Blockade of Human Cardiac Potassium Channel Human Ether-a-go-go-Related Gene (HERG) by Macrolide Antibiotics

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ABSTRACT
Several macrolides have been reported to cause QT prolongation and ventricular arrhythmias such as torsades de pointes. To clarify the underlying ionic mechanisms, we examined the effects of six macrolides on the human ether-a-go-go-related gene (HERG)-encoded potassium current stably expressed in human embryonic kidney-293 cells. All six drugs showed a concentration-dependent inhibition of the current with the following IC_{50} values: clarithromycin, 32.9 \mu M; roxithromycin, 36.5 \mu M; erythromycin, 72.2 \mu M; josamycin, 102.4 \mu M; erythromycinylamine, 273.9 \mu M; and oleandomycin, 339.6 \mu M. A metabolite of erythromycin, des-methyl erythromycin, was also found to inhibit HERG current with an IC_{50} of 147.1 \mu M. These findings imply that the blockade of HERG may be a common feature of macrolides and may contribute to the QT prolongation observed clinically with some of these compounds. Mechanistic studies showed that inhibition of HERG current by clarithromycin did not require activation of the channel and was both voltage- and time-dependent. The blocking time course could be described by a first-order reaction between the drug and the channel. Both binding and unbinding processes appeared to speed up as the membrane was more depolarized, indicating that the drug-channel interaction may be affected by electrostatic responses.

Considerable evidence has accrued that a variety of noncardiac drugs may prolong the QT interval of the surface electrocardiogram, which represents ventricular depolarization and repolarization, imparting an increased risk of developing a potentially fatal cardiac arrhythmia known as torsades de pointes (TdP) (De Ponti et al., 2001). This has stimulated intense discussions and focused attention on methodological issues involved in the cardiac risk-benefit assessment of noncardiac pharmaceuticals, including antimicrobial agents, which has resulted in withdrawals of approved antibiotics such as grepafloxacin. So far, numerous electrophysiological studies have been conducted with respect to individual drugs to delineate the cellular basis of this electrophysiological phenomenon. Interestingly, although in theory the prolongation of action potential can result from disturbance of any of the cardiac ion channels, almost all reported QT-prolonging drugs that have been tested so far appear to inhibit I_{Kr}, the rapidly activating delayed rectifier current (De Ponti et al., 2001). This highlights the importance and uniqueness of I_{Kr} channel in cardiac repolarization. In humans, I_{Kr} is most likely carried by the potassium channel encoded by HERG (Sanguinetti et al., 1995). Drugs associated with QT prolongation and, occasionally, TdP have been reported to significantly inhibit the HERG-encoded channel current (Crumb and Cavero, 1999; Cavero et al., 2000). Due to inherent difficulties associated with I_{Kr} recording in native cardiac myocytes, mammalian cell lines expressing HERG have been widely used to assess the potency of drugs in inhibiting this channel and the cardiac electrophysiological safety of pharmaceuticals.

Macrolides are a group of closely related compounds characterized by a macrocyclic lactone ring (usually containing 14 to 16 atoms) to which sugars are attached. They have been widely used as effective antibiotics against Gram-positive organisms. However, numerous reports have been published regarding the cardiac adverse effects of macrolides, especially erythromycin (Katapadi et al., 1997). In fact, this prototype macrolide is also the most thoroughly characterized with respect to the effects on cardiac repolarization that seems to be mediated by cardiac potassium channel blockade (Antzelevitch et al., 1996; Rampe and Murawsky, 1997; Drici et al., 1998). In contrast, little is known about the effects of these macrolides on the HERG channel. With increasing reports of QT prolongation and arrhythmias associated with these drugs (Sekkarie, 1997; Lee et al., 1998; Kamochi et al., 1999; Woywodt et al., 2000), it is of particular importance to

**ABBREVIATIONS:** TdP, torsades de pointes; DME, des-methyl erythromycin; HEK, human embryonic kidney; HERG, human ether-a-go-go-related gene; I_{Kr}, rapidly activating delayed rectifier potassium current; DDI, drug-drug interaction; R_s, series resistance; E-4031, N-[4-{[1-[2-[6-methyl-2-pyridinyl]ethyl]-4-piperidinyl]carbonyl[phenyl]-methanesulfonamide, dihydrochloride.
elucidate their electrophysiological characteristics. Therefore, we examined the effects of six macrolides on HERG currents expressed in HEK-293 cells and used clarithromycin to better understand the mechanism and relative role of HERG blockade by these macrolides.

