Antiarrhythmic effects and potential mechanism of WenXin KeLi in cardiac Purkinje cells

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BACKGROUND Previous studies have demonstrated that WenXin KeLi (WXKL), a traditional Chinese medicine, can exert antiarrhythmic properties through complex multichannel inhibition, but its pharmacologic effect remains to be elucidated, especially in the cardiac conductive system.

OBJECTIVE To explore the antiarrhythmic property of WXKL in cardiac Purkinje cells (PCs).

METHODS PCs were isolated from rabbit hearts and action potentials (APs) and ion currents were recorded by whole-cell patch clamp technique. Anemonia toxin II (ATX-II) and isoproterenol (ISO) were used to induce early or delayed afterdepolarizations (EADs, DADs) or triggered activities (TAs).

RESULTS WXKL (1 g/L and 5 g/L) significantly abbreviated the action potential duration (APD) of PCs in a dose- and rate-dependent manner. Treatment of PCs with ATX-II (2 nM) prolonged APD and induced EADs, which were significantly suppressed by WXKL. WXKL (1, 5 g/L) also inhibited ISO-induced EADs, DADs, and TAs. To reveal the ionic mechanisms, we studied the effects of WXKL on late sodium current (I_{NaL}), peak sodium current (I_{NaP}), and L-type calcium currents (I_{CaL}) in PCs. WXKL-attenuated ATX-II (5 nM) induced I_{NaL} augmentation and blocked I_{NaL} with an IC_{50} of 4.3 $\pm$ 0.5 g/L, which is 3- to 4-fold more selective than that of I_{NaP} (13.3 $\pm$ 0.9 g/L) and I_{CaL} (17.6 $\pm$ 1.4 g/L). Moreover, WXKL exerted significantly less use-dependent block of I_{NaP} than that of flecainide, indicating its lower proarrhythmic effect.

CONCLUSIONS WXKL exhibits antiarrhythmic properties in cardiac PCs via selective inhibition of I_{NaL}.

KEYWORDS Antiarrhythmic drugs; Cardiac Purkinje cells; Electrophysiology; WenXin KeLi; Late sodium current

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Introduction

Cardiovascular diseases are the major causes that threaten human health; structural heart disease is often related to arrhythmias, some of which can be serious or even deadly.[1] Although nonpharmacologic therapies such as implantable devices and ablation have been used to treat a variety of arrhythmias, there remain many conditions where antiarrhythmic drugs are still needed, as in the cases of sinus rhythm control of patients with atrial fibrillation, spontaneous arrhythmia control after nonpharmacologic therapies, etc. However, the therapeutic effects of current antiarrhythmic medicines are far from satisfactory, because of the life-threatening proarrhythmic effect in some antiarrhythmic drugs.[2]

WenXin KeLi (WXKL), a traditional Chinese medicine consisting of *Radix codonopsis pilosulae*, *Rhizoma polygonati*, *Radix notoginseng*, succinum, and *Rhizoma nardostachyos*, has been reported to be effective in the treatment of heart failure, cardiac inflammation, and arrhythmias.[3–5] One study[3] showed that WXKL could suppress atrial fibrillation by selective inhibition of peak sodium current (I_{NaP}) in atria. Another study[4] demonstrated that the antiarrhythmic mechanism of WXKL in suppressing ventricular arrhythmia was mainly mediated by inhibition of late sodium current (I_{NaL}). However, it remains unknown if the antiarrhythmic effect of WXKL is also related to its impact on the cardiac conductive system. It is known that cardiac Purkinje fibers (PFs) play a crucial role in the propagation of impulse from atrioventricular node to ventricular muscle and may initiate a variety of ventricular arrhythmias, such as postinfarction ventricular tachycardia,[6] catecholaminergic polymorphic ventricular tachycardia,[7] and long-duration ventricular fibrillation.[8] Unlike ventricular muscle, PFs show an unstable electrophysiological property that is susceptible to developing early

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afterdepolarizations (EADs) and delayed afterdepolarizations (DADs).[9] We thus speculate that at least part of the antiarrhythmic effects of WXKL may be mediated by its impact on PFs.

Moreover, it is reported that choice of species and cardiac tissues play an important role in evaluating the pharmacologic property of an antiarrhythmic drug. The electrophysiological parameters of different cardiac tissues show regional heterogeneity,[10,11] among which PFs seem to be the most sensitive to drug-induced arrhythmia.[12] Thus, we extend investigation of antiarrhythmic effects of WXKL previously conducted in atria and ventricle to isolated Purkinje cells (PCs), so as to explore its potential PC-related antiarrhythmic mechanism.

