Quinolones are a new class of antibiotic agent that have demonstrated clinical efficacy against a range of bacterial infections (Alcala et al., 1999; Vacher et al., 1999). Although this class of antibiotics is generally well tolerated and effective, the use of these agents is problematic because of recent reports of proarrhythmic actions. Recently, sparflaxacin, was reported to cause QT interval prolongation (Carlsson et al., 1990, 1991, 1992). IKr antagonist actions can be assayed in cardiac cells, or in cells transfected with HERG, the gene encoding IKr activity. Na+ currents present in AT-1 cells were inactivated by a holding potential of −40 mV, and L-type calcium current was blocked by adding the blocker nisoldipine (0.5–1 μM). To obtain current-voltage relations for IKr, activating currents were elicited with depolarizing

Materials and Methods

Cellular Electrophysiology. Experiments were conducted in whole cell voltage-clamp mode in mouse atrial tumor (AT-1) cells, which express a robust IKr (Yang and Roden, 1996). Na+ and T-type Ca2+ currents present in AT-1 cells were inactivated by a holding potential of −40 mV, and L-type calcium current was blocked by adding the blocker nisoldipine (0.5–1 μM). To obtain current-voltage relations for IKr, activating currents were elicited with depolarizing

ABBREVIATIONS: TdP, torsade de pointes; Ikr, rapid component of the delayed rectifier potassium current; SPX, sparflaxacin; MOX, moxifloxacin; GAT, gatifloxacin; GRX, grepafloxacin; AT-1, mouse atrial tumor cells; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid; QTc, rate-corrected QT interval; PVC, premature ventricular contraction; VT, ventricular tachycardia; HERG, human ether-a-go-go-related gene. 

Potassium Current Antagonist Properties and Proarrhythmic Consequences of Quinolone Antibiotics

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Received July 11, 2000; accepted October 30, 2000

This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

Quinolones are clinically important antibiotic drugs. One quinolone antibiotic, sparflaxacin (SPX), has been recently reported to increase the QT interval, and another quinolone, grepafloxacin (GRX), was withdrawn because it induced torsade de pointes (TdP), a polymorphic ventricular tachycardia (VT) linked to excessive QT interval prolongation. To determine whether SPX, GRX, and other recently developed quinolones, gatifloxacin (GAT), and moxifloxacin (MOX), have similar, potentially deleterious, properties we compared these agents in two ways. First, we measured their relative antagonist potency against the rapid component of the delayed rectifier K+ current (Ikr), and second we determined the QT interval prolongation and inducibility of VT and TdP using a well established in vivo rabbit arrhythmia model. All of these agents are Ikr antagonists with the following IC50 values (mean ± S.E.) for Ikr block: SPX, 0.23 ± 0.07 μM; MOX, 0.75 ± 0.31 μM; GAT, 26.5 ± 13.4 μM; and GRX, 27.2 ± 11.6 μM. All agents also increased the maximum QT interval (mean ± S.E.) from baseline (241 ± 10 ms): SPX, 370 ± 30 ms; MOX, 270 ± 30 ms; GRX, 280 ± 25 ms; and GAT, 255 ± 23 ms. No agents caused TdP during a standard 30-min observation period, but SPX-treated animals developed nonsustained VT (three of six) and TdP (one of six) during an extended 60-min observation period. These findings show that Ikr block may be a common feature of many quinolone antibiotics, and that the proarrhythmic consequences vary according to Ikr antagonist potency, but are also influenced by additional, unidentified factors.
pulses from a holding potential of −40 mV to +60 mV in 10-mV steps, and deactivating tail currents were recorded upon repolarization to −40 mV to mimic a cell membrane potential relevant to terminal (phase 3) action potential repolarization. When drug was added to the cell bath, the currents were monitored by pulses administered every 15 s, and “on-drug” current traces were recorded only when steady state was reached. The concentration dependence of drug block of $I_{Kr}$ was determined by first normalizing on-drug tail current as $I_{drug}/I_{control}$, and then fitting these data to the Hill equation $I_{drug}/I_{control} = 1/(1 + ([drug]/IC_{50})^n)$, where $IC_{50}$ is the concentration producing 50% block, and $n$ is the Hill coefficient.

