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Short communication

Effects of sparfloxacin, grepafloxacin, moxifloxacin, and ciprofloxacin on cardiac action potential duration

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Abstract

Fluoroquinolone antibiotics have been associated with QT prolongation following administration to humans. This study compares the effects of four fluoroquinolones, sparfloxacin, grepafloxacin, moxifloxacin and ciprofloxacin on action potential duration recorded from canine isolated cardiac Purkinje fibres. Left and right ventricular Purkinje fibres were isolated from canine hearts and continuously superfused with physiological salt solution. Action potential duration at 90% repolarization was recorded via intracellular microelectrodes. Sparfloxacin, grepafloxacin, moxifloxacin and ciprofloxacin prolonged action potential duration in a concentration dependent manner. Mean concentrations causing a 15% prolongation of action potential duration recorded at a stimulation frequency of 1 Hz were: sparfloxacin 4.2 ± 0.7 μg/ml; grepafloxacin 9.3 ± 0.9 μg/ml; moxifloxacin 9.9 ± 1.6 μg/ml and ciprofloxacin 72.8 ± 26.4 μg/ml. Prolongation was inverse frequency dependent with larger increases in action potential duration occurring when the stimulation frequency was reduced to 0.5 Hz. These results indicate that effects on action potential duration vary within this class of compound. Rank order of potency was sparfloxacin > grepafloxacin = moxifloxacin > ciprofloxacin.

Keywords: Fluoroquinolones; Sparfloxacin; Moxifloxacin; Grepafloxacin; Ciprofloxacin; (Canine); Purkinje fibre; Cardiac action potential

1. Introduction

Antibiotics such as the macrolide erythromycin and quinolone sparfloxacin have been associated with risk of QT prolongation and arrhythmias (Thomas, 1994; Dupont et al., 1996). Both compounds are known to prolong the cardiac action potential (Lancaster et al., 2000; Adamantidis et al., 1998) and to block the delayed rectifier K^+ channel, $K_{(Vr)}$ which is a known target for compounds which prolong QT interval. In animal studies, sparfloxacin and moxifloxacin have been reported to prolong QT interval in dog (Jaillon et al., 1996; Von Keutz and Schluter, 1999). Quinolone antibiotics have been suggested to have a class effect of blocking the human *ether-a go-go* related gene (HERG) K^+ channel (the α-subunit of $K_{(Vr)}$), prolonging action potential duration with an associated prolongation of QT interval. In the present study, we have compared the effects of four fluoroquinolone antibiotics sparfloxacin, grepafloxacin, moxifloxacin and ciprofloxacin on the cardiac action potential duration recorded from canine isolated Purkinje fibres. Concentrations of the compounds were chosen which reflect the plasma concentration range achieved therapeutically in humans around 3 μg/ml extending to 100 μg/ml.

2. Methods and materials

2.1. Recording of cardiac action potentials

Purkinje fibres were isolated from the left and right ventricles of hearts taken from male and female beagle dogs killed by overdose of sodium pentobarbitone (euthatal). Fibres were pinned out in a perspex chamber and superfused with physiological salt solution (PSS) maintained at 35–36°C. Composition of PSS was (in mM): NaCl 125; KCl 5.4; $CaCl_2$ 1.8; $MgCl_2$ 1.0; NaH_2P0_4 1.2; $NaHCO_3$ 25; D-glucose 5.5 gassed with 95% O_2/5% CO_2. Intracellular recordings of membrane potential were made

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with conventional glass microlectrode techniques using an Axoclamp 2A amplifier (Axon Instruments) and data was recorded and analysed using Notocord HEM v3.2 data capture/analysis system. Action potential duration was calculated to the point of repolarization to 90% of the resting membrane potential. Fibres were perfused with PSS for around 1 h to washout any remaining anaesthetic and then for a further 1 h, pre-administration of compounds, to establish stable baseline recordings. Throughout the experiment, the preparation was stimulated via silver bipolar electrodes at suprathreshold levels to evoke cardiac action potentials. The basal stimulation frequency was 1 Hz. At the end of the period of superfusion with each concentration of compound, action potentials were recorded at 0.5 Hz to investigate potential inverse use-dependent prolongation of action potential duration.

2.2. Compounds

Grepafloxacin was provided by Otsuka Pharmaceutical Company. Sparfloxacin, moxifloxacin and ciprofloxacin were extracted from commercially available formulations of these drugs. The compounds were dissolved in water to achieve a stock solution of 10 mg/ml and aliquots added to the PSS to achieve the required concentrations.

