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ORIGINAL ARTICLE

Canagliflozin Pretreatment Attenuates Myocardial Dysfunction and Improves Postcardiac Arrest Outcomes After Cardiac Arrest and Cardiopulmonary Resuscitation in Mice

Feng Ju[1] · Geoffrey W. Abbott[2] · Jiaxue Li[1] · Qifeng Wang[1] · Ting Liu[1] · Quanhua Liu[1] · Zhaoyang Hu[1]

Abstract

Objective The SGLT2 inhibitor, canagliflozin, not only reduces glycemia in patients with type 2 diabetes but also exerts cardioprotective effects in individuals without diabetes. However, its potential beneficial effects in cardiac arrest have not been characterized. The purpose of this study was to examine the protective effect of canagliflozin pretreatment on postresuscitation-induced cardiac dysfunction in vivo.

Methods Male C57/BL6 mice were randomized to vehicle (sham and control) or canagliflozin treatment groups. All mice except for the sham-operated mice were subjected to potassium chloride-induced cardiac arrest followed by chest compressions and intravenous epinephrine for resuscitation. Canagliflozin therapy efficacies were evaluated by electrocardiogram, echocardiography, histological analysis, inflammatory response, serum markers of myocardial injury, protein phosphorylation analysis, and immunohistological assessment.

Results Canagliflozin-pretreated mice exhibited a higher survival rate ($P < 0.05$), a shorter return of spontaneous circulation (ROSC) time ($P < 0.01$) and a higher neurological score ($P < 0.01$ or $P < 0.001$) than control mice after resuscitation. Canagliflozin was effective at improving cardiac arrest and resuscitation-associated cardiac dysfunction, indicated by increased left ventricular ejection fraction and fractional shortening ($P < 0.001$). Canagliflozin reduced serum levels of LDH, CK-MB and α-HBDH, ameliorated systemic inflammatory response, and diminished the incidence of early resuscitation-induced arrhythmia. Notably, canagliflozin promoted phosphorylation of cardiac STAT-3 postresuscitation. Furthermore, pharmacological inhibition of STAT-3 by Ag490 blunted STAT-3 phosphorylation and abolished the cardioprotective actions of canagliflozin.

Conclusions Canagliflozin offered a strong cardioprotective effect against cardiac arrest and resuscitation-induced cardiac dysfunction. This canagliflozin-induced cardioprotection is mediated by the STAT-3-dependent cell-survival signaling pathway.

Keywords Cardiac arrest · Cardiopulmonary resuscitation · Sodium-glucose co-transporter 2 (SGLT-2) inhibitors · Canagliflozin · STAT-3

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Introduction

Cardiac arrest is a major public health issue that causes over 300,000 deaths in the United States each year. It has been reported that cardiac arrest accounts for approximately 50% of all cardiovascular deaths [1]. Early cardiopulmonary resuscitation and defibrillation are vital for rescuing lives. However, patients who are successfully rescued from cardiac arrest may eventually die due to postresuscitation syndrome. The mortality in these individuals may be attributed to cardiovascular instability, sepsis, and multi-organ failure after resuscitation [2]. Therefore, it is of great importance to develop effective therapeutic approaches for patients with cardiac arrest.

Sodium-glucose co-transporter-2 (SGLT2) inhibitors are a new class of prescription medicines that have been approved as oral antidiabetic drugs for patients with type 2 diabetes mellitus (T2DM). SGLT2 inhibitors exert their glucose-lowering effect by reducing glucose reabsorption in the renal proximal tubule [3]. Now, four types of SGLT2 inhibitors, including empagliflozin, canagliflozin, dapagliflozin, and ertugliflozin are commercially available. In addition to their promising antidiabetic effects, large clinical trials such as the EMPA-REG OUTCOME trial [4] and the CANVAS trial [5] have shown that SGLT2 inhibitors produce cardiovascular benefits in patients with T2DM. Moreover, the EMPEROR-Reduced [6], EMPEROR-Preserved trials [7], and DAPA-HF [8] trials as well as the follow-up meta-analysis [9] have demonstrated that SGLT2 inhibitors can play a significant role in reducing major adverse cardiovascular outcomes and hospitalization in patients with heart failure (irrespective of diabetes). Thereafter, in a large number of animal studies it was also demonstrated that SGLT2 inhibitors exert cardioprotective effects by improvement of cardiac function, limitation of infarct size, or attenuation of heart failure development in a glucose-independent manner. Although the cardioprotective effects of SGLT2 inhibitors have been confirmed in both large clinical trials and preclinical studies, knowledge of their potential as treatment options for postresuscitation syndrome, specifically myocardial dysfunction, is incomplete. A very recent EMMY trial showed that administration of empagliflozin is effective in reducing NT-proBNP levels for acute myocardial infarction patients treated with PCI [10]. Consistent with this, scattered preclinical studies have indicated that canagliflozin is capable of limiting infarction against myocardial ischemia and reperfusion (MI/R) injury in non-diabetic animals. In contrast to regional MI/R from a coronary occlusion, cardiac arrest is a global but transitory ischemic event, and emergency resuscitation from this global ischemia may bring a sudden recover of oxygenation, which may ultimately lead to postresuscitation syndrome.

Therefore, it remains unclear whether canagliflozin has beneficial effects against postresuscitation-induced cardiac dysfunction. Furthermore, a better understanding of the underlying mechanisms by which canagliflozin protects the heart after cardiac arrest may provide novel therapeutic targets for the prevention of postresuscitation syndrome.

Although many large clinical trials have indicated the cardiovascular protective effects of SGLT2 inhibitors, the underlying mechanisms are incompletely understood. It has been suggested that the JAK (Janus tyrosine Kinase)-STAT (Signal Transducer and Activator of Transcription) cell survival signaling pathway is involved in ischemia/reperfusion injury, among which, the signaling transducer and activator of transcription 3 (STAT3) plays a vital role. The activation of STAT-3 (via phosphorylation) by protective stimuli such as ischemic preconditioning or postconditioning results in cardioprotection [11, 12]. However, pharmacological inhibition of STAT3 phosphorylation or gene deletion of STAT3 negated the protective effects [13, 14]. In this current study, we also explored the role of STAT-3 in canagliflozin-induced cardioprotection against postresuscitation-induced cardiac dysfunction.

In the present study, we investigated the hypothesis that canagliflozin may attenuate myocardial injury after resuscitation using a non-diabetic mouse model of sudden cardiac arrest. Furthermore, we explored the underlying molecular mechanism for this canagliflozin-induced cardioprotection.

Materials and Methods

Animals

All animal experiments were conducted in conformity with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH Publication 8th edition, 2011). The experiments were approved by the Institutional Animal Care and Use Committee of Sichuan University (Sichuan, China, approval number: 20211201A). Male C57/BL6 mice (20–25g, 5-6 months of age) were purchased from Chengdu Dashuo Experimental Animal Research Center (Chengdu, China) and were housed in standard SPF-grade facilities. Mice were given free access to food and water before experiments.

Experimental Protocols

Figure 1 shows the experimental protocol. Mice were randomized to vehicle or canagliflozin treatment and underwent experimental procedures. Sham-operated group (sham, n = 6): mice in the sham group received physiological saline (0.9% NaCl) and sham surgeries. Control group (CON, n =

Fig. 1 Experimental protocols. Mice in all groups except for the sham mice underwent 5 min of cardiac arrest followed by 5 min of cardiopulmonary resuscitation. Mice in the canagliflozin group were given canagliflozin (20 mg/kg dissolved in saline) daily for 4 days. A similar volume of saline was given to mice in the control or sham group. Ag490, a STAT3 inhibitor was intravenously injected via the jugular vein at a dose of 5 mg/kg 10 min prior to the induction of cardiac arrest. CPR, cardiopulmonary resuscitation; sham, sham-operated group; CON, control
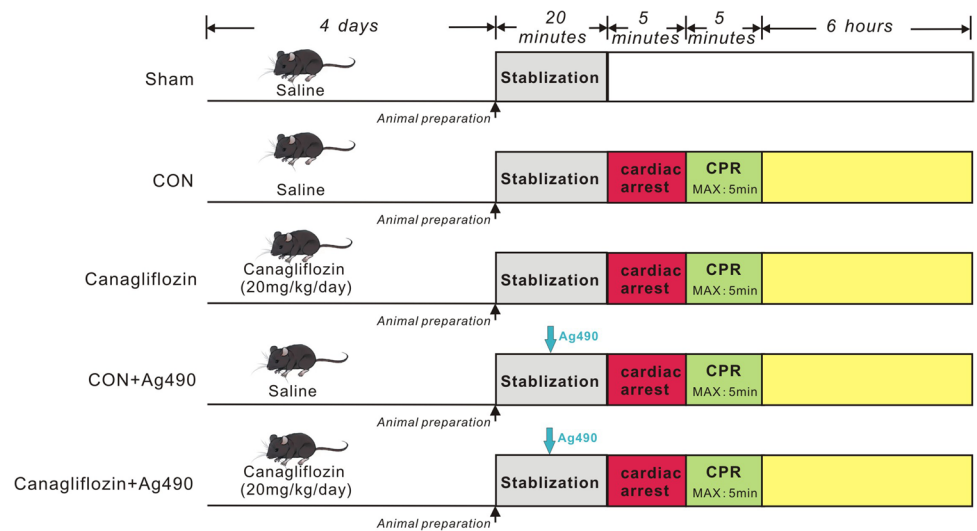
22): mice were given saline as vehicle. Canagliflozin group: canagliflozin (Janssen Pharmaceuticals, Inc., UK) was dissolved in saline solution and was administrated to the mice (20 mg/kg, n = 21). The dose of canagliflozin was chosen based on previously published studies with modification [15]. An equal amount of vehicle (saline and canagliflozin) was given orally once a day to all animals for 4 days before cardiac arrest. Control with Ag490 group (CON+Ag490, n = 22): Ag490 (5 mg/kg) was intravenously given to the mice via the jugular vein in the control group 10 min prior to the induction of cardiac arrest. Canagliflozin with Ag490 group (canagliflozin+Ag490, n = 24): Ag490 was given to mice in the canagliflozin group.

