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European Journal of Pharmacology 502 (2004) 117–122

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Comparison of sensitivity of surrogate markers of drug-induced torsades de pointes in canine hearts

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Received 11 June 2004; received in revised form 16 August 2004; accepted 20 August 2004
Available online 13 September 2004

Abstract

Given a limited information regarding the difference of the sensitivity of surrogate markers of drug-induced torsades de pointes, including early afterdepolarization, ectopic beats, phase 3 repolarization and dispersion of ventricular repolarization, we simultaneously analyzed them in the halothane-anesthetized canine model (n=5). A non-specific I_{Kr} channel blocker sparfloxacin, which has been known to induce torsades de pointes in animals and clinical patients, prolonged the repolarization process in a dose-related and reverse use-dependent manner. No significant change was detected in any of the proarrhythmic markers except for the backward parallel shift of phase 3 repolarization in the cardiac cycle with the QT interval prolongation, which would be the most sensitive marker in predicting the potential arrhythmogenic property of sparfloxacin in the "non-remodeled" normal heart.

Keywords: Fluoroquinolone; Interventricular dispersion of repolarization; Electrically vulnerable period; Phase 3 repolarization

1. Introduction

Non-cardiovascular drug-induced prolongation of the QT interval is often associated with the onset of torsades de pointes resulting in a life-threatening ventricular arrhythmia (Belardinelli et al., 2003; Tamargo, 2000). Several electrophysiological changes are known to be closely associated with the development of torsades de pointes (Belardinelli et al., 2003; Tamargo, 2000). For example, a drug that prolongs action potential duration induces early afterdepolarizations and ectopic beats, prolongs phase 3 repolarization and increases dispersion of ventricular repolarization is likely to cause torsades de pointes (Kirchhof et al., 1998; Satoh et al., 1999; Sugiyama and Hashimoto, 2002; Sugiyama et al., 2003; Van Opstal et al., 2001; Verduyn et al., 1997; Vos et al., 1998; Yoshida et al., 2002). Although the exact relationship between these electrophysiological events and the development of torsades de pointes has not been fully defined, the potential of a drug to elicit these events might predict its proarrhythmic risk (Belardinelli et al., 2003; Tamargo, 2000).

The purpose of this study was to compare the sensitivity of these proarrhythmic markers simultaneously using the in vivo animal model, since previous data were obtained from studies that varied in species, gender, diseased versus non-diseased state and experimental conditions (Belardinelli et al., 2003; Tamargo, 2000). We selected a fluoroquinolone antibacterial agent sparfloxacin as a typical proarrhythmic compound, which was reported to prolong the QT interval and to cause the lethal ventricular arrhythmias, including torsades de pointes, in both experimental and clinical in vivo studies (Anderson et al., 2001; Chiba et al., 2000, 2004; Demolis et al., 1996; Dupont et al., 1996; Satoh et al., 2000). Moreover, in vitro studies have indicated that sparfloxacin markedly prolongs the action potential duration of the isolated guinea pig ventricular myocytes (Hagiwara et al., 2001), and inhibits human ether-à-go-go-related gene

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doi:10.1016/j.ejphar.2004.08.035

(HERG)-mediated K^+ currents (Anderson et al., 2001; Kang et al., 2001).

We propose that current study will provide an important information for researchers particularly in the field of Safety Pharmacology to better understand the sensitivity of multiple surrogate markers of proarrhythmia, since they are now using such experimental animal models that possess "non-remodeled" normal heart to evaluate the potential for delayed ventricular repolarization by human pharmaceuticals according to a recently announced ICH S7B guideline (The ICH Steering Committee, 2004).

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. Cardiohemodynamic and electrophysiological parameters

Experiments were carried out using female beagle dogs weighing 8–11 kg (n=5). The 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 systemic blood pressure was continuously monitored at the right femoral artery. The surface lead II electrocardiogram (ECG) was obtained from the limb electrodes. A bi-directional steerable monophasic action potential (MAP) recording/pacing combination catheter (EP Technologies, 1675P, Sunnyvale, CA, USA) was placed in the right ventricle through the right femoral vein, whereas another MAP recording catheter was positioned in the left ventricle through the left femoral artery. We estimated the extent of the interventricular dispersion of repolarization by measuring the MAP duration of both ventricles based on previous reports (Van Opstal et al., 2001; Verduyn et al., 1997; Vos et al., 2000). The MAP signal was amplified with a DC preamplifier (EP Technologies, Model 300) and its 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 values of MAP_{90} of both ventricles were measured during the sinus rhythm ($MAP_{90(sinus)}$) and at pacing cycle lengths of 500 ms ($MAP_{90(CL500)}$), 400 ms ($MAP_{90(CL400)}$) and 300 ms ($MAP_{90(CL300)}$) to get rid of the influences of the heart rate variation. The extent of interventricular dispersion of repolarization was calculated at each pacing cycle length besides during the sinus rhythm using the following equation: interventricular dispersion of repolarization=left ventricular MAP_{90}−right ventricular MAP_{90}.

