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Effects of Dronedarone on HERG and KCNQ1/KCNE1 Channels

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Abstract: Dronedarone, a noniodinated and methanesulfonanilide derivative of amiodarone, is under evaluation as a potentially less toxic anti-arrhythmic agent alternative to amiodarone. However, effects of this drug on I_{Kr} and I_{Ks} channels, two major cardiac repolarizing potassium channels, had not been determined yet.

To clarify the effects on I_{Kr} and I_{Ks}, we studied the effects of dronedarone on HERG channels and KCNQ1/KCNE1 channels heterologously expressed in Xenopus Oocytes by 2-electrodes voltage clamp technique. Because I_{Kr} channel is coded by HERG gene, and I_{Ks} is coded by co-expression of both KCNQ 1and KCNE1 genes.

Dronedarone potently blocked both HERG channel (IC_{50}: 3.8±1.0 μM, 0 mV, n=3) and KCNQ1/KCNE1 channels (IC_{50}: 19.1±2.1 μM, 0 mV, n=2). Amiodarone, a structurally similar compound of dronedarone, had already reported not to block KCNQ1/KCNE1 channels. These findings together with previous reports suggest that 1) dronedarone potently inhibits both HERG channels and KCNQ1/KCNE1 channels. 2) Insertion of methanesulfonanilide compound to dronedarone might result in an inhibitory action on KCNQ1/KCNE1 channels. 3) Dronedarone might be a potential antiarrhythmic drug.

Key words: Dronedarone, Amiodarone, HERG channel, KCNQ1/KCNE1 channels

Dronedarone, a newly-developed antiarrhythmic agent, is a noniodinated and methanesulfonanilide derivative of amiodarone. Although amiodarone is widely used in the treatment of various types of arrhythmia (Chatelain et al, 1986), clinical use of amiodarone had long been more limited because of its significant extracardiac toxicity involving the liver, lungs, and thyroid (Singh et al, 1983; Heger et al, 1984; Mason, 1987; Singh, 1990; Gill et al, 1992; Nattel et al, 1992). Dronedarone was developed to avoid these side effects of amiodarone. Dronedarone has been reported to prolong action potential duration (APD) in canine (Varro et al, 2001) and rabbit (Sun et al, 1999) ventricular myocytes and clarified as class III anti-arrhythmic drugs. The ionic mechanisms of these APD prolongations, however, have not been clarified.

Most of class III drugs exert their anti-arrhythmic action by inhibitions of two major repolarizing currents, I_{Kr} and I_{Ks}. The pharmacological profile of each class III drug, however, depends on the relative potency of block between I_{Kr} and I_{Ks}. Methanesulfonanilide class III drugs, for example, dofetilide and dl-sotalol inhibit I_{Kr} selectively leading to reversed use dependency in APD prolongation.

In the present study, to clarify the pharmacological profile of dronedarone, we investigated the relative potency of inhibitions by dronedarone on I_{Kr} and I_{Ks}. HERG and KCNQ1/KCNE1 channels heterologously expressed in Xenopus Oocytes were used because these genes are encoding I_{Kr} and I_{Ks} channels, respectively.

Materials and Methods

Plasmid Construct. The full-length cDNA for HERG, KCNQ1 and KCNE1 were kindly provided by Dr. Michael C Sanguinetti (University of Utah, Salt Lake City, UT). The cDNA expression construct in the pSP64 transcription vector (Promega, Madison, WI) and synthesis of cRNA were conducted as described previously (Sanguinetti and Xu, 1999).

cRNA Injection and Voltage Clamp of Oocytes. Isolation and maintenance of Xenopus laevis oocytes and injection with cRNA were performed as described previously (Stuhmer, 1992). Xenopus laevis frogs were anesthetized by immersion in 0.2% tricane (Sigma, St. Louis, MO) for 15 to 30 min. Ovarian lobes were removed and digested with 2 mg/ml type IA collagenase (Sigma) in Ca^{2+} free ND96 solution for 1.5 h to remove follicle cells. Stage V and VI oocytes were injected with 15 ng of cRNA encoding HERG for I_{Kr} analysis. To study I_{Ks}, KCNQ1 (5 ng) and KCNE1 (1 ng) cRNA were coinjected into oocytes. Oocytes were cultured in Barth's solution supplemented with 50 μg/L gentamicin and 1 mM pyruvate at 18°C.

