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Effects of Psoralen Derivatives on hKv1.5 Current

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Abstract – We examined the effects of psoralen derivatives on a rapidly activating delayed rectifier K^+ channel (hKv1.5) cloned from human heart and stably expressed in Ltk^- cells. Using the whole cell configuration of the patch-clamp technique, we found that the five psoralen derivatives inhibited hKv1.5 current. Especially, 4-(2-Propenyloxy)-7H-furo[3,2-g][1]benzopyran-7-one (compound 5) was more potent than the inhibition of the hKv1.5 current of psoralen. The compound 5 inhibited the hKv1.5 current in a concentration-, time-, and voltage-dependent manner. These results suggest that the compound 5 is an excellent candidate as an antiarrhythmic drug for atrial fibrillation.

Keywords □ hKv1.5 channel, antiarrhythmic drug, Psoralen derivatives

INTRODUCTION

A major obstacle to the widespread use of drugs to manage cardiac arrhythmias has been a relatively high incidence of extracardiac side effects. It is well known that various K^+ channels regulate action potential durations and K^+ channel genes differentially express depending on the regions of the heart. K^+ channels represent the most diverse class of ion channels in heart. K^+ currents in the myocardium can be classified into two categories: 1) inward K^+ currents such as I_{K1} (inward rectifying K^+ current), I_{KACh} (acetylcholine-activated K^+ current), and I_{KATP} (ATP-sensitive K^+ current); and 2) voltage-gated K^+ (Kv) currents (Roden and George, 1999). The Kv channels contribute to cell repolarization and regulate the action potential duration. Accordingly, Kv channels become major targets for the treatment of arrhythmias. The hKv1.5 channel is known to have the same electrophysiological and pharmacological properties as I_{KUR}, a current specific in human atrium (Fedida, *et al.*, 1998). Thus, the hKv1.5 may form an important molecular target for the treatment of atrial tachy-arrhythmias, which represent a major clinical problem with serious morbidity (Cobbe, 1994). We previously reported that psoralen inhibited the hKv1.5 current in a concentration-, and voltage-dependent manner (Eun *et al.*, 2005).

In the present study, we synthesized the 5 derivatives of psoralen with 5-hydroxypsoralen or 8-hydroxypsoralen and examined the effects of psoralen derivatives on cardiac K^+ channels expressed in Ltk^-cells.

MATERIALS AND METHODS

Materials

The NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. The chemical shifts are reported as parts per million (delta) and the signals are quoted as a s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and dd (doublet of doublets). Thin Layer Chromatography (TLC) was carried out on precoated silicagel F_{254} plates (Merck, Darmstadt, Germany). 5-hydroxypsoralen, 8-hydroxypsoralen and other reagents were obtained from Aldrich Chemical Co.

Cell culture and transfection

The method used to establish hKv1.5 expression in a clonal mouse Ltk^- cell line is the same as it was described previously

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(Snyders *et al.*, 1992; 1993). The expression vector contains a dexamethasone-inducible murine mammary-tumor virus promoter that controls transcription of the inserted cDNA and a gene that confers neomycin resistance driven by the simian virus 40 early promoter. The cDNA-containing expression vector was transfected into mouse Ltk$^-$ cells. After 24 hours, the cells selection with 0.5 mg/ml of G418 was performed for 2 weeks or until discrete foci formed. Individual foci were isolated and maintained in 0.25 mg/ml of G418, and screened by Northern blotting and electrophysiological analysis (Tamkun *et al.*, 1991). 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 5% CO_2 atmosphere. Before experiments, subconfluent cultures were incubated with 2 μM dexamethasone for 12 hours to induce expression of hKv1.5 channels. The cells were removed from the dish with a rubber policeman, a procedure that left the majority of the cells intact.

Electrical recordings

The currents were recorded by using the whole cell configuration of the gigaohm-seal patch clamp techniques (Kwak *et al.*, 1999). The electrical signals were amplified with a patch clamp amplifier (Axopatch-1D, Axon Instruments, Foster, USA). The currents were digitized by a signal converter (Digidata 1200, Axon Instruments) and stored on the hard disk of a computer. The micropipette with a resistance of 1-2 MΩ (Kimax-51, 1.5-1.8x10mm) for current recording was pulled out by a 2-stage pipette puller (Narishige, PP-83, Tokyo, Japan). 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).

