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Decursin from *Angelica gigas* Nakai Blocks hKv1.5 Channel

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Abstract

Decursin was purified from *Angelica gigas* Nakai, and its effects on the human Kv1.5 (hKv1.5) currents were recorded in mouse fibroblasts (Ltk^- cells) by whole-cell patch-clamp technique. Decursin inhibited hKv1.5 current in a concentration-dependent manner, with an IC_{50} value of 2.7 μM at +60 mV. Decursin accelerated the inactivation kinetics of the hKv1.5 channel, and slowed the deactivation kinetics of the hKv1.5 current, resulting in a tail crossover phenomenon. Also, decursin inhibited the hKv1.5 current in a use-dependent manner. These results strongly suggest that decursin is a kind of open-channel blocker of the hKv1.5 channel.

Key Words: hKv1.5 channel blocker, Decursin, *Angelica gigas* Nakai

INTRODUCTION

Atrial fibrillation is the most frequent cardiac arrhythmia that can result in serious morbidity (Chung *et al.*, 2001). It is well known that various K^+ channels regulate the action potential duration and K^+ channel genes are differentially expressed depending on the regions of the heart. The main Kv channel genes expressed in the human heart are the hKv1.4, hKv1.5, hKv4.3 and HERG genes. All these genes are highly expressed in both the atrium and ventricle, whereas the hKv1.5 gene is preferentially expressed in the human atrium. Furthermore, the electrophysiological and pharmacological characteristics of the current generated by hKv1.5 channels is similar to the ultra-rapid delayed rectifier K^+ current (I_{KUR}) recorded in human atrial myocytes (Fedida *et al.*, 1998). Thus, the hKv1.5 channel is thought to be an unique target for atrial fibrillation. In the present study, we tested decursin from the roots of *Angelica gigas* Nakai (Umbelliferae) on K^+ currents expressed in Ltk^- cells. *A. gigas* Nakai has been used as a traditional medicine for treatment of anemia, a sedative and tonic agent (Yook, 1990). Earlier investigations on the chemical constituents of *A. gigas* mainly dealt with the isolation of coumarins (Ryu *et al.*, 1990; Jung *et al.*, 1991; Pachaly *et al.*, 1996; Lee *et al.*, 2002). The literature survey revealed that several pharmacological works, anti-tumor, inhibition of hepatic microsomal drug metabolizing enzyme, and inhibition of acetylcholinesterase activities, have been carried out on *A. gigas* (Shin *et al.*, 1996; Kang *et al.*, 2001; Lee *et al.*, 2003; Lee *et al.*, 2009; Kim *et al.*, 2010). Our studies have focused on the development of antiarrhythmic drug, and we previously reported that papaverine (Choe *et al.*, 2003), oxypeucedanin (Eun *et al.*, 2005b), psoralen and their derivatives (Eun *at al.*, 2005a; Eun *et al.*, 2007) and torilin (Kwak *et al.*, 2006) inhibited the hKv1.5 current. The present study was examined to investigate the effect of decursin from *A. gigas* on hKv1.5 channels using the whole-cell patch-clamp technique.

MATERIALS AND METHODS

General procedure

All the ^1H- and ^{13}C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. The EI/MS (70eV) spectrum was determined on a VG-VSEQ mass spectrometer (VG Analytical, UK). The TLC was carried out on precoated silica gel F_{254} plates (Merck, Darmstadt, Germany), and the silica gel for column chromatography was Kiesel gel 60 (230-400 mesh, Merck). The column used for LPLC was the Lobar A (Merck Lichroprep Si 60, 240-10 mm). All the other chemicals and solvents were of analytical grade and they were used without further purification.

Plant materials

The roots of *A. gigas* were purchased from Namchangdang, Jeonju, Korea. A voucher specimen is deposited in the herbarium of college of pharmacy, Woosuk University (WSU-

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Extraction and isolation

The air-dried plant materials (300 g) were ground and extracted with MeOH under 50°C. The resultant MeOH extract (52 g) followed by the successive solvent partition to give methylene chloride (20 g), *n*-BuOH (18 g) and H_2O soluble fractions. Methylene chloride soluble fraction showed the most significant hKv1.5 current inhibitory activity. This fraction was chromatographed over silica gel column using a solvent system *n*-hexnae-CH_2Cl_2-EtOAc (3:2:1) as an eluent to give three subfractions, Subfraction 2 was purified by JAI-ODS column (MeOH) to give compound 1 (150 mg).