Materials and Methods

Cell Preparation and Chemicals. HEK-293 cells stably expressing HERG potassium channels (Zhou et al., 1998) (licensed from Wisconsin Alumni Research Foundation) were cultured in minimal essential medium (Invitrogen, Carlsbad, CA) supplemented with 0.1 mM nonessential amino acid (Invitrogen), 1.0 mM sodium pyruvate, 10% fetal bovine serum and 0.05% gentamicin (G418). On the day of the experiment, cells were dissociated using 0.05% trypsin-EDTA and stored at room temperature in M199 medium (Hanks’ salt) for electrophysiological study. Erythromycin was purchased from Sigma-Aldrich (St. Louis, MO). All other drugs were provided by Pfizer Global Research and Development (Groton, CT). Drugs were dissolved in dimethyl sulfoxide at 10 to 30 mM as a stock solution, and then directly added into Tyrode’s solution to a desired concentration. Dimethyl sulfoxide at the maximal concentration in this study (0.3%) did not have any detectable effect on the HERG current.

Patch-Clamp Recording. Aliquots of cells were allowed to settle on the bottom of the recording chamber (≈5 ml in volume) mounted on an inverted microscope (Axiovert S100; Carl Zeiss, Inc., Thornwood, NY). Cells were superfused with Tyrode’s solution containing 137 mM NaCl, 4 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 10 mM glucose, and 10 mM HEPES (pH 7.4 with NaOH). Ionic currents were measured in the whole-cell configuration using a patch-clamp technique (Hamill et al., 1981). Recording electrodes with a resistance of 2 to 3 MΩ when filled with the internal solution were connected to an EPC-9 patch-clamp amplifier controlled by the Pulse + PulseFit program (HEKA Elektronik, Lambrecht/Pfalz, Germany). The internal solution was composed of 130 mM KCl, 5 mM MgATP, 1.0 mM MgCl2, 10 mM HEPES, and 5 mM EGTA (pH 7.2 with KOH). Seal resistances in all of the experiments were more than 1 GΩ; therefore, leakage subtraction was not performed. Series resistance (Rs, generally between 3 and 6 MΩ before compensation) was routinely compensated by at least 80% and checked periodically during the experiment. The anticipated voltage errors resulted from the uncompensated Rs in each experiment were limited to ±5 mV. All experiments were performed at 35 ± 1°C. The bath temperature was maintained by a TC-344B temperature controller (Warner Instruments, Hamden, CT).

In concentration-response experiments, HERG potassium currents were elicited by 1-s voltage pulses to +20 mV from a holding potential of −80 mV, followed by a repolarizing ramp (0.5 V/s) to −80 mV (frequency, 0.25 Hz), eliciting a large outward “tail” current. The effects of the macrolides were studied on the peak tail current observed at −40 mV. Recordings were started 8 to 10 min after membrane rupture to allow cell dialysis with the pipette solution. Compounds were administered after the recordings became stable for at least 3 min. At the end of each experiment, 10 μM dofetilide, a specific I\textsubscript{Kr} blocker, was given to evaluate the endogenous outward currents, which were subsequently subtracted off-line. Dofetilide at 10 μM did not have any noticeable effect on the endogenous currents in nontransfected HEK-293 cells (data not shown). Our previous studies on the time course of the HERG current under control conditions indicated that this current declined over time. This phenomenon, known as “run-down” (Belles et al., 1988; Duchatelle-Gourdon et al., 1989), appeared to reach steady state and to become linear after 3 to 4 min of recording. To obtain an accurate estimate of the blockade, drug data were corrected by applying a linear regression through the linear portion of control values and extrapolated over the entire experiment. Recordings with >15% rundown during the course of the experiment were rejected. Steady-state block of the macrolides was determined as the difference between the amplitude of the current measured in the presence of the drug and its corresponding control value when extrapolated by the linear regression. The concentration-response relationship was quantified by fitting the data using the Hill equation: $I_{drug}/I_{control} = 1/(1 + (D/IC_{50})^p)$, where $D$ is the drug concentration, $IC_{50}$ is the concentration for 50% inhibition, and $p$ is the Hill coefficient.

