Acacetin, a Natural Flavone, Selectively Inhibits Human Atrial Repolarization Potassium Currents and Prevents Atrial Fibrillation in Dogs

Gui-Rong Li, PhD; Hong-Bing Wang, PhD; Guo-Wei Qin, PhD; Man-Wen Jin, PhD; Qiang Tang, PhD; Hai-Ying Sun, BSc; Xin-Ling Du, MD, PhD; Xiuliong Deng, PhD; Xiaohua Zhang, MSc; Jing-Bo Chen, MSc; Lei Chen, MB; Xiaohui Xu, BSc; Lik-Cheung Cheng, MD; Shui-Wah Chiu, MD; Hung-Fat Tse, MD; Paul M. Vanhoutte, MD, PhD; Chu-Pak Lau, MD

Background—The development of atrium-selective antiarrhythmic agents is a current strategy for inhibiting atrial fibrillation (AF). The present study investigated whether the natural flavone acacetin from the traditional Chinese medicine Xuelianhua would be an atrium-selective anti-AF agent.

Methods and Results—The effects of acacetin on human atrial ultrarapid delayed rectifier K⁺ current (IKur) and other cardiac ionic currents were studied with a whole-cell patch technique. Acacetin suppressed IKur and the transient outward K⁺ current (IC₅₀ 3.2 and 9.2 μmol/L, respectively) and prolonged action potential duration in human atrial myocytes. The compound blocked the acetylcholine-activated K⁺ current; however, it had no effect on the Na⁺ current, L-type Ca²⁺ current, or inward-rectifier K⁺ current in guinea pig cardiac myocytes. Although acacetin caused a weak reduction in the hERG and hKCNQ1/hKCNE1 channels stably expressed in HEK 293 cells, it did not prolong the corrected QT interval in rabbit hearts. In anesthetized dogs, acacetin (5 mg/kg) prolonged the atrial effective refractory period in both the right and left atria 1 to 4 hours after intraduodenal administration without prolongation of the corrected QT interval, whereas sotalol at 5 mg/kg prolonged both the atrial effective refractory period and the corrected QT interval. Acacetin prevented AF induction at doses of 2.5 mg/kg (50%), 5 mg/kg (85.7%), and 10 mg/kg (85.7%). Sotalol 5 mg/kg also prevented AF induction (60%).

Conclusions—The present study demonstrates that the natural compound acacetin is an atrium-selective agent that prolongs the atrial effective refractory period without prolonging the corrected QT interval and effectively prevents AF in anesthetized dogs after intraduodenal administration. These results indicate that oral acacetin is a promising atrium-selective agent for the treatment of AF. (Circulation. 2008;117:2449-2457.)

Key Words: arrhythmia • drugs • electrophysiology • pharmacology • ion channels

Atrial fibrillation (AF) is the most common form of cardiac dysrhythmia, and the occurrence of AF increases with age: The prevalence rises from 0.5% of people in their 50s to 5% of people over the age of 65 years and nearly 10% of the population over 80 years of age.¹² AF is a major cause of morbidity and mortality because it increases the risk of death, congestive heart failure, and embolic phenomena, including stroke.¹² AF is believed to be a lifetime risk in an aging population, and therefore, it is emerging as a major public health concern.³⁴ Antiarrhythmic drug therapy remains the principal approach for suppressing AF and its recurrence. Class III antiarrhythmic agents are effective in treating AF⁵⁶ but have major limitations, such as inducing severe ventricular arrhythmia (ie, long-QT syndrome).⁷ Therefore, a key objective among the current strategies for suppressing AF is the development of antiarrhythmic agents that preferentially affect atrial rather than ventricular electrical parameters.⁸⁹

Received January 28, 2008; accepted March 19, 2008.
From the Department of Medicine (G.-R.L., H.-Y.S., X.-L. Du, X.-L. Deng, H.-F.T., C.-P.L.) and Department of Physiology (G.-R.L.). Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong SAR, China; Shanghai Institute of Materia Medica (H.-B.W., G.-W.Q.), Chinese Academy of Science, Shanghai, China; Department of Pharmacology (M.-W.J., Q.T., X.-H.Z., J.-B.C., L.C., X.-H.X.), Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; Cardiothoracic Unit (L.-C.C., S.-W.C.), Grantham Hospital, University of Hong Kong, Hong Kong SAR, China; and Department of Pharmacology (P.M.V.), Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong SAR, China.

