Hirsutenone Directly Blocks Human *ether-a-go-go* Related Gene K^+ Channels

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Received May 12, 2011; accepted September 8, 2011; published online September 26, 2011

The aim of the present study was to investigate whether hirsutenone affects the human *ether-a-go-go* related gene (hERG) K^+ channels. Many drugs promote formation of the acquired form of long QT syndrome (LQTS) by blocking the hERG K^+ channels. Hirsutenone, a new candidate for the treatment inflammatory skin lesions, induced a concentration-dependent decrease in hERG K^+ current amplitudes. Hirsutenone significantly decreased the time constants at the onset of inactivation. However, the reductions in the time constants of steady-state inactivation and the recovery from inactivation after hirsutenone treatment were not significant. In addition, the drug had no effect on the voltage-dependent activation curve or the steady-state inactivation curve. In summary, hirsutenone potentially acts as a blocker of hERG K^+ channels functioning by modifying the channel inactivation kinetics.

Key words human *ether-a-go-go* related gene; hirsutenone; long QT syndrome; patch clamp

The K^+ channel controlled by the human *ether-a-go-go* related gene (hERG) conducts the rapidly activating delayed rectifier K^+ current (I_{Kr}) in cardiac myocytes, which plays a central role in the repolarization of action potentials.[1—3] Loss of function or blockade of the hERG K^+ channel can induce long QT syndrome (LQTS), which is characterized by a prolonged QT interval on an electrocardiogram and fatal arrhythmias such as torsade de pointes (TdP). Many drugs exert cardiotoxic effects by blocking I_{Kr} and the hERG K^+ channel and cause an excessive prolongation of the QT interval, leading to an acquired form of the LQTS.[4,5] Therefore, the regulatory guidelines [CPMP/986/96 (1997) and ICH S7B (2005)] recommend the use of *in vivo* and *in vitro* preclinical studies to detect the QT prolonging effects and arrhythmogenic potential of new chemical entities.[4,6,7] The hERG K^+ channel assay is one of the recommended *in vitro* studies for detection of potential drug-induced LQTS.[4] I_{Kr} reduction can be caused by acute inhibition of hERG K^+ channels or chronic drug-induced inhibition of hERG K^+ channel trafficking.[8—10] The disruption of hERG K^+ channel trafficking may reduce the hERG K^+ protein density on the cell membrane, which can reduce I_{Kr} leading to prolongation of cardiac repolarization.[9,11] Currently, it is well accepted to combine electrophysiological recording and Western blot analysis to assess drug safety.[4,12,13]

Hirsutenone is an active form of diarylheptanoids compound isolated from the bark of *Alnus japonica*, which has been used for fever, hemorrhage, diarrhea, gastroenteric disorder, lymphatic disease, and cancers in traditional oriental medicine.[14] Previous studies have demonstrated that hirsutenone has a wide range of biological activities, including anti-inflammatory and anti-tumor promoting effects.[15—19] Recently, topical application and intraperitoneal injection treatment with hirsutenone on NC/Nga mice with *Dermatophagoies farina* containing cream-induced skin lesions showed the therapeutic effects.[20] However, there is no information and no study about arrhythmogenic potential of hirsutenone.

The present study was designed to investigate whether hirsutenone affects the electrophysiological properties and the protein trafficking of hERG K^+ channels.

MATERIALS AND METHODS

Cell Cultures hERG cDNA was a generous gift from Dr. Tong Mook Kang, Ph.D. (Professor, Department of Physiology, College of Medicine, Sungkyunkwan University, Suwon, Korea). hERG cDNA was subcloned into mammalian expression vector pCDNA3.1 (Invitrogen, U.S.A.) for transfection into cells. Transfection of hERG into Chinese Hamster Ovary (CHO) cells was achieved *via* the lipofectamin method (Life Technologies, U.S.A.). The stably transfected hERG-CHO cells were generated through G418 (Gibco, U.S.A.) antibiotic selection. The transfected clones were selected through the use of 1000 μg/ml of G418. The CHO cells stably expressed hERG (hERG-CHO) were maintained in F-12 supplemented with 10% fetal bovine serum and 500 μg/ml G418.

Hirsutenone Hirsutenone was kindly provided from Dr. Min Won Lee, Ph.D. (Professor, College of Pharmacy, Chung-Ang University, Seoul, Korea) and was dissolved in dimethyl sulfoxide (DMSO) to obtain a 100 mM stock solution, which was stored at −20 °C. Hirsutenone was diluted in bath solution to achieve the final concentration. The highest concentration of DMSO used in this study was 0.05%.

Electrophysiological Recordings hERG K^+ currents were recorded using the whole-cell patch clamp technique. For the hERG K^+ current recording, the bath was perfused with *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES)-buffered Tyrode solution containing (in mM) NaCl 155, KCl 5, $MgCl_2$ 1, HEPES 10, and glucose 10 (pH 7.3 with NaOH). The bath solution was perfused at a rate of 0.7 ml/min. The pipette solution contained (in mM) KCl 140, Mg-ATP 5, $MgCl_2$ 1, KH_2PO_4 10, ethylene glycol bis(2-

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aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) 0.1, and HEPES 5 (pH 7.2 with KOH). The electrodes were constructed from borosilicate glass (WPI) using a micropipette puller (Narishige, Japan) and were heat-polished with a microforge (Narishige). The resistance of the electrode when filled with the pipette solution was 2—3 MΩ. Membrane currents were recorded with an Axopatch 200B amplifier (Axon Instruments, U.S.A.) and a Digidata 1322A (Axon Instruments). Currents were filtered at 2 kHz and digitized at 10 kHz. Capacitance and series resistance compensations were optimized. The pCLAMP 9.2 software (Axon Instruments) was used to generate voltage clamp protocols and analyze current traces.

