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In vivo experimental approach for the risk assessment of fluoroquinolone antibacterial agents-induced long QT syndrome

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Abstract

The proarrhythmic effects of fluoroquinolone antibacterial agents, sitafloxacin, gatifloxacin and moxifloxacin, were compared using three in vivo models. In the halothane-anesthetized dogs ($n=5$), intravenous 10-min infusion of gatifloxacin and moxifloxacin (1–3 mg/kg) prolonged the ventricular effective refractory period and the repolarization period to a similar extent, whereas sitafloxacin (1–3 mg/kg) prolonged the former only. No significant change was detected in other cardiovascular parameters. In the chronic complete atrioventricular block dogs ($n=4$), oral administration of 100 mg/kg of gatifloxacin (2 of 4) and moxifloxacin (3 of 4) induced torsades de pointes, which was not observed by sitafloxacin. In the α-chloralose-anesthetized rabbits ($n=5$), intravenous 20-min infusion of 60 mg/kg of gatifloxacin induced torsades de pointes (1 of 5) in the presence of methoxamine infusion, which was not observed by sitafloxacin or moxifloxacin. Thus, the halothane-anesthetized model is suitable for assessing QT prolongation, whereas the chronic complete atrioventricular block model is sensitive for detecting torsadogenic action of drugs. The α-chloralose-anesthetized model is the simplest and least expensive method, but its sensitivity to detect proarrhythmic action may be less great.

Keywords: Fluoroquinolone; Monophasic action potential; QT interval; Torsades de pointes; Atrioventricular block; α_1-Adrenoceptor stimulation

1. Introduction

Since noncardiovascular drug-induced prolongation of the QT interval is often associated with the onset of torsades de pointes resulting in life-threatening ventricular arrhythmias (De Ponti et al., 2001; Haverkamp et al., 2000; Tamargo, 2000), worldwide regulatory authorities have raised a heightened awareness on the submission of data surrounding the ventricular repolarization process. Moreover, general nonclinical testing strategy for delayed ventricular repolarization by human pharmaceuticals is being discussed in draft stage guideline ICH S7B for safety pharmacology studies (The ICH Steering Committee, 2002).

In the case of fluoroquinolone antibacterial agents, it has been reported that sparfloxacin and grepafloxacin can prolong the QT interval to cause lethal ventricular arrhythmias (Bertino and Fish, 2000; Demolis et al., 1996; Dupont et al., 1996; Owens, 2001), which were withdrawn in most countries. Recently, gatifloxacin and moxifloxacin were developed as third generation of fluoroquinolones (Ball, 2000). However, in vitro studies have indicated that gatifloxacin and moxifloxacin markedly prolonged the action potential duration of the isolated guinea pig ventricular myocardium and canine Purkinje fibers (Gintant et al., 2001; Hagiwara et al., 2001; Patmore et al., 2000). Also, gatifloxacin and moxifloxacin inhibited the human cardiac repolarizing K^+ current (Anderson et al., 2001; Bischoff et al., 2000; Kang et al., 2001). Clinical studies on the safety pharmacology of gatifloxacin and moxifloxacin indicated that these fluoroquinolones may induce QT prolongation and ventricular arrhythmias (Bertino et al., 2002; Démolis et al., 2000; Iannini and Circiumaru, 2001; Noel et al., 2003; Siepmann and Kirch, 2001; Von Keutz and Schlüter, 1999).

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Since in vivo animal models of proarrhythmia have not taken part in the routine preclinical testing system, we developed two types of canine models, namely, a halothane-anesthetized, closed-chest model (Chiba et al., 2000; Satoh et al., 2000; Sugiyama and Hashimoto, 1998; Sugiyama et al., 2001, 2002b, 2003) and a chronic complete atrioventricular block model (Chiba et al., 2000; Sugiyama et al., 2002a,b, 2003; Volders et al., 1998; Vos et al., 1998). In this study, we tested the usefulness of these models in predicting the arrhythmogenecity of fluoroquinolones in humans in comparison with an α-chloralose-anesthetized rabbit model which has been reported to be suitable in detecting torsadogenic action of QT prolonging drugs (Anderson et al., 2001, Carlsson et al., 1990, 1993). Gatifloxacin and moxifloxacin were used as proarrhythmic fluoroquinolones, whereas sitafloxacin, another third generation fluoroquinolone, was selected as a reference drug that did not prolong the action potential duration in the in vitro study (Hagiwara et al., 2001).

2. Materials and methods

All experimental procedures were performed in accordance with the in-house guidelines of the Institutional Animal Care and Use Committee of Daiichi Pharmaceutical and those of University of Yamanashi.

2.1. Experiment 1: halothane-anesthetized canine model

Experiments were carried out using female beagle dogs weighing 7.5–12.0 kg ($n = 5$ for each group) according to a previous report (Chiba et al., 2000). Dogs were initially anesthetized with thiopental sodium (30 mg/kg, i.v.). After intubation with a cuffed endotracheal tube, 1% halothane vaporized with 100% oxygen was inhaled with a volume-limited ventilator (Shinano, SN-480-3, Tokyo, Japan). Tidal volume and respiratory rate were set at 20 ml/kg and 15 strokes/min, respectively. To prevent blood clotting, heparin sodium (200 IU/kg) was intravenously administered. The surface lead II electrocardiogram (ECG) was obtained from the limb electrodes. The systemic blood pressure was measured at the right femoral artery. A pig tail catheter was positioned at the left ventricle through the right femoral artery to measure the left ventricular pressure. The maximum upstroke velocity of the ventricular pressure ($LVdP/dt_{max}$) and left ventricular end-diastolic pressure (LVEDP) were recorded during the sinus rhythm to estimate the cardiac contractility and preload to the left ventricle, respectively.

A bi-directional steerable monophasic action potential (MAP) recording/pacing combination catheter (EP Technologies, 1675P, Sunnyvale, CA, USA) was positioned at the endocardium of the interventricular septum of the right ventricle through the left femoral vein to obtain MAP signals. The signals were amplified with a DC preamplifier (EP Technologies, Model 300) and the interval (ms) at 90% repolarization level was defined as MAP_{90}. The heart was electrically driven using a cardiac stimulator (Nihon Kohden, SEC-3102, Tokyo, Japan) via the pacing electrodes of the combination catheter placed in the right ventricle. The stimulation pulses were rectangular in shape, 2 V of amplitude (about twice the threshold voltage) and 1 ms of duration. The MAP_{90} was measured during the sinus rhythm ($MAP_{90(sinus)}$) and at a pacing cycle length of 400 ms ($MAP_{90(CL400)}$) and 300 ms ($MAP_{90(CL300)}$) to get rid of the influences of the heart rate variation. The effective refractory period of the right ventricle was assessed by a programmed electrical stimulation. The pacing protocol consisted of five beats of basal stimuli in a cycle length of 400 ms followed by an extra stimulus of various coupling intervals. Starting from the late diastole, the coupling interval was shortened in 5 ms decrements until refractoriness occurred. The terminal repolarization period ($= MAP_{90(CL400)} -$ effective refractory period) of the right ventricle was calculated to estimate the extent of electrically vulnerability of the ventricular muscle (Franz, 1994; Franz and Costard, 1988; Kirchhof et al., 1998; Sugiyama and Hashimoto, 2002).

