Research Article

Antiarrhythmic effects of ginsenoside Rg2 on calcium chloride–induced arrhythmias without oral toxicity

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1. Introduction

Ginsenosides, the primary active ingredients in ginseng, exhibit various pharmacological and biological effects on cardiovascular diseases (CVDs) [1], central nerve system (CNS) disorders [2], immune function [3], and so on. To date, more than 180 ginsenosides have been identified, and almost 50 of them have been isolated [4]. Ginsenoside Rg2 is one of the protopanaxatriol-type (PPT) ginsenosides with a glucose (2-1) rhamnose moiety attached to α-OH at C-6 position in the aglycon PPT (Fig. 1A). Several previous studies have reported that Rg2 possesses various pharmaceutical activities. For instance, Rg2 has neuroprotective effects due to its anti-apoptosis property that can attenuate the impairment of learning and memory disorders in rat dementia models [5]. Furthermore, our research group found that Rg2 could improve cognitive behavior in a mouse model of Alzheimer disease [6] and prevented high-fat diet–induced insulin resistance via activation of autophagy [7]. In addition, Rg2 could attenuate myocardial ischemia/reperfusion injury by reducing oxidative stress and inflammatory response via SIRT1 signaling [8]. We also found that Rg2 could inhibit H2O2-induced injury and apoptosis in H9c2 cells via its antioxidative and antiapoptotic functions [9], suggesting its potential cardioprotective effects.

CVD affects populations throughout the world. It is now the leading cause of mortality in the world [10]. Arrhythmia is a common manifestation of CVD. Malignant arrhythmia, such as...
ventricular tachycardia (VT) and ventricular fibrillation (VF) may even cause sudden death. Clinical antiarrhythmic drugs are divided into four categories according to different phases of their action potential, including Na$^+$ channel blockers (Class I), β-receptor blockers (Class II), K$^+$ channel blockers (Class III), and Ca$^{2+}$ channel blockers (Class IV). Most of them have severe side effects (e.g. proarrhythmia) and narrow window between their therapeutic and toxic effects [11]. As natural products, ginsenosides are regarded as potential candidates for arrhythmia treatment, because of their cardioprotective effects and low toxicity [12]. So far, there are no reports about the toxicity of Rg2 and its impact on arrhythmias. In the present study, we investigated single-dose acute oral toxicity in mice and 28-day repeated dose oral toxicity of Rg2 in rats. We also assessed the antiarrhythmic effects of Rg2 and studied the possible mechanism in calcium chloride (CaCl$_2$)-induced arrhythmic rats and H9c2 cells.

2. Materials and methods

2.1. Reagents and materials

Ginsenoside Rg2 was prepared in our laboratory by a modified biotransformation method as previously reported [13]. The purity of Rg2 was 98% determined by an external standard method using reverse-phase high performance liquid chromatography (HPLC) (Fig. 1B) [14]. Standard Rg2 was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Pentobarbital sodium was purchased from Merck & Co., Inc. (Kenilworth, NJ, USA). Verapamil hydrochloride was obtained from Sanofi Pharmaceutical co., LTD (Hangzhou, China). Sodium carboxymethylcellulose (CMC-Na) was purchased from Aladdin biochemical technology co. LTD (Shanghai, China). Fluo-4 AM and Bay-K8644 were purchased from Dalian Meilun biotechnology co. LTD (Dalian, China). Anti-GAPDH was purchased from Cell Signaling Technology (Danvers, MA, England); anti-phospho-CaMKII and anti-CaMKII were obtained from Affinity Biosciences (Changzhou, China). All other reagents were purchased from Sigma Aldrich (St. Louis, MO, USA) and used as received.

2.2. Animals and management

Animal experiments were carried out in compliance with the Animal Management Rules of the Ministry of Health of the People's Republic of China and approved by the Animal Care and Use Committee of Northeast Normal University. Kunming mice and Sprague Dawley (SD) rats were purchased from Liaoning Changsheng Biological Technology co., LTD. All animals were housed in a controlled environment (temperature 20 ± 2 °C, humidity 50% ± 20%, 12/12 h light-dark cycle) with standard pelleted diet and tap water.

