Interactions of the Antimalarial Drug Mefloquine with the Human Cardiac Potassium Channels KvLQT1/minK and HERG

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ABSTRACT

Mefloquine is a quinoline antimalarial drug that is structurally related to the antiarrhythmic agent quinidine. Mefloquine is widely used in both the treatment and prophylaxis of Plasmodium falciparum malaria. Mefloquine can prolong cardiac repolarization, especially when coadministered with halofantrine, an antagonist of the human ether-a-go-go-related gene (HERG) cardiac K⁺ channel. For these reasons we examined the effects of mefloquine on the slow delayed rectifier K⁺ channel (KvLQT1/minK) and HERG, the K⁺ channels that underlie the slow (Iₖs) and rapid (Iₖr) components of repolarization in the human myocardium, respectively. Using patch-clamp electrophysiology we found that mefloquine inhibited KvLQT1/minK channel currents with an IC₅₀ value of approximately 1 μM. Mefloquine slowed the activation rate of KvLQT1/minK and more block was evident at lower membrane potentials compared with higher ones. When channels were held in the closed state during drug application, block was immediate and complete with the first depolarizing step. HERG channel currents were about 6-fold less sensitive to block by mefloquine (IC₅₀ = 5.6 μM). Block of HERG displayed a positive voltage dependence with maximal inhibition obtained at more depolarized potentials. In contrast to structurally related drugs such as quinidine, mefloquine is a more effective antagonist of KvLQT1/minK compared with HERG. Block of KvLQT1/minK by mefloquine may involve an interaction with the closed state of the channel. Inhibition by mefloquine of KvLQT1/minK in the human heart may in part explain the synergistic prolongation of QT interval observed when this drug is coadministered with the HERG antagonist halofantrine.

Voltage-dependent K⁺ channels play an important role in the repolarization of the human myocardium. As such, their activity is a main determinant of the QT interval on the electrocardiogram. Several human cardiac K⁺ channels have now been discovered and cloned, allowing for detailed study of their physiology and pharmacology. For example, the human ether-a-go-go-related gene (HERG), possibly in combination with the MiRP1 subunit, expresses the K⁺ channel that underlies the slow component of the delayed rectifier current (Iₖs) in the human heart (Sanguinetti et al., 1995; Abbott et al., 1999). HERG activity appears to be an especially important component of cardiac repolarization. Mutations in HERG are the cause of the type 2 form of congenital long QT syndrome (Curran et al., 1995). Furthermore, it is now established that HERG is the main molecular target for drugs that produce a prolongation of the QT interval (acquired long QT syndrome), and that this interaction may contribute to the generation of the ventricular arrhythmia torsades de pointes. Drugs that prolong QT interval via block of HERG include the antihistamines terfenadine (Roy et al., 1996) and astemizole (Zhou et al., 1999), the antipsychotic agent sertindole (Rampe et al., 1998), and the gastric prokinetic drug cisapride (Mohammad et al., 1997; Rampe et al., 1997).

Another prominent K⁺ channel in the human myocardium is KvLQT1. This channel complexes with the minK subunit to form the K⁺ channel that underlies the slow component of the delayed rectifier current, Iₖs (Barhanin et al., 1996; Sanguinetti et al., 1996). Mutations in KvLQT1 or minK cause the hereditary long QT syndromes LQT1 and LQT5, respectively (for review, see Priori et al., 1999). This channel also functions in the inner ear because mutations in KvLQT1 are associated with at least some forms of the Jervell and Lange-Nielsen cardioauditory syndrome (Neyroud et al., 1997). However, compared with HERG, relatively little is known about the pharmacology of KvLQT1/minK.

