

Original Article

State-dependent blockade of human *ether-a-go-go*-related gene (hERG) K⁺ channels by changrolin in stably transfected HEK293 cells

Wei-hai CHEN^{1,2}, Wen-yi WANG¹, Jie ZHANG¹, Ding YANG^{1,2}, Yi-ping WANG^{1,*}

¹State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; ²Graduate University of Chinese Academy of Sciences, Beijing 100049, China

Aim: To study the effect of changrolin on the K⁺ channels encoded by the human *ether-a-go-go*-related gene (hERG).

Methods: hERG channels were heterologously stably expressed in human embryonic kidney 293 cells, and the hERG K⁺ currents were recorded using a standard whole-cell patch-clamp technique.

Results: Changrolin inhibited hERG channels in a concentration-dependent and reversible manner (IC₅₀=18.23 μmol/L, 95% CI: 9.27–35.9 μmol/L; Hill coefficient=-0.9446). In addition, changrolin shifted the activation curve of hERG channels by 14.3±1.5 mV to more negative potentials ($P<0.01$, $n=9$) but did not significantly affect the steady-state inactivation of hERG ($n=5$, $P>0.05$). The relative block of hERG channels by changrolin was close to zero at the time point of channel opening by the depolarizing voltage step and quickly increased afterwards. The maximal block was achieved in the inactivated state, with no further development of the open channel block. In the “envelope of tails” experiments, the time constants of activation were found to be 287.8±46.2 ms and 174.2±18.4 ms, respectively, for the absence and presence of 30 μmol/L changrolin ($P<0.05$, $n=7$). The onset of inactivation was accelerated significantly by changrolin between -40 mV and +60 mV ($P<0.05$, $n=7$).

Conclusion: The results demonstrate that changrolin is a potent hERG blocker that preferentially binds to hERG channels in the open and inactivated states.

Keywords: changrolin; human *ether-a-go-go*-related gene; *torsades de pointes*; whole-cell patch-clamp; anti-arrhythmic agent

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Introduction

The human *ether-a-go-go*-related gene (hERG or KCNH2) encodes the hERG K⁺ channels, which conduct the rapid delayed rectifier K⁺ current (I_{Kr})^[1]. Although hERG is expressed in multiple tissues and cell types, including neural, smooth muscle and tumor cells, it is most highly expressed in the heart^[1]. In the human heart, I_{Kr} is the most prominent component of phase 3 repolarization of the action potential. Inhibition of hERG channels tends to lengthen the cardiac action potential and the duration from the start of the QRS complex to the end of the T wave in the electrocardiogram (QT interval), and this prolongation of the action potential duration can be useful as an anti-arrhythmic property. However, excessive prolongation of the action potential duration leads to acquired long QT syndrome and life-threatening *torsades de*

pointes arrhythmias (TdP)^[2]. Many different types of drugs, including some anti-arrhythmics, antihistamines, antibiotics, gastrointestinal prokinetics and antipsychotics, have been shown to cause QT prolongation through the interference with hERG channels^[3]. The assessment of a direct hERG channel block has proven useful for the evaluation of drugs suspected of causing delays in cardiac repolarization and TdP^[2].

Changrolin [2,6-bis(pyrrolidin-1-ylmethyl)-4-(quinazolin-4-ylamino) phenol] (Figure 1) is derived from β-dichroine, an active component of the Chinese medicinal herb, *Dichroa febrifuga* Lour, and is used as a class I anti-arrhythmic agent^[4]. Changrolin has beneficial effects on the premature beats caused by coronary heart disease, the sequelae of myocarditis and the atrial premature beats caused by acute myocarditis^[5]. Its effectiveness against ventricular premature beats and paroxysmal supraventricular tachycardia is also supported by clinical trials^[6,7]. Class I anti-arrhythmic drugs normally block hERG channels and prolong the QT interval to a moderate extent, and thus they are pro-arrhythmic^[8,9]. Although

* To whom correspondence should be addressed.

E-mail ypwang@mail.shcnc.ac.cn

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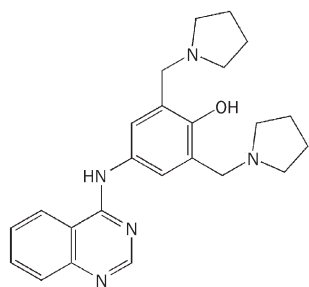


Figure 1. Chemical structure of changrolin [2,6-bis(pyrrolidin-1-ylmethyl)-4-(quinazolin-4-ylamino) phenol].

changrolin had been considered to have a good clinical safety profile, especially with respect to cardiac function^[5], a previous study reported that changrolin prolonged the QT interval in some patients^[6]. This result suggested that changrolin may block hERG channels. Also, available anti-arrhythmic drugs and their narrow therapeutic index have led researchers to explore the safety profile and effectiveness of alternative drugs, such as changrolin and its derivatives^[10, 11]. However, little information is available about the effect of changrolin on hERG channels, and this effect must be further clarified to avoid potentially life-threatening side effects in clinic practice.

Therefore, in this study we aimed to characterize the electrophysiological actions of changrolin on hERG channels expressed heterologously in human embryonic kidney (HEK) 293 cells by using the whole-cell patch-clamp technique. Our findings provide detailed insight into the biophysical mechanism of hERG channel blockade by changrolin.