The online-only Data Supplement, consisting of an expanded Methods section, tables, and figures, is available with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.108.769554/DC1.

Correspondence to Gui-Rong Li, Department of Medicine, The University of Hong Kong, 21 Sassoon Rd, Pokfulam, Hong Kong SAR, China (e-mail gri@hkucc.hku.hk) or Guo-Wei Qin, Shanghai Institute of Materia Medica, Chinese Academy of Science, Shanghai, China (e-mail gqwqin@mail.shcnc.ac.cn).

© 2008 American Heart Association, Inc.

Circulation is available at http://circ.ahajournals.org

DOI: 10.1161/CIRCULATIONAHA.108.769554

2449
Clinical Perspective p 2457

Inhibition of the ultrarapid delayed rectified potassium current (I_{Kur}), present in atria but not ventricles in human heart,\textsuperscript{10} is an example of an atrium-selective approach. I_{Kur} block selectively prolongs atrial repolarization and can suppress AF.\textsuperscript{9,11} Hence, pharmaceutical investigations have focused on developing selective inhibitors of the human atrial I_{Kur} or hKv1.5 channels;\textsuperscript{12} however, no such therapeutic agent is commercially available. Traditional Chinese medicine may be a great resource that can be used to develop this type of drug. The present study used traditional Chinese medicine to find selective I_{Kur} blockers for the treatment of AF. It examined whether or not the natural flavone acacetin, initially isolated from the traditional Chinese medicine Xuelianhua (Saussurea tridactyla), is atrium selective and effective in preventing AF.

Methods

Human and Guinea Pig Cardiac Myocyte Preparation

Human atrial cells and guinea pig cardiac myocytes were enzymatically dissociated as described previously\textsuperscript{13,14} and in the online-only Data Supplement.

Cell Line Culture

Established HEK 293 cell lines stably expressing the hERG (human ether-a-go-go related gene) channel gene\textsuperscript{15} and recombinant human cardiac KCNQ1/KCNEL channel current (I_{Ks})\textsuperscript{16} were maintained separately in DMEM (Invitrogen, Carlsbad, Calif) supplemented with 10% fetal bovine serum and containing 400 μg/mL G418 (for hERG channels) or 100 μg/mL hygromycin (for I_{Ks}).

Solutions and Drugs

Solutions used in the present study are described in the online-only Data Supplement. Acacetin, which was initially isolated and purified from the traditional Chinese medicine Xuelianhua and then synthesized in the laboratory, was dissolved in dimethyl sulfoxide as a 100 mmol/L stock solution and stored at −20°C. Other chemicals were purchased from Sigma-Aldrich (St Louis, Mo).

Patch-Clamp Recording

Whole-cell patch voltage or current clamp techniques were used to record membrane currents or action potentials as described in the online-only Data Supplement.

Isolated Rabbit Heart Preparation

New Zealand White rabbits (weight 2 to 3 kg) of either gender were anesthetized with pentobarbital (30 mg/kg IV), and their hearts were removed quickly, placed into oxygenated (95% O\textsubscript{2}-5% CO\textsubscript{2}) Krebs-Henseleit solution, and mounted in a Langendorff system and perfused with 37°C oxygenated solution as a 100 mmol/L stock solution and stored at −20°C. Other chemicals were purchased from Sigma-Aldrich (St Louis, Mo).

In Vivo Cardiac Electrophysiology and Experimental AF Model in Anesthetized Dogs

Adult mongrel dogs (weight 12 to 15 kg, n=44) were used to determine the atrial effective refractory period (ERP) and generate AF as described in the online-only Data Supplement.

