Effects of Sparfloxacin on Delayed Rectifier Potassium Current of Ventricular Myocyte in Guinea Pig

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Abstract: Administration of fluoroquinolone antibiotics in human has proven to induce prolongation of QT interval, which is mainly assumed the blockade of some kind of potassium channel(s). This study is to examine the effect of sparfloxacin on delayed rectifier potassium current of ventricular myocyte in guinea pig. Single ventricular cells were dissociated from guinea pig heart and continuously superfused with sparfloxacin solution of different concentrations. Delayed rectifier potassium current (I_K) was recorded with whole-cell patch clamp technique. Results showed that sparfloxacin decreased the current amplitude of I_K in a concentration dependent manner. When the concentration was from 0.1 μM to 1,000 μM, the inhibitory capacity of drug on I_K peak current gradually enhanced; and depressed rates of the tail currents increased too. Sparfloxacin displayed an IC_{50} value of 14.89 μM. These results indicate that sparfloxacine inhibits I_K concentration-dependently to the ventricular myocyte. [Life Science Journal. 2006;3(4):33-36] (ISSN: 1097-8135).

Keywords: sparfloxacin; ventricular myocyte; delayed rectifier potassium current; whole-cell patch-clamp

Abbreviations. IC_{50}: 50% inhibitory concentration; I_K: delayed rectifier potassium current

1 Introduction

There is considerable interest in ventricular repolarization, since prolonged repolarization is associated largely with ventricular tachyarrhythmia, syncope and sudden death, known as long-QT syndrome. It is the delayed rectifier potassium current (I_K) that plays a critical role in cardiac repolarization. Therefore any congenial or environmental variation that interferes with I_K might underlie long QT syndrome. Recently, it has been suggested that some antibiotics, such as fluoroquinolone antibacterial drugs, are a hint to the occurrence of long QT syndrome[1,2]. Sparfloxacin belongs to fluoroquinolone class and has been widely prescribed in clinic since 1993 for the treatment of infection. The present study was designed to examine *in vitro* the effects of sparfloxacine on I_K of isolated ventricular myocyte, in order to explore the pharmacological mechanism of cardiac inhibition, and to provide support for clinical drug application.

2 Materials and Methods

2.1 Cardiomyocyte isolation

Adult guinea-pigs (300±50 g) were adopted. The operation followed previous reports with slight modification[3]. In brief, animals were sacrificed by blunt trauma to the head. Hearts were rapidly excised, rinsed and mounted on a Langendorff system. Hearts were perfused for 10 minutes with Ca^{2+}-free Tyrode solution containing: NaCl 140 mM, KCl 5.4 mM, $MgCl_2$ 1 mM, HEPES 10 mM, and glucose 10 mM(pH 7.4). Then the perfusion was switched to normal Tyrode solution (25 μM $CaCl_2$) complemented with 0.36 g/L collagenase (type B, Roche) and 0.26 g/L protease (type XIV, Sigma). When the flow-out appeared viscous and the heart was obviously dilated, digestion process was ceased by perfusing solution consisting of: Glutaminc acid 120 mM, KOH 80 mM, KCl 20 mM, $MgCl_2$ 1 mM, EGTA 0.3 mM, HEPES 10 mM, and glucose 10 mM(pH 7.4±0.5). Ventricular free walls were removed, cut into pieces, gently agitated in Ca^{2+}-free Tyrode solution and filtrated through 200 μm nylon sieve. Harvested myocytes were placed at room temperature (18-22℃) in Ca^{2+}-free Tyrode solution.

2.2 Whole-cell patch clamp recording

Microelectrodes (hard, thin-wall glass capillaries with inner diameter of 1.5 mm) were pulled with the micropipette puller (Narishige PP-83,

Japan). Resistance when filled with inner solution was 2 – 3 MΩ. Inner solution of the pipette contained: KCl 140 mM, Mg-ATP 4 mM, $MgCl_2$ 1 mM, HEPES 10 mM, and EGTA 5 (pH 7.3). Isolated cells were bathed and continuously perfused with oxygen-saturated Tyrode solution containing: NaCl 140 mM, KCl 5.4 mM, $MgCl_2$ 1 mM, $CaCl_2$ 0.025 mM, HEPES 10 mM, and glucose 10 mM (pH 7.4). Rod-shaped ventricular cells with smooth edge and clear striates were selected for recording.