Materials and methods

Cell culture

A conventional protocol was used to construct a cell line that expressed hERG channels^[12]. Briefly, pcDNA3 [a plasmid encoding the hERG gene (GeneBank accession No U04270)] was generously donated by Dr G ROBERTSON (University of Wisconsin). Human embryonic kidney (HEK) 293 cells were transfected with hERG/pcDNA3 using the Lipofect Transfection Reagent (Tiangen Biotech Co, Ltd, Beijing, China). The HEK293 cells that stably expressed hERG channels were cultured in Dulbecco's modified Eagle medium (DMEM; Invitrogen Corporation, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen Corporation, Carlsbad, CA, USA) and 0.4 g/L geneticin (G418) in an atmosphere of 95% air and 5% CO₂^[13].

Solutions and drug administration

The external bath solution for whole-cell patch-clamp studies contained (in mmol/L): NaCl 137, KCl 4, CaCl₂ 1.8, MgCl₂ 1, HEPES 10 and glucose 10 (pH adjusted to 7.4 with NaOH). The pipette solution contained (in mmol/L): KCl 130, CaCl₂ 1, MgCl₂ 5, Na₂-ATP 5, EGTA 5 and HEPES 10 (pH adjusted to 7.4 using KOH)^[12].

Chemical products used to prepare the external and inter-

nal solutions were purchased from Sigma-Aldrich Chemical Company (St Louis, MO, USA) or Sinopharm Chemical Reagent Corporation (Shanghai, China). Changrolin was kindly provided by the Department of Medicinal Chemistry at the Shanghai Institute of Materia Medica (Shanghai, China). Changrolin was diluted in bath solution to the desired concentration (1–300 μmol/L) from a stock solution (100 mmol/L in diluted hydrochloric acid, stored at -20 °C) before use.

Electrophysiological recordings

Whole-cell patch-clamp recordings were made at room temperature (22 °C to 26 °C) using a conventional patch-clamp technique. Currents were recorded using an Axopatch 200 A amplifier (Axon Instruments, Foster City, CA, USA) with a 2 kHz low-pass filter, digitized (at 50 μs intervals) and stored using the Digidata 1322 interface and the Pclamp/Clempex software (Axon Instruments, Foster City, CA, USA). For the description of tails, currents were expressed relative to the baseline at the holding potential (*ie*, -80 mV). Electrodes were constructed from borosilicate glass using a micropipette puller (P-97; Sutter Instrument Co, Novato, CA, USA). The final resistance of the electrode was 2 MΩ to 3 MΩ when filled with the pipette solution.

Statistical analysis

Data from patch-clamp experiments are expressed as mean±SEM, where n represents the number of experiments performed. Statistical significance was evaluated using a paired Student's *t*-test. A *P*-value < 0.05 was considered statistically significant. The concentration of changrolin needed to yield a 50% blockade of the hERG current (IC₅₀) was obtained by fitting the data to a Hill equation: $I/I_0 = 1/[1 + ([C]/IC_{50})^{nH}]$, where *I*₀ and *I* are the current amplitudes measured in the absence and presence of changrolin, respectively, [C] is the concentration of changrolin in the external solution and *nH* is the Hill coefficient.

Results

hERG channels were blocked by changrolin

The hERG channels of HEK293 cells were blocked by changrolin in a concentration-dependent manner. Figure 2A depicts the concentration-dependent inhibition of hERG channels by changrolin. The current was elicited by a 2-s depolarizing step to +20 mV, followed by a 1.6-s repolarizing step to -40 mV, which produced a large, slowly decaying, outward tail current that is the feature of hERG channels^[14]. In preliminary experiments, hERG tail current amplitudes after 5 min, 10 min, 20 min and 30 min of external bath solution application were 101.9%±1.3%, 101.2%±1.1%, 99.7%±1.3% and 97.8%±1.6%, respectively, of the current immediately after rupture (*n*=6). The holding potential was -80 mV in all experiments performed in this study, unless otherwise indicated. To assess the concentration dependence of hERG current inhibition by changrolin, peak tail currents in the presence of the drug were normalized to their respective control values and plotted as relative current amplitudes in Figure 2B (*n*=7). The mean data

