Allocryptopine and benzyltetrahydropalmatine block hERG potassium channels expressed in HEK293 cells

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Aim: Allocryptopine (ALL) is an alkaloid extracted from Corydalis decumbens (Thunb) Pers Papaveraceae, whereas benzyltetrahydropalmatine (BTHP) is a derivative of tetrahydropalmatine extracted from Corydalis ambigua (Pall) Cham et Schlecht. The aim of this study was to investigate the effects of ALL and BTHP on the human ether-a-go-go related gene (hERG) current expressed in HEK293 cells.

Methods: Cultured HEK293 cells were transiently transfected with hERG channel cDNA plasmid pcDNA3.1 using Lipofectamine. The whole-cell current $I_{\text{HERG}}$ was evoked and recorded using Axon MultiClamp 700B amplifier. The drugs were applied via superfusion.

Results: Both ALL and BTHP reversibly suppressed the amplitude and density of $I_{\text{HERG}}$ in concentration- and voltage-dependent manners (the respective IC$_{50}$ value was 49.65 and 22.38 μmol/L). BTHP (30 μmol/L) caused a significant negative shift of the steady-state inactivation curve of $I_{\text{HERG}}$, while ALL (30 μmol/L) did not affect the steady-state inactivation of $I_{\text{HERG}}$. Furthermore, BTHP, but not ALL, shortened the time constants of fast inactivation and slow time constants of deactivation of $I_{\text{HERG}}$. But both the drugs markedly lengthened the time constants for recovery of $I_{\text{HERG}}$ from inactivation. Using action potential waveform pulses, it was found that both the drugs at 30 μmol/L significantly suppressed the current densities in the late phase of action potential, but did not significantly affect the current densities in the early phase of action potential.

Conclusion: Both ALL and BTHP derived from Chinese herbs potently block hERG current.

Keywords: allocryptopine; benzyltetrahydropalmatine; hERG current; whole-cell patch-clamp recording; anti-arrhythmic agent
Electrophysiological action of ALL and BTHP on hERG channels expressed heterologously in human embryonic kidney (HEK) 293 cells using the whole-cell patch-clamp technique. Our findings provided detailed insight into the biophysical mechanism of hERG channel blockade by these two drugs.

Materials and methods

Transfection of HEK 293 cells

HEK 293 cells (American Type Culture Collection, Manassas, VA, USA) were maintained under 5% CO₂ in humidified air at 37°C for biochemical analysis. Transient transfection of hERG channel cDNA plasmid pcDNA3.1 2.0 µg into the cultured cells was performed using Lipofectamine (Life Technologies, Gaithersburg, MD, USA) and the manufacturer’s instructions. CD8 cDNA was co-transfected as a reporter gene (EBo-pCD vector, American Type Culture Collection). The CD8-positive cells were identified using Dynabeads (M-450 CD8, Invitrogen Co, CA, USA). The cells were harvested 48–72 h after transfection, and 25%–30% of transfection-positive cells were identified.

Reagents and solutions

Alpha-allocryptopine (ALL) was supplied by the Pharmaceutical Department of Lanzhou University (molecular weight 365, melting point 168°C, a white crystal powder, 99.0% purity; its structure is shown in Figure 1A). It was dissolved in dimethyl sulfoxide (DMSO) to make a stock solution of 5.0 mmol/L. Benzyltetrahydropalmatine (BTHP) was supplied by China Pharmaceutical University (molecular weight 478.5, melting point 204°C, white crystalline powder, over 99.0% purity; its structure is shown in Figure 1B). It was dissolved in distilled water to make a stock solution of 5 mmol/L, and the drug stock was added to a bath solution to produce the final concentrations mentioned in the Results section.

Patch clamp experiments

The HEK293 cells were bathed in a solution containing (in mmol/L) NaCl 140, KCl 4, CaCl₂ 2, MgCl₂ 1, HEPES 10, and glucose 5, and adjusted to a pH of 7.4 with NaOH. The current was recorded using the whole cell patch-clamp technique and a MultiClamp 700B amplifier (Axon Instruments). All of the signals were acquired at 5 kHz (Digidata 1322A, Axon Instruments). Patch pipettes were pulled from borosilicate glass on a P-97 horizontal puller (Sutter Instruments). The electrodes had a resistance of 1–3 MΩ.