2.3. Experimental design and data analysis

Five groups of fibres were exposed to either vehicle (water) ($n = 8$), sparfloxacin ($n = 4$), grepafloxacin ($n = 4$), moxifloxacin ($n = 4$) or ciprofloxacin ($n = 4$). Action potential duration was recorded at baseline and after 30 min superfusion with vehicle or compound. Statistical comparisons were made between changes from baseline in compound treated and vehicle treated fibres. This approach compensates for any effects of vehicle and time dependent changes in action potential duration. Replicates in the vehicle (control) were increased in order to optimise the power of multiple comparisons between compound treated groups and the control group. Data were analysed using one-way analysis of variance, followed by a Levene's test, which indicated that variances were heterogeneous and therefore a non-parametric analysis was performed (Conover's test followed by Dunnett's test). Additionally, concentrations, which were associated with a 15% increase of action potential duration, were estimated for each fibre and mean values were calculated.

3. Results

At a stimulation frequency of 1 Hz, sparfloxacin, grepafloxacin, moxifloxacin and ciprofloxacin prolonged action potential duration in a concentration dependent manner (Fig. 1A). These effects were statistically significant at concentrations of 3 μg/ml and above for sparfloxacin and moxifloxacin and at 10 μg/ml and above for grepafloxacin and ciprofloxacin. The magnitude of prolongation of action potential duration varied between compounds with the following rank order sparfloxacin > grepafloxacin = moxifloxacin > ciprofloxacin. The mean concentration associated with a 15% increase in action potential duration for each compound is shown in Table 1, together with the prolongation of action potential achieved at 100 μg/ml.

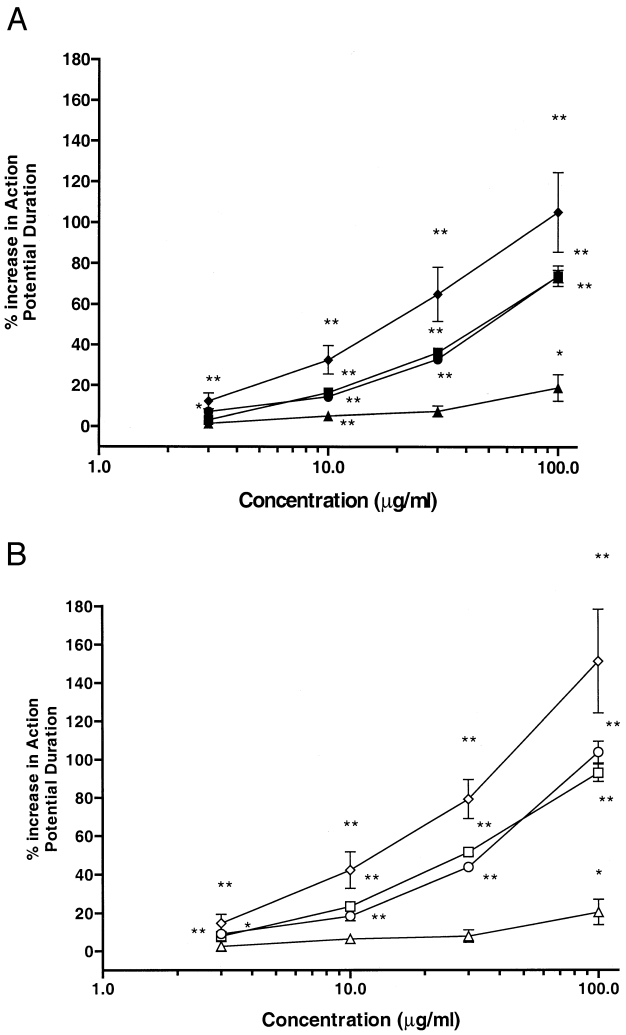

Fig. 1. Mean prolongation of action potential duration at 1 Hz (A) (closed symbols) and 0.5 Hz (B) (open symbols) by sparfloxacin (◆◇), grepafloxacin (■□), moxifloxacin (●○) and ciprofloxacin (▲△). Error bars represent S.E.M.; $^{*}P < 0.05$; $^{**}P < 0.01$.