The ECG, systemic blood pressure, left ventricular pressure and MAP signals were continuously monitored using a polygraph system (Nihon Kohden, RMP-6018M) and analyzed using an ECG processor (Softron, SBP-8, Tokyo, Japan). Corrected QT interval (QTc) was obtained using Bazett's formula (Bazett, 1920). After control assessment, each fluoroquinolone in a low dose of 1 mg/kg was administered via the right femoral vein over 10 min and each cardiovascular variable was recorded at 5, 10, 15, 20 and 30 min after the start of infusion. Then, a high dose of 3 mg/kg was additionally administered over 10 min and each variable was recorded in the same manner. A volume of 2 ml of blood was drawn from the left femoral artery to measure the plasma drug concentration at each recording point. Each cardiovascular variable of this model has been shown to be stable >3 h in the absence of active drug (Sugiyama and Hashimoto, 1998).

2.2. Experiment 2: chronic atrioventricular block canine model

Experiments were carried out using beagle dogs of either sex weighing 8.0–12.5 kg ($n = 4$ for each group) according to a previous report (Chiba et al., 2000). Dogs were anesthetized with pentobarbital sodium (30 mg/kg, i.v.). After intubation with a cuffed endotracheal tube, the respiration was controlled using a volume-limited ventilator (Shinano, SN-480-3) with room air. Tidal volume and respiratory rate were set at 20 ml/kg and 15 strokes/min, respectively. The surface lead II ECG was continuously monitored. A quad-polar electrodes catheter with a large tip of 4 mm (Cordis-Webster, D7-DL-252, CA, USA) was inserted through the right femoral vein using the standard percutaneous technique under sterile conditions and positioned across the tricuspid valve under the guide of bipolar electrograms from the distal electrode pair.

The optimal site for the atrioventricular node ablation was determined on the basis of the intracardiac electrogram, of which a very small His deflection was recorded and atrium/ventricular voltage ratio was >2. The site was usually found at 1–2 cm proximal from the position where the largest His bundle electrogram was recorded. The power source for the atrioventricular node ablation was obtained from an electrosurgical generator (Silver Medical, SL-1PR, Tokyo, Japan), which delivers continuous unmodulated radiofrequency energy at a frequency of 500 kHz. After determining the location, the radiofrequency energy of 20 W was delivered for 10 s from the tip electrode to an indifferent patch electrode positioned on the animal's back, which was continued then for 30 s if junctional ectopic complexes were induced. The endpoint of this procedure was the development of the atrioventricular block with an onset of stable idioventricular escaped rhythm. Proper care was taken for the animals. Proarrhythmic properties of the drugs were assessed >4 weeks after the onset of the atrioventricular block based on the previous reports (Chiba et al., 2000; Sugiyama et al., 2002a,b, 2003; Vos et al., 1998).

Holter recording system (Del Mar Avionics, Model 461, CA, USA) was used to monitor the ECG for about 24 h. The ECG was analyzed with the Holter analyzing software (Del Mar Avionics, StrataScan Model 563). QT interval represents the mean of three consecutive complexes. QTc was calculated using the Bazett's formula (Bazett, 1920). Proarrhythmic effects of two different doses of each drug were assessed in one atrioventricular block animal without anesthesia. Namely, about 2 h after the start of ECG monitoring, a low dose of 10 mg/kg was orally administered using gelatin capsules. On the next day, a high dose of 100 mg/kg was administered in the same manner. A volume of 2 ml of venous blood was sampled before and 2, 4, 6 and 24 h after the administration of the drug to measure its plasma concentration.

2.3. Experiment 3: α-chloralose anesthetized rabbit model

Experiments were carried out using female New Zealand White rabbits weighing 2.1–3.4 kg ($n = 5$ for each group) according to previous reports (Carlsson et al., 1990, 1993). After the animals were anesthetized with thiopental sodium (30 mg/kg, i.v. via marginal ear vein), α-chloralose (90 mg/kg) was administered for 20 min. Then, a tracheotomy was performed to control the respiration using a volume-limited ventilator (Shinano, SN-480-6) with room air. Tidal volume and respiratory rate were set at 5–10 ml/kg and 35 strokes/min, respectively. Catheters were inserted into the right femoral artery and vein to monitor the systemic blood pressure and to infuse the drugs, respectively. The surface lead II ECG was obtained from the limb electrodes.

A left thoracotomy was performed at the fourth intercostal space. The pericardium was opened and a MAP recording catheter (EP Technologies, Model 220) was attached on the epicardium of the left ventricle to obtain MAP signals. The signals were amplified with a DC preamplifier (EP Technologies, Model 300) and the interval (ms) at 90% repolarization level was defined as MAP_{90}. Intrathoracic temperature was maintained within a physiological range by covering the chest with a plastic sheet in addition to the use of an overhead light source.

The ECG, MAP signals and systemic blood pressure were continuously monitored using a polygraph system (Nihon Kohden, RMP-6008M), and recorded on a thermal array recorder (Nihon Kohden, RTA-1300M) at a paper speed of 100 mm/s at each time point. Each value of ECG and MAP signals represents the mean of three consecutive complexes. Corrected QT interval (QTc) was obtained using the following formula (Carlsson et al., 1993): $QTc = QT - 0.175 (RR - 300)$. After control assessment, α_1-agonist methoxamine at 1.7 mg/4 ml/kg/h was continuously infused into the marginal ear vein. Ten minutes later, a fluoroquinolone of 60 mg/kg was intravenously administered over 20 min into the right femoral vein. Each cardiovascular variable was recorded every 10 min after the start of methoxamine infusion for 40 min. Before assessing the proarrhythmic effects of sitafloxacin, gatifloxacin and moxifloxacin, we validated currently used α-chloralose-anesthetized model using sparfloxacin, a nonspecific I_{Kr} channel inhibitor ($n = 5$) that has been demonstrated to prolong repolarization period and to induce torsades de pointes in the same model as used in this study (Anderson et al., 2001).

2.4. Definition of arrhythmia in experiments 2 and 3

Torsades de pointes was defined as polymorphic ventricular tachycardia, of which QRS complex twisted around the baseline, lasting more than six consecutive beats (Satoh and Zipes, 1996). Ventricular fibrillation was defined as disorganized, irregular ventricular complexes with changing contours. In experiment 3, onset of ventricular fibrillation was further confirmed by the disappearance of pulsation of arterial pressure.