Compound 1 (Decursin, Fig. 1)

Colorless needles (MeOH); 95°C, the ^{1}H-NMR and ^{13}C-NMR data we obtained were in good agreement with the literature values (Lee *et al.*, 2003).

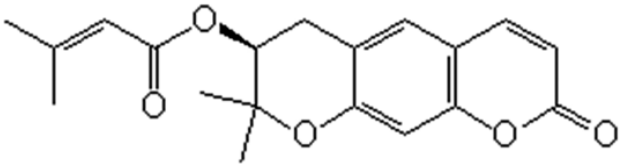
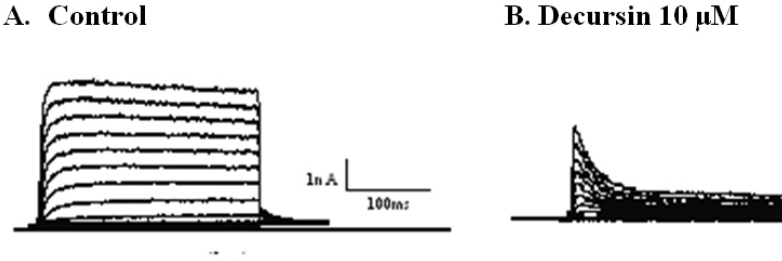
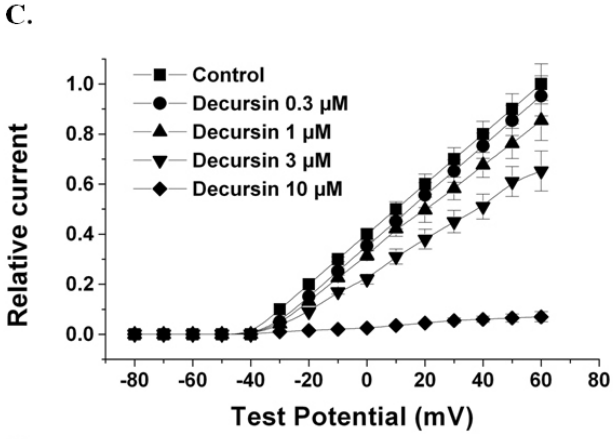


Fig. 1. Chemical structure of Decursin.





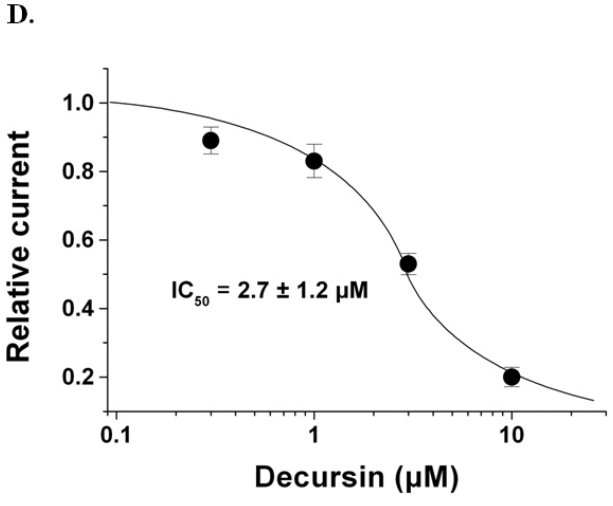


Fig. 2. Effects of decursin on the hKv1.5 current expressed in Ltk$^-$ cell line. hKv1.5 current traces were recorded before (A) and 20 min after exposure to 1 μM decursin (B). (C) The resultant I-V relationship of the steady-state current taken at the end of the depolarizing pulses. (D) Concentration-response relationship of hKv1.5 block by decursin. Each point with a vertical bar denotes the mean ± S.E.M.

Cell culture and transfection

The method is the same as that described previously (Snyders *et al.*, 1993). The transfected cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% horse serum and 0.25 mg/ml of G418, under a 5% CO_2 atmosphere. Before the experimentals, the subconfluent cultures were incubated with 2 μM dexamethasone for 12 h to induce expression of hKv1.5 channels.