Synthesis of 9-(2-propenyl)-7H-furo[3,2-g][1]benzopyran-7-one (compound 1)

A solution of 9-hydroxypsoralen (0.88 g, 4.353 mmol) in anhydrous dichloromethane (22 mL) was cooled to 0°C. Freshly distilled N,N-diisopropyl ethylamine (0.83 mL, 4.788 mmol) was added dropwise, followed by the addition of trifluoromethanesulfonyl chloride (0.8 mL, 4.788 mmol). The mixture was stirred for 0.5 h at 0°C and quenched with saturated aqueous NH_4Cl solution. The organic layer was separated and washed with H_2O, brine. The organic layer was dried over $MgSO_4$ and concentrated in vacua. The residue was purified by flash chromatography (Hexane : EtOAc = 2 : 1) to give [9- trifluoromethanesulfoxy-7H-furo[3,2-g][1]benzopyran-7-one as white needles. To a solution of trifluoromethanesulfoxy-7H-furo[3,2-g][1]benzopyran-7-one (1.27 g, 3.8 mmol) in THF (19 mL) was added $Pd(PPh_3)_4$ (0.44 g, 0.38 mmol), allyltributyltin (2.22 mL, 7.6 mmol). The resulting mixture was stirred for 18 h at 60 °C and the poured into ice water. After extraction with EtOAc and washing with H_2O, the organic layer was dried over $MgSO_4$ and concentrated in vacua. The residue was purified by flash chromatography (Hexane : EtOAc = 3 : 1) to give compound 1 (0.60 g, 74% yield) as white crystals. R_f = 0.30 (Hexane : EtOAc = 3 : 1), ^{1}H-NMR (500 MHz, $CDCl_3$); δ 3.90 (dt, 2H, *J* = 1.5, 1.5, 6.5 Hz), 5.08 (dd, 1H, *J* = 1.5, 10 Hz), 5.17 (dd 1H, *J* = 1.5, 17 Hz), 6.11 (ddt, 1H, *J* = 6.5, 10, 17 Hz), 6.38 (d, 1H, *J* = 9.5 Hz), 6.83 (d, 1H, *J* = 2 Hz), 7.58 (s, 1H), 7.72 (d, 1H, *J* = 2 Hz), 7.80 (d, 1H, *J* = 9.5 Hz)

Synthesis of 4-(2-Propenyloxy)-7H-furo[3,2-g][1]benzopyran-7-one (compound 5)

A solution of 5-hydroxypsoralen (0.015 g, 0.074 mmol) in DMF (3 mL) was cooled to 0°C. NaH (0.005 g, 0.119 mmol, 60% in mineral oil) was added slowly for 10 min and then the mixture was stirred for 2 h at 0°C. Allyl bromide (0.026 mL, 0.297 mmol) was added dropwise and the reaction mixture was stirred for 19 h at room temperature and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacua. The residue was purified by flash chromatography (Hexane : EtOAc = 4 : 1) to give compound 5 (0.010 g, 56% yield) as a white solid. R_f= 0.26 (Hexane : EtOAc = 4 : 1), ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.21-8.17 (m, 1Hz), 7.62-7.60 (m, 1H), 7.18 (dd, 1H, *J*=0.5, 2.5Hz), 6.96-6.94 (m, 1H), 6.32-6.29 (m, 1H), 6.17-6.09 (m, 1H), 5.51-5.49 (m, 0.5H), 5.46 (dd, 0.5H, *J* =1.5, 3Hz), 5.38 (dd, 0.5H, *J* =1.5, 2.5Hz), 5.37-5.35 (m, 0.5H), 4.96-4.93 (m, 2H).

Synthesis of 9-acetoxy-7H-furo[3,2-g][1]benzopyran-7-one (compound 2), 9-(2-propenyloxy)-7H-furo[3,2-g][1]benzopyran-7-one (compound 3), 4-acetoxy-7H-furo[3,2-g][1]benzopyran-7-one (compound 4)

9-acetoxy-7H-furo[3,2-g][1]benzopyran-7-one (compound 2), 9-(2-propenyloxy)-7H-furo[3,2-g][1]benzopyran-7-one (compound 3) and 4-acetoxy-7H-furo[3,2-g][1]benzopyran-7-one (compound 4) were synthesized by the experimental method of Loutfy, M.A. *et al.* (1957).

