 4$. Scale bar, 100 μm . (C–E) Western blot and quantitative analysis of protein expressions of Bax and Bcl-2. $n = 6$ mice for each treatment. (F,G) Representative images of IHC staining against cleaved-Caspase-3 (F) and CD68 (G). Scale bar, 50 μm .

positive) cells following LPS treatment, which was substantially reduced upon ART administration (Figure 4D,E and Supplementary Figure S3F,G). In addition to its anti-apoptotic effects, ART exhibited anti-inflammatory activity in LPS-challenged cardiomyocytes. Immunofluorescence staining demonstrated that LPS stimulation markedly increased the expression of phosphorylated NF- κB p65 (p-P65), a key indicator of NF- κB pathway activation. ART treatment significantly decreased p-P65 fluorescence intensity, suggesting suppression of inflammatory signaling (Figure 4F,G and Supplementary Figure S3H,I). Furthermore, intracellular ROS levels were evaluated using DCFH-DA staining. LPS exposure led to a significant elevation in ROS in H9c2 cells compared to the control group. Consistent with the *in vivo* findings, ART treatment effectively reduced ROS accumulation (Supplementary Figure S2C,D). Collectively, these results confirm that ART mitigates LPS-induced apoptosis, inflammation, and oxidative stress in cardiomyocytes *in vitro*.

ART ameliorates FoxO3-mediated inflammation and apoptosis

To explore the underlying mechanisms of ART's protective effects in SICM, integrated metabolomic and transcriptomic analyses were performed on heart tissues collected from LPS-treated mice administered either vehicle (saline; $n = 5$) or ART ($n = 5$). The metabolomics analysis revealed substantial differences in the metabolic profiles between the ART-treated and vehicle groups, with a large number of metabolites exhibiting significant upregulation or downregulation following ART treatment (Figure 5A and Supplementary Figure S4A,B). Similarly, transcriptomic profiling demonstrated distinct gene expression patterns between the two groups (Figure 5B and Supplementary Figure S4C,D). Pathway enrichment analysis of the significantly altered metabolites using the KEGG database indicated that metabolic pathways, the sphingolipid signaling pathway, and the FoxO signaling pathway were among the most significantly enriched (Figure 5C and

