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Short communication

Prulifloxacin: in vitro (HERG current) and in vivo (conscious dog) assessment of cardiac risk

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Abstract

Prulifloxacin, a new thiazeto-quinoline derivative with antibiotic properties, was evaluated for cardiac risk both in vitro on the ether-à-go-go-related gene (HERG) K^+ channel, and in vivo in the conscious dog monitored by telemetry. HERG current was measured from stably transfected human embryonic kidney (HEK) 293 cells by means of the patch-clamp technique. Application of AF 3013, the active metabolite of prulifloxacin, produced only minor reduction of HERG current amplitude (tail current = – 40 mV), producing a maximum blockade of 12.3 ± 3.3% at the highest concentration tested (335 μM). In comparison, ciprofloxacin also failed to produce a 50% inhibition of HERG current amplitude, although the maximum blockade was greater than that observed with prulifloxacin (47.6 ± 1.9% at the highest concentration tested (335 μM). In contrast, moxifloxacin blocked HERG current amplitude with an IC_{50} value of 74.7 μM. Prulifloxacin had no effect on the QTc interval (Fridericia's) following 5 days of repeated oral administration (150 mg/kg/day) in the conscious dog monitored by telemetry. These findings suggest that prulifloxacin is not likely to prolong the QT interval.
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Keywords: Prulifloxacin; Cardiac risk assessment; QT prolongation; HERG current; ECG (electrocardiogram)

1. Introduction

Fluoroquinolones are clinically established potent antibiotics. Sparfloxacin, however, was the first of these compounds reported to prolong cardiac repolarization in the rabbit Purkinje fibre (Adamantidis et al., 1998), and grepafloxacin was withdrawn worldwide because it induced torsades de pointes, a polymorphic ventricular tachycardia clearly linked to excessive QT interval prolongation. Bischoff et al. (2000) showed that moxifloxacin, sparfloxacin and grepafloxacin inhibited ether-à-go-go-related gene (HERG) currents with IC_{50} values of about 34.4 to 104.3 μM (10 to 50 μg/ml), whereas ciprofloxacin did not affect HERG currents at concentrations up to 301.8 μM (100 μg/ml). Kang et al. (2001) showed that sparfloxacin was the most potent blocker of HERG currents with an IC_{50} of 18 μM, whereas ofloxacin was the least potent compound with an IC_{50} of 1424 μM. Other compounds showed IC_{50} values of 50 μM (grepafloxacin), 129 μM (moxifloxacin), 130 μM (gatifloxacin), 915 μM (levofloxacin) and 966 μM (ciprofloxacin). Anderson et al. (2001) showed that sparfloxacin and moxifloxacin blocked the delayed rectifier K^+ current (I_{Kr}) in mouse atrial tumor cells, not HERG, with IC_{50} values of 0.23 and 0.75 μM, respectively, whereas gatifloxacin and grepafloxacin blocked I_{Kr} with IC_{50} values of 26.5 and 27.2 μM, respectively. Using the in vivo rabbit arrhythmia model described by Carlsson et al. (1990), all agents caused some prolongation of the QT and QTc interval following i.v. infusion. Sparfloxacin was clearly the most active and was the most potent in developing non-sustained ventricular tachycardia and torsades de pointes.

Prulifloxacin is the prodrug of AF 3013, a new thiazeto-quinoline broad spectrum antibacterial agent (Montanari et al., 2001; Ozaki et al., 1991). After oral administration, prulifloxacin is absorbed by the intestine and enters the circulation where it is immediately and quantitatively transformed into its active metabolite AF 3013 (Okuhama et al., 1997), other metabolites accounting for less than 15% of the administered dose (Nakashima et al., 1994). In light of the findings reported with the other fluoroquinolones, pruliflox-

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acin was evaluated in the present experiments for cardiac risk both in vitro, testing AF 3013 on the HERG potassium channel, and in vivo in the conscious dog monitored by telemetry.

