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Oriental Pharmacy and Experimental Medicine 2004 **4(3)**, 157-161

Traditional oriental herbal medicine, *Jukyeoondam-tang*, occludes aconitine-induced ventricular arrhythmia in hearts

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SUMMARY

We showed the effects of the traditional herbal medicine, *Jukyeoondam-tang* (JO-T, *Zhu-ru-Wen-Dan-Tang* in Chinese), on ventricular arrhythmia induced by aconitine. Electrophysiological experiments with conventional microelectrode techniques revealed that JO-T potently suppressed the aconitine-induced arrhythmias in ventricular strips of the rat. In the aconitine-induced arrhythmia model of the rat, pretreatment with JO-T (100 μg/ml) completely occluded the appearance of ventricular tachyarrhythmia (VT) or ventricular fibrillation (VF) induced by aconitine. Furthermore, the aconitine-induced ventricular arrhythmia was occluded by Na^+ channel blocker quinidine but was not occluded by K^+ channel blocker glibenclamide (3 μmol/L) and Ca^{2+} channel blocker nifedipine (10 μmol/L). We also confirmed the effect of JO-T in the ischemia-reperfusion (I/R)-induced arrhythmia model of the rat. JO-T did not affect the I/R-induced arrhythmias in rats. JO-T may alleviate the risk of ventricular arrhythmias following aconitine. These results suggest that JO-T is a potent antiarrhythmic drug having a Na^+ channel-blocking action.

Key words: Jukyeoondam-tang (JO-T, 竹茹溫膽湯, Zhu-ru-Wen-Dan-Tang); Aconitine; Arrhythmia; Na^+ channel; Ischemia-reperfusion (I/R)

INTRODUCTION

Sudden cardiac death is a major cause of mortality, and most sudden cardiac deaths are caused by ventricular fibrillation (VF) (Wever *et al.*, 1993). The mechanism of such arrhythmia is not yet well defined. It has been reported that the interaction between an abnormal substrate and an imbalance of autonomic nervous control, characterized by either decreased vagal or increased sympathetic activity, would facilitate life-threatening arrhythmias after myocardial infarction (MI) patients (Schwartz *et al.*, 1992; Gill *et al.*, 1993).

Jukyeoondam-tang (JO-T) (Chinese name: *Zhuru-Wen-Dan-Tang*) is a traditional Korean herbal medicine that originated in China and it is composed of 14 species of medicinal plants. It has long been used, mainly for the treatment of insomnia, pneumonia, and palpitation of the heart, but the mechanisms of its action are unclear like other herbal medications. Therefore,

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exploring their therapeutic effect and clarifying their mode of action in the laboratory are important for medical application. This study was carried out to measure the effects of JO-T in our animal model of ventricular arrhythmia induced by aconitine. Therefore, in the present study, we investigated whether JO-T can block aconitine-induced ventricular arrhythmia in hearts. We found that JO-T could occlude the aconitine-induced ventricular arrhythmia via blocking the sodium channels.

MATERIALS AND METHODS

The JO-T preparation of extract

An extract of *Jukyeoondam-tang* (JO-T) was prepared by decocting the dried prescription of the herbs with boiling distilled water. The extraction was decocted for approximately 3 h, filtered, lyophilized, and then kept at 4 ℃. The JO-T water extract powder was dissolved in phosphate buffered saline (PBS) and filtered through a 0.22 mm syringe filter. JO-T include 8 g of *Radix Bupleuri*, 4 g of *Tuber Pinelliae*, 4 g of *Poria*, 3.2 g of *Rhizoma Cyperi*, 4 g of *Radix Platycodi*, 4 g of *Pericarpium Citri Nobilis*, 4 g of *Caulis In Taeni Phyllostachyos*, 2 g of *Radix Ginseng*, 6 g of *Rhizoma Coptidis*, 1.2 g of *Radix Glycyrrhizae*, 1.2 g of *Radix Ophiopogonis*, 4 g of *Fructus Immaturus Ponciri*, 2 g of *Fructus Zizyphi Jujubae*, and 6 g of *Rhizoma Zingiberis recens*, respectively.