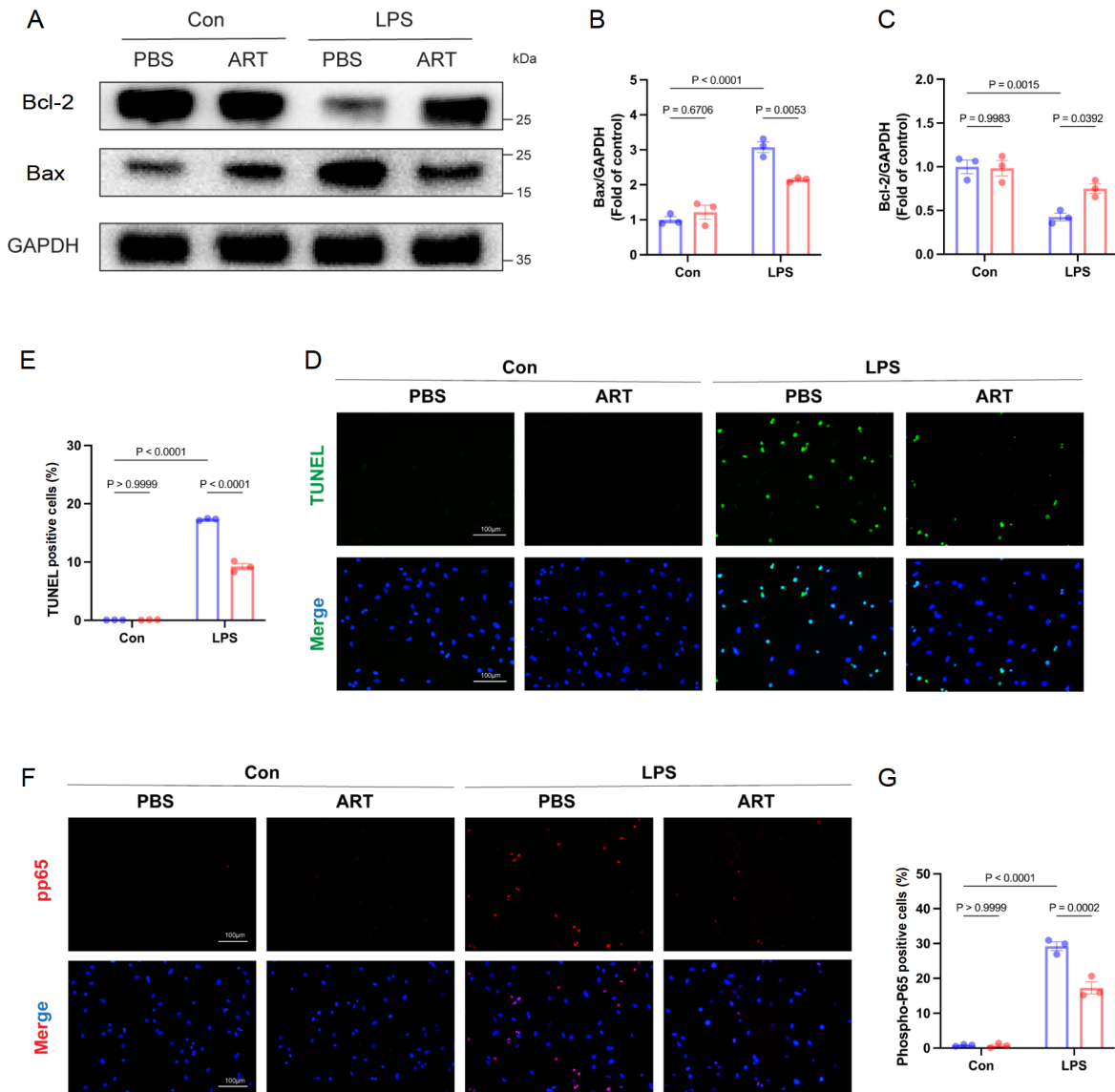


Figure 4. ART attenuates inflammation and apoptosis in LPS-challenged cells (A–C) Western blot and quantitative analysis of protein expressions of Bax and Bcl-2 in NRCMs. $n = 3$. (D,E) Representative images of TUNEL staining and qualification of positive cells in the experimental groups. $n = 3$. Scale bar, 100 μm . (F,G) Representative images of immunofluorescence staining against p-P65 and qualification of positive cells in the experimental groups. $n = 3$. Scale bar, 100 μm .

Supplementary Figure S4E,F). Cross-analysis of the enriched pathways from both metabolomic and transcriptomic datasets identified 15 overlapping pathways with potential involvement (Figure 5D). Among these, the FoxO signaling pathway was of particular interest due to its well-established role in regulating apoptosis and the oxidative stress response. Further analysis of the transcriptomic data revealed that FoxO3, a key member of the FoxO family, was notably affected by ART treatment. Based on this observation, we hypothesized that ART may exert its cardioprotective effects in SICM through the modulation of FoxO3 signaling.

Consistent with this hypothesis, LPS administration significantly decreased the phosphorylation level of FoxO3 (p-FoxO3) in cardiac tissues, indicating impaired FoxO3 regulation during septic insult. ART treatment effectively reversed this reduction, restoring p-FoxO3 levels (Figure 5E,F). These findings were further corroborated

in vitro using both NRCMs and H9c2 cardiomyocytes, in which ART consistently rescued the LPS-induced decrease in p-FoxO3 expression (Figure 5H–I and Supplementary Figure S5A,B). IHC analysis further provided additional spatial confirmation, showing that ART treatment restored p-FoxO3 expression in myocardial tissues, which was otherwise diminished by LPS exposure (Figure 5G). Notably, the total FoxO3 protein levels remained largely unchanged across all experimental groups, both *in vivo* and *in vitro*, suggesting that ART primarily influences the activation state of FoxO3 rather than its overall expression. Given that FoxO3 activity is tightly regulated by its phosphorylation-dependent subcellular localization, we next examined the intracellular distribution of FoxO3. IF analysis demonstrated that ART treatment significantly altered FoxO3 localization in cardiomyocytes (Figure 5M and Supplementary Figure S5C). Consistently,

