COMPARATIVE ASSESSMENT OF PRURIFLOXACIN, SPARFLOXACIN, GATIFLOXACIN AND LEVOFLOXACIN IN THE RABBIT MODEL OF PROARRHYTHMIA

Megumi AKITA, Yoshiaki SHIBAZAKI, Masaaki IZUMI, Kazuyuki HIRATSUKA, Toki SAKAI, Tohru KUROSAWA and Yasuhiro SHINDO

Toxicology Laboratory, Pharmaceutical Development Department, Meiji Seika Kaisha, Ltd., 760 Morooka-cho, Kohoku-ku, Yokohama 222-8567, Japan

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ABSTRACT — The administration of certain quinolone antibiotics has been associated with a prolongation of the QT interval on electrocardiogram, and in rare cases ventricular arrhythmias such as torsades de pointes. In this in vivo study using a rabbit arrhythmia model, we assessed the proarrhythmic effects and changes in the QT interval elicited by the administration of NM394 (UFX), an active metabolite of the new quinolone antibiotic prulifloxacin, and three representative quinolones, sparfloxacin (SPFX), gatifloxacin (GFLX) and levofloxacin (LVFX). Chloralose-anesthetized rabbits were co-administered a continuous infusion of methoxamine (15 µg/kg/min) together with NaOH (vehicle, 0.2 mol/L), SPFX (2, 3, 4 mg/kg/min), GFLX (4 mg/kg/min), LVFX (4 mg/kg/min) or UFX (4 mg/kg/min) via the ear vein, and then the effects on electrocardiogram were examined. SPFX and GFLX both prolonged the QT and QTc intervals. GFLX also induced premature ventricular contractions in all 6 rabbits that received it, and subsequently it induced torsades de pointes (TdP) in 3 of the 6 rabbits. SPFX infused at the dose of 4 mg/kg/min induced conduction blocks without inducing TdP, whereas that infused at the lower dose of 3 mg/kg/min induced both conduction blocks and TdP. The infusions with LVFX and UFX did not elicit remarkable prolongations in the QT interval, and none of the animals infused with the agents developed arrhythmia. These findings suggested that LVFX and UFX were less potent than SPFX and GFLX in prolonging the QT interval and inducing life-threatening arrhythmias.

KEY WORDS: Quinolones, QT interval, Torsades de pointes, Arrhythmia, Rabbit

INTRODUCTION

Quinolone antibiotics have a broad spectrum of antimicrobial activity and are widely prescribed in the treatment of infections. However, the administration of certain quinolone antibiotics has recently been linked with cardiac events, giving rise to clinical concerns over the cardiotoxicity of these agents. For instance, sparfloxacin (SPFX) and grepafloxacin (GPFX) have been shown to prolong QT interval on electrocardiogram (ECG) at clinical doses (Morganroth et al., 1999a, 1999b; Lipsky and Baker, 1999), as well as to induce torsades de pointes (TdP), a life-threatening ventricular arrhythmia (Dupont et al., 1996). The proarrhythmic potential of the latter, GPFX, has even warranted the agent’s withdrawal from the market. Further, concern over the proarrhythmic effects of many other quinolone antibiotics continues to grow. In non-clinical studies, the proarrhythmic effects of quinolone antibiotics used clinically have been assessed by measuring the duration of the action potential and other electrophysiological signs in vitro. However, in vitro studies using myocardium and cardiomyocyte are only of limited use in detecting the torsadogenic activities of drugs, and there have been gaps between clinically reported proarrhythmic effects and in vitro observations.

Several in vivo animal models have recently been developed to assess the proarrhythmic consequences of agents prescribed to prolong the QT interval. In one
widely accepted model using anesthetized rabbits (Carlsson et al., 1990), arrhythmias including TdP were evoked by co-administration of a compound possessing proarrhythmic properties together with the selective α₁-agonist methoxamine that increases intracellular calcium attributable to early after-depolarizations (Anderson et al., 2001; Carlsson et al., 1990, 1996). In the present study we used this anesthetized rabbit model to assess the proarrhythmic effects and changes in the QT interval elicited by the administration of NM394 (UFX), an active metabolite of the new quinolone antibiotic prulifloxacin (PUFX), and three representative quinolones, sparfloxacin (SPFX), gatifloxacin (GFLX) and levofloxacin (LVFX).