Cardiac Arrest and Cardiopulmonary Resuscitation Model

Anesthesia was induced with 3% isoflurane and 95% oxygen in a gas chamber, followed by oropharyngeal airway incubation with a 22-gauge cannula. The mice were ventilated by a mouse ventilator (Harvard Apparatus, Holliston, MA, USA). Anesthesia was maintained with 1–2% isoflurane. The depth of anesthesia was verified by the respiration rate and heart rate as well as the loss of corneal reflex. ECGs were continuously recorded in anaesthetized mice using a PowerLab/8sp data acquisition system (AD Instruments Colorado Springs, CO, USA). A heparin-refilled PE-10 micro tube was placed in the right internal jugular vein for fluid administration. After stabilization for 20 min, cardiac arrest was induced by a bolus injection of potassium chloride (0.08 mg/g) into the jugular vein and further verified by the disappearance of electrical conduction from ECGs and absence of cardiac contraction on the chest wall. Four and half minutes after the initiation of cardiac arrest, mechanical ventilation

resumed. Mice were ventilated with a tidal volume of 8–10 ul/g at a rate of 150 strokes/min (FiO$_2$:100%). Five minutes after the initiation of cardiac arrest, resuscitation was initiated at a frequency of 350–400 bpm using index finger. Simultaneously, 0.4 μg/g epinephrine was administrated via right internal jugular vein through micro-PE catheter. Return of spontaneous circulation (ROSC) was confirmed by the presence of spontaneous regular electrical activity visible on the ECG and visible heart beat on the chest wall. Resuscitation was terminated when ROSC was achieved, or it was declared a failure when there was no ROSC after 5 min of CPR. Mechanical ventilation was withdrawn when spontaneous respiratory rate was beyond 60 breaths after ROSC. Mice were returned to a recovery cage after the surgery and observed for 6 h postcardiopulmonary resuscitation. Nalbuphine (2 mg/kg, S.C.) was used for analgesia after surgery. Rectal temperature was monitored and maintained at 36-37 °C during surgery with a heating blanket.

Arrhythmia Analysis

After anesthesia, the standard limb lead II configuration electrocardiographic system was used for continuously monitoring cardiac conduction activity throughout the entire experiment (Powerlab/8sp system, AD Instruments, Colorado Springs, CO, USA). ECG parameters were analyzed offline with LabChart7.2.1 software (AD Instruments, Colorado Springs, CO, USA). Early cardiopulmonary resuscitation-induced arrhythmia parameters such as the incidence of cardiac arrhythmia, ventricular premature beats (VPB), ventricular tachycardia (VT), and AV block (AVB) were recorded for 5 min after ROSC and analyzed.
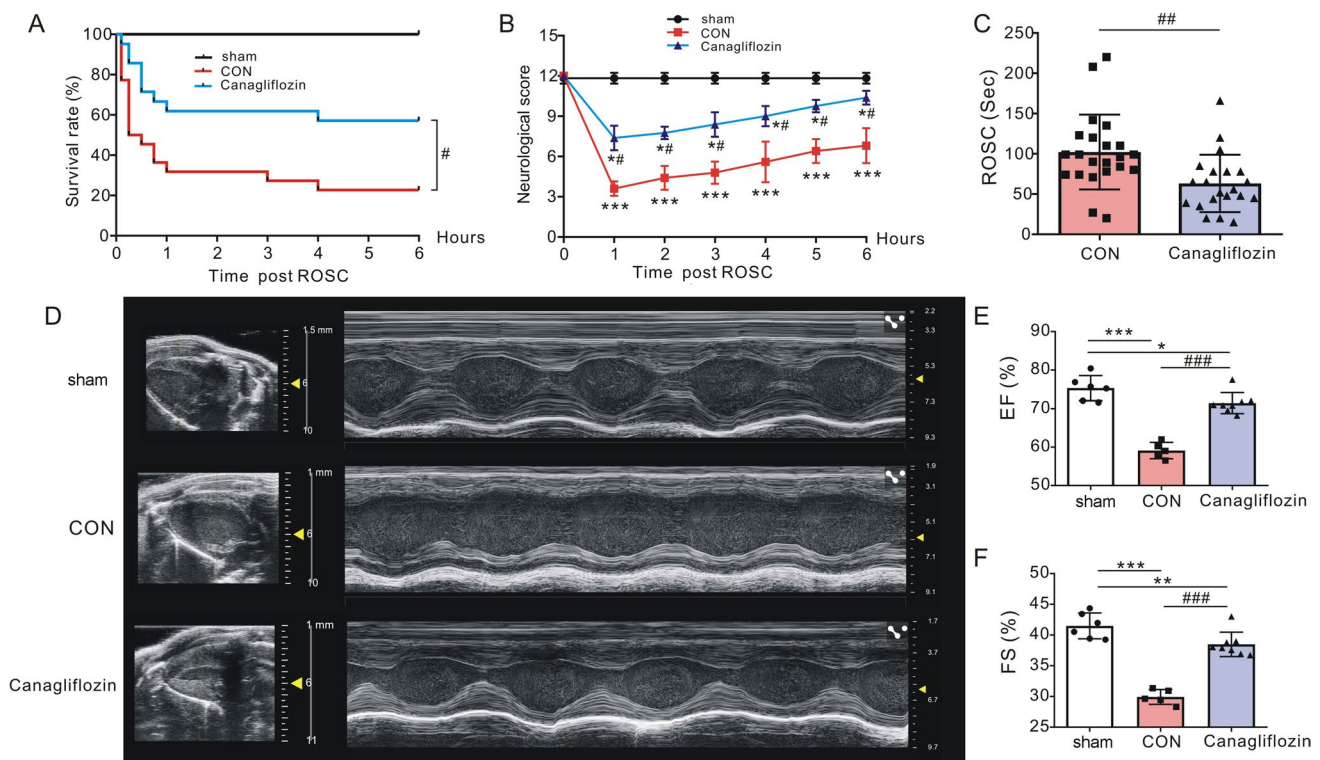
Fig. 2 The effect of canagliflozin on postcardiopulmonary resuscitation outcomes (**A**) Survival of the CON (n = 22) and canagliflozin-treated mice (n = 21) after cardiopulmonary resuscitation. CON, control. ROSC: return of spontaneous circulation. $^{\#}P < 0.05$ vs. CON (by Kaplan–Meier survival analysis test). (**B**) Neurologic scores following cardiopulmonary resuscitation (n = 5–8). $^{*}P < 0.05$ $^{***}P < 0.001$ vs. sham, $^{\#}P < 0.05$ vs. CON (by Kruskal–Wallis test). (**C**) ROSC rates following 5 min of cardiac arrest. CON, control. ROSC: return of spontaneous circulation. n = 21-22 per group. $^{\#\#}P < 0.01$ vs. CON (by unpaired two-tailed student's t-test). (**D**) Exemplar echocardiograms for mice with or without canagliflozin. (**E**) Left ventricular ejection fraction (EF) at 6 h after ROSC. n = 5–8 per group. $^{*}P < 0.05$ $^{***}P < 0.001$ vs. sham, $^{\#\#\#}P < 0.001$ vs. CON (by one-way ANOVA). (**F**) Percent left ventricular fractional shortening (FS) at 6 h after ROSC. n = 5–8 per group. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. sham, $^{\#\#\#}P < 0.001$ vs. CON (by one-way ANOVA)

Mouse Echocardiography

Six hours after ROSC, mice were anesthetized with isoflurane and were placed onto a Vevo 3100 (VisualSonics, Toronto, ON, Canada) platform in the supine position. The limbs were taped onto metal ECG leads. Transthoracic echocardiography was performed. The two-dimensional B-mode parasternal long axis imaging was obtained at the mid-ventricular level. Later, the M-mode view was obtained for measurements of cardiac contractility. The left ventricular (LV) ejection fraction (EF) and LV fractional shortening (FS) were measured and calculated. All measurements were conducted by the same investigator who was blinded to the experimental groups.

Neurological Function

Neurological function postcardiopulmonary resuscitation was determined using an established neurological scoring system [16]. Briefly, the neurological function was scored from 0 to 2 according to the following six aspects: the level of consciousness, corneal reflex, respirations, righting reflex, coordination, and movement/activity. The maximum possible score is 12 (no neurological damage). A total score for each variable was determined for each mouse. Assessment was performed independently by two investigators blind to the experimental groups.

Blood Serum

At the end of the experiment, mice were euthanized by an overdose of sodium pentobarbital (200 mg/kg,i.p.). Death was confirmed by observation of cessation of cardiac activity and respiration. Blood was taken from the left ventricle immediately after euthanasia, followed by centrifugation (10 min, 4000 g, 4 ℃). The supernatant was separated and stored in a –20 ℃ freezer until use.