To record K⁺ currents, pipettes were filled with (in mmol/L) K-aspartame acid 140, MgATP 4, MgCl₂ 1, EGTA 10, GTP 0.1, and HEPES 10 and were adjusted to a pH of 7.3 with KOH. To ensure reproducibility, after the whole-cell conditions were established by rupturing the cell membrane, we allowed a dialysis period of 4 min before measuring any control records. During the dialysis period, we monitored the current-voltage relationships to ensure the stability and consistency of the recordings. The holding potential was -90 mV, and the interpulse interval was at least 15 s.

Data analysis and statistical methods

Off-line leak correction was performed on all of the amplitude data. Data are presented as the mean±SEM, with n representing the number of cells analyzed. pCLAMP version 9.2 (Axon Instruments) and Origin (Microcal Software) software were used for data analysis. Statistical significance was evaluated using a paired Student’s t-test.

A P<0.05 was considered statistically significant. The concentration of drugs needed to yield a 50% blockade of the hERG current (IC₅₀) was obtained by fitting the data to a Hill equation: \[ I/I_{\text{max}} = 1/[1+(C/IC_{\text{50}})^{nH}], \] where \( I \) and \( I_{\text{max}} \) are the current amplitudes measured in the absence and presence of drugs, respectively, \([C]\) is the concentration of drugs in the presence of the drugs, respectively, and \( nH \) is the Hill coefficient.

Results

Concentration-dependent and time characteristics of \( I_{\text{hERG}} \) inhibition by ALL and BTHP

To define the concentration dependence of hERG channel current blockade by ALL and BTHP, we examined the effects of the two drugs on step and tail currents created from a holding potential of -90 mV. The membrane potential was stepped to +60 mV for 2000 ms and then repolarized to -40 mV for 3000 ms to elicit an outward tail current. The standard protocol was applied with a start-to-start interval of 15 s. The amplitude of the outward tail current in the reversal pulse partially exceeded the amplitude of the activating current due to slow activation, fast inactivation, and rapid recovery from inactivation. The current characteristics were stable by the fifth application of the protocol, which was considered the control for comparison with the currents recorded in the presence of the drugs. Figure 2A and 2B show representative...
traces recorded from a single cell in which a range of concentrations of drugs was applied sequentially. During superfusion, the cell was continually stimulated to achieve saturation inhibition. Three different concentrations of ALL and BTHP, including 10, 30, and 100 µmol/L, were tested. Similar experiments were performed using seven different concentrations of the two drugs (i.e., 0.3, 1.0, 3.0, 10.0, 30.0, 100.0, and 300.0 µmol/L). The percent inhibition of the peak tail current was measured, and the mean data points were fitted with the Hill equation, as described in the Materials and Methods section. Figure 2C and 2D show plots of half-maximum inhibitory concentration (IC₅₀) values obtained for each drug; the IC₅₀ value for BTHP was lower than that obtained for ALL. For ALL, IC₅₀ was 49.65 µmol/L [95% confidence interval (CI): 34.75–64.54 µmol/L], and the Hill coefficient for the fit was 1.21; the IC₅₀ of BTHP was 22.38 µmol/L (95% CI: 13.56–31.20 µmol/L) with a Hill coefficient of 1.34. (E and F) Time course of hERG tail current inhibition by 30 µmol/L ALL and BTHP and washout effect (n=15). (G) The hERG current amplitude was decreased by dofetilide 10 nmol/L.

