Glycyrrhetinic Acid Protects the Heart from Ischemia/Reperfusion Injury by Attenuating the Susceptibility and Incidence of Fatal Ventricular Arrhythmia During the Reperfusion Period in the Rat Hearts

Hong-Jin Wu a Ji-Yuan Yang b Min Jin b Sheng-Qi Wang c De-Lin Wu b Yu-Na Liu b Xu Yan a,d Cui Yang a Ge Zhang a Jing He a

a Beijing Haidian Hospital, Haidian Section of Peking University Third Hospital, Beijing, b Beijing Hospital of Integrated Traditional Chinese and Western Medicine, Beijing, c Beijing Institute of Radiation Medicine, Beijing, d Postdoctoral Workstation of the Zhongguancun Haidian Science Park, Beijing, China

Key Words
Glycyrrhetinic acid • Ischemia/reperfusion • Arrhythmias • Ion channel

Abstract
Background/Aims: Licorice has been used to treat many diseases, including palpitations, in both Eastern and Western societies for thousands of years. It has been reported that glycyrrhetinic acid (GA), an aglycone saponin extracted from licorice root, exerts protective effects on the cardiovascular system, limits infarct sizes and protects against the development of arrhythmia. However, the mechanisms underlying the effects of glycyrrhetinic acid on the cardiovascular system remain poorly understood. This study aimed to determine the mechanisms underlying the protective effects of GA against lethal cardiac arrhythmias induced via ischemia-reperfusion in rat hearts, and to examine its electropharmacological properties.

Materials and Methods: Anesthetized rats were divided into control (CTL), GA5, GA10, and GA20 groups. GA was administered intravenously 15 min before the occlusion of the left anterior descending coronary artery, at dosages of 5, 10 and 20 mg/kg, respectively. Single ventricular myocytes were isolated using enzymolysis. The whole-cell patch clamp technique was utilized to record Ica, L, Ito and action potentials (APs).

Results: During reperfusion, the incidence of ventricular fibrillation (VF) was decreased in each of the groups compared with the CTL group (p<0.05). The ventricular tachycardia (VT)/VF score was significantly decreased in

H.-J. Wu, J.-Y. Yang and M. Jin contributed equally to this paper.

Hong-Jin Wu
Beijing Haidian Hospital, Haidian Section of Peking University Third Hospital, 29th Zhongguancun Setreet Beijing, 100080 (China)
E-Mail whjhdyyz@yeah.net
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**Introduction**

Ischemic heart disease is one of the most significant problems facing clinicians in the industrialized world [1, 2]. Reperfusion injury following a period of ischemia has recently drawn more attention, as thrombolytic therapy and primary percutaneous coronary intervention (PCI) are the most effective strategies with which to improve patients' clinical outcomes. Reperfusion arrhythmia (RA) is one of the consequences of myocardial reperfusion injury and is responsible for sudden cardiac death, most likely as a result of ion-channel dysfunction [3, 4]. The electrophysiological characteristics of the ion channels located within the cardiomyocyte membrane are the primary targets of antiarrhythmic pharmacological research [5, 6]. However, the available antiarrhythmic drugs have clinical limitations because of their proarrhythmic effects. These effects are often related to highly selective single channel blocking actions. The inhibition of multiple channels has been suggested as both a safe and effective means of utilizing either amiodarone or potassium blockers, which prolong action potential durations (APDs) and the QT interval [7, 8].

Licorice is derived from the root extract of a perennial herb and has been used to treat a variety of diseases, including heart palpitations, asthma, cough, angina, and stomach pain [9]. The early documented medicinal use of licorice may be traced back to ancient Assyrian, Egyptian, Chinese and Indian cultures [10]. GA, an aglycone saponin extracted from licorice root, is known for its antitumor, antiinflammatory and antihepatitic effects [11-14], and has been used worldwide. GA reportedly exerts protective effects on the cardiovascular system, including gap junction uncoupling [15], vasodilatory effects, negative inotropic effects [16, 17], and limiting infarct sizes during ischemia/reperfusion (I/R) [18]. The available evidence also suggests that GA exhibits antiarrhythmic activity in animal models of arrhythmias induced by chloroform adrenaline and CaCl₂ [19]. Our previous study demonstrated that GA reduced peak I Na and late I Na in Xenopus oocytes [20]. It has also been reported that GA exerts antiarrhythmic effects in the setting of myocardial ischemia via the blockage of Ina and Ica-L [21]. Additionally, GA inhibited potassium currents in both ventricular myocytes and HEK293 cells [22]. Although previous authors have reported that GA may be involved in the uncoupling of gap junctions, either prior to or during ischemia [23], the evidence pertaining to arrhythmias induced by I/R is scarce.