Data are expressed as mean ± S.E. Statistical significance was evaluated by applying a paired t test for comparing the data before and after administration of a drug, or an analysis of variance, followed by a Tukey-Kramer test for multiple comparisons. A p value <0.05 was considered significant. Curve fitting was performed using a nonlinear least-squares regression analysis in Origin v6.0 (Microcal Software, MA) or Igor Pro v4.01 (WaveMetrics Inc., OR).

Results

Inhibitory Effect of Macrolides. The effects of six marketed macrolides and a metabolite of erythromycin, desmethyl erythromycin, on the HERG current are illustrated in Fig. 1, and typical recordings obtained with clarithromycin are shown in Fig. 1A and B. The HERG current blockade by these macrolides was investigated in four to six cells at each drug concentration using the voltage protocol shown in Fig. 1A. Drugs were administered in a cumulative fashion up to a maximal concentration of 100 μM to eliminate the potential concerns of drug solubility. Concentration-response relationships, shown in Fig. 1D, were obtained by fitting the data with a Hill equation to yield IC_{50} values. The resulting data are as follows (Hill coefficient in parentheses): clarithromycin, 32.9 μM (0.98); erythromycin, 72.2 μM (1.04); roxithromycin, 36.5 μM (1.16); josamycin, 102.4 μM (1.14); erythromycylamine, 273.9 μM (0.96); oleandomycin, 339.6 μM (1.13); and DME, 147.1 μM (0.88). The potencies of these compounds can therefore be ranked ordered as follows: clarithromycin > roxithromycin > erythromycin > josamycin > DME > erythromycylamine > oleandomycin. A Hill coefficient close to 1.0 was obtained for each compound, indicating a single binding site to the HERG channel.

Voltage- and Time-Dependent Block by Clarithromycin. Given the similarity in the chemical structure to other macrolides and the increasing attention to its cardiac side effects, clarithromycin was chosen for further mechanistic exploration. Figure 2A shows representative current recordings in response to a series of depolarization steps before and after the administration of 30 μM clarithromycin. Cells were held at −80 mV and stimulated with a series of 1-s depolarizing pulses ranging from −70 to +60 mV with 10-mV increments at 0.1 Hz. Tail currents were recorded upon repolarization to −60 mV. When measured at the end of the depolarizing steps, the time-dependent current started to activate at voltages between −60 and −50 mV, peaked at −20 mV, and then showed inward rectification because of the rapid voltage-dependent C-type inactivation (Smith et al., 1996). Addition of 30 μM clarithromycin significantly inhibited the HERG current at voltages positive to −30 mV. Current reductions at −30, 0, and 40 mV were 18 ± 10, 45 ± 5, and 56 ± 7%, respectively (n = 12; Fig. 2C). In 8 of 12 cells studied, increases in the steady-state current amplitude of 97 ± 31 and 36 ± 15%, respectively, were observed at −50 and −40 mV, which accounted for the elevation and the large variation of the total averaged currents at these two voltages.
the “voltage-dependent” reduction of the current amplitude, averaged data from the other four observations were plotted in Fig. 2C (open symbols). Increased inhibition was again observed as the membrane was depolarized (from 25% inhibition at −50 mV to 65% at +60 mV, respectively).

The voltage dependence of the steady-state activation of HERG currents was evaluated by plotting normalized tail current amplitude as a function of voltage (Fig. 2D). Data were fitted with a Boltzmann function: 

\[ I_{\text{max}} / I = 1 / [1 + \exp((-V_{1/2} - V_m)/S)], \]

where \( I \) represents the tail current, \( V_m \) is the test membrane potential, \( V_{1/2} \) is the half-maximal activation voltage, and \( S \) is the slope factor, which reflects the steepness of the voltage dependence. At 30 \( \mu \)M, clarithromycin caused a small but significant shift in the voltage required to half-maximally activate HERG from \(-33.4 \pm 0.9 \) mV to \(-38.2 \pm 0.8 \) mV (\( p < 0.001 \); Fig. 2D), and the slope factor was slightly decreased (5.7 \( \pm 0.1 \) mV in control versus 5.4 \( \pm 0.1 \) mV with clarithromycin, \( p = 0.035 \)).

