Toxicology and Applied Pharmacology 322 (2017) 89–96

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Inhibition of potassium currents is involved in antiarrhythmic effect of moderate ethanol on atrial fibrillation

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ARTICLE INFO

Article history:
Received 22 December 2016
Revised 1 March 2017
Accepted 7 March 2017
Available online 09 March 2017

Keywords:
Ethanol
Atrial fibrillation
Action potential duration
Ultra-rapid delayed rectifying potassium current

ABSTRACT

Excessive consumption of alcohol is a well-established risk factor of atrial fibrillation (AF). However, the effects of moderate alcohol drinking remain to be elucidated. This study was designed to determine the effects of moderate ethanol ingestion on atrial fibrillation and the electrophysiological mechanisms. In acetylcholine-induced canine and mouse AF models, the moderate ethanol prevented the generation and persistence of AF through prolonging the latent period of AF and shortening the duration of AF. The action potential duration (APD) was remarkably prolonged under the concentration range of 12.5–50.0 mM ethanol in guinea pig atrial myocytes. Ultra-rapid delayed rectified potassium currents ($I_{Kv1.5}$) were markedly inhibited by 12.5–50.0 mM ethanol in a concentration-dependent manner. Ethanol with 50.0 mM could inhibit rapid delayed rectifier potassium currents (I_{hERG}). Ethanol under 6.25–50.0 mM did not affect on inward rectifier potassium currents ($I_{Kir2.1}$). Collectively, the present study provided an evidence that moderate ethanol intake can prolong the APD of atrial myocytes by inhibition of $I_{Kv1.5}$ and I_{hERG}, which contributed to preventing the development and duration of AF.

1. Introduction

Atrial fibrillation (AF) is the most popular type of arrhythmia. In 2010, the number of individuals with AF was about 33.5 million, and the incidence was 0.5% in global (Chugh et al., 2014). Several regional studies revealed the rising prevalence and incidence of AF with a noticeable variation in different areas (Conen et al., 2011; Ruwald et al., 2013). The prevalence of AF was higher in developed world, especially in North America (Kim et al., 2011). Extensive focus has been shifted to the pathogenesis and treatment of AF.

AF resulted from a variety of conditions that caused atrial remodeling, including structural and electrical remodeling (Nattel and Harada, 2014). Recently, more and more researches investigated on the mechanisms that initiated episodes of AF. Firstly, rapid ectopic activity might trigger AF. The triggers could be localized to particular sites, especially the pulmonary veins. Secondly, AF itself caused electrical remodeling, which promoted the duration of AF in pathophysiology. The electrical remodeling altered the normal atrial electrophysiological characteristics, which increased AF sustainability and enhanced atrial vulnerability to AF induction (Narayan et al., 2011). The risk factors for AF included myocardial infarction, congestive heart failure, hypertension, valvular heart disease, even heavy drinking (Andrade et al., 2014; Kuriachan et al., 2015).

Current treatments of AF include drug therapy, catheter ablation and maze operation. Drug therapy plays a pivotal role in controlling rhythm and preventing thromboembolic events in AF. Novel antiarrhythmic drugs transiently block rapid Na^+ currents, K^+ outward currents and slow Ca^{2+} inward currents, resulting in cardiomyocyte action potential duration (APD) prolonged, heart rate controlled, and the risk of ventricular arrhythmia reduced (Dobrev and Nattel, 2010).

Alcohol, as a special small molecule, takes effect on cardiovascular system. Several studies had reported that alcohol consumption might change the cardiac electrophysiologic characteristics (Moulin et al., 2015; Qiao et al., 2015; Horakova et al., 2016). It has been known that alcohol altered APD and inhibited ionic currents such as the L-type calcium current ($I_{Ca\text{-}L}$), sodium current (I_{Na}). Moreover, heavy drinking is considered as one of the risk factors of AF (Sano et al., 2014).

Potassium currents play a critical role in the repolarization of atria. These currents participate in controlling the frequency of pacemaker

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http://dx.doi.org/10.1016/j.taap.2017.03.006
0041-008X/

cells, the resting membrane potential, the shape and duration of the cardiac action potential (Schmidt et al., 2015; Tang et al., 2015). The abnormal activities of potassium currents might cause the alteration of APD, which could induce the arrhythmia. Cardiac potassium currents have been recognized as potential therapeutic targets for arrhythmia (Ravens and Wettwer, 2011). Even some of new compounds could selectively inhibit different kinds of potassium currents (Abramochkin et al., 2013; Flaherty et al., 2014; Melgari et al., 2015). Although there are some evidences documenting the effects of alcohol on ionic currents, the effects on potassium currents have not been elucidated.

It is well known that alcohol exerts a biphasic effect on many organ systems, especially on cardiovascular system, with toxic effects in heavy drinking and benefit effects in moderate drinking (De Gaetano et al., 2016). However, the association between AF and moderate alcohol intake is still poorly understood. In the present study, the acetylcholine-induced animal AF models were used to evaluate the effects of moderate alcohol on AF. Furthermore, in order to shed some light on the mechanism of alcohol on AF, this study was undertaken to explore the effects of alcohol on cardiomyocyte action potential and several potassium currents.

2. Materials and methods

2.1. Animals and reagents

This study was carried out in accordance with the guide for animal handling and experimentation of the Ethical Committee of Xi'an Jiaotong University. Male ICR mice (body weight 18–22 g), male guinea pigs (body weight 300–350 g) and adult male mongrel dogs (body weight 12–15 kg) were obtained from the Experimental Animal Center of Xi'an Jiaotong University. Acetylcholine was obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol, $CaCl_2$, and other chemical reagents were all analytical pure, obtained from Xi'an Chemical Agent Factory (Xi'an, Shaanxi, PRC). The lead IIECG was recorded by BL-420F data acquisition and analysis system (Taimeng Co., Chengdu, China).

