Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2015, **13**(5): 0361–0367
Chinese Journal of Natural Medicines

Antiarrhythmic ionic mechanism of Guanfu base A —Selective inhibition of late sodium current in isolated ventricular myocytes from guinea pigs

JIN Si-Si[1], GUO Qiao[1], XU Jing[1], YU Peng[1], LIU Jing-Han[2], TANG Yi-Qun[1*]

[1]*Research Division of Pharmacology, China Pharmaceutical University, Nanjing 210009, China;*

[2]*Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China*

Available online 20 May 2015

[ABSTRACT] The present study was designed to determine the effects of Guanfu base A (GFA) on the late sodium current ($I_{Na.L}$), transient sodium current ($I_{Na.T}$), HERG current (I_{HERG}), and Kv1.5 current ($I_{Kv1.5}$).The values of $I_{Na.L}$, $I_{Na.T}$, I_{HERG} and $I_{Kv1.5}$ were recorded using the whole-cell patch clamp technique. Compared with other channels, GFA showed selective blocking activity in late sodium channel. It inhibited $I_{Na.L}$ in a concentration-dependent manner with an IC_{50} of (1.57 ± 0.14) μmol·L^{-1}, which was significantly lower than its IC_{50} values of (21.17 ± 4.51) μmol·L^{-1} for the $I_{Na.T}$. The inhibitory effect of GFA on $I_{Na,L}$ was not affected by 200 μmol·L^{-1} H_2O_2. It inhibited I_{HERG} with an IC_{50} of (273 ± 34) μmol·L^{-1} and has slight blocking effect on $I_{Kv1.5}$, decreasing $I_{Kv1.5}$ by only 20.6% at 200 μmol·L^{-1}. In summary,GFA inhibited $I_{Na.L}$ selectively and remained similar inhibition in presence of reactive oxygen species.. These findings may suggest a novel molecular mechanism for the potential clinical application of GFA in the treatment of cardiovascular disorders.

[KEY WORDS] Guanfu base A; Late sodium current; Transient sodium current; Kv1.5 current; Arrhythmia

[CLC Number] R965 **[Document code]** A **[Article ID]** 2095-6975(2015)05-0361-07

Introduction

Arrhythmias are primarily resultant from disturbance in transmembrane ion flux of Na^+, Ca^{2+}, and/or K^+. As ion channels play a critical role in regulating the electrical activity of cardiac cells and many arrhythmias are due to abnormalities of channel expression or function, ion channels are obvious targets for antiarrhythmic therapy. To date, most antiarrhythmic agents target at ion channels.

Aconitum Coreamum. Rapaics is a classic Chinese traditional herbal medicine. Guanfu base A (GFA, Fig. 1A), a main component of Guanfu total base, is isolated from the root of *Aconitum coreanum*. Previous studies have shown that GFA produces protective effects against arrhythmia induced by $CaCl_2$, aconitine, and coronary ligation [1-2]. It also inhibits atrial fibrillation (AF) in a rat model of inducible AF [3]. GFA has passed phases II and III clinical studies and has been approved for the treatment of paroxysmal supraventricular tachycardia in China since 2005 [4]. However, the exact ionic effects of GFA are not fully understood. Recently, it has been reported that GFA blocks the L-type calcium current (I_{CaL}) [5], delayed rectifier potassium current (I_K) [6], and pacemaker current (I_f) [7]. But the current-blocking concentrations are much higher than its antiarrhythmic level. Up to date, there is no reported study investigating the effect of GFA on the ultra-rapid potassium channel (I_{Kur}/ Kv1.5). I_{Kur} is regarded as a new therapeutic target to develop new anti-AF drugs.

Pharmacologically, the delayed rectifier potassium current (I_{Kr}) is the principal current responsible for cardiac repolarization. The α-subunit of I_{Kr} is encoded by the human ether-a-go-go-related gene (HERG) and the HERG product was found to be a functional K+ channel with properties resembling those of I_{Kr} [8]. Blockade of I_{HERG} increases the action potential duration (APD) and the effective refractory period (ERP). It is also well known that suppression of HERG function can lead to long-QT syndrome. HERG channel may be one of the key target sites responsible for both therapeutic effects and potential cardiotoxicity. In the present study, we examined the pharmacological effects of GFA on the

[Received on] 28-Apr.-2014

[Research funding] This work was supported by the Fundamental Research Funds for the Central Universities (No. JKZ2011007) and the National "Key Program for New Drug Research Development" (No. 2009 ZX09103-345).

[*Corresponding author] Tel: 86-25-83271070, Fax: 86-25-83271299, E-mail: tyq@cpu.edu.cn

These authors have no conflict of interest to declare.