Fig. 3 Canagliflozin alleviates cardiac damage after cardiopulmonary resuscitation. Post-cardiopulmonary resuscitation mean serum levels of CK-MB (**A**), LDH (**B**),α-HBDH (**C**), and cTnI (**D**) in mice subjected to 5 min of cardiac arrest followed by 5 min of cardiopulmonary resuscitation. n = 5–8 each group. ***$P < 0.001$ vs. sham, ##$P < 0.01$, ###$P < 0.001$ vs. CON (by one-way ANOVA). CK-MB, creatine kinase-MB; LDH, lactate dehydrogenase; α-HBDH, α-hydroxybutyrate dehydrogenase; cTnI: cardiac troponin
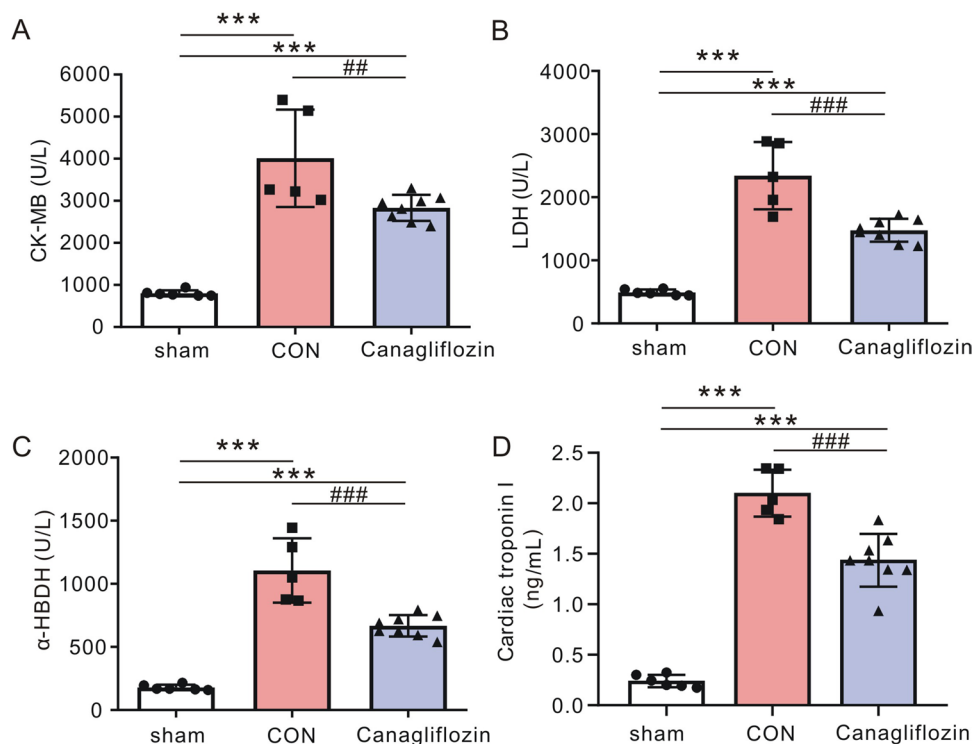
Serum Tests

Serum levels of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and α-hydroxybutyrate dehydrogenase (α-HBDH) at 6 h after ROSC were measured by an automatic BS-120 biochemical analyzer (Mindray, Shenzhen, China). Serum levels of cardiac troponin I (cTnI) were detected using an enzyme-linked immunosorbent assay (ELISA) kit (Quanzhou Ruixin Biological Technology Co., Ltd., Fujian, China) according to the manufacturer's instructions.

Inflammatory Cytokines Measurements

The levels of inflammatory cytokines (interleukin-6, IL-6 and tumor necrosis factor-α, TNF-α) were measured in blood serum in duplicate using ELISA kits (Neobioscience, Shenzhen, China) according to the manufacturer's instructions.

Tissue Collection

At the end of the experiment, the heart was taken and cut into two halves parallel to the atrioventricular groove. The upper half of the whole heart, including right ventricle, left ventricle, and septum, was immersed in 10% phosphate-buffered formalin solution overnight at room temperature. The heart was then fixed in formaldehyde and embedded in paraffin wax. The heart was sectioned into serial heart slices (3 μM thickness) and mounted on glass for morphology

(hematoxylin and eosin (H&E) staining), apoptosis, and immunohistochemistry studies. The lower half of the heart was stored in a –80 ℃ freezer for western blot analysis.

TUNEL Assay

Myocardial apoptosis was determined by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay kit (DeadEnd™ Fluorometric TUNEL system, Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. TUNEL-positive nuclei were stained green and DAPI nuclei were stained blue. Ten random fields were recorded for each heart. The apoptotic index was calculated as the percentage of the number of TUNEL-positive nuclei to the total number of DAPI nuclei. Images were obtained and analyzed using a Nikon Eclipse Ni-E upright fluorescence microscope (Nikon, Tokyo, Japan). Assessment was performed independently by two investigators blind to the experimental groups.

Immunohistochemical Staining

The heart sections were deparaffinized and rehydrated. Then, antigen retrieval was performed using an EDTA antigen retrieval reagent (Beijing Zhongshan Jinqiao Biological Technology Co., Ltd. Beijing, China). The slices were treated with H_2O_2 for 20 min at room temperature before blocking at 37 ℃. Then, the slices were incubated overnight at 4℃ with rabbit phosphorylated STAT-3 (Tyr705),
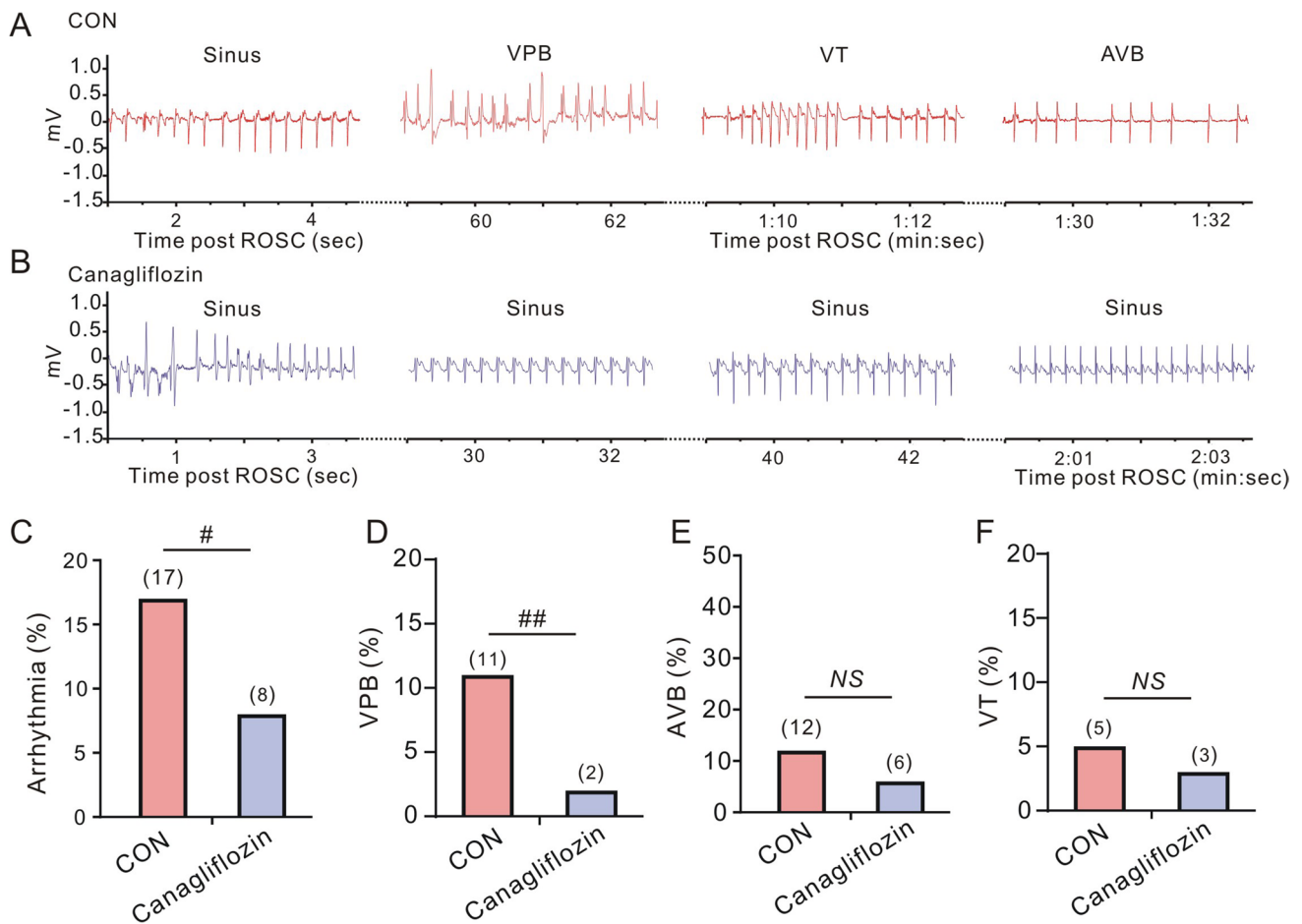
Fig. 4 Canagliflozin-treated mice are less susceptible to early post-cardiopulmonary resuscitation arrhythmias. Representative ECG traces from control (**A**) and canagliflozin-treated mice (**B**) after ROSC. CON, control; VPB, ventricular premature beats; VT, ventricular tachycardia; AVB, AV block. The incidence of arrhythmia (**C**), AVB (**D**), VT (**E**), and VPB (**F**) after ROSC for mice with or without canagliflozin. Values in parentheses indicate the numbers of mice per category. n = 21–22 per group. [#]$P < 0.05$, [##]$P < 0.01$ vs. CON. NS, no significant difference (by Fisher's exact test)

IL-6, TNF-α, Bcl-2, and Bax IgG (Affinity bioscience, Cincinnati, OH, USA). Sections were washed in PBS three times, and peroxidase-conjugated goat anti-rabbit secondary antibody solution (Santa Cruz Biotechnology, Santa Cruz, CA) was applied for 45 min at 37 °C. The sections were then incubated in 3,3-diaminobenzidine solution (DAB, Beijing Zhongshan Golden Bridge Biotechnology, Beijing, China) for chromogen staining and counterstained with hematoxylin. Brown staining in the cytoplasm of myocytes was recognized as positive expression. For negative controls, the primary antibody was replaced by a phosphate buffer solution. All images were taken using a CAST system (Olympus A/S, Ballerup, Denmark) and analyzed by Image-pro plus software (Media Cybernetics Inc., Carlsbad, CA, USA). Ten views were randomly selected for each heart and analyzed by independent researchers in a blinded manner.

Westernblot Analysis

Western blot analysis was performed as previously stated [17, 18]. Briefly, after homogenization, heart lysates were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein was transferred onto a nitrocellulose blotting membrane (Pall Corporation, Pensacola, USA). The membrane was blocked with 5% non-fat milk before being incubated at 4 °C overnight with specific primary antibodies including IL-6, TNF-α (all 1:1000, Affinity Biosciences, Cincinnati, OH, USA), GAPDH (1:2000, YEASEN Bio. Inc., Shanghai, China), phosphorylated Akt (ser473) (p-AKT), total Akt, phosphorylated extracellular signal-regulated kinase1/2 (ERK1/2) (Thr202/Tyr204)(p-ERK), and total ERK1/2, phosphorylated glycogen synthase kinase-3β(Ser9) (p-GSK-3β), total GSK-3β, phosphorylated STAT-3 (Tyr705)(p-STAT3), and

total STAT-3, (all, 1:1000, Cell Signaling, Danvers, MA, USA). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG secondary antibody was applied (1:5000, Bio-Rad, Hercules, CA, USA) for 2 h at room temperature. The membranes were incubated in ECL solution (GE healthcare, Little Chalfont, UK) and imaged on an Amersham Imager 600 system (GE healthcare, Little Chalfont, UK). Band densities were determined by ImageJ Data Acquisition Software (National Institutes of Health, Bethesda, MD, USA). The densities of phosphorylated bands were normalized to their corresponding total protein. The densities of IL-6 and TNF-α were normalized to GAPDH.