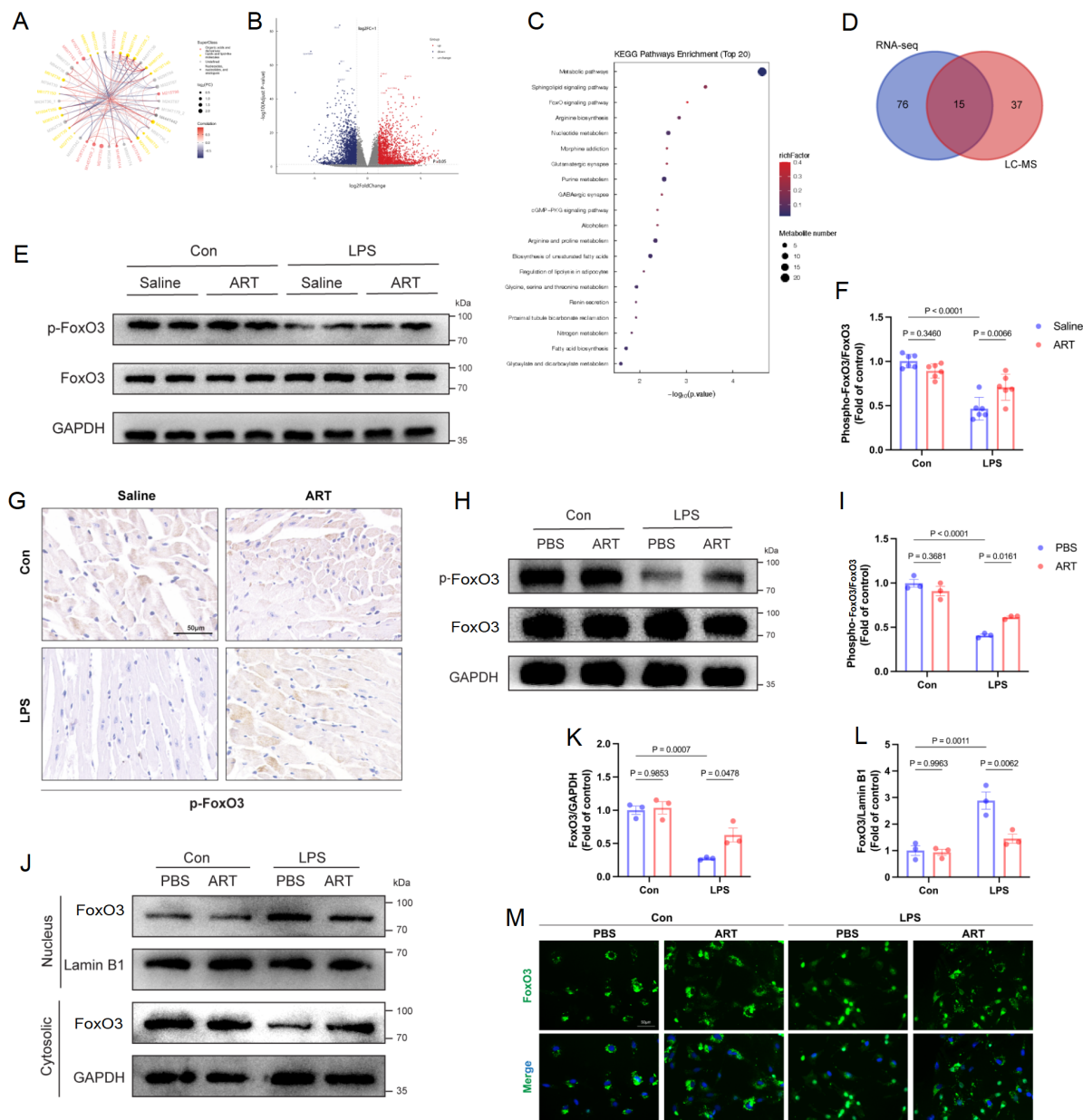


Figure 5. ART ameliorates inflammation and apoptosis via FoxO3 signaling (A) Network of different metabolites between saline- and ART-treated septic mice groups. $n = 5$. (B) Volcano plot of differentially expressed genes between the Saline and ART groups. $n = 5$. (C) Scatter plot of KEGG enrichment analysis for differential metabolites between the saline and ART groups. $n = 5$. (D) Venn plot of the intersected pathways enriched in both metabolomic and transcriptomic analyses. (E,F) Western blot and quantitative analysis of protein expression of FoxO3 and p-FoxO3 in septic mice. $n = 6$. (G) Representative images of IHC staining against FoxO3. Scale bar, 50 μm . (H,I) Western blot and quantitative analysis of protein expressions of FoxO3 and p-FoxO3. $n = 3$. (J-L) Western blot and quantitative analysis of protein expression of FoxO3 through nuclear versus cytoplasmic fractionation. $n = 3$. (M) Representative images of IF staining against FoxO3 in NRCMs. Scale bar, 50 μm .

nuclear versus cytoplasmic fractionation followed by western blot analysis further confirmed ART-induced changes in FoxO3 sub-cellular distribution in both cardiac tissues and cultured cardiomyocytes (Figure 5J–L and Supplementary Figure S5D–F).

ART inhibits FoxO3 transcriptional activity via Akt activation

The transcriptional activity of FoxO3 is primarily regulated through phosphorylation. It is well established that phosphorylation by Akt

leads to FoxO3 inactivation, resulting in its cytoplasmic retention and reduced transcription of pro-apoptotic target genes [18–20]. In addition to Akt, FoxO3 activity can also be modulated by other upstream kinases, including AMPK, JNK, and SIRT1, in a context-dependent manner. To determine whether these parallel regulatory pathways are involved in our model, we examined the expressions and activation status of AMPK, JNK, and SIRT1 by western blot analysis (Supplementary Figure S4G–J). While LPS stimulation significantly altered the protein levels of SIRT1 as well as the

phosphorylation ratios of AMPK and JNK, ART treatment did not result in obvious changes in these pathways. These findings suggest that the modulatory effect of ART on FoxO3 activity in SICM is unlikely to be mediated through AMPK-, JNK-, or SIRT1-dependent mechanisms. Notably, the Akt signaling pathway was identified as one of the enriched pathways in the integrated pathway analysis, which suggests the potential involvement of the Akt signaling pathway in this context. Thus, we hypothesize that ART may exert its protective effects by activating Akt, thereby inhibiting FoxO3 transcriptional activity.

In heart tissues from SICM mice, the phosphorylation level of Akt (p-Akt) was significantly reduced compared to that in the Con group. ART treatment effectively restored p-Akt expression, indicating reactivation of Akt signaling (Figure 6A,B). This observation was further validated *in vitro* using LPS-challenged NRCMs and H9c2 cardiomyocytes, where LPS exposure led to a marked decrease in p-Akt levels, while ART administration significantly reversed this effect (Figure 6C,D and Supplementary Figure S5G,H). The total Akt protein levels remained unchanged across all groups, both *in vivo* and *in vitro*, suggesting that ART

specifically influences the phosphorylation status of Akt rather than its total expression.

To further explore the potential direct interaction between ART and Akt, molecular docking analysis was performed as a computational, hypothesis-generating approach. As shown in Figure 6E–G, the molecular docking results showed that ART exhibited favorable predicted binding energies with all three Akt isoforms, with the strongest affinities observed for Akt3 (−8.6 kcal/mol) and Akt1 (−8.2 kcal/mol). Notably, the binding energies for all Akt isoforms were lower than −5.0 kcal/mol, a commonly accepted threshold indicative of stable and reliable ligand-protein interactions in docking studies. These results suggest that ART has the ability to interact with Akt proteins, although such computational predictions do not establish functional binding or isoform selectivity in a biological context.