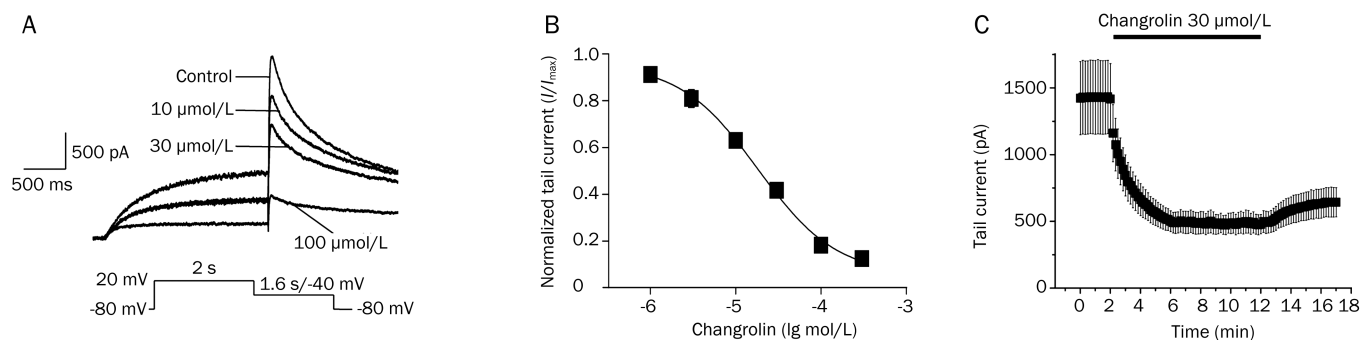


Figure 2. Inhibition of hERG channels by changrolin. (A) Representative current traces recorded from the same cell under control conditions and after superfusion with changrolin (10 $\mu\text{mol/L}$, 30 $\mu\text{mol/L}$ and 100 $\mu\text{mol/L}$). (B) Concentration-response relationship of the effects of changrolin on hERG peak tail currents ($n=7$). The IC_{50} was 18.23 $\mu\text{mol/L}$ (95% CI: 9.27–35.9 $\mu\text{mol/L}$) with a Hill coefficient of -0.9446. (C) Time course of hERG tail current inhibition by 30 $\mu\text{mol/L}$ changrolin ($n=6$).

points were fitted with the Hill equation. According to the concentration-response relationship, the IC_{50} was 18.23 $\mu\text{mol/L}$ (95% CI: 9.27–35.9 $\mu\text{mol/L}$) with a Hill coefficient of -0.9446. After a 2-min control recording, changrolin was applied to the bath. Inhibition of the hERG channels at 30 $\mu\text{mol/L}$ changrolin reached steady-state conditions after 4 min. Upon washout for 5 min, the blocking effects of changrolin on the hERG channels were partially reversed to $48.3\pm 6.8\%$ (Figure 2C, $n=6$).

Effect of changrolin on hERG channels activation

The effect of changrolin on the hERG current-voltage ($I-V$) relationship is shown in Figure 3. hERG currents were elicited from a holding potential of -80 mV by 2-s depolarizing steps from -80 mV to +80 mV applied in 10-mV increments at 0.1 Hz to produce activation currents, followed by a repolarizing step to -40 mV for 1.6 s to elicit outward tail currents. Representative currents were recorded under control conditions and 5 min after the application of 30 $\mu\text{mol/L}$ changrolin in the same cell (Figure 3A and 3B, respectively). Under control conditions, hERG currents had an activation threshold of -40 mV, and the activating currents reached a current maximum at +10 mV. A considerable current reduction at higher test pulse potentials was observed due to inward rectification. Figure 3C shows the $I-V$ curve at the end of the activating currents. After the application of 30 $\mu\text{mol/L}$ changrolin, the current maximum (at 0 mV) was reduced by $42.1\pm 7.7\%$ ($n=6$). Tail currents were saturated after a test pulse potential of +30 mV or above (Figure 3D), and changrolin at a concentration of 30 $\mu\text{mol/L}$ reduced the peak tail current amplitude (after a test pulse to +80 mV) by $59.9\pm 3.1\%$ ($n=6$). Activating and tail currents normalized to the maximum currents in the absence (control) and presence of changrolin (30 $\mu\text{mol/L}$) as shown in Figure 3E and 3F. Changrolin caused a 10-mV shift in the peak activating currents toward more negative potentials. The mean half-maximal activation voltage ($V_{1/2}$) of the activation curve of the hERG tail currents was shifted by 13.3 ± 2.2 mV toward negative potentials ($P<0.01$, $n=6$).

A second voltage protocol was used to study the activation curves of hERG tail currents^[15], where variable test pulses

ranging from -100 mV to +100 mV (400 ms) were applied in 10-mV increments to measure the corresponding inward tail current amplitude at -120 mV (400 ms) in the absence and presence of changrolin (30 $\mu\text{mol/L}$) (Figure 4A and 4B). The large inward tail currents elicited by the voltage step to -120 mV were normalized to the maximum currents, plotted as a function of the preceding test pulse potential and fitted to a Boltzmann equation to obtain activation curves (Figure 4C). Changrolin shifted the $V_{1/2}$ by 14.3 ± 1.5 mV toward more negative potentials ($P<0.01$, $n=9$).

hERG channels were blocked by changrolin in a state-dependent manner

Three approaches were used to examine the state-dependent block of hERG channels by changrolin. In the first protocol, the voltage was stepped from -80 mV to 0 mV for 7.5 s to induce a large activation current, which was first recorded under control conditions. After the control measurement, the cells were incubated with 100 $\mu\text{mol/L}$ changrolin for 5 min without any intermittent test pulse while holding the channels in the closed state at -80 mV. Measurements with changrolin were then performed. As illustrated in the representative experiment shown in Figure 5A, at the end of the depolarization pulse (0 mV), changrolin (100 $\mu\text{mol/L}$) inhibited the hERG outward current by $70.0\pm 6.5\%$ ($n=5$); however, the initial portion of the current trace in the presence of changrolin (100 $\mu\text{mol/L}$) appears to be the same as the current trace recorded under control conditions. Figure 5B illustrates the time-dependent increase of relative blockade, as determined by the normalization and division of current traces before and after exposure to changrolin. The block was phasic from the beginning of the test pulse (*ie*, the relative block was not observed at the onset of depolarization and developed rapidly within 1 s). Analysis of the depolarizing pulse after exposure to changrolin revealed that the blockade occurred very quickly, with more than 80% of the block developing within the first second. Similar results were obtained from five experiments.