Western Blot Analysis Cells were solubilized at 4 °C in radio immunoprecipitation assay (RIPA) buffer (Sigma, U.S.A.) containing a protease inhibitor. Protein concentrations were determined with the Bradford protein assay (BIO-RAD). Equal amounts of proteins were separated on a 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and were transferred to nitrocellulose membranes (Whatman, Germany). Membranes were blocked in 10% skim milk overnight and incubated with anti-hERG antibody (Santa Cruz Biotechnology, U.S.A.), followed by horseradish peroxidase conjugated secondary antibody (Santa Cruz Biotechnology). The membranes were then developed using Western blotting luminal reagent (Santa Cruz Biotechnology) for electrochemiluminescence detection in LAS-1000 V1.5 (Fujifilm, Japan).

qRT-PCR (Quantitative Reverse Transcriptase Polymerase Chain Reaction) RNA isolation and reverse transcription reaction were performed using the PureLink™ RNA Mini kit (Invitrogen) and SuperScript™ II (Invitrogen), respectively. The hERG mRNA expression levels were quantified using qRT-PCR using LightCycler 1.0 (Roche, Germany). cDNA derived from hERG-CHO cells was amplified using LightCycler FastStart DNA Master SYBR Green (Roche) in the presence of primers specific for hERG and β-actin. The qRT-PCR was performed with an initial pre-incubation step for 10 min at 95 °C, followed by 40 cycles for denaturation at 95 °C for 20 s, annealing at 60 °C for 20 s and extension at 72 °C for 20 s. The primers, 5′-TCAACCTGCGAGATACCAACATG-3′; 5′-CTGGCTGCTCCGTGTCCTT-3′ and 5′-CTTCAACACCCCAGCCATGT-3′; 5′-GTGGTACGACCAGAGGCATACA-3′ were used for amplification of hERG[21] and β-actin,[22] respectively.

Statistical Analysis Data were expressed as mean±standard error of the mean (S.E.M.). Data analysis and curve fitting were performed with Clampfit 9.2 (Axon Instruments) and Origin 8.0 software (OriginLab). Statistical comparisons were made using a two-tailed Student's *t*-test. *p*-Values <0.05 were considered statistically significant.

RESULTS

Inhibition of hERG K^+ Currents by Hirsutenone The effect of hirsutenone on the hERG K^+ current was assessed in CHO cells expressing hERG K^+ channels using the whole-cell patch clamp technique. The hERG K^+ currents were elicited from a holding potential of −80 mV by test pulses ranging from −60 to +40 mV in 10 mV steps. Each test pulse had a duration of 4 s duration and was applied every 8 s. Each test pulse was followed by a repolarization step to −50 mV for 2 s to evoke tail current. Twenty micromolar hirsutenone was applied to the bath for 20 min after control currents were recorded. Hirsutenone reduced both hERG currents during I_{step} (the depolarizing test pulse) and I_{tail} (the repolarizing pulse) (Fig. 1A). Normalized current–voltage (*I–V*) relationships of both I_{step} (left panel) and I_{tail} (right panel) are illustrated in Fig. 1B, which shows that I_{step} increased from −60 to +10 mV, peaked around +10 mV and then decreased with further depolarization. I_{tail} was increased with depolarizing voltages and reached a plateau around +30 mV. Hirsutenone reduced both I_{step} and I_{tail} amplitudes at all test potentials.

Concentration Dependence of hERG K^+ Channel Blockade At the hirsutenone concentrations of 5, 10, 20, 30 and 50 μM, the peak amplitudes of I_{step} and I_{tail} were measured in the same cell and were normalized to the control value. Hirsutenone blocked both hERG I_{setp} and I_{tail} in a concentration-dependent manner. The fractional blockages in I_{step} were 9.1±1.5%, 16.9±7.1%, 28.0±6.6%, 42.1±4.7% and 68.4±9.6%, and those in I_{tail} were 13.3±3.1%, 29.5±2.9%, 42.2±10.2%, 55.89±8.8% and 83.7±10.8% in the 5, 10, 20, 30 and 50 μM hirsutenone groups, respectively ($n=10$, Fig. 1C insert). Compared with the control, hirsutenone showed significant concentration-dependent inhibitory effects on I_{step} and I_{tail}. To obtain the IC_{50} of hirsutenone, concentration–response relationships of I_{tail} were fitted using the Hill equation. The IC_{50} of hirsutenone was 14.9±2.0 μM ($n=10$, Fig. 1C). In addition, an irreversible inhibitory effect of hirsutenone was observed (data not shown).

Effects of Hirsutenone on hERG K^+ Channel Kinetics We evaluated the voltage dependence of activation by plotting normalized I_{tail} as a function of voltage (Fig. 1D). Data were fitted with the Boltzmann function: $I=I_{max}[1+\exp(V_{1/2}-V)/k]^{-1}$, where I_{max}, $V_{1/2}$ and k are the maximum amplitude, half-activation voltage and slope factor, respectively. In this protocol, hirsutenone changed neither the half-activation voltage nor the slope factor ($V_{1/2}$: −3.6±2.8 mV and −2.9±1.8 mV; k: 9.3±1.9 and 10.3±1.8; in controls and after hirsutenone, respectively; $n=10$, $p>0.05$). These results indicated that the rate of activation was not changed after treatment with of 20 μM hirsutenone.