Mefloquine is an antimalarial drug that is used both in the prophylaxis and in the treatment of malaria (Palmer et al., 1993). Mefloquine is structurally related to quinine and quindine, two drugs that are associated with QT prolongation (Jaeger et al., 1987; Karbwang et al., 1993). Mefloquine, when administered alone, has been shown to produce either no significant effect on the QT interval (Bindschedler et al., 2000) or to cause an approximately 10- to 20-ms prolongation

ABBREVIATIONS: HERG, human ether-a-go-go-related gene; KvLQT1/minK, slow delayed rectifier K⁺ channel; CHO, Chinese hamster ovary; CL, confidence limits.
Mefloquine Block of Cardiac K⁺ Channels

Results

Mefloquine and quinidine are both quinoline derivatives. Their structures are shown in Fig. 1. We tested the effects of quinidine on cloned KvLQT1/minK but found it to be a weak antagonist of the channel, displaying an IC₅₀ value of 44 μM (27–69 μM, 95% CL). These data are similar to a previous report showing quinidine has an IC₅₀ value for native Iₖ₅ currents of approximately 50 μM (Balser et al., 1991). In contrast to quinidine, we found mefloquine to be a much more...
potent antagonist of KvLQT1/minK. Figure 2 shows the effects of mefloquine on the human K⁺ channel KvLQT1/minK. In these experiments, cells were held at −80 mV and depolarized to +20 mV for 4 s to elicit KvLQT1/minK currents. Mefloquine inhibited KvLQT1/minK currents in a dose-dependent manner over the concentration range of 300 nM to 10 μM. The effects of mefloquine were mainly reversible upon washout of the drug (Fig. 2A). In addition to reducing KvLQT1/minK current amplitude, mefloquine also appeared to alter the current waveform. Specifically, activation appeared to be slowed with more block apparent at the beginning of the 4-s depolarizing pulse compared with the end. Thus, when measured 1 s into the depolarizing pulse, the IC₅₀ for mefloquine block of KvLQT1/minK was 0.88 μM (0.72–1.10 μM, 95% CL; Fig. 1B). When measured at the end of the 4-s depolarization, this value increased to 1.43 μM (1.26–1.66 μM, 95% CL). Depolarization-dependent unblocking of the channel is illustrated in Fig. 2C. Here, the block of KvLQT1/minK by 1 μM mefloquine is plotted as a function of time. Single exponential fit of the data yield a t₁/₂ value of 2293 ms.

The effects of mefloquine on KvLQT1/minK currents, measured over a wide range of test potentials, are illustrated in Fig. 3. Cells were held at −80 mV and currents were elicited by 4-s depolarizing pulses to potentials ranging from −60 to +30 mV. Current traces in the absence and presence of 3 μM mefloquine are shown in Fig. 3, A and B, respectively. The resultant current-voltage relationships are presented in Fig. 3C. Although mefloquine reduced current amplitude at all test potentials, greater inhibition was observed at lower membrane potentials compared with that observed at more depolarized ones. Inhibition of KvLQT1/minK current ranged from 96 ± 2% at −30 mV to 71 ± 5% at +30 mV in the presence of 3 μM mefloquine, and from 54 ± 5 to 24 ± 3% over the same voltage range in the presence of 1 μM drug (Fig. 3D). Figure 4 plots the time to half-maximal activation (t₁/₂) of KvLQT1/minK current at various test potentials. In the absence of drug, the t₁/₂ values ranged from 2084 ± 57 ms at −10 mV to 1062 ± 36 ms at +30 mV. In the presence of 3 μM mefloquine, these values were significantly (p < 0.05, paired t test) increased and now ranged from 2404 ± 56 ms at −10 mV to 1645 ± 58 ms at +10 mV.

The effects of mefloquine on KvLQT1/minK currents, in the absence of depolarizing pulses, are shown in Fig. 5. In this experiment cells were held at −80 mV and depolarized to +20 mV for 4 s to activate KvLQT1/minK. Cells were then held at −80 mV without depolarization for 3 min while mefloquine (3 μM) was allowed to equilibrate with the channel. After this 3-min period, depolarizing pulses were resumed. Under these conditions, block of KvLQT1/minK current by mefloquine was immediate and essentially complete during the first pulse with little additional block apparent upon subsequent depolarizations (Fig. 5, A and B).

Figure 6 shows the effects of mefloquine on KvLQT1/minK currents under conditions that were designed to more closely mimic those encountered in cardiac tissue in vivo. Cells were held at −80 mV and depolarized to +20 mV for 350 ms at a rate of 0.5 Hz. Currents were allowed to equilibrate for several minutes prior to the addition of mefloquine (3 μM). After the addition of drug, current amplitude decreased over the next several minutes (Fig. 6A), reflecting the equilibration of the drug with the cell. Under these conditions the average reduction in current measured 69 ± 6% in three cells tested. The effects of mefloquine were reversible upon washing the cells for several minutes with drug-free solution (Fig. 6B).