Collectively, these data support a model in which ART activates Akt signaling in the septic myocardium, subsequently inhibiting the transcriptional activity of FoxO3 and contributing to its anti-apoptotic and anti-inflammatory effects in SICM. While molecular docking suggests a potential interaction between ART and Akt,

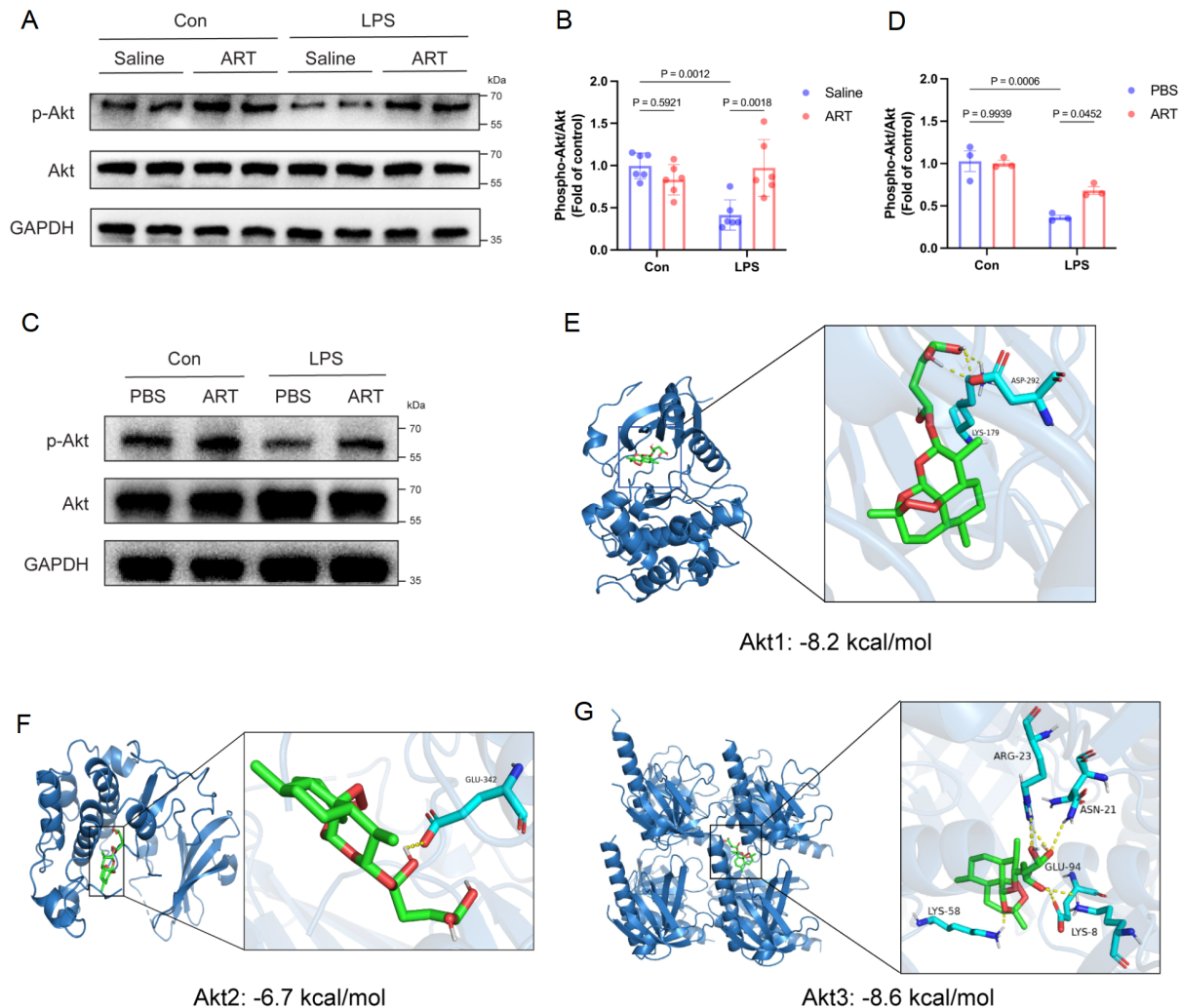


Figure 6. ART protects against SICM via docking with AKT (A,B) Western blot and quantitative analysis of protein expressions of Akt and p-Akt in septic mice. $n = 6$. (C,D) Western blot and quantitative analysis of protein expressions of Akt and p-Akt in NRCMs. $n = 3$. (E–G) Docking diagram between ART and Akt1 (E), Akt2 (F), Akt3 (G).

further biochemical and genetic studies will be required to definitively establish the directness and functional relevance of this interaction.

FoxO3 knockdown reverses the cardioprotective effects of ART in SICM

To establish stronger evidence, experiments using *FoxO3* knockdown models were further conducted. Cardiomyocytes exposed to LPS were infected with control siRNA (NC) or rat *FoxO3* siRNA. As shown in Figure 7A–C and Supplementary Figure S6A–C, knockdown of *FoxO3* markedly attenuated the cardioprotective effects of ART. The ART-induced increase in Bcl-2 expression and the reduction in Bax protein levels were substantially diminished following siFoxO3 infection, as assessed by western blot analysis. Consistently, TUNEL staining revealed that *FoxO3* knockdown reversed the anti-apoptotic effect of ART, resulting in an increased number of apoptotic cells despite ART administration (Figure 7D,E

and Supplementary Figure S6D,E). The ART-mediated suppression of p-P65 immunofluorescence intensity was also significantly reduced in the presence of siFoxO3, further supporting the effect of FoxO3 in mediating the anti-inflammatory effect of ART (Figure 7F,G and Supplementary Figure S6F,G). Collectively, the above results further confirm the role of FoxO3 in the cardioprotective effects of ART in SICM.