To measure steady-state inactivation, a special protocol was used to inactivate the channel at a holding potential of +20 mV for 2 s, recover the channel from inactivation at various potentials from −120 to +60 mV for 15 ms in 10 mV steps, and measure the resulting peak outward current at a constant +20 mV in the absence and presence of 20 μM hirsutenone (Fig. 2A). In Fig. 2B, the inactivating outward current amplitude was normalized and plotted against the test pulse potential, producing the steady-state inactivation curve. This curve was fitted with a Boltzmann function, in which the $V_{1/2}$ values were −43.5±4.5 mV in the control and −37.4±4.9 mV in hirsutenone-treated samples; the corresponding k values were 19.6±4.2 and 19.8±4.2, respectively ($n=10$). Statistical significance was not detected with $V_{1/2}$ or k values. Hirsutenone did not significantly affect the half-maximal inactivation voltages of the hERG channels; no significant shift was observed in the inactivation curve. The time course of channel inactivation was also assessed. To obtain the time constants of steady-state inactivation, the de-

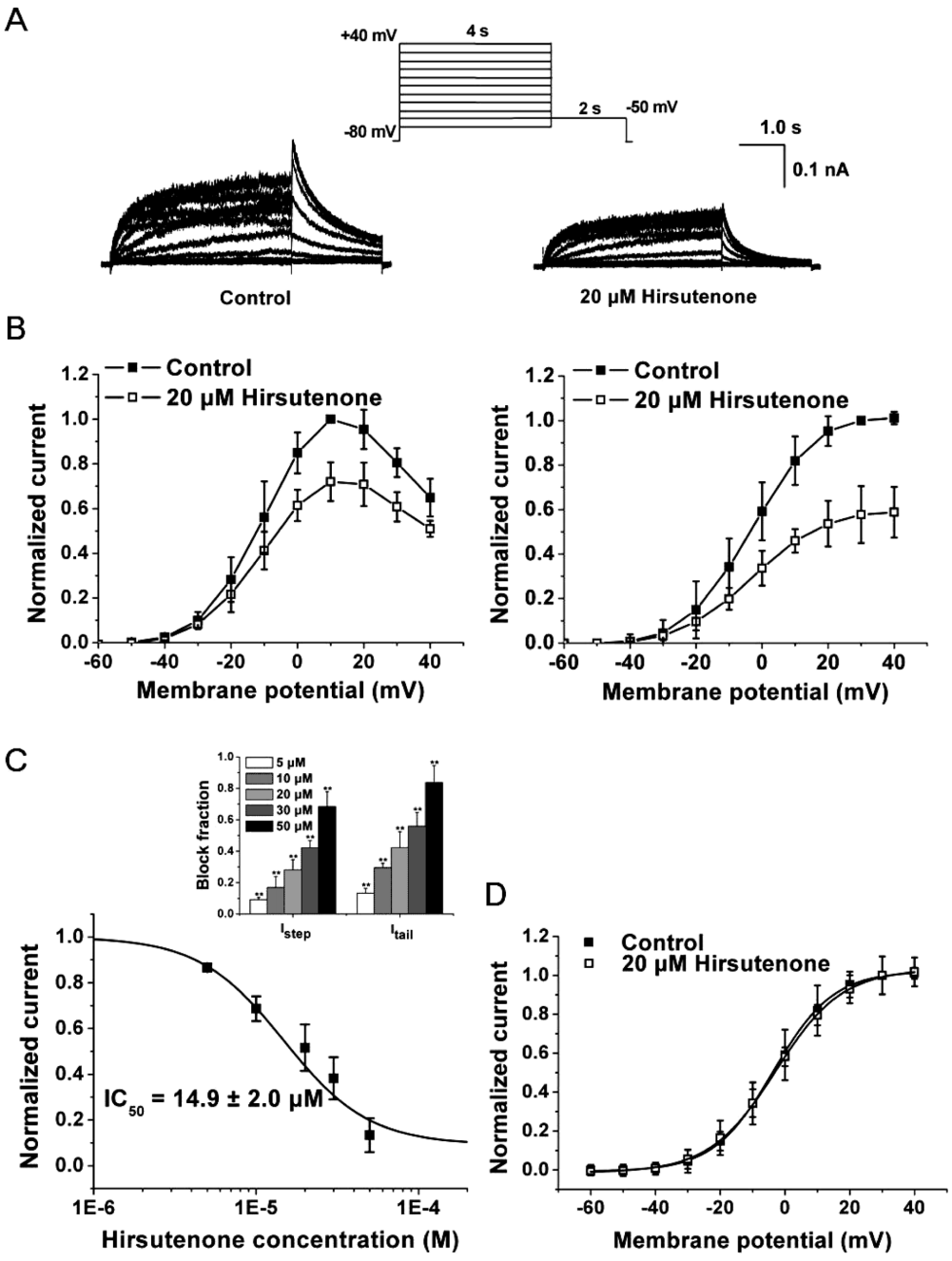

Fig. 1. Effects of Hirsutenone on hERG K^+ Currents

(A) Representative hERG K^+ current traces in control and in the presence of 20 μM hirsutenone (lower panel) were recorded using a two-stage voltage protocol (upper panel). (B) Normalized *I–V* relationships for current measured at the peak of depolarizing steps current (I_{step}, left panel) and repolarizing tail current (I_{tail}, right panel) in the absence and presence of 20 μM hirsutenone ($n=10$). (C) Concentration–response relationships for hirsutenone in I_{tail}. Data were fitted with the Hill equation and IC_{50} was 14.9±2.0 μM ($n=10$). (C, insert) Mean fractional blocks of I_{step} (at +10 mV) and I_{tail} (at +30 mV). At the hirsutenone concentrations of 5, 10, 20, 30, 50 μM, the peak current amplitudes were measured, normalized to the control amplitude, and then averaged ($n=10$, $^{**}p<0.01$). (D) Voltage-dependent activation curves in the absence and presence of 20 μM hirsutenone, as calculated from the normalized peak tail current amplitudes ($n=10$). Smooth curves were fitted with the Boltzmann function.