Statistical Analysis

Data were expressed as the mean $\pm$ standard deviation (SD) and analyzed with GraphPad Prism version 8.0 (GraphPad, La Jolla, CA, USA) and SPSS 26.0 software for Windows (SPSS Inc., Chicago, IL, USA). Two-way repeated-measures ANOVA was used to analyze neurological data. Difference in survival rate was analyzed by Kaplan–Meier survival analysis test. Fisher's exact test was used to compare arrhythmogenesis. Two independent values were compared using unpaired two-tailed student's t-test. Kruskal–Wallis test was applied to compare the neurological scores. For multiple group comparison, the Levene's test was used to test homogeneity of variance. One-way ANOVA followed by Newman–Keuls test was applied if the variance was equal, or Dunnett's T3 test was used. All P values were two-sided. Statistical significance was set as $P < 0.05$

Results

Canagliflozin Improves Postcardiopulmonary Resuscitation Outcomes

A total of 101 mice were used in this current study. Six mice had sham surgeries. In the CON group, 25 mice had CPR and 22 mice achieved ROSC. However, only 5 mice survived at 6 h after ROSC. In the canagliflozin group, 23 mice underwent CPR; 21 mice achieved ROSC and 12 mice survived at the end of the experiment. Twenty-six mice experienced CPR in the CON+Ag490 group; 22 achieved ROSC and 5 survived. In the canagliflozin+Ag490 group, 27 mice suffered cardiac arrest, 24 achieved ROSC, and 6 survived. Those who failed to achieve ROSC were excluded from the study. No differences were detected in terms of baseline body weight, temperature, and heart rate (data not shown). Notably, Kaplan and Meier curve showed that canagliflozin-treated mice exhibited a higher survival rate than control mice after CPR within 6 h ($P < 0.05$, Fig. 2A). Neurological function was evaluated at 6 h after CPR. We found that all of the surviving animals

showed impaired neurological function as compared to sham-operated mice ($P < 0.001$). However, the values of neurological scores in the canagliflozin group were markedly higher than those in the non-canagliflozin-treated control mice ($P < 0.05$, Fig. 2B). Importantly, we observed that mice in the canagliflozin group (63.2 ± 35.7 s) had a shorter ROSC time than that in the control group (102.2 ± 46.5 s, $P < 0.01$, Fig. 2C). Parameters of cardiac function including LV ejection fraction (EF) and fractional shortening (FS) were measured 6 h after ROSC (Fig. 2D). Canagliflozin was effective at improving cardiac arrest and resuscitation-associated cardiac dysfunction, indicated by increased EF ($P < 0.001$, Fig. 2E) and FS ($P < 0.001$, Fig. 2F) in the canagliflozin group compared with those in the control group. The levels of LDH, CK-MB, and α-HBDH are biomarkers of cardiac damage. We found in the current study that mice in CON and canagliflozin groups had elevated serum levels of LDH, CK-MB, and α-HBDH following successful CPR after cardiac arrest (all $P < 0.001$ vs. sham). However, the elevations of post-CPR LDH, CK-MB, α-HBDH, and cardiac troponin I levels were markedly reduced in canagliflozin-treated mice as compared to corresponding controls ($P < 0.01$ or $P < 0.001$, Fig. 3A–D).

Canagliflozin Diminishes Spontaneous Ventricular Premature Beats After CPR

It is known that early stages of resuscitation-associated arrhythmia can cause sudden cardiac death and that the administration of antiarrhythmic drugs improves resuscitation outcomes after ROSC [19]. Here, we also examined the effect of canagliflozin on arrhythmogenesis after cardiac arrest and resuscitation in our study. Representative ECG tracings from control and canagliflozin-treated mice are shown in Fig. 4A and B. Importantly, we found that most of the control mice (17 out of 22, 77%) exhibited one or more arrhythmias, including ventricular premature beats (VPB), atrioventricular block (AVB), or ventricular tachycardia (VT), at the early stage of ROSC. In contrast, only 8 out of 21 (38%) canagliflozin-treated mice had arrhythmia, while the remainder remained in sinus rhythm after ROSC ($P < 0.05$, Fig. 4C). Notably, although the incidence of AVB (Fig. 4D) or VT (Fig. 4E) was similar in control and canagliflozin groups after ROSC, there was a striking protective effect of canagliflozin against early postcardiopulmonary resuscitation-induced VPB (2/21 canagliflozin mice, compared to 11/22 control mice ($P < 0.01$, Fig. 4F).

Effect of Canagliflozin on Myocardial Morphology and Apoptosis After CPR

Next, we sought to determine whether canagliflozin affects cardiac morphology, inflammatory response or apoptosis post-CPR. As shown in Fig. 5A, H&E staining revealed no
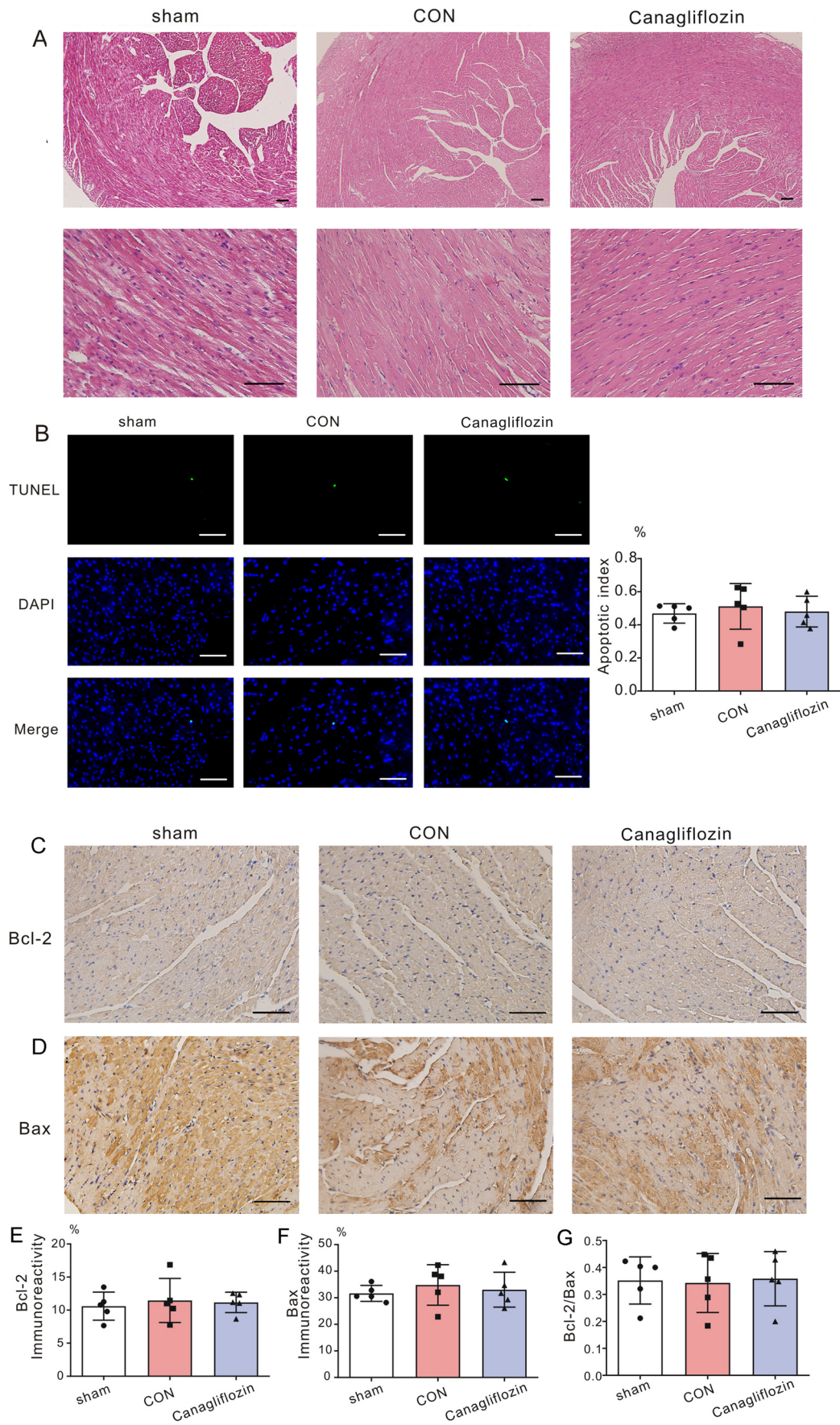
◄Fig. 5 Canagliflozin did not affect cardiac morphology or apoptosis after CPR (**A**) Representative H&E-stained heart sections after cardiopulmonary resuscitation. Scale bars, 50 µm. n = 5 mice per group. (**B**) *Left,* Representative TUNEL stained heart sections after cardiopulmonary resuscitation. Scale bars, 50 µm. n = 5 mice per group. *Right*, Apoptotic index in each group. (n = 5 mice per group). CON: control (by one-way ANOVA). Immunostaining for apoptotic related Bcl-2 (**C**) and Bax (**D**) proteins in hearts with or without canagliflozin treatment. Scale bars, 50 µm. The analysis of Bcl-2 (**E**), Bax (**F**), and Bcl-2/Bax (**G**) protein immunoreactivity in mice hearts after cardiopulmonary resuscitation. Scale bars, 50 µm. Each group, n = 5 (by one-way ANOVA)

evidence of myocardial structural changes in groups. Moreover, there was no difference in the percentage of apoptotic cells between sham-operated mice and mice treated with canagliflozin or vehicle after CPR (Fig. 5B). Consistent with this, the expression of apoptotic-related proteins such as Bcl-2 and Bax was not different among groups (all $P >$ 0.05, Fig. 5C–G). These findings suggest that canagliflozin improves postcardiopulmonary resuscitation outcomes via mechanisms other than modifying myocardial morphology or apoptosis.