Voltage-dependent block of the HERG channel has been reported as a common property of open-channel blocking compounds such as dofetilide (Snyders and Chaudhary, 1996). These drugs require channel opening to access the channel pore and block the current. Therefore, to determine whether clarithromycin displays open-channel blocking properties, cells were held at −80 mV for 3 min to keep the channel in the closed conformation during the wash-in of 30 \( \mu \)M clarithromycin or (as a comparator) 10 nM E-4031, followed by a series of depolarization steps to +20 mV from a holding potential of −80 mV. Tail currents were recorded at −60 mV (Fig. 3). In the experiment with E-4031, the first depolarization after a 3-min pause yielded little or no reduction in current amplitude, and the subsequent block of HERG developed on a step-by-step basis, requiring an additional 12 to 15 min to reach a steady-state block (Fig. 3, A and B). In contrast, the first tracing for 30 \( \mu \)M clarithromycin showed a significant inhibition of the HERG current, and no further blockade was observed with subsequent stimuli (Fig. 3, C and D). The effect of clarithromycin was reversible, as illustrated in Fig. 3D. Less than 3 min was sufficient for a full recovery of the HERG current from drug blockade. Similar observations were obtained in three to four more experiments.

We also studied the effects of clarithromycin (30 \( \mu \)M) on the kinetics of channel activation, deactivation, inactivation, and recovery from inactivation by using methods described previously (Zhou et al., 1998), and the data are summarized in Fig. 4. The activation time course was described by fitting a single-exponential function to the envelope of tail currents obtained from depolarizing steps to +40 mV for varying durations, followed by a repolarizing step to −60 mV to elicit the tail current. The HERG deactivation time course was obtained by fitting a double-exponential function to the decay of tail currents elicited by a 1-s depolarization to +50 mV, followed by a series of 3-s repolarization steps ranging from −100 to 40 mV. The kinetics of HERG inactivation was measured by applying a three-pulse protocol. The current was first activated and inactivated by 200-ms depolarization steps to +60 mV. Then the cells were repolarized to −100 mV for 2 ms to allow channels to shift from inactivated to open state without significant deactivation. The test steps were applied to different voltages to observe the inactivation of the HERG current, and the traces were fitted with a single-exponential function. To measure the recovery kinetics from

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**Fig. 1.** Effects of macrolides on the HERG current. A, superimposed current traces in an experiment with clarithromycin. Currents were elicited by a 1-s depolarization step to +20 mV from a holding potential of −80 mV, followed by a repolarizing ramp (0.5 V/s) to −80 mV. Stimulation frequency, 0.25 Hz. B, time course of the effect of clarithromycin. A linear regression was performed through the linear portion of control current traces in an experiment with clarithromycin. Currents were averaged from the other four observations were plotted in Fig. 2C (open symbols). Increased inhibition was again observed as the membrane was depolarized (from 25% inhibition at −50 mV to 65% at +60 mV, respectively).

This phenomenon could not be explained by experimental artifacts, such as changes in pipette-membrane sealing conditions and series resistance. Moreover, the increase in amplitude was only observed at voltages close to the threshold of channel activation (−50 and −40 mV). In contrast, when a series of repetitive depolarization steps to −50 mV were applied in the presence of the drug, clarithromycin only caused a reduction of the current amplitude (data not shown). These observations are similar to those described previously for azimilide (Jiang et al., 1999), which may reflect two separate drug actions on the HERG channel through different sites. To estimate the potential contribution of the enhancing effect of clarithromycin to the pattern of...
inactivation, a two-pulse protocol was used. Cells were depolarized to $-60 \text{ mV}$ for 200 ms to activate HERG channels and then repolarized to different voltages to elicit tail currents. The rising phase of the tail current was then fitted with a monoexponential function, and the time constant was obtained. As shown in Fig. 4, clarithromycin (30 $\mu$M) had no significant effects on the kinetics of HERG activation, deactivation, inactivation, and recovery from inactivation.

**Kinetics of Block by Clarithromycin.** The blockade of the HERG current by clarithromycin, as shown in Figs. 2A and 5A, appeared to develop gradually during the depolarization steps, indicating that the inhibitory effect of clarithromycin is time-dependent. To further investigate the blocking time course, currents recorded in the presence of the drug were normalized to the control currents, and the resulting ratio of relative current amplitudes at each membrane potential was plotted (Fig. 5B). In this figure, the resulting relative currents from a representative cell are shown following depolarizing steps to $-40$, $-20$, $+20$, and $+50 \text{ mV}$, respectively. These data could be well described by a single-exponential function (Fig. 5B, solid line), and the resulting time constants and the steady-state relative amplitudes at different potentials are summarized in Fig. 6, A and B, respectively. The results again show that the inhibition of HERG current by clarithromycin is both voltage- and time-dependent.