Electrical recording

hKv1.5 currents in Ltk^- cells were recorded at room temperature (20-22°C) using the whole-cell configuration of the patch clamp technique (Kwak *et al.*, 1999) with an Axopatch-200B patch clamp amplifier (Axon Ins, Foster City, CA, USA). Currents were sampled at 1 to 10 kHz after an anti-alias filtering was done at 0.5 to 5 kHz. Data acquisition and command potentials were controlled by pClamp 6.0.3 software (Axon Ins). Junction potentials were zeroed with the electrode in the standard bath solution. Gigaohm seal formation was achieved by suction and, after establishing the whole cell configuration, the capacitive transients were elicited by a symmetrical 10 mV voltage clamp in steps from −80 mV, and they were recorded at 50 kHz for the calculation of cell capacitance. Whole cell currents of −1 to 4 nA and series resistances of 2 to 3 MΩ were used for the analysis. The intracellular pipette-filling solution for whole cell mode contained 100 mM KCl, 10 mM HEPES, 5 mM K_4BAPTA, 5 mM K_2ATP and 1 mM $MgCl_2$ (pH 7.2). The extracellular solution contained 130 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES and 10 mM glucose (pH 7.35). The current traces were recorded with a depolarizing pulse of +50 mV from a holding potential of −80 mV, followed by a repolarizing pulse of −50 mV in the Ltk^- cells. To observe the concentration-dependent block of hKv1.5 channel currents by decursin, steady-state currents taken at the end of the depolarizing pulse of +50 mV were normalized to the control obtained in the absence of decursin.

Statistical analysis

All of the data are presented as mean ± S.E.M. The Student's *t*-test and analysis of variance (ANOVA) were used to calculate the statistical significant. Differences were considered to be statistically significant if a *p*-value of <0.05 was obtained.

RESULTS AND DISCUSSION

A selective block of the hKv1.5 current results in a significant prolongation of the action potential (Wang *et al.*, 1994). Fig. 2 demonstrates a representative experiment to examine the effects of decursin on the hKv1.5 channel currents expressed in the Ltk^- cells. The membrane potentials were held at −80 mV and 250-ms depolarizing pulses from −80 to +60 mV in 10 mV steps were applied every 20 s. The outward currents were followed by decaying outward tail currents upon repolarization to −50 mV. In control conditions, a depolarization positive to −40 mV elicited outward. At +60 mV, after the current reached the maximum, it declined slowly during the maintained depolarization (Fig. 2A). The peak current amplitude was affected much less than the steady-state current amplitude. The inhibition of hKv1.5 appeared within 20 s of the application of the drug and reached a new steady state within 2 min. The washout of decursin was obtained within 4 min, and the currents recovered to 92.1 ± 4.7% (n=8) of the control value. Fig. 2C shows the steady-state I-V relationship for the hKv1.5 channels under control conditions and in the presence of decursin (10 μM), and the I-V relations were constructed by plotting the current amplitudes measured at the end of 250-ms depolarizations as a function of the test pulse voltages. The hKv1.5 current was blocked by decursin over the whole potential range for activation. Thus, decursin significantly shifted the voltage dependence of channel opening to more hyperpolarized potentials, as reported for the action of two other drugs (Perchenet *et al.*, 2000; Choe, *et al.*, 2003). The block of hKv1.5 by decursin was in a concentration-dependent manner, as shown in Fig. 2D. Steady-state currents were measured at the end of depolarizing pulse of +60 mV to construct the concentration-response curve. Plots of steady-state current as a function of decursin concentration were fitted to the Hill equation. For decursin-induced block, a half-maximal inhibitory concentration (IC_{50}) and Hill coefficient were 2.7 ± 1.2 μM and 1.31, respectively (n=8)(Fig. 2D). Drugs that block ion channels often alter the voltage dependence. Voltage-dependence of the drugs acting on ion channels is sometimes very useful in evaluating the clinical applications of the drugs. The voltage dependence of activation (Fig. 3A) and inactivation (Fig. 3B) were analyzed in the absence or presence of decursin (3 μM). The activation