I_K was recorded using conventional whole cell clamp protocol at room temperature. Whole-cell current records were performed with an amplifier (2300E, Axon Instruments, America), filtered at 1 kHz and sampled at 0.25 kHz. After reaching "sealing", series resistance and capacitive resistance were compensated to minimize the resistance impact and to ensure the recording stability. Both voltage pulses delivering and current signals collecting were processed automatically by pClamp 5.51 software (Axon Instruments, America). Step-voltage depolariztions were fired from – 10 mV to + 80 mV with + 10 mV step, maintaining 5 seconds each. Before recording, 0.3 mM $CdCl_2$ was added to external solution to block L-type Ca^{2+} channels. A pre-stimulating pulse of – 40 mV was applied to ensure inactivation of Na^+ channel and T-type Ca^{2+} channels.

2.3 Group and data analysis

I_K recorded in normal Tyrode solution were the control group. Five test groups included I_K recorded when the cells were for 20 minutes each immersed in drug of different concentrations from 0.1 μM to 1,000 μM. Data were noted as current density (pA/pF) (that is, current on each unit of membrane surface, I_K/C_m). I_K value came from real measurement using pClamp 6.0 Software (Axon Instrument); and C_m, the capacitance of cellular membrane which represents the superficial area, was calculated from formula $C_m = \tau \times I/V$, among which I(pA) stands for the amplitude of the capacity current, τ(ms) is for the attenuation constant of uncompensated capacitance current, and V(mV) is for a constant of 10 mV depolarization. In this experiment the mean membrane capacitance obtained was 61.12 pF ± 2.64 pF ($n = 30$), which is consistent with the published data[4].

All data were presented as mean ± SD. Difference significance was statistically determined by Student's t test ($\alpha = 0.05$). Statistical analysis was performed using Origin 6.0 Software.

3 Results

Sparfloxacine reduced the current amplitude of I_K. As shown in Figure 1, the comparison of current amplitudes before and after drug administration indicated that sparfloxacine had inhibitory effect on I_K (both peak current and tail current). The inhibition occurred steadily after the cells were exposed to drug superfusion more than 20 minutes. In the span of testing voltages the inhibitory capacity increased with the voltage steps, which can be observed from the I-V (current-voltage) curves (Figure 2). The compounds couldn't be easily washed-out.

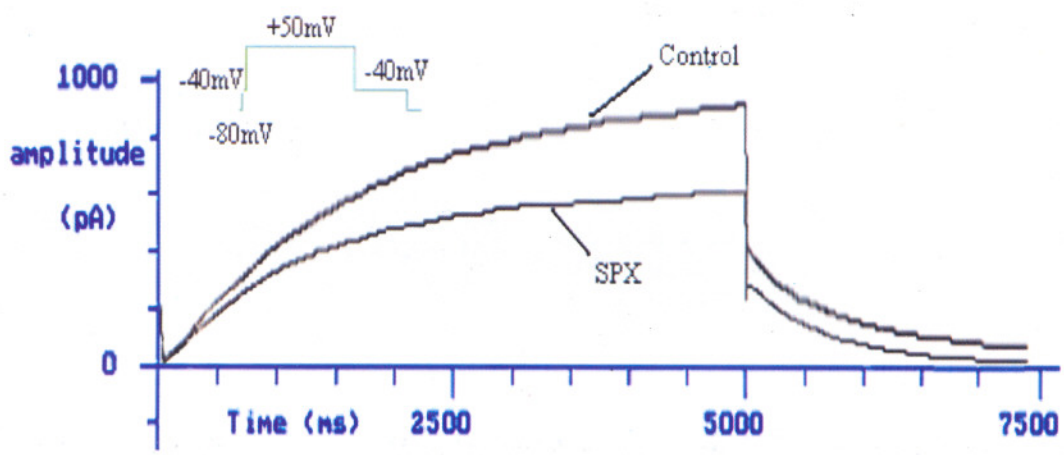

Figure 1. Inhibitory effect of sparfloxacine on I_K. Whole-cell I_K were elicited by a 5-second depolarizing pulse to + 50 mV from a holding potential of – 80 mV. Then potential was returned to – 40 mV to generate a large outward tail current. The effect of 100 μM sparfloxacine was displayed to reduce the I_K significantly ($P < 0.05$).

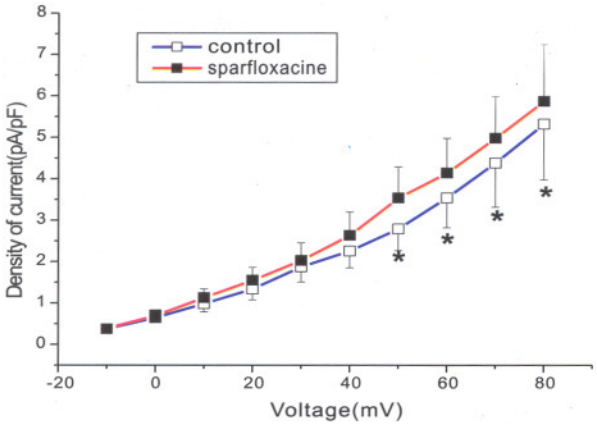

Figure 2. I-V curves of I_K

Cells were held at −80 mV and depolarized for 5 seconds to potentials ranging from −10 mV to +80 mV with +10 mV increments, and then returned to −40 mV to generate outward tail currents. Currents influenced with and without 100 μM sparfloxacine were measured and pictured. It showed that the inhibitory capacity increased with the voltage steps ($P<0.05$).