Materials and methods

Solutions and drugs

All solutions were made with Milli-Q-grade water. The standard Tyrode solution contained (in mM) 135 NaCl, 5.4 KCl, 1.0 $MgCl_2$, 10 glucose, 0.33 NaH_2PO_4, 10 HEPES, 1.8 $CaCl_2$ with pH adjusted to 7.4 with NaOH. The Ca^{2+}-free Tyrode solution was devoid of Ca^{2+}. The KB solution contained 85 KOH, 50 K-glutamate, 30 KCl, 20 taurine, 10 glucose, 10 HEPES, 0.5 EGTA, 1.0 $MgCl_2$ with pH adjusted to 7.4 with KOH. All chemicals were purchased from Sigma-Aldrich (St Louis, USA) unless indicated. WXKL was provided by Buchang Pharmaceuticals Corporation, Shandong, China, and dissolved in dimethylsulfoxide and added to the referred external solution to reach the desired concentration. The final concentration of dimethylsulfoxide was less than 0.1%. The stock solutions of nifedipine, tetrodotoxin (TTX), ranolazine, anemonia toxin II (ATX-II), and flecainide were diluted according to the manufacturer's instruction.

Cell isolation

The use of animals in this investigation conformed to the Guide for the Care and Use of Laboratory Animals of Shanghai, China and was approved by the Institutional Animal Care and Use Committee of Xinhua Hospital. Single PCs were isolated from rabbit hearts as described by Scamps and Carmeliet.[13] After the PFs had been dissected from both ventricles, ventricular muscle cells (VMs) were dissociated from digested ventricles by gentle mechanical dissociation. Cells were then placed in KB solution and kept at 4°C until use.

Action potential recording

Cells were maintained in standard Tyrode solution for 5–10 minutes after perfusion and the data were recorded after entering the cell for 5 minutes to stabilize the parameter. All experiments were performed at 37°C. For action potential (AP) recordings, patch pipettes (resistance 2–5 M) were filled with pipette solution containing (in mmol/L) 110 K-aspartate, 30 KCl, 5 NaCl, 10 HEPES, 0.1 EGTA, 5 MgATP, 5 creatine phosphate, 0.05 CAMP, pH 7.2 with KOH. APs were elicited at different basic cycle lengths (BCLs) by 2-ms duration, 2× diastolic threshold current pulses. Resting membrane potential (RMP), AP amplitude, maximum upstroke velocity (Vmax), and action potential duration (APD) at 50% and 90% repolarization (APD_{50} and APD_{90}) were analyzed.

Ion current recording

To record I_{NaL}, patch pipettes (1–2 M) were filled with an internal solution containing 110 Cs-aspartate, 30 CsCl, 10 HEPES, 0.5 EGTA, 0.2 Na_3-GTP, 5 Na_2-phosphocreatine, 5 MgATP; pH was adjusted to 7.2 with CsOH. Myocytes were bathed with a modified Tyrode solution in which KCl was replaced with equivalent CsCl. Nifedipine (30 μM) was added to the bath solution to block calcium current. I_{NaL} was elicited by 300 ms voltage-clamp pulses from -90 to -20 mV, the amplitude of I_{NaL} was measured at 200 ms, and 10 μM TTX was used to quantify I_{NaL}. The same pipette solution was used to record I_{NaP}; the bath solution contained the following (in mmol/L): 10 NaCl, 130 CsCl, 1.0 $MgCl_2$, 1.0 $CaCl_2$, 5 HEPES, 10 glucose, and 0.3 $CdCl_2$. I_{NaP} was recorded during an 80-ms-duration depolarizing voltage step from -90 to 35 mV in increments of 5 mV. For L-type calcium current (I_{CaL}) recording, the pipette solution contained (in mmol/L) 20 CsCl, 100 Cs-aspartate, 1 $MgCl_2$, 20 TEACl, 10 EGTA, 10 HEPES, and 5 Mg-ATP; pH was adjusted to 7.2 with CsOH. The bath solution was the same as that used for I_{NaL}. I_{CaL} was recorded using a series of 200-ms steps from -40 mV to +30 mV with increments of 10 mV from a holding potential of -80 mV prior to a 100 ms prepulse to -40 mV.

Data analysis

The pCLAMP 10.1 software, Statistical Package for Social Sciences (version 13.0), GraphPad Prism 5, and Origin 8 software were used for the data acquisition and analysis. All data were presented as mean ± standard error of mean, unless otherwise noted; n represents the number of cells analyzed. The statistical comparisons between different groups were performed with 1-way analysis of variance followed by Bonferroni test. Fisher's exact test was used for the comparison of event incidence between different groups. $P < .05$ was considered statistically significant.

Results

Electrophysiological property of single isolated cardiac Purkinje cells

Figure 1A presents representative morphology of PCs and VMs; compared with VMs, rod-shaped PCs had no typical staircase configuration and striations. The AP of PCs was also different, usually had a rapid phase 1 repolarization, a relative negative plateau, and a longer APD_{90} (Figure 1B) compared with that of VMs. A summary of transmembrane AP values is shown in Table 1.

Electrophysiological effects of WenXin KeLi in Purkinje cells

In single PCs isolated from both ventricles, we investigated the rate-dependent repolarization of AP in the presence and absence of WXKL. WXKL shortened APD in a dose-