Effects of equol on multiple K⁺ channels stably expressed in HEK 293 cells

Xiu-Ling Deng¹*, Yan Wang², Guo-Sheng Xiao²*

¹ Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Xi’an Jiaotong University Health Science Center, Xi’an, Shaanxi, China, ² Xiamen Cardiovascular Hospital, Xiamen University, Xiamen, Fujian, China

* dengxl@mail.xjtu.edu.cn (XLD); xgs71@163.com (GSX)

Abstract

The present study investigated the effects of equol on cardiovascular K⁺ channel currents. The cardiovascular K⁺ channel currents were determined in HEK 293 cells stably expressing cloned differential cardiovascular K⁺ channels with conventional whole-cell patch voltage-clamp technique. We found that equol inhibited hKv1.5 (IC₅₀: 15.3 μM), hKv4.3 (IC₅₀: 29.2 μM and 11.9 μM for hKv4.3 peak current and charge area, respectively), Iₖs (IC₅₀: 24.7 μM) and I₅₀ (IC₅₀: 31.6 and 56.5 μM for I₅₀ tail and I₅₀ step, respectively), but not hKir2.1 current, in a concentration-dependent manner. Interestingly, equol increased BKCa current with an EC₅₀ of 0.1 μM. It had no significant effect on guinea pig ventricular action potentials at concentrations of ≤3 μM. These results demonstrate that equol inhibits several cardiac K⁺ currents at relatively high concentrations, whereas it increases BKCa current at very low concentrations, suggesting that equol is a safe drug candidate for treating patients with cerebral vascular disorders.

Introduction

Equol [7-hydroxy-3-(4’-hydroxyphenyl)-chroman] is an active metabolite of the soy isoflavone daidzein generated in the intestinal microbial flora in some, but not all, individuals after consuming daidzein[1–3]. Biological activities of equol are greater than daidzein in non-vascular systems[4, 5] with superior antioxidant activity[6–10]. An earlier study from Sobey and colleagues demonstrated that equol dilates carotid artery in vitro and basilar artery of normotensive rats in vivo with similar potency to its parent compound daidzein. Equol retains its vasorelaxant activity in carotid arteries from hypertensive rats, whereas effects of daidzein were insignificant, suggesting that equol may be a useful therapeutic agent to treat cerebral vascular disorders[11]. A recent study demonstrates that equol producers showed lower serum uric acid, triglyceride, and the ratio of waist and hip, as well as higher HDL-cholesterol, suggesting that equol may reduce cardiovascular risk[12]. Therefore, it is believed that equol rather than daidzein itself may contribute to the beneficial effects of soy foods in preventing cardiovascular disorders.

Our recent study showed that equol increases large-conductance Ca²⁺-activated K⁺ (BKCa) current by acting on its auxiliary β1-subunits and contributes to the equol-mediated...
vasodilation and increase of cerebral blood flow[13]. However, it is unknown whether equol could affect other cardiovascular K+ currents or whether it could prolong ventricular action potentials. The present study was therefore designed to investigate the effects of equol on cloned hKv1.5 (encoding for IKur, ultra-rapidly-delayed rectifier K+ current), hKv4.3 (encoding for cardiac Ito, transient outward K+ current), recombinant cardiac IKs (hKCNQ1/hKCNE1, slowly-delayed rectifier K+ current), hERG (human Ether-a-go-go-related gene encoding for cardiac IKr, rapidly-delayed rectifier K+ current), stably expressed in HEK 293 cells, and ventricular action potential of guinea pig hearts. Our results demonstrated that equol inhibited these cardiac K+ currents at relatively high concentrations, whereas it increased BKCa current at a low concentration range without prolonging action potential duration in guinea pig ventricular myocytes.