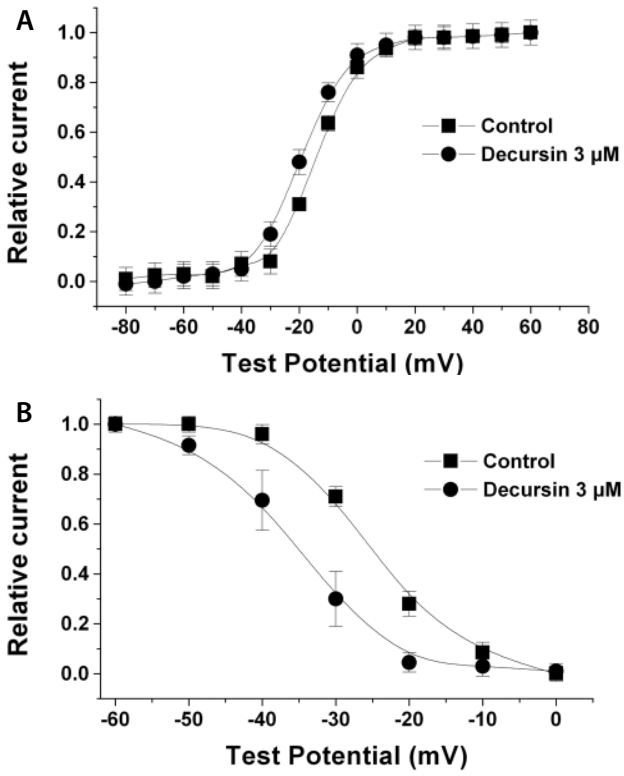


Fig. 3. Effects of decursin on the activation and inactivation of the hKv1.5 current. The activation curve (A) was obtained from the deactivating tail current amplitudes at −50 mV, following 250 ms depolarizing steps to potentials between −80 to +60 mV. The inactivation curve (B) was obtained from the depolarizing steps to potentials between −60 mV to 0 mV, in steps of 10 mV. Each point with a vertical bar denotes the mean ± S.E.M.

curve was unchanged in the presence of decursin: The values of $V_{1/2}$ for the activation were −13.7 ± 1.5 and −17.5 ± 1.8 mV (n=8), without and with decursin, respectively. The values of *k* were not significantly different 7.6 ± 0.9 mV for control and 9.1 ± 1.2 mV for decursin (n=8). Under control conditions, $V_{1/2}$ and *k* of the steady-state inactivation curve measured −24.3 ± 1.3 mV and 4.8 ± 0.9 mV (n=8), respectively. After the addition of decursin, the $V_{1/2}$ and *k* of the steady-state inactivation curve changed to −37.1 ± 2.1 mV and 4.3 ± 1.2 mV (n=8), respectively.

To quantify the voltage dependence of the decursin-induced block of hKv1.5, the relative current $I_{cytochalasin\ B}/I_{control}$ was plotted as a function of membrane potential (n=5)(Fig. 4). In the presence of decursin, the blockade increased steeply between −30 and 0 mV, which corresponds to the voltage range of the channel opening (Snyders *et al.*, 1993). However, there was no additional inhibition of hKv1.5 by decursin in the range of voltages between 0 and +60 mV, a range where the channels are fully activated. These data suggest that decursin binds primarily to the open state of the hKv1.5 channel. We tested the channel state-dependency of hKv1.5 block by decursin (Fig. 5). Fig. 5 shows the superposition of the tail currents obtained with a depolarizing pulse of +50 mV from a holding potential of −80 mV, followed by a repolarizing pulse of −50 mV under control conditions and in the presence of decursin. In the presence of decursin, the initial tail current was decreased, with the subsequent slower decline resulting in a "crossover" phenomenon with the control tracing. This suggests that decursin acts as an open channel blocker on the hKv1.5 channel. On the basis of all these results, we suggest that decursin blocks hKv1.5 currents not by its known-mechanism, but rather via a direct one-to-one interaction between the drug and the channel in the open state, like as papaverine (Choe *et al.*, 2003), oxypeucedanin (Eun *et al.*, 2005b), psoralen (Eun *et al.*, 2005a; Eun *et al.*, 2007) and torilin (Kwak *et al.*, 2006). The use-dependence of decursin-induced inhibition of the hKv1.5 channel was investigated. The current traces were produced by 20 repetitive applications of depolarizing pulses at two different frequencies, 1 and 2 Hz. As shown in Fig. 6, the peak amplitude of the hKv1.5 current decreased slightly at a frequency of 1 and 2 Hz under control conditions. In the presence of decursin (3 μM), the peak amplitude of the hKv1.5 current progressively decreased at 1 and 2 Hz Hz (n=4) respectively. Thus, the degree of inhibition of decursin on hKv1.5 increased at