2.3. Single-dose acute oral toxicity study

This study was carried out in compliance with the Testing Guidelines for Safety Evaluation of Drugs (notification [H] GPT2-1 issued by China Food and Drug Administration on March 2005). Twenty Kunming mice (~ 20 g, 4 weeks; half male and half female) were randomly divided into two groups: Rg2-treated group and control group. Rg2 was suspended in 0.5% (w/v) CMC-Na aqueous solution at concentration of 0.25 g/mL. Mice in the Rg2-treated group were given a single dose of Rg2 at the maximal feasible dose of 10 g/kg by gavage, whereas the control group was treated with equal volume of 0.5% CMC-Na solution. Clinical observations were performed, and body weight, food consumption, and mortality were recorded daily for 14 days.

2.4. Repeated dose 28-day oral toxicity study

Repeated dose 28-day oral toxicity test was conducted according to the Organization for Economic Cooperation and Development Guideline 407 [15]. Forty SD rats (~ 200 g, 8 weeks) were randomly placed into four groups: three Rg2-treated groups of different doses and a control group (half male and half female). Rats in the Rg2-treated groups were orally administrated with Rg2 suspension in 0.5% CMC-Na aqueous solution at concentration of 0.25 g/mL. Mice in the Rg2-treated group were given a single dose of Rg2 at the maximal feasible dose of 10 g/kg by gavage, whereas the control group was treated with equal volume of 0.5% CMC-Na solution. Clinical observations were performed, and body weight, food consumption, and mortality were recorded daily for 14 days.

Fig. 1. Structure and purity analysis of ginsenoside Rg2. (A) Chemical structure and (B) HPLC analysis of ginsenoside Rg2 prepared by a biotransformation method in comparison with reference standard Rg2.
2.5. Antiarrhythmic effect of ginsenoside Rg2

Forty SD rats were randomly divided into five groups (half male and half female): (1) the control group treated with 0.5% CMC-Na solution, (2) the positive control group treat with verapamil (30 mg/kg/d), (3) the low-dose Rg2-treated group (8 mg/kg/d), (4) the mid-dose Rg2-treated group (40 mg/kg/d), and (5) the high-dose Rg2-treated group (80 mg/kg/d). Each group was orally administrated with drugs daily for 14 days. The selection of Rg2 doses was based on its cardiac protective function [8,16]. One hour after the final administration on day 14, the rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Lead II electrocardiogram (ECG) of each animal was monitored by PowerLab system (AD-instrument, Australia). To induce arrhythmia, 10% CaCl2 aqueous solution (100 mg/kg) was injected into sublingual vein of rat within 10 s. The occurrence time and duration of arrhythmia, the mortality and incidence of ventricular premature (VP), VT, VF, and atrioventricular block (AVB) of each group within 20 minutes were used to evaluate antiarrhythmic effects. Heart tissues of each group were isolated immediately after death or 20-minutes' recording for hematoxylin and eosin staining assay.

2.6. Cell culture and measurement of intracellular calcium

Rat embryonic cardiomyoblast-derived H9c2 cell line was obtained from Shanghai Institute of Cell Biology, Chinese Academy of Science (Shanghai, China). Cells were cultured in Dulbecco’s modified eagle media (GIBCO, Grand Island, NY, USA) with 10% fetal bovine serum (GIBCO, Grand Island, NY, USA) under standard cultured conditions (37 °C, 95% humidified air, and 5% CO2).

H9c2 cells were pretreated with different concentrations of Rg2 (10, 20, 40 and 80 μM) and verapamil (10 μM) for 24 h, respectively. Thereafter, cells were incubated with Ca2+ indicator Fluor-4AM (2 μM) in Krebs-Ringer (KR) solution (118 mM NaCl, 4.75 mM KCl, 1.18 mM KH2PO4, 1.18 mM MgSO4, 10 mM CaCl2, 25 mM NaHCO3, and 11 mM glucose) for 60 min at 37 °C. After washing for three times, H9c2 cells were incubated with Bay-K8644 (10 μM) in KR solution for 5 min to evoke Ca2+ influx. Fluorescent intensity was measured using an automatic microplate reader (Tecan, Männedorf, Switzerland) at 486-nm excitation and 515-nm emission. The changes in intracellular Ca2+ concentration was calculated by relative fluorescence intensity (F - F0)/F0 (F and F0 represent the fluorescence intensity of drug-treated cells and control cells, respectively). Fluorescent imaging was performed by fluorescent microscope (EVOS FL Auto, Life Technologies, Carlsbad, CA) immediately after incubation of cells with Bay-K8644.