Materials and methods
Cell culture
The HEK 293 cell lines stably expressing hKv4.3 (KCND3, encoding Ito)[14], hKv1.5 (KCNA5, encoding IKur)[15], hERG (KCNH2, encoding IKr)[16], hKCNQ1/hKCNE1 (encoding IKs)[17], Kir2.1 (KCNJ2, encoding IK1)[18] or KCa1.1/β1 (KCNMA1/KCNMB1, encoding BKCa) [19] were generously provided by Dr. Gui-Rong Li, the University of Hong Kong, and maintained in Dulbecco’s modified eagle medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and 400 μg/ml G418 (Sigma-Aldrich, St Louis, MO, USA) for the cell line expressing hKv4.3, hKv1.5, hERG, Kir2.1 and KCa1.1/β1, or 100 μg/ml hygromycin (Invitrogen) for the cell line expressing recombinant cardiac IKs. Cells were seeded on a glass cover slip for electrophysiological recordings.

Preparation of guinea pig ventricular myocytes
Guinea pigs weighing 250–300 g of either sex were used in the present study. The experimental procedure was approved by the Institutional Ethic Committee of Animal Use for Teaching and Research. After anesthetization of the animal with sodium pentobarbital (40 mg/kg, i.p.), ventricular myocytes were enzymatically dissociated from guinea pig hearts as described previously[20, 21]. The isolated cardiomyocytes were maintained in a high-K+ storage medium at room temperature for 2 h before action potential recording.

Solution and chemicals
Tyrode’s solution contained (in mM): 140 NaCl, 5.4 KCl, 1 MgCl2, 1.8 CaCl2, 0.33 NaH2PO4, 10 HEPES, 10 glucose, pH was adjusted to 7.4 with NaOH. The pipette solution contained (in mM): 20 KCl, 110 K-aspartate, 1 MgCl2, 10 HEPES, 5 EGTA, and 0.1 GTP, 5 Na2-phosphocreatine, and 5 Mg-ATP (or K2-ATP for IKs or action potential recording), pH was adjusted to 7.2 with KOH. MgCl2 in pipette solution was replaced by NaCl, when recording IKs. Equol (50% R-equol and 50% S-equol) was obtained from Nanjing Laiyin Medicine Technology Limited Company (Nanjing, Jiangsu, China), and was prepared as 50 mM stock solutions in dimethyl sulfoxide (DMSO, Sigma-Aldrich) and added to the bath solution at the indicated final concentrations. The DMSO concentration in the working solution was <0.2% and did not affect the membrane currents.

Electrophysiology
Coverslips with HEK 293 cells expressing corresponding ion channels or ventricular myocytes were transferred to an open perfusion chamber (1 ml) mounted on the stage of an inverted
microscope (IX50, Olympus, Japan), and superfused at 2–3 ml/min with Tyrode’s solution. The whole-cell patch-clamp technique was used for electrophysiological recording. Borosilicate glass electrodes (1.2-mm OD) were pulled with a Brown-Flaming puller (model P-97, Sutter Instrument Co., Novato, CA, U.S.A.) and had tip resistances of 2–3 MΩ when filled with pipette solution. A 2-M KCl-agar salt bridge was used as reference electrode. The series resistance was compensated by 70–80% to minimize voltage errors. The membrane currents were recorded with an EPC-10 amplifier and Pulse software (HEKA, Lambrecht, Germany). Command pulses were controlled by Pulse software. Current signals were low-pass filtered at 5 kHz. The ionic currents were recorded at room temperature (23–25˚C), and cardiac action potentials were recorded at 36–37˚C.

Data analysis
Group data are presented as mean±SEM. Nonlinear curve fitting was performed using Pulsefit (HEKA) and SigmaPlot 12.0 (SPSS Science, Chicago, IL, USA). Paired and/or unpaired Student’s t-tests were used to evaluate the statistical significance of differences between two group means. ANOVA was used for multiple groups. Values of P<0.05 were considered statistically significant.