Tissue preparation

Male New Zealand white rabbits (1.5-2.0 kg) were anesthetized by injection of thiopental sodium (10 mg kg^{-1}) into the marginal ear vein. After chest was open, the hearts were rapidly excised and transferred to Tyrode's solution. The composition (in mmol/L) of the Tyrode's solution were: (NaCl 137, KCl 5.4, $MgCl_2$1.05, $CaCl_2$1.8, glucose 5, HEPES 10, pH 7.4) oxygenated with 100 % O_2 at room temperature. Ventricular muscles (1.5-2 mm in width and 2-4 mm in length) were carefully dissected from left ventricular wall and mounted in a narrow channel of tissue chamber that allowed continuous superfusion (5 mL/min) with normal Tyrode's solution (37 ℃). Each side of ventricular muscle was fixed by an insect pin to the bottom of the chamber coated with Sylgard. The tissue next to the insect pin was pressed against the floor by stimulation electrodes, which elicited action potentials and contractions.

Action potential recordings

For action potential recording, ventricular muscle was stimulated by square pulses (2 Hz, 1 ms duration, 20 - 30 % above threshold voltage) using a stimulator with a stimulus isolation unit (WPI, Sarasota, FL, USA). After 2 h of stabilization, action potentials were recorded with a 3 M KCl-filled conventional microelectrode (20-30 megaohms) connected to an amplifier (KS-700, WPI, Sarasota, FL, USA) and displayed on an oscilloscope (Tektronix, Beaverton, OR, USA).Action potential duration was measured at 90 % of repolarization (APD_{90}). It took usually 0.5 to 1 h for resting membrane potential (RMP) to be recovered from penetration of the electrode into membrane and to be stabilized. There was little change of APD_{90} during this period. After another 1 h of stabilized period, drugs were applied. When no drug was treated, the RMP and the APD_{90} remained constant for more than 6 h. The tissue chamber and micromanipulators were mounted on a vibration-free table.

Perfusion of isolated rat heart

Male Sprague-Dawley rats (200-250 g) were utilized for the preparation of Langendorff-perfused hearts. The animals were anesthetized

and the hearts were rapidly excised and mounted onto a Langendorff heart perfusion apparatus using a protocol adapted from (Tsuchida *et al.*, 1994). The hearts were retrograde perfused on a Langendorff apparatus with Krebs-Henseleit solution (in mmol/L: 118 NaCl, 4.75 KCl, 1.18 $MgSO_4$, 1.18 KH_2PO_4, 25 $NaHCO_3$, 11 Glucose, 2.5 $CaCl_2$, 10 HEPES) with a gas mixture of 95 % O_2 and 5 % CO_2 at 37 ℃ in a constant perfusion pressure of 100 cm H_2O. Perfused hearts were stabilized for 15 min. Through a left atria incision, a latex balloon connected to a pressure transducer was inserted into the left ventricular (LV) cavity for measurement of the LV isovolumic pressure. The LV isovolumic pressure and electrocardiogram (ECG) were continuously monitored using a polygraph and a computer analysis system (PolyView, GRASS, West Warwick, RI, USA).

RESULTS

The effect of JO-T on ventricular APDs

Aconitine promotes sodium channel opening, induces depolarization of the resting membrane potential, and thus drastically affects the excitability of cardiac tissues. It was reported that the activation of sodium channels by aconitine plays an important role in the aconitine-induced tachyarrhythmia in murine heart (Kimura *et al.*, 1994).