A modified protocol was used to determine if the hERG channels were blocked by changrolin in the inactivated state.

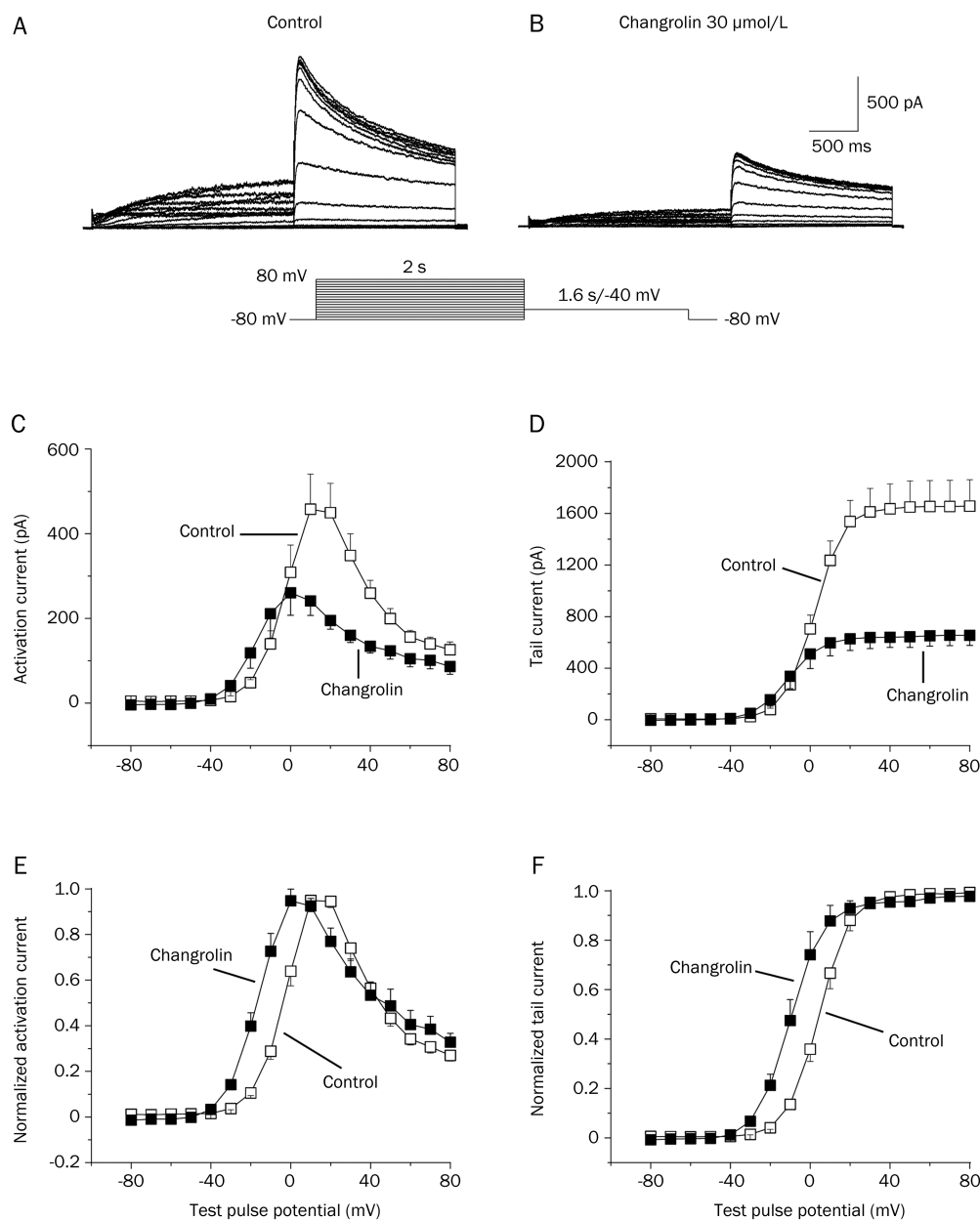


Figure 3. Effects of changrolin on the voltage dependence of hERG channel activation. Control measurement (A) and the inhibitory effect of 30 $\mu\text{mol/L}$ changrolin (B) are shown in the same cell. (C) The corresponding activating current amplitude at the end of the first test pulse is shown as a function of the test pulse potentials. Following the application of 30 $\mu\text{mol/L}$ changrolin, the peak current amplitude was reduced by $42.1\pm 7.7\%$ ($n=6$). (D) The tail current amplitude as a function of the preceding test pulse potentials. The application of 30 $\mu\text{mol/L}$ changrolin reduced the peak tail current amplitude by $59.9\pm 3.1\%$ ($n=6$). (E and F) Normalized activating and tail currents in the absence and presence of 30 $\mu\text{mol/L}$ changrolin. Changrolin shifted the peak activating currents by 10 mV toward more negative potentials. The activation curves of hERG tail currents were also shifted by 13.3 ± 2.2 mV toward more negative potentials ($P<0.01$).