Data Analysis

All results are expressed as mean±SEM. Statistical comparisons were analyzed by paired Student t test or repeated ANOVA where appropriate. Categorical data were analyzed with the χ\textsuperscript{2} test. A value of P<0.05 was considered statistically significant. Nonlinear curve fitting was performed with Pulsefit (HEKA, Lambrecht/Pfalz, Germany) and Sigmaplot (SPSS, Chicago, Ill).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Initial Finding

We initially studied the effects of extracts from the traditional Chinese medicine Xuelianhua (Saussurea tridactyla) on membrane currents in human atrial myocytes and found that 1 of the extracts (XLH-I) showed remarkable inhibition of both I_{Kur} and the transient outward K\textsuperscript{+} current (I_{to}) in human atrial myocytes (online-only Data Supplement Figure 1). Then, 4 compounds were isolated from the extract XLH-I, and compound A was found to block I_{Kur} and I_{to} in human atrial myocytes. The chemical structure of compound A was verified to be acacetin (Figure 1), which then was synthesized in the laboratory. Because the effects of this compound on ion channels and cardiovascular diseases are not understood, we studied the effects of acacetin on I_{Kur}, I_{to}, and other cardiac ion channel currents and evaluated its in vivo anti-AF action.

Effects of Acacetin on I_{Kur}, I_{to} and Action Potential in Human Atrial Myocytes

To determine the effects of acacetin on I_{Kur}, the time course of the current was recorded in human atrial cells, as described previously,\textsuperscript{13,14} in the absence and presence of acacetin (Figure 2A). Acacetin 3 μmol/L gradually inhibited I_{Kur}, and the effect recovered (by 94%) on washout. Voltage-dependent I_{Kur} was determined with the voltage protocol shown in the inset of Figure 2B. Acacetin 3, 10, or 30 μmol/L substantially inhibited both tail and step currents of I_{Kur} (Figure 2B). The concentration-response relationship for the inhibition of I_{Kur} by acacetin from 0.3 to 100 μmol/L was evaluated at 40 mV (Figure 2C). The IC\textsubscript{50} was fitted to the Hill equation as described in the online-only Data Supplement. The IC\textsubscript{50} of acacetin for inhibiting I_{Kur} was 3.2 μmol/L, and its Hill coefficient was 0.8.

Figure 3A displays the time course of I_{to} recorded in a typical experiment in the absence and presence of acacetin. Acacetin 3 μmol/L reduced I_{to}, and the effect recovered (by 95%) on washout; however, the sustained current (ie, I_{Kurb}) was simultaneously reduced by acacetin. Although the I_{to} measured was peak to the quasi–steady state level, we suspected that the evaluation of the effect of acacetin on I_{to} might not be accurate. We have recently found that verapamil inhibits I_{Kur} without causing a reduction of I_{to} amplitude, whereas it induces an increase of measured I_{to} in human atrial

![Figure 1. Chemical structure of acacetin.](Image)
myocytes. Therefore, verapamil 10 μmol/L was used to separate I\textsubscript{to} as shown in Figure 3B. I\textsubscript{to} amplitude was actually increased by verapamil. Voltage-dependent I\textsubscript{to} was substantially inhibited by acacetin (3 or 10 μmol/L) in the presence of verapamil 10 μmol/L (Figure 3B). The inhibitory effect of I\textsubscript{to} by acacetin was concentration dependent, with an IC\textsubscript{50} of 9.3 μmol/L (Figure 3C) and a Hill coefficient of 0.9.

Acacetin 10 μmol/L did not affect the voltage dependence of either inactivation or activation of I\textsubscript{Kur} (online-only Data Supplement Figure II A), whereas it slowed the recovery of I\textsubscript{Kur} from inactivation (τ=102±12 ms in control and 136±7 ms in the presence of acacetin; P<0.01; online-only Data Supplement Figure II B).

The inhibition of I\textsubscript{Kur} and I\textsubscript{to} by acacetin suggests that this compound prolongs action potential duration (APD) in human atrial myocytes. We therefore recorded action potentials at 36°C with the perforated patch configuration in current-clamp mode to determine the effect of acacetin on human atrial APD. Figure 4A illustrates action potentials recorded at 2 Hz in representative human atrial myocytes in the absence and presence of acacetin or 4-aminopyridine (4-AP, a well-known blocker of I\textsubscript{Kur}). Acacetin (5 or 10 μmol/L) prolonged the APD in a parallel fashion without affecting the resting membrane potential or the amplitude of the action potential. This effect recovered on washout. The APD at 50%, 75%, and 90% repolarization was increased significantly (Figure 4B). Acacetin induced a slight rate-dependent increase in 50%, 75%, and 90% APD repolarization (APD\textsubscript{50}, APD\textsubscript{75}, and APD\textsubscript{90}, respectively). A concentration of 50 μmol/L 4-AP prolonged APD\textsubscript{50} more than APD\textsubscript{90} (Figure 4A) and induced a reverse rate-dependent prolongation of APD\textsubscript{50}, APD\textsubscript{75}, and APD\textsubscript{90} (online-only Data Supplement Figure III). These results suggest that the prolongation of human atrial APD by acacetin is likely not limited to the inhibition of I\textsubscript{Kur} and I\textsubscript{to}.