Akt inhibition by MK2206 abolishes the anti-inflammatory and anti-apoptotic effects of ART in SICM

To confirm that the protective effect of ART against SICM is mediated via the Akt/FoxO3 signaling pathway, NRCMs and H9c2 cells exposed to LPS were treated with the Akt-specific inhibitor MK2206. As shown in Figure 8A–D and Supplementary Figure S7A–D, inhibition of Akt activation markedly attenuated the protective effects of ART. Specifically, the ART-induced increase in p-FoxO3 and Bcl-2 expression, along with the reduction in Bax protein levels,

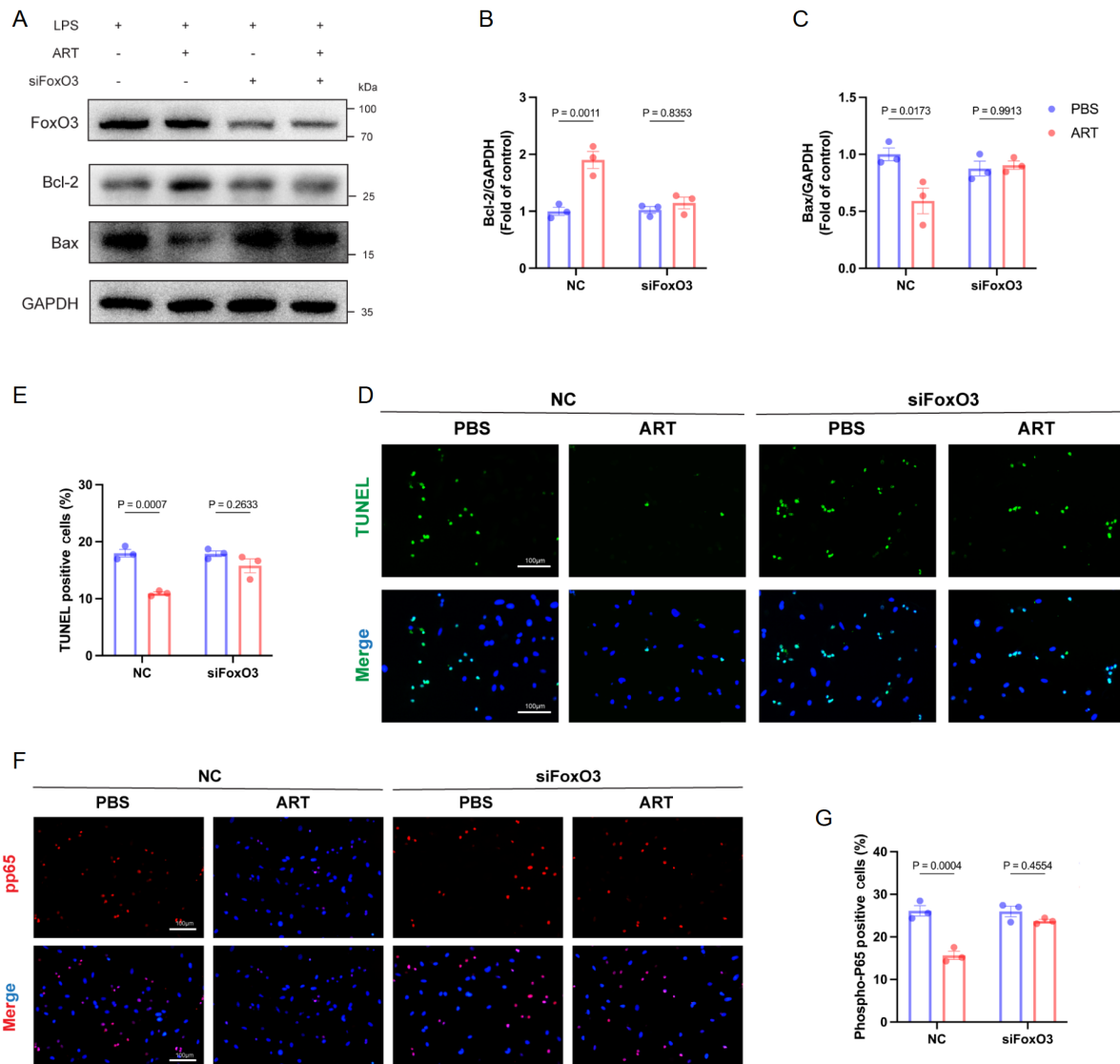


Figure 7. *FoxO3* knockdown abolishes the cardioprotective effect of ART in the context of LPS-challenged models *in vitro* (A–C) Western blot and quantitative analysis of protein expressions of FoxO3, Bax, and Bcl-2 in LPS-challenged NRCMs with siFoxO3 or NC. *n* = 3. (D,E) Representative images of TUNEL staining and qualification of positive cells in the experimental groups. *n* = 3. Scale bar, 100 μ m. (F,G) Representative images of immunofluorescence staining against p-P65 and qualification of positive cells in the experimental groups. *n* = 3. Scale bar, 100 μ m.

was substantially diminished following MK2206 treatment. We further assessed the subcellular localization of FoxO3 via IF and western blot analysis, as presented in Figure 8E–H and Supplementary Figure S7E–H. Consistently, TUNEL staining revealed that Akt inhibition reversed the anti-apoptotic effect of ART, resulting in an increased number of apoptotic cells despite ART administration (Figure 8I,J and Supplementary Figure S7I,J). Additionally, the ART-mediated suppression of p-P65 immunofluorescence intensity—a marker of NF- κ B-driven inflammation—was significantly reduced in the presence of MK2206, further supporting the involvement of the Akt/FoxO3 signaling axis in mediating the anti-inflammatory effect of ART (Figure 8K,L and Supplementary Figure S7K,L).

Taken together, these results from the rescue experiments demonstrate that ART exerts its protective effects against LPS-induced apoptosis and inflammation in SICM models primarily through regulation of the Akt/FoxO3 signaling pathway.