Figure 2. Inhibition of hERG channels by ALL and BTHP. (A and B) Representative current traces recorded from the same cell under control conditions and after superfusion with ALL and BTHP (10, 30, and 100 µmol/L). (C and D) Concentration-response relationship of the effects of ALL and BTHP on hERG peak tail currents (n=20). The IC₅₀ of ALL was 49.65 µmol/L (95% CI: 34.75–64.54 µmol/L) with a Hill coefficient of 1.21 and the IC₅₀ of BTHP was 22.38 µmol/L (95% CI: 13.56–31.20 µmol/L) with a Hill coefficient of 1.34. (E and F) Time course of hERG tail current inhibition by 30 µmol/L ALL and BTHP and washout effect (n=15). (G) The hERG current amplitude was decreased by dofetilide 10 nmol/L.
demonstrated the stability of the experimental conditions, 30 µmol/L ALL was applied to perfuse the bath. The maximal inhibition with 38% of the control current occurred within 4 min (n=15), and a steady-state block was obtained with a small further increase in inhibition (less than 5%, Figure 2E). With the drug-free solution washout, the amplitude of $I_{hERG}$ recovered to 90.0% of the control level in approximately 10 min. In another experiment, after a control period of 2 min, 30 µmol/L BTHP was applied instead of ALL. The maximal inhibition with 55% of the control current occurred within 4 min (n=15), and a steady-state block was obtained. The drug was washed out, and the inhibitory effect of BTHP was reversed with 78.5% recovery within 10 min (Figure 2F). When the cells were repetitively stimulated at a frequency of 0.1 Hz, the development of the hERG current blockade by ALL and BTHP occurred very rapidly; on washout, the effects recovered rapidly. This indicated an irreversible blockade.

**Voltage dependence of $I_{hERG}$ inhibition by ALL and BTHP**

Drugs that block ion channels often alter the voltage dependence or kinetics of channel gating. Therefore, we examined the effects of the drugs on the voltage dependence of activation and rectification and their effects on the kinetics of inactivation and deactivation. The voltage dependence of the drug-induced blockage of $I_{hERG}$ was investigated by applying voltage commands of 5000 ms duration from a holding potential of -90 mV to a range of test potentials, followed by a repolarizing step to -40 mV for 3000 ms to elicit tail currents. The measurements of step and tail currents were made in the control group and in the presence of 30 µmol/L ALL or 30 µmol/L BTHP. Both the step and tail current amplitudes were dramatically reduced by the two drugs (Figure 3A). At a test potential of +60 mV, the step current was reduced from 51.2±3.2 pA/pF to 34.6±2.9 pA/pF by ALL and to 24.5±2.4 pA/pF by BTHP. The tail current was reduced from 143.5±7.8 pA/pF to 97.1±5.6 pA/pF by ALL and to 65.3±4.2 pA/pF by BTHP (P<0.01, n=15, Figure 3B). The peak amplitudes of the tail currents were reduced by 32.3±1.6% (ALL) and 54.5±2.3% (BTHP).

Figure 3C and 3D show a representative current-voltage relationship for $I_{hERG}$ step and tail currents before and after using the drugs. The hERG currents were activated at potentials greater than -40 mV. The amplitude of the outward step current increased with depolarization up to 0 mV and decreased with further depolarization, which is typical for an inward rectifying property of the hERG current. The $I$–$V$ curve of the step current showed a bell-shaped relationship, and the tail current amplitude was steady-state after test pulses of +30 mV or above. The step current densities were...

![Image](https://example.com/image.png)

**Figure 3.** Inhibition effect of ALL and BTHP on step and tail currents by repolarizing pulse of -40 mV. (A) Representative current traces recorded under control conditions and after superfusion with ALL and BTHP (30 µmol/L). (B) The inhibitory effect of drugs (30 µmol/L) on repolarizing pulse of -40 mV is shown and the peak amplitude of step current was reduced. At test potential of +60 mV, the step current was reduced from 51.2±3.2 pA/pF to 34.6±2.9 pA/pF by ALL and to 24.5±2.4 pA/pF by BTHP and the magnitude of $I_{hERG}$ tail was from 143.5±7.8 to 97.1±5.6 pA/pF by ALL and to 65.3±4.2 pA/pF by BTHP. (C and D) Summary of ALL and BTHP on current density-voltage relationship of step and tail currents. *P<0.05, **P<0.01, n=15.
decreased by the two drugs between -20 mV and +20 mV. At a potential of -10 mV, the tail current densities were significantly decreased by both drugs (P<0.05 or P<0.01), while the blocking effects of the drugs on the tail current resulted in a slight change at more positive potentials. A reason for this might be the low amplitude of the current at these potentials.