The ability of mefloquine to block the HERG cardiac K⁺ channel is shown in Fig. 7. HERG currents were elicited by a
Mefloquine Block of Cardiac $K^+$ Channels

Discussion

Mefloquine is an antimalarial agent that is structurally related to the antiarrhythmic drug quinidine. Quinidine is known to block cardiac potassium channels and produce QT prolongation on the electrocardiogram. Quinidine has been shown to inhibit cloned HERG current with an IC$_{50}$ value of 5.61 µM (3.63–8.51 µM, 95% CL). Under these same conditions quinidine blocked HERG currents with an IC$_{50}$ value of 547 nM (433–690 nM, 95% CL, n = 4) in good agreement with previously published data (Po et al., 1999). Figure 8 shows the effects of mefloquine on HERG current measured over a wide range of test potentials. Current traces in the absence and presence of 10 µM mefloquine are shown in Fig. 8, A and B, respectively, whereas the corresponding current-voltage relationships are shown in Fig. 8C. At test potentials of −40 and −30 mV, mefloquine caused a small increase in current that was reversible upon washout of drug. At all other test potentials, mefloquine inhibited HERG current with greater inhibition occurring at more positive potentials. When inhibition of current is plotted as a function of test potential, a statistically significant (p < 0.05, analysis of variance) positive correlation is observed with inhibition ranging from 32% at −20 mV to 71% at +30 mV (Fig. 8D).

2-s depolarizing pulse to +20 mV from a holding potential of −80 mV followed by repolarization to −40 mV to produce large, slowly deactivating tail currents characteristic of HERG (Sanguinetti et al., 1995). Mefloquine reduced tail current amplitude in a dose-dependent manner, resulting in an IC$_{50}$ value of 5.61 µM (3.63–8.51 µM, 95% CL). Under these same conditions quinidine blocked HERG currents with an IC$_{50}$ value of 547 nM (433–690 nM, 95% CL, n = 4) in good agreement with previously published data (Po et al., 1999). Figure 8 shows the effects of mefloquine on HERG current measured over a wide range of test potentials. Current traces in the absence and presence of 10 µM mefloquine are shown in Fig. 8, A and B, respectively, whereas the corresponding current-voltage relationships are shown in Fig. 8C. At test potentials of −40 and −30 mV, mefloquine caused a small increase in current that was reversible upon washout of drug. At all other test potentials, mefloquine inhibited HERG current with greater inhibition occurring at more positive potentials. When inhibition of current is plotted as a function of test potential, a statistically significant (p < 0.05, analysis of variance) positive correlation is observed with inhibition ranging from 32% at −20 mV to 71% at +30 mV (Fig. 8D).

Fig. 4. Effects of mefloquine on KvLQT1/minK activation. Data generated from the current-voltage relationships described in Fig. 3 were used. The time to reach half-maximal current (T$_{1/2}$) during a 4-s pulse is plotted against test potential in the absence and presence of 3 µM mefloquine. Mefloquine significantly slowed T$_{1/2}$ at all test potentials measured (p < 0.05, paired t test). Error bars indicate S.E.M. (n = 5).

Fig. 5. Block by mefloquine of KvLQT1/minK in the absence of depolarizing pulses. Cells were held at −80 mV and stepped to +20 mV at 40-s intervals. After three such pulses, cells were held at −80 mV for 3 min while mefloquine (3 µM) was washed in. After this 3-min incubation period, depolarizing pulses were resumed. Figure 4A shows KvLQT1/minK current before and after drug exposure. Inhibition of KvLQT1/minK current was virtually complete with the first drug pulse (pulse 1) and little additional block was evident upon further depolarization (pulse 6). Data from five such experiments are shown in B. Current was sampled at the end of the 4-s pulses and normalized to the first pulse of the series. Error bars indicate S.E.M.

Fig. 6. (A) Current-voltage relationships generated from Fig. 4. The time to reach half-maximal current (T$_{1/2}$) was calculated from the currents measured during 4-s depolarizing pulses. Data are plotted as a function of test potential, a statistically significant (p < 0.05, analysis of variance) positive correlation is observed with inhibition ranging from 32% at −20 mV to 71% at +30 mV (Fig. 8D).