2.7. Determination of CaMKII-δ phosphorylation

The level of phosphorylation of CaMKII-δ was determined in heart tissues and in H9c2 cells. Rats were pretreated with Rg2 (80 mg/kg) or verapamil (30 mg/kg) by gavage as mentioned previously. One hour after the final administration, CaCl2 (100 mg/kg) or a mixture of Bay-K8644 (3.6 mg/kg) and CaCl2 (50 mg/kg) were injected into the sublingual vein of anesthetized rats. Hearts of rats in each group (n = 3) were isolated immediately after death or 20-minute recording. After washing three times in ice-cold phosphate buffered saline (PBS), ventricular myocardium was homogenized to extract total protein. In addition, H9c2 cells were pretreated with Rg2 (20 μM) and verapamil (10 μM) for 24 h. Then, the cells were incubated with Bay-K8644 in KR solution for 5 minutes and lysed in lysis buffer (50 mM Tris/acetate, pH 7.4, 1 mM ethylene diamine tetraacetic Acid (EDTA), 0.5% Triton X-100, 150 mM sodium chloride, 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and protease inhibitor cocktail) to extract total protein. The protein concentration in tissue homogenates and cell lysates was measured by the Bradford method. Proteins (50 μg per sample) were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then transferred to a polyvinylidene fluoride (PVDF) membrane and blotted with anti-phospho-CaMKII-δ, anti-CaMKII, and anti-GAPDH. The immunoreactive bands were visualized using enhanced chemiluminescence reagent.

2.8. Statistical analysis

Results are expressed as the mean ± standard deviation. Statistical significance was assessed by the Student’s t-test between two groups in SPSS 23.0 (IBM, Armonk, USA).

3. Results

3.1. Acute oral toxicity of Rg2

Acute oral toxicity was assessed by using the Limit Test method in which mice were orally administered Rg2 at the maximal feasible dose of 10 g/kg. Throughout the study, there were no signs of toxicity, and all mice survived. As shown in Fig. S1, body weight and food consumption in all groups was essentially the same, with no significant difference among groups over a 6-week period.

Most hematological and biochemical parameters of the three Rg2-treated groups showed no statistical differences compared with the control group (Table 1 and Fig. 2). These results demonstrated that 28-day repeated dose oral administration of Rg2 (~5 g/kg/d) had no obvious toxicity effects on hematology, kidney, or liver function, as well as the electrolyte balance in rats. While some abnormal findings were noted in the high-dose group, the lymphocyte count of high-dose group was significantly reduced (2.48 ± 1.12 versus 4.65 ± 0.64, p < 0.05), but this change was considered not to be of toxicological significance. The prothrombin time was significantly increased (16.9 ± 1.4 versus 13.5 ± 1.9, p < 0.05), and the total cholesterol (TC) level was dramatically decreased (0.34 ± 0.16 versus 0.89 ± 0.39 p < 0.05, Fig. 2A) compared with the control group, implying that high-dose of Rg2 might impact on lipid metabolism and blood coagulation function.

As shown in Fig. 3, no lesions in any of the main organs were observed, even in the Rg2 high-dose cohort. Relative organ weights (organ weights/body weights) showed no statistical difference between groups (Table S2). Fig. 4 showed HE-stained tissue sections from the lung, liver, spleen, kidney, and heart. No inflammatory or necrotic changes in these organs were observed either in the control group or in the high-dose Rg2 treatment group. Our results indicated that Rg2 had no apparent oral toxicity in rats treated at doses < 5 g/kg/d for 28 days.