2.2. Effects of ethanol on acetylcholine-induced AF in canine

Twenty male mongrel dogs were randomly divided into four groups with five dogs in each: control group, ethanol groups (0.2, 0.4 and 0.8 g/kg). The canines were anaesthetized intravenously with sodium pentobarbital (30 mg/kg), and trachea intubation was made for artificially ventilation. The surface lead IIECG was recorded with subcutaneous needle electrodes during the entire experiment using data acquisition and analysis system. The thoracic cavity was opened via a midsternal thoracotomy, and exposed the heart. The dogs were firstly administrated normal saline, 0.2, 0.4 and 0.8 g/kg ethanol, via femoral vein, respectively. Then five minutes later, a cotton ball (dipped in 5% acetylcholine solution) was attached to atrionector area, the change of ECG was observed. After 1 min with acetylcholine, the atrium was lightly pinched with smooth forceps to trigger AF. The latent period of AF (the period between AF triggering and f waves appearing on ECG) and the duration of AF were recorded via ECG.

2.3. Effects of ethanol on acetylcholine-$CaCl_2$-induced AF in mouse

Thirty-two mice were randomly divided into four groups with eight mice in each: control group, ethanol groups (0.4, 0.8 and 1.6 g/kg). All mice were anaesthetized intraperitoneally with urethane (0.12 g/kg), and fixed in supine position. The surface lead IIECG was recorded using limb electrodes. The normal saline, 0.4, 0.8 and 1.6 g/kg ethanol were tardily injected via the caudal vein in mice, respectively. Five minutes after treatment of normal saline or ethanol, the mixture of acetylcholine and $CaCl_2$ (consisting 6 mg $CaCl_2$ and 25 μg acetylcholine per ml) was administrated intravenously (10 ml/kg). The latent period of AF (the period between drug administration and f waves appearing on ECG) and the duration of AF were recorded for statistical analysis.

2.4. Measurement of blood ethanol concentration

Five minutes after treatment of different doses of ethanol, the arterial blood samples were obtained to determine blood ethanol levels by GC-2014C gas chromatography (Shimadzu Co., Kyoto, Japan).

2.5. Effects of ethanol on action potential in guinea pig atrial myocytes

Guinea pigs were anaesthetized with sodium pentobarbital (30 mg/kg). The hearts were rapidly excised and immersed in 37 °C oxygenated (100%) rectified Tyrode's solution (in mM: NaCl 137.00, KCl 5.40, $CaCl_2$ 1.80, $MgCl_2$ 1.05, Glucose 11.1, Tris 10.00, pH 7.4). The left and right atrium were isolated and placed between two stimulating electrodes in a special perfusion chamber (volume 2 ml), which was perfused with 10 ml/min rectified Tyrode's solution and oxygenated with 100% O_2. Equilibrium was maintained for 60 min before the experiment. The sample was then stimulated with a stimulator (SEN-3201 Nihon Kohden, Japan), and the action potential was induced by glass microelectrode technology. Action potential amplitude (APA), action potential duration (APD), action potential duration of 50% repolarization action (APD_{50}) and action potential duration of 90% repolarization action (APD_{90}) were recorded after administrating different concentrations of ethanol (6.25, 12.5, 25.0 and 50.0 mM).

2.6. Effects of ethanol on potassium currents ($I_{Kv1.5}$, I_{hERG}, $I_{Kir2.1}$)

The hKv1.5/pBK_{CMV} plasmid, hERG/pcDNA3 plasmid and hKir2.1/pcDNA3 plasmid were transfected respectively into HEK293 cells (ATCC, VA, USA) by using Lipofectamine 2000™ (Invitrogen, CA, USA), and selected with 1000 μg/ml G418. The HEK293 cells stably expressing current channels were maintained in DMEM with 400 μg/ml G418 and 10% fetal bovine serum. One day before electrophysiological experiment, cells were seeded on the glass coverslips. The glass coverslip with cells was moved to an opened cell chamber superfused with Tyrode solution. Glass microelectrodes were made by a P-97 puller (Sutter instrument, Nato, CA, USA) and controlled tip resistances of 2–3 MΩ by filling the pipette solution. The reference electrode was a 3 mol/l KCl-agar bridge. The tip potential was adjusted zero before the pipette contacted the cell. After a gigaohm seal was successed, ruptured the cell membrane to execute whole-cell configuration to record ultra-rapid delayed rectifier potassium current ($I_{Kv1.5}$), rapid delayed rectifier potassium current (I_{hERG}) or inward rectifier potassium current ($I_{Kir2.1}$) and observe the effects of different concentrations of ethanol (6.25, 12.5, 25.0 and 50.0 mM). The potassium currents were recorded with an EPC-10 amplifier and Pulse software (HEKA, Lambrecht, Germany).

2.7. Statistical analysis

Data are expressed as mean ± SEM, and n equals the number of animals (or cells) used. For comparisons between two groups. The Student's *t*-test or one-way ANOVA was used. All data were analyzed by the SPSS of windows version 18.0. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of ethanol on acetylcholine-induced AF in canine

Five minutes after administration of 0.2, 0.4 and 0.8 g/kg ethanol, the concentrations of blood ethanol in the treated canines were 11.21 ± 0.57, 20.62 ± 1.19 and 43.83 ± 2.23 mM, respectively. All canines were generated AF after administrating of acetylcholine (Fig. 1A, B). After administration of ethanol, the present data showed that 0.2 g/kg