Results
Effect of equol on hKv1.5 current
Fig 1A shows the time course of the hKv1.5 current recorded in a representative cell in the absence and presence of 10 μM equol with the voltage protocol shown in the inset (300-ms voltage step to +50 mV from −80 mV and then back to −40 mV). Equol gradually inhibited the Kv1.5 current, and the inhibitory effect was partially reversed on washout. Similar results were obtained in 5 other cells. Fig 1B displays the voltage-dependent hKv1.5 current recorded in a typical experiment with the voltage protocol shown in the inset in the absence and presence of 3, 10, and 30 μM equol. Fig 1C illustrates the current-voltage (I-V) relationships of hKv1.5 current measured at end of depolarization voltage steps during control and after application of 3, 10 and 30 μM equol. The current was inhibited by 10 and 30 μM equol (n = 7, P<0.05 or P<0.01 vs. control at 0 mV to +60 mV). The concentration-response relationship of equol (1 to 100 μM) for inhibiting hKv1.5 current at +50 mV was fitted to a Hill equation (Fig 1D). The IC50 of equol for inhibiting hKv1.5 current was 15.3 μM with a Hill coefficient of 2.3.

Inhibition of hKv4.3 current by equol
Fig 2A shows the time course of hKv4.3 current recorded in a HEK 293 cell stably expressing KCND3 in the absence and presence of 30 μM equol. Equol reversibly inhibited Kv4.3 current. Similar results were obtained in another typical cell for voltage-dependent Kv4.3 current recorded with the voltage protocol shown in the inset (Fig 2B). I-V relationships of equol (10, 30, and 100 μM) for inhibiting hKv4.3 current are illustrated in Fig 2C. Equol inhibited hKv4.3 current in a concentration-dependent manner. Significant inhibition of Kv4.3 current was seen at test potentials of −10 mV to +60 mV (n = 7, P<0.05 or P<0.01 vs. control). The IC50 of equol for inhibiting hKv4.3 current was 29.2 μM with a Hill coefficient of 1.3 (Fig 2D).

Equol remarkably increased hKv4.3 inactivation (or decay) (Fig 2A and 2B), indicating a typical of open channel blocking effect as described previously[22, 23], and charge area of hKv4.3 current was decreased more than hKv4.3 peak current. We therefore analyzed the charge area[24] of hKv4.3 current at +50 mV by integrating the area under current curve (Fig
with different concentrations of equol. The IC50 of equol for reducing the current charge area was 11.9 μM with a Hill coefficient of 1.5 (Fig 2F).

Fig 3A illustrates the representative current and voltage-protocol used for determining the availability of hKv4.3 current in the absence and presence of 30 μM equol. The variables (Fig 3B) of I/Imax and g/gmax were fitted to a Boltzmann equation: \( g = \frac{1}{1 + \exp[(V1/2 − Vt)/K]} \), where \( V1/2 \) is the voltage of 50% channel availability or maximal activation of the channel, \( Vt \) is the test potential, and \( K \) is slope factor. The \( V1/2 \) of hKv4.3 inactivation was −43.8 ± 2.1 mV in control, and was negatively shifted to −50.4 ± 1.9 mV by 30 μM equol (n = 7, \( P < 0.01 \)), while the slope factor K was −5.7 ± 1.2 mV in control and −6.2 ± 1.5 mV in the presence of 30 μM equol, respectively (\( P = \text{NS} \)). Steady-state activation of hKv4.3 was determined using tail
current at −40 mV after a brief depolarization step (8 ms) to between −40 and +60 mV from holding potential of −80 mV as described previously[15]. The V1/2 of hKv4.3 activation conductance was positively shifted from 0.7 ± 1.5 mV in control to 9.8 ± 1.7 mV by 30 μM equol (n = 7, P<0.01 vs. control).
Time-dependent recovery of hKv4.3 current from inactivation was analyzed with a paired-pulse protocol by varying P1–P2 interval as shown in the inset of Fig 3C. The normalized recovery curves were fitted to a mono-exponential function in the absence and presence of equol (Fig 3D). Recovery time constant was 103.4 ± 3.7 ms in control, and 105.6 ± 3.1 ms in the presence of 30 μM equol, respectively (n = 6, P = NS). The result suggests that recovery of hKv4.3 current from inactivation is not affected by equol.