cays of the outward currents in Fig. 2A were fitted to single exponential functions and plotted against voltages (Fig. 2C). Although hirsutenone caused a slight decrease in the time constants of direct channel inactivation, the changes were not statistically significant.

To assess the onset rate of channel inactivation, a three-pulse protocol was used. The channels were first inactivated by clamping the membrane at +20 mV for 2 s, followed by a prepulse to −120 mV for 15 ms. This prepulse was sufficiently long to allow for the rapid recovery of channels from inactivation but short enough to prevent significant channel deactivation. Following the recovery prepulse, a series of test pulses were delivered to potentials ranging from −80 to +60 mV, resulting in large, outward inactivating currents. The current traces at the onset of inactivation in the absence and presence of 20 μM hirsutenone are shown in Fig. 3A. The time constants for the onset of inactivation were obtained by fitting a single exponential function to the decaying current traces during the third pulse of the protocol. The time constants were significantly decreased in the presence of 20 μM hirsutenone ($n=10$, Fig. 3B).

To determine the recovery from inactivation, the fully acti-

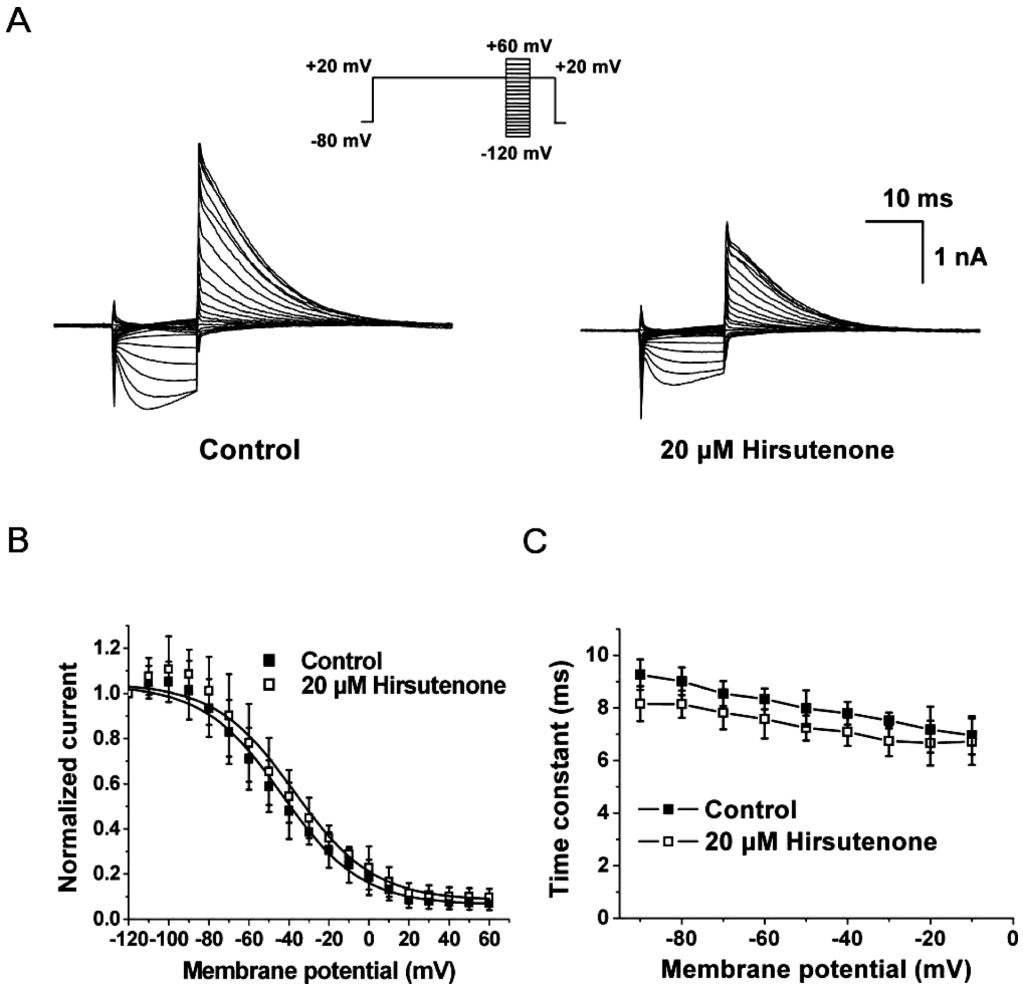

Fig. 2. Effects of Hirsutenone on the Steady-State Inactivation of the hERG K^+ Channels