Canagliflozin Decreases Systemic Inflammatory Response Postcardiopulmonary Resuscitation

Resuscitation can produce inflammatory responses [20]. Inflammatory cytokines such as IL-6 and TNF-α are vital mediators in the pathogenesis of post-cardiac arrest syndrome, whose release may increase the burden of tissue injury. Notably, we found that vehicle-treated control mice had a greater elevation in serum levels of IL-6 and TNF-α after CPR when compared to sham-operated mice ($P < 0.001$). However, canagliflozin significantly suppressed these expressions ($P < 0.01$ or $P < 0.001$ vs. CON, Fig. 6A and B). However, we did not detect any difference regarding cardiac expression levels of IL-6 and TNF-α among groups using westernblot ($P > 0.05$, Fig. 6C–E) or immunohistology ($P > 0.05$, Fig. 6F and G) methods.

Canagliflozin Promotes Myocardial STAT-3 Phosphorylation After CPR

We previously showed that empagliflozin exerted its antiarrhythmic effect by activating the reperfusion injury salvage kinase (RISK) pathway but not the survivor activating factor enhancement (SAFE) pathway [18]. To further understand the protective role of canagliflozin in the current study, we investigated whether or not canagliflozin may affect the RISK pathway after CPR, specifically the vital signaling molecules in the RISK pathway, such as ERK1/2, AKT, and GSK-3β. The phosphorylation levels of these proteins were evaluated via normalization to their total protein levels. We found no difference regarding the

total protein levels among experimental groups ($P > 0.05$). After cardiac arrest and cardiopulmonary resuscitation, AKT was similarly phosphorylated among groups ($P > 0.05$, Fig. 7A). Meanwhile, the ratios of phosphorylated to total myocardial ERK1/2 (1.10±0.2) and GSK-3β (0.7±0.2) protein were higher in control hearts than in sham-operated hearts (ERK1/2:0.3±0.1, GSK-3β: 0.2±0.1, all $P <0.001$), and not further enhanced in canagliflozin-treated hearts (ERK1/2:1.1±0.2, GSK-3β: 0.8±0.2, all $P > 0.05$ vs CON, Fig. 7B and C). These results suggest that the RISK pathway is not associated with canagliflozin-induced postresuscitation cardioprotection. Activation of the SAFE signaling pathway, including the signal transducer and activator of transcription 3 (STAT-3) signaling protein, has been shown to be involved in cardioprotection [21]. We next evaluated the influence of canagliflozin on the phosphorylation status of STAT-3. We found that the phosphorylation level of STAT-3 was increased in control hearts (0.5±0.3) after CPR when compared with sham-operated hearts (0.2±0.1, $P <$ 0.05). Surprisingly, canagliflozin-treated hearts exhibited increased phospho-STAT3 levels (1.0±0.2) when compared to controls ($P < 0.001$, Fig. 7D). Consistent with these westernblot findings, our immunohistochemistry results showed that phosphorylated STAT-3 immunoreactivity in the control group was increased when compared to those in the sham hearts ($P < 0.01$), while STAT-3 was further phosphorylated upon canagliflozin treatment after CPR ($P < 0.001$ vs. CON, Fig. 7E). These results suggest that STAT-3 may be responsible for the canagliflozin-induced cardioprotection after CPR.

Inhibition of STAT-3 Abolishes the Beneficial Effect of Canagliflozin After CPR

We found increased STAT-3 phosphorylation in canagliflozin-treated hearts after CPR. To further confirm the role of STAT-3, Ag490, a STAT-3 inhibitor, was administrated intravenously ahead of cardiac arrest in our study. We had previously observed that canagliflozin-treated mice had a higher survival rate than those in the control group (Fig. 2A). However, Ag490 pretreatment eliminated this difference, resulting in similar mortality rates between control and canagliflozin-treated mice ($P > 0.05$, Fig. 8A). Next, we quantified the effects on postcardiopulmonary resuscitation neurological function of pretreatment with Ag490 in both vehicle or canagliflozin-treated mice. Strikingly, AG490 pretreatment abolished the beneficial effect of canagliflozin on neurological function after CPR, such that canagliflozin-treated mice exhibited reduced neurological scores with the presence of Ag490 when compared with non-Ag490-treated canagliflozin mice ($P < 0.05$, Fig. 8B). Furthermore, Ag490 eliminated the reduction of ROSC time in canagliflozin group (107.0±45.8 s in canagliflozin+Ag490 group
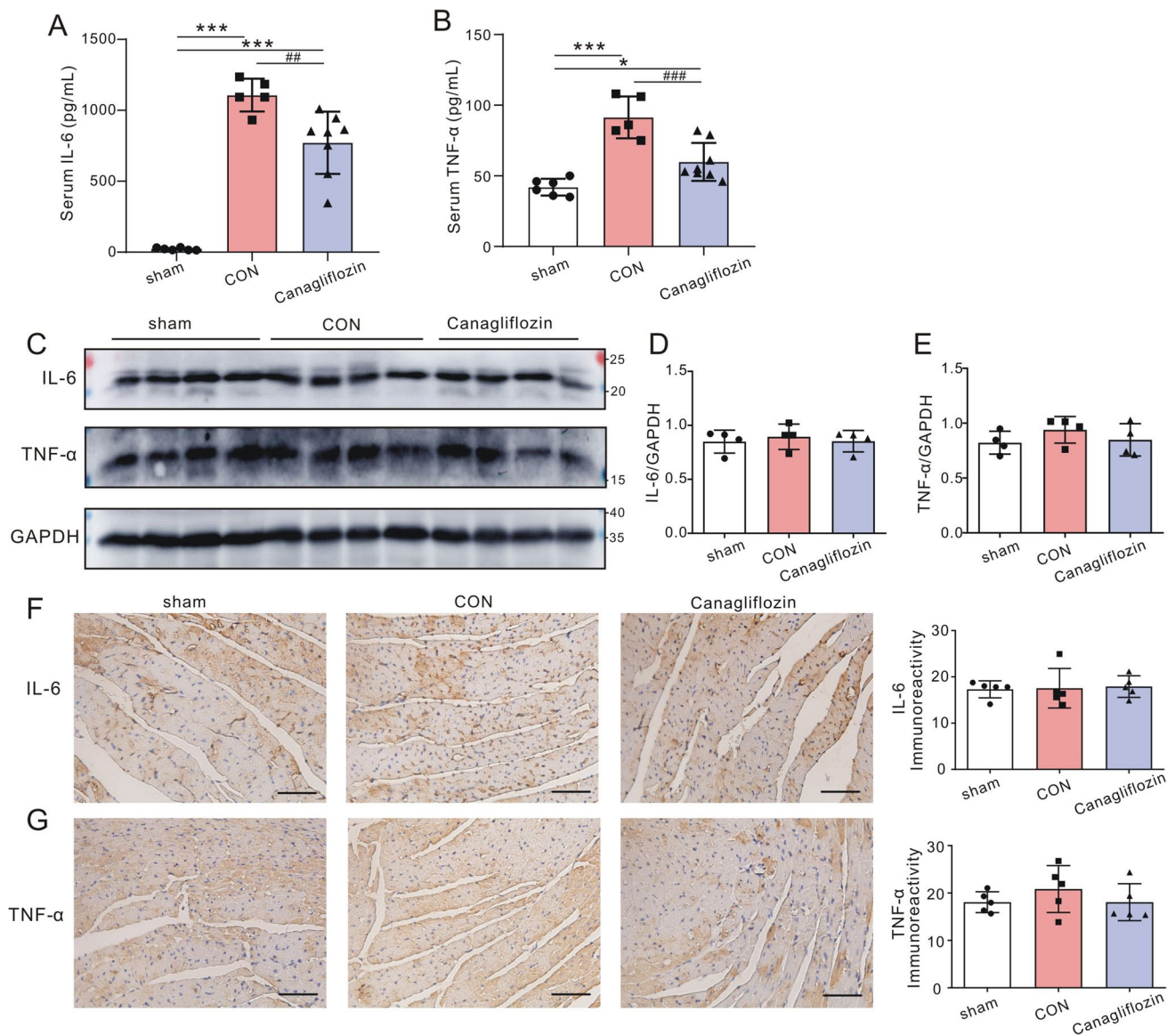
Fig. 6 The effect of canagliflozin administration on inflammatory response. (**A**) Serum levels of interleukin-6 (IL-6) after 6 h of ROSC. n = 5–8 per group. ***P < 0.001, versus sham-operated mice; ##P < 0.01, versus control mice (by one-way ANOVA). CON: control (**B**) Serum levels of tumor necrosis factor-α (TNF-α) after 6 h of ROSC. n = 5–8 per group. **P < 0.01, ***P < 0.001, versus sham-operated mice; ##P < 0.01, versus control mice (by one-way ANOVA). (**C**) western blots of IL-6, TNF-α, and GAPDH in heart tissues after cardiac arrest and resuscitation. n = 4 per group. Bar graph showing mean ratio of IL-6/GAPDH (**D**) and TNF-α/GAPDH (**E**) band densities (by one-way ANOVA). (**F**) *Left* The immunostaining for interleukin-6 (IL-6) in hearts from mice treated with saline or canagliflozin after cardiopulmonary resuscitation. Scale bars, 50 μm. *Right* The level of immunoreactivity of IL-6 in mouse hearts. Each group, n = 5 (by one-way ANOVA). (**G**) *Left* The immunostaining for tumor necrosis factor-α (TNF-α). Scale bars, 50 μm. *Right* The level of immunoreactivity of TNF-α. Each group, n = 5 (by one-way ANOVA)