Currents were recorded with a Dagan TEV-200 amplifier (Dagan, Minneapolis, MN) using standard two-microelectrode voltage-clamp techniques (Stuhmer, 1992) 2 to 4 days after injection of cRNA. Currents were recorded at room tempera-

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ture (22–24°C). Glass microelectrodes were filled with 3 M KCl, and their tips were broken to obtain a resistance of 0.5 to 1.0 MΩ. To attenuate endogenous chloride currents, Cl^- was replaced with 2-(N-morpholino) ethanesulfonic acid (MES) in the external solution that contained 96 mM NaMES, 2 mM KMES, 2 mM $CaMES_2$, 5 mM HEPES, and 1 mM $MgCl_2$, adjusted to pH 7.6 with methanesulfonic acid. Voltage commands were generated using pCLAMP software (version 6.0.4; Axon Instruments, Burlingame, CA). Specific voltage-clamp protocols used to elicit currents are described under Results.

Dronedarone was obtained from Sanofi-synthelabo. Co (Tokyo, Japan). A 100 μM stock solution was prepared in methanol and diluted with extracellular solution to the desired final concentrations immediately before each experiment.

Data Analysis. Data are presented as mean ± S.E.M. unless otherwise specified. Concentration-response relationships were fit to the Hill equation to determine the drug concentration required for 50% inhibition (IC_{50}). A nonlinear least-squares curve-fitting program (Clampfit 6.0.4) was used to analyze the kinetics of current deactivation.

Results

HERG currents were measured with a 2-step pulse protocol at a frequency of 0.03 Hz. From a holding potential at –90 mV, a 2-second depolarization (–80 to 50 mV) was applied to activate outward currents, followed by return of the membrane potential to –80 mV to evoke tail currents. The amplitude of outward tail currents exceeded the amplitude of the activating currents, characteristic of HERG channel behavior. Bath application of dronedarone (3 to 100 μM) for 20 minutes resulted in a concentration-dependent decrease in outward currents during depolarization and tail currents (Figure. 1A). The IC_{50} for dronedarone block of the HERG channel tail current was 3.8±1.0 μM (n=3) (Figure. 1B).

Injection of oocytes with cRNA encoding KCNQ1 and KCNE1 subunits induced I_{Ks}, and currents were measured during a 5-second depolarizing pulse to potentials ranging from –90 to 40 mV, with tail currents measured at –70 mV. Pulses were applied at a frequency of 0.03 Hz. Bath application of dronedarone for 20 minutes resulted in a concentration-dependent decrease in outward currents during depolarization and tail currents (Figure. 2A). The IC_{50} for dronedarone block of the KCNQ1/KCNE1 channel tail current was 19.1±2.1 μM (n=2) (Figure. 2B).

Discussion

In our study, dronedarone potently blocked both HERG current and KCNQ1/KCNE1 current. Effects of amiodarone, a structurally similar compound of dronedarone, on these currents were also demonstrated by Kamiya et al (Kamiya et al,