Effects of equol on recombinant cardiac I_{KS}

Fig 4A displays the time course of recombinant I_{KS} traces recorded in a representative cell in the absence and presence of 10, 30, and 100 μM equol using a 3-s voltage step to +30 mV every 10 s. Equol inhibited I_{KS} in a concentration-dependent manner. Fig 4B shows the voltage-dependent I_{KS} in another typical experiment with the voltage protocol shown in the inset. Equol at 30 μM substantially suppressed I_{KS}, and the inhibitory effect was reversed by wash-out. Fig 4C illustrates the I-V relationships of I_{KS}.step in the absence and presence of 10, 30 and 100 μM equol. Equol significantly inhibited I_{KS} at test potentials from 0 to +50 mV (n = 6, P<0.05, P<0.01 vs. control). The IC50 of equol for inhibiting I_{KS}.step was 24.7 μM with a Hill coefficient of 1.5 (Fig 4D).

The steady-state activation (g/g_{max}) of cardiac I_{KS} was determined using tail current in the absence and the presence of 30 μM equol (Fig 4E). The values were fitted to the Boltzmann
function. The $V_{1/2}$ of $I_{KS}$ activation was $14.5\pm3.1$ mV in control and $22.3\pm3.7$ mV in $30\,\mu M$ equol ($n=6$, $P<0.01$ vs. control).

Effect of equol on cardiac hERG channels

Fig 5A shows the time course of hERG tail current ($I_{hERG,tail}$) traces recorded in a HEK 293 cell stably expressing (KCNH2) in the absence and presence of 10, 30, and 100 μM equol. Equol decreased $I_{hERG,tail}$ in a concentration-dependent manner, and the inhibition was reversed by
Fig 5. Effects of equol on hERG channels. (A) Time course of $I_{\text{hERG, tail}}$ recorded in a representative HEK 293 cell stably expressing KCNH2 with the voltage protocol shown in the inset in the absence and presence of 10, 30, and 100 μM equol. Original current traces at corresponding time points are shown in the right. (B) Voltage-dependent $I_{\text{hERG}}$ traces recorded in a typical experiment in the absence and presence of 30 μM equol and upon washout. (C) I-V relationships of $I_{\text{hERG, tail}}$ (left) and $I_{\text{hERG, step}}$ (right) in the absence and presence of 10, 30, and 100 μM equol ($n = 7$, *$P<0.05$, **$P<0.01$ vs. control). (D) Concentration-response relationships of equol for inhibiting $I_{\text{hERG, tail}}$ and $I_{\text{hERG, step}}$ were fitted to the Hill equation. The numbers in the parentheses represent experimental number. (E) Variables of normalized $I_{\text{hERG, tail}}$ in the absence and presence of 30 μM equol were fitted to the Boltzmann equation.

washout. Similar inhibition and washout were obtained with 30 μM equol for voltage-dependent hERG current ($I_{\text{hERG}}$) with the voltage protocol shown in the inset (Fig 5B). Equol at 10 μM decreased $I_{\text{hERG, tail}}$ at +10 mV to +60 mV ($n = 6$, $P<0.05$, $P<0.01$ vs. control), but not $I_{\text{hERG, step}}$ (Fig 5C). Significant reduction for both $I_{\text{hERG, tail}}$ and $I_{\text{hERG, step}}$ was observed at 30 and 100 μM equol (0 mV to +60 mV, $n = 7$, $P<0.05$, $P<0.01$ vs. control). The IC$_{50}$ of equol was 31.6 μM for inhibiting $I_{\text{hERG, tail}}$ and 56.5 μM for inhibiting $I_{\text{hERG, step}}$ (Fig 5D).

