Inhibitory Effect of Nicardipine on hERG Channel

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Abstract — Drug-induced long QT syndrome is known to be associated with the onset of torsades de pointes (TdP), resulting in a fatal ventricular arrhythmia. QT interval prolongation can result from blocking the human ether-a-go-go-related gene (hERG) channel, which is important for the repolarization of cardiac action potential. Nicardipine, a Ca-channel blocker and antihypertensive agent, has been reported to increase the risk of occasional serious ventricular arrhythmias. We studied the effects of nicardipine on hERG K+ channels expressed in HEK293 cells and Xenopus oocytes. The cardiac electrophysiological effect of nicardipine was also investigated in this study. Our results revealed that nicardipine dose-dependently decreased the tail current of the hERG channel expressed in HEK293 cells with an IC50 of 0.43 μM. On the other hand, nicardipine did not affect hERG channel trafficking. Taken together, nicardipine inhibits the hERG channel by the mechanism of short-term channel blocking. Two S6 domain mutations, Y652A and F656A, partially attenuated (Y652A) or abolished (F656A) the hERG current blockade, suggesting that nicardipine blocks the hERG channel at the pore of the channel.

Keywords: Nicardipine, HERG, LQTS, HEK293, Xenopus oocyte

INTRODUCTION

A few blockbuster drugs such as terfenadine, an anti-histamine drug, were withdrawn from the market due to drug-induced QT prolongation (Redern et al., 2004). These cases of drug withdrawals not only caused heavy damage in the pharmaceutical industry, but also unexpected clinical adverse effects, such as sudden death in humans. Recently, testing for drug-induced QT prolongation has been mandatory during drug development in pharmaceutical companies and is required by drug regulatory authorities (Fermini and Fossa, 2003; Picard and Lacroix, 2003; Ajay et al., 2004; Finlayson et al., 2004).

Drug-induced long QT syndrome (LQTS) is known to result from blocking of IKr, which is “delayed rectifier potassium current” (Finlayson et al., 2004). The rapid component of IKr, which is involved in repolarization of cardiac action potential, and can induce polymorphic ventricular tachycardia Torsades de Pointes (TdP) and sudden death, is encoded by human ether-a-go-go-related-gene (hERG) (Roden and Spooner, 1999). The S6 domain located in the pore region of the channel has been reported as a binding site of drugs (Sanguinetti et al., 2005). In addition to this direct mechanism, an indirect mechanism for disrupting hERG protein trafficking to the cell surface membrane must also be considered in the hERG channel blocking activity of drugs (Sanguinetti et al., 2005).

Nicardipine, 2-[benzyl(methyl)amino]ethyl methyl 2,6-di-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, is an antihypertensive agent classified with the dihydropyridine derivatives, and is a potent calcium channel blocker with vasodilating properties. Nicardipine has been categorized with drugs of possible risk for Torsades de Pointes (TdP), which means drugs that may prolong the QT interval, but currently lack substantial evidence for causing torsades de pointes, by Arizona Center for Education and Research on Therapeutics (www.azcert.org).

In this study, we have investigated the direct and indirect effects of nicardipine on hERG K+ channels stably...
expressed in HEK293 cells and Xenopus oocytes.

**MATERIALS AND METHODS**

**Cell culture**
Stably transfected hERG-expressing HEK293 cells were obtained from Dr. Choe. These cells were cultured in minimum essential medium containing 10% fetal bovine serum and 400 μg/ml genetin (G418) in an atmosphere of 5% CO₂ and at 37°C.

**Whole cell patch-clamp recording from hERG - HEK293 cells**

hERG - HEK293 cells were collected using trypsin-EDTA and were suspended and settled in a chamber filled with normal tyrode solution on an inverted microscope (Carl Zeiss, Germany). The normal tyrode solution consisted of 143 mM NaCl, 5.4 mM KCl, 2.7 mM CaCl₂, 0.4 mM MgCl₂, 10 mM HEPES, 0.33 mM NaH₂PO₄, 5.0 mM glucose and 1.8 mM CaCl₂ at pH 7.4. The internal solution consisted of 130 mM KCl, 1 mM MgCl₂, 5 mM EGTA, 5 mM Mg-ATP and 10 mM HEPES at pH 7.2. All reagents were purchased from Sigma (St. Louis, MO, USA). A stock solution of nicardipine (100 mM) was prepared in DMSO and diluted in the bath solutions at suitable concentrations. The solution of nicardipine was prepared in distilled water and added to the external solutions at suitable concentrations shortly before each experiment. Solutions were applied to oocytes by continuous perfusion of the chamber while recording. Solution exchanges were completed within three min, and the hERG currents were recorded 5 min after the solution exchange. Currents were measured at room temperature (20-23°C) with a two-microelectrode voltage clamp amplifier (Warner Instruments, Hamden, CT, USA). Electrodes were filled with 3 M KCl and had a resistance of 2-4 MΩ for voltage-recording electrodes and 0.6-1 MΩ for current-passing electrodes. Stimulation and data acquisition were controlled with an AD-DA converter (Digidata 1200, Axon Instruments, USA) and pCLAMP software (v 5.1, Axon Instruments).

**Western blot analysis**

Whole-cell lysates were prepared in lysis buffer (50 Mm Tris-HCl at pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% Nonidet P-40 (NP-40) and 10% glycerol) containing a protease inhibitor mini-tablet (Roche Diagnostics, Mannheim, Germany). Protein concentration was determined by the BCA (bicinchoninic acid) method (Pierce Chemical, Rockford, IL). Each 10 μg protein sample was separated on SDS (sodium dodecylsulfate) polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were probed with hERG (Alomone Labs, APC-062 rabbit anti-human hERG), HSP70, HSP90 and GAPDH antibody (Cell Signaling Technology, USA), and developed using ECL Plus (GE Healthcare, Piscataway, NJ). The bands were captured on a Biorad Photomager (Biorad, USA).