We studied the effects of *Jukyeoondam-tang* (JO-T) on aconitine-induced arrhythmia in rabbit ventricular muscles. To confirm whether the ventricular APD exhibited ventricular arrhythmic properties in response to aconitine, the drug was added to isolated rabbit ventricular strips in an organ bath. Figure 1A shows a typical effect of aconitine on action potentials elicited by applying suprathreshold-depolarizing currents (duration 1 ms) at a rate of 2 Hz. The ventricular muscle was perfused with normal Tyrode's solution at a rate of 5 mL/min. After the control action potential was recorded in the normal Tyrode's solution, perfusate was switched to aconitine-containing solution. Control APD_{90} were 1926.9 ms (n = 6) with a regular rhythm (Fig. 1Aa). When aconitine (10 nmol/L) was applied for about 30 min, the aconitine induced the tachyarrhythmias rapidly (Fig. 1Bb). To investigate the effects of JO-T on ventricular arrhythmia induced by aconitine, we observed the effect of JO-T (100 μg/mL) in the presence of aconitine. As shown in Figure 1Ac, JO-T completely occluded the arrhythmia induced by aconitine in rabbit ventricular muscles. The aconitine-induced ventricular arrhythmia was occluded by quinidine (Fig. 1Ba) but was not occluded by glybenclamide and nifedipine (Fig. 1B, b and c). These results suggest that the antiarrhythmic effect of JO-T exerts via blocking of sodium channels.

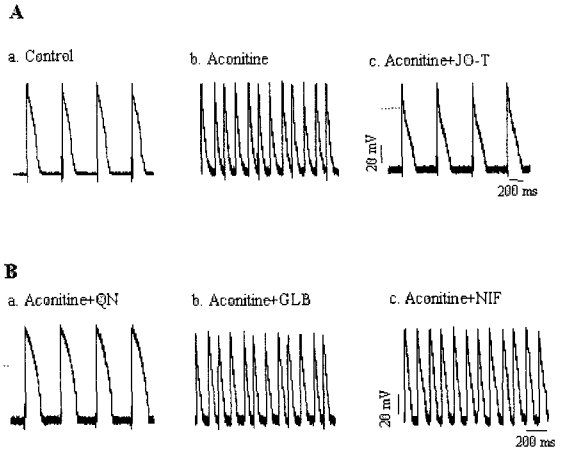

Fig. 1. JO-T occludes the ventricular arrhythmia in rabbit ventricular APDs. (A) Ventricular muscles were treated with aconitine (10 nmol/L) for 30 min or aconitine plus JO-T (100 μg/ml) for 60 min. (a) control. Aconitine induced the ventricular arrhythmia (b) but was completely occluded by application of JO-T (c). (B) Aconitine-induced arrhythmias were occluded by Na^+ channel blocker quinidine (QN, 30 μmol/L) (a) but were not occluded by K^+ channel blocker glybenclamide (GLB, 3 μmol/L) (b) or Ca^{2+} channel blocker nifedipine (NIF, 10 μmol/L) (c).

The effect of JO-T on aconitine-induced ventricular arrhythmia

To investigate the effect of JO-T in the myocardial response to aconitine-induced ventricular arrhythmia, we used Sprague-Dawley rats. As shown in Figure 2, when aconitine was added to perfusate during Langendorff perfusion, rat hearts were induced ventricular arrhythmia (Fig. 2A). However, aconitine-induced ventricular arrhythmia completely abolished by pretreated JO-T (100 μg/mL) (Fig. 2B). We also confirmed the effect of JO-T in the ischemia-reperfusion (I/R)-induced arrhythmia of the rat. To evaluate the effect of JO-T on the I/R-induced arrhythmias, hearts were subjected to 30 min of global ischemia and 50 min of reperfusion. Incidence of arrhythmias during I/R, including ventricular premature beats or ventricular fibrillation, was not occluded by treatment of JO-T. These results suggest that the effect of JO-T exerts via blocking of Na^+ channels only in aconitine- induced ventricular arrhythmias.