Discussion

In this study, we systematically investigated the cardioprotective effects of ART against SICM using both *in vivo* and *in vitro* models. Our findings demonstrate that ART significantly improves cardiac function, alleviates cardiomyocyte apoptosis, and reduces inflammatory responses under septic conditions, likely through modulation of the Akt/FoxO3 signaling pathway.

Sepsis is a life-threatening clinical syndrome characterized by overwhelming systemic inflammation and subsequent multi-organ dysfunction due to a dysregulated host immune response [21]. Among the complications associated with sepsis, SICM represents a particularly severe yet often underrecognized form of cardiac dysfunction, which markedly worsens the overall prognosis and increases mortality in septic patients. Despite its clinical relevance, to date, there is no consensus on effective therapeutic strategies for the prevention or treatment of SICM [4,22]. A variety of pathophysiological factors, such as uncontrolled inflammation, apoptosis, and oxidative stress, are thought to contribute to the development and progression of SICM [5,6]. However, the exact pathophysiological mechanisms of SICM remain incompletely understood.

ART, a semisynthetic derivative of artemisinin, is widely recommended as a first-line therapy for severe malaria [8,9]. Beyond its antimalarial action, ART has demonstrated anti-tumor [23,24], antiviral [25,26], and anti-inflammatory [27] properties, alongside favorable safety, low toxicity, and good tolerability profiles in clinical settings [8]. In the context of cardiovascular disease, ART has been shown to attenuate pathological processes such as cardiac fibrosis [12], hypertrophy [28,29], myocardial infarction-related injury [30], and heart transplantation-induced acute rejection [31]. Furthermore, recent studies have elucidated its anti-inflammatory effects in sepsis-induced multi-organ injury [13,14]. However, despite these advancements, the specific impact of ART on SICM, a critical complication characterized by acute cardiac dysfunction in septic patients, had not been previously established. This study aimed to address this gap by investigating the potential protective mechanisms of ART in SICM.

Apoptosis is a predominant form of programmed cell death contributing to organ damage during sepsis. Excessive cardiomyocyte apoptosis has been widely recognized as a key contributor to the pathogenesis of SICM. ART, beyond its established antimalarial

efficacy, has shown therapeutic potential in a variety of apoptosis-related conditions, including diabetic neuropathy [15], lung injury [32], and malignancies [33]. Consistent with the predictions of our pharmacological network analysis, the present study confirmed that ART treatment significantly reduced cardiomyocyte apoptosis in SICM models. In addition to its anti-apoptotic effects, ART was also found to mitigate inflammatory responses and oxidative stress in septic myocardium—two interrelated pathological processes that are closely linked to sepsis-induced cardiac injury. Notably, while the anti-inflammatory and antioxidative properties of ART have been reported in other disease settings, this study is the first to provide direct evidence of these protective effects in the context of SICM.

Importantly, in our present study, all experiments were conducted exclusively in male mice, which seemed to limit the generalizability of our findings. However, we chose to use male mice primarily due to methodological considerations in this study. Based on our pilot experiments, male mice exhibited greater resilience to severe septic challenge in our specific model, resulting in a more consistent and survivable level of injury. In contrast, preliminary data indicated significantly higher and more variable mortality in female mice under the same conditions, which could have compromised group sizes, statistical power, and the interpretation of therapeutic outcomes. Given the recognized sex-specific differences in sepsis and sepsis-induced cardiomyopathy, future studies incorporating both sexes are essential. Moreover, the ART dosing regimen employed in this study does not fully reflect clinical practice, in which therapeutic interventions are initiated after the onset of sepsis symptoms. Although this design was appropriate for mechanistic proof-of-concept, it limits immediate clinical translatability. The therapeutic efficacy of ART when administered after the full establishment of sepsis remains to be determined and warrants systematic investigation in future studies. Furthermore, cardiac function and molecular endpoints were assessed at a single 24-h time point, a time point that is widely used to capture the peak of acute inflammatory injury and myocardial dysfunction [34–36]. However, we observed extremely low survival rates beyond this acute phase in the LPS model, which limited the feasibility of reliable functional assessments at later time points. Consistent with this, several previous studies [37,38] using LPS-induced models have focused on earlier observation windows, such as 6 or 12 h, to interrogate acute cardiac injury. Nonetheless, we fully acknowledge that assessment at a single 24-h time point cannot reveal whether the beneficial effects of ART are sustained over time, nor can it address critical long-term outcomes such as maladaptive cardiac remodeling or survival. Extended observation periods (48–72 h or even longer) will be necessary to determine whether the protective effects of ART are sustained and whether they influence longer-term outcomes such as myocardial remodeling or survival. Additionally, we also fully acknowledge that the lack of a comparative control with a standard therapy limits the immediate translational impact of our conclusions. At present, however, effective targeted therapies for SICM remain limited, and current management strategies, including beta-blockers [39], levosimendan [40], and anti-inflammatory agents [41], are largely supportive rather than disease-modifying, with SICM continuing to contribute substantially to sepsis-related mortality. Accordingly, our work was designed as an initial mechanistic investigation to define the potential cardioprotective effect of ART against SICM,