MATERIALS AND METHODS

Animals

Thirty-seven male New Zealand white rabbits (10-15 weeks old) were used in this study. The animals were obtained from Kitayama Labes Co., Ltd. (Nagano, Japan) and handled according to a protocol approved by the Ethical Committee for Animal Welfare at the Pharmaceutical Research Center of Meiji Seika Kaisha, Ltd., Tokyo, Japan.

Drugs

SPFX, GFLX and LVFX were extracted from the commercial products of Spara® (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), GATIFLO® (Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan), and Cravit® (Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan), respectively. UFX was synthesized at Nippon Shinyaku Co., Ltd. (Kyoto, Japan). The following drugs were purchased: methoxamine hydrochloride (Sigma Chemical Co., St. Louis, Mo, U.S.A.), α-chloralose (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and pentobarbital sodium (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan). The quinolones were dissolved in NaOH solution (0.2 mol/mL) at concentrations of 30, 45, and 60 mg/mL (SPFX, 30, 45 and 60 mg/mL; GFLX, LVFX, UFX, 60 mg/mL) for intravenous administration. All solutions were prepared fresh daily. In order to achieve a delivery rate of 4 mg/kg/min, a 60 mg/mL dose of the drug solution was infused at a speed of 4 mL/kg/hr. Vehicle (NaOH solution) was also infused at the same speed. Additional doses of 2 and 3 mg/kg/min SPFX were administered by infusing 30 and 45 mg/mL of the solution at the same speed, respectively.

Animal preparations

The proarrhythmic activity of quinolone antibiotics was examined using the method developed by Carlsson et al. (1990). Pentobarbital sodium (5 mg/kg, i.v.) and α-chloralose (100 mg/kg i.v. at an infusion volume of 10 mL/kg and rate of 1 mL/min) anesthetics were infused through catheters placed in the marginal veins of the ear. After tracheal cannulation, the animals were mechanically ventilated with room air at 7 mL/kg per stroke and 40 strokes/min. The catheter for recording blood pressure was inserted into the femoral artery. ECG (standard limb leads I and II) was taken with subcutaneous enamel wire electrodes and amplified by 1270A or 1804 preamplifiers (NEC San-ei Instruments, Ltd., Tokyo, Japan) with 1253A driver amplifiers (NEC San-ei Instruments, Ltd., Tokyo, Japan). ECG and blood pressure were recorded throughout the experiments by a digital tape recorder (PC208Ax, Sony Precision Technology Inc., Tokyo, Japan) and HEM 3.4 data acquisition system (NOTOCORD SYSTEMS, Croissy-sur-Seine, France).

Experimental protocol

Once the animals were prepared, methoxamine was continuously infused to each rabbit at an infusion dose of 15 µg/kg/min via the marginal vein of the right ear until the end of the experiment (infusion rate 2 mL/kg/hr). Ten min after the beginning of methoxamine infusion, SPFX, GFLX, LVFX, UFX or NaOH solution (0.2 mol/mL) was co-administered by intravenous infusion via the marginal vein of the left ear for 30 min (4 mg/kg/min infusion) or 60 min (2 or 3 mg/kg/min infusion). ECGs were continuously monitored on a monitor screen of the HEM system throughout the experiment. If TdP or sustained ventricular fibrillation (VF) occurred before termination of the administration period, the experiment was finalized at that time.