**Effects of Acacetin on Acetylcholine-Activated Potassium Currents in Guinea Pig Atrial Myocytes**

To further investigate the effects of acacetin on other cardiac ionic currents, guinea pig left atrial myocytes were used to study the effect on acetylcholine-activated K\textsuperscript{+} current (I\textsubscript{KACCh}), because 4-AP–sensitive I\textsubscript{Kur} or I\textsubscript{to} channels are not expressed in the atria of this species. The membrane currents recorded with a ramp protocol (Figure 5A) and voltage-step protocol (Figure 5B) showed that carbachol 5 μmol/L augmented membrane conductance, and acacetin 3 μmol/L significantly reversed the increased membrane conductance. Figure 5C illustrates current-voltage (I-V) relationships of carbachol-
Effects of Acacetin on Other Cardiac Ionic Currents
The effects of acacetin on other cardiac ionic currents ($I_{Na}$, $I_{Ca}$, and $I_{K}$) were studied in guinea pig ventricular myocytes. Acacetin 30 μmol/L had no inhibitory effect on $I_{Na}$ (Figure 5D), $I_{Ca}$ (Figure 5E), or $I_{K}$ (Figure 5F), and at 100 μmol/L, it also did not inhibit $I_{Na}$, $I_{Ca}$, or $I_{K}$ (data not shown).

The effect of acacetin on $I_{Kr}$ or $I_{Ks}$ was determined in HEK 293 cells stably expressing hKCNQ1/hKCNE1. Acacetin 30 μmol/L inhibited the amplitude of hERG channels (α-subunit of human cardiac $I_{Kr}$) or $I_{Ks}$ channels (hKCNQ1/hKCNE1). Acacetin 30 μmol/L inhibited the amplitude of hERG channels elicited by the voltage protocol (inset of Figure 6A) in a representative cell, and this effect disappeared on washout (Figure 6A). The IC$_{50}$ of acacetin for inhibition of $I_{hERG,tot}$ was 32.4 μmol/L (Figure 6B), and its Hill coefficient was 0.9.

Figure 6C displays the effects of acacetin on $I_{Ks}$ stably expressed in an HEK 293 cell line. Acacetin 30 μmol/L significantly inhibited the amplitude of $I_{Ks}$, and the effect partially recovered (by 83%) on washout. Acacetin decreased $I_{Kr}$ at 20 to 60 mV in a concentration-dependent manner. The IC$_{50}$ of acacetin for inhibition of $I_{Kr}$ (40 mV) was 81.4 μmol/L (Figure 6D), and its Hill coefficient was 0.8.

Effects of Acacetin on Corrected QT Interval in Isolated Rabbit Hearts
The suppression of $I_{hERG}$ and $I_{Ks}$ suggests that acacetin has the potential to prolong the QT interval. Rabbit hearts express significant $I_{Ks}$ channels$^{18}$ and have been used for the evaluation of the proarhythmic effects of cardioactive agents.$^{19}$ Therefore, the isolated rabbit heart was used to study whether or not acacetin would increase the corrected QT interval (QTc). The hearts were perfused with a hypokalemic (3 mmol/L K$^+$) solution. Representative ECG recordings are shown in Figure 7A for acacetin and in Figure 7B for quinidine. Acacetin 30 μmol/L had no effect on heart rate or the QTc, whereas quinidine 10 μmol/L slowed the heart rate and significantly increased the QTc. Mean values of heart rate and the QTc are illustrated in Figure 7C and 7D. These results suggest that acacetin does not prolong the QTc of the isolated rabbit heart under hypokalemic conditions.

Acute Toxicity in Mice
Acute in vivo toxicity was assessed in mice with a maximal concentration of acacetin obtainable in suspension of a maximal volume after starvation of the animal for 14 hours. The dose of acacetin 0.3 g/kg was administered 3 times at intervals of 1.5 hours. No animal deaths occurred within a 2-week observation period, and no abnormal activity was observed compared with vehicle-control animals. This result suggests that oral administration of acacetin has low or no acute toxicity.