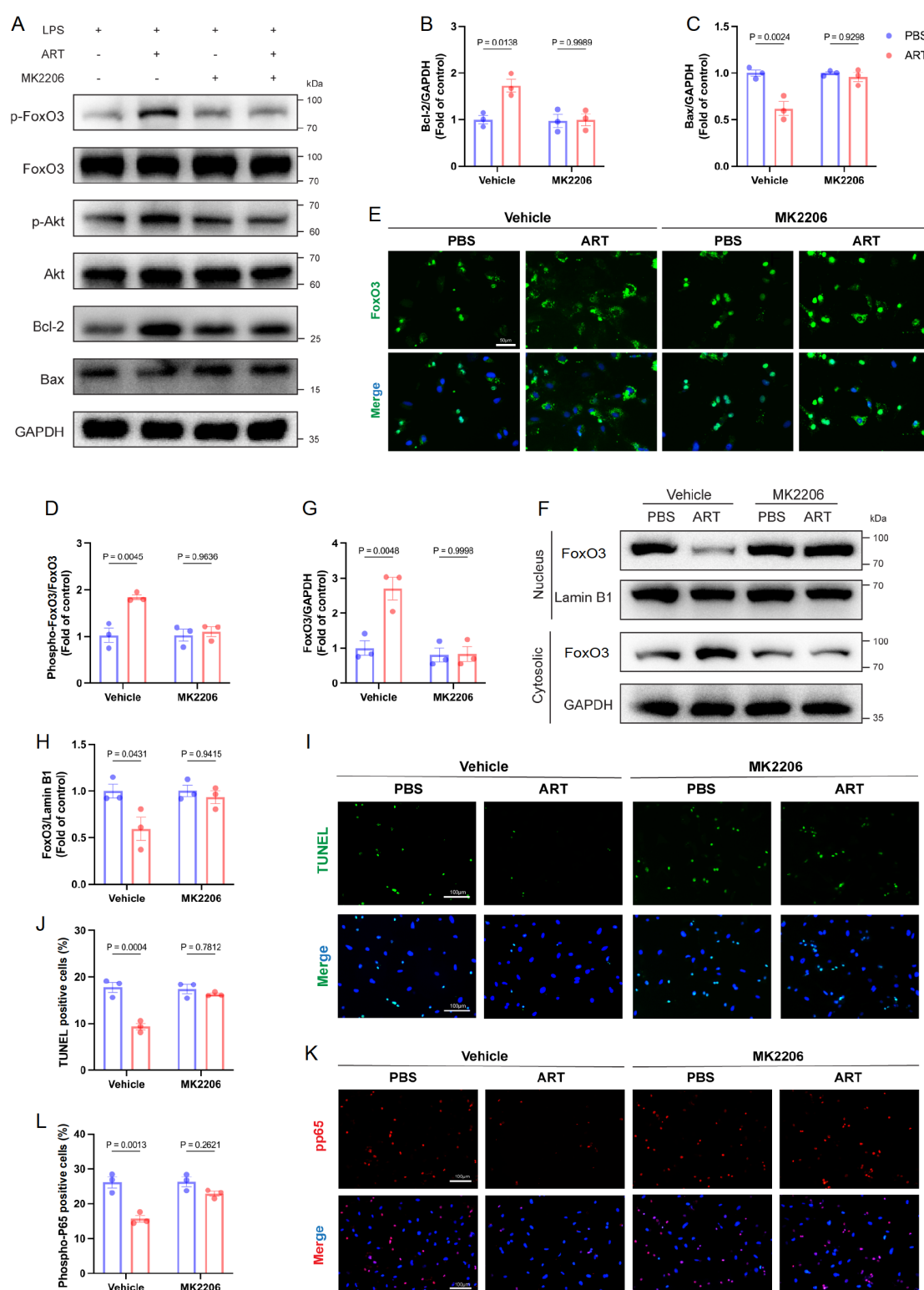


Figure 8. AKT inhibition abolishes the anti-inflammatory and anti-apoptotic effects of ART in SICM (A–D) Western blot and quantitative analysis of protein expressions of Akt, p-Akt, FoxO3, p-FoxO3, Bax, and Bcl-2 in LPS-challenged NRCMs with or without Akt inhibitor. $n = 3$. (E) Representative images of IF staining against FoxO3 in NRCMs. Scale bar, 50 μ m. (F–H) Western blot and quantitative analysis of protein expression of FoxO3 through nuclear versus cytoplasmic fractionation. $n = 3$. (I, J) Representative images of TUNEL staining and qualification of positive cells in the experimental groups. $n = 3$. Scale bar, 100 μ m. (K, L) Representative images of immunofluorescence staining against p-P65 and qualification of positive cells in the experimental groups. $n = 3$. Scale bar, 100 μ m.

providing a novel therapeutic candidate for SICM.

By performing integrated transcriptomic and metabolomic analyses as well as validation experiments, we demonstrated that ART exerts its cardioprotective effects potentially by modulating the Akt/FoxO3 axis. The Akt signaling pathway is a well-established regulator of cell survival and plays a central role in orchestrating anti-apoptotic signaling cascades in septic conditions [42,43]. Previous studies have shown that ART can modulate Akt signaling in multiple pathological conditions, such as diabetes [44], acute lung injury [32], and myocardial infarction [30]. In line with these observations, our data demonstrated that ART treatment enhances Akt phosphorylation in sepsis-induced myocardial injury, suggesting that the cardioprotective effects of ART are, at least in part, mediated through activation of the Akt signaling pathway. In addition to biochemical validation, we employed molecular docking analysis as a computational, hypothesis-generating approach to explore the potential direct interaction between ART and Akt. Our docking results showed that the binding energies for all Akt isoforms were lower than -5.0 kcal/mol, a threshold generally considered indicative of stable and reliable ligand-protein interactions, which demonstrated that ART is capable of directly binding to all three Akt isoforms, with preferential affinity for Akt3 and Akt1, which may contribute to its observed regulatory effects on Akt phosphorylation. While these findings do not establish direct functional binding or isoform selectivity in a biological context, they raise the possibility that ART may interact with Akt proteins and thereby influence Akt phosphorylation and signaling activity. Given that Akt1 is widely recognized for its cardioprotective role in maintaining myocardial structure and function and that Akt3 has also been implicated in cardiovascular development and cardiac physiology [45,46], further exploration is necessary to delineate the isoform-specific contributions of Akt signaling in the context of SICM treated with ART. In addition, molecular docking analysis was used as a computational, hypothesis-generating tool to explore potential interactions between ART and Akt isoforms. While these results suggest a possible interaction, they do not constitute conclusive evidence of functional binding, isoform selectivity, or direct modulation of Akt phosphorylation. Additional biochemical and genetic studies are still needed to verify the mechanism by which ART influences Akt activation.