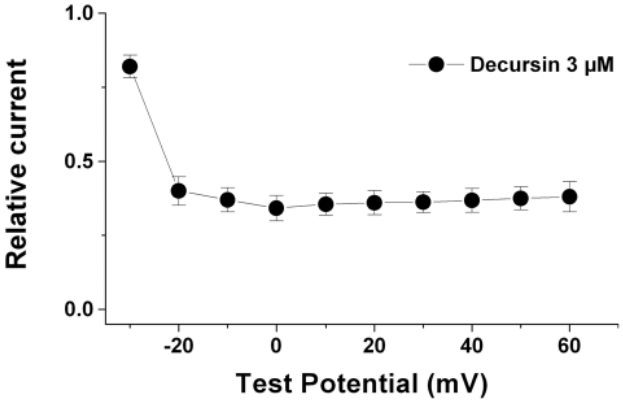


Fig. 4. Voltage-dependent block of hKv1.5 expressed in Ltk⁻ cells by decursin. The voltage protocol consisted of 250-ms depolarizing pulses from −20 to +60 mV, in 10-mV increments, from a holding potential of −80 mV, with repolarizing to −50 mV for 400 ms. Relative currents were obtained from the $I_{decursin}/I_{control}$ ratio at each depolarizing potential in the absence and presence of decursin. Each point with a vertical bar denotes the mean ± S.E.M.

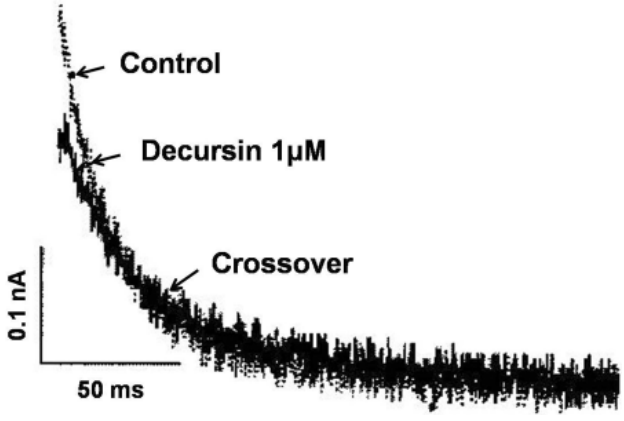


Fig. 5. Effects of decursin on the deactivation kinetics of hKv1.5 current expressed in Ltk⁻ cells. Deactivation kinetics were investigated during a repolarizing step of -50 mV for 400 ms, after a 250 ms depolarizing step to +60 mV. From a holding potential of -80 mV. By superimposing the tail currents in the absence and presence of decursin, a tail crossover phenomenon (indicated by the arrow) was observed.

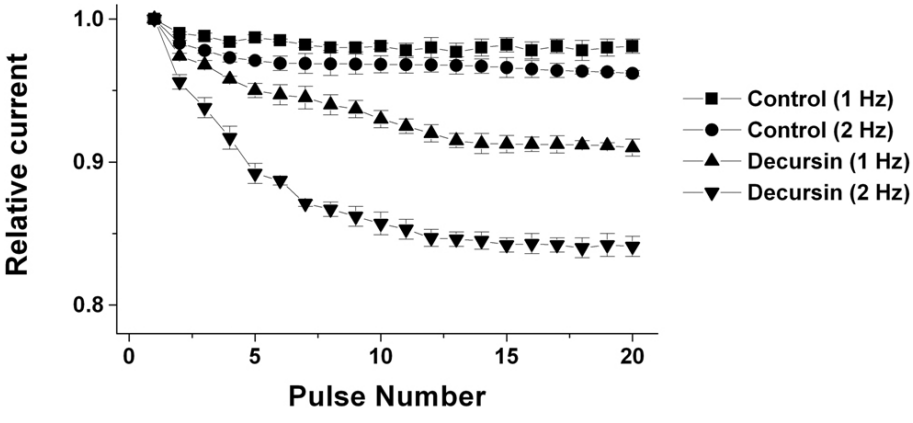


Fig. 6. Use-dependent inhibition of hKv1.5 expressed in Ltk⁻ cells by decursin. The plot of the normalized peak amplitudes of the currents, under control conditions and in the presence of decursin (3 μM), at every pulse versus the pulse numbers in the pulse train.

the pulse frequency increased, showing that decursin blocks hKv1.5 channels in a frequency-dependent manner.

The main Kv channel genes expressed in the human heart are the hKv1.4, hKv1.5, hKv4.3 and HERG genes. All these genes are highly expressed in both the atrium and ventricle, whereas the hKv1.5 gene is preferentially expressed in the human atrium. Furthermore, the electrophysiological and pharmacological characteristics of the current generated by hKv1.5 channels is similar to the I_{KUR} recorded in human atrial myocytes. Thus, the block of the hKv1.5 channels by decursin would be an ideal antiarrhythmic drug specific for atrial fibrillation without the risk of Torsades de Pointes. The present study may provide some important idea for future development of antiarrhythmic drugs specific for atrial fibrillation.

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