Journal of Molecular and Cellular Cardiology 51 (2011) 966–973

Contents lists available at SciVerse ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Original article

Acacetin causes a frequency- and use-dependent blockade of hKv1.5 channels by binding to the S6 domain

Hui-Jun Wu [a], Wei Wu [a], Hai-Ying Sun [a], Guo-Wei Qin [b,*], Hong-Bing Wang [c], Panwen Wang [d], Hari Krishna Yalamanchili [d], Junwen Wang [d], Hung-Fat Tse [a], Chu-Pak Lau [a], Paul M. Vanhoutte [e], Gui-Rong Li [a,f,**]

[a] Department and Medicine, Li Ka Shing Faculty of Medicine, the University of Hong Kong, Pokfulam, Hong Kong SAR, China
[b] Shanghai Institute of Materia Medica, Chinese Academy of Science, Shanghai, China
[c] School of Life Science and Technology, Tongji University, Shanghai, China
[d] Department of Biochemistry, Li Ka Shing Faculty of Medicine, the University of Hong Kong, Pokfulam, Hong Kong SAR, China
[e] Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, the University of Hong Kong, Pokfulam, Hong Kong SAR, China
[f] Department of Physiology, Li Ka Shing Faculty of Medicine, the University of Hong Kong, Pokfulam, Hong Kong SAR, China

ARTICLE INFO

Article history:
Received 2 June 2011
Received in revised form 1 August 2011
Accepted 21 August 2011
Available online 27 August 2011

Keywords:
Acacetin
hKv1.5
Tonic blockade
Open channel blockade
Rate-dependent blockade

ABSTRACT

We have demonstrated that the natural flavone acacetin selectively inhibits ultra-rapid delayed rectifier potassium current (I_{Kur}) in human atria. However, molecular determinants of this ion channel blocker are unknown. The present study was designed to investigate the molecular determinants underlying the ability of acacetin to block hKv1.5 channels (coding I_{Kur}) in human atrial myocytes using the whole-cell patch voltage-clamp technique to record membrane current in HEK 293 cells stably expressing the hKv1.5 gene or transiently expressing mutant hKv1.5 genes generated by site-directed mutagenesis. It was found that acacetin blocked hKv1.5 channels by binding to both closed and open channels. The blockade of hKv1.5 channels by acacetin was use- and frequency-dependent, and the IC_{50} of acacetin for inhibiting hKv1.5 was 3.5, 3.1, 2.9, 2.1, and 1.7 μM, respectively, at 0.2, 0.5, 1, 3, and 4 Hz. The mutagenesis study showed that the hKv1.5 mutants V505A, I508A, and V512A in the S6-segment remarkably reduced the channel blocking properties by acacetin (IC_{50}, 29.5 μM for V505A, 19.1 μM for I508A, and 6.9 μM for V512A). These results demonstrate the novel information that acacetin mainly blocks open hKv1.5 channels by binding to their S6 domain. The use- and rate-dependent blocking of hKv1.5 by acacetin is beneficial for anti-atrial fibrillation.

1. Introduction

Atrial fibrillation is a major clinical problem that results in an increased mortality in aging population [1] due to the risk of congestive heart failure and embolic stroke [2,3]. It is emerging as a public-health concern [4]. Anti-arrhythmic drug therapy remains the principal approach for suppressing atrial fibrillation and its recurrence. However, although class III antiarrhythmic drugs are effective in managing atrial fibrillation, their potentially adverse effects including proarrhythmia limit the utility for treating atrial arrhythmias due to their prolongation of the ventricular action potential duration (APD) by blocking the major cardiac repolarizing potassium currents [i.e. the rapidly activating delayed rectifier potassium current (I_{Kr}) and/or the slowly activating delayed rectifier potassium current (I_{Ks})], which may result in QT interval prolongation and/or torsades de pointes (Tdp) [5]. The current strategy in the development of more effective and safer drugs to treat atrial arrhythmias is to develop selective agents that target ion channels that are mainly expressed in human atrial tissue [6,7].