Data analysis and arrhythmia definition

ECGs recorded on digital tapes were analyzed for RR and QT intervals by a Softron ECG processor (Softron, Tokyo, Japan) at the following points: just before methoxamine infusion, 10 min after the start of methoxamine infusion, and every 5 min after the start of co-administration. The rate-corrected QT interval (QTc) was subsequently calculated by using the equation of Carlsson et al. (1993) (QTc(C)=QT-0.175(RR-300) (sec)) and Bazett’s formula (QTc(B)=QT/square root RR (msec)). The values of blood pressure were obtained by HEM at the same time points as ECGs. The data was not taken if the frequent recurrence of
arrhythmia made it difficult to measure RR and QT intervals and blood pressure.

The incidence of ventricular arrhythmias was obtained from the ECG waveform recorded by the HEM system. Premature ventricular contraction (PVC), bigeminy, salvo, ventricular tachycardia (VT) and ventricular fibrillation (VF) were defined in accordance with the definitions in the Lambeth Conventions (Walker et al., 1988). TdP was defined as an arrhythmia in which five or more repetitive PVCs were coupled and for which the QRS complex showed a cyclic variation in size and shape. Runs of four PVCs and runs of five or more PVCs without the torsade-like twisting QRS morphology were differentiated from TdP and defined as VT. Atrioventricular conduction blocks were also defined.

Statistical analysis
Data are presented as the mean ± S.E.M. except for the data from SPFX infusion (2 mg/kg/min). Changes from baseline (the data at 10 min after the start of methoxamine infusion) were statistically compared in the animals administered with each quinolone antibiotic and animals administered with NaOH alone. This approach was adopted to compensate for any effects of NaOH (vehicle) and time-dependent changes in the ECG interval. The comparisons of the quinolone infusion groups with the NaOH infusion group were analyzed using Dunnett’s test (Dunnett, 1964). The values of p<0.05 and p<0.01 were considered significant.

RESULTS
The effects on QT interval
Fig. 1 summarizes the time-course changes in RR, QT, QTc(B) and QTc(C) intervals after the administration of each quinolone antibiotic (4 mg/kg/min) and NaOH (vehicle) alone. The NaOH induced a remarkable prolongation in the interval, which in turn induced a less pronounced prolongation in the QT interval. The effects of UFX on RR and QT intervals were similar to those of NaOH (vehicle) and time-dependent changes in the ECG interval. The comparisons of the quinolone infusion groups with the NaOH infusion group were analyzed using Dunnett’s test (Dunnett, 1964). The values of p<0.05 and p<0.01 were considered significant.

Arrhythmia induction
Table 1 shows arrhythmias observed within 30 min after the start of co-administration of methoxamine with each test agent (4 mg/kg/min). No arrhythmia was observed in any of the animals administered with LVFX or UFX. Administration with SPFX induced atrioventricular conduction blocks in 4 animals, although PVC and other arrhythmias had been observed in 1 of those animals before the block. All of the animals administered with GFLX exhibited PVC, and half of them exhibited TdP (Fig. 3).

The effects on blood pressure
Fig. 4 shows the time-course changes in systolic blood pressure after the administration of each quinolone antibiotic (4 mg/kg/min) or NaOH (vehicle) alone. Systolic blood pressure decreased after the start of LVFX infusion.

The low rate infusion study of SPFX
Table 2 shows the arrhythmias that occurred during SPFX infusion (2 mg/kg/min or 3 mg/kg/min). SPFX infused at a rate of 2 mg/kg/min failed to induce arrhythmia in any animals (including TdP), whereas that infused at a rate of 3 mg/kg/min induced at least one type of arrhythmia in 4 out of 5 animals administered with the agent. TdP was detected in 1 animal that showed no evidence of atrioventricular conduction block.

The time-course changes in RR, QT, QTc(B) and QTc(C) intervals during SPFX infusion at the dose of 2 mg/kg/min were compared to those at 4 mg/kg/min (Fig. 5). The change in each interval at the dose of 2 mg/kg/min was similar to that at 4 mg/kg/min.
Fig. 1. Time course changes of RR, QT, QTc(B), and QTc(C) intervals during infusions with NaOH and quinolone antibiotics. The arrows indicate methoxamine and NaOH or quinolone antibiotics infusion. (Met: methoxamine, X: NaOH, SPFX, GFLX, LVFX or UFX). −10 min values and 0 min values indicate time points just before methoxamine infusion and just before X infusion, respectively. Values are mean ± S.E.M. NaOH (n=6), SPFX (n=5), GFLX (n=6), LVFX (n=6), UFX (n=6). The data of SPFX at 25 and 30 min and GFLX at 30 min are not expressed because the data in some animals could not be taken due to arrhythmia.

