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Interactions of a Series of Fluoroquinolone Antibacterial Drugs with the Human Cardiac K^+ Channel HERG

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ABSTRACT

Administration of certain fluoroquinolone antibacterials has been associated with prolongation of the QT interval on the electrocardiogram and, on rare occasions, ventricular arrhythmia. Blockade of the human cardiac K^+ channel HERG often underlies such clinical findings. Therefore, we examined a series of seven fluoroquinolones for their ability to interact with this channel. Using patch-clamp electrophysiology, we found that all of the drugs tested inhibited HERG channel currents, but with widely differing potencies. Sparfloxacin was the most potent compound, displaying an IC_{50} value of 18 μM, whereas ofloxacin was the least potent compound, with an IC_{50} value of 1420 μM. Other IC_{50} values were as follows: grepafloxacin, 50 μM; moxifloxacin, 129 μM; gatifloxacin, 130 μM; levofloxacin, 915 μM; and ciprofloxacin, 966 μM. Block of HERG by sparfloxacin displayed a positive voltage dependence. In contrast to HERG, the KvLQT1/minK K^+ channel was not a target for block by the fluoroquinolones. These results provide a mechanism for the QT prolongation observed clinically with administration of sparfloxacin and certain other fluoroquinolones because free plasma levels of these drugs after therapeutic doses approximate those concentrations that inhibit HERG channel current. In the cases of levofloxacin, ciprofloxacin, and ofloxacin, inhibition of HERG occurs at concentrations much greater than those observed clinically. The data indicate that clinically relevant HERG channel inhibition is not a class effect of the fluoroquinolone antibacterials but is highly dependent upon specific substitutions within this series of compounds. HERG channel affinity should be an important criterion for the development of newer fluoroquinolones.

Acquired or drug-induced long QT syndrome is a potentially dangerous side effect that is associated with the administration of certain prescription medications. This syndrome is characterized by a prolongation in the QT interval on the electrocardiogram and is thought to contribute to the generation of the ventricular arrhythmia torsades de pointes (Ben-David and Zipes, 1993). Block of voltage-dependent K^+ channels in the human heart is one means by which drugs can prolong cardiac repolarization and precipitate ventricular arrhythmia. Advances in cellular electrophysiology and molecular biology have lead to the discovery and cloning of several human cardiac K^+ channels. KvLQT1, for example, complexes with the minK subunit to form the K^+ channel that underlies the slow component of the delayed rectifier current I_{Ks} (Barhanin et al., 1996: Sanguinetti et al., 1996). The *human ether-a-go-go*-related gene, HERG, expresses the K^+ channel that underlies the rapid component of the delayed rectifier current (I_{Kr}) in the human heart (Sanguinetti et al., 1995). Mutations in HERG lead to the type 2 form of congenital long QT syndrome, LQT2 (Curran et al., 1995). Moreover, blockade of the HERG channel seems to be the main mechanism through which a number of drugs act to produce acquired long QT syndrome and associated ventricular arrhythmia. These drugs include the antihistamines terfenadine and astemizole (Roy et al., 1996; Zhou et al., 1999), the antipsychotic drugs sertindole and pimozide (Rampe et al., 1997; Kang et al., 2000), and the gastric motility agent cisapride (Rampe et al., 1997; Mohammad et al., 1997).

The fluoroquinolone class of antibacterials is widely prescribed for the treatment of infections. Development of this class of drugs is ongoing and seeks not only to improve antimicrobial activity but also to reduce adverse events, including CNS toxicity and phototoxicity (Domagala, 1994; Lipsky and Baker, 1999). Electrocardiographic changes, manifested by a prolongation in the QT interval, represent another adverse effect that is observed with some fluoroquinolones. For instance, sparfloxacin produces a dose-dependent increase in QT interval with significant prolongation apparent at normal clinical doses (Morganroth et al., 1999a,b). Therapeutic doses of grepafloxacin have also been shown to prolong QT interval (Ball et al., 1999; Lipsky and Baker, 1999; Stahlmann and Lode, 1999). Concerns over the potential for proarrhythmia have recently prompted the withdrawal of grepafloxacin from the market (Medwatch, 1999). On the other hand, no reports have linked ciprofloxa-

ABBREVIATIONS: HERG, *human ether-a-go-go*-related gene; SAR, structure-activity relationship.

cin and ofloxacin to prolongation in the QT interval despite extensive therapeutic use of these drugs. In light of these findings, the present study was undertaken to examine the effects of a series of fluoroquinolones on the HERG cardiac K^+ channel and to determine their relative potencies for inhibiting this channel.