2.5. Determination of plasma drug concentration in experiments 1 and 2

The blood samples were centrifuged at $1000 \times g$ for 30 min at 4 °C. Supernatant plasma was removed and stored at −80 °C until the drug concentration was measured. A sensitive and specific determination of the concentration of each drug was performed using a bioassay method, which employed an agar plate diffusion technique with *Bacillus subtilis* as the test strain (Stass and Dalhoff, 1997). The limit of quantification of each drug was 0.02–0.04 μg/ml.

2.6. Drugs

Sitafloxacin, gatifloxacin and moxifloxacin were synthesized at Daiichi Pharmaceuticals. Sparfloxacin was extracted from a commercial source (Spara™, Dainippon Pharmaceuticals, Tokyo, Japan). The following drugs were purchased:

halothane (Takeda chemical Industries, Tokyo, Japan), heparin sodium (Shimizu Pharmaceuticals, Shizuoka, Japan), methoxamine hydrochloride (Sigma, MO, USA), pentobarbital sodium (Nembutal, Dainippon Pharmaceuticals) and thiopental sodium (Tanabe Seiyaku, Osaka, Japan). Drugs for intravenous administration were dissolved in 1% lactate solution. All solutions were daily prepared fresh.

2.7. Statistical analyses

Data are presented as the mean ± S.E.M. The statistical differences of paired data within a group were evaluated by paired *t*-test or one-way repeated measures analysis of variance (ANOVA) followed by contrasts for mean value comparison. The statistical differences among the three groups were evaluated using Tukey's test. A *P*-value less than 0.05 was considered significant.

3. Results

3.1. Experiment 1: effects on the halothane-anesthetized model

The time courses of changes in the heart rate, mean blood pressure, LVdP/dt_{max} and LVEDP are summarized in Fig. 1 ($n = 5$ for each group). The heart rate (beats/min), mean blood pressure (mm Hg), LVdP/dt_{max} (mm Hg/s) and LVEDP (mm Hg) at the pre-drug control (C) were 98 ± 7, 121 ± 10, 1959 ± 307 and 9 ± 2 in the sitafloxacin-administered group, 100 ± 3, 131 ± 5, 1992 ± 147 and 15 ± 5 in the gatifloxacin-administered group, and 94 ± 5, 124 ± 13, 1930 ± 215 and 8 ± 1 in the moxifloxacin-administered group, respectively. There was no significant difference in the respective control values among the three groups. No significant change was observed in these parameters during the study.

The time courses of the plasma concentration of sitafloxacin, gatifloxacin and moxifloxacin are summarized in Fig. 1 ($n = 5$ for each group). The peak plasma concentration after the administration of 1 and 3 mg/kg was 2.4 ± 0.3 and 8.2 ± 0.9 μg/ml for sitafloxacin, 2.8 ± 0.1 and 11.6 ± 1.2 μg/ml for gatifloxacin, and 2.3 ± 0.2 and 7.0 ± 0.4 μg/ml for moxifloxacin, respectively.

Typical tracings of ECG during the sinus rhythm before and after the administration of 3 mg/kg of gatifloxacin are shown in Fig. 2, whereas the time courses of changes in the QT interval, QTc, PR interval and QRS width are summarized in Fig. 3 ($n = 5$ for each group). The PR interval, QRS width, QT interval (ms) and QTc (ms/$s^{1/2}$) at the pre-drug control were 114 ± 8, 58 ± 3, 314 ± 11 and 399 ± 21 in the sitafloxacin-administered group, 107 ± 4, 61 ± 2, 306 ± 4 and 394 ± 7 in the gatifloxacin-administered group, and

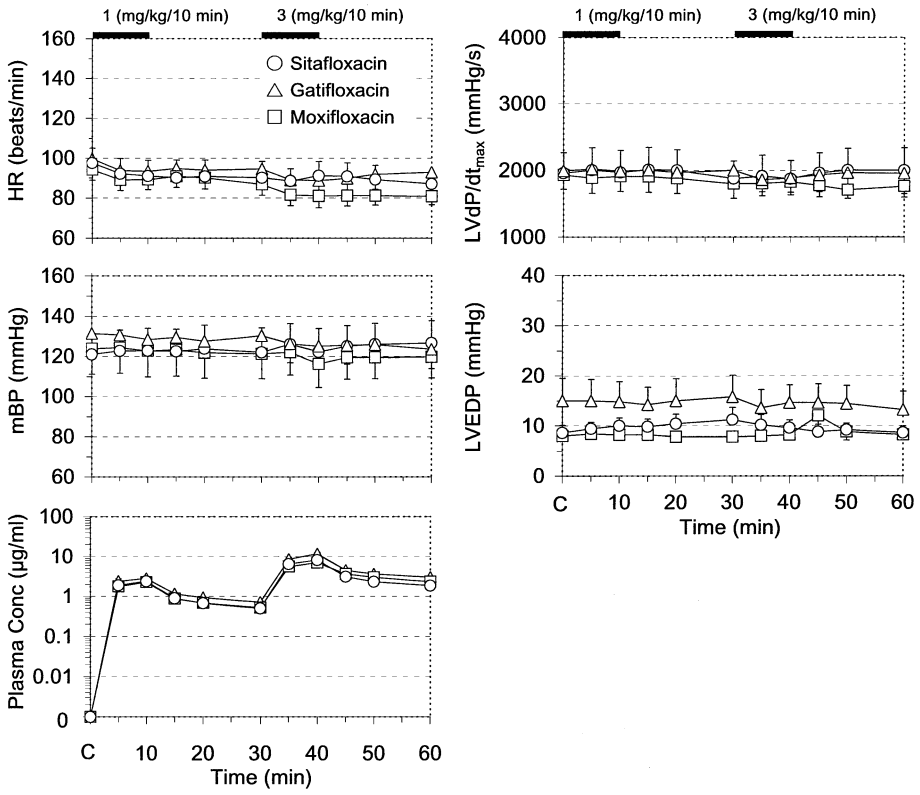

Fig. 1. Time courses of the heart rate (HR), mean blood pressure (mBP), plasma drug concentration (Plasma Conc), maximum upstroke velocity of the left ventricular pressure (LVdP/dt_{max}) and left ventricular end-diastolic pressure (LVEDP) assessed in the canine halothane-anesthetized model ($n = 5$ for each group). Sitafloxacin (circles), gatifloxacin (triangles) and moxifloxacin (squares) were infused intravenously for 10 min. Data are presented as the mean ± S.E.M. C: pre-drug control.