(A) Representative current traces for steady-state inactivation in control and 20 μM hirsutenone-treated samples. (B) Normalized steady-state inactivation curves before and after exposure to 20 μM hirsutenone. Smooth curves were fitted with the Boltzmann function ($n=10$). (C) The time constants of steady-state inactivation. The time constants obtained from a single exponential fit the decay of the outward current ($n=10$).

vated *I–V* protocol was used. Each current was obtained through depolarization to +20 mV for 2 s to inactivate the hERG channels, followed by varying repolarizing pulses to test potentials between −120 to +30 mV in 10 mV steps. The prepulse potential at +20 mV was sufficiently positive to induce full conductance of the hERG channels but also rendered a large number of the channels inactive. The recovery from inactivation current traces in the absence and presence of 20 μM hirsutenone are shown in Fig. 3C. The increasing phase of the tail current represented the rapid recovery of hERG channels from inactivated to open states. The time constants were obtained by fitting a single exponential function to the initial increase in tail current amplitude ($n=10$, Fig. 3D). The time constants of recovery from inactivation were slightly small in the presence of 20 μM hirsutenone, but did not demonstrate significant changes.

Effect of Protein Kinase A (PKA), Protein Kinase C (PKC) and Protein Tyrosine Kinase (PTK) inhibitors on I_{tail} Blockade by Hirsutenone To further understand hERG K^+ current blockade by hirsutenone and to investigate the involvement of PKA, PKC and PTK pathways in mediating hirsutenone-induced I_{tail} depression, the effects of PKA, PKC and PTK inhibitors were investigated. Respectively, pre-treatment with 1 μM H-89, 1 μM Chelerythrine Chloride (CTC) and 80 μM Genestein (Gen) for 24 h did not alter the effects of hirsutenone on I_{tail} in hERG-CHO cells (Fig. 4).

Delayed Effects of Hirsutenone on hERG K^+ Channel Expression To study the effects of hirsutenone on hERG K^+ channel protein trafficking, we investigated the effects of hirsutenone on channel expression using the Western blotting. hERG-CHO cells were incubated with either control or hirsutenone-containing medium (5, 10, 20, 30, 50 μM) for 24 h. In control medium, hERG channels showed protein bands at 135 kDa (immature, core-glycosylated channel protein) and 155 kDa (mature, fully glycosylated channel protein). There were no bands present at either size in non-transfected CHO cells (Fig. 5A). Incubation with hirsutenone produced a concentration-dependent increase in the intensity of fully glycosylated channel protein (155 kDa), whereas the core-glycosylated channel protein (135 kDa) remained unchanged except at 30 and 50 μM, where the intensity of the 135 kDa band was increased (Fig. 5A).

To further analyze the effect of hirsutenone on hERG K^+ channel processing, the influence of hirsutenone on hERG mRNA levels was quantified using qRT-PCR. hERG-CHO cells were treated with either control or hirsutenone-containing medium (5, 10, 20, 30, 50 μM) for 24 h. The quantification of *hERG* mRNA levels was performed using the com-