The present study was designed to investigate whether GA exerts antiarrhythmic effects in the setting of I/R in rats, as well as the possible electrophysiological mechanisms underlying these effects. Moreover, the electrophysiological effects of GA on cardiac APDs and membrane currents were recorded in individual myocytes from rat hearts.

**Materials and Methods**

Ethics statement: All experiments were approved by the Institutional Animal Care and Use Committee of Beijing Haidian Hospital (Haidian Section of Peking University Third Hospital).

**Materials**

GA was provided by the Chinese Biological Product Assay Institute (Beijing, China). Taurine, L-glutamic acid, ethylene glycol bis (2-N b ether) tetraacetic acid (EGTA), 4-(2-hydroxyethyl) piperazine-ethanesulfonic
acid (HEPES), NaCl, KCl, CaCl₂, MgCl₂, CsCl, NaH₂PO₄, Na₂ATP, KH₂PO₄, DMSO, CsOH, KOH, KOH, and NaOH were each purchased from Sigma-Aldrich. Collagenase II was purchased from the Worthington Biochemical Corporation. Male adult Sprague-Dawley (SD) rats (220 - 250 g) obtained from Vital River Laboratories were used for each of the experiments.

Sample preparations
A Tyrode solution was prepared by mixing 137 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 0.33 mM NaH₂PO₄, 1.0 mM MgCl₂, 10 mM HEPES and 10 mM glucose. The pH was adjusted to 7.4 with NaOH. The isolated cardiomyocytes were stored in Krebs buffer (KB) solution containing the following (in mM): 40 KCl, 20 KH₂PO₄, 20 taurine, 3.0 MgCl₂, 70 KOH, 50 L-glutamic acid, 0.5 EGTA, 10 HEPES, and 10 glucose; the pH was adjusted to 7.4 with KOH. To record whole-cell I_{ca,L}, a pipette solution containing the following was used (in mM): 120 CsCl, 1 CaCl₂, 5 MgCl₂, 10 HEPES, 11 EGTA, 5 Na₂ATP, and 11 glucose; the pH was adjusted to 7.2 with CsOH. In order to record whole-cell I acquainted, a bath solution containing the following was used (in mM): 137 NaCl, 5.4 KCl, 1.8 CaCl₂, 0.33 NaH₂PO₄, 1.0 MgCl₂, 10 HEPES, and 10 glucose; the pH was adjusted to 7.4 with NaOH. In order to record whole-cell Iₜ, a bath solution containing the following was used (in mM): 140 KCl, 1 MgCl₂, 5 K_2ATP, 5 EGTA, and 10 HEPES; the pH was adjusted to 7.2 with KOH. To isolate Iₜ, a bath solution containing the following was used (in mM): 140 NaCl, 4 KCl, 1.5 CaCl₂, 1 MgCl₂, 0.5 CdCl₂, 5 HEPES, and 10 glucose; the pH was adjusted to 7.4 with NaOH. In order to record whole-cell AP, a pipette solution containing the following was used (in mM): 120 KCl, 1 CaCl₂, 1 MgCl₂, Na₂ATP (3H,O), 11 EGTA, HEPES, and 11 glucose; the pH was adjusted to 7.2 with KOH. In order to record APs, a bath solution containing the following was used (in mM): 137 NaCl, 5.4 KCl, 1.8 CaCl₂, 0.33 NaH₂PO₄, 1.0 MgCl₂, 10 HEPES, and 10 glucose; the pH was adjusted to 7.4 with NaOH. For the whole cell patch clamp experiments, GA powder was dissolved in dimethylsulfoxide (DMSO) as stock. The percentage of DMSO in the final solution was less than 0.1%.