(in contrast to its effects on HERG). During 4-s depolarizing pulses to +20 mV, mefloquine block of KvLQT1/minK was more pronounced early in the pulse (1 s) compared with the end of the pulse. Furthermore, the data in Fig. 5 show that channel opening is not required for mefloquine to fully block KvLQT1/minK. Taken together, these results suggest that mefloquine may inhibit KvLQT1/minK current by binding to or stabilizing a closed state of the channel. However, we cannot exclude a component of open channel block occurring from the outside of the channel by the positively charged mefloquine molecule. Further studies at the single channel level will be necessary for determining the exact mechanism of action of mefloquine.

Mefloquine has a modest effect on QT interval when administered alone. In some studies no significant prolongation in QT interval is observed following mefloquine treatment (Bindschedler et al., 2000), whereas in other studies a 10- to 20-ms increase has been reported (Coyne et al., 1996; Davis et al., 1996). However, it is well known that overlapping therapy with mefloquine and halofantrine produces a prolon-
gation in the QT interval that is greater than that observed for either drug alone (Nosten et al., 1993; Coyne et al., 1996). Thus, the QT prolongation observed for any given plasma concentration of halofantrine is increased when mefloquine is also present in the blood (Nosten et al., 1993). In these studies the total average plasma concentrations of mefloquine measured approximately 1.5 μM (Nosten et al., 1993; Coyne et al., 1996) similar to the IC50 value observed here for block of KvLQT1/minK. Furthermore, the maximal increase in QT interval obtainable with halofantrine alone is less than that observed for the combination of the two drugs in humans (Nosten et al., 1993) and rabbits (Lightbown et al., 2001). Halofantrine has now been shown to be a potent antagonist of the HERG cardiac potassium channel, displaying an IC50 value of 21 nM (Mbai et al., 2000). Although we do not rule out additive effects of these two drugs on HERG, or some metabolic interactions (Lightbown et al., 2001), we believe that at least some of these synergistic effects on QT interval result from the block of KvLQT1/minK by mefloquine superimposed upon HERG block by halofantrine. This may lead to a significant reduction in the ability of the myocardium to repolarize, resulting in rather extreme prolongation of the QT interval (Nosten et al., 1993; Coyne et al., 1996). A similar synergism has been noted for the development of early afterdepolarizations in canine ventricular cells upon concomitant block of IKs and IKr (Burashnikov and Antzelevitch, 1997). These results suggest caution when using mefloquine concurrently with other drugs that block HERG/IKr.

At present, little is known about the pharmacology of KvLQT1/minK or the therapeutic consequences of its inhibition. Experimental compounds, including the benzodiazepine L-768,673 and the chromanol derivative 293B have been synthesized that preferentially block KvLQT1/minK relative to other K+ channels such as HERG (Lynch et al., 1999; Yang et al., 2000). In some studies these compounds have been shown to prolong cardiac repolarization in vitro, whereas in other studies no effects were observed (Bosch et al., 1998; Lengyel et al., 2001). However, no clinical data are available on these or other drugs that selectively inhibit KvLQT1/minK. The present study demonstrates that certain quinoline derivatives such mefloquine can also block KvLQT1/minK. After therapeutic administration, total plasma levels of mefloquine range from about 1 to 5 μM (Karbwang and White, 1990). Free levels of the drug in plasma are considerably less due to its protein binding (ca. 98%). However, the drug is very lipophilic and extensively distributed into tissues (Karbwang and White, 1990). Free levels of the drug in plasma are considerably less due to its protein binding (ca. 98%). However, the drug is very lipophilic and extensively distributed into tissues (Karbwang and White, 1990). Thus, the levels of mefloquine in the human heart may easily approximate those that significantly inhibit KvLQT1/minK (approximately 300 nM and higher). Although having only modest effects on QT interval when administered alone, we believe block of KvLQT1/minK by mefloquine can contribute to the excessive QT prolongation observed with the overlapping administration of halofantrine. Although speculative, it is possible that block of KvLQT1/minK could contribute to other rare side effects, such as hearing loss, that have been reported during mefloquine treatment (Lobel et al., 1998; Fusetti et al., 1999).
Further clinical studies with the use of mefloquine, or other antagonists of KvLQT1/minK, are necessary to explore these possibilities.

References


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