2. Materials and methods

2.1. Herg K^+ channel

The method was described by Crumb (2000). Human embryonic kidney (HEK) 293 cells were stably transfected through the lipofectamine method (Saldeen et al., 1996) with the HERG clone. Cells were maintained in a CO_2 incubator at 37 °C in minimum essential medium with Earle's salts supplemented with nonessential amino acids, sodium pyruvate, penicillin, streptomycin, geneticin and fetal bovine serum. The external solution (solution bathing the cell) used for recording HERG currents had the following ionic composition (in mM): 137 NaCl, 4 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 11 dextrose, 10 HEPES, adjusted to a pH of 7.4 with NaOH. The internal (pipette) solution had the following ionic composition (in mM): 130 KCl, 1 $MgCl_2$, 10 NaATP, 5 EGTA, 5 HEPES, adjusted to a pH of 7.2 using KOH. Experiments were performed at 37 ± 1 °C. Currents were measured using the whole-cell variant of the patch-clamp method (Hamill et al., 1981). Glass pipettes were pulled from borosilicate glass and had tip resistances of approximately 1–2 μm for K^+ current recordings. Pipette tip resistance was approximately 1.0–2.0 MΩ. An Axopatch 1-B amplifier (Axon Instruments) was used for whole-cell voltage clamping. Creation of voltage clamp pulses and data acquisition was controlled by a PC running pClamp software (Axon Instruments). After rupture of the cell membrane (entering whole-cell mode), current amplitude and kinetics were allowed to stabilize as the cell was dialyzed with internal solution and paced at 0.1 Hz. Pulse protocol was a 500-ms pulse to + 10 mV from a holding potential of − 75 mV followed by a 500-ms pulse to − 40 ms. Currents were considered stable if currents elicited by a series of voltage pulses given at 0.1 Hz were superimposed. Rate-dependent effects were determined by a train of 20 depolarizing voltage steps using pulses to + 10 mV for 200 ms followed by pulses to − 40 mV for 200 ms, at 0.7–1.4 Hz from a holding potential of − 75 mV. Peak HERG current was measured for the first and last pulse of the train under control conditions and in the presence of the test compound. To avoid overestimating the degree of current reduction in the presence of test compound, rate-dependent drug effects were calculated by subtracting the current reduction in control from that observed in the presence of test compound in the same cell at the same pacing rate. Peak HERG current was measured as the maximum outward deflection of the tail current elicited upon return to − 40 mV. The test compound (AF 3013) and two comparison compounds were screened against HERG current amplitude from 0.1 to 335 μM at 0.1 Hz. The rate-dependence behaviour of the test and the reference compounds at 0.7, 1.1 and 1.4 Hz at 30 μM was also studied. The positive control substance E-4031 was evaluated in one cell at 1–100 nM, at 0.1 Hz.

2.2. Conscious dog monitored by telemetry

The method followed was described by Lacroix and Provost (2000). Dogs were anaesthetised with sodium pentobarbital (30 mg/kg i.v.) Following an incision in the flank and in the inguineal area, an implantable telemetric device (TL11M2-D70-PCT, Data Sciences International) was introduced into a pouch under the skin and the catheter of the device was passed and then inserted facing upstream into a femoral artery. The biopotential positive and negative leads were coiled into a loop, after having been passed subcutaneously, and anchored to surrounding tissue in lead II configuration. The tab located on the device body was sutured to the inner abdominal wall. The skin incisions were then closed. The animals were given 1 g amoxicilline i.m. and returned individually to their cages. They were given 400 mg amoxicilline p.o., once daily over the 3 subsequent days. Two weeks later, a telemetry receiver was positioned nearby each animal's home cage to record mean, systolic and diastolic arterial blood pressure (mm Hg) and heart rate (HR, bpm), which was derived from pulse blood pressure. The PR and the QT interval (ms) were also measured and the QTc interval (ms) was calculated according to Fridericia's formula $= \text{QT (ms)}/\sqrt[3]{[60/\text{HR (bpm)}]}$. Recordings were taken in blocks of 30 s every 15 min, for 30 min before and for 24 h after administration, and reported at time points 0, 15, 30 min before and then 1, 2, 3, 4, 5, 6, 8, 12, 16, 20 and 24 h after administration. Prulifloxacin was evaluated at 150 mg/kg, administered p.o. in gelatin capsules once daily for 5 days. Each animal received the vehicle, i.e. an empty gelatin capsule (single administration) on Day 0, then prulifloxacin (repeated administration for 5 days, i.e. from Days 1 to 5, at approximately 10.00 h), with a washout period of at least 48 h between the vehicle treatment and prulifloxacin treatment. The evaluation of prulifloxacin was performed following the fifth administration. Intra-group comparison was performed using a one-way analysis of variance (time) with repeated measures at each time point, followed by Dunnett's *t*-tests in case of significant time effect, to compare each time value with the *T*0 value (i.e. basal value before each treatment). Inter-group comparison was performed using a two-way analysis of variance (group, time) with repeated measures at each time, followed by a one-way analysis of variance (group) at each time in case of significant group × time interaction.