3.2. Subchronic oral toxicity from Rg2

During the experimental period, all rats survived in four groups. No signs of toxicity or abnormal behaviors were observed in any of the groups. As shown in Table S1, body weights of both male and female rats gradually increased over time, and food consumption in all groups was essentially the same, with no significant difference among groups over a 6-week period.

Most hematological and biochemical parameters of the three Rg2-treated groups showed no statistical differences compared with the control group (Table 1 and Fig. 2). These results demonstrated that 28-day repeated dose oral administration of Rg2 (~5 g/kg/d) had no obvious toxicity effects on hematology, kidney, or liver function, as well as the electrolyte balance in rats. While some abnormal findings were noted in the high-dose group, the lymphocyte count of high-dose group was significantly reduced (2.48 ± 1.12 versus 4.65 ± 0.64, p < 0.05), but this change was considered not to be of toxicological significance. The prothrombin time was significantly increased (16.9 ± 1.4 versus 13.5 ± 1.9, p < 0.05), and the total cholesterol (TC) level was dramatically decreased (0.34 ± 0.16 versus 0.89 ± 0.39 p < 0.05, Fig. 2A) compared with the control group, implying that high-dose of Rg2 might impact on lipid metabolism and blood coagulation function.

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3.3. Antiarrhythmic activity of Rg2 on CaCl2-induced arrhythmia in rats

Intravenous injection of high dose of CaCl2 leads to hypercalcemia and increases Ca2+ influx, which induces arrhythmia by
excitation of myocardial tissue and also by an indirect action mediated through the automatic nervous system [17]. As shown in Table 2 and Fig. 3, in the control group, arrhythmias occurred approximately 8 s after CaCl₂ injection and lasted up to more than 1000 s. This resulted in high mortality (∼50%) and incidence of malignant arrhythmias. PR interval represents atrioventricular conduction time that associates with extracellular Ca²⁺ influx in pacemaker cells [18]. CaCl₂ dramatically shortened the PR interval of the control rats (from 46.3 ± 6.4 ms to 41.0 ± 3.5 ms) by increasing Ca²⁺ influx within 1 min after injection (Fig. 5A). Positive-control drug verapamil exhibited remarkable antiarrhythmic efficacy by blocking Ca²⁺ channels. It could prolong the PR interval and decrease the duration time (120 s, p < 0.01), mortality (16.7%), and incidence of malignant arrhythmias (Table 2). However, pretreatment with verapamil resulted in the PR interval prolongation and heart rate reduction of normal rats (Fig. 5A and D).

In the three Rg2 pretreated groups, the duration of arrhythmia in low- and mid-dose groups showed no significant difference compared with the control group, although the incidence of VFs and VPs was reduced. When Rg2 was administered at 80 mg/kg/d (high-dose group), the duration of arrhythmia decreased to 620 s, which had significant difference (p < 0.05) compared with the control group. The mortality and incidence of VPs, VFs, and AVBs in the high-dose group were dramatically reduced, similar to that in the verapamil-treated group. Rg2 could prolong the PR interval and reduce heart rate of CaCl₂-induced arrhythmic rats within 1 min after injection (Fig. 5A and D), probably by inhibiting Ca²⁺ influx. However, Rg2 had little influence on the electrophysiological parameters of normal rats (Fig. 5), implying its low cardiotoxicity. Moreover, histopathologic examination further confirmed that there was no apparent lesion in the heart, liver, spleen, lung, and kidney of arrhythmic rats pretreated with high-dose Rg2 (Fig. S2). Overall, our results demonstrate that pretreatment with Rg2 at 80 mg/kg/d could shorten the duration of CaCl₂-induced arrhythmia and remarkably reduce mortality and the incidence of malignant arrhythmias in rats.