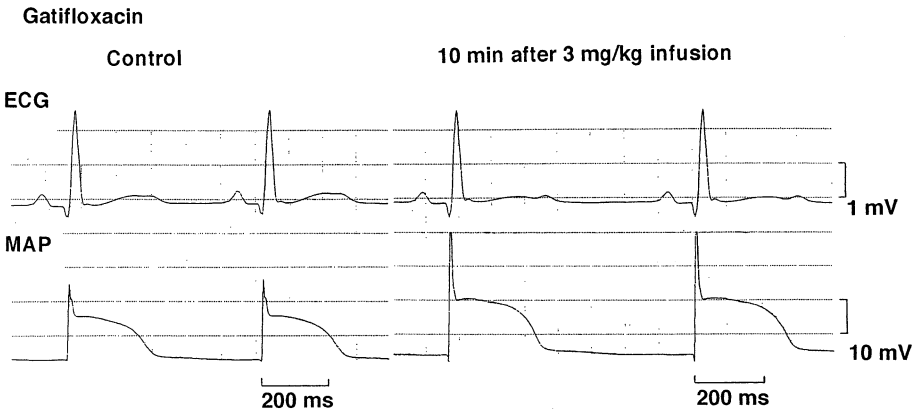

Fig. 2. Typical tracings of lead II surface ECG (ECG) and monophasic action potentials recorded from the right ventricular endocardium (MAP) during the sinus rhythm at pre-drug control (Control) and 10 min after the start of 3 mg/kg of gatifloxacin infusion (10 min after 3 mg/kg infusion) of the canine halothane-anesthetized model. Marked prolongation of QT interval and MAP duration were observed.

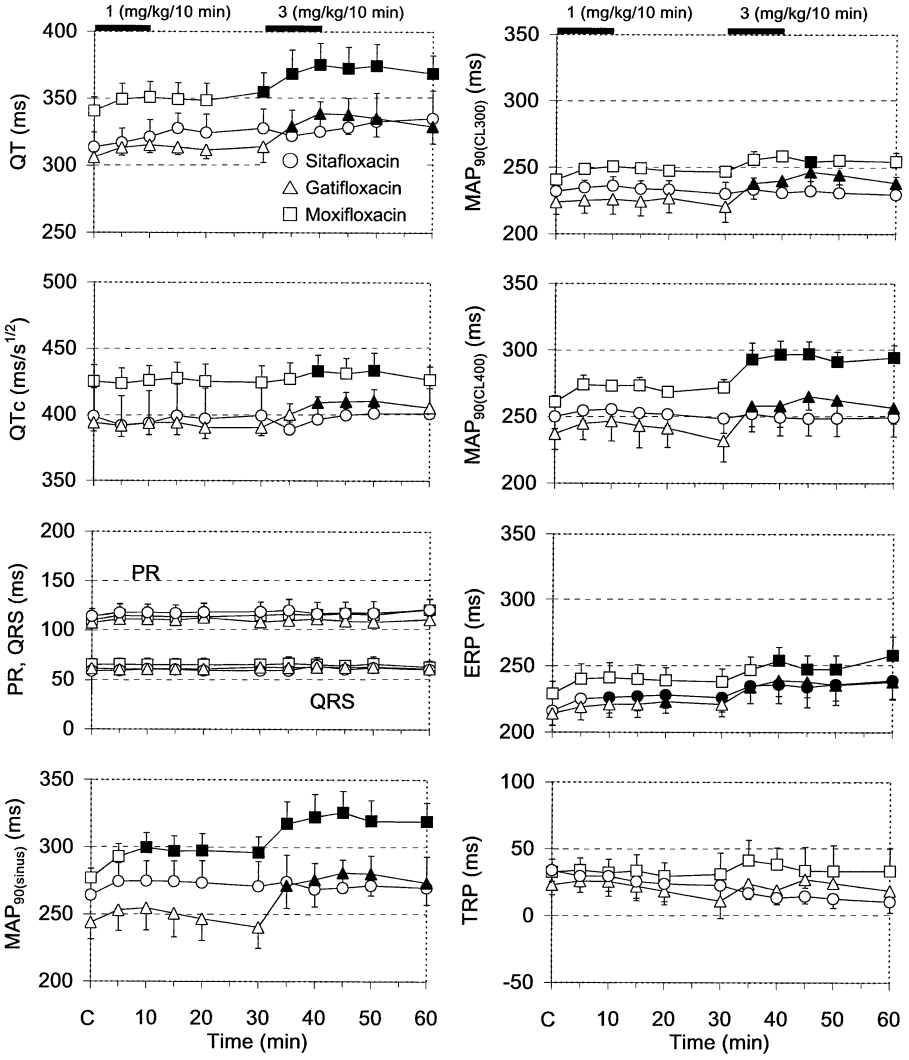

Fig. 3. Time courses of the QT interval (QT), QTc, PR interval (PR) and QRS width (QRS) in lead II ECG. Those of the monophasic action potential duration at 90% recovery level during the sinus rhythm ($MAP_{90(sinus)}$), and at a pacing cycle length of 400 ms ($MAP_{90(CL400)}$) and 300 ms ($MAP_{90(CL300)}$); the effective refractory period of the right ventricle (ERP); and terminal repolarization period ($TRP = MAP_{90(CL400)} - ERP$) assessed in the canine halothane-anesthetized model ($n = 5$ for each group). Sitafloxacin (circles), gatifloxacin (triangles) and moxifloxacin (squares) were infused intravenously for 10 min. Data are presented as the mean ± S.E.M. The closed symbols represent the significant differences from the respective control values (C) by $P < 0.05$.

110 ± 6, 65 ± 5, 340 ± 11 and 425 ± 13 in the moxifloxacin-administered group, respectively. There was no significant difference in the respective control values among the three groups. In the sitafloxacin-administered group, no significant change was observed in these parameters during the study. In the gatifloxacin and moxifloxacin-administered groups, the QT interval and QTc were prolonged significantly, while no significant change was detected in the PR interval or QRS width. In the gatifloxacin-administered group, significant changes were observed in the QT interval for 5–30 min and the QTc for 10–20 min after the start of the high dose of 3 mg/kg infusion. In the moxifloxacin-administered group, significant changes were observed in the QT interval at 30 min after the start of the low dose of 1 mg/kg infusion and for 5–30 min after the high dose, and in the QTc at 10 and 20 min after the high dose. Ventricular arrhythmia was not observed during the study in any group.