A long test pulse to +80 mV (4000 ms) from a holding potential of -80 mV was applied to inactivate the channels, followed by a second test pulse to 0 mV (3750 ms) to rapidly recover the channels from inactivation. Figure 5C displays the current traces from a typical experiment before and after incubation with changrolin for 5 min. The normalized relative blockade upon channels during the second test pulse (0 mV) is illustrated in Figure 5D. The inhibition of hERG channels was obtained during the preceding inactivation (+80 mV pulse). No additional time-dependent blockade of the open channels was observed during the 0 mV pulse, suggesting that the maximal inhibition of the hERG channels was obtained during the first inactivation test pulse to +80 mV, whereas the vast majority of channels remained in the inactivated state. Similar results were obtained from six experiments.

An "envelope of tails" protocol was performed to assess the development of hERG channel blockade by changrolin during depolarization^[16]. Cells were depolarized to +30 mV from a holding potential of -80 mV for a variable duration from 25 ms to 1600 ms, followed by a repolarizing step to -50 mV to elicit hERG tail currents every 10 s. Tail currents at -50 mV were measured after each test pulse. The "envelope of tails" test was performed in the same cell both before and after the addition of 30 $\mu\text{mol/L}$ changrolin. Original current traces in the absence and presence of 30 $\mu\text{mol/L}$ changrolin are shown in Figure 6A and B. Both in the absence and the presence of changrolin, the hERG tail current amplitude increased with the depolarizing pulse duration over the first few pulses and then reached a steady maximal amplitude. To evaluate the hERG current activation, the time constants of the activation

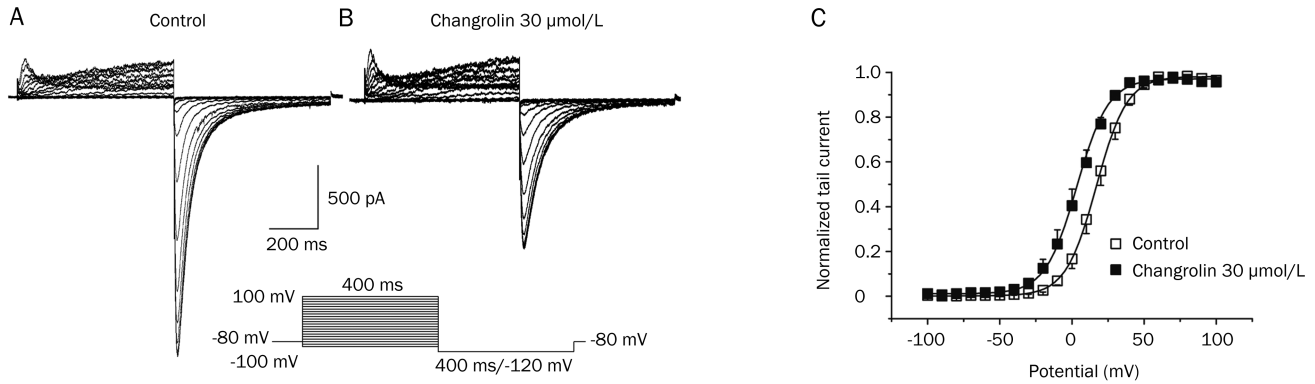


Figure 4. Changrolin shifted the hERG current activation curves to more negative potentials. The activation of the hERG current curves was studied in detail using a two-step protocol (lower panel) that gave rise to large inward tail currents. (A and B) A representative experiment in the absence and presence of 30 μmol/L changrolin. (C) Peak inward tail currents were normalized to the maximum currents, plotted as a function of the preceding test pulse potential and fitted to a Boltzmann equation to obtain activation curves. Changrolin caused a significant shift in the mean half-maximal activation voltage by 14.3 ± 1.5 mV toward more negative potentials ($P < 0.01$, $n = 9$).