Materials and Methods

Molecular Biology. The cDNA encoding the HERG K^+ channel was cloned from a human neuroblastoma cell line. Chinese hamster ovary cells (CHO cells; American Type Culture Collection, Manassas, VA) were transfected with the cDNA as described previously (Rampe et al., 1997; Kang et al., 2000). Cells were grown in Ham's F-12 media supplemented with 10% fetal bovine serum and 500 μg/ml G418 (Life Technologies, Gaithersburg, MD) in an atmosphere of 95% air/5% CO_2. KvLQT1 and minK were cloned from human heart and stably expressed in CHO cells as described previously (Kang et al., 2000). Cells used for electrophysiological experiments were seeded onto glass or plastic coverslips 24 h before use.

Electrophysiology. HERG and KvLQT1/minK channel currents were recorded using the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). Electrodes (2–6 MΩ resistance) were fashioned from TW150F glass capillary tubes (World Precision Instruments, Sarasota, FL). Electrodes were filled with the following solution: 120 mM potassium aspartate, 20 mM KCl, 4 mM Na_2ATP, 5 mM HEPES, 1 mM $MgCl_2$, pH 7.2, with KOH. For KvLQT1/minK current recordings, the internal solution was further supplemented with 14 mM sodium phosphocreatine, 0.3 mM sodium GTP, and 50 U/ml creatine phosphokinase. The external solution contained 130 mM NaCl, 5 mM KCl, 2.8 mM sodium acetate, 1.0 $MgCl_2$, 10 mM HEPES, 10 mM glucose; 1.0 mM $CaCl_2$, pH 7.4, with NaOH. Currents were recorded at room temperature using an Axopatch 1-D or Axopatch 200 B amplifier (Axon Instruments, Foster City, CA) and were conditioned by a 4-pole, low-pass filter with a cutoff frequency of between one quarter and one half the sampling frequency. Currents were analyzed using the pCLAMP suite of software (Axon Instruments). IC_{50} values were obtained by nonlinear least-squares fit of the data (GraphPAD Software, San Diego, CA).

Chemicals. All antibiotics used in the study were obtained from Aventis Pharmaceuticals, Inc (Romanville, France) except ofloxacin, which was obtained from Sigma Chemical (St. Louis, MO), and gatifloxacin, which was a generous gift of R.W. Johnson Pharmaceuticals, Inc. (New Brunswick, NJ). All other chemicals were obtained from Sigma.

Results

Figure 1 shows the chemical structures for the fluoroquinolones used in this study and Fig. 2 illustrates the effects of these drugs on HERG K^+ channel currents. In these experiments, a 2-s depolarizing pulse to +20 mV from a holding potential of −80 mV was followed by repolarization of the cell to −40 mV to produce large, slowly deactivating tail currents characteristic of HERG (Sanguinetti et al., 1995). The effect of these antibiotics on HERG is typified by moxifloxacin and shown in Fig. 2A. All of the drugs tested reduced peak tail current amplitude measured at −40 mV in a dose-dependent manner. Dose-response relationships (Fig. 2B) generated from this protocol yielded IC_{50} values (95% confidence limits) as follows: sparfloxacin, 18 μM (13–26 μM); grepafloxacin, 50 μM (37–66 μM); moxifloxacin, 129 μM (99–167 μM); gatifloxacin, 130 μM (87–192 μM); levofloxacin, 915 μM (724–1150 μM); ciprofloxacin, 966 μM (562–1670 μM) and ofloxacin, 1420 μM (794–2500 μM).

Figure 3 shows the effects of sparfloxacin on HERG channel currents measured over a wide range of test potentials. In these experiments, cells were held at −80 mV and currents were elicited by 2-s depolarizing pulses to potentials ranging from −40 mV to +30 mV in 10 mV increments. The membrane potential was then returned to −100 mV and peak inward tail currents were recorded. Current traces in the absence and presence of 30 μM sparfloxacin are shown in Fig. 3, A and B, respectively. The resultant current-voltage relationship averaged from five cells is presented in Fig. 3C. Although sparfloxacin reduced the current amplitude at all test potentials, greater inhibition was observed at more depolarized levels. When inhibition of HERG current is plotted as a function of test potential, a statistically significant ($p < 0.05$, ANOVA) correlation between voltage and drug effect was observed with inhibition ranging from 24% at −30 mV to 58% at +30 mV (Fig. 3D).