The steady-state activation ($g/g_{\text{max}}$) of $I_{\text{hERG}}$ was determined by normalizing $I_{\text{hERG, tail}}$ in control and in the presence of 30 μM equol (Fig 5E). The values were fitted to the Boltzmann
function. The \( V_{1/2} \) of \( I_{\text{hERG}} \) activation was 0.15±2.5 mV and −10.5±2.8 mV in the absence (control) and presence of 30 μM equol, respectively (n = 6, \( P < 0.01 \) vs. control).

**Effect of equol on hKir2.1 current**

Fig 6A shows the typical hKir2.1 current recorded in a HEK 293 cell expressing \( KCNJ2 \) with the voltage protocol shown in the inset in control and in the presence of 30 μM equol. Equol in the concentration from 3 to 30 μM had no effect on Kir2.1 current (Fig 6B).

**Effect of equol on BK\(_{\text{Ca}}\) current**

Our recent study has demonstrated that equol induces vasodilation and increases rat cerebral blood flow by stimulating BKCa channels by acting on its auxiliary β1-subunits[13]. However, the efficacy of equol on BKCa channels was not determined. We therefore determined the EC50 (50% of effective maximum concentration) of equol for increasing BKCa current in HEK 293 cells stably expressing both the core α-subunit (\( KCNMA1 \)) and the auxiliary β1-subunit (\( KCNMB1 \)) of the channel. Fig 7A shows the BKCa current traces recorded in a representative cell with the voltage protocol shown in the inset. BKCa current was increased by 0.1, 0.3 and 1 μM equol. The BKCa channel blocker paxilline (1 μM) almost fully suppressed the current. Fig 7B illustrates the concentration-response curve of equol (0.01–3 μM) for stimulating BKCa current (+70 mV) fitted to the Hill equation. The EC50 of equol for eliciting BKCa current was 0.1 μM with a Hill coefficient of 1.3.

**Effect of equol on ventricular action potential**

Equol at 10 μM significantly inhibited both hERG current and \( I_{\text{Ks}} \), which implies a potential to cause prolongation of QT interval by increasing ventricular action potential duration, a well-known cardiac toxicity. We therefore determined the effect of equol (1, 3 and 10 μM) on action potential duration (APD) in guinea pig ventricular myocytes (37˚C). Fig 8A illustrates the ventricular action potentials recorded (2 Hz) in a representative myocyte in control and in the presence of 1, 3, and 10 μM equol. APD was clearly shortened by 10 μM equol. The effect was partially reversed on drug washout. Fig 8B shows the individual and mean values of APD at 50% and 90% repolarization (APD50 and APD90) in ventricular myocytes of guinea pig hearts. Equol at 1 and 3 μM had no effect on APD50 and APD90. Although 10 μM equol reduced APD50 and APD90 from 141.4±25.4 ms and 155.1±28.4 ms in control to 131.1±20.7 ms and 142.2±25.1 ms, no statistical significance was observed (n = 8, \( P > 0.05 \)). Changes in resting membrane potential and action potential amplitude were not observed with equol (1–10 μM, data not shown) treatment, suggesting that equol does not significantly inhibit inward rectifier potassium current (\( I_{\text{K1}} \)).

**Discussion**

The present study demonstrates for the first time that equol inhibits cloned hKv1.5, hKv4.3, hKCNQ1/hKCNE1, and hERG channels, while stimulating BK\(_{\text{Ca}}\) channels, stably expressed in HEK 293 cells. Equol has no effect on hKir2.1 current. The inhibitory effect of equol on K\(^+\) channels is as follows: hKv4.3>hKv1.5>hKCNQ1/hKCNE1>hERG. Interestingly, the efficacy of equol for stimulating BK\(_{\text{Ca}}\) channels is 150-fold greater than for inhibiting cardiac K\(^+\) channels.