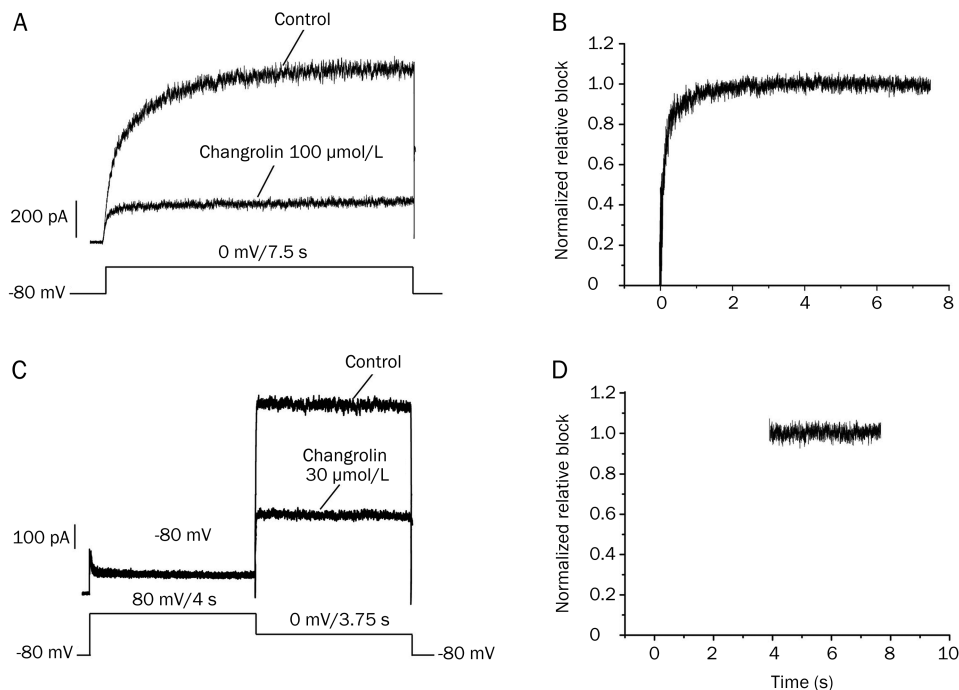


Figure 5. Changrolin blocked hERG channels that were in the open and inactivated states. To determine if hERG channels are blocked by changrolin in the open or the closed states, the voltage was stepped from -80 mV to 0 mV for 7.5 s to induce a large activation current. Shown are overlay experiments (A) for control and after incubation with 100 μmol/L changrolin (for 5 min, without intermittent test pulses). (B) The normalized relative block was plotted versus time after a voltage step to 0 mV, indicating a rapid open channel block without a major blockade of closed channels. Similar results were obtained in five experiments. (C) Inhibition of inactivated channels by 30 μmol/L changrolin. To investigate whether hERG channels were blocked by changrolin in the inactivated state, a holding potential of -80 mV kept hERG channels in their closed state while a 4000-ms test pulse to +80 mV led to channel inactivation, which was followed by channel opening at 0 mV. The corresponding normalized relative block during the test pulse to 0 mV is shown in (D). Maximum inhibition was achieved at the beginning of the second pulse, and no further time-dependent blockade occurred upon channel opening during the second voltage step. Similar results were obtained in six experiments.

were obtained by fitting the peak amplitude of the tail currents with a single exponential function (Figure 6C). The blockade of the hERG channels yielded time constants of 287.8 ± 46.2 ms and 174.2 ± 18.4 ms for the absence and presence, respectively,

of 30 μmol/L changrolin ($P < 0.05$, $n = 7$; Figure 6D).