When the frequency of stimulation was reduced to 0.5 Hz, equivalent to a heart rate associated with a marked bradycardia in man (30 bpm), baseline action potential duration was prolonged. Increases in action potential duration from baseline caused by the compounds were more marked with larger percentage increases achieved for all of the compounds tested, the mean concentrations associated

Table 1

Compound (oral therapeutic dose)	C_{max} (μg/ml)	% Plasma protein binding	Estimated free plasma concentration	1 Hz [Δ + 15%] (μg/ml)	% ↑APD at 100 (μg/ml)	0.5 Hz [Δ + 15%] (μg/ml)	% ↑APD at 100 (μg/ml)
Sparfloxacin (400mg)	1.3[a]–1.5[b]	45	0.7–0.8	4.2 ± 0.7	105	3.5 ± 0.6	151
Grepafloxacin (400mg)	0.9[c]–1.5[d]	50	0.45–0.75	9.3 ± 0.9	74	4.9 ± 0.6	93
Moxifloxacin (400mg)	2.5[e]–5.0[f]	50	1.25–2.5	9.9 ± 1.6	74	7.1 ± 1.7	104
Ciprofloxacin (500mg)	2.2[g]–3.0[h]	20–40	1.5–2.1	72.8 ± 26.4	19	61.0 ± 20.0	20

% ↑APD — % increase from baseline action potential duration.
[Δ + 15%] — mean (±S.E.M.) concentration associated with a 15% increase in action potential duration.
C_{max} range is the highest and lowest mean C_{max} values reported in the literature. Plasma protein binding data are taken from Clinical Pharmacology 2000 web site. For ciprofloxacin, a value of 30% plasma protein binding has been used in the estimation of free plasma concentrations.

[a] Zix et al. (1997).
[b] Montay (1996).
[c] Efthymiopoulos et al. (1997).
[d] Child et al. (1995).
[e] Stass et al. (1998).
[f] Web site http://cp.gsm.com.
[g] Naber et al. (1999).
[h] Wise (1991).

with a 15% increase in action potential duration were also slightly lower (Fig. 1B; Table 1).

4. Discussion

The effects of the fluoroquinolones sparfloxacin, moxifloxacin, grepafloxacin and ciprofloxacin on the K^+ channel encoded by the HERG has been reported by Bischoff et al. (2000). Ciprofloxacin was found to have little interaction with the HERG channel at concentrations up to 100 μg/ml, whilst sparfloxacin, grepafloxacin and moxifloxacin inhibited HERG currents with IC_{50} values of 13, 37 and 41 μg/ml, respectively. This rank order of potency correlates well with the functional prolongation of action potential duration observed in this study. The more modest prolongation of action potential duration by ciprofloxacin in this study is associated with a little or no block of HERG at concentrations up to 100 μg/ml.

All of the compounds tested prolonged action potential duration supporting the idea of a class effect described by Ball (2000), however, within this family of fluoroquinolones, marked differences in potency and maximum prolongation of action potential duration were noted. The compounds tested have similar antimicrobial potency (Blondeau, 1999) and have similar target therapeutic dose levels (Table 1), (Curran et al., 1995; Geddes, 1999; Appelbaum, 1999; Ball, 2000). Ciprofloxacin was least effective in prolonging action potential duration achieving a significant prolongation of action potential duration of 5% at 10 μg/ml with a stimulation frequency of 1 Hz. The effects of moxifloxacin and grepafloxacin were similar, prolonging action potential duration by 14–16% at 10 μg/ml. At 3 μg/ml, moxifloxacin caused a significant prolongation of 7% whilst grepafloxacin prolonged action potential duration by only 3% (n.s.). Sparfloxacin was most potent achieving a 15% prolongation at approximately half the concentration of moxifloxacin and grepafloxacin and a 12% increase in action potential duration at the lowest concentration tested 3 μg/ml. The maximum prolongation achieved by sparfloxacin was also much greater than that of moxifloxacin and grepafloxacin.

Comparison of the concentrations at which these agents prolong action potential duration with the estimated therapeutic free plasma levels suggest that sparfloxacin may cause prolongation of action potential duration and therefore QT interval at plasma levels associated with antimicrobial efficacy. For moxifloxacin and grepafloxacin, prolongation of action potential duration and QT interval may be noted at the upper end of the plasma level range required for antibiotic activity and at a level over threefold higher. For ciprofloxacin, a wider safety window is evident with more modest but statistically significant prolongation of the action potential duration apparent at 3–10 times the anticipated therapeutic plasma level.

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