Myocardial Ischemia Reperfusion Model
All surgical procedures were performed as described previously [24]. Briefly, the SD rats were weighed and anesthetized via an intraperitoneal injection of pentobarbital (50 mg/kg). The rats were intubated and ventilated with 100% oxygen (220 µl stroke volume, 60 strokes/min) using a respirator (BL-420E, Taimeng, China). A thoracotomy was performed, and the left anterior descending coronary artery (LAD) was visualized and ligated proximally using a 6-0 silk suture. A piece of PE-10 tubing was placed between the left coronary artery and the 6-0 silk suture to minimize the coronary artery trauma induced via occlusion and facilitate reperfusion. The LAD was completely occluded for 10 min; the reperfusion (30 min) was subsequently initiated via the removal of the 6-0 suture. This study conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S National Institutes of Health.

The evaluation of the arrhythmias
The arrhythmias were defined and analyzed based on the criteria of the Lambeth Conventions [25]. The ECG recordings were analyzed for ventricular arrhythmias during both ischemia and reperfusion. The arrhythmia scores were measured as previously described [26]. The arrhythmias were categorized into 5 groups and assigned the following point values: no arrhythmias, 0 points; premature atrial or ventricular beats, 1 point; supraventricular tachycardia or paired premature ventricular beats, 2 points; bigeminal or trigeminal premature ventricular beats or nonsustained ventricular tachycardia (≥3 consecutive premature ventricular beats), 3 points; and sustained ventricular tachycardia (> 10 consecutive premature ventricular beats) or polymorphic ventricular tachycardia, 4 points.

Measurements of Ca²⁺ content in the myocardial cells
After 30 mins, the rats with myocardial ischemia and reperfusion were killed; their chests were immediately opened, and the hearts were removed; the anterior wall myocardial infarct areas were flushed with iced saline, and excess moisture was absorbed with filter paper; the specimens were refrigerated at -70°C before being weighed and placed in tissue homogenizer, to which ice-saline was added at a 1:9 weight ratio. The homogenizer was placed into an ice water mixture, after which the tissues were ground and centrifuged at 2500 rpm/min. Following 20 min of centrifugation, the supernatant was removed, and an electrolyte analyzer was used to measure the concentration of Ca²⁺.
The isolation of the cardiac ventricular myocytes

The cardiac ventricular myocytes were enzymatically isolated from the SD rats. Briefly, the rats were anesthetized via the administration of chloral hydrate at a dose of 3.5 ml kg\(^{-1}\) and heparinized with 1000 U heparin. Under anesthesia, the hearts were rapidly removed and mounted on a Langendorff apparatus before being retrogradely perfused through the aorta using Ca\(^{2+}\)-free Tyrode solution at a constant rate of 5 ml/min. After allowing the blood to drain from the heart (over approximately 5 min), the perfusate was replaced with a low calcium enzyme solution containing 0.05 mmol/L CaCl\(_2\), 0.4 mg/ml collagenase (Type II; Worthington, USA), 0.04 mg/ml protease (Type XVI) and 0.25 mg/ml bovine serum albumin, pH 7.4. Following 25-30 min of perfusion with an enzyme solution, the ventricles were cut from the heart and minced. The resulting myocyte suspension was subsequently filtered and stored in fresh KB solution at room temperature. The myocytes were used for the experiments within 10 h following the isolation procedure. All solutions were gassed with 100% O\(_2\) at 37°C.

Electrophysiological recording

Individual rod-shaped cells with well visible striations were used for the membrane voltage and current recordings, using the whole-cell patch clamp technique. The patch pipettes were pulled from borosilicate glass capillary tubes using a horizontal micropipette puller (P-97; Sutter Instruments, USA). The resistances of the filled glass electrodes ranged from 1.5 - 2 MΩ. The currents were monitored using a patch clamp amplifier (Axopatch 200B; Molecular Devices, USA) interfaced with a digitizer (Digidata 1440A; Molecular Devices, USA) and a computer. The data acquisition and analyses were conducted using pClamp 10.1 (Molecular Devices), and additional analyses were performed using Origin 7.0 (Origin-lab, Northampton, MA). All experiments were performed at room temperature. The n denotes the number of myocytes from which the current recordings were obtained.

Experimental protocols

The rats were randomly placed in five experimental groups. The control animals were infused with saline through the femoral vein for 3 min before being subjected to a 10 min occlusion period and a 30 min reperfusion period involving the LAD. Glycyrrhetinic acid dissolved in saline to final concentrations of 5, 10, 20 mg/kg was administered to an additional 10 rats under similar conditions.