We also examined the effects of the fluoroquinolones on the KvLQT1/minK K^+ channel that underlies I_{Ks} in the human heart. Even at high concentrations, none of the drugs tested produced substantial block of this channel. Sparfloxacin (100

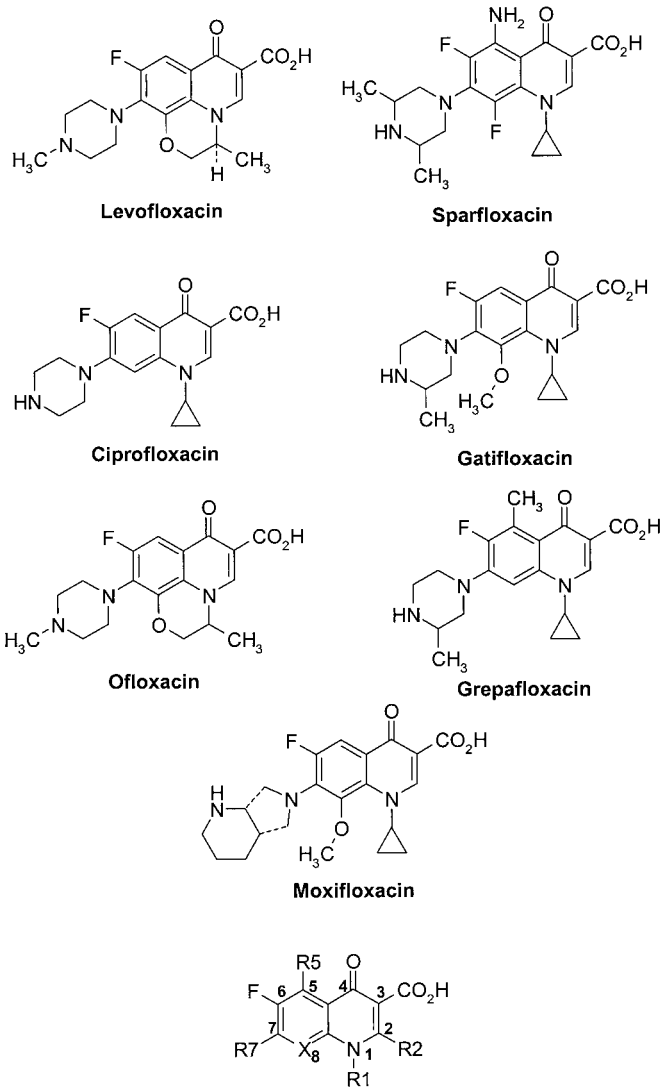

Fig. 1. Chemical structures of the fluoroquinolone antibiotics. The numbering convention of the fluoroquinolone structure is also shown.

μM) reduced KvLQT1/minK current by 3 ± 5% (n = 3; Fig. 4). At 1 mM concentration, moxifloxacin, gatifloxacin, levofloxacin, ciprofloxacin, and ofloxacin reduced KvLQT1/minK channel current by 12 ± 6, 4 ± 7, 6 ± 2, 6 ± 9, and 14 ± 5%, respectively (n = 3–4). Grepafloxacin was not tested because of lack of compound supply.

Table 1 compares the inhibition of HERG channel current recorded in this study to the peak free (unbound) plasma levels of these drugs measured in clinical settings. All of the drugs listed in the table are clinically available with the exception of grepafloxacin, which was recently withdrawn from the market. For all drugs, we sought to report the highest free plasma levels after therapeutic doses. The plasma concentrations and protein binding values were obtained from the Physicians' Desk Reference (1999) or, in the case of gatifloxacin and moxifloxacin, from product information (package insert) supplied for the drugs by the manufacturers. For those drugs where both oral and intravenous dosing is available, the plasma levels for both routes of administration are reported.