Since the time course of the effect of clarithromycin could be well described by a single-exponential function, the kinetics of the block was further analyzed by using a first-order reaction model (Katayama et al., 2000).

$$O \xrightleftharpoons{\beta}{\alpha} I$$

where $O$ is the conducting HERG channel without clarithromycin binding, $I$ is the nonconducting state of the channel bound by the drug, $\beta$ is the apparent binding rate constant of the drug to the channel, and $\alpha$ is the unbinding rate constant. In this first-order reaction model, the channel's steady-state open probability ($P_o$) and the time constant ($\tau$) could be described by $P_o = \beta/\alpha + \beta$ and $\tau = 1/(\alpha + \beta)$, respectively, whereas $P_o$ is equivalent to the normalized current ratio ($I_{\text{clarithromycin}}/I_{\text{control}}$) at steady state and $\tau$ could be obtained by fitting the relative current curves (Fig. 5B). The unbinding and binding rate constants $\alpha$ and $\beta$ were then calculated at each membrane potential and summarized in Fig. 6, C and D. It appeared that both the unbinding and binding processes were...
facilitated when the membrane was more depolarized, which could be described by a single Boltzmann function. The resulting voltages at the half-maximum value of the slope factors were $-13.1$ and $5.2$ mV for $\alpha$, and $-9.0$ and $9.9$ mV for $\beta$, respectively. It is noted that the unbinding rate constant $\alpha$, unlike $\beta$, slightly declined at membrane potentials more positive to $+10$ mV.

**Discussion**

The pharmacokinetic parameters of the macrolides tested in this study following oral administration at relatively high doses, as illustrated in Table 1, are compared with their potencies ($IC_{50}$) to inhibit the HERG channel. Calculated ratios of $IC_{50}$ to $C_{max}$ for individual drugs range from 15- to 642-fold. However, inhibition of $I_{Kr}$ and QT prolongation by these drugs may still occur at clinically relevant concentrations for the following reasons. First, QT prolongation has been reportedly induced by drugs at concentrations causing $<20\%$ inhibition of HERG. Cisapride, for instance, produced an average of 6 ms of QTc prolongation over 24 h in healthy volunteers at 40 ng/ml (van Haarst et al., 1998). At this concentration (free drug, 1.7 nM), it only inhibited the HERG current by 10 to 20% (Mohammad et al., 1997; Rampe et al., 1997). Several other drugs, including E-4031, dofetilide, terfenadine, and risperidone, are also reported to produce QT prolongation at clinical concentrations significantly lower than the $IC_{50}$ values reported to inhibit the HERG current.
Second, clinical exposure of macrolides can often exceed the concentrations listed in Table 1, in cases such as intravenous administration, DDIs, and poor metabolism (Rubart et al., 1993; Sekkarie, 1997; van Haarst et al., 1998). The peak serum concentrations of erythromycin, for example, can average ~30 μg/ml after a 900-mg i.v. infusion compared with 2 to 4 μg/ml after a 500-mg oral dose (Rubart et al., 1993). Finally, most of the macrolides studied here are found to accumulate in tissues, including the heart, and the resulting local concentrations can be significantly higher than in the plasma (Yoshida and Furuta, 1999). Nonetheless, clarithromycin, roxithromycin, and erythromycin (IC50 values were ≤50-fold Cmax) would then be expected to have a higher propensity than the other three drugs to cause QT prolongation in humans according to the rank order of potency. In fact, they all have been associated with long QT syndrome and arrhythmias, whereas no adverse cardiac events have yet been reported for josamycin, oleandomycin, and erythromycylamine. Therefore, although inhibition of HERG current appears to be a common feature for these macrolide antibiotics, differences in their potencies and therapeutic windows seem to be the key to the clinical outcomes.