Fig. 2. Percent changes from 0 min value after NaOH or quinolone antibiotic infusion in the QT interval corrected for RR interval using Bazett’s formula or Carlsson’s formula. Values are mean ± S.E.M. NaOH (n=6), SPFX (n=5), GFLX (n=6), LVFX (n=6), UFX (n=6). The data of SPFX at 25 and 30 min and GFLX at 30 min are not expressed because the data in some animals could not be taken due to arrhythmia. *: p<0.05, **: p<0.01
pH values of the test agent solutions

Table 3 shows pH values of each test agent solution. The LVFX solution had a lower pH than the other solutions. NaOH had the highest pH among the test agents.

DISCUSSION

The purpose of this study was to assess the potential risk of cardiac event in the administration of UFX, an active metabolite of the new quinolone antibiotic.

![ECG traces showing typical changes](image)

**Fig. 3.** A typical change of ECG in an animal infused with methoxamine and GFLX. Panels are ordered chronologically and show ECG before co-administration of methoxamine and GFLX (A), QT prolongation at 17 min after the start of co-administration (B), and TdP induced at 22 min after the start of co-administration (C).

![SBP changes over time](image)

**Fig. 4.** Time course changes in systolic blood pressure (SBP) during NaOH or quinolone antibiotics infusion. The arrows indicate methoxamine and NaOH or quinolone antibiotics infusion. (Met: methoxamine, X: NaOH, SPFX, GFLX, LVFX or UFX). −10 min values and 0 min values indicate just before methoxamine infusion and X infusion, respectively. Values are mean ± S.E.M. NaOH (n=6), NM394 (n=6), SPFX (n=6), GFLX (n=6), LVFX (n=6). The data of SPFX at 25 and 30 min and GFLX at 30 min are not expressed because the data in some animals could not be taken due to arrhythmia.
PUFX, and three representative quinolones, SPFX, GFLX and LVFX, using the rabbit arrhythmia model. This model is a widely accepted method for testing the proarrhythmic and QT-interval-prolonging effects of drugs possessing a potency in blocking the delayed rectifier potassium current (I_{Kr}) (Buchanan et al., 1993; Carlsson et al., 1990). We found that LVFX and UFX had no significant effect on electrocardiographic parameters in this model, whereas GFLX and SPFX had a significant potential to prolong the QT interval and induce TdP and other forms of arrhythmia.

Both SPFX and GFLX significantly prolonged QT interval and induced arrhythmia, whereas LVFX and UFX showed almost no prolongation of the QT interval and induced no arrhythmia whatsoever. Earlier studies have suggested that SPFX blocks the human ether-a-go-go related gene (HERG) K^+ channel (Jiesheng et al., 2001), prolongs the action potential duration of the Purkinje fibers or ventricular myocardia (Adamantidis et al., 1998, Hagiwara et al., 2001, Patmore et al., 2000), and increases the QTc interval at clinical doses (Morganroth et al., 1999a, 1999b). Moreover, GFLX has also been reported to block the HERG channel (Jiesheng et al., 2001), prolong the action potential duration (Hagiwara et al., 2001), and prolong the QTc interval (Iannini and Circiumaru, 2001). In contrast, LVFX has been reported to be a less potent inhibitor of HERG than SPFX and GFLX (Jiesheng et al., 2001), and to scarcely prolong the action potential duration (Adamantidis et al., 1998, Hagiwara et al., 2001). Similarly, UFX has been suggested to elicit only slight inhibition of the HERG current (Lacroix et al., 2003). Given these earlier findings, we can speculate that the remarkable prolongation in the QT interval and the induction of arrhythmia by the SPFX and GFLX infusions in this study may have been related to the stronger efficacy of these two drugs in inhibiting the HERG current compared with LVFX and UFX.