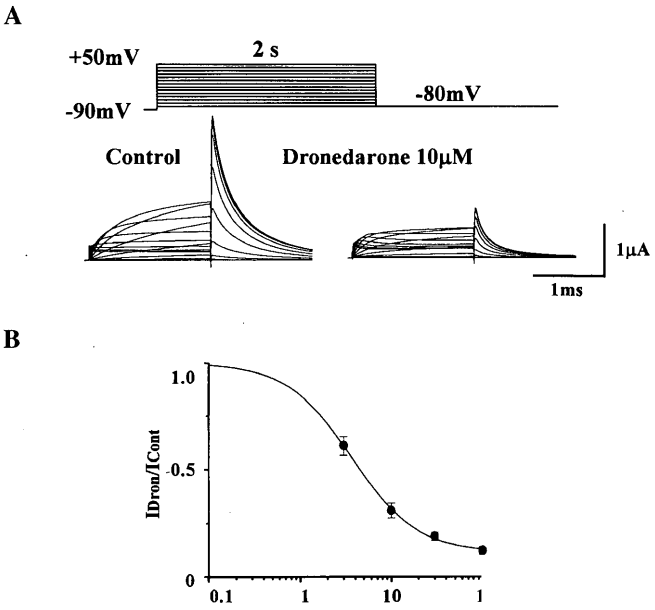

Fig. 1 Effects of dronedarone on HERG currents in Xenopus oocytes.
A, Representative HERG current traces recorded from oocyte before and after application of 10 μM dronedarone. Voltage-clamp pulse protocol is shown above current traces.
B, Concentration-response relationship for block of current recorded at repolarizing pulse of –80 mV followed by 0 mV depolarizing pulse. IC_{50} values were 3.8±1.0 μM (mean ± SE, n=3).

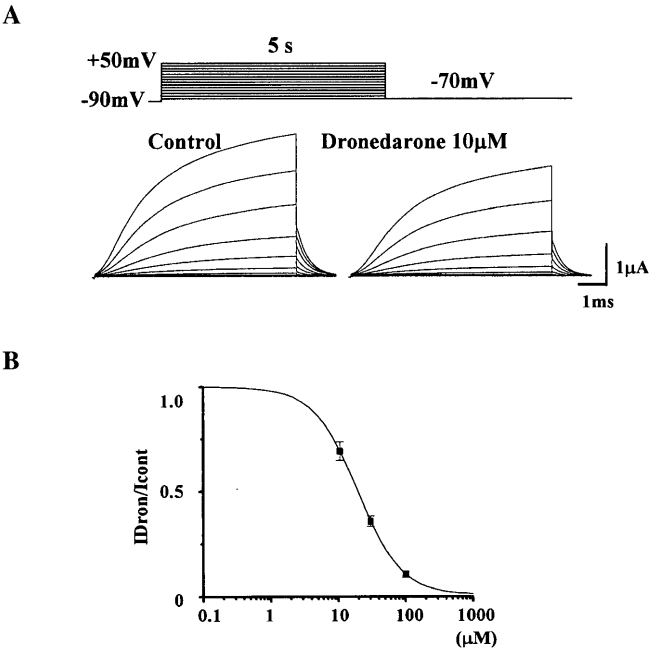

Fig. 2 Effects of dronedarone on KCNQ1/KCNE1 currents in Xenopus oocytes.
A, Representative KCNQ1/KCNE1 current traces recorded from oocyte before and after application of 10 μM dronedarone. Voltage-clamp pulse protocol is shown above current traces.
B, Concentration-response relationship for block of current recorded at the end of a 5-s depolarization step (n=2) to 0 mV for KCNQ1/KCNE1. IC_{50} values were 19.1±2.1 μM (mean ± SE, n=2).

2001). In their study, different from dronedarone, amiodarone preferentially blocked HERG current and did not affect KCNQ1/KCNE1 current. In short, dronedarone inhibits KCNQ1/KCNE1 channel but amiodarone does not. These differential effects on KCNQ1/KCNE1 between dronedarone and amiodarone might be explained by different chemical structures of two drugs. Dronedarone contained a methanesulfonanilide compound and amiodarone did not. The methanesulfoanilide compound is a common chemical structure for KCNQ1/KCNE1 blockers. We speculated that the insertion of a methanesulfonanilide structure to amiodarone molecule might result in KCNQ1/KCNE1 channel block by dronedarone. Further studies will be necessary to confirm this hypothesis.

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