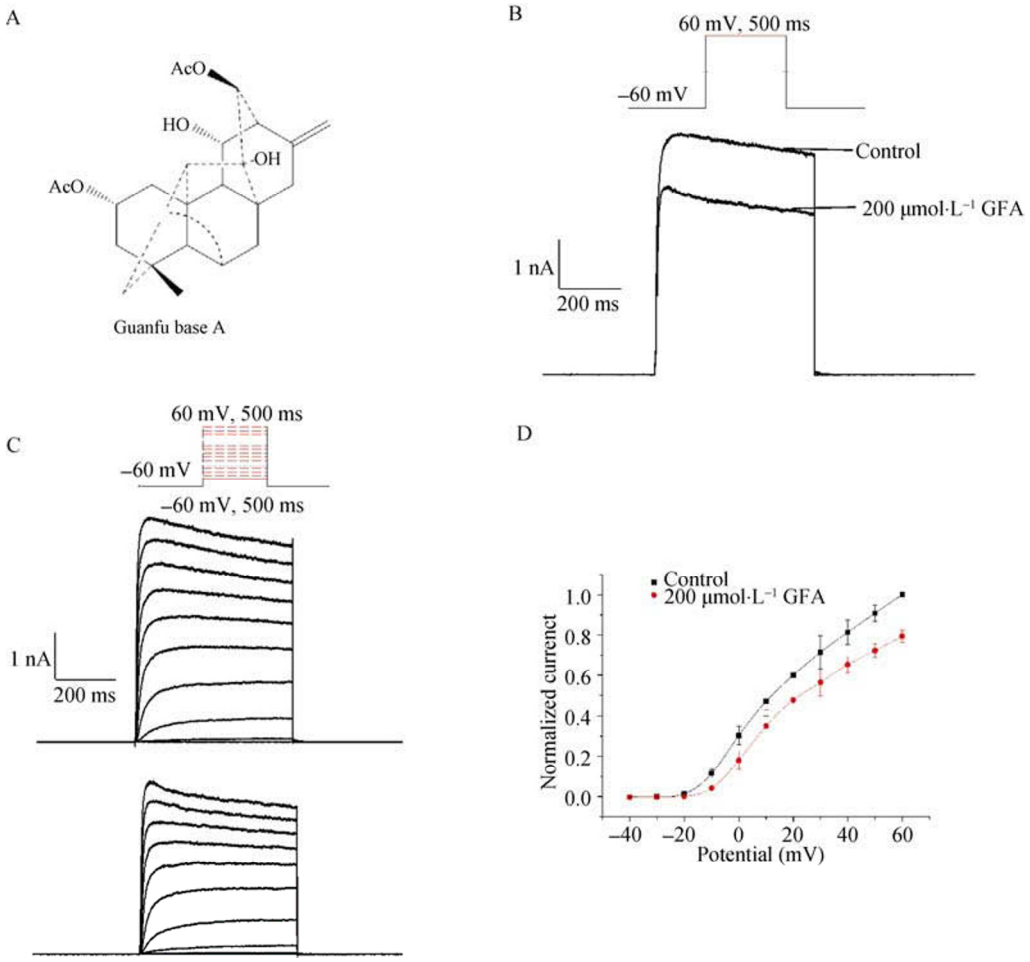

Fig. 1 Chemical structure of GFA and the effects on the $I_{Kv1.5}$ in HEK293 cell. (A) Chemical structure of the GFA. (B) Kv1.5 currents obtained in the absence and presence of 200 μmol·L^{-1} of GFA. (C) Representative voltage-dependent tracings $I_{Kv1.5}$ in a HEK293 cell under control conditions and in the presence of 200 μmol·L^{-1} of GFA. The current was elicited by 500-ms voltage steps between –40 and +60 mV starting from a holding potential of –60 mV. (D) Normalized current-voltage (I–V) relationships for the steady-state currents before and after the treatment with 200 μmol·L^{-1} of GFA

electrophysiology of cloned HERG potassium channels.

In the clinic, most arrhythmias occur in diseased heart. Cardiomyopathies are associated with metabolic stress and oxidative stress. Several ion channels expressed in the heart, including the sodium channel, L-type calcium channel, and potassium channel, are quite sensitive to redox state. Moreover, the activity of a variety of kinases and phosphatases that regulate ion channels and other proteins via phosphorylation are also redox sensitive [9]. Therefore, it is not surprising some new antiarrhythmic agents have been shown to have varying antiarrhythmic efficacy and safety in clinical trials [10].

Increased oxidative stress is believed to be an important factor predisposing the diseased heart to lethal arrhythmias. There are increasing data suggesting that an increase in $I_{Na.L}$ is a major ionic mechanism underlying the cardiac actions of ROS. Reducing $I_{Na.L}$ may be a critical step to attenuate ROS-induced myocardial electrical dysfunction [11]. Previous studies have indicated that GFA blocks the $I_{Na.T}$, but there is no report regarding the effect of GFA on cardiac $I_{Na.L}$.

The aim of the present study was to determine the GFA electrophysiological profiles with respect to $I_{Na.L}$, $I_{Na.T}$, I_{Kur}, and I_{HERG} currents. We also examined the ability of GFA to inhibit $I_{Na.L}$ current under conditions of excessive ROS formation. It was hoped that to the present study would unveil the ionic mechanism of this drug for the treatment of cardiac arrhythmia.

Materials and Methods

Animals

Guinea pigs (either gender, weighing 250 ± 20 g) were purchased from Qinglongshan Experiment Animal Centre (SCXK (Su) 2012-0008, Nanjing, Jiangsu Province, China). The experimental protocol was approved by the Animal Research and Care Committee of China Pharmaceutical University, Nanjing, China.