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The effects of allitridi and amiodarone on the conduction system and reverse use-dependence in the isolated hearts of rats with myocardial infarction

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ABSTRACT

Ethnopharmacological relevance: Allium sativum L. (*DaSuan* in Mandarin) is a traditional Chinese herb that has been used to prevent and heal cardiovascular diseases.
Objective: To study the effects of allitridi (an active constituent of *Allium sativum* L.) and amiodarone on the conduction system and on reverse use-dependence in the isolated hearts of normal rats and rats with myocardial infarction (MI).
Materials and methods: Male Sprague Dawley rats, with a ligated left anterior descending coronary artery, were used as myocardial infarction models to investigate the biological effects of the traditional Chinese herb. A single-phase electrode assay and isolated heart perfusion administration methods were employed to study and compare the electrophysiological effects of allitridi and amiodarone on normal and MI rats. Monophasic action potential (MAP) in vitro, effective refractory period (ERP) and monophasic action potential duration (MAPD)/ERP were measured to investigate reverse use-dependence (RUD) with allitridi and amiodarone. Moreover, bundle maps and heart rates were analyzed to evaluate the electrophysiological effects of allitridi on the conduction system of the cardiac muscles. Coronary flow was used to study the beneficial effects of the two drugs on the bundle of His in myocardial infraction.
Results: (1) Allitridi and amiodarone can reduce the infarction model of the His bundle (A-H, H-V) conduction and cardiac sinus rhythm in normal rats and isolated rat hearts. After washing in physiological solution (AK-H) for 15 min, the allitridi group partially recovered, but the amiodarone group did not recover. (2) Allitridi and amiodarone had no significant effects on the change of $MAPD_{90}$ or ERP in normal and MI rat hearts at different pacing frequencies (200, 250 and 300 beats/min), which indicated no RUD. In addition, the effects of allitridi on prolonging $MAPD_{90}$ and ERP were weaker than those of amiodarone ($P < 0.01$). The effects of allitridi on myocardial repolarization and its variation rate were also weaker than those of amiodarone ($P < 0.01$). However, the prolonged administration of allitridi still did not cause RUD. Allitridi and amiodarone can significantly increase the ERP/APD_{90} rate of the isolated heart ventricles of normal rats and rats with MI.
Conclusion: We propose that allitridi and amiodarone have similar effects on the cardiac conduction system and on the electrophysiology without RUD, which may be the result of the use of multi-channel blockers, such as calcium channel blockers and IKr and IKs channel blockers. Allitridi may be a promising antiarrythmic drug.

Abbreviations: AP, action potential; LVH, left ventricular hypertrophy; APD, action potential duration; RUD, reversed use dependence; MI, myocardial infarction; MAP, monophasic action potential; ERP, effective refractory period; EAD, early after-depolarization; MAPD, monophase action potential duration; BK-H, before drug perfusion; AK-H, after drug perfusion.

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1. Introduction

Cardiovascular diseases are the most common threat to human health worldwide and are the leading cause of morbidity in China. Cardiac hypertrophy is a common pathological process in many cardiovascular diseases. Clinical epidemiology indicates that the incidence of sudden cardiac death in patients with cardiac hypertrophy is much higher than in the normal population. The occurrence of sudden cardiac death is significantly associated with ventricular arrhythmias. Currently, electrophysiological research on cardiac hypertrophy mainly focuses on changes in action potentials and related ionic mechanisms. The most consistent finding in animal models of left ventricular hypertrophy (LVH) is the significant prolonging of action potential duration (APD), especially in low-frequency stimulation or pacing, which has shown that APD of cardiac hypertrophy is of significant frequency-dependence (McIntosh et al., 1998; Davey et al., 2001).

Rials et al. (1997) discovered that captopril reverses LVH and recovers prolonged APD repolarization dispersion, as well as reduces the threshold of ventricular fibrillation. Thus, for the first time, it was proved that drugs that reverse left ventricular hypertrophy could also reverse electrophysiological abnormalities. Significant prolonging of APD would increase the incidence of early after-depolarization (EAD) and triggered activity, thereby increasing the incidence of ectopic self-regulation and arrhythmia (Xu et al., 2001; Yan et al., 2001). The argument for AP repolarization dispersion would promote the occurrence of reentrant arrhythmias (Gomez et al., 1997; Volders et al., 1999), while the significant frequency-dependence of APD in hypertrophic cardiomyopathy would help to explain the phenomenon that arrhythmia is more likely to occur in patients with lower heart rates.