Table 1
The changes of hematological parameters of rats orally administrated with 0.5% CMC-Na (control) and Low-, mid-, and high-dose ginsenoside Rg2 for 28 days

| Hematological parameter | Control group (0.5% CMC-Na) | Low-dose group (1.75 g/kg/d) | Mid-dose group (3.5 g/kg/d) | High-dose group (5 g/kg/d) |
|-------------------------|-----------------------------|-----------------------------|-----------------------------|---------------------------|
| RBC (×10¹²/L) | 7.13 ± 0.02 | 7.24 ± 0.34 | 7.34 ± 0.30 | 7.30 ± 0.21 |
| PLT (×10¹⁰/L) | 8.25 ± 0.30 | 8.35 ± 0.94 | 7.32 ± 0.95 | 7.96 ± 0.64 |
| WBC (×10⁹/L) | 5.65 ± 0.35 | 5.85 ± 1.09 | 5.50 ± 0.16 | 4.85 ± 0.16 |
| LYM (×10⁹/L) | 4.65 ± 0.64 | 3.78 ± 2.26 | 4.87 ± 0.21 | 2.48 ± 1.12* |
| PMN (×10⁹/L) | 0.50 ± 0.14 | 0.20 ± 0.08* | 0.47 ± 0.05 | 0.40 ± 0.03 |
| HCT (%) | 40.45 ± 0.92 | 39.80 ± 2.81 | 39.57 ± 2.10 | 40.68 ± 1.18 |
| MCV (%) | 57.00 ± 1.41 | 55.00 ± 1.63 | 52.40 ± 2.11 | 55.75 ± 1.30 |
| HGB (g/L) | 141.00 ± 14.1 | 137.67 ± 8.26 | 138.33 ± 9.10 | 137.50 ± 4.39 |
| MCHC (g/L) | 348.51 ± 12.00 | 345.67 ± 4.99 | 351.33 ± 13.52 | 338.50 ± 3.20 |
| AST (U/L) | 13.5 ± 1.9 | 14.8 ± 1.1 | 14.4 ± 0.5 | 16.9 ± 1.4* |
| ALT (U/L) | 7.13 ± 0.02 | 7.24 ± 0.34 | 7.34 ± 0.30 | 7.30 ± 0.21 |
| ALP (U/L) | 14.8 ± 1.1 | 14.4 ± 0.5 | 16.9 ± 1.4* | 19.8 ± 2.1 |

RBC, red blood cell count; PLT, platelet count; WBC, white blood cell count; LYM, lymphocyte count; PMN, polymorphonuclear neutrophils; HCT, hematocrit; MCV, mean corpuscular volume; HGB, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PT, prothrombin time.

Data are expressed as the mean ± standard deviation.

* Represents significant difference to the control group (p < 0.05).

Fig. 2. The changes of serum biochemistry of rats orally administrated with 0.5% CMC-Na (control) and low-, mid-, and high-dose ginsenoside Rg2 (1.75, 3.5, and 5 g/kg/d, respectively) for 28 days. (A) triglyceride (TG) and total cholesterol (TC); (B) total protein (TP) and albumin (ALB); (C) alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP); (D) blood urea nitrogen (BUN) and creatinine (CREA); (E) potassium (K⁺), sodium (Na⁺), and chloride (Cl⁻). * represents significant difference to the control group (p < 0.05).
3.4. Effects of Rg2 on Ca^{2+} influx via L-type Ca^{2+} channels

The induction of arrhythmias by high-dose CaCl_{2} might be associated with abnormal calcium influx [19,20]. Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) plays an important role for regulation of Ca^{2+} current through interaction with L-type Ca^{2+} channels [21]. Therefore, we examined the phosphorylation level of CaMKII-δ in heart tissues. As shown in Fig. 6A, compared with normal rats, the phosphorylation of CaMKII-δ (p-CaMKII-δ) in hearts was significantly enhanced in CaCl_{2} (100 mg/kg)-induced arrhythmic rats (p < 0.01). By contrast, the upregulation of p-CaMKII-δ induced by CaCl_{2} was dramatically suppressed in verapamil (30 mg/kg) and Rg2 (80 mg/kg) pretreatment groups (p < 0.01). This was confirmed in H9c2 cells triggered by an L-type Ca^{2+} channel agonist Bay-K8644, which can enhance Ca^{2+} influx and transiently increase intracellular Ca^{2+} concentration. As displayed in Fig. 6B, Bay-K8644 (10 μM) induced a significant increase of p-CaMKII-δ in cells at high extracellular Ca^{2+} concentration (10 mM), whereas Rg2 (20 μM) and verapamil (10 μM) could remarkably attenuate the upregulation of p-CaMKII-δ triggered by Bay-K8644 (p < 0.01 compared with the model group). Similar results were obtained in Bay-K8644 (3.6 mg/kg) plus CaCl_{2} (50 mg/kg)-induced arrhythmic rats (Fig. S3), which indicated that Bay-K8644 could enhance Ca^{2+} influx by activating L-type Ca^{2+} channels at high blood Ca^{2+} level and thereby induced arrhythmias.