Typical tracings of the MAP during the sinus rhythm before and after the administration of 3 mg/kg of gatifloxacin are shown in Fig. 2, whereas the time courses of changes in the MAP_{90} during the sinus rhythm and ventricular pacing are summarized in Fig. 3 ($n = 5$ for each group). The $MAP_{90(sinus)}$, $MAP_{90(CL300)}$ and $MAP_{90(CL400)}$ (ms) at the pre-drug control were 264 ± 11, 232 ± 7 and 250 ± 9 in the sitafloxacin-administered group, 244 ± 12, 224 ± 9 and 237 ± 12 in the gatifloxacin-administered group, and 277 ± 7, 241 ± 4 and 261 ± 5 in the moxifloxacin-administered group, respectively. There was no significant difference in the respective control values among the three groups. In the sitafloxacin-administered group, no significant change was observed in these parameters during the study. In the gatifloxacin and moxifloxacin-administered groups, the $MAP_{90(sinus)}$ and $MAP_{90(CL300)}$ and $MAP_{90(CL400)}$ were prolonged significantly. In the gatifloxacin-administered group, significant changes were observed in the $MAP_{90(sinus)}$, $MAP_{90(CL300)}$ and $MAP_{90(CL400)}$ for 5–30 min after the high dose. In the moxifloxacin-administered group, significant changes were observed in the $MAP_{90(sinus)}$ from 10 min after the low dose to the end of the experiment, and in the $MAP_{90(CL300)}$ at 15 min after the high dose, and in the $MAP_{90(CL400)}$ for 5–30 min after the high dose. The time courses of the increment in the $MAP_{90(CL300)}$ and $MAP_{90(CL400)}$ were also calculated (not shown in the figure). Increment of the $MAP_{90(CL400)}$ was greater than that of the $MAP_{90(CL300)}$ for 5–10 min after the low dose and for 5–15 min after the high dose of gatifloxacin, and for 5–30 min after the high dose of moxifloxacin, indicating that both drugs can prolong the repolarization period in a reverse use-dependent manner.

The time courses of changes in the effective refractory period and terminal repolarization period are summarized in Fig. 3 ($n = 5$ for each group). The effective refractory period and terminal repolarization period (ms) at the pre-drug control were 229 ± 9 and 34 ± 8 in the sitafloxacin-administered group, 214 ± 9 and 23 ± 7 in the gatifloxacin-administered group, and 229 ± 9 and 33 ± 10 in the moxifloxacin-administered group, respectively. There was no significant difference in the respective control values among the three groups. The effective refractory period was prolonged significantly in each group. In the sitafloxacin-administered group, significant changes were observed in the effective refractory period from 10 min after the low dose to the end of the experiment. In the gatifloxacin-administered group, significant changes were observed in the effective refractory period at 20 min after the low dose and for 5–30 min after the high dose. In the moxifloxacin-administered group, significant changes were observed in the effective refractory period for 10–30 min after the high dose. No significant change was observed in the terminal repolarization period during the study in any group.

3.2. Experiment 2: effects on the chronic atrioventricular block model

The proarrhythmic effects of sitafloxacin, gatifloxacin and moxifloxacin are summarized in Fig. 4A, and the typical tracings of ECG demonstrating the torsadogenic action of moxifloxacin are shown in Fig. 4B. After the oral administration of the high dose of 100 mg/kg as well as the low dose of 10 mg/kg of sitafloxacin, torsades de pointes was not induced in any animal. On the other hand, after the high dose of gatifloxacin, torsades de pointes was observed in two out of four animals, which was not induced by the low dose. The initial torsades de pointes was observed at 3.2 and 6.4 h ($n = 2$) after the high dose. Meanwhile, after the high dose of moxifloxacin, torsades de pointes was observed in three out of four animals, which was not induced by the low dose. The initial torsades de pointes was observed at 2.6–8.4 h ($n = 3$) after the high dose. In addition, in two animals torsades de pointes degenerated into ventricular fibrillation at 2.6 and 14.1 h. The elapsed time between the first torsades de pointes and the latest one that degenerated into ventricular fibrillation was 0 and 8.1 h, respectively. Onset of torsades de pointes was closely related to "R on T" phenomenon as shown in Fig. 4B. Among all animals that induced torsades de pointes, no malignant ventricular arrhythmias, including sustained or monomorphic ventricular tachycardias, or both, were observed before the onset of torsades de pointes. The duration and rate of nonlethal torsades de pointes were 1–5 s and >300 beats/min, respectively. The number of episodes of torsades de pointes was 1–6 per each animal ($n = 5$).

Idioventricular escaped rate during the study were 30–50 (beats/min) in each animal. The time courses of changes in QT interval (ms) and QTc ($ms/s^{1/2}$) are summarized in Fig. 5 ($n = 4$ for each group). The QT interval and QTc at the pre-drug control (C) were 321 ± 14 and 281 ± 21 in the low-dose sitafloxacin-administered group, 305 ± 6 and 246 ± 3 in the low-dose gatifloxacin-administered group, and 314 ± 11 and 251 ± 15 in the low-dose moxifloxacin-administered group, respectively. Meanwhile, those were 315 ± 19 and 268 ± 15 in the high-dose sitafloxacin-administered group, 325 ± 17