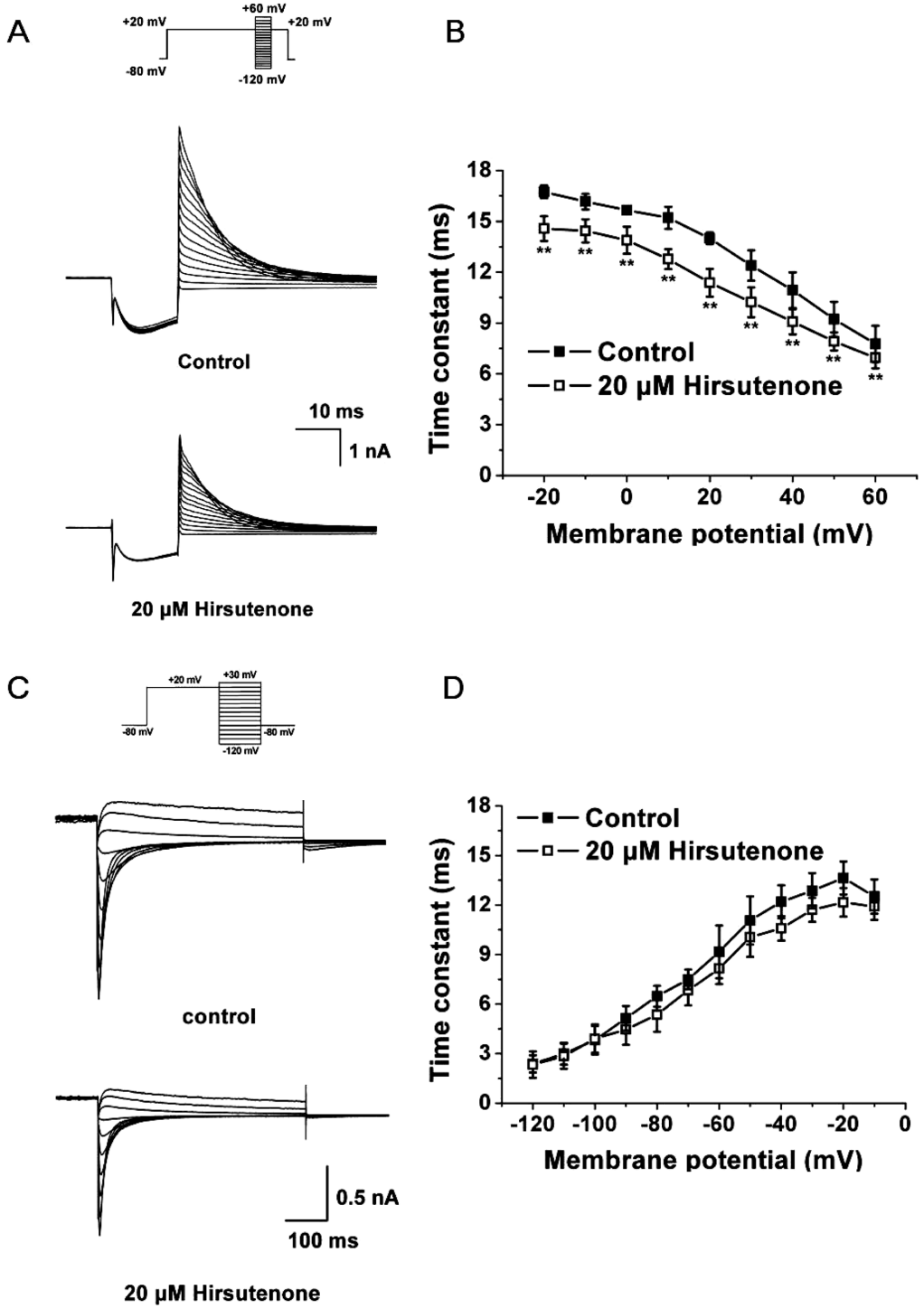

Fig. 3. Effects of Hirsutenone on the hERG K^+ Channel Kinetics

(A) Representative current traces for the onset of inactivation in control and 20 μM hirsutenone-treated samples. (B) The time constants of inactivation were measured by fitting a single exponential function to the decaying current ($n=10$, $^{**}p<0.01$). (C) Representative current traces for recovery from inactivation in control and 20 μM hirsutenone-treated samples. (D) The time constants of recovery from inactivation were obtained by fitting a single exponential function to the initial increase in tail current amplitude ($n=10$).

parative cycle threshold method, with the β-actin expression level as an internal control, and through subsequent ratio determination relative to samples from untreated hERG-CHO cells. In these analyses, 5, 10 and 20 μM hirsutenone did not affect gene expression, whereas 30 and 50 μM hirsutenone resulted in significant increases of *hERG* mRNA ($n=4$, Fig. 5B).

To confirm that incubations with high concentrations (30, 50 μM hirsutenone) result in increases of hERG K^+ channel surface and mRNA expression, current density was measured in hERG-CHO cells. The cells were incubated with control and 30 μM hirsutenone-containing medium for 24 h and current density was measured. On the day of experiment, cells were washed and placed in standard bath solution (control) and 30 μM hirsutenone containing bath solution (acute, 24 h pre-incubation) for electrophysiological recording. Current