Statistical analysis

All data were analyzed using SPSS 11.5 software. The group data were expressed as means ± SEs. Paired sample t-tests were used to compare the electrophysiological data among the groups. A two-tailed P < 0.05 was indicative of a statistically significant difference. Compared with the control rats, GA significantly diminished the incidence of both ventricular tachycardia and ventricular fibrillation. The GA group (5, 10 and 20 mg/kg) also exhibited reduced total VT and VF durations following reperfusion. The doses administered to the rats from the GA group, (5, 10 and 20 mg/kg) resulted in significantly lower frequencies of I/R-induced VT, frequencies of 16±6, 13±6, and 10±5, respectively (n = 8, P < 0.05), and VF (5±3, 4±3, 3±1, n = 8, P < 0.05) (Fig. 1D).

Results

The effects of GA on the ventricular arrhythmias induced by I/R in the rats

Figure 1 depicts the protective effects exerted by GA against I/R-induced ventricular tachycardia (VT) and ventricular fibrillation (VF). The rats received intravenous GA (GA group: 5, 10 and 20 mg/kg) and were subjected to 10 min of ischemia via coronary artery ligation, followed by 30 min of reperfusion. The representative ECGs depicting sinus rhythm, VT and VF are included in Figure 1A. The corresponding details are included in Figures 1B, 1C and 1D.

Compared with the control rats, GA significantly diminished the incidence of both ventricular tachycardia and ventricular fibrillation. The concentrations of 5-20 kg/mg significantly reduced the incidences of VT (12.5-35.5%) and VF (12.5-50%) (Fig. 1B).

The GA group (5, 10 and 20 mg/kg) also exhibited reduced total VT and VF durations following reperfusion, as follows: 168±47 to 161±53, 135±46 and 113±48 s (n = 8, p < 0.05),
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and decreased the VT durations from 104±59 to 96±53, 87±46 and 78±39 s (n = 8, p < 0.05), and reduced the VF durations from 75±42 to 68±31, 47±33 and 32±16 s (n = 8, p < 0.05) (Fig. 1C).

During the 30 min reperfusion period, the hearts in the control group developed VT and VF at frequencies of 18±9 and 5±2, respectively. Compared with the control group, GA at the doses of 5, 10 and 20 mg/kg significantly decreased the frequencies of I/R-induced VT to 16±6, 13±6 and 10±5, respectively (n = 8, P < 0.05), as well as the frequencies of VF (5±3, 4±3, 3±2, n = 8, P < 0.05) (Fig. 1D).
We also quantified arrhythmias via modified arrhythmia scores; the GA group exhibited a lower arrhythmia score than the control group (4.25 ± 0.89, 3.88 ± 0.84, 3.38 ± 0.92 vs. 5 ± 0.46, n = 8, P < 0.05) (Fig. 1E).

The effects of GA on the [Ca\(^{2+}\)]\(_i\) levels in the cardiomyocytes

Substantial calcium overload induces reperfusion injury; the inhibition of calcium overload may interrupt the cascade of events leading to reperfusion-induced arrhythmias. We investigated the effects of GA on reperfusion-induced [Ca\(^{2+}\)]\(_i\) overload in rat hearts. Figure 2 depicts the calcium concentrations of the hearts before and after the addition of GA. In

<table>
<thead>
<tr>
<th>Group</th>
<th>RMP (mV)</th>
<th>APA (mV)</th>
<th>APD50 (ms)</th>
<th>APD90 (ms)</th>
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</thead>
<tbody>
<tr>
<td>control</td>
<td>-70.4 ± 2.5</td>
<td>91.7 ± 5.1</td>
<td>45.9 ± 1.6</td>
<td>79.2 ± 3.4</td>
</tr>
<tr>
<td>GA</td>
<td>-68.4 ± 2.6</td>
<td>49.2 ± 6.4*</td>
<td>106.9 ± 2.7*</td>
<td>161.2 ± 3.7*</td>
</tr>
<tr>
<td>washout</td>
<td>-69.7 ± 3.1</td>
<td>80.1 ± 6.3</td>
<td>49.8 ± 1.9</td>
<td>86.7 ± 2.6</td>
</tr>
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Table 1. The effects of GA (10 μM) on APD in rat ventricular myocytes. The data are expressed as means ± S.E.M., *P < 0.05, control vs. GA 10 μM; RMP, resting membrane potential; APA, action potential amplitude; APD50 and APD90, action potential duration at 50% and 90% repolarization, respectively.