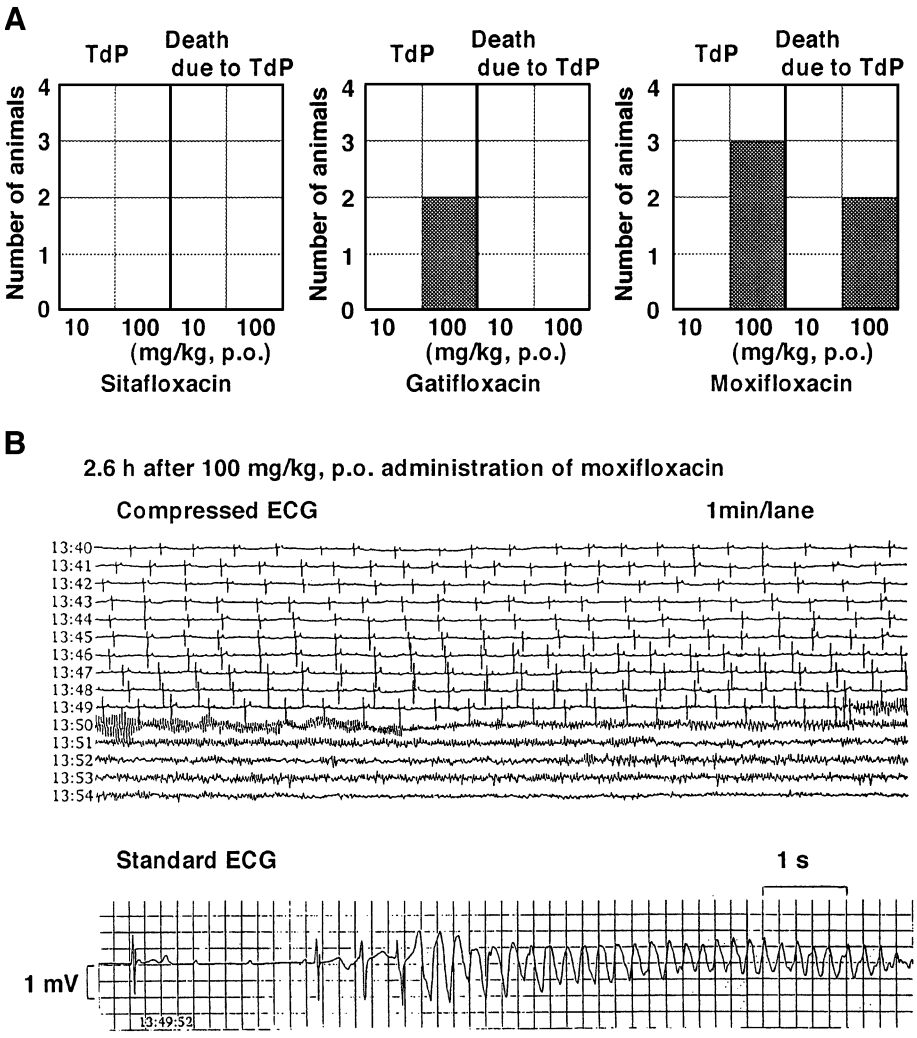

Fig. 4. Summary of the proarrhythmic effects of sitafloxacin, gatifloxacin and moxifloxacin in the chronic complete atrioventricular block dogs ($n = 4$ for each group) (A) and typical tracings of ECG demonstrating the torsadogenic action of moxifloxacin (B). Typical episode of torsades de pointes (TdP) was recorded 2.6 h (13:49) after the oral administration of 100 mg/kg of moxifloxacin, which degenerated into ventricular fibrillation.

and 246 ± 20 in the high-dose gatifloxacin-administered group, and 349 ± 18 and 261 ± 10 in the high-dose moxifloxacin-administered group, respectively. There was no significant difference in the respective control values among the three groups. No significant prolongation was observed in the QT interval or QTc in any group.

The time courses of the plasma concentration of sitafloxacin, gatifloxacin and moxifloxacin are summarized in Fig. 5 ($n = 4$ for each group). Peak plasma concentrations after the administration of low and high doses were 1.7 ± 0.4 and 9.8 ± 1.7 μg/ml for sitafloxacin, 4.1 ± 0.3 and 11.3 ± 1.6 μg/ml for gatifloxacin, and 2.1 ± 0.3 and 12.6 ± 1.0 μg/ml for moxifloxacin, respectively.

3.3. Experiment 3: effects on the α-chloralose-anesthetized model

After the administration of sparfloxacin, torsades de pointes was induced in two out of five animals. The initial episode was observed at 14 and 15 min (for cumulative doses of 42 and 45 mg/kg), respectively. In the former animal, the initial torsades de pointes degenerated into the ventricular fibrillation, leading to the animal's death, whereas in the latter animal, torsades de pointes was induced seven times, which returned to the normal sinus rhythm every time. Typical tracings of MAP, ECG and blood pressure demonstrating the torsadogenic action of sparfloxacin are depicted in Fig. 6.

The time courses of changes in MAP_{90}, QT and QTc are summarized in Fig. 7 ($n = 5$ for each group). The MAP_{90}, QT interval and QTc (ms) at 10 min before the start of methoxamine infusion were 119 ± 5, 133 ± 11 and 152 ± 9 in the sitafloxacin-administered group, 131 ± 5, 131 ± 8 and 149 ± 7 in the gatifloxacin-administered group, and 122 ± 4, 149 ± 13 and 168 ± 12 in the moxifloxacin-administered group, respectively. There was no significant difference in the respective control values among the three groups. Infusion of methoxamine decreased the heart rate

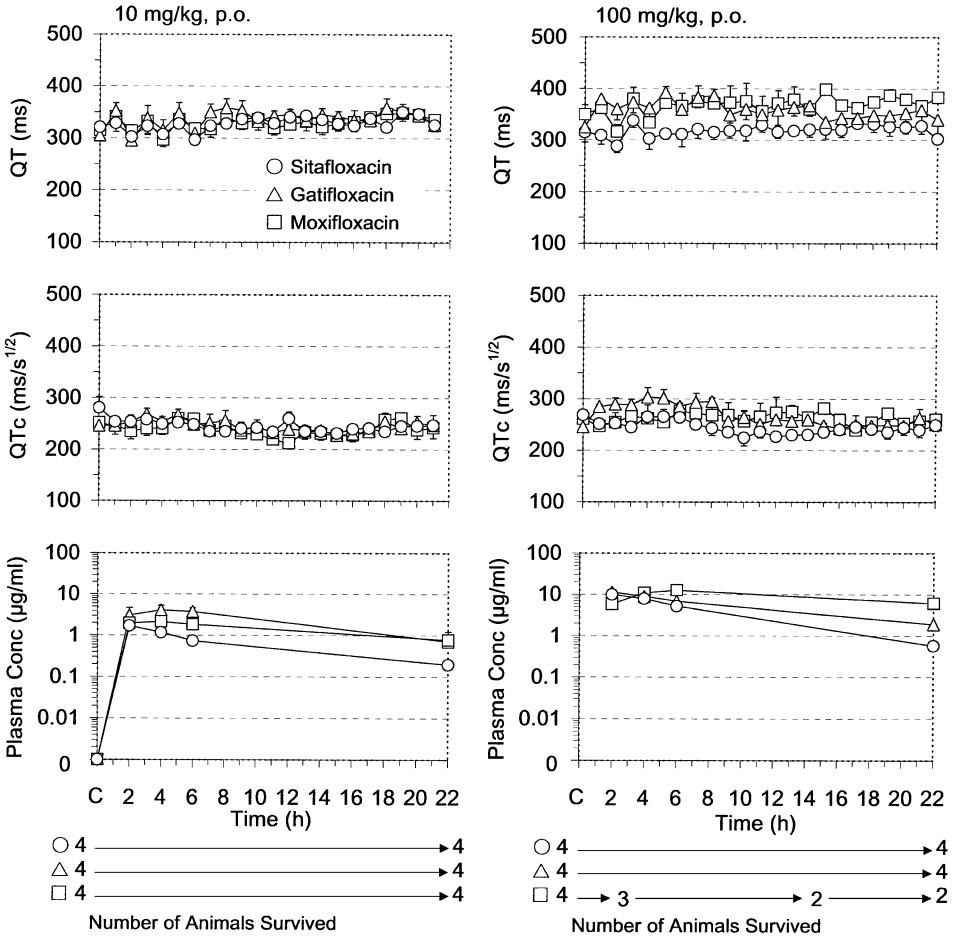

Fig. 5. Time courses of the QT interval (QT), QTc and plasma drug concentration (Plasma Conc) after the oral administration of 10 mg/kg (left) and 100 mg/kg (right) of sitafloxacin (circles), gatifloxacin (triangles) and moxifloxacin (squares) assessed in the chronic complete atrioventricular block dogs ($n = 4$ for each group). Data are presented as the mean ± S.E.M. The number of animals survived after the administration of each drug was shown at the bottom of the figure. C: pre-drug control.