To further confirm that Rg2 could suppress p-CaMKII-δ to inhibit Ca^{2+} influx, we investigated the effects of Rg2 on intracellular Ca^{2+} concentration in H9c2 cells (Fig. 6C and D). Green fluorescence from Ca^{2+} indicator Fluo-4AM was significantly enhanced in Bay-K8644 (10 μM) triggered cells, while it did not obviously change in verapamil or Rg2 pretreated cells (Fig. 6C). Quantitative analysis results (Fig. 6D) showed that the relative fluorescence intensity was increased two-fold by Bay-K8644. As expected, pretreatment with verapamil (10 μM) could completely inhibit Bay-K8644–triggered fluorscin.
Ca\(^{2+}\) influx by blocking L-type Ca\(^{2+}\) channels. However, pretreatment with Rg2 showed inhibitory effects on Bay-K8644–triggered Ca\(^{2+}\) influx in a dose-dependent manner. These results suggest that Rg2 could regulate L-type Ca\(^{2+}\) channels activity by suppressing the phosphorylation of CaMKII-\(\delta\) in CaCl\(_2\)-induced arrhythmic rats and H9c2 cells.

4. Discussion

Previous studies have evaluated the oral toxicity of ginsenosides in vivo. For instance, Lu et al.\(^{[22]}\) found that rats could tolerate ginsenoside Re up to an oral dose of 0.375 g/kg/d for 26-weeks. Jeong et al.\(^{[23]}\) reported that rats could tolerate a dose as high as 2 g/kg/d of ginsenoside Rh2 for 90 days, and its lethal dose was 4 g/kg/d based on acute oral toxicity. These results indicate the low oral toxicity of ginsenosides in animals. Our results further demonstrated minimal toxicity from ginsenoside Rg2 in mice and rats. While some abnormal findings were noted, TC levels in the high-dose group (5 g/kg/d) were significantly decreased compared with the control. In general, high levels of TC increase the risk of CVDs, especially atherosclerosis and stroke.\(^{[24,25]}\) 3-Hydroxy-3-methyl glutaryl coenzyme A reductase (HMG CoA reductase) is a rate-limiting enzyme in endogenous cholesterol synthesis, which plays an important role in maintaining the homeostasis of blood cholesterol. A previous report showed that Korean Red Ginseng water extract, primarily consisting of ginsenoside Rg2, Rg3, Rh1, and Rh2, could reduce intracellular triglyceride and cholesterol levels in HepG2 cells by inhibition of fatty acid synthase and HMG CoA reductase expression.\(^{[26]}\) Based on these previous studies and our results, we speculated that Rg2 might have a potential to regulate lipid metabolism in vivo. In addition, prothrombin time in the high-dose group (5 g/kg/d) was significantly increased. Li et al.\(^{[27]}\) found that Rg2 had strong anticoagulation activity in vitro. Our results further demonstrated that Rg2 might have an anticoagulant effect in vivo. Owing to the low oral toxicity and the potential antihyperlipidemic and anticoagulant effects, Rg2 is a promising drug candidate for cardiovascular protection.