**Solutions.** The intracellular pipette filling solution contained 110 mM KCl, 5 mM K$_2$BAPTA, K$_2$ATP, 1 mM MgCl$_2$, and 10 mM HEPES. The solution was adjusted to pH 7.2 with KOH, yielding a final intracellular K$^+$ concentration of approximately 145 mM. The extracellular Tyrode’s solution contained 130 mM NaCl, 4 mM KCl, 1.8 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM HEPES, and 10 mM glucose, and the solutions was adjusted to pH 7.35 with NaOH. The quinoline antibiotics (MOX, SPX, GAT, and GRX) used in these studies were provided by the Bayer Corporation (Wuppertal, Germany). The solutions were prepared each day from stocks and stored in 4°C.

**AT-1 Cells.** The preparations of AT-1 cells have been described in detail by our laboratory (Yang et al., 1994). In brief, tumors were minced and digested with collagenase-containing PC-1 culture medium. After gentle centrifugation, supernatants were collected, plated, and cultured for at least 7 days at 37°C. In the voltage-clamp experiments described here, cells were cultured for 7 to 14 days, desegregated by a brief trypsinization procedure, and stored until use in culture medium at room temperature (22–23°C). Cells that appeared round were then used for electrophysiological study.

**Rabbit Arrhythmia Model: Animal Preparation.** The in vivo rabbit model of TdP was implemented as described by Carlsson et al. (1990) with minor modifications described by us (Mazur et al., 1999). Methoxamine (70 nmol/kg/min i.v.) was infused for 10 min before starting SPX, MOX, GAT, or GRX (2 mg/kg/min i.v.), after which both agents continued simultaneously for 30 min (GAT and GRX) or 60 min (SPX and MOX). Animals were euthanized with pentobarbital after the study (50 mg/kg i.v.). All procedures performed in this study were approved by the Vanderbilt University Animal Care Committee.

**Electrocardiography.** Standard surface ECG limb leads (I, II, III, aVF, aVL, and aVR) were continuously monitored and recorded at 100 mm/s paper speed (Electronics for Medicine; Honeywell Inc., Pleasantville, NY), as previously described (Mazur et al., 1999). **QT and QTc Interval Measurements.** ECG intervals were measured as the average from three consecutive beats using a single lead providing the clearest end of the QT interval (usually lead II or III), as previously published (Mazur et al., 1999). The QT interval was
corrected (QTc) according to the method of Carlsson et al. (1993) for rabbits by the formula: 
\[ \text{QTc} = \frac{\text{QT}}{0.175(\text{RR} + 30)} \]
where RR indicates the RR interval that was measured from the onset of consecutive QRS complexes.

**Arrhythmia Definition.** Premature ventricular contractions (PVCs) were defined as aberrant QRS complexes not preceded by a normal PR interval. TdP was defined as \( \geq 4 \) consecutive beats of polymorphic ventricular tachycardia (VT) and nonsustained VT was \( < 6 \) beats.

**Statistics.** Mean ± S.E.M. was calculated for continuous variables, and absolute and relative frequencies were measured for discrete variables. Continuous variables were compared between groups using one-way analysis of variance and Bonferroni’s correction for repeated measures, as appropriate. \( P \) values \( \leq 0.05 \) were considered statistically significant.

**Results**

**Quinolones Are \( I_{Kr} \) Antagonists.** Voltage-clamp studies demonstrated that all of the quinolone antibiotic agents tested were \( I_{Kr} \) antagonists (Fig. 1). The potency of \( I_{Kr} \) blockade varied by approximately an order of magnitude between the four agents with SPX being the most (IC\(_{50}\) = 0.23 ± 0.07 \( \mu \)M) and GRX (IC\(_{50}\) = 27.2 ± 11.6 \( \mu \)M) the least potent. None of the quinolone agents appeared to alter the voltage dependence of \( I_{Kr} \) activation (Fig. 2). Although these findings are consistent with the hypothesis that all quinolones are \( I_{Kr} \) antagonists, there is a wide range of \( I_{Kr} \) blocking potency among members of this drug class.