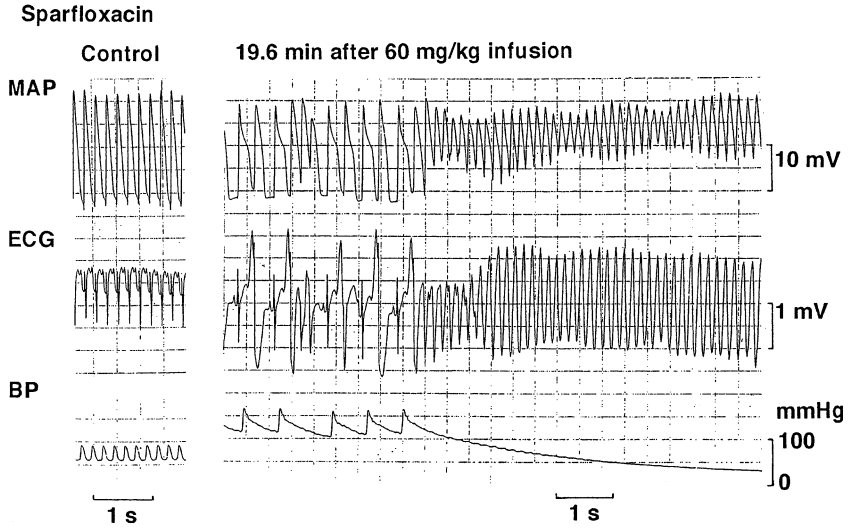

Fig. 6. Typical tracings of monophasic action potentials recorded from the left ventricular epicardium (MAP), lead II surface ECG (ECG) and systemic blood pressure (BP) of the α-chloralose-anesthetized rabbit, demonstrating the torsadogenic action of sparfloxacin. Third episode of torsades de pointes was induced at 19.6 min after the start of sparfloxacin infusion in this animal. C: pre-drug control.

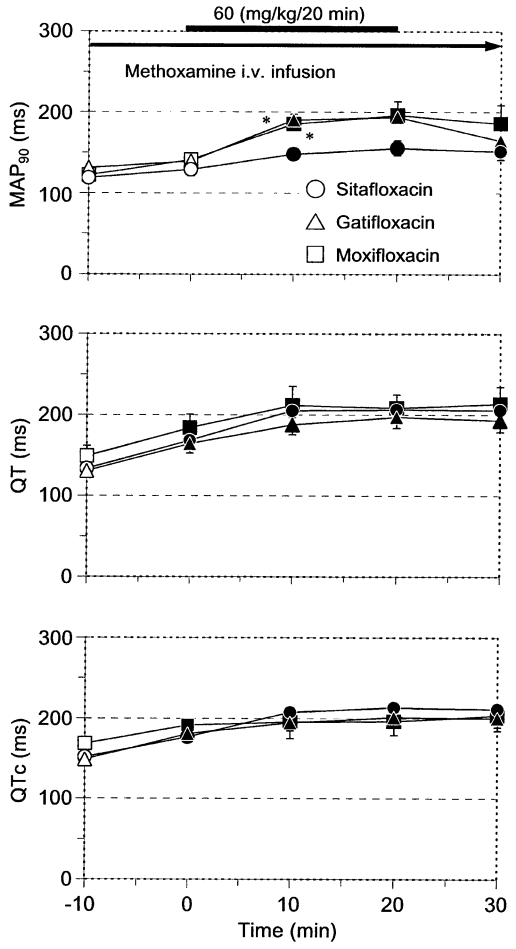

Fig. 7. Time courses of the monophasic action potential duration at 90% recovery level during the sinus rhythm (MAP_{90}), QT interval (QT) and QTc in lead II ECG assessed in the α-chloralose-anesthetized rabbits ($n=5$ for each group). Sitafloxacin (circles), gatifloxacin (triangles) and moxifloxacin (squares) were infused for 20 min. Methoxamine infusion (3 mg/kg/min) was started 10 min before the start of each fluoroquinolone infusion. Data are presented as the mean ± S.E.M. The closed symbols represent the significant differences from the respective control values (− 10 min) by $P<0.05$. Asterisks indicate the significant differences among the groups at each time point by $P<0.05$.

and increased the mean blood pressure (not shown in the figure). Also, methoxamine prolonged the QT interval and QTc in each group during the initial 10 min of infusion, of which no significant difference was detected in the magnitude of changes among the three groups. The MAP_{90}, QT interval and QTc were further prolonged after the administration of the fluoroquinolones in each group. Gatifloxacin and moxifloxacin caused greater prolongation in MAP_{90} compared with sitafloxacin at 10 min. Torsades de pointes was not induced by sitafloxacin or moxifloxacin, whereas gatifloxacin induced torsades de pointes in one of five animals at 20 min (for a cumulative dose of 60 mg/kg), which immediately degenerated into the ventricular fibrillation (>300 beats/min), lasted for 2.4 min with a simultaneous marked drop in blood pressure, but then returned to the normal sinus rhythm.

4. Discussion

The purpose of this study was to assess the usefulness of the halothane-anesthetized canine model and chronic complete atrioventricular block model in predicting arrhythmogenecity of sitafloxacin, gatifloxacin and moxifloxacin in humans in comparison with that of the α-chloralose-anesthetized rabbit model.

4.1. Plasma drug concentration

In previous clinical studies (Aminimanizani et al., 2001; Grasela, 2000; Lubasch et al., 2000; O'Grady et al., 2001), single oral administration of therapeutically relevant doses of 400–500 mg/body of sitafloxacin, gatifloxacin and moxifloxacin provided peak plasma concentrations (C_{max}) of 4.7, 3.8 and 4.3 μg/ml, respectively. In addition, in a limited number of preliminary experiments, we observed higher doses of the drugs induced hypotension via nonspecific mechanisms, which significantly modified the electrophysiological profile of the drugs in vivo. Based on these previous knowledge, we intravenously infused the drugs at a speed of 1 and 3 mg/kg/10 min to the halothane-anesthetized dogs, whereas 10 and 100 mg/kg were orally administered to the atrioventricular block animals. As shown in the results, the C_{max} values after the administration of the low dose of each drug in both models were close to their therapeutic plasma concentration, whereas those after the high dose were about 10 μg/ml in both models which was >2 times higher than the therapeutic concentration. Thus, the currently observed effects of the fluoroquinolones may reflect the cardiovascular profile at therapeutic to supra-therapeutic levels of plasma drug concentration in both the halothane-anesthetized dogs and the atrioventricular block animals. Although we did not measure the plasma drug concentrations in experiment 3, the observations in the canine models together with previous reports (Aminimanizani et al., 2001; Grasela, 2000; Lubasch et al., 2000; O'Grady et al., 2001) indicate that intravenous infusion of 60 mg/kg/20 min of the fluoroquinolones to the rabbit model could provide supra-therapeutic levels of plasma drug concentrations.