Figure 4 shows the inhibition effects of ALL and BTHP on the tail currents using a repolarizing pulse of -110 mV. The current traces were recorded under control conditions and after superfusion with ALL 30 μmol/L and BTHP 30 μmol/L. At a test potential of +60 mV, the tail current was reduced from -190.3±11.7 pA/pF to -137.4±9.9 pA/pF by ALL and to -105.4±7.8 pA/pF by BTHP (P<0.01, n=15). The peak amplitude of the tail current was reduced by 27.8%±3.1% (ALL) and 45.6%±2.8% (BTHP) (Figure 4A–4C). A significant reduction in the tail current was first observed at -10 mV, and the inhibition gradually became more significant with increasingly positive potentials, which was different from the inhibition feature of the tail current elicited at a repolarization potential of -40 mV. Neither drug showed the maximal blockade of the tail current at a repolarization potential of -110 mV within the range of test potentials from -10 mV to +60 mV.

The effects of ALL and BTHP on the rectification characteristics of hERG currents are shown in Figure 5A and 5B. The I–V relationship for the steady-state exposure of the two drugs exhibited a similar pattern to the inward rectification characteristics of the control between -70 mV to +20 mV. Conversely, the rectification curve by 30 μmol/L BTHP diverged slightly lower over +10 mV of test potentials (Figure 5C and 5D). The rectification characteristics of hERG may be increased by BTHP in the range of these potentials.

The effects of ALL and BTHP on the activation and deactivation of the I\textsubscript{hERG} channel

To understand the apparent effect of the drugs in greater detail, the steady-state activation of I\textsubscript{hERG} was studied and fitted with the Boltzmann equation: \(G/G_{\text{max}}=1/(1+\exp[(V_m-V_{1/2})/k])^{[22]}\). The voltage dependence of the current activation was assessed using standard tail current analysis. The cells were depolarized to potentials in the range of -50 mV to +60 mV for 2000 ms and the tail current was recorded at -40 mV for 2000 ms. The voltage-dependent activation curves for I\textsubscript{hERG} for the control and the drugs are plotted in Figure 6A and 6B. Neither ALL nor BTHP exerted significant effects on the half-activated voltage (\(V_{1/2}\)) and activated curve slope (k) when compared with the control. Neither drug significantly altered the slope factor for the fitted relationships. The small and similar effects of both drugs suggest that the effects on the voltage dependence of I\textsubscript{hERG} activation cannot account for the greater variation when using ALL compared to BTHP. The I\textsubscript{hERG} tails were fitted with a single-exponential equation (\(I=\text{Aexp}(-t/\tau)+C\)). The mean values of the activation time constants in the control and the presence of 30 μmol/L ALL or 30 μmol/L BTHP were not significantly different (Figure 6C and 6D). It is suggested that neither drug has a statistically significant effect on the voltage dependence of hERG activation time constants.

![Figure 4. Inhibitory effect of ALL and BTHP on step and tail currents by repolarizing pulse of -110 mV.](image-url)

(A) Representative current traces recorded under control conditions and after superfusion with ALL and BTHP (30 μmol/L). (B) The inhibitory effect of drugs (30 μmol/L) on repolarizing pulse of -110 mV is shown and the magnitude of I_{hERG} tail was reduced from -190.3±11.7 to -137.4±9.9 pA/pF by ALL and to -105.4±7.8 pA/pF by BTHP. (C) Summary of ALL and BTHP on current density-voltage relationship of tail currents. \(^{a}P<0.05, ^{b}P<0.01, n=15.\)
under our experimental conditions.

The deactivation procedure of the \(I_{\text{hERG}}\) tails were fitted with a bi-exponential equation \(I=A_1\exp(-t/\tau_1)+A_2\exp(-t/\tau_2)+C\)
[23, 24]. The slow deactivation time course of the \(I_{\text{hERG}}\) tails was accelerated by BTHP, but not by ALL (Figure 6E). At a test potential of -40 mV, the mean values of the slow deactivation time constants (\(\tau_2\)) in the control and in the presence of 30 μmol/L BTHP were 1450.2±43.6 ms and 1060.3±47.5 ms, respectively (\(P<0.01, n=15\)). The mean values of the fast deactivation time constants (\(\tau_1\)) before and after 30 μmol/L BTHP were 315.3±21.7 ms and 298.8±14.3 ms, respectively (Figure 6F). The results suggested that BTHP mainly accelerated the slow deactivation procedure of the hERG channel. The shortened deactivation in the presence of BTHP suggested that the closure of the activation gate was delayed when the drug was bound to the channel. The results showed that neither the fast nor slow deactivation time course of the \(I_{\text{hERG}}\) tails was significantly altered by 30 μmol/L ALL (Figure 6E). To determine whether the deactivated kinetics were changed, the fast and slow time constant proportions \([A_1/(A_1+A_2)\) and \(A_2/(A_1+A_2)\)] under each test potential were calculated. The results showed that the fast and slow time constant proportions under each test potential did not change before or after exposure to the two drugs (Figure 6G and 6H).