**QT Interval Prolongation by Quinolone Agents.** In vivo studies were next performed to determine the functional consequences of \( I_{Kr} \) inhibition by these quinolone agents. All of the quinolone agents caused some QT and QTc interval prolongation, and an example of QT prolongation seen after GRX is shown in Fig. 3. However, there were marked differences in the magnitude of these effects among the different agents. SPX caused the greatest absolute increase in both the QT and the QTc intervals (Fig. 4). In contrast, neither MOX nor GRX caused significant increases in the QT interval at any of the time points tested (Fig. 4), although QTc was prolonged at some time points. The lack of clear association between \( I_{Kr} \) antagonist potency and QT interval prolongation, suggested that factors independent of \( I_{Kr} \) block may be important for QT interval prolongation.

**Arrhythmia Induction.** Both \( I_{Kr} \) block and QT interval prolongation are known to favor induction and maintenance of the life threatening arrhythmia TdP (Roden et al., 1996). Previous studies by us (Mazur et al., 1999) and others (Carlsson...
son et al., 1990, 1991) have shown that IKr antagonist agents can induce TdP in this rabbit model within a 30-min time frame. To enhance the sensitivity of detecting arrhythmia induction, the SPX and MOX infusions were continued for a total of 60 min in an attempt to further distinguish the proarrhythmic potential of these two agents, given their more potent IKr antagonist actions (Figs. 1 and 2). None of the agents resulted in significant numbers of premature ventricular contractions or arrhythmias within the initial 30-min observation window (Table 1) (i.e., after 60 mg/kg cumulative infusion for each drug). SPX-treated animals were more likely to develop PVCs and nonsustained VT during the infusion period and SPX was the only agent that resulted in TdP (Fig. 5; Table 1). The TdP induction that followed QT interval prolongation and PVC development in an SPX-treated animal is shown in Fig. 5. Overall, these findings support the hypothesis that increased IKr antagonist potency of SPX compared with MOX translates into greater QT interval prolongation with significant proarrhythmic consequences. In contrast, differences in QT interval and QTc interval prolongation in response to GAT and GRX did not

**TABLE 1**

Arrhythmia induction rates

The numerator is the number of animals with PVC, NSVT, or TdP and the denominator is the number of animals studied with each drug.

<table>
<thead>
<tr>
<th></th>
<th>SPX</th>
<th>MOX</th>
<th>GAT</th>
<th>GRX</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVC</td>
<td>4/6</td>
<td>1/6</td>
<td>2/5</td>
<td>1/6</td>
</tr>
<tr>
<td>NSVT</td>
<td>3/6</td>
<td>0/6</td>
<td>1/5</td>
<td>1/6</td>
</tr>
<tr>
<td>TdP</td>
<td>1/6</td>
<td>0/6</td>
<td>0/5</td>
<td>0/6</td>
</tr>
</tbody>
</table>

NSVT, nonsustained ventricular tachycardia.

Fig. 4. QT and QTc interval prolongation in response to quinolone antibiotics. A–D, QT (○) and QTc (●) responses to serial and parallel methoxamine (10 μg/kg/min) and quinolone (2 mg/kg/min) infusion, as indicated by the arrows. The specific quinolone agent is indicated at the top of each panel: MOX (n = 6) (A), SPX (n = 6) (B), GAT (n = 4) (C), and GRX (n = 5) (D). *p < 0.05 compared with the value at 0 min.

Fig. 5. TdP induction after infusion of methoxamine and SPX. Panels are ordered chronologically and show baseline ECG (A); a PVC (marked by the arrow) after 46 min combined infusion of methoxamine and SPX (B); a PVC initiates TdP (marked by the arrow) (C) followed by continuous recordings of TdP (D) and spontaneous resolution to sinus rhythm (E). Scale bar, 400 ms.
translate into differences in arrhythmia induction during the 30-min observation period.