vs. 63.2±35.7 s in canagliflozin group, P < 0.01, Fig. 8C). We observed that canagliflozin could reduce the severity of postresuscitation myocardial dysfunction, as reflected by improved LV, EF, and FS. However, Ag490 pretreatment diminished the values of EF and FS to a level similar to those of hearts in the vehicle-treated control group (all P < 0.001 vs. canagliflozin group without inhibitor, Fig. 9A–C). Meanwhile, Ag490-pretreated canagliflozin mice were more susceptible to post-cardiopulmonary resuscitation-induced arrhythmias (Fig. 9D) or VPB (Fig. 9E) when compared to canagliflozin mice without inhibitor (P < 0.05). Additionally, AG490 eliminated the decrease in serum levels of CK-MB (Fig. 9F), LDH (Fig. 9G), α-HBDH (Fig. 9H), cTnI (Fig. 9I), IL-6 (Fig. 9J), and TNF-α (Fig. 9K) that were achieved by canagliflozin treatment in the absence of inhibitor (P < 0.01 or P < 0.001 vs. canagliflozin group without
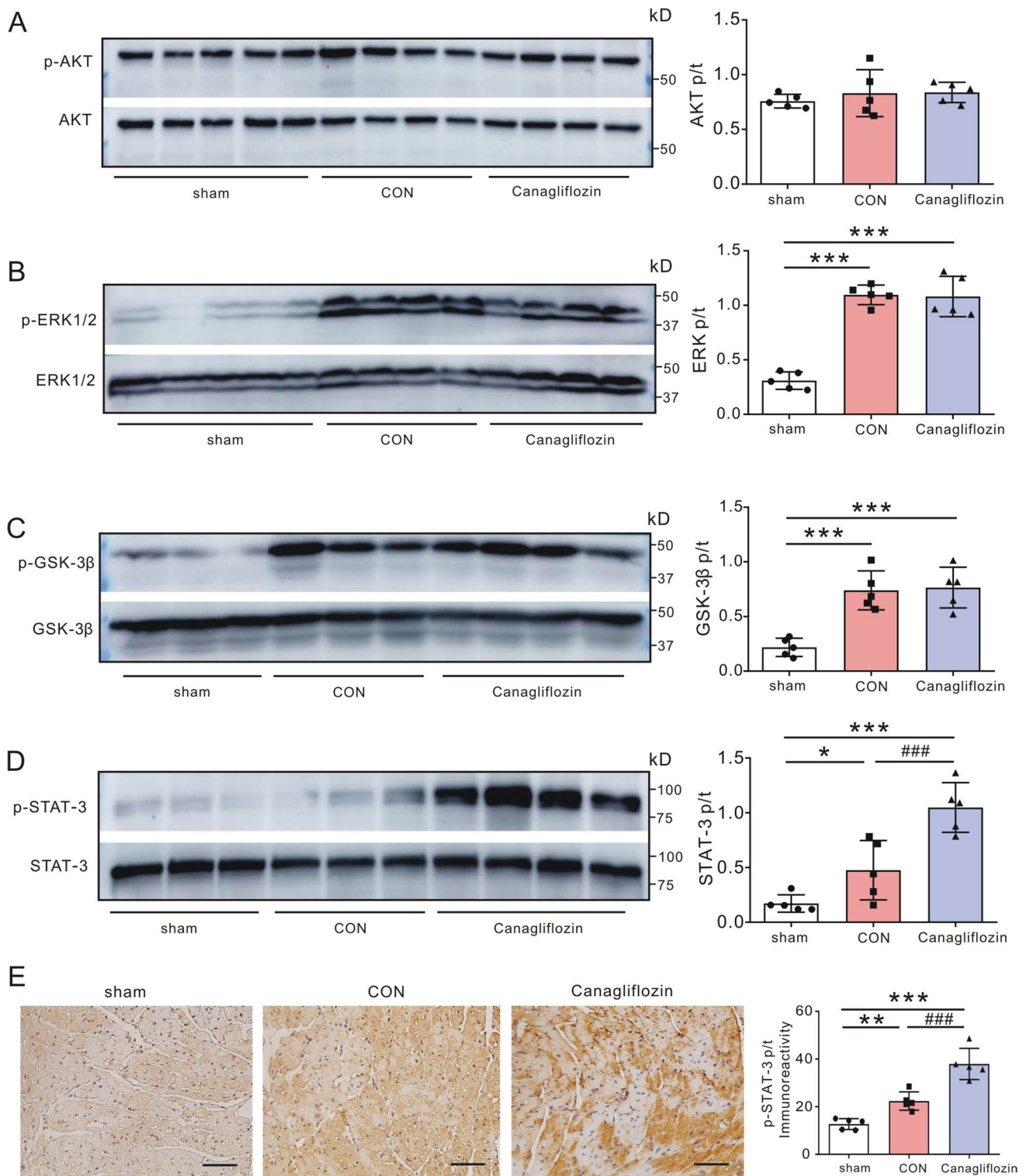
Fig. 7 Canagliflozin promotes STAT-3 phosphorylation after cardiopulmonary resuscitation Myocardial expression (*Left*) and band densities (*Right*) of phosphorylated ERK1/2 and total ERK1/2 (**A**), phosphorylated AKT and total AKT (**B**), phosphorylated GSK-3β and total GSK-3β (**C**), and phosphorylated STAT3 and total STAT3 (**D**) in sham, control and canagliflozin-treated mice (n = 5 per group).

*P < 0.05, ***P < 0.001 vs. sham, ###P < 0.001 vs. CON (by one-way ANOVA). sham, sham-operated group; CON, control. (**E**) *Left* Typical Immunostaining images (for p-STAT-3 in heart sections after cardiopulmonary resuscitation. Scale bars, 50 µm. *Right* Analysis of p-STAT-3 immunoreactivity levels. n=5 per group. **P < 0.01, ***P < 0.001 vs. sham, ###P < 0.001 vs. CON (by one-way ANOVA)
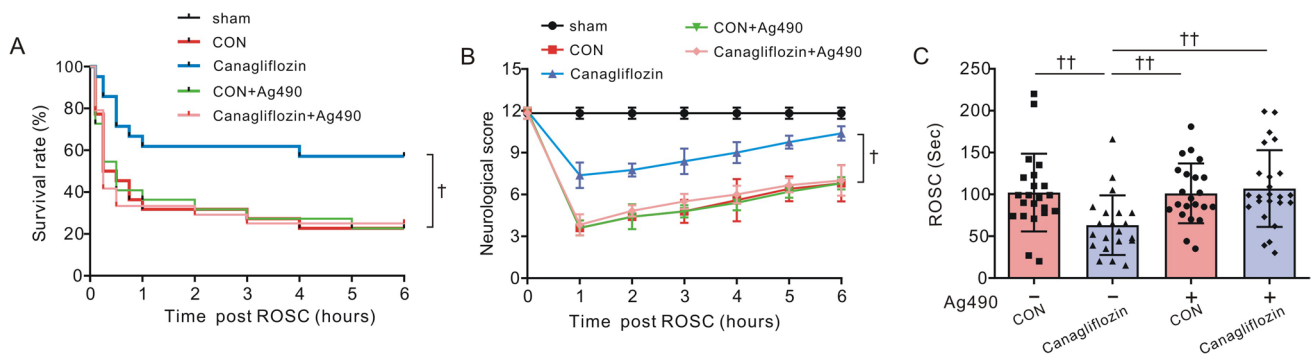
Fig. 8 The effect of Ag490 on post-cardiopulmonary resuscitation outcomes. (**A**) The effect of STAT-3 inhibition on mortality in CON (n = 22) and canagliflozin-treated mice (n = 24) upon administration of Ag490 prior to cardiac arrest. CON, control. ROSC, return of spontaneous circulation. $^{†}P < 0.05$ vs. canagliflozin-treated mice without Ag490 (by Kaplan–Meier survival analysis test). Values for CON and canagliflozin mice are repeated from Fig. 2A for comparison. (**B**) Neurologic scores following cardiopulmonary resuscitation of the mice with (+) or without (–) Ag490 (n = 5–8). $^{†}P < 0.05$ vs. canagliflozin-treated mice without Ag490 (by Kruskal–Wallis test). Values for sham, CON, and canagliflozin mice are repeated from Fig. 2B for comparison. (**C**) ROSC rates following 5 min of cardiac arrest of the mice with (+) or without (–) Ag490. CON, control. ROSC, return of spontaneous circulation. n = 21–24 per group. $^{††}P < 0.01$ vs. canagliflozin-treated mice without Ag490 (by one-way ANOVA). Values for CON and canagliflozin mice are repeated from Fig. 2C for comparison

inhibitor). Furthermore, Ag490 did not alter cardiac histology or apoptosis after CPR (supplementary Fig. 1)

Ag490 Diminished Canagliflozin-Induced STAT-3 Phosphorylation After CPR

We next investigated whether STAT-3 inhibition could alter canagliflozin-induced STAT-3 phosphorylation after CPR. Western blots results indicated that the phosphorylation level of STAT-3 in canagliflozin+Ag490 group (0.6 ± 0.2) was largely decreased when compared with non-Ag490-treated canagliflozin group (1.1 ± 0.1, $P < 0.01$, Fig. 10A). Meanwhile, no difference regarding phosphorylation level of STAT-3 was detected between CON+Ag490 and canagliflozin+Ag490 groups ($P > 0.05$); Our immunohistochemistry data also showed similar results. We found that phosphor-STAT-3 was decreased in Ag490-treated canagliflozin hearts when compared to mice exposed to canagliflozin alone ($P < 0.001$, Fig. 10B). Taken together, these results indicate that the beneficial effects of canagliflozin after CPR may be mediated by a STAT-3 dependent mechanism.