Effects of changrolin on hERG channel inactivation kinetics

Drugs that block ion channels often affect the kinetics of chan-

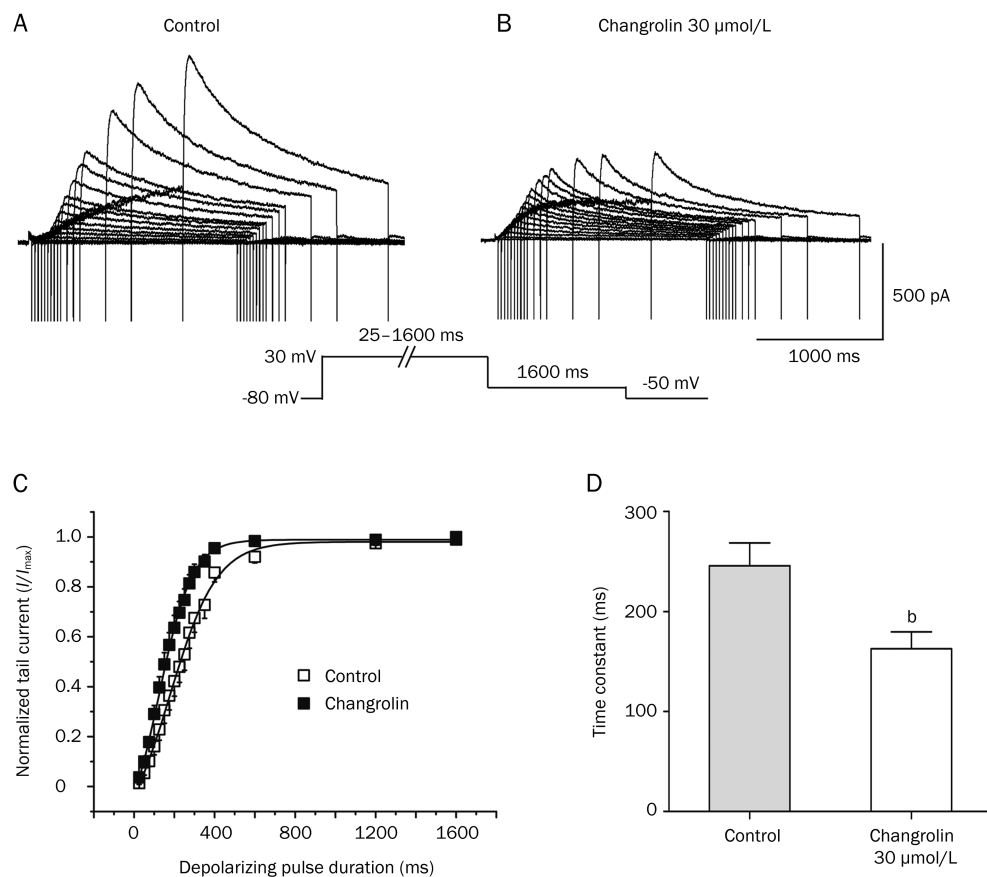


Figure 6. Development of hERG channel blockade during depolarization was assessed using the “envelope of tails” protocol (lower panel). (A and B) Representative original current traces of the hERG channel, elicited by the “envelope of tails” protocol in the absence and presence of 30 μmol/L changrolin. (C) The peak tail current elicited by a repolarizing step to -50 mV was plotted as a function of the test pulse duration. Solid lines were obtained by fitting with a single exponential function. (D) The pooled time constants of hERG current activation in the absence and presence of 30 μmol/L changrolin were 287.8±46.2 ms and 174.2±18.4 ms, respectively (differences were considered significant, with ^b $P < 0.05$, $n = 7$).

nel gating^[17]. A three-pulse protocol was used to study the effects of changrolin on the onset of hERG channel inactivation. The cells were first depolarized from the holding potentials at -80 mV to +60 mV for 200 ms to promote inactivation, followed by recovery from inactivation at -100 mV for 10 ms, which is a sufficient time for recovery from inactivation, as indicated in Figure 7A. Then, the recovered channels were forced to re-inactivate at potentials between -40 mV and +60 mV in 10-mV increments for 150 ms at 0.1 Hz. The time constants of inactivation were measured by fitting a single exponential function to the decaying current traces. The onset of inactivation was accelerated significantly at potentials between -40 mV and +60 mV ($P < 0.05$, $n = 7$; Figure 7B).

To investigate the effects of changrolin on steady-state inactivation, the channels were inactivated at +60 mV for 200 ms and were then recovered from inactivation at various potentials ranging from -100 mV to +10 mV in 10-mV increments for 10 ms at 0.1 Hz. Finally, the channels were re-inactivated at +60 mV, and the resulting peak outward currents at +60 mV were recorded as a measure of steady-state inactivation (Figure 7C). After obtaining control measurements, the potential was clamped at -80 mV, 30 μmol/L changrolin was applied to the cells for 5 min and the protocol was repeated. The inactivating outward current amplitude was measured at +60 mV, normalized and fitted to a Boltzmann function to obtain inactivation curves (Figure 7D). The half-maximal inactivation voltage was shifted slightly by 3.1±2.1 mV to negative potentials,

but this shift was not statistically significant ($n = 5$, $P > 0.05$).

Discussion

In the present study, we investigated the effects of changrolin on hERG K⁺ channels expressed heterologously in HEK293 cells for the first time. Our findings demonstrated that changrolin potently inhibited hERG channels heterologously expressed in HEK293 cells in a concentration- and state-dependent manner. The onset of the block was fast, and the inhibition was partly reversible upon washout. Changrolin blocked hERG potassium channels mainly in the open and inactivated states.

Blockade of hERG channels by changrolin has several features that are typical of blockade in open and inactivated states, as demonstrated using specially designed voltage protocols to discriminate between the different states. We found that channel inhibition by changrolin was voltage dependent, increasing at positive potentials, and was associated with a hyperpolarizing shift in the voltage dependence of activation (Figures 3 and 4). In addition, the blockade by changrolin was close to zero at the time point of channel opening by the depolarizing voltage step and quickly increased afterwards, and the maximal block was achieved in the inactivated state with no further development of open channel blockade (Figure 5). These results indicate that changrolin blocks the open and inactivated states of hERG channels rather than the closed state, which is a common pattern among hERG