FoxO3, a member of the FoxO transcription factor family, is highly expressed in the heart, skeletal muscle, spleen, and ovary [47]. FoxO3 is known to regulate a wide range of cellular processes, including apoptosis [48], oxidative stress [49], proliferation [50], and tumorigenesis [51]. The activity of FoxO3 is tightly controlled by phosphorylation, particularly via Akt-mediated signaling, which retains FoxO3 in the cytoplasm, limiting its pro-apoptotic nuclear functions [18]. In this study, we observed that ART administration effectively reversed cardiac dysfunction and injury by restoring both p-Akt and p-FoxO3 levels in septic myocardium, which correlated with a significant reduction in apoptosis and inflammation. Furthermore, pharmacological inhibition of Akt using MK2206 not only negated the ART-induced increase in p-FoxO3 but also diminished its anti-apoptotic and anti-inflammatory effects, highlighting the central role of the Akt/FoxO3 axis in mediating the cardioprotective effect of ART in SICM and this pathway as a potential therapeutic target for future intervention strategies. Although changes in FoxO3 phosphorylation and subcellular localization were assessed, direct evaluation of FoxO3 transcrip-

tional activity, such as measurement of canonical FoxO3 target gene expression, was not performed. Such analyses will be important for definitively establishing the downstream transcriptional consequences of Akt/FoxO3 modulation.

Interestingly, the role of FoxO3 in cardiovascular pathophysiology appears to be highly context-dependent and dynamically regulated. During cardiac development, FoxO3 is abundantly expressed, and its deletion leads to cardiac hypertrophy in adult mice, highlighting its critical involvement in myocardial growth regulation and homeostasis [52]. In the aging heart, enhanced FoxO3 activity can reduce collagen deposition and fibrosis [53]. Conversely, phosphorylation of FoxO3 has been shown to counteract diabetes-induced hyperacetylation and mitochondrial dysfunction in diabetic cardiomyopathy [54]. Additionally, in laminopathy models, excessive nuclear FoxO3 activation has been linked to heightened apoptosis and shortened survival, while suppression of FoxO3 improved outcomes [55]. Collectively, these findings emphasize that FoxO3 functions as a double-edged sword, with its biological impact largely dictated by the specific pathological context, the nature of upstream signaling events, and the stage of disease progression. Understanding this complex regulatory landscape is essential for the rational design of FoxO3-targeted therapeutic strategies in cardiovascular diseases.

Despite the promising results, several limitations must be acknowledged. First, our current research focused exclusively on the Akt/FoxO3 pathway. While our data support a central role for this pathway in cardiomyocytes, there remains potential crosstalk between the Akt/FoxO3 axis and the NF- κ B signaling pathway *in vivo*, where inflammatory cells such as macrophages may introduce additional layers of regulation through paracrine signaling and reciprocal pathway activation. Second, the untargeted metabolomics analysis was conducted with a sample size typical of exploratory omics; nevertheless, the relatively small cohort limits statistical power. These findings should therefore be interpreted as preliminary and require validation in larger, independent cohorts. In addition, metabolomic analysis was conducted as an exploratory, hypothesis-generating approach to identify pathways potentially involved in ART-mediated cardioprotection. While enrichment of the FoxO signaling pathway was observed, specific metabolites directly linking ART treatment to FoxO3 regulation were not identified. Thus, the metabolomic findings should be interpreted as indirect associations rather than evidence of direct metabolic regulation. Third, our *in vivo* findings were derived from an LPS-induced endotoxemia model, which represents an acute inflammatory component of sepsis but does not fully capture the complexity of clinical sepsis. Accordingly, our findings should be interpreted within this context and validated in more clinically relevant models, such as the cecal ligation and puncture model. Fourth, mechanistic rescue experiments were performed only *in vitro*, and the absence of *in vivo* genetic or pharmacological rescue limits definitive causal inference at the organismal level.

In summary, this study demonstrated that ART exerts significant protective effects against SICM by mitigating cardiomyocyte apoptosis and suppressing inflammatory responses, both *in vivo* and *in vitro*. Mechanistically, these protective effects were mediated, at least in part, through modulation of the Akt/FoxO3 signaling pathway. These findings provide evidence for the cardioprotective potential of ART and highlight its promise as a candidate therapeutic agent for the treatment of SICM, yet further

validation needs to be addressed in future studies.

Supplementary Data

Supplementary data are available at *Acta Biochimica et Biophysica Sinica* online.

Data Availability

The RNA-seq data have been uploaded to the GEO database (accession No. GSE317118). The metabolomics data have been publicly released via the OMIX (accession No. OMIX014306). All other data supporting the findings of this study, including raw data and original images, are available from the corresponding author upon reasonable request.

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Author contributions

X.J. and D.Y. wrote the main manuscript text. Y.C., Y.Z., and Y.C. participated in experiments. Z.L. supervised the whole procedure. All authors reviewed the manuscript.

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Conflict of Interest

The authors declare they have no conflict of interest.

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