Fig. 4. GA inhibited I\(_{\text{ca-L}}\) in the rat ventricular myocytes. I\(_{\text{ca-L}}\) was activated by 400 ms depolarizing steps from -40 to +60 mV, with a holding potential of -40 mV and an interpulse interval of 10 s. (A) Representative raw current tracings under controlled conditions. (B) An example of the inhibitory effects of GA on I\(_{\text{ca-L}}\). (C) I-V relationships under both controlled conditions and in the presence of GA (n = 7). (D) Examples of experimental recordings depicting changes in I\(_{\text{ca-L}}\) at different concentrations of GA as a function of time. I\(_{\text{ca-L}}\) was elicited via a depolarizing pulse at a holding potential of -40 to 0 mV.
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**Fig. 5.** GA inhibited $I_o$ in rat ventricular myocytes. $I_o$ was activated by 400 ms depolarizing steps from -40 to +60 mV, with a holding potential of -40 mV at an interpulse interval of 10 s. (A) Representative raw current tracings under controlled conditions. An example of the inhibitory effect of GA on $I_o$ and I-V relationships under controlled conditions in the presence of GA (n = 7). (B) Examples of experimental recordings depicting changes in $I_o$ at different concentrations of GA as a function of time. $I_o$ was elicited via a depolarizing pulse at a holding potential of -40 to +60 mV.

**Fig. 6.** A schematic model demonstrating the protective effects of GA via the prolongation of the APD as a result of the inhibition of both Ica-L and Ito.

This figure, compared with the sham group, the Ca$^{2+}$ content of I/R group was significantly improved ($P < 0.05$), indicating that the heart muscle exhibits severe injury following I/R. Compared with the control group, the Ca$^{2+}$ content in each of the GA groups was inhibited in a dose-dependent manner ($P < 0.05$), a finding indicative of the protective effects of GA.

The effects of GA on action potential duration in the rat ventricular myocytes

Figure 3 depicts the action potentials recorded in the ventricular myocytes isolated from the rat hearts using the current-clamp technique. A 5 ms depolarizing stimulatory pulse was administered in order to trigger the action potential. Resting potential (RP), 50% ($APD_{50}$) and 90% ($APD_{90}$) repolarization were used to describe the action potential changes. GA (10
µM) was perfused to the cells after the normal action potential was recorded. The results indicated that GA (10 µM) resulted in prolonged APDs in the myocytes.

Table 1 demonstrated the effect of GA (10 µM) on APD in the rat ventricular myocytes. In this Table, the APD_{50} was prolonged from 45.9 ± 1.6 to 106.9 ± 2.7 ms (n = 7, p < 0.05), and the APD_{90} was also prolonged from 79.2 ± 3.4 to 161.2 ± 3.7 ms.

The effects of GA on Ica-L in the rat ventricular myocytes

The cardiomyocytes were placed in an experimental chamber and continuously perfused using extracellular solution. I_{Na} was inactivated at a holding potential of -50 mv. Figure 2A depicts the superimposed Ica-L tracings recorded by applying 400 ms pulses from -50 to + 60 mv before the addition of GA and following the administration of GA. GA reduced the Ica-L at every potential, as illustrated by the current-voltage (I-V) curves included in Figure 4A. The peak was slightly shifted to a more positive value.

The effects of GA on Ito in the rat ventricular myocytes

The I_{to} currents were elicited via square test pulses of 300 ms ranging from -40 to +50 mv, from a holding potential of -80 mv. The outward peak amplitude was measured as I_{to}. I_{to} was the predominant repolarizing potassium current in the rat ventricular myocytes. At a testing potential of +50 mv, 10 µM GA reduced I_{to} from 4.1 ± 0.9 nA to 2.3 ± 0.6 nA (n = 8, P < 0.05). Figure 3 depicts the effects of GA on I_{to}.