The effective refractory period of the right ventricle was measured 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 duration of phase 3 repolarization of action potential, which would reflect the extent of electrical vulnerability of the ventricular muscles (Kirchhof et al., 1998; Satoh et al., 1999; Sugiyama and Hashimoto, 2002).

2.2. Experimental protocol

The systemic blood pressure, ECG and MAP signals were continuously monitored using a polygraph system (Nihon Kohden, RMP-6018M), and analyzed with an ECG processor (SBP-8, Softron, Tokyo, Japan). Corrected QT interval (QTc) was obtained using Bazett's formula (Bazett, 1920). The values of MAP_{90} of both ventricles and ECG parameters represent the mean of three consecutive complexes at each time point of assessment. After control assessment, 3 mg/kg of sparfloxacin, which can provide clinically relevant antibiotic plasma concentration of 1–2 μg/ml (Chiba et al., 2000; Satoh et al., 2000), was administered over 10 min into the right femoral vein and each cardiovascular variable was recorded at 5, 10, 20 and 30 min after the start of infusion. Then, supratherapeutic dose of 10 mg/kg of sparfloxacin was additionally administered over 10 min and each variable was recorded in the same manner.

2.3. Drugs

Sparfloxacin was extracted from the commercial source (Spara™, Dainippon Pharmaceuticals, Tokyo, Japan), which was dissolved with 1% lactate solution in concentrations of 2.0 and 6.7 mg/ml as previously described (Chiba et al., 2000). The solutions were prepared freshly for each experiment. The following drugs were purchased: halothane (Takeda Chemical Industries, Tokyo, Japan), heparin sodium (Shimizu Pharmaceuticals, Shizuoka, Japan) and thiopental sodium (Tanabe Seiyaku, Osaka, Japan).

2.4. Statistical analyses

Data are presented as the mean±S.E.M. The statistical differences within a parameter were evaluated by one-way

repeated-measures analysis of variance (ANOVA) followed by contrasts for mean values comparison. A P-value less than 0.05 was considered significant.

3. Results

The time courses of the changes in the heart rate, mean blood pressure, QT interval, QTc, effective refractory period and terminal repolarization period are summarized in Fig. 1A (n=5). Meanwhile, those of the MAP_{90} of both ventricles and interventricular dispersion of repolarization are shown in Fig. 1B (n=5). Typical tracings of the ECG and MAP of both ventricles during the sinus rhythm are depicted in Fig. 2. Neither ventricular arrhythmia nor early afterdepolarization was observed during the study.

Intravenous administration of 3 mg/kg of sparfloxacin hardly affected any of the heart rate, mean blood pressure, terminal repolarization period or interventricular dispersion of repolarization, whereas it prolonged the QT interval, QTc, effective refractory period and MAP_{90} of both ventricles, as shown in Fig. 1 (closed symbols; P<0.05 versus pre-drug control). Additional administration of 10 mg/kg of sparfloxacin decreased the heart rate in addition to the potentiation of those observed by the low dose, whereas no significant change was detected in any of the mean blood pressure, terminal repolarization period or interventricular dispersion of repolarization.

The increment of MAP_{90} of both ventricles was calculated for each pacing cycle length to analyze the use-dependent prolongation of repolarization. The extent of the drug-induced prolongation of the MAP_{90} was more prominent at slower heart rate for both ventricles. In the right ventricle, such reverse use-dependency was detected at 10 min after the low dose and for 5–10 min after the high dose, whereas in the left ventricle, it was observed from 10 min after the low dose to the end of the experiment (data not shown in the figure).

After the pharmacological assessment, the animals were sacrificed to confirm the location of the tip of each catheter. One catheter was placed at the endocardium of the interventricular septum of the right ventricle, whereas the