At +50 mV, I_K reduction (depressed rate) was calculated by dividing the depressed current value with the current amplitude of the control group. As listed in Table 1, in the concentration of 0.1 μM, the depressed rate of sparfloxacine on I_K peak current was (12.94 ± 3.09)%, and that of tail current was (7.66 ± 2.72)%. While the concentration increased to 10^4 times, depressed rates of I_K peak and tail currents reached to (38.40 ± 5.13)% and (60.64 ± 6.53)% separately, which were obviously different with those in low dose.

So concentration/response relationship was calculated by a non-linear squares fit of equation: $f = 1/(1 + (x/IC_{50})^{nH})$ to the data (Figure 3). Sparfloxacine inhibited I_K with an IC_{50} of 14.89 μM.

Table 1. Depressed rates of I_K impacted by different doses of sparfloxacine solutions(%)($n=25$)

0Concentration (μM)	$I_{K,step}$(%)	$I_{K,tail}$(%)
0.1	12.94 ± 3.09	7.66 ± 2.72
1	16.26 ± 2.21	10.03 ± 1.48
10	24.70 ± 2.78	17.78 ± 3.21
100	32.23 ± 3.19	32.90 ± 3.91
1000	38.40 ± 5.13	60.64 ± 6.53

4 Discussion

The results indicated that sparfloxacine did inhibit ionic current of I_K in even a relatively low concentration. I_K is a current conducted by two types of potassium channels-one is rapid activating channel; the other slow activating channel. In this study, two kinds of potassium channels had not been further identified, but surely one or both of them might be blocked by sparfloxacine, which led directly to abatement of I_K. Furthermore, sparfloxacine blocked I_K in a dose (concentration) dependent manner. The inhibitory impact occurred slightly in lower dose; with the raise of concentration, depressed effect could be greatly enhanced.

The QT interval is an electrocardiographic measurement of the period between ventricular depolarization and the completion of repolarization. The acquired form of the long QT interval syndromes is caused by various agents that reduce the magnitude of outward repolarizing K^+ currents, enhance inward depolarizing Na^+ or Ca^{2+} currents, thereby triggering the arrhythmia. The electrophysiological action of I_K blockade observed in this study, may translate into prolongation of the QT interval and then may predispose to development of torsades de pointes in clinic. According to the available reports, the fluoroquinolones in the market present a low risk of drug-induced QT prolongation, with a frequency approximately 0.2−2.7 per million prescriptions[5]. The safest member of the class appears to be ciprofloxacin[6]. Sparfloxacine represents comparably less secure one.

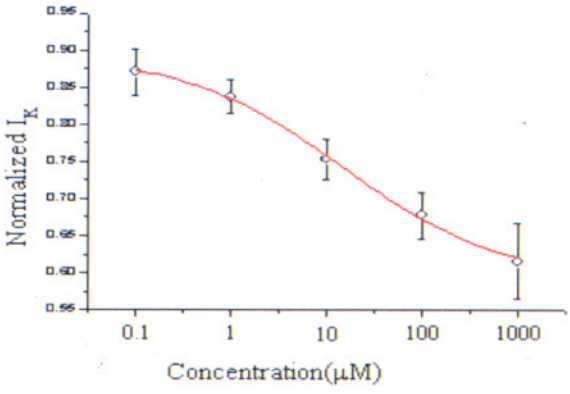

Figure 3. Concentration-response relationship of sparfloxacine response of peak currents at +50 mV was used to generate the concentration-response curve. The points represented the rates of remained currents to non-pressed ones. The slopes of the curves ranged from 87.06% for 0.1 μM to 61.60% for 1,000 μM.

Fortunately, judged from the relationship between drug concentration and inhibition response, the IC_{50} value of sparfloxacine was 14.89 μM, which fell far above the therapeutic peak concentration in patient blood (1.8 μM)[7]. So sparfloxacine is still reliable in clinical treatment of infection, ex-

cept that extra attention should be paid to the monitoring of electrocardiograph, especially when heavy dose is applied or excessive accumulation happens because of liver or kidney damage.

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