Effects of Acacetin on Atrial ERP and Acute AF in Anesthetized Dogs
To demonstrate whether acacetin would exhibit an anti-AF action in anesthetized dogs, we first determined the effect of the compound on atrial ERP (online-only Data Supplement) by introducing S1–S2 stimuli via a programmed cardiac stimulator (Figure 8A). We found that left and right atrial ERPs were significantly prolonged after the duodenal administration of acacetin (5 mg/kg) or sotalol (5 mg/kg) during a 4-hour observation at basic cycle lengths of 300, 250, and 200 ms (online-only Data Supplement Tables I and II). Figure 8B shows an example of mean values of the percent changes in atrial ERP (online-only Data Supplement). Figure 8A shows an example of mean values of the percent changes in atrial ERP (online-only Data Supplement).

We found that the S2 stimulus would trigger sustained AF (lasting >1 minute) when right ERP was measured with a 200-ms basic cycle length in anesthetized dogs. In 1 animal from the acacetin group, AF was induced by S2 stimulus before drug administration (Figure 8C) but not after 2 hours of acacetin administration (Figure 8D). In another animal from the vehicle group, AF was always induced by S2 stimulus when right atrial ERP was determined during the
observation period. This suggests that acacetin likely has an anti-AF effect.

Sotalol 5 mg/kg showed a reverse rate-dependent prolongation of ERP and increased QTc, as reported previously.\textsuperscript{5,6} However, acacetin had no reverse rate-dependent effect on ERP (Figure 8E) and did not prolong the QTc (Figure 8F). These results suggest that acacetin is likely an anti-AF agent that does not cause QTc prolongation.

The effect of acacetin on experimental AF was then evaluated in anesthetized dogs. AF was induced by S1–S2 stimulation at 100-ms basic cycle length with bilateral vagal stimulation (online-only Data Supplement, Methods and Figure IV) at certain time points during the 0.5- to 4-hour period after intraduodenal administration. AF lasted for 10 minutes and terminated once vagal stimulation was stopped. Either no AF occurrence or a shortened AF duration during the continuous vagal stimulation was considered to represent prevention of AF. The incidence of AF was reduced in drug-treated groups (Figure 8G). Sustained AF was observed in 100% of the animals (n = 5) during each AF induction test in the vehicle group but not in 50% (3 of 6), 57% (4 of 7), and 57% (4 of 7) of the animals in the acacetin 2.5-, 5-, and 10-mg/kg groups, respectively, or in 40% (2 of 5) of the animals in the sotalol group (5 mg/kg).

In addition, in the acacetin 5-mg/kg group, 2 dogs showed a shorter duration of AF (1 lasted for 5 minutes 31 seconds, and another lasted for 6 minutes 12 seconds) 2 hours after drug administration. In the acacetin 10-mg/kg group, AF lasted for 4 minutes 30 seconds in 1 animal, and 7 minutes 11 seconds in another animal. In the sotalol group, shortened AF duration (8 minutes 5 seconds) was observed in 1 animal. The summarized anti-AF efficacy was 0%, 50%, 85.7%, 85.7%, and 60% in the vehicle group, acacetin 2.5-mg/kg group, acacetin 5-mg/kg group (P < 0.05), acacetin 10-mg/kg group (P < 0.05), and sotalol 5-mg/kg group, respectively (Figure 8H).

**Discussion**

Acacetin is a flavone compound (5,7-dihydroxy-4′-methoxyflavone) that is broadly distributed in plant pigments, universally present in vascular plants, and responsible for many of the colors in nature.\textsuperscript{20} Acacetin has been reported to possess antiperoxidative, antiinflammatory, and antiplasmodial effects,\textsuperscript{21,22} to enhance differentiation-inducing activity in HL-60 cells,\textsuperscript{23} and to exert an anticancer action in several types of cancers, including human prostate cancer, lung cancer, and HepG2.\textsuperscript{24–26} In addition, acacetin can inhibit glutathione reductase and cytochrome P450.\textsuperscript{27,28} The present study provides new evidence that the flavone acacetin preferentially inhibits I_{Kur} and I_{Na} and prolongs APD in human atrial myocytes and that it prolongs ERP and prevents AF induction in anesthetized dogs.