The effects of ALL and BTHP on \(I_{\text{hERG}}\) availability and inactivation time-course

Two different protocols were used to analyze the effects of ALL and BTHP on the steady-state and fast inactivation of the hERG current. First, the voltage dependence of the steady-state inactivation/availability was measured using a condition pulse depolarization to +20 mV for 1000 ms and short pulses to potentials ranging from -120 mV to +20 mV for 20 ms with 10 mV-increments to recover the channels from inactivation, followed by a test potential of +20 mV for 1500 ms. In the protocol, we set the second voltage step pulse duration to 20 ms, which was well beyond 4 times the time constants of the recovery from inactivation for hERG channels; this ensured almost full recovery from inactivation by the end of the pulse. The current amplitudes were normalized and fitted to a Boltzmann function,
\[
\frac{I}{I_{\text{max}}} = \frac{1}{1 + \exp \left[ \frac{(V_{1/2} - V_m)}{k} \right]},
\]
where \(I/I_{\text{max}}\) is the relative current, \(V_{1/2}\) is the half-maximum inactivation voltage, \(V_m\) is the test membrane potential, and \(k\) is the slope factor. The experiment was performed in the absence and presence of the drug. Compared to the control, \(V_{1/2}\) of the steady-state inactivation curve of the hERG current shifted to a more negative potential (from -54.13±2.42 mV to -78.89±2.38 mV) with 30 μmol/L BTHP (\(P<0.01, n=17\)), while it showed...
only a slight change with 30 μmol/L ALL (from -55.33±3.14 mV to -57.38±2.52 mV) (Figure 7A–7D).

In the second approach, the voltage dependence of the time course of fast inactivation was investigated. From a holding potential of -90 mV, a condition test pulse of +50 mV for 1500 ms was applied to inactivate the channel; short pulses to -100 mV for 10 ms and voltage steps to potentials ranging from -20 mV to +60 mV for 1500 ms in 10-mV increments were then used to elicit large outward inactivating currents. The inactivating currents were fitted with single exponential functions to obtain a time constant\[25, 26\]. Compared to the control, the time constants of fast inactivation were significantly shortened by 30 μmol/L BTHP at a range of the various potentials, except at +60 mV (\(P<0.01, n=17\)). Similarly, the time constant of fast inactivation was shortened by 30 μmol/L ALL at -20 and 0 mV, but there was a slight change at +10 mV (Figure 7E–7H). Considered collectively, these results suggested that the two drugs had similar effects on the availability or inactivation time-course. Furthermore, BTHP caused a more negative potential shift of \(V_{1/2}\) and a shorter time constant than ALL.

The effects of ALL and BTHP on \(I_{hERG}\) recovery of channel inactivation

Recovery from inactivation was observed as a time-dependent initial increase in current amplitude at potentials from -120 mV to -50 mV for 3000 ms after a conditioning pulse of +50 mV for 1500 ms. The effects of ALL and BTHP on \(I_{hERG}\) recovery of channel inactivation are shown in Figure 8A and 8B. The tail
Figure 7. Effects of ALL and BTHP on steady-state inactivation and fast inactivation of hERG current. $V_{1/2}$ of the steady-state inactivation curve of hERG current showed more negative shift by 30 μmol/L BTHP (from -54.13±2.42 mV to -78.89±2.38 mV) ($P<0.01$, $n=17$), while showed only slight change by 30 μmol/L ALL (from -55.33±3.14 mV to -57.38±2.52 mV) (A–D). Inactivating currents were fitted with single exponential functions to obtain time constants. Voltage dependence of time course of fast inactivation was investigated. Compared with control, time constant of fast inactivation was significantly shortened by 30 μmol/L BTHP ($P<0.01$, $n=17$) but was only slightly shortened by 30 μmol/L ALL over +10 mV (E–H).
currents were fitted by a single exponential function\[^{[23]}\]. Compared with the control, the time constants for recovery from inactivation were significantly lengthened over -80 mV potentials after exposure to ALL. For BTHP, the time constants for recovery from inactivation were significantly lengthened over -90 mV potentials. Meanwhile, a more pronounced prolongation of the time constant was observed with BTHP (4.35 times the control) than with ALL (2.45 times the control) at a test potential of -50 mV (Figure 8C and 8D).