Traditional Chinese medicine has been used for thousands of years to heal cardiovascular diseases. *Allium sativum* L. (Da-Suan in Mandarin) has been used in herbal form for thousands of years to cure cardiovascular diseases. Because of its good cardiovascular pharmacology, which has been confirmed by modern research, it has also been widely used in other countries to prevent cardiovascular diseases (Aqel et al., 1991). Allitridi is a stable active constituent extracted from *Allium sativum* L. Its chemical structure is $CH_2{=}CH{-}CH_2{-}S{-}S{-}S{-}CH_2{-}CH{=}CH_2$, which has been synthesized and produced worldwide (Lang and Gy, 1981). Its anti-arrhythmic effects have also been reported and have thus attracted the attention of an increasing number of researchers. Martin et al. (1992) found that allitridi has antiarrhythmic effects and pharmacological effects, such as slowing sinus rhythm and inhibiting myocardial contraction. Several Chinese researchers have used ordinary microelectrode in atrial muscle recording to observe that allitridi can reduce self-discipline and excitement, extend the ERP, and increase ERP/APD_{90} (Cheng et al., 1997, 2002). Theoretically, the action potential duration (APD) and effective refractory period (ERP) of ventricular cells are similar to the electrophysiological characteristics of class-III antiarrhythmic drugs. An ideal drug for the treatment of ventricular arrhythmias should prolong cardiac repolarization without a reverse use-dependence (RUD) effect (Hondeghem and Snyders, 1990). In the Class III of antiarrhythmic drugs, amiodarone is ideal because it provides control with which to evaluate the pharmacological effects of TCM.

The goal of this paper was to systematically investigate the anti-arrhythmic effects of *Allium sativum* L. (allitridi) using electrophysiological technology and an evaluation system. Monophasic action potential (MAP) technology was used to evaluate and compare the electrophysiological effects and frequency-dependent effects of allitridi and amiodarone on the isolated hearts of normal rats and rats with myocardial infarction, under without the sympathetic nerves and breathing in vitro. The research efforts presented here provide better insight for the screening of anti-arrhythmic drugs.

2. Materials and methods

2.1. Animal grouping

Normal male Sprague-Dawley (SD) rats that weighed 250 ± 20 g (n = 10) were chosen to establish an MI model; the amiodarone myocardial infarction group weighed 250 ± 20 g (n = 5), and the allitridi myocardial infarction group weighed 250 ± 20 g (n = 5). After the establishment of the MI model for 1 month, another group of Sprague-Dawley (SD) male rats (n = 10) that were similar to the MI group in weight were chosen as normal controls, including an normal amiodarone group weighing 330 ± 20 g (n = 5) and a normal allitridi group weighing 350 ± 20 g (n = 5).

2.2. Establishment of myocardial infarction model

Sodium pentobarbital 1% (50 mg/kg) was intraperitoneally injected. The procedures performed included endotracheal intubation, ventilator positive pressure ventilation, preoperative recording of 12-lead ECG, 1-lead monitoring, local skin disinfection, chest opening, thoracotomy device setup and opening of the pericardium, the pulmonary cone and the left atrial appendage 2–3 mm from the bottom of the left anterior descending coronary artery ligation. Twelve-lead ECG was recorded after the experiments. MI rats were fed normally for 4 weeks, and then the normal rats and rats with MI were sacrificed to isolate the hearts for the electrophysiological experiments.

2.3. Determination of experimental doses of allitridi and amiodarone

When 7.5 mg/l allitridi was used to perfuse the isolated hearts of rats for 10 min, we observed the gradual prolongation of the action potential repolarization time and the His bundle A-H and H-V, as well as the occurrence of atrioventricular blockade. Similarly, when 5 mg/l amiodarone was used to perfuse the isolated hearts for 15 min, we observed the gradual prolongation of the action potential repolarization time and the His bundle A-H and H-V, as well as the occurrence of atrioventricular blockade. Therefore, the doses of allitridi and amiodarone were determined as 7.5 mg/l and 5 mg/l, respectively.

2.4. Langendorff heart perfusion

Urethane (10%) at a concentration of 1.39 g/kg was used to anesthetize the rats; then, thoracotomy and isolation of the hearts was performed. By conventional physiological techniques, reverse perfusion of heart via the aortic was conducted, during which an experimental perfusate composed of NaCl 6.93, KCl 0.35, $MgSO_4$ 7, H_2O 4.6, $CaCl_2$ 0.28, glucose 2.0, and sodium bicarbonate 2.1 (all g/L). HCl was used to adjust the pH value to between 7.2 and 7.4 (room temperature 29 °C). During the experiment, mixed gas with 95% O_2 and 5% CO_2 was maintained at a flow rate of 2 L/min.

2.5. Measurement of electrophysiological indices

Three electrodes were inserted into the left atrium, right ventricle and left ventricle of the isolated hearts from normal rats and into the left atrium, left ventricle close to the infarction, and right ventricle away from the infarction of the isolated hearts from rats with myocardial infarction. The procedure for allitridi perfusion in the myocardial infarction group and the normal group was that both groups were infused with the physiological fluid for 15 min. Once