Discussion

Sudden cardiac arrest (SCA) causes about 325,000 adult deaths in the United States annually. In North America and Europe, the incidence of SCA is between 50 to 100 per 100,000 persons every year. SCA is responsible for half of all heart disease deaths. It is estimated that 50% of the SCAs happens to those without previously diagnosed heart disease [22]. Cardiopulmonary resuscitation (CPR) is a lifesaving

procedure that effectively restores circulation and cardiac function in patients with SCA. However, the outcome of resuscitation is often not satisfactory. The survival rate of out-of-hospital cardiac arrest (OHCA) patients is poor [23] and among those in-hospital cardiac arrest (IHCA) patients with sustainable return of spontaneous circulation (ROSC), the mortality rate is also high, varying from 60% [24] to 80% [25] in the intensive care unit. The deaths are due to post-cardiac arrest syndrome after resuscitation [26], which frequently leads to multiple-organ failure and high mortality. Post-cardiac ischemia/reperfusion response, cardiac arrest brain injury and myocardial dysfunction, and persistent precipitating pathology may account for its pathophysiology of post-resuscitation deterioration [20]. Pathophysiological processes triggered by cardiac arrest are exacerbated by reperfusion. Among these adverse responses, hemodynamic instability and myocardial dysfunction are among the most severe and common alterations. It has been shown that over 68% of patients developed myocardial dysfunction post-resuscitation [27] and left ventricular dysfunction is the most general pattern [28]. Therefore, it is of great value to identify novel therapeutic strategies to improve myocardial tolerance for patients at high risk of cardiac arrest.

Cardiac arrest can be induced by asphyxia, ventricular fibrillation, or potassium in experimental animals. Based on a systematic review discussing the animal models of cardiac arrest, for mouse models of cardiac resuscitation, 97% of the publications used potassium chloride injections (the other 3% did not report the method) [29]. KCl induction has several advantages for small animals, for example, less heart damage and easy control of the ischemic duration. Most importantly, KCl can induce immediate circulatory arrest,
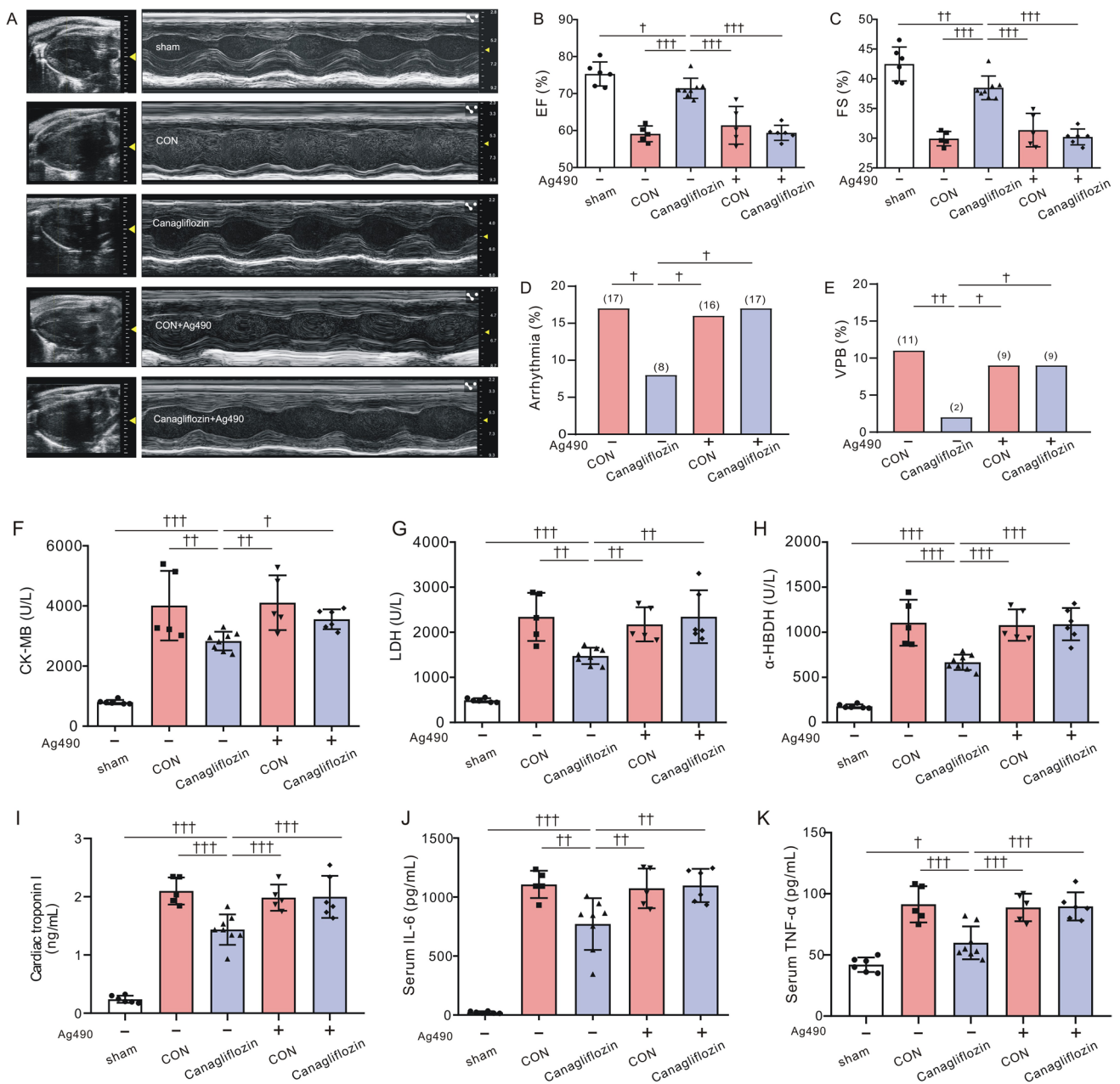
Fig. 9 Ag490 abolishes canagliflozin-induced cardioprotection. (**A**) Exemplar echocardiograms for mice with or without STAT-3 inhibition after CPR. Left ventricular ejection fraction (**B**) and fractional shortening (**C**) at 6 h after ROSC of the mice with (+) or without (–) Ag490. n = 5–8 per group. † $P < 0.05$, $^{††}P < 0.01$,$^{†††}P < 0.001$ vs. canagliflozin-treated mice without Ag490 (by one-way ANOVA). Values for sham, CON and canagliflozin mice are repeated from Fig. 2E and F for comparison. The incidence of arrhythmia (**D**) and VPB (**E**) after ROSC for mice with (+) or without (–) Ag490 Values in parentheses indicate the numbers of mice per category. n = 21–24 per group. $^†P<0.05$, < 0.05, $^{††}P < 0.01$ vs. canagliflozin-treated mice without Ag490 (by Fisher's exact test). Values for CON and canagli-

flozin mice are repeated from Fig. 4C and F for comparison. Serum levels of CK-MB (**F**), LDH (**G**),α-HBDH (**H**) and cTnI (**I**) at 6 h after ROSC of the mice with (+) or without (–) Ag490. n = 5–8 per group. † $P < 0.05$, $^{††}P < 0.01$, $^{†††}P < 0.001$ vs. canagliflozin-treated mice without Ag490 (by one-way ANOVA). Values for sham, CON and canagliflozin mice are repeated from Fig. 3 for comparison. Serum levels of IL-6 (**J**) and TNF-α (**K**) at 6 h after ROSC of the mice with (+) or without (–) Ag490. n = 5–8 per group. $^{††}P < 0.01$, $^{†††}P < 0.001$ vs. canagliflozin-treated mice without Ag490 (by one-way ANOVA). Values for sham, CON and canagliflozin mice are repeated from Fig. 6A–B for comparison

Fig. 10 The effect of Ag490 on myocardial STAT-3 phosphorylation (**A**) Myocardial expression (*left*) and band densities (***right***) of phosphorylated STAT3 and total STAT3 in groups (n = 5 per group). $^{††}P < 0.01$, $^{†††}P < 0.001$ vs. canagliflozin-treated mice without Ag490 (by one-way ANOVA). (**B**) *Upper left* Typical immunostaining images (for p-STAT3 in heart sections. Scale bars, 50 μm. *Lower right* Analysis of p-STAT-3 immunoreactivity levels. $^{††}P < 0.01$, $^{†††}P < 0.001$ vs. canagliflozin-treated mice without Ag490 (by one-way ANOVA). n = 5 per group. Values for sham, CON, and canagliflozin mice are repeated from Fig. 7E for comparison
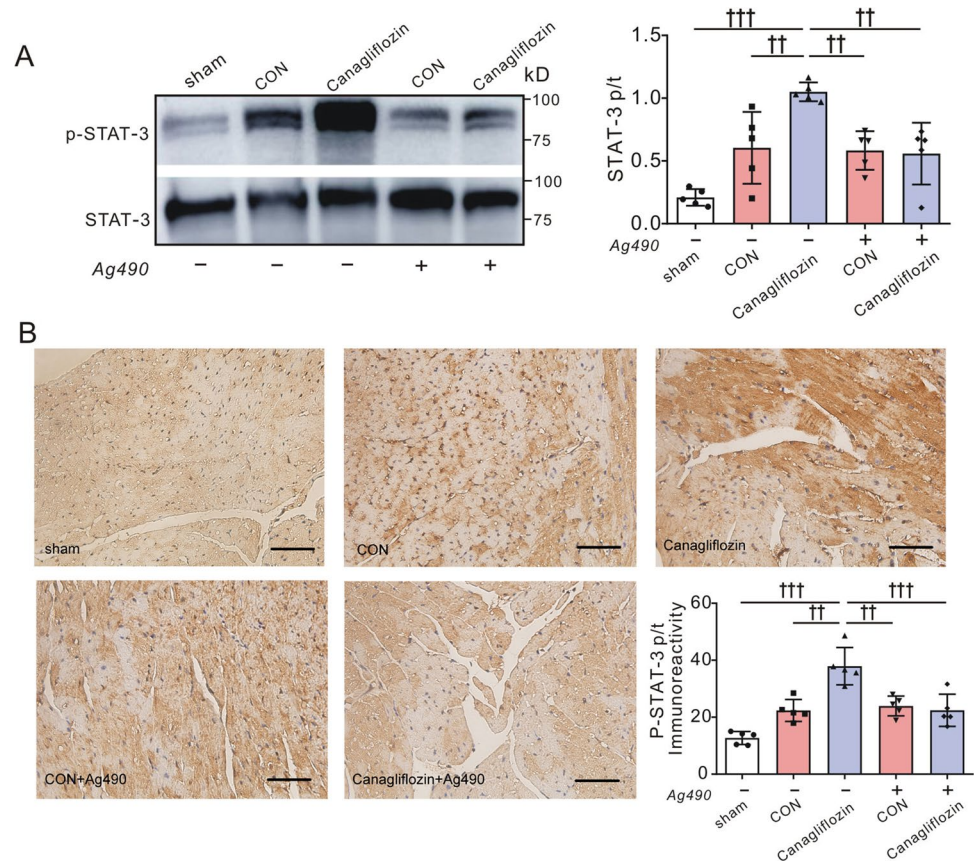
which is the most common cause of cardiac arrest seen in patients with coronary artery disease in clinical settings. In comparison, the asphyxial model requires the application of neuromuscular blockade and mimics non-cardiac causes of cardiac arrest in the clinic. It may not reflect well the pathophysiologic sequence after sudden onset cardiac arrest in most patients. In addition, pacing-induced cardiac arrest can be achieved by implantation of electrodes on ventricles. However, this procedure requires direct intervention upon the heart, thus it may produce myocardial injury. In the present study, we therefore used a standard in vivo mouse potassium chloride-induced cardiac arrest model followed by chest compressions and intravenous epinephrine for resuscitation. The present study is the first to characterize the anti-post-resuscitation cardiac dysfunction activity of canagliflozin in vivo. We found that canagliflozin significantly protected the heart against resuscitation-induced cardiac depression; for example, canagliflozin increased survival rate, improved neurological and ventricular function, ameliorated the systemic inflammatory response, and diminished the incidence of resuscitation-induced spontaneous ventricular premature beats.