Previous studies reported that plasma equol concentrations in human were ranged from ~0 to 130 nM depending upon the type of diet[25]. In a cohort study of healthy adult, serum equol concentration was found to be from 10.3–139 nM (2.5–33.6 mg/L) after consuming...
2×250 ml/d soy milk for 3 days[26]. However, little information is available in the literature for plasma protein binding ratio of equol. A recent study reported the plasma levels of free equol and its conjugates in a 24-h period after single oral administration of dietary daidzein (20 mg) in ovariectomized Sprague-Dawley rats using a LC-MS/MS approach[27]. The maximum plasma concentration (Cmax) for total equol (conjugated and unconjugated), unconjugated equol, equol monosulfate and equol glucuronides were 3682±2675 nM, 1.21±0.64 nM, 3.15±3.04 nM and 3349±2799 nM, respectively. This suggests that most of the circulating equol was conjugated-equol, specifically equol glucuronides.

It is well recognized that equol is an active metabolite of daidzein and exists in two enantiomeric forms, R-(+)-equol and S-(-)-equol. Although both are biologically active, only S-equol is the natural diastereoisomer produced by intestinal bacteria in 20%–30% population in Western countries and 50%–60% in Asian population consuming soy-containing diets[2, 28].

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Fig 6. Effect of equol on hKir2.1. (A) Voltage-dependent hKir2.1 current recorded in a representative HEK 293 cell expressing KCNJ2 in the absence and presence of 30 μM equol. (B) Percentage values of hKir2.1 current at −100 mV in the absence and presence of 3, 10, 30 μM equol (n = 5).
potential cardiovascular benefits of equol have been widely reported[29–33]; however, information related to ion channel involvement is limited. Only one study reported that equol (40 μM) significantly inhibited L-type Ca2+ current (I\(\text{Ca,L}\)) in guinea pig cardiac ventricular myocytes[34]. Our recent study demonstrated that equol increased cerebral blood flow by vasodilation via activating BKCa channels[13]. The present study provides novel experimental information that equol decreases several cardiovascular K+ channels including hKv1.5, hKv4.3, hKCNQ1/hKCNE1, and hERG channels.

Kv1.5 channels are expressed not only in human atrial myocytes for encoding human atrial IKur responsible for atrial repolarization[35, 36], but also in human pulmonary artery smooth muscle cells in which it plays an important role in regulating membrane potential and vascular tone[37]. IKur/hKv1.5 is considered an atrial-selective target for developing anti-atrial fibrillation drug[38, 39]. The present study demonstrated that equol significantly suppressed hKv1.5 stably expressed in HEK 293 cells (IC50, 15.3 μM). The efficacy of equol for inhibiting hKv1.5
Equol decreased hKv4.3 current stably expressed in HEK 293 cells with IC50s of 11.9 μM (for current charge area) and 30.3 μM (for peak current), and negatively shifted the inactivation.

Fig 8. Effects of equol on action potentials in isolated ventricular myocytes from guinea pig hearts.
(A) Action potentials recorded (2 Hz) in current clamp mode in a representative myocyte in the absence (control) and presence of 1, 3, and 10 μM equol, and washout. (B) Individual (triangle symbols) and mean (thick horizontal lines) values of APD50 and APD90 in the absence and presence of 1, 3, and 10 μM equol (n = 8–11, P = NS vs. control).

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is greater than the parent compound daidzein for inhibiting Kv current in rabbit coronary arterial smooth muscle cells (26% inhibition with 30 μM) and in mouse Schwann cells (no inhibition with 100 μM). Whether equol can be used to treat atrial fibrillation remains to be studied.