**The effects of ALL and BTHP on the \(I_{\text{hERG}}\) current with an action potential waveform pulse**

The characteristics of hERG currents during an action potential (AP) were tested using the AP-clamp technique\[^{[27, 28]}\]. An AP waveform of a human ventricular cell was stimulated at a 400 ms cycle length, digitized at 10 kHz and stored. The AP waveform was used as a command signal under voltage-clamp conditions. At least 10 consecutive waveforms were applied to reach stable electrical activity. The peak current densities, as a function of the voltage applied, showed a decrease of 43.22%±5.3% with ALL and 56.78%±2.7% with BTHP during the late phase of the action potential (\(n=6, P<0.01\)). The densities were not significantly different between the control and the two drugs during the early phase of the action potential (Figure 9).

**Discussion**

The inhibition effects of ALL and BTHP on \(I_{\text{hERG}}\) and clinical implications

It is very important to search for new antiarrhythmic agents because many antiarrhythmic drugs increase the tendency for heart arrhythmias and the occurrence of TdP ventricular tachycardias. It is of particular interest to investigate the effects of antiarrhythmic drugs on the hERG potassium channel. Likewise, a blockade of hERG channels by various drugs has been investigated previously (such drugs include azimilide, amiodarone, terikalant, dofetilide, clofilium and its analogue LY97241, haloperidol, terfenadine and carvedilol)\[^{[29–35]}\].

The major finding of this study is that two commonly prescribed Chinese herb drugs, \(ie\), ALL and BTHP, potently block the hERG current in a voltage- and concentration-dependent manner. This is, to our knowledge, the first study showing that ALL and BTHP are potent hERG blockers. Previous studies have indicated that BTHP has antiarrhythmic effects in various animal models\[^{[17–19]}\]. The electrophysiological effect of BTHP is related to the prolongation of the action potential duration, similar to that of class III antiarrhythmic agents. In previous studies, we found that BTHP could inhibit both...
The effects of ALL and BTHP on the \( I_{\text{hERG}} \) channel gating mechanism

As repolarization proceeds, a transient increase in the hERG current occurs due to the fast recovery from inactivation and slow deactivation, which electively repolarizes the cardiac cell\[38, 40\]. At negative repolarizing voltages, prominent tail currents are produced after the channels reopen and rapidly recover from inactivation, before closing at a slow rate\[38-39\]. Rapidly inactivation characteristics of channel makes hERG operate as an inward rectifier, although it has 6 membrane-spanning domains and regions typical of depolarization-activated channels\[31\]. It is necessary to investigate the effect of a new drug on hERG kinetic characteristics. In our experiment, a blockade of the hERG channel by both drugs led to several features in the inactivation state, deactivation states and during recovery of fast inactivation. The activation state and inward rectifier channel were not markedly affected. The \( V_{1/2} \) of the steady-state inactivation curve of the hERG current showed a significantly negative shift with BTHP (BTHP: \(-78.89±2.38 \text{ mV}\) vs Ctrl: \(-54.13±2.42 \text{ mV}\), \( P<0.001 \)) but not ALL (ALL: \(-57.38±2.52 \text{ mV}\) vs Ctrl: \(-55.33±3.14 \text{ mV}\)). The time constants of fast inactivation were significantly shortened with BTHP, while the time constants were slightly changed by 30 \( \mu \text{mol/L} \) ALL from \(-20 \text{ mV}–0 \text{ mV}\). Meanwhile, the slow time constants of deactivation were shortened with BTHP, but not with ALL. Furthermore, the time constants for recovery from inactivation were significantly lengthened by both drugs at all potentials compared to the control. This may be the major reason for the blockade effect of ALL on the hERG current, despite the more significant acceleration with BTHP. Collectively, these reasons may explain the stronger inhibition of BTHP on the hERG current, while the hERG currents were blocked by both drugs. BTHP blocked the tail currents (-40 mV) by 45.5%±2.3%, and ALL blocked the tail currents by 33.3%±1.6%. BTHP blocked the tail currents (-110 mV) by 45.6%±2.8%, and ALL blocked the tail currents by 27.8%±3.1%.