3. Results

AF 3013 produced very little reduction of HERG current amplitude at 0.1 Hz. The maximum blockade reached

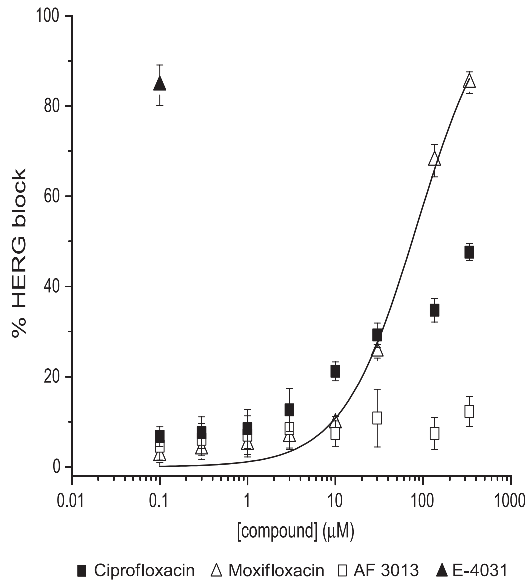

Fig. 1. Dose–response curve for blockade of HERG current amplitude by AF 3013 at 0.1 Hz. Comparison with ciprofloxacin and moxifloxacin. Data are mean ± S.E.M.

12.3 ± 3.3% (NS), at 335 μM, the highest concentration tested (Fig. 1). In comparison, ciprofloxacin also produced little reduction of HERG current amplitude, although the inhibitory effect was more marked (47.6 ± 1.9% max, $P<0.05$, at 335 μM. In contrast, moxifloxacin clearly blocked HERG current amplitude with an IC_{50} value of 74.7 μM. The IC_{50} value for the reference compound E-4131 was 26.4 nM. The reduction in HERG current amplitude observed at 30 μM with AF 3013, ciprofloxacin and moxifloxacin (0.1 Hz) was not further enhanced upon rapid stimulation rates of 0.7–1.4 Hz (additional reduction beyond that observed at 0.1 Hz: 1.7 ± 0.9%, 5.3 ± 2.2% and 2.4 ± 1.3% at 1.4 Hz, for AF 3013, ciprofloxacin and moxifloxacin, respectively).

In the conscious dog (Table 1), a progressive decrease in arterial blood pressure and heart rate was observed following the administration of vehicle (22% maximum decrease in mean arterial blood pressure at time point 3-h post-administration, 26% maximum decrease in heart rate at time point 16-h post-administration, $P<0.05$). Arterial blood pressure and heart rate tended to remain reduced during the dark period, as is usually observed in this test because of the reduction in spontaneous locomotor activity and sleep occurring during this period. Similar changes occurred following the fifth administration of prulifloxacin, but prulifloxacin had no effect on arterial blood pressure or heart rate, as compared with vehicle (inter-group comparison). Similarly, a progressive lengthening of the PR and the QT interval was observed following the administration of vehicle (16% max lengthening of the PR interval at time points 3- and 4-h post-administration, $P<0.01$, and 8% max lengthening of the QT

Table 1
Effects of prulifloxacin (150 mg/kg) on arterial blood pressure, heart rate and the parameters of the electrocardiogram following repeated[a] oral administration in the conscious dog monitored by telemetry