The ultra-rapidly activating delayed rectifier potassium current I_{Kur} [8] is present in the atria, but not the ventricles of the human heart [9]. The human atrial I_{Kur} encoded by hKv1.5 (or hKCNA5) gene [8,10] mediates an outward potassium current with fast activation and slow inactivation kinetics [11], and therefore plays a crucial role in human atrial repolarization. Therefore, human atrial I_{Kur} and/or hKv1.5 channels appear to be ideal targets for developing atrial selective agents potentially used for treating atrial fibrillation [6,7].

Acacetin is a flavone compound broadly distributed in plant pigments, responsible for many of the colors in nature [12]. In addition to inhibiting transient outward potassium current (I_{to}) and acetylcholine-activated potassium current ($I_{K,ACh}$), acacetin effectively block human

Abbreviations: I_{Kur}, ultra-rapidly delayed rectifier potassium current; HEK 293 cells, human embryonic kidney 293 cells; Kv channels, voltage-gated potassium channels; WT, wild type.

* Corresponding author.
** Correspondence to: G.-R. Li, L4-59, Laboratory Block, FBM, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong SAR, China. Tel.: +86 2819 9513; fax: +86 2855 9730.
E-mail addresses: gwqin@mail.shcnc.ac.cn (G.-W. Qin), grli@hkucc.hku.hk (G.-R. Li).

0022-2828/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.yjmcc.2011.08.022

atrial I_{Kur}. Importantly, acacetin increased the atrial effective refractory period and prevented the occurrence of atrial fibrillation in anesthetized dogs without prolonging the QT interval [13]. The present study was designed to determine the electrophysiological characteristics of the hKv1.5 channel blockade by acacetin and to investigate the molecular determinants of this action. The present results suggest that acacetin is an open and close channel blocker with use- and frequency-dependent blocking properties resulted from its binding to the S6 domain of hKv1.5 channels.

2. Materials and methods

2.1. Cell line culture and gene transfection

A HEK 293 cell line stably expressing the human Kv1.5 (KCNA5) gene [14,15] was maintained in Dulbecco's modified eagle's medium (DMEM, Invitrogen, Hong Kong) supplemented with 10% fetal bovine serum and 400 μg/mL G418 (Sigma-Aldrich). Cells used for electrophysiology were seeded on a glass cover slip.

Polymerase chain reaction-based site-directed mutagenesis was used to produce mutations of the hKv1.5/pBK$_{CMV}$ vector provided by Dr. M Tamkun (Colorado State University, CO, USA). Primers used to generate the channel mutants were synthesized by the Genome Research Center, the University of Hong Kong (Hong Kong), and the mutants were generated using a QuikChange kit (Stratagene, La Jolla, CA) and confirmed by DNA sequencing. The mutant was transiently coexpressed with 4 μg of hKv1.5 mutant cDNA plasmid and green fluorescence plasmid (0.4 μg) in HEK 293 cells using 10 μL of Lipofectamine 2000™. The cells with green fluorescence were identified using a fluorescence microscope (Nikon, Japan) for the mutant hKv1.5 current recording.

2.2. Drugs and solutions

Acacetin used in the present study was synthesized in the laboratory as described previously in the US patent (http://www.patentstorm.us/patents/7816400.html) [16]. The compound was analyzed by LC/MS with purity of 99.5% (Fig. 1) and dissolved in dimethyl sulfoxide (DMSO) as a 100 mM stock solution. Aliquot stock was stored at −20 °C. Tyrode solution contained (in mM) 140 NaCl, 5.4 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 0.33 NaH_2PO_4, 10 HEPES, and 10 glucose; pH was adjusted to 7.4 with NaOH. The pipette solution contained (in mM) 20 KCl, 110 K-aspartate, 1 $MgCl_2$, 10 HEPES, 5 EGTA, 0.1 GTP, 5 Na_2-phosphocreatine, and 5 Mg-ATP; pH was adjusted to 7.2 with KOH.

2.3. Patch-clamp recording

Currents were recorded at room temperature (22–23 °C). Coverslips with adherent cells on the surface were transferred to an open cell chamber mounted on the stage of an inverted microscope and superfused at 2–3 mL/min. The whole-cell patch-clamp technique was used for electrophysiological recording as described [14,17]. Borosilicate glass electrodes (1.2-mm OD) were pulled using a Brown-Flaming puller (model P-97, Sutter Instrument Co, Novato, CA, USA). They had tip resistances of 2–3 MΩ when filled with the pipette solution. Membrane currents were recorded in voltage-clamp mode using an EPC-10 amplifier and Pulse software (HEKA, Lambrecht, Germany). A 3-M KCl–agar salt bridge was used as the reference electrode. The tip potential was zeroed before the patch pipette contacted the cell. After a gigaohm seal was obtained, the cell membrane was ruptured by gentle suction to establish the whole-cell configuration. The series resistance (Rs) was 3–5 MΩ and was compensated by 50–70% to minimize voltage errors, and membrane capacitance (12–18 pF) was electrically compensated. The current signal was sampled at 10 kHz, recorded and stored in the hard disk of an IBM compatible computer.

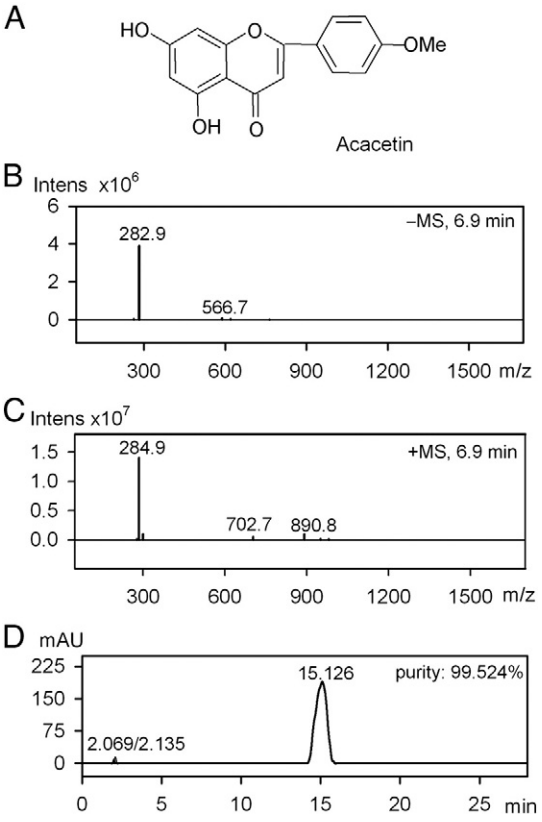

Fig. 1. Analysis of the synthesized acacetin. A. Chemical structure of acacetin. B and C. Mass spectrum (MS) analysis for identification (282.9 with −MS and 284.9 with +MS) of acacetin. D. Liquid chromatography (LC) analysis of acacetin.

2.4. Data analysis

The hKv1.5 current was measured at the end of voltage steps in the absence and presence of different concentrations of acacetin, and normalized to the control current. The concentration–response relationship curves were fitted to the Hill equation: $E = E_{max}/[1 + (IC_{50}/C)^b]$, where E is the inhibition of the current in percentage at drug concentration C, E_{max} is the maximal (completed) inhibition, IC_{50} is the concentration producing half-maximal inhibition, and b is the Hill coefficient.

The results are expressed as means ± S.E.M. Non-linear curve fitting was performed with Pulsefit (HEKA) and Sigmaplot (SPSS, Chicago, IL). Statistical comparisons were analyzed by Student's *t* test for two group data or analysis of variance for multiple group data. P values less than 0.05 were considered to indicate statistically significant differences.

2.5. Molecular modeling and docking

Interactions between acacetin and hKv1.5 channels were investigated by computational molecular docking using the software autodock 4.0 (http://autodock.scripps.edu/). Based on the crystal structure of hKv1.2 potassium channel subunit complex [18] from PDBe Protein Interfaces Surfaces and Assemblies (http://pdbe.org/pisa/) as described previously [19], the hKv1.5 tetramer was developed. The structure of acacetin was obtained from the website database (http://v1.inpacdb.org/acd0046/acd0046.pdb). The consistent valence force field was used to calculate conformational energies of acacetin and Kv1.5 model. The lowest free energy of docked conformation was between −5.39 kcal/mol and −5.34 kcal/mol. The random conformations of acacetin in the cavity of Kv1.5 tetramer were produced with all parameters default for 27,000 generations of the genetic algorithm.