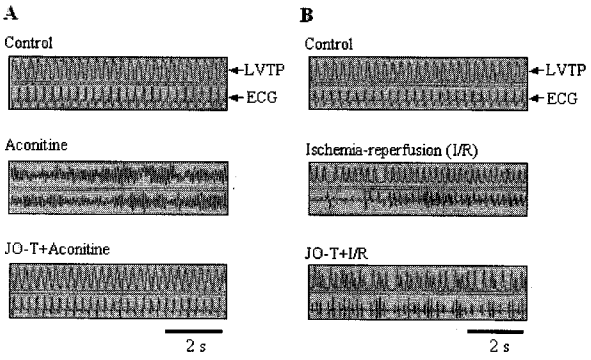

Fig. 2. JO-T occludes the ventricular arrhythmia in Langendorff perfused hearts. (A) Tracing from continuous monitor recordings obtained during aconitine (10 nmol/L) or aconitine plus JO-T (100 μg/ml) perfusion. Aconitine induced the ventricular arrhythmia (b) but it was completely occluded by JO-T (c). (B) Tracing from continuous monitor recordings obtained during 30 min of ischemia and 50 min of reperfusion (I/R). JO-T did not occlude the I/R-induced arrhythmia (b and c). Each tracing demonstrates the left ventricular transduced pressure (LVTP) and electrocardiogram (ECG) over time.

DISCUSSION

The present study was undertaken to assess the effect of JO-T on ventricular arrhythmia induced by aconitine in rabbit or rat hearts. We investigated the antiarrhythmic effect of JO-T in aconitine-induced tachyarrhythmic models using a conventional microelectrode technique and Langendorff heart perfusion apparatus. Aconitine is a cardiotoxic agent widely used for producing ventricular arrhythmia in rats, targets sodium current and plays an important role in the assessment of new anti-arrhythmic drugs (Shu *et al.*, 2004). It was reported that activation of sodium channels by aconitine plays an important role in the aconitine-induced tachyarrhythmia in murine atria (Matsuda *et al.*, 1959; Kimura *et al.*, 1994). In the present study, the application of aconitine induced tachyarrhythmia or ventricular fibrillation (Fig. 1); these cardiac arrhythmias may be caused mainly by activation of sodium channels. Voltage-gated sodium channels are primarily responsible for action potential propagation in excitable tissues. As a crucial component in the physiology of excitable tissues, sodium channels are primary targets for several naturally occurring neurotoxins (Strichartz *et al.*, 1987; Catterall *et al.*, 1992). It is generally accepted that aconitine affects cardiac rhythm by delaying the inactivation of sodium channels. This leads to a delay in the final repolarization phase of the action potential and initiates premature excitations (Matsuda *et al.*, 1959). Consistent with these properties of aconitine provoked a transient increase in force of contraction in isolated atria and induced a similar pattern of arrhythmia, which may result

from an activation of sodium channels. JO-T has been used in traditional Korean medicine for its reversal effect on palpitation of the heart. However, the cardiovascular effects of JO-T have not been systematically studied and any criteria for effective treatments has not been established. In the present study, JO-T was effective against aconitineinduced ventricular arrhythmias, but had no effect on ischemia-reperfusion-induced arrhythmias. Furthermore, the aconitine-indued ventricular arrhythmia was occluded by Na^+ channel blocker quinidine but was not occluded by K^+ channel blocker glybenclamide and Ca^{2+} channel blocker nifedipine. These results suggest that the JO-T effect exerts via blocking of sodium channels. In conclusion, our study suggests that the attenuation of aconitineinduced ventricular arrhythmia by JO-T may be due, at least in part, to blocked Na^+ channels, and subsequent affected excitability of cardiac tissues. Therefore, the present study supports the clinical usefulness of JO-T for treating cardiac arrhythmias.

ACKNOWLEDGEMENTS

This work was supported by the Korea Research Foundation Grant (KRF-EX 0001).

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