4.2. Cardiohemodynamic effects

Cardiohemodynamic effects of the fluoroquinolones were assessed in the halothane-anesthetized dogs, since we did not measure such variables in the atrioventricular block animals, and co-administration of methoxamine in the α-chloralose-anesthetized rabbits may have modified the cardiohemodynamic effects of the fluoroquinolones. As described in the results, we did not observe any cardiohemodynamic effects of the fluoroquinolones in the halothane-anesthetized dogs during the observation period, indicating that these three drugs can be used safely in patients with a limited reserve of cardiohemodynamic function.

4.3. Electrophysiological effects

Electrophysiological profile of the fluoroquinolones was assessed using the three models. In the halothane-anesthetized dogs, intravenous administration of gatifloxacin and moxifloxacin prolonged the repolarization period in a reverse use-dependent manner, whereas sitafloxacin hardly affected it. These observations were essentially in accordance with the previous clinical studies (Bertino et al., 2002; Démolis et al., 2000; Iannini and Circiumaru, 2001; Noel et al., 2003; Siepmann and Kirch, 2001; Von Keutz and Schlüter, 1999). In the in vitro study, gatifloxacin and moxifloxacin have been reported to inhibit the human ether-à-go-go-related gene (HERG) I_{Kr} current with IC_{50} of 26.5 and 0.75 μM, respectively (Anderson et al., 2001). Since the C_{max} values of gatifloxacin and moxifloxacin after the high dose were 11.3–12.6 μg/ml (=about 30 μM), currently observed changes in the repolarization process may be largely exerted through the I_{Kr} channel inhibition in the in vivo heart. On the other hand, lack of QT prolongation by sitafloxacin may be explained by the previous experimental observations, in which sitafloxacin neither inhibited HERG I_{Kr} current (unpublished data) nor prolonged action potential duration (Hagiwara et al., 2001) at concentrations of up to 100 μM. The fluoroquinolones tested in this study hardly affected the atrioventricular nodal or intraventricular conduction during the sinus rhythm, indicating that these drugs will not inhibit the Na^+ or Ca^{2+} channels at relatively slow heart rate of <100 beats/min. Meanwhile, sitafloxacin may inhibit the Na^+ channels at faster heart rate, since the drug prolonged the effective refractory period without affecting MAP duration, which is a typical manifestation of the Na^+ channel inhibitory action of drugs in the halothane-anesthetized canine model (Sugiyama et al., 2001).

In the atrioventricular block animals, we could not detect a significant change in the ECG parameters by any fluoroquinolone during the observation period except the onset of torsades de pointes by gatifloxacin and moxifloxacin. In the α-chloralose-anesthetized rabbit model, greater prolongation of MAP duration was transiently induced by gatifloxacin and moxifloxacin compared with that of sitafloxacin. However, this model may be inappropriate for assessing the extent of drug-induced QT prolongation, since infusion of methoxamine itself may prolong QT interval significantly. These results of the electrophysiological effects of the drugs obtained from the three models indicate that the halothane-anesthetized model will be the most suitable for assessing the electrophysiological as well as the cardiohemodynamic profile of QT prolonging drugs in vivo.

4.4. Proarrhythmic effects

Before assessing the proarrhythmic effects of the third generation fluoroquinolones, we validated our α-chloralose-anesthetized rabbit model using a typical proarrhythmic fluoroquinolone sparfloxacin (Chiba et al., 2000; Dupont et al., 1996). As described in the results, torsades de pointes was induced in two animals out of five by sparfloxacin, which is in good accordance with a previous observation (Anderson et al., 2001), indicating the reproducibility and reliability of our rabbit arrhythmia model.

The proarrhythmic potential was examined using the three animal models. Torsades de pointes was induced in the atrioventricular block canine model by the high dose of gatifloxacin (2 out of 4 animals) and moxifloxacin (3 out of 4 animals), and in the rabbit model by gatifloxacin (1 out of 5 animals), whereas no new onset arrhythmia was observed by any drug in the halothane-anesthetized canine model. These results suggest that the chronic atrioventricular block model is the most sensitive for detecting torsades de pointes. α-Chloralose-anesthetized model may be the simplest and least expensive method, but its sensitivity to detect proarrhythmic action may be less great. We and others have reported that higher sensitivity of the atrioventricular block model for detecting the drug-induced torsades de pointes can be explained by the reduction of the delayed rectifier K^+ current (I_{Kr} and I_{Ks}), bradycardia and structural remodeling in addition to the neurohumoral responses (Sugiyama et al., 2002a; Volders et al., 1998, 1999; Vos et al., 1998). In addition, torsades de pointes occurred several hours after the Tmax of the p.o. drug administration in the atrioventricular block model, suggesting accumulation of the drug in the heart and/or production of QT prolonging active metabolites, which needs to be determined by further studies.

Potential mechanisms of proarrhythmic effects of gatifloxacin and moxifloxacin can be estimated by their electrophysiological effects on the halothane-anesthetized canine model. It has been demonstrated that prolongation and/or backward shift of the electrically vulnerable period of the ventricular muscle can increase the risk of aberrant excitation, which may provide an ideal substrate for reentry arrhythmias (Chiba et al., 2000; Franz, 1994; Kirchhof et al., 1998; Sugiyama and Hashimoto, 2002). In this study, we simultaneously measured the ventricular MAP and effective refractory period at the same site to estimate the extent of the electrically vulnerable period. Gatifloxacin and moxifloxacin prolonged the MAP_{90} in parallel with the effective refractory period, resulting in little change in the terminal repolarization period. However, one can speculate that backward shift of the terminal repolarization period in the cardiac cycle itself by gatifloxacin and moxifloxacin may at least in part explain the onset of torsades de pointes, and/or that more drug doses may be needed to demonstrate the prolongation of the terminal repolarization period in the halothane-anesthetized model like sparfloxacin in our previous studies (Chiba et al., 2000; Satoh et al., 2000).

4.5. Conclusion

The present in vivo experimental studies indicate that gatifloxacin and moxifloxacin may delay the repolarization

process possibly via I_{Kr} channel inhibition, which could be closely related with the onset of torsades de pointes. Since sitafloxacin lacks such profile, proarrhythmia may not be a class effect of the fluoroquinolones. Thus, the use of in vivo analytical strategy proposed in this study will make it possible to predict the possible proarrhythmic effects of wide variety of new compounds before they are introduced into the clinical studies.

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