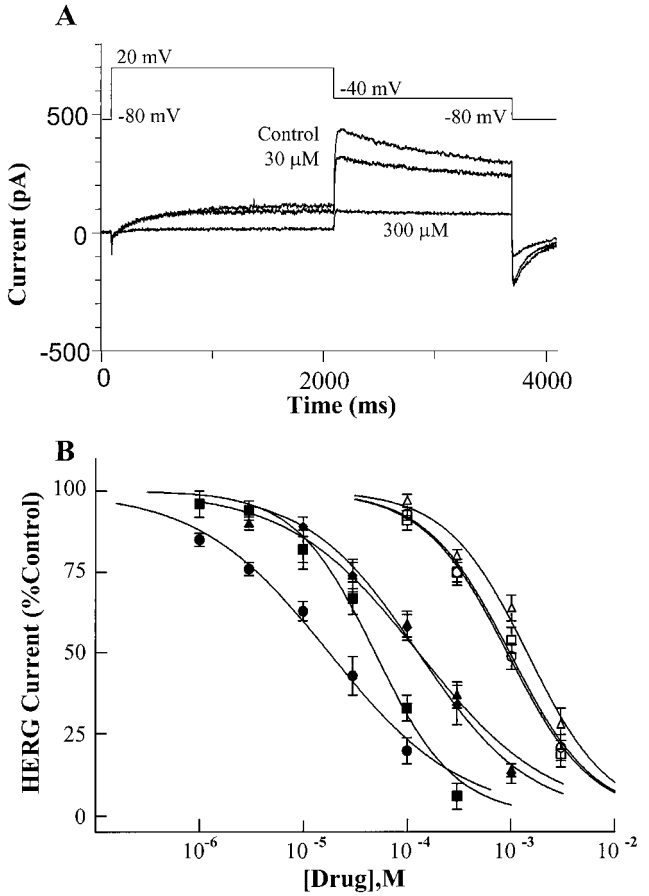

Fig. 2. Effects of fluoroquinolone antibiotics on HERG. A, whole-cell HERG currents were elicited by a 2-s depolarizing pulse to +20 mV from a holding potential of −80 mV at 40-s intervals. The cell was then returned to −40 mV to generate large outward tail currents. The effects of 30 and 300 μM moxifloxacin are shown. B, dose-response relationships for sparfloxacin (●), grepafloxacin (■), moxifloxacin (♦), gatifloxacin (▲), levofloxacin (○), ciprofloxacin (□), and ofloxacin (△) are shown. Inhibition of peak outward tail currents at −40 mV was used to generate the dose-response relationships. The slopes of the curves ranged from −0.70 for sparfloxacin and gatifloxacin to −1.13 for grepafloxacin. Error bars indicate S.E.M. (n = 4–8).

Discussion

The present study is the first to examine the effects of the fluoroquinolone antibacterials on human cardiac K^+ channels. KvLQT1/minK was not a target for block by the fluoroquinolones because high concentrations of these drugs produced only modest (~10%) reductions in KvLQT1/minK