Discussion

Acute myocardial infarction is caused by the death of myocardial cells as a result of the thrombotic occlusion of coronary arteries. Acute myocardial infarction is the leading cause of death in the setting of human cardiovascular disease [27, 28]. Swift restoration of the normal blood supply is the only effective means of minimizing cardiac injury. However, reperfusion itself may also cause myocardial injury and cardiac dysfunction, a phenomenon known as "reperfusion injury." Therefore, mitigating myocardial ischemia–reperfusion (I/R) injury is an important means of treating ischemic heart disease [29-31].

Although reperfusion is essential for preventing irreversible cellular injury and preserving ventricular function, reperfusion and recovery from ischemia-induced metabolic, ionic, electrical, and signaling transduction changes cause ventricular arrhythmia, cellular injury and sudden death [33-37].

The mechanisms underlying ventricular arrhythmia in acute myocardial ischemia and reperfusion have been studied primarily in animal models. Consistent with the findings of previous studies, the present study determined that left main coronary artery occlusion and release resulted in ventricular arrhythmias, including premature ventricular beats, ventricular tachycardia and ventricular fibrillation in rats. The effects of GA on the ischemia-reperfusion arrhythmias were studied in a rat model of myocardial ischemia-reperfusion injury. The administration of GA prior to the onset of ischemia resulted in a dose-dependent reduction in the incidence of ischemia-reperfusion induced arrhythmia, including both ventricular tachycardia and ventricular fibrillation.

The reperfusion of the ischemic myocardium is associated with several pathologic derangements, including reperfusion arrhythmias [35]. Malignant ventricular arrhythmias resulting in cardiovascular collapse are believed to the primary mechanism underlying sudden death. The mechanism of arrhythmogenesis caused by I/R injury has not yet been elucidated. Ischemia/reperfusion (IR) injury refers to the tissue damage caused when blood returns to the affected tissue following a period of ischemia. The absence of both oxygen and nutrients from the blood during ischemia creates a condition in which the restoration of circulation results in both inflammation and oxidative damage via the induction of oxidative stress as opposed to the restoration of normal function [38, 39], which means that the progressive and irreversible damage incurred during ischemia may only be
stopped via immediate reperfusion. Otherwise, severe and irreversible myocardial damage may result from reperfusion itself, a phenomenon referred to as reperfusion injury. An important consequence of myocardial I/R is the disturbance of cardiac rhythm, including the development of VF, a potentially lethal arrhythmia. [Ca$^{2+}$], homeostasis plays a central role in the cardiovascular system, particularly where arrhythmias are concerned[40]. The pathophysiological mechanisms underlying the development of VT and VF include the overproduction of oxygen-derived free radicals and calcium overload during the initial stages of reperfusion.

During myocardial reperfusion, there is an abrupt increase in intracellular Ca$^{2+}$, which overwhelms the normal mechanisms of Ca$^{2+}$ regulation in cardiomyocytes and results in both intracellular and mitochondrial Ca$^{2+}$ overload, which causes cardiomyocyte death via the hypercontracture of the affected cardiac cells. Attenuating intracellular Ca$^{2+}$ overload with pharmacologic antagonists of sarcolemmal Ca$^{2+}$ ion channels decreases myocardial infarct sizes in experimental studies. During ischemia/reperfusion, the cardiac myocytes encounter adenosine triphosphatase (ATP) depletion; therefore, the Na$^+$-K$^+$-ATPase is inhibited, which results in increased intracellular Na$^+$ concentrations and the activation of the Na$^+$-Ca$^{2+}$ exchanger, which promotes Ca$^{2+}$ entry and the subsequent development of intracellular Ca$^{2+}$ overload [41-43]. Additionally, reperfusion injury may cause the release of metabolites, which may affect resting membrane potential, intracellular Ca$^{2+}$ concentrations and ion channel function [44, 45]. In the present study, we observed that GA decreases the incidence of I/R injury-induced arrhythmia.

In cardiac myocytes, there exist various ion channels, including sodium channels, calcium channels, and potassium channels. Cardiac electrical activity is well organized as a result of the functional and structural equilibrium of these various channels[46]. Any abnormality in ion channel function may result in changes in the action potential durations of the ventricular myocytes, which may cause arrhythmia.