Discussion

All of the quinoline antibiotics were I_{Kr} antagonists at a cell membrane potential relevant to cardiac action potential repolarization, in the AT-1 atrial tumor cell line. This cell type has been previously demonstrated by us (Yang et al., 1994; Yang and Roden, 1996) to be a useful tool for determining I_{Kr} block in cardiac tissue. It is important to note, however, that I_{C_{50}} measurements are critically dependent on specific experimental conditions such as cell membrane potential, pulse duration, and extracellular K^+ concentration (Yang and Roden, 1996). I_{Kr} is a current that is blocked by a wide range of drugs with disparate chemical structures and is the most common repolarizing current linked to TdP following the use of drugs in patients (Roden, 1998b). The HEG R^+ channel, which underlies I_{Kr}, has a large vestibule that may facilitate trapping of chemically diverse drugs to produce current block (Mitcheson et al., 2000). Recently, it has become clear that quinoline antibiotic agents may prolong action potentials (Adamantidis et al., 1998) and cause QT interval prolongation and TdP. One such agent, GRX, was withdrawn from further clinical use because of this finding. Thus, it is important to better define the safety profile of agents of this important antibiotic class.

The plasma concentrations were not measured as part of this study. Quinolone antibiotics all have modest protein binding (~50%), high bioavailability (>90%), and similar metabolism (primarily glucuronide and sulfate conjugation with mixed renal and fecal excretion), suggesting that plasma concentrations were comparable across experimental groups. Human plasma concentrations following a single oral dose (200–600 mg) of SPX, MOX, or GRX is ~1 μg/ml, within the range of I_{C_{50}} values for I_{Kr} block by SPX and MOX (Fig. 1), but may be much higher in critically ill patients receiving parenteral therapy.

All of the agents tested increased QT and QTc intervals in the methoxamine-treated rabbit model. As was the case with measurements of I_{Kr} blocking potency, the four quinolone agents tested here exhibited a range of potency for prolonging cardiac repolarization. Interestingly, the increased tendency for QT and QTc interval prolongation correlated with greater I_{Kr} antagonist potency for SPX over MOX, but this correlation between QT and QTc interval prolongation and I_{Kr} antagonist potency did not hold up consistently across the whole range of agents studied. Overall, MOX, GAT, and GRX resulted in similar patterns of QT interval prolongation. Thus, it seems likely that parameters other than I_{Kr} block are important determinants of QT interval prolongation by some quinolone agents.

None of the agents tested resulted in arrhythmias within the standard 30-min window in the methoxamine-treated rabbit model. Because of the markedly higher I_{Kr} antagonist potency of SPX and MOX over the other agents tested, rabbits treated with these agents were studied for a prolonged interval. Using the longer interval, there was an increased tendency for PVCs, nonsustained VT, and TdP in SPX-treated compared with MOX-treated animals, but these differences were not statistically significant. Thus, for these two more potent I_{Kr} antagonist drugs, there was a correlation between I_{Kr} antagonist potency and an increased tendency to prolong the QT interval and to cause arrhythmic disturbances known to be related to QT interval prolongation. Although the methoxamine-pretreated rabbit model has been shown to be a useful guide to clinical outcomes for drugs that cause arrhythmias related to excessive QT interval prolongation, other factors not accounted for by this model are likely also important determinants of proarrhythmia in the clinical setting. Some of these factors include gender (Makkar et al., 1993), concomitant use of other drugs that may affect the disposition of the primary action potential-prolonging agent, and genetically determined repolarization reserve (Roden, 1998a). Thus, an important challenge is to develop models for evaluating proarrhythmic consequences of drugs that can incorporate more of these important variables.

Acknowledgments

We thank Dr. Katy Topadze and Holly Waldrop for excellent technical assistance.

References


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