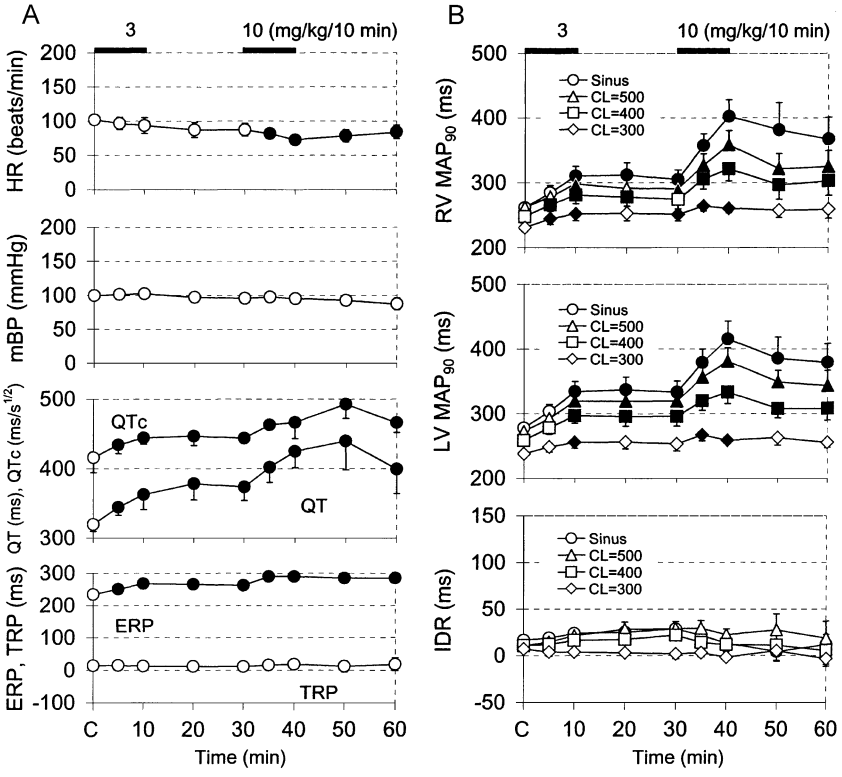

Fig. 1. Time courses of the cardiovascular effects of sparfloxacin (n=5). (A) Time courses of the heart rate (HR), mean blood pressure (mBP), QT interval (QT), QTc, effective refractory period (ERP) and terminal repolarization period (TRP=$MAP_{90(CL400)}$−ERP) of the right ventricle. (B) Time courses of the MAP_{90} of the right ventricle (RV MAP_{90}) and left ventricle (LV MAP_{90}) and interventricular dispersion of repolarization (IDR=LV MAP_{90}−RV MAP_{90}) during the sinus rhythm and ventricular pacing. MAP_{90} represents the duration of the monophasic action potential at 90% repolarization level. $MAP_{90(sinus)}$: MAP_{90} during the sinus rhythm. $MAP_{90(CL500)}$, $MAP_{90(CL400)}$ and $MAP_{90(CL300)}$: MAP_{90} at pacing cycle lengths of 500, 400 and 300 ms, respectively. Sparfloxacin was intravenously infused 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. CL=pacing cycle length.

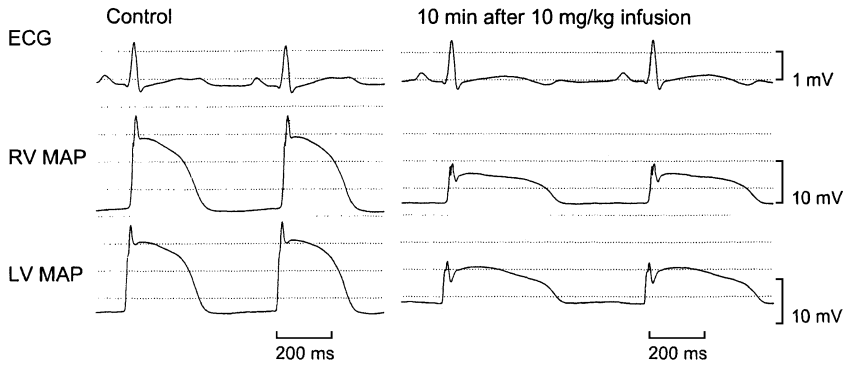

Fig. 2. Typical tracings of lead II surface ECG (ECG) and monophasic action potentials (MAP) of the right ventricle (RV MAP) and left ventricle (LV MAP) during the sinus rhythm at pre-drug control (Control) and 10 min after the start of 10 mg/kg of sparfloxacin infusion (10 min after 10 mg/kg infusion). Marked prolongation of QT interval as well as MAP duration was observed, but no significant difference was observed between the left and right ventricles in the extent of prolongation of MAP_{90}. In addition, it is difficult to realize if the drug produced any triangulation of the action potential morphology because of the reduced amplitude of the signals in the presence of sparfloxacin, suggesting that the triangulation of the action potential morphology may be less sensitive.

other was positioned at the free wall of the left ventricle (n=5).

4. Discussion

Given a limited information regarding the difference of the sensitivity of surrogate markers of drug-induced torsades de pointes, including early afterdepolarization, ectopic beats, phase 3 repolarization and dispersion of ventricular repolarization, we simultaneously analyzed them using the well-established halothane-anesthetized canine model (Chiba et al., 2000, 2004; Satoh et al., 1999, 2000; Sugiyama et al., 2003).

A non-specific I_{Kr} channel blocker, fluoroquinolone antibacterial agent sparfloxacin at supra-therapeutic dose decreased the heart rate, whereas no significant change was detected in the mean blood pressure during the study. On the other hand, sparfloxacin prolonged the QT/QTc interval as well as the MAP duration in a dose-related manner. The extent of prolongation was more prominent at slower pacing rate in both ventricles, indicating that sparfloxacin can delay the repolarization process in a reverse use-dependent manner, which may reflect characteristics of in vitro I_{Kr} channel blocking property of the drug (Anderson et al., 2001; Kang et al., 2001). Therefore, the negative chronotropic action of sparfloxacin may further potentiate