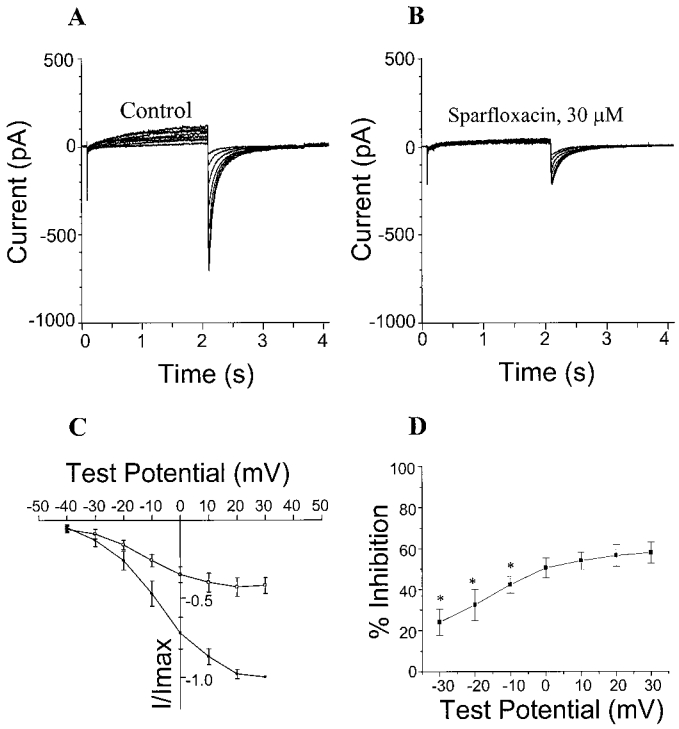

Fig. 3. Effects of membrane potential on sparfloxacin block of HERG. Cells were held at −80 mV and depolarized for 2 s to potentials ranging from −40 mV to +30 mV in 10-mV increments. The cells were then returned to −100 mV to generate inward tail currents. Traces in the absence and presence of 30 μM sparfloxacin are pictured in A and B, respectively. C, peak tail current amplitudes at −100 mV were normalized to those obtained after the +30 mV pulse in the absence of drug. The normalized tail currents are plotted as a function of the test potential. Data in the absence of drug (●) and after the addition of 30 μM sparfloxacin (○) are shown. Error bars indicate S.E.M. (n = 5). D, inhibition of peak tail current amplitude is plotted as a function of test potential. Asterisks denote statistical significance compared with inhibition observed after the +30 mV test pulse ($p < 0.05$, analysis of variance). Error bars indicate S.E.M. (n = 5).

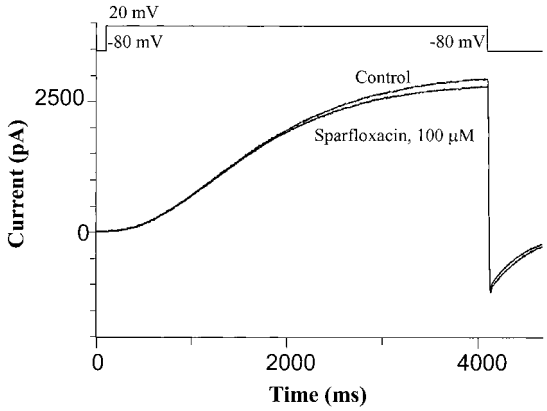

Fig. 4. The effects of sparfloxacin on KvLQT1/minK. KvLQT1/minK currents were elicited by 4-s depolarizing pulses to +20 mV from a holding potential of −80 mV. The effects of 100 μM sparfloxacin are shown.

currents. Conversely, we found that each of the compounds tested inhibit HERG channel currents in a dose-dependent fashion, albeit with widely differing potencies. IC_{50} values ranged from 18 μM for sparfloxacin to 1420 μM for ofloxacin. The effects of sparfloxacin were voltage-dependent with the greatest block observed at more depolarized potentials. This suggests that sparfloxacin interacts with an activated state of the HERG channel. In this respect, sparfloxacin is similar to other drugs that are known to block HERG and produce QT prolongation including cisapride (Mohammad et al., 1997; Rampe et al., 1997) and sertindole (Rampe et al., 1998).

The series of fluoroquinolones reported here demonstrate a wide range of potencies against HERG—approximately a 100-fold difference from sparfloxacin to ofloxacin. Fairly extensive structure-activity relationships are already available for the antibiotic activity of these agents (Domagala, 1994) and the data presented here offer the first, albeit limited, opportunity to compare that SAR with the anti-HERG SAR. For antibacterial potency, substitution at C_6 by F and C_3 by COOH is known to be important, as is the quinolone carbonyl functionality, but significant substituent variation is permissible at C_5, C_7, C_8, and N_1. It is thus of interest that levofloxacin, ciprofloxacin, and ofloxacin all lack C_5 substituents, whereas those agents most potent against HERG—sparfloxacin and grepafloxacin—have substituents at this position. Interestingly, at C_8, the least potent compounds against HERG are either unsubstituted (ciprofloxacin) or are substituted in a conformationally restricting manner with a bridge to N_1 (levofloxacin and ofloxacin). Of note also are the equipotent gatifloxacin and moxifloxacin (IC_{50} values $\sim$ 130 μM) which both have —OMe substitutions at C_8. The small series and their nonhomologous relationship make it difficult to speculate more about the different SARs for antibacterial and anti-HERG activities, save that there are important apparent differences and that a more comprehensive analysis may further delineate these.

Excellent clinical data are available correlating plasma levels of sparfloxacin with concomitant changes in the corrected QT interval (QTc). After an oral dose of 400 mg, peak plasma levels average about 1.3 μg/ml or about 2 μM free drug (Morganroth et al., 1999a,b). At this plasma concentration, QTc is increased by an average of 16 ms (approximately 4%). After a dose of 1600 mg, free plasma levels of sparfloxacin are approximately 7 μM and result in a mean increase in QTc of 55 ms (14%) (Morganroth et al., 1999b). We found that sparfloxacin inhibited HERG channel current at concentrations of 1 μM and above (15 $\pm$ 2% inhibition at 1 μM; $p <$ 0.05, paired t test). Thus, plasma levels of sparfloxacin that are associated with QT prolongation correspond to those that block HERG in the present set of experiments by about 15 to 30%. This makes HERG/I_{Kr} blockade the most likely mechanism to account for the QT interval prolongation observed with sparfloxacin treatment and for torsades de pointes arrhythmia that has been reported with its use (Dupont et al., 1996).