The pathophysiology of post-resuscitation-related cardiac dysfunction is complex. It may be attributed to the results of immediate establishment of circulation, resumption of oxygenation, or resuscitation manoeuvers, which may cause reduced left ventricular compliance and contractility. Different from patients undergoing reperfusion treatments for acute infarction from a regional coronary occlusion, people with cardiac arrest experience a transient whole-body ischemia followed by emergency resuscitation from global ischemia. Accordingly, whether the reperfusion may lead to apoptosis and necrosis after CPR remains controversial. In the current study, we did not detect any apoptosis or morphological alterations among groups, indicating that in the experimental setting we employed, 5 min of global ischemia with resuscitation was not capable of producing myocardial necrosis or damage. Reperfusion injury-induced myocardial necrosis or apoptosis is initiated in a time-dependent manner. It is reported that in the heart, ischemia lasting less than 15 min is not sufficient to cause irreversible injury, and only prolonged ischemic insult shifts the cell toward death [30]. In agreement with our findings, previous observations have demonstrated that myocardial apoptosis does not arise in murine models of cardiac arrest and cardiopulmonary resuscitation [31, 32]. It is known that systemic inflammatory response, acts as part of the postcardiac-arrest syndrome, occurs after ROSC [20]. Elevated serum levels of inflammatory cytokines were associated with increased mortality in patients [33]. Previous investigations using either rodent or swine models

have demonstrated that IL-6 is an essential component of the postresuscitation inflammatory cascade [34] and blockade of TNF-α improved survival after CPR [35]. Moreover, a recent clinical study further showed that treatment with IL-6 receptor antibodies (directed against IL-6 itself) resulted in reduction in systemic inflammation and myocardial injury in OHCA patients [36]. Consistent with these previous reports, we found in the current study that 6h after resuscitation, serum levels of IL-6 and TNF-α were increased, and that canagliflozin decreased the elevation. However, it is important to note that cardiac expression levels of IL-6 and TNF-α were not altered after CPR among groups in our study, indicating that 5 min of cardiac arrest followed by 5 min (maximum) of resuscitation was not capable of inducing local cardiac inflammatory response. Therefore, the beneficial effect of canagliflozin observed in our study may be attributed to the alleviation of systemic inflammatory response.

SGLT2 inhibitors are newly approved anti-diabetic therapies for patients with T2DM. They effectively improve glycemic control by inhibiting the absorption of glucose from the proximal tubule of the kidney. In addition, SGLT2 inhibitors have favorable effects on blood pressure, body mass, endothelial function, and arterial function [37]. Importantly, large clinical trials have repeatedly shown that they improve a wide range of cardiovascular and renal outcomes without increasing hypoglycemic risk. Large randomized outcome trials assessing cardiovascular efficacy outcomes of SGLT2 inhibitors showed the strong cardioprotective effect of SGLT2 inhibitors in patients with T2DM; for example, empagliflozin [38] reduced cardiovascular death and the risk of major adverse cardiovascular events (MACE) (EMPA-REG OUTCOME trial). Dapagliflozin [39] lowered the rate of cardiovascular death or hospitalization for heart failure (DECLARE-TIMI 58 trial). Canagliflozin [5] reduced the risk of cardiovascular events (CANVAS trial). Importantly, further cardiovascular outcomes trials revealed that empagliflozin (EMPA-REG OUTCOME trial) [4] and dapagliflozin (DAPA-HF trial) [8] protected hearts in a glucose-independent manner. A follow-up meta-analysis also supported these clinical findings[9]. Meanwhile, a very recent CHIEF-HF remote, patient-centered randomized trial confirmed the cardioprotective effect of canagliflozin in patients without diabetes [40]. Additionally, the beneficial effects of SGLT2 inhibitors on cardio-endpoints observed in clinical trials have been confirmed in animal studies [41]. SGLT2 inhibitors may also have SGLT2 independent effects in vitro [42, 43]. Focusing on acute cardiac I/R injury, the recently published EMMY trial revealed that patients treated with empagliflozin after a PCI for acute myocardial infarction had better cardiac outcomes [10]. In agreement with this clinical finding, recent experimental studies have shown that canagliflozin had infarct-sparing effects independent of glycemic control in in vivo [44] or ex vivo [45] animal

models of regional ischemia. Our study expands these findings by showing that canagliflozin effectively increases survival rate, improves cardiac function, reduces inflammatory response, and postresuscitation-induced cardiac arrhythmia in a cardaic arrest and resuscitation mice model. However, cardiac contractile dysfunction but no necrosis, apoptosis, or histological alterations post-I/R was observed in the current study, indicating that a short period of cardiac arrest (5 min) followed by resuscitation is insufficient to induce permanent myocardial injury. The myocardium may experience a so-called short-term myocardial hibernation, which may be an adaptation of contractile function to the reduced blood flow [46]. Our investigations may also provide experimental evidence for optimizing current resuscitation strategies for patients with cardiac arrest, to reduce postresuscitation complications and mortality.

The cardioprotective effects of canagliflozin have been confirmed by clinical and preclinical studies; however, the underlying molecular mechanism is not fully elucidated. Its mechanism of ameliorating postresuscitation-induced cardiac dysfunction remains unclear. The JAK (Janus tyrosine kinase)-STAT (signal transducer and activator of transcription) cell survival signaling pathway is well-recognized as a crucial mediator in myocardial I/R injury, whose activation confers cardioprotection. The STAT family has STAT1-6 members. It has been shown that protective strategies, such as ischemic preconditioning or postconditioning, exert potent cardioprotection via activation of STATs, specifically, STAT-3, a major signal component in the survivor activating factor enhancement (SAFE) pathways [47]. In the current in vivo study, using a 5-min cardiac arrest followed by resuscitation protocol, we found that canagliflozin had potent cardioprotective effects against resuscitation-induced complications and promoted myocardial STAT-3 phosphorylation. Moreover, inhibition of STAT-3 activity by Ag490 abolished the beneficial effect of canagliflozin after CPR. This study provides the first direct evidence of the involvement of STAT-3 in canagliflozin-induced cardioprotection against postresuscitation myocardial dysfunction.

In addition, we also examined another reperfusion injury-associated cell survival signaling pathway, named the reperfusion injury salvage kinase (RISK) pathway, which was first proposed by the Yellon group [48]. The RISK pathway consists of protein kinase B (AKT), extracellular signal-regulated kinases (ERK1/2), and their downstream target glycogen synthase kinase-3β (GSK-3β). The RISK pathway can be activated (via phosphorylation) upon protective stimuli during reperfusion injury [49]. We previously found that empagliflozin recruited ERK1/2 in the RISK pathway to protect the heart against myocardial reperfusion injury-induced lethal arrhythmia [18]. However, in the current study, the RISK pathway was not responsible for canagliflozin-induced cardioprotection.

The present study contains certain limitations. First, our model replicates resuscitation-induced early cardiac dysfunction after cardiac arrest, which is of clinical relevance but does not incorporate long-term chronic cardiac remodeling after resuscitation. Meanwhile, canagliflozin was given prior to cardiac arrest; our intention was to demonstrate that canagliflozin could be cardioprotective. Future studies will be needed to elucidate whether canagliflozin administered after ROSC can also improve post resuscitation outcomes, and determine the long-term benefits of canagliflozin. Second, although the mouse model of cardiac arrest provides a better tool to explore new therapeutic strategies and understand the pathophysiology of cardiac arrest, it may not completely reflect all aspects of cardiac arrest in clinical settings. Third, the aim of our study was to determine whether canagliflozin can improve postresuscitation outcomes in non-diabetic mice, yet it remains unknown whether canagliflozin also has beneficial effects in the diabetic animals. Furthermore, SGLT2 is mainly expressed in the human lumen of the small intestine and kidneys, and since we did not examine the role of SGLT2 or sodium ion in the kidney or heart in our current study, it will be interesting to study in the future whether canagliflozin may affect the sodium balance or this canagliflozin-mediated cardioprotection is SGLT2-independent or mediated by renal effects.

In summary, our data demonstrated that canagliflozin protected hearts against cardiac arrest and resuscitation-induced cardiac dysfunction in vivo, which was associated with phosphorylation of cardiac STAT-3. Furthermore, inhibition of STAT-3 activation abolished the canagliflozin-induced cardioprotection. Owing to its potent protective efficacies, future treatment and prevention strategies could include SGLT2 inhibitors designed for cardioprotection for patients with a high risk of cardiac arrest.

Data Availability All the data in this study are available upon reasonable request from the corresponding author.

Declarations

Ethics Approval All animal experiments were conducted in conformity with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH Publication 8th edition, 2011). The experiments were approved by the Institutional Animal Care and Use Committee of Sichuan University (Sichuan, China, approval number: 20211201A).

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare that they have no conflicts of interest.

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