Almost all types of clinical antiarrhythmic drugs have significant side effects, especially if taken for long periods of time.\(^{[28]}\) Several studies have shown that ginseng stem leaf saponins and ginsenoside Re could treat arrhythmias after a single intravenous injection.\(^{[29]}\) Re could suppress L-type Ca\(^{2+}\) current through cyclic

### Table 2

The antiarrhythmic effects of ginsenoside Rg2 on CaCl\(_2\)-induced arrhythmia in rats (n = 6)

| Group            | Occurrent time (s) | Duration (s) | Mortality (%) | VP (%) | VT (%) | VF (%) | AVB (%) |
|------------------|--------------------|--------------|---------------|--------|--------|--------|---------|
| Control (CMC-Na) | 7.8 ± 3.3          | 1044 ± 242.2 | 50.0          | 50.0   | 66.7   | 83.3   | 83.3    |
| Verapamil (30 mg/kg/d) | 10.1 ± 4.6     | 119 ± 129.6** | 16.7          | 16.7   | 33.3   | 16.7   | 50.0    |
| Low-dose Rg2 (8 mg/kg/d) | 12.9 ± 2.9    | 781 ± 325.9  | 50.0          | 16.7   | 33.3   | 33.3   | 50.0    |
| Mid-dose Rg2 (40 mg/kg/d) | 11.6 ± 4.8    | 920 ± 368.5  | 50.0          | 33.3   | 66.7   | 50.0   | 83.3    |
| High-dose Rg2 (80 mg/kg/d) | 11.4 ± 4.4   | 620 ± 390.2** | 16.7          | 0      | 66.7   | 16.7   | 50.0    |

Values are expressed as the mean ± standard deviation. Drug effects are expressed as occurrent time, duration of arrhythmia, mortality, and group incidence (n = 6) of one or more episodes of ventricular premature (VP), ventricular tachycardia (VT), ventricular fibrillation (VF), and atrioventricular block (AVB).

* represents p < 0.05 for significant difference to the control group.

** represents p < 0.01 for significant difference to the control group.
guanosine monophosphate (cGMP) pathway in ventricular myocytes [30]. To our knowledge, this is the first study demonstrating that oral administrated Rg2 exhibited antagonistic effects on arrhythmic rats. Our results indicate that pretreatment with Rg2 could reduce the duration time, mortality, and incidence of malignant arrhythmias induced by CaCl2. Although the effective dose of Rg2 was higher than verapamil, pretreatment with Rg2 had no influence on the electrophysiological parameters of normal animals and major organs of both normal and arrhythmic rats, implying its low toxicity.

Electrocardiogram interval analysis showed that Rg2 could prolong the PR interval of arrhythmic rats after CaCl2 injection, which indicates that Rg2 might help to regulate L-type Ca2+ channels activity. Accumulating evidence reveals that CaMKII can regulate CaV1.2 L-type Ca2+ channel activity through interaction with the alpha1C C terminus of CaV1.2. Autophosphorylation of CaMKII is required for initiating the interaction with the CaV1.2 and CaMKII [31]. Among various CaMKII isofoms, CaMKII-δ is abundant in the myocardium. Western blot results showed that CaMKII-δ phosphorylation in hearts was dramatically enhanced in arrhythmic rats induced either by CaCl2 or Bay-K8644 plus CaCl2, resulting in the increased Ca2+ influx through L-type Ca2+ channels. Rg2 or verapamil pretreatment could significantly attenuate the upregulation of p-CaMKII-δ. This was confirmed in vitro that Rg2 could significantly inhibit CaMKII-δ phosphorylation and Ca2+ influx in H9c2 cells evoked by L-type Ca2+ channel agonist Bay-K8644. Our results demonstrate that the antiarrhythmic mechanism of Rg2 might be the inhibition of Ca2+ influx through L-type Ca2+ channels by suppressing CaMKII-δ phosphorylation. On the basis of these results and previous research, ginsenoside Rg2 has superiority over conventional antiarrhythmic drugs for the prevention and treatment of arrhythmia, owing to its multifaceted protective effects on cardiovascular system [1–8].

5. Conclusion

Acute and subchronic oral toxicity results indicate that oral administration of the ginsenoside Rg2 does not have any apparent toxic effects in mice or rats. Moreover, Rg2 shows antiarrhythmic effects on a rat model of CaCl2-induced arrhythmia. The possible antiarrhythmic mechanism is that Rg2 could inhibit Ca2+ influx through L-type Ca2+ channels by suppressing CaMKII-δ phosphorylation. Our findings support the development of Rg2 as a promising antiarrhythmic drug for clinic use.

Conflicts of interest

The authors declared no conflicts of interest regarding the publication of this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2019.06.005.

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