Grepafloxacin, moxifloxacin, and gatifloxacin displayed intermediate potencies for inhibiting HERG channel current. The ratio of the HERG IC_{50} values to peak free plasma concentrations ranged from 13- to 22-fold (Table 1). Unlike sparfloxacin, detailed clinical studies relating plasma levels to QT interval changes are not available for these drugs. However, grepafloxacin is well known to prolong cardiac repolarization and has been reported to increase the QT interval by an average of 10 ms in clinical trials (Ball et al., 1999; Lipsky and Baker, 1999; Stahlmann and Lode, 1999). Recently, grepafloxacin was withdrawn from the market because of concerns over QT interval prolongation and rare cases of ventricular arrhythmia, including torsades de pointes, in patients receiving the drug (Medwatch, 1999). Less clinical information is available for moxifloxacin and gatifloxacin, two newer fluoroquinolones that have only recently been approved by the Food and Drug Administration. Clinical trials have shown both drugs can prolong QT interval by several milliseconds (Ball et al., 1999; gatifloxacin product information, Bristol-Myers Squibb Company, Princeton NJ). The clinical significance of these findings relative to their affinity for HERG awaits further clinical study and postmarketing surveillance.

Levofloxacin, ciprofloxacin, and ofloxacin were significantly less potent inhibitors of HERG compared with the other fluoroquinolones tested. One case study has associated levofloxacin with QTc prolongation and ventricular arrhythmia in an elderly patient with structural heart disease and atrial fibrillation (Samaha, 1999). However, no changes in QTc have been detected with levofloxacin in numerous clinical trials (Ball et al., 1999; Lipsky and Baker, 1999; Stahlmann and Lode, 1999) and, to our knowledge, there are no other published reports associating levofloxacin with QT prolongation. Similarly, we know of no published reports that causally link QT prolongation or ventricular arrhythmia with the use of ciprofloxacin or ofloxacin despite the fact that these drugs have been clinically available for more than 10 years. This probably reflects the lower potency of these drugs for HERG both in absolute terms (IC_{50} valuess $\sim$ 1000 μM)

TABLE 1
Plasma levels and HERG affinities of the fluoroquinolone antibiotics

Drug	Dose	Peak Free [Plasma]	HERG IC_{50}	Ratio: IC_{50}/[Plasma]
Sparfloxacin	400 mg p.o.	1.8 μM	18 μM	10
Grepafloxacin	600 mg p.o.	3.1 μM	50 μM	16
Moxifloxacin	400 mg p.o.	5.9 μM	129 μM	22
Gatifloxacin	400 mg i.v.	9.8 μM	130 μM	13
Gatifloxacin	400 mg p.o.	9.0 μM	130 μM	14
Levofloxacin	500 mg i.v.	13 μM	915 μM	70
Levofloxacin	500 mg p.o.	12 μM	915 μM	76
Ciprofloxacin	400 mg i.v.	11 μM	966 μM	88
Ciprofloxacin	750 mg p.o.	10 μM	966 μM	97
Ofloxacin	400 mg i.v.	14 μM	1420 μM	101
Ofloxacin	400 mg p.o.	8.7 μM	1420 μM	163

p.o., by mouth.

and relative to their peak free plasma levels (9–14 μM). Indeed, statistically significant inhibition of HERG channel current was detected for these drugs only at concentrations $>$ 100 μM.

In conclusion, the present report is the first to detail the effects of the fluoroquinolone antibacterials on human cardiac K^+ channels. We found that these drugs were ineffective at blocking KvLQT1/minK but inhibited HERG with widely differing potencies. Clinically relevant blockade of HERG and the attending proarrhythmic potential do not seem to be a class effect but instead indicate the existence of specific structural features in these molecules that contribute separately to their antimicrobial activity and to HERG blockade. Separation of these structural features will be very important to the development of new fluoroquinolones.

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