The effects of ALL and BTHP on the \( I_{\text{hERG}} \) current with the action potential clamp

The action potential clamp technique offers a valuable approach to the study of the dynamics of specific currents because the currents actually occur during the action potential. This tool is also used to study the dynamic properties of membrane ionic currents\[28, 53, 54\]. Similar to results of the voltage-clamp, the current densities showed a marked decrease of 49.2% with ALL blockade and 61.5% with BTHP blockade during the late phase of the action potential. The densities were not significantly different between the control group and after exposure to two drugs. (A) Representative current tracings before and after exposure to two drugs. (B) The current densities by two drugs, as a function of the voltage applied, showed marked decrease compared to control during late phase of action potential, especially for peak current. The peak current densities, as a function of the voltage applied, showed a decrease of 43.22%±5.3% of ALL and 56.78%±2.7% of BTHP during the late phase of action potential \( (n=6, B) \), while they were not significantly different during early phase of action potential. \( ^nP<0.01 \) vs control.

\( I_{\text{Kr}} \) and the “slow” delayed rectifier potassium current \( (I_{\text{Kr}}) \) without any obvious selectivity\[36-39\]. In other reports, we demonstrated that the threshold doses of aconitine and calcium chloride that induced ventricular ectopic beats, ventricular tachycardia and ventricular fibrillation in rats were increased by ALL. The electrophysiological measurements of ALL showed a prolongation of APD in the rabbit heart\[14, 15\]. We have also found that ALL exerted an inhibition effect on the transient outward potassium current \( (I_{\text{to}}) \) in a frequency-dependent manner and decreased the transmural gradient of \( I_{\text{to}} \[16\]. Both ALL and BTHP have a basic isoquinoline constitution (Figure 1) and belong to isoquinoline alkaloid categorization. Other reports showed that several isoquinoline alkaloid agents also had antiarrhythmic effects as a series of quaternary ammonium type of antiarrhythmic drugs\[40-47\]. Therefore, we can speculate that ALL and BTHP, as potential antiarrhythmic agents, may be brought into use in the near future.

**Figure 9.** Effects of ALL and BTHP on characteristics of hERG current with action potential waveform pulse. Digitized action potential waveform was used as command potential. (A) Representative current tracings before and after exposure to two drugs. (B) The current densities by two drugs, as a function of the voltage applied, showed marked decrease compared to control during late phase of action potential, especially for peak current. The peak current densities, as a function of the voltage applied, showed a decrease of 43.22%±5.3% of ALL and 56.78%±2.7% of BTHP during the late phase of action potential \( (n=6, B) \), while they were not significantly different during early phase of action potential. \(^nP<0.01 \) vs control.
α-subunit, which is modulated in vitro by single transmembrane domain ancillary subunits MiRP1 (encoded by KCNE2, respectively). KCNE2 has been linked to hereditary arrhythmias and pro-arrhythmic drug sensitivity. In this article, we have focused on the functional electrophysiological effects of ALL and BTHP on the HERG current. It is necessary to investigate the effects of the two drugs on co-expression experiments with HERG and KCNE2.

Acknowledgements
This study was supported by grants from the National Natural Science Foundation of China (No 81170177, No 30770901).

The authors would like to thank Prof Silvia G PRIORI from Molecular Cardiology, Fondazione Salvatore Maugeri IRCCS, at the University of Pavia in Italy for generously providing the hERG plasmid.

Author contribution
Yang LI designed the research; Kun LIN, Yu-qi LIU, Jin-liao GAO, and Bin XU performed research; Yi-cheng FU and Yu CHEN contributed new reagents or analytic tools; Qiao XUE and Yang LI analyzed data; Yang LI and Yu-qi LIU wrote the paper.

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