Previous studies have demonstrated that electrophysiological changes in AP profiles, changes such as APD prolongation and modifications in both potassium and calcium current, are accompanied by the prolongation of either the QT interval or the APD by medicinal compounds, which has become a major concern among medical professionals and within the pharmaceutical industry, as the medications in question may exert proarrhythmic effects. The use of specific Ikr blockers such as E-4031 in the treatment of various ventricular arrhythmias is accompanied by an increased likelihood of proarrhythmic episodes, particularly TdP. Combination therapy with quinidine (Ina-f, Ikr and Ina-l blocker) and mexiletine (Ina blocker) is more effective at preventing ventricular tachycardia and ventricular fibrillation in both animal models and humans compared with mono-drug therapy. Therefore, the inhibition of multiple channels has been suggested as a means of improving both the safety and the efficacy of potassium blockers, which prolong APDs and the QT interval, without inducing EADs or triggering TdP. The efficacy of amiodarone and its low incidence of proarrhythmic effects may be attributed to this complex multi-channel inhibition. Our data suggest that GA fits this electrophysiological profile and induces the progressive prolongation of APDs in isolated ventricular myocytes in a concentration-dependent manner. The observed alterations were accompanied by specific changes in membrane current as determined via voltage-clamp measurements. Perfusion with GA resulted in decreased I$_n$ and I$_{Ca^{2+}}$. Taken together, these results demonstrate that GA exerts antiarrhythmic effects in the setting of rat I/R and induces electrophysiological alterations in ventricular myocytes.

GA may exert antiarrhythmic effects via the prolongation of APDs and effective refractory periods (ERPs) by blocking potassium channels. The prolongation of APDs via GA administration results primarily from the blockade of I$_{Ca^{2+}}$. The blockade of I$_{Ca^{2+}}$Land I$_{Ca-L}$ by GA attenuated APD prolongation. The inhibition of Ito was greater than the inhibition of both Ica-L and Ina. As a result, the net outward current decreased, prolonging the APD. GA is similar to ranolazine and amiodarone in terms of the inhibitory effects it exerts on multiple cardiac ion channels, including Ina-L, Ito and Ica-L. Our previous study suggested...
that glycyrrhetinic acid blocks cardiac sodium currents, particularly late $I_{Na-L}$. Penkoske’s results suggested that the cardiac electrophysiology underlying reperfusion arrhythmias was characterized by refractory period shortening. A pure $I_{ca-L}$ blocker may aggravate this tendency; however, it may also counter-balance the contradiction via the simultaneous inhibition of $I_{to}$. Under these conditions, the blockade of multi-channels may be more useful than the inhibition of a single type of ion channel.

Our results indicate that GA acts on both calcium and potassium channels and exerts effects similar to those of class III drugs such as amiodarone. These effects give rise to voltage-dependent effects. Intravenous amiodarone is the drug of choice for the treatment of ventricular tachycardia/fibrillation (VT/VF) in emergency medicine. However, the onset of amiodarone’s effects occurs at 6-8 h following its administration. Furthermore, its intravenous use is occasionally accompanied by hypotension and bradycardia, effects that make its use as a rescue medication in life-threatening situations difficult.

Recently, researchers have focused on newer class III antiarrhythmic agents such as nifekalant hydrochloride, which acts by increasing the time course of myocardial repolarization. Nifekalant is available only as an intravenous agent and is used in the treatment of ventricular tachycardia/fibrillation (VT/VF) in emergency medicine. Life-threatening ventricular tachyarrhythmias such as VT or VF are likely to develop during the acute phase of ACS (acute coronary syndrome); these arrhythmias exert important effects on the prognoses of affected patients. Recent studies have demonstrated that agents with multiple channel (including $I_{Na-L}$) blocking actions may be a viable approach to treating various arrhythmias induced via I/R and may be less proarrhythmic than selective ion channel inhibitors [47]. Therefore, GA may represent a novel therapeutic intervention in the treatment of I/R injury-induced arrhythmia.

In conclusion, the results of this study suggest that treatment with GA, particularly at a dosage of 20 mg/kg, attenuates both the susceptibility to and the incidence of fatal ventricular arrhythmia during reperfusion in rats. This protective effect is apparently mediated via the prolongation of APDs as a result of the inhibition of Ica-L and Ito. Therefore, GA may be a promising antiarrhythmic agent in the setting of ischemia/reperfusion.

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Disclosure Statement

No conflicts of interest, financial or otherwise, are declared by the authors.

References


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