The effect of a drug on QT interval can be influenced by various factors, such as multiple ion channel activity, electrolyte disturbances (e.g., hypokalemia or hypomagnesemia), bradycardia, genetic defects underlying the congenital long QT syndrome, female gender, and concomitant medications that may prolong the QT interval and/or affect the pharmacokinetics of the drug. Extrapolation of the in vitro study on a single ionic current, therefore, has to be made cautiously. In this study, we observed that a metabolite of erythromycin, DME, also blocked the HERG channel with an IC50 of 147.1 μM, implying that only comparing the potencies among parent drugs may be an oversimplification. Importantly, all these macrolides can inhibit and/or be metabolized by liver enzymes, especially cytochrome P450 subtype CYP3A4, with a tendency to cause DDIs in the following order: erythromycin > clarithromycin > josamycin ≈ roxithromycin ≈ erythromycylamine ≈ oleandomycin (Periti et al., 1992). DDIs resulting in relevant cardiac events have indeed been reported between macrolides and multiple agents (Pai et al., 2000; Westphal, 2000). Moreover, mutant forms of MinK-related peptide 1, an accessory peptide likely coassembling with the pore-forming HERG subunit to form the native Ik, (Abbott et al., 1999), has reportedly demonstrated diminished potassium currents and/or increased channel blockade by clarithromycin (Abbott et al., 1999; Sesti et al., 2000). Therefore, some instances of antibiotic-associated QT prolongation and TdP may in fact represent the unmasking of otherwise silent genetic channelopathies. Furthermore, lack of clinical information resulting from limited utilization of a drug and insufficient awareness among physicians of prescribed QT-prolonging drugs (Yap and Camm, 2000) may also make the correlation difficult. Among macrolides, clarithromycin was listed with the highest report-utilization ratio of 0.34 cardiac event reports per million prescriptions by the Adverse Event Report System of the U.S. Food and Drug Administration, whereas erythromycin accounts for 53% of the total reports among macrolides because of its long cumulative time on market. Roxithromycin, which is not registered in the U.S. market, has only a few clinical reports, so far, on QT-related adverse effects (Ortqvist et al., 1996; Woywodt et al., 2000), although it is a relatively potent HERG blocker, as shown in this study. All things considered, our data do not exclude the possibility of QT prolongation and cardiac arrhythmias being associated with josamycin, erythromycylamine, and oleandomycin.

The aromatic residues (e.g., Y652 and F656) of the S6 domain of the HERG channel have recently been demonstrated to interact hydrophobically with class III antiarrhythmic drugs such as dofetilide (Mitcheson et al., 2000a). In a competitive ligand-binding study, we observed that macrolide compounds, including erythromycin, showed a concentration-dependent inhibition of [3H]dofetilide binding to the HERG channel in micromolar ranges, indicating that they may interact with the same site(s) as dofetilide (data not
shown). Interestingly, erythromycin, clarithromycin, and roxithromycin have higher lipophilicity (E log Ds are 1.3 to 1.6 at physiological pH) than the other macrolides (E log Ds of 0.31 and 0.43 for erythromycylamine and oleandomycin, respectively) (McFarland et al., 1997), which may favor interactions with the binding site and correlate with their relatively higher potencies. We also showed that clarithromycin block of the HERG channel was slightly voltage-dependent, a property commonly seen for the classical I\textsubscript{Kr} blockers such as E-4031. These blockers require activation of the channel to access the inner binding site and get "trapped" when the channel is closed (Mitcheson et al., 2000a,b). Therefore, the kinetics of both blocking and unblocking are normally very slow (>10 min to reach a steady state in our experiment). However, clarithromycin, despite its large molecular size, can inhibit the current even when channels are closed and the recovery from blocking is fairly fast (<3 min washout in this study), indicating that it may be able to bypass the activation gate or the "trapping" mechanism through a hydrophobic pathway. Certainly, more experiments may be needed to reach a definitive conclusion on its interaction with the channel. Since clarithromycin has a pK\textsubscript{a} of 8.99, it may be positively charged at physiological pH and, therefore, may have an electrostatic response to the depolarized voltages. This may account for, at least in part, the voltage dependence of the HERG channel blockade. Our kinetic analysis on clarithromycin block demonstrated a first-order reaction between the channel and drug molecules. This reaction is apparently independent of channel gating because a single-exponential fit is sufficient to describe the time course of the effect during depolarization steps. Besides, the difference between the voltage dependence of the binding rate constant (V\textsubscript{1/2} of −13.1 and s of 5.2 mV) and that of the HERG activation (V\textsubscript{1/2} of −33.4 and s of 5.7 mV) also indicates that channel blocking and activation gating are likely two separate processes. Because both binding and unbinding rate constants increase as membrane potential is more depolarized, it is likely that the drug-channel interaction may be affected by electrostatic responses in both ways (facilitating or hindering). We also observed that clarithromycin exhibited a small enhancing effect on the HERG current at voltages close to its activation threshold (−50 to −40 mV), an effect similar to that of azimilide.
reported previously (Jiang et al., 1999). Further studies are warranted to investigate its mechanism and implications.

References


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