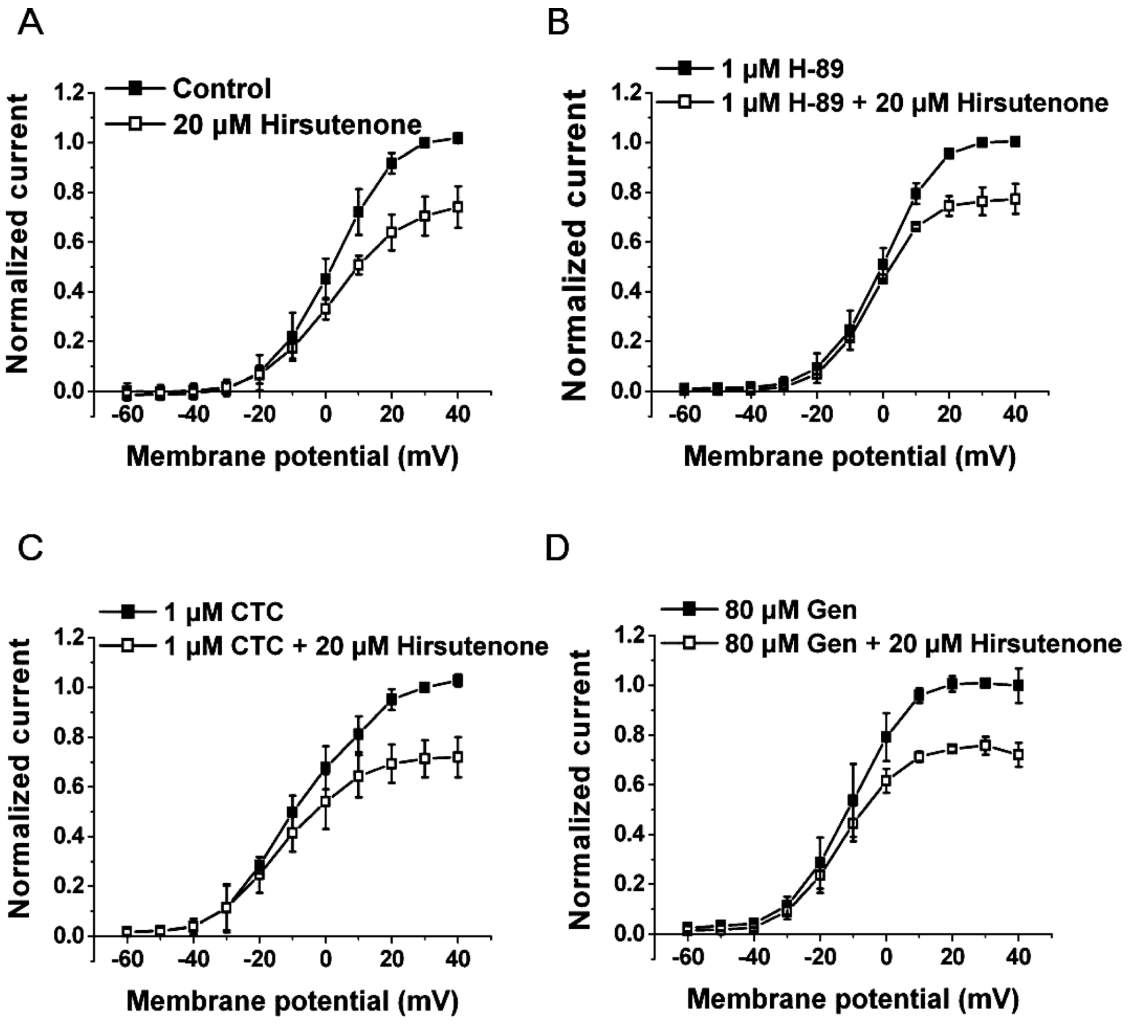

Fig. 4. Effects of Protein Kinase Inhibitors on hERG I_{tail} Blockade by Hirsutenone

Normalized *I–V* relationships; (A) control and 20 μM hirsutenone, (B) 24 h pre-incubation with PKA inhibitor (1 μM H-89) and 1 μM H-89 +20 μM hirsutenone, (C) 24 h pre-incubation with PKC inhibitor (1 μM CTC) and 1 μM CTC +20 μM hirsutenone, (D) 24 h pre-incubation with PTK inhibitor (80 μM Gen) and 80 μM Gen +20 μM hirsutenone.

density was 30.09±11.13, 19.06±3.6 and 29.94±4.63 in cells treated with control, acute, and 24 h pre-incubation with 30 μM hirsutenone respectively. Acute treatment with 30 μM hirsutenone significantly reduced current density ($n=8$, $p<0.01$), although 24 h pre-incubation with 30 μM hirsutenone did not significantly affect current density ($n=6$, $p>0.05$) (Fig. 5C). This same 24 h pre-incubation with 30 μM hirsutenone significantly affected hERG K^+ channel expression but did not significantly affect current density.

DISCUSSION

To assess the cardiovascular safety of new drugs, their effects on hERG K^+ channels are generally examined using a whole cell patch clamp method. Because hERG K^+ channel blockade has the potential to cause extensive side effects, in addition to LQTS and arrhythmias, the test for drug-induced hERG K^+ channel blockade is essential to drug development before doing the *in vivo* toxicological studies.[23] A number of drugs have been withdrawn from the market or failed in late-stage clinical trials due to a side effect inducing cardiac arrhythmia. Therefore, understanding drug-hERG K^+ channel interaction at a molecular level is necessary for rational drug design.

The present study showed that hirsutenone directly blocks hERG K^+ channels in hERG-CHO cells. The blockage was significantly increased at the voltage (+30 mV) at which the hERG K^+ channel was fully activated (Fig. 1B). These results suggested that hirsutenone blockage might occur *via* open-state blockage. However, no channel kinetics related to channel activation were changed (Fig. 1D), indicating that hirsutenone may not block the hERG K^+ channel when it is open. So, we also investigated the effects of hirsutenone on hERG K^+ channel inactivation kinetics. Hirsutenone significantly decreased the time constants of onset of inactivation (Fig. 3B) although steady-state inactivation and recovery from inactivation properties were not significantly changed (Figs. 2, 3D). These results indicate that the inactivation of hERG K^+ channels was accelerated by hirsutenone and hirsutenone might also exhibit high affinity for the inactivated state.

Some drugs inhibit voltage-gated K^+ channels *via* tyrosine phosphorylation of the channel protein.[24,25] Therefore, we investigated whether phosphorylation mechanism was involved in hERG K^+ channel blockade by hirsutenone. Pre-incubation with PKA, PKC and PTK inhibitor for 24 h did