Based on our estimations of the changes in the plasma concentrations of GFLX, LVFX and UFX induced by fixed-rate infusion on the basis of the transition after intravenous administration of each drug (Okuyama et al., 1996, Ooie et al., 1999, Destache et al., 2001), we concluded that the changes in the plasma concentrations of the three drugs were similar within the initial 30 min. Moreover, electrolyte abnormalities such as hypokalaemia are likely to prolong the QT interval and induce TdP. In this study, there was some concern that the balance of plasma electrolytes might have been altered by the solutions obtained by dissolving the test agents with high alkalinity in the vehicle of 0.2 mol/mL NaOH. However, we found that the vehicle, the most alkaline of all the test solutions used, did

| Incidence of arrhythmias in rabbits treated with NaOH or quinolone antibiotics. |
|--------------------------------|---------|---------|---------|---------|---------|---------|
| Arrhythmia                  | PVC  | bigeminy| salvo  | VT      | VF      | TdP     | block   |
| NaOH                        | 1/6  | 0/6     | 0/6    | 0/6     | 0/6     | 0/6     | 0/6     |
| SPFX                        | 1/6  | 0/6     | 1/6    | 1/6     | 0/6     | 0/6     | 0/6     |
| GFLX                        | 4/6  | 6/6     | 4/6    | 2/6     | 1/6     | 3/6     | 0/6     |
| LVFX                        | 0/6  | 0/6     | 0/6    | 0/6     | 0/6     | 0/6     | 0/6     |
| UFX                         | 0/6  | 0/6     | 0/6    | 0/6     | 0/6     | 0/6     | 0/6     |

The numerator is the number of animals with arrhythmia and the denominator is the number of animals studied with each drug. PVC=premature ventricular contraction, VT=ventricular tachycardia, VF=ventricular fibrillation, TdP=torsades de pointes, block=atrioventricular conduction block.

<table>
<thead>
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<th>Incidence of arrhythmias in rabbits treated with SPFX.</th>
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<tr>
<td>infusion rate</td>
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<td>2 mg/kg/min (30 mg/mL)</td>
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<td>3 mg/kg/min (45 mg/mL)</td>
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The numerator is the number of animals with arrhythmia and the denominator is the number of animals studied with each dose. PVC=premature ventricular contraction, VT=ventricular tachycardia, VF=ventricular fibrillation, TdP=torsades de pointes, block=atrioventricular conduction block, none=no arrhythmia.
Quinolone-induced arrhythmia in the rabbit model.

not notably prolong the QT interval, and induced only one type of arrhythmia, PVC, in only one animal once within the 30 min of observation. Thus, we could confirm that the high pH of the test solutions hardly influenced the prolongation of the QT interval or induction of proarrhythmic activity.

In the present study, the infusion of SPFX (4 mg/kg/min) elicited atrioventricular conduction blocks in many animals, whereas it induced severe arrhythmias such as VT in only one. This was not consistent with a report by Dupont et al. describing repeated episodes of TdP in patients administered with SPFX (1996). It was also somewhat discrepant with a study by Anderson et al. (2001), who found that SPFX induced TdP at infusion doses lower than the ones used in the present study, whereas GFLX did not. We suspected that these discrepancies between our data and those earlier findings were due to the infusion dose of the test agents. To certify whether this was true, we performed additional experiments with SPFX at the lower infusion doses of 2 mg/kg/min and 3 mg/kg/min. In the 2 animals administered with SPFX at the dose of 2 mg/kg/min, no arrhythmias were observed at any time point. At the dose of 3 mg/kg/min, 2 of 5 animals developed PVCs followed by either TdP or VF, whereas 3 of 5 animals exhibited atrioventricular conduction blocks. Thus, we concluded that the induction of atrioventricular conduction blocks had prevented the development of other