It is generally accepted that cardiac repolarization and refractoriness are determined by the balance of inward Ca\textsuperscript{2+} and outward K\textsuperscript{+} currents. I_{Kur} and I_{Na} are major outward currents in the human atrium and thus play important roles in human atrial repolarization.\textsuperscript{29} Previous work has demonstrated that I_{Kur} is functionally present in the atrium but not in the ventricle of the human heart.\textsuperscript{10} Therefore, I_{Kur} is believed to be a target for development of selective anti-AF agents.\textsuperscript{30} The present study showed that acacetin inhibits human atrial I_{Kur} in a concentration-dependent manner with an IC_{50} of 3.2 μmol/L (Figure 2). This concentration is lower than those observed previously in antiperoxidative, antiinflammatory, and antimutagenic studies.\textsuperscript{22,25,31} In addition, acacetin blocked I_{Na} with an IC_{50} of 9.2 μmol/L (Figure 3) and slowed the recovery of I_{Na} from inactivation without affecting the voltage dependence of the current (online-only Data Supple-
The inhibition of \( I_a \) by acacetin should also contribute to the prolongation of APD in human atrium.\(^{29}\) Acacetin (5 or 10 \( \mu \)mol/L) significantly prolonged APD at 50%, 75%, and 90% repolarization at 0.5, 1.0, and 2.0 Hz (Figure 4). The prolongation of APD would increase the ERP and terminate AF.

The effect of potassium channel blockers on action potential was different in post-AF remodeling from that in normal atrium.\(^{32}\) A limitation of the present study was that we did not perform a comparative study of the effects of acacetin on action potential in cells from patients in sinus rhythm and those with AF, because we were unable to obtain consent/approval from AF patients to use their atrial specimens for this purpose.

Vagal stimulation shortens the atrial APD and ERP, and therefore, vagal nerve tone plays a crucial role in AF.\(^{33,34}\) The acetylcholine-activated potassium current \( I_{KAC} \) mediates much of the cardiac response to vagal nerve stimulation via muscarinic M-receptor activation.\(^{35,36}\) \( I_{KAC} \) and M-receptor expression are upregulated in AF patients\(^{37}\) and in AF induced by experimental heart failure in dogs.\(^{38}\) Therefore, blockage of \( I_{KAC} \) should terminate AF induced by increased vagal nerve tone. The selective \( I_{KAC} \) blocker tertiapin, a bee venom peptide, terminated AF caused by stimulation of the vagal nerve in dogs.\(^{35}\) The present findings demonstrate that acacetin 3 and 10 \( \mu \)mol/L substantially inhibits carbachol-elicited \( I_{KAC} \) in guinea pig atrial myocytes (Figure 5), which indicates that acacetin is most likely effective in terminating AF induced by \( I_{KAC} \) activation.

Acacetin 30 to 100 \( \mu \)mol/L demonstrated no inhibition of cardiac \( I_{Na} \), \( I_{CaL} \), or \( I_{K1} \) in guinea pig ventricular myocytes (Figure 5). These results suggest that acacetin should not reduce cardiac conduction velocity or contractility and should not depolarize the cell membrane, as observed in class I or class IV antiarrhythmic drugs.

On the other hand, acacetin inhibited \( I_{hERG} \) with an \( IC_{50} \) of 32.4 \( \mu \)mol/L in the HEK 293 cell line and suppressed human cardiac \( I_{Ks} \) with an \( IC_{50} \) of 81.4 \( \mu \)mol/L in HEK 293 cells stably expressing hKCNQ1/hKCNE1 channels (Figure 6). These results suggest that acacetin may have the potential to cause QT prolongation.\(^{7}\) However, acacetin (30 \( \mu \)mol/L), in contrast to quinidine (10 \( \mu \)mol/L), did not affect heart rate and did not cause QTc prolongation in isolated hypokalemic rabbit hearts (Figure 7).
Blockade of the ultrarapid delayed rectifier current \(I_{\text{Kur}}\) (or \(K_v1.5\)) channel has been proposed as a novel target for the development of safer and potentially more effective atrial antiarrhythmic agents.\(^3\) Four structurally distinct synthesized antiarrhythmic agents have been described as possessing \(I_{\text{Kur}}\) block as part of their spectrum of actions: NIP-141,\(^4\) AVE0118,\(^4\) RSD1235,\(^4\) and DPO-1.\(^4\) The present study demonstrates that acacetin, a natural flavone \(I_{\text{Kur}}\) blocker, also blocks \(I_{\text{to}}\) and \(I_{\text{KACh}}\). These properties are favorable for termination of AF. In addition to the blockade of \(I_{\text{Kur}}\), \(I_{\text{to}}\), and \(I_{\text{KACh}}\), previous work has demonstrated that the compound possesses an antiperoxidative effect,\(^2\) which may exert an additional beneficial effect for the treatment of AF, because oxidant damage is believed to contribute to the genesis of AF in humans.\(^4\)\(^5\)\(^6\)