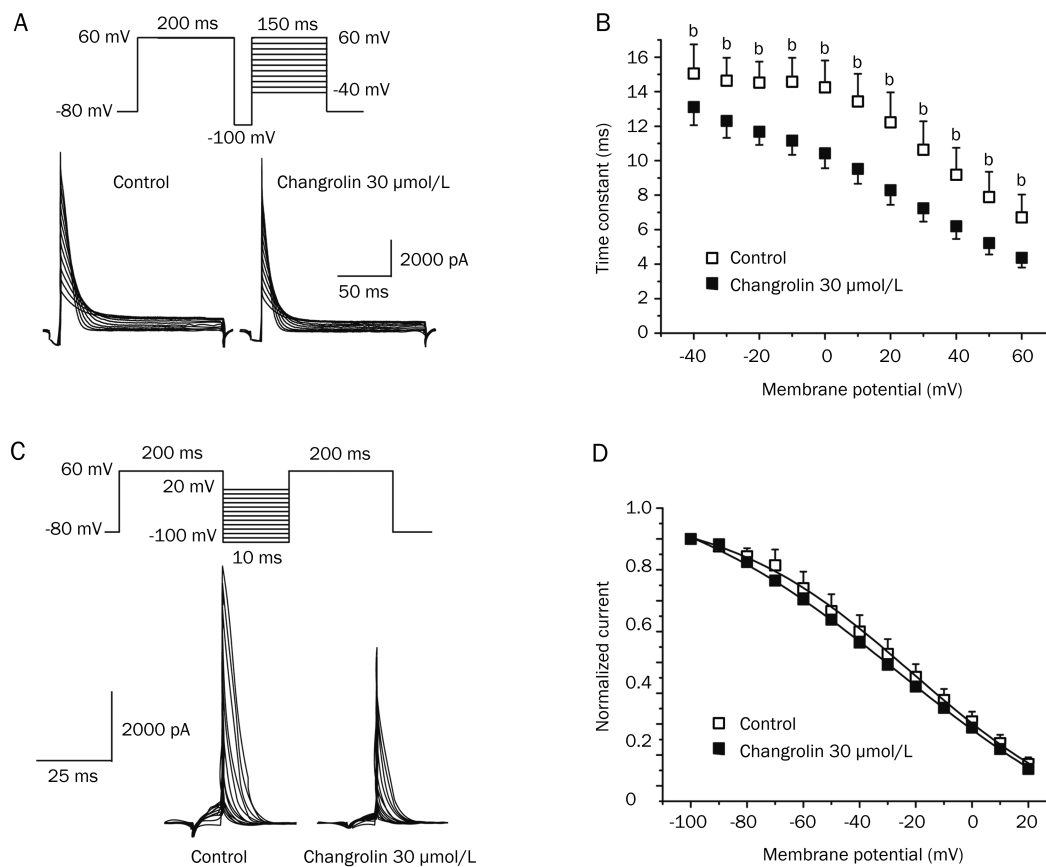


Figure 7. Effects of changrolin on hERG current inactivation. (A) Time constants of inactivation were investigated using a three-stage voltage protocol (inset). The original traces in the absence and presence of 30 $\mu\text{mol/L}$ changrolin are shown. (B) Voltage dependence of the time constants for the onset of inactivation in the absence and presence of changrolin. The onset of inactivation was accelerated significantly at potentials between -40 mV and +60 mV (differences were considered significant with $^bP < 0.05$, $n = 7$). (C) Representative current traces of steady-state inactivation were elicited by the protocol (inset), and the region of interest is magnified for clarity. (D) The inactivating outward current amplitude measured at +60 mV was normalized and fitted to a Boltzmann function to obtain steady-state inactivation curves. Only a small shift was observed in the steady-state inactivation curves without significance ($n = 5$, $P > 0.05$).

antagonists^[18, 19].

We used an “envelope of tails” protocol to find that the blockade was enhanced by the further activation of currents (Figure 6), which further supports the hypothesis that changrolin blocks the hERG channels primarily in the open and/or inactivated states rather than in the closed state. To further characterize the state-dependence blockade, we assessed the effects of changrolin on the kinetics of inactivation of the hERG channel. Changrolin accelerated the onset of inactivation at potentials within the range of normal channel activation, but we saw no evidence that changrolin affected the steady-state inactivation of hERG channels (Figure 7). The electrophysiological mechanism of hERG blockade by changrolin is similar to the mechanisms of other drugs known to induce acquired long QT syndrome through the inhibition of hERG channels^[17, 20, 21].

The IC_{50} of changrolin in HEK293 cells was 18.23 $\mu\text{mol/L}$. In a previous clinical study, the effective plasma concentration of changrolin was approximately 6.0 $\mu\text{mol/L}$ to 6.7 $\mu\text{mol/L}$ after continuous intravenous infusion^[6]. In light of the rela-

tionship between the hERG current blockade, QT prolongation and TdP, a 30-fold safety margin has been provisionally recommended between the maximal effective plasma concentration and the hERG IC_{50} *in vitro*^[22]. These safety requirements were not achieved by changrolin. In addition, the risk of QT prolongation of ventricular repolarization or TdP is increased in patients with organic heart diseases (*eg*, congenital long QT syndrome, myocardial infarction, congestive heart failure, dilated cardiomyopathy, hypertrophic cardiomyopathy, bradycardia, hypokalemia and hepatic impairment)^[3]. Therefore, clinicians must practice constant vigilance when changrolin is used in patients with pre-existing heart disease, any of the risk factors listed above, previous ventricular arrhythmias and electrolyte imbalances, such as hypokalemia. Also, physicians should be aware of the potential risks of interactions with other medications that prolong the QT interval, inhibit hepatic cytochrome P450, or cause electrolyte disturbances^[3]. We suggest monitoring of these patients routinely during the initiation of changrolin therapy and during any change in the dosage and discontinuation of therapy in cases of QT interval

prolongation.

Our data demonstrate that the hERG channels expressed in HEK293 cells are blocked by changrolin in a concentration-dependent manner. Blockade of hERG potassium channels by changrolin is preferentially dependent on the open and inactivated states. This study is to reveal how changrolin affects hERG channels and furthermore suggests that changrolin treatment should be administered with caution.

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Author contribution

Yi-ping WANG and Wei-hai CHEN designed the research; Wei-hai CHEN and Wen-yi WANG performed the research; Jie ZHANG and Ding YANG analyzed the data; and Wei-hai CHEN and Yi-ping WANG wrote and revised the paper.

Abbreviations

HEK, human embryonic kidney; hERG, human *ether-a-go-go*-related gene; TdP, *torsades de pointes*

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