Kv4.3 (KCND3) encodes cardiac Ito (transient outward K+ current) or IA (voltage-activated A-type K+ current) in smooth muscles or neuronal cells. Ito is responsible for early rapid repolarization of cardiac action potential in several mammalian species including humans, and the gain-of-function mutation of hKv4.3 is implicated Brugada Syndrome. Equol decreased hKv4.3 current stably expressed in HEK 293 cells with IC50s of 11.9 μM (for current charge area) and 30.3 μM (for peak current), and negatively shifted the inactivation.
potential and positively shifted activation potential of hKv4.3 current. The efficacy for inhibiting hKv4.3 by equol is greater than daidzein (only 30%–40% inhibition with 300 μM)[46]. Equol, as quinidine for blocking open channel of rat cardiac Ito[22], significantly increased inactivation phase, so that the open channel blockade of equol caused a great reduction of the current charge area at a low concentration range (IC50 = 11.9 μM), which suggests potential effect of treating Brugada Syndrome if plasma concentrations reach 3–10 μM.

It is interesting to note that our recent studies demonstrates that equol provides significant neuroprotection against ischemia/reperfusion injury[33], and increases cerebral blood flow [13] in rat models. Further experiments show that equol dilates rat ex vivo cerebral basilar artery by eliciting BKCa channels via acting its auxiliary β1-subunits[13]. The present study showed that the effective concentrations of equol for activating BKCa channels were as low as 0.03 to 3 μM (EC50, 0.1 μM), which is very close to equol’s plasma concentrations. The efficacy was much greater than daidzein (little effect at 1 μM) for stimulating BKCa in vascular smooth cells from rat cerebral basilar arteries[47]. Therefore, equol may be a promising drug candidate for treating patients with brain ischemia.

It is well known that IKr and IKs are encoded respectively by hERG[48] and hKCNQ1/ hKCNE1[49], and both are important for repolarization in human heart[35, 50]. The genetic or acquired dysfunction of IKr or IKs may cause cardiac arrhythmias due to prolongation of electrocardiogram (ECG) QT interval induced by delayed ventricular repolarization; therefore, blockade of IKr (hERG) and/or IKs is implicated to be a potential risk of cardiac arrhythmia [51, 52]. In the present study, we found that equol significantly decreased hERG and IKs. However, the concentrations for inhibiting hERG (IC50s, 31.6 μM for IhERG.tail and 56.2 μM for IhERG.step) and recombinant IKs (IC50, 24.7 μM) are higher than plasma concentrations[25–27]. In addition, equol had no effect on Kir2.1 channels. Interestingly, equol did not prolong ventricular APD at 1 and 3 μM, which are effective concentrations for increasing BKCa current, and decreased cardiac APD at 10 μM, which may be resulted from the L-type Ca2+ current inhibition[34]. Therefore, equol would have little cardiac toxicity potential of prolonging ventricular APD thereby QT interval on ECG.

Conclusion
Collectively, the present study provides the novel information that equol has a stimulating effect on BKCa channels at very low concentrations, inhibitory effects on hKv1.5 and hKv4.3 at relatively low concentrations, and inhibitory effects on IKr and IKs at high concentrations. These results suggest that equol may be a promising and safe drug candidate for treating patients with cerebral vascular disorders with limited cardiac toxicity potential.

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Author Contributions
Conceptualization: Xiu-Ling Deng, Yan Wang, Guo-Sheng Xiao.
Data curation: Xiu-Ling Deng, Guo-Sheng Xiao.
Formal analysis: Xiu-Ling Deng, Guo-Sheng Xiao.
**Funding acquisition:** Xiu-Ling Deng, Yan Wang.

**Investigation:** Xiu-Ling Deng, Guo-Sheng Xiao.

**Methodology:** Xiu-Ling Deng, Guo-Sheng Xiao.

**Project administration:** Xiu-Ling Deng, Yan Wang.

**Resources:** Xiu-Ling Deng.

**Software:** Guo-Sheng Xiao.

**Supervision:** Xiu-Ling Deng.

**Validation:** Xiu-Ling Deng, Guo-Sheng Xiao.

**Visualization:** Xiu-Ling Deng, Yan Wang, Guo-Sheng Xiao.

**Writing – original draft:** Xiu-Ling Deng, Guo-Sheng Xiao.

**Writing – review & editing:** Xiu-Ling Deng.

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