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**Fig. 5.** Time course changes in RR, QT, QTc(B) and QTc(C) intervals during SPFX infusion (2 mg/kg/min or 4 mg/kg/min). The arrows indicate methoxamine and SPFX infusion. (Met; methoxamine, X; NaOH, SPFX, GFLX, LVFX or UFX). The values at -10 min and 0 min indicate the values just before methoxamine infusion and SPFX infusion, respectively. Values in 4 mg/kg/min and 2 mg/kg/min are mean ± S.E.M. and mean, respectively. 2 mg/kg/min (n=2), 4 mg/kg/min (n=6). The data of SPFX (4 mg/kg/min) at 25 and 30 min are not expressed because the data in some animals could not be taken due to arrhythmia.

**Table 3.** pH values of NaOH and quinolone antibiotics solutions.

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<tr>
<th></th>
<th>NaOH</th>
<th>LVFX</th>
<th>SPFX</th>
<th>GFLX</th>
<th>UFX</th>
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<tr>
<td>pH</td>
<td>12.2 ± 0.1</td>
<td>8.4 ± 0.1</td>
<td>11.2 ± 0.0</td>
<td>11.4 ± 0.1</td>
<td>11.0 ± 0.1</td>
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Values are mean ± S.E.M. NaOH (n=6), SPFX (n=6), GFLX (n=6), LVFX (n=6), UFX (n=6).
severe arrhythmias in the animals administered with SPFX at the dose of 4 mg/kg/min, while the lower-dose infusion made it possible to detect other arrhythmias such as TdP.

SPFX at the dose of 2 mg/kg/min prolonged the QT interval without inducing arrhythmia, and the time course of the increase in the QT interval was similar to that at 4 mg/kg/min. This finding suggested that the increase in QT interval did not always have a direct relationship to the incidence of arrhythmia, although the prolongation of QT interval increased the risk of arrhythmia induction.

The mean maximal plasma concentration (Cmax) of each quinolone tested in this study was 1.5-3.0 µg/mL, when given orally at a clinically effective dose. The plasma concentrations of quinolone at the point when the arrhythmias emerged were considered to be 15-30 times higher than the Cmax values for the agents tested. Moreover, adrenergic stimulation was given together with quinolone administration in this model to increase the free cytosolic calcium level, a factor that has been shown to be important in the induction of early after-depolarizations and triggered activities (Carlsson et al., 1996). Therefore, SPFX and GFLX were not always considered to prolong QT interval or induce arrhythmias in clinical use. However, in clinical studies comparing the incidences of TdP associated with quinolones, the incidence of TdP induced by LVFX was lower than that induced by GFLX (Frothingham, 2001).

RR interval was less prolonged in the animals administered with LVFX than in the animals with NaOH or other quinolones. LVFX inhibited hypertension induced by methoxamine infusion. Therefore, LVFX was considered to inhibit baroreflex-mediated bradycardia. Many compounds with quinolone structure possess a vasodilator or hypotensive action (Paton and Reeves, 1991). In the hypotensive action of quinolones, α1-adrenoceptor-blocking activity and histamine liberation are partly involved (Ito et al., 1993). LVFX was observed to induce hypotension in dogs, but had no effect on the pressor response to norepinephrine at 20 mg/kg or lower (Takasuna et al., 1992). However, the LVFX dose in this study was more than that in Takasuna’s study. In this study, the inhibition of α1-adrenoceptors may be partly concerned with LVFX-induced hypotension. Moreover, inhibition of calcium movements mediated by α1-adrenoceptors may be partly related to low proarrrhythmic potential of LVFX.

In conclusion, the present study demonstrates that LVFX and UFX have lower proarrrhythmic poten-

tials than SPFX and GFLX. In addition, this anesthetized rabbit model showing the tendency of quinolone antibiotics to induce arrhythmia may also be useful for detecting the proarrrhythmic potential of those drugs.

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REFERENCES


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