More importantly, acacetin (5 mg/kg) significantly prolonged atrial ERP without prolonging the QTc in anesthetized dogs (Figure 8; online-figures only Data Supplement Tables I and II) and thus differs from sotalol, which prolonged both atrial ERP and QTc. These results suggest that acacetin has a potential anti-AF effect with no proarhythmic potential. Indeed, the anti-AF effect was proven in anesthetized dogs, because acacetin 5 and 10 mg/kg significantly prevented AF induction (Figure 8). In addition, in the present study, no animal deaths occurred in mice with a maximal oral administration of acacetin (900 mg/kg) during a 2-week observation period, which indicates that acacetin has low or no acute toxicity when administered orally.

In conclusion, the present results suggest that the natural flavone acacetin is likely a novel, promising, orally effective atrium-selective antiarrhythmic agent for the treatment of AF. In addition to the blockade of atrial \(I_{\text{Kur}}\), \(I_{\text{to}}\), and \(I_{\text{KACh}}\), the antiperoxidative and antiinflammatory effects of acacetin would be of benefit. These results should encourage the development of acacetin for treatment of atrial tachyarrhythmias, especially AF.
Acknowledgments
The authors thank Professor Tak-Ming Wong for his support, Dr Heather J. Ballard for her critical reading of the manuscript, and Yan Hu for her excellent technical assistance.

Sources of Funding
This study was supported by grants from the Research Grant Committee of the University of Hong Kong, Biopharmaceutical Development Centre of University of Hong Kong, and Sun Chieh Yeh Heart Foundation of Hong Kong.

Disclosures
None.

References
The present study demonstrated that the natural flavone acacetin from the Chinese medicine *Xuelianhua* selectively inhibited the human atrial ultrarapid delayed rectifier K⁺ current (I_{Kur}) and the transient outward K⁺ current (I_{to}) and prolonged action potential duration in human atrial myocytes. The compound blocked the acetylcholine-activated K⁺ current; however, it had no effect on the Na⁺ current, L-type Ca²⁺ current, or inward-rectifier K⁺ current in guinea pig ventricular myocytes. Although acacetin had a weak reduction in the hERG and hKCNQ1/hKCNE1 channels stably expressed in HEK 293 cells, it did not prolong the corrected QT interval in rabbit hearts. In anesthetized dogs, acacetin prolonged the atrial effective refractory period 1 to 4 hours after intraduodenal administration without prolongation of the corrected QT interval, whereas sotalol prolonged both. In addition, acacetin prevented atrial fibrillation induction in anesthetized dogs. The present study shows that acacetin is an atrium-selective agent and effectively prevents atrial fibrillation in anesthetized dogs after intraduodenal administration, which indicates that oral acacetin is a promising agent for the treatment of atrial fibrillation in humans.
Acacetin, a Natural Flavone, Selectively Inhibits Human Atrial Repolarization Potassium Currents and Prevents Atrial Fibrillation in Dogs
Gui-Rong Li, Hong-Bing Wang, Guo-Wei Qin, Man-Wen Jin, Qiang Tang, Hai-Ying Sun, Xin-Ling Du, Xiu-Ling Deng, Xiao-Hua Zhang, Jing-Bo Chen, Lei Chen, Xiao-Hui Xu, Lik-Cheung Cheng, Shui-Wah Chiu, Hung-Fat Tse, Paul M. Vanhoutte and Chu-Pak Lau

_Circulation_. 2008;117:2449-2457; originally published online May 5, 2008;
doi: 10.1161/CIRCULATIONAHA.108.769554
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/117/19/2449

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2008/05/05/CIRCULATIONAHA.108.769554.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/