	Measurements time following the last administration (h)								ANOVA[b]
	0	1	2	3	4	6	12	24	
Mean arterial blood pressure (mm Hg)									
Vehicle	88 ± 6	80 ± 9	75 ± 7	69 ± 9*	80 ± 5	86 ± 7	85 ± 7	89 ± 5	
Prulifloxacin	90 ± 4	93 ± 11	91 ± 6	87 ± 6	70 ± 2	68 ± 6	74 ± 4	86 ± 9	NS
Heart rate (bpm)									
Vehicle	117 ± 12	106 ± 11	97 ± 11	88 ± 8	96 ± 6	96 ± 9	86 ± 9	106 ± 16	
Prulifloxacin	113 ± 1	84 ± 5	103 ± 9	99 ± 5	77 ± 6*	67 ± 4**	74 ± 12**	113 ± 4	NS
PR interval (ms)									
Vehicle	96 ± 4	101 ± 2	105 ± 5	111 ± 4**	111 ± 3**	99 ± 7	102 ± 4	99 ± 4	
Prulifloxacin	96 ± 3	102 ± 5	95 ± 7	97 ± 6	112 ± 4	111 ± 11	107 ± 6	96 ± 5	NS
QTc interval (ms)									
Vehicle	234 ± 6	237 ± 4	237 ± 4	247 ± 2	244 ± 6	236 ± 8	250 ± 1	233 ± 7	
Prulifloxacin	228 ± 4	233 ± 7	229 ± 6	241 ± 6	258 ± 2**	261 ± 3**	256 ± 3**	234 ± 5	NS
QTc interval (ms)[c]									
Vehicle	289 ± 8	285 ± 6	277 ± 7	280 ± 7	284 ± 4	274 ± 7	281 ± 11	278 ± 10	
Prulifloxacin	282 ± 5	260 ± 8	273 ± 10	284 ± 7	280 ± 7	270 ± 7	273 ± 14	289 ± 3	NS

Data are presented as mean ± S.E.M. ($n=4$).

[a] Prulifloxacin (150 mg/kg) administered orally once daily for 5 days. Dark period from the 7th to the 19th h post-administration.

[b] Inter-group comparison performed using a two-way ANOVA with repeated measures.

[c] Corrected QT interval (QTc) calculated according to Fridericia's formula: $\mathrm{QT\ (ms)}/\sqrt[3]{[60/\mathrm{HR\ (bpm)}]}$.

* $P<0.05$, intra-group comparison (versus $T=0$) performed using a one-way ANOVA with repeated measures, followed by Dunnett's *t*-tests in case of significant time effect.

** $P<0.01$, intra-group comparison (versus $T=0$) performed using a one-way ANOVA with repeated measures, followed by Dunnett's *t*-tests in case of significant time effect.

interval at time point 4-h post-administration, NS). This effect can be attributed to the decrease in heart rate. Prulifloxacin had no effect either on the PR and the QT interval, as compared with vehicle (inter-group comparison). Prulifloxacin had no effect on the QTc interval, as compared with vehicle (inter-group comparison).

4. Discussion

AF 3013, the active metabolite of prulifloxacin, induced only minor reduction in HERG current amplitude, with a maximum blockade of 12.3 ± 3.3% at the highest concentration tested (335 μM), > 70 times higher than its human plasma concentration (4.3 μM) (Nakashima et al., 1994). Ciprofloxacin also failed to produce a 50% inhibition of HERG current amplitude, although the maximum blockade (47.6 ± 1.9% at 335 μM, the highest concentration tested) was greater than that observed with AF 3013. In contrast, moxifloxacin blocked HERG current amplitude with an IC_{50} value of 74.7 μM. The results observed in the present study, indicating that moxifloxacin was much more active than ciprofloxacin in inhibiting HERG current amplitude, are similar to those reported by Bischoff et al. (2000), Kang et al. (2001) and Anderson et al. (2001). In our study, prulifloxacin was also found to be devoid of any effect on the QTc interval of the electrocardiogram following a repeated oral administration of 150 mg/kg for 5 days. Interestingly, Hart et al. (2002) found that moxifloxacin dose-dependently prolonged the QTc interval (Fridericia's) following intravenous administration in the restrained conscious dog, i.e. 6.1% (250 ms), 12.7% (265 ms), 22.9% (289 ms) and 39.2% (328 ms) at 1, 10, 25 and 50 mg/kg, respectively, in a fashion clearly consistent with the in vitro electrophysiology results with moxifloxacin.

The very modest blockade of HERG current amplitude by AF 3013 (45% bound to plasma protein; Nakashima et al., 1994) together with the absence of QTc interval prolongation in vivo following 5 days of repeated oral treatment suggest that prulifloxacin is not likely to prolong the QT interval.

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