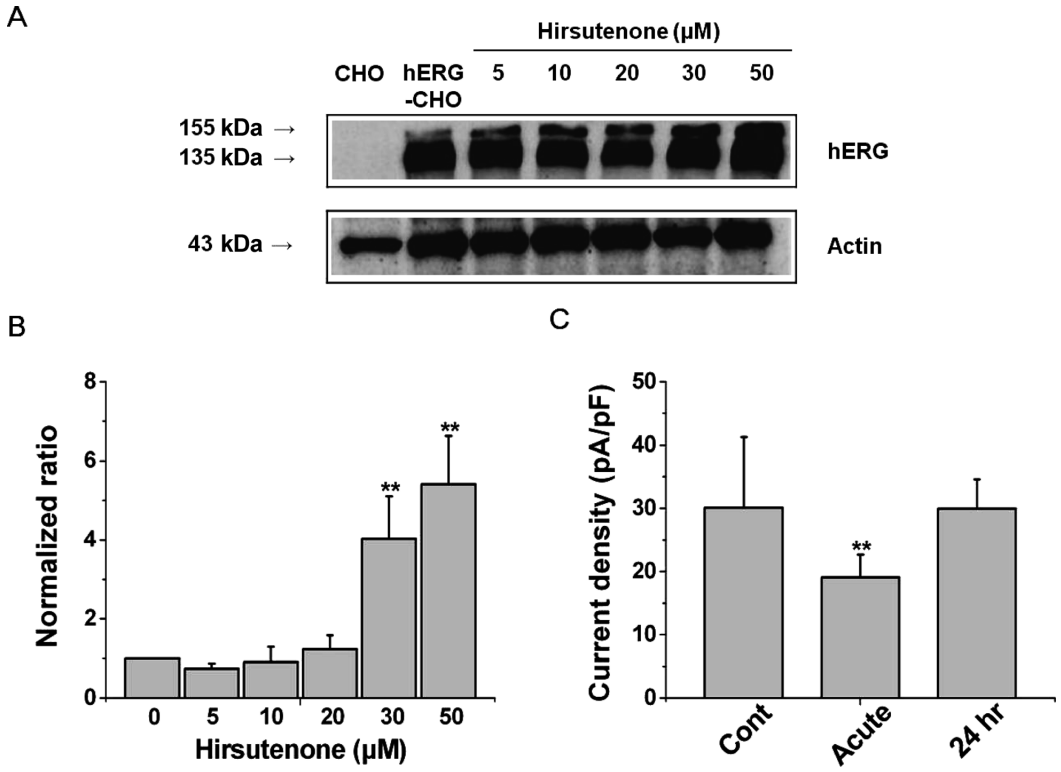

Fig. 5. Delayed Effects of Hirsutenone on the hERG K^+ Channel Expression

(A) Western blot analysis of the expression of hERG K^+ channel protein in untransfected CHO cells and stably expressed hERG-CHO cells treated with 0, 5, 10, 20, 30 and 50 μM hirsutenone. Two hERG K^+ channel protein bands were detected in the hERG-CHO cells: immature form (the core-glycosylated; 135 kDa) and mature form (the fully glycosylated; 155 kDa). β-Actin was used in each reaction as a positive control. (B) Effects of hirsutenone on *hERG* mRNA expression analyzed *via* the qRT-PCR. hERG-CHO cells treated with 0, 5, 10, 20, 30 and 50 μM hirsutenone for 24 h ($n=4$, $^{**}p<0.01$). (C) Effects of 30 μM hirsutenone on current density. Thirty micromolar hirsutenone was acutly treated and incubated for 24 h. Current density measured under control (cont), 30 μM hirsutenone (acute, $n=8$) and 24 h pre-incubation with 30 μM hirsutenone (24 h, $n=6$).

not prevent I_{tail} blockade by hirsutenone (Fig. 4). The above results suggest that the hERG K^+ channel may be directly blocked by hirsutenone *via* promoting the inactivation process.

Many drugs induce action potential prolongation by indirectly reducing the hERG K^+ channel protein trafficking rather than directly inhibiting the channel current.[10,26] Some drugs inhibit both hERG K^+ current and protein trafficking.[26] To investigate whether hirsutenone affects hERG K^+ channel protein trafficking, hERG-CHO cells were incubated with hirsutenone for 24 h. We did not find any significant effects of hrisutenone on the immature form at 5, 10 and 20 μM, but the mature form at all test concentrations and the immature form at 30 and 50 μM was significantly increased (Fig. 5A). These increases at high concentrations may be caused by an increase in gene expression.

We also investigated whether hirsutenone affects hERG K^+ channel processing. Analyzing *hERG* mRNA levels, we found an increase of *hERG* gene expression after incubation with 30 and 50 μM hirsutenone (Fig. 5B). A significant increase in *hERG* gene and surface expression was detected *via* qRT-PCR and Western blotting. However, these results are not consistent with the electrophysiological results. Therefore, to investigate the contradictory results, we compared hERG K^+ current density in control, 30 μM hirsutenone (acute), and after 24 h pre-incubation (chronic) with 30 μM hirsutenone. The current density was significantly reduced in acute treatment, but was unaffected by chronic treatment (Fig. 5C). These findings indicate that hirsutenone affects the hERG K^+ channels *via* dual mechanisms; acute inhibition of hERG K^+ channels and chronic drug-induced increase of hERG expression. Further studies are necessary to determine, whether increased hERG K^+ channel expression could be compensated the current density reduction.

In this study, we found that hirsutenone directly inhibited hERG K^+ currents in a concentration-dependent manner, with an IC_{50} of 14.9 μM (Fig. 1C). With regard to the relationship among the hERG K^+ current blockade, QT prolongation and fatal arrhythmia, Redfern *et al.*[27] recommended a 30-fold safety margin between the maximal effective therapeutic plasma concentration and the IC_{50} of hERG K^+ channel. Based on this recommendation, the maximal therapeutic plasma concentration should be less than 0.5 μM. However, hirsutenone has demonstrated anti-inflammatory and immune modulatory effects between 10 to 50 μM concentrations in *in vitro* experiments.[15,17—19] *In vitro* effective concentrations are higher than the recommended therapeutic plasma concentrations; therefore, if hirsutenone is used as an injected or orally administered drug there is an expected risk of QT prolongation and fatal arrhythmia. Although hirsutenone has the possibility of inducing a cardiac arrhythmia, its positive anti-inflammatory and immune modulatory effects are too strong to recommend discontinuation of the drug. If we use hirsutenone as a topical ointment to treat inflammatory skin lesions like atopic dermatitis and contact dermatitis, we could minimize the side effects because the transcutaneous absorption rate is lower than 1% that of oral delivery or injection.

In conclusion, hirsutenone was shown to be a potent direct

blocker of hERG K^+ channels which functions by changing channel inactivation kinetics. In addition, hirsutenone affected hERG K^+ channel trafficking and processing. Our results provide an understanding of the cardiovascular risk profile and safety margin of the new drug hirsutenone.

Acknowledgements This study was supported by a Grant from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare and Family Affairs, Republic of Korea (A091121).

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