Statistical analysis

The results are expressed as mean ± S.E.M. The student's *t*-

test and analysis of variance (ANOVA) were used to calculate the statistical analysis. Value of $p<0.05$ was considered statistically significant.

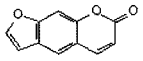
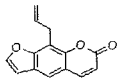
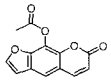
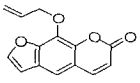
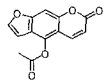
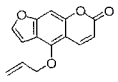
RESULTS AND DISCUSSION

In view of the above findings, it was considered of interest to undertake the synthesis of new psoralen derivatives, especially 5- and 8- substituted psoralen. In the preliminary results, 5- and 8-hydroxypsoralen were known to be inactive on hKv1.5 current expressed in the Ltk$^-$ cells. Therefore, hydroxyl moiety was converted to various functional groups.

Table I shows the effects of psoralen derivatives on hKv1.5 current expressed in the Ltk$^-$ cells. Inhibitory effects of compound 5 were more potent than psoralen. Under control conditions, depolarization positive to -20 mV elicited outward currents that progressively increased with further depolarizations. At +60 mV, after the current reached the maximum, it declined slowly during the maintained depolarization. In the presence of compound 5 (3 μM), both outward current during depolarizing steps and tail current were reduced compared to the control group (Fig. 1). Figure 1 shows the effects of compound 5 on the steady-state current-voltage relationship for the hKv1.5 channel constructed by plotting the current amplitudes at the end of 250-ms depolarizations as a function of the test pulse voltage. In the presence of compound 5, an inhibition of steady-state currents was observed through the whole voltage range over which hKv1.5 was activated ($n = 6$).

To quantify the voltage dependence of compound 5 block, the relative current $I_{compound}/I_{control}$ was plotted as a function of membrane potential (Fig. 2, n=5). In the presence of compound 5 (3 μM), the action of block on hKv1.5 current did not show

Table I. Effects of psoralen derivatives on hKv1.5 current expressed in Ltk$^-$ cell line.

Samples	Structures	Inhibitory rate (%)
Psoralen		72.8±9.7
Compound 1		75.2±4.7
Compound 2		15.5±3.8
Compound 3		45.5±6.2
Compound 4		10.3±2.1
Compound 5		95.3±8.7

The hKv1.5 current traces were recorded before and 20 min after exposure to 10 μM psoralen derivatives. Voltage protocol consisted of 250-ms depolarizing pulses from -80 to +60 mV with 10 mV increments from a holding potential of -80 mV and repolarization to -50 mV for 400 ms. Steps were repeated at 20-s intervals. Each data represents the mean ± S.E.M.

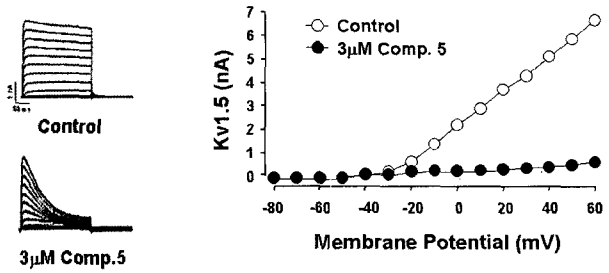


Fig. 1. Effects of compound 5 on hKv1.5 current expressed in Ltk$^-$ cell line. hKv1.5 current traces were recorded before and 20 min after exposure to 3 μM compound 5. Voltage protocol consisted of 250 ms depolarizing pulses from -80 to +60 mV with 10 mV increments from a holding potential of -80 mV and repolarization to -50 mV for 400 ms. Steps were repeated at 20-second intervals. Resultant current-voltage (I-V) relationship of steady-state current taken at the end of the depolarizing pulses in the absence and the presence of 3 μM compound 5.

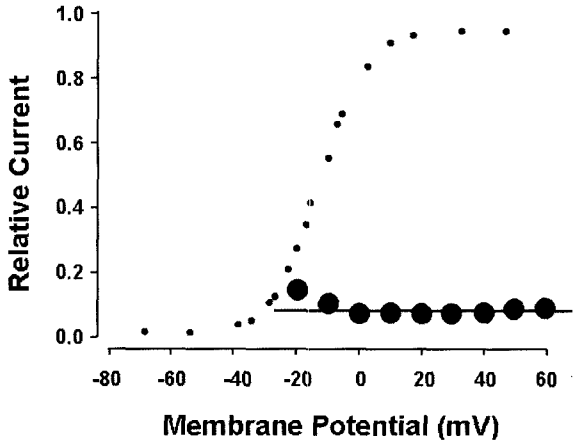


Fig. 2. Voltage-dependent block of hKv1.5 expressed in Ltk$^-$ cells by compound 5. Voltage protocol consisted of 250-ms depolarizing pulses from -80 to +60 mV with 10 mV increments from a holding potential of -80 mV and repolarization to -50 mV for 400 ms. Steps were repeated at 20-s intervals. Relative currents were obtained by $I_{compound}/I_{control}$ at each depolarizing potential in the absence and presence of 3 μM compound 5. Steady-state current amplitude, normalized to control, was plotted as a function of test potential.

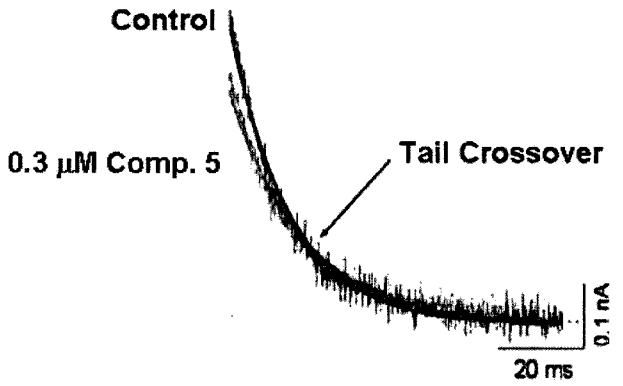


Fig. 3. Effects of compound 5 on the deactivation kinetics of hKv1.5 current expressed in Ltk⁻ cells. Deactivation kinetics was investigated during a repolarizing step of -50 mV after a depolarizing step to +60 mV. By superimposing the tail currents in the absence and presence of 0.3 μM compound 5, tail crossover phenomenon (indicated by the arrow) was observed.

between -30 and 0 mV, which corresponds to the voltage range of channel opening (Snyders et al., 1993). Also, we quantified the deactivation kinetics. In the presence of compound 5 (0.3 μM), the initial tail current was reduced and the subsequent slower decline resulted in a "crossover" phenomenon with the control tracing (Fig. 3). The mammalian Kv channel are divided into nine subfamilies, Kv1~Kv9. Among them, Kv1 subfamily is the most diverse one, and includes at least eight subclasses, Kv1.1~Kv1.8 (Grissner, 1997). Kv1.1, Kv1.2, Kv1.4, Kv1.5, Kv2.1, Kv4.2 and Kv4.3 of Kv channels genes have been cloned from cardiac tissue (Deal, *et al.*, 1996). Main Kv channel genes expressed in human heart are hKv1.4, hKv1.5, hKv4.3 and HERG genes. All these genes are highly expressed in both atrium and ventricle, and in particular, the hKv1.5 gene is preferentially expressed in human atrium. The current generated by hKv1.5 channels is similar in physiological and pharmacological sensitivity to the very rapidly activating rectifier K^+ current recorded in human atrial myocytes (I_{KUR}) (Wang *et al.*, 1993). In particular, selective block of hKv1.5-like current in human atrial myocytes results in a significant prolongation of the action potential (Wang *et al.*, 1994). In the present study, compound 5 among psoralen derivatives preferentially interacts with the open state of the hKv1.5 channel with the following results. The compound 5 accelerated the rate of hKv1.5 current decay with little effect on the initial activation kinetics. Also, the compound 5 was voltage-dependent and increased steeply in the voltage range of channel activation. The compound 5 slowed the deactivation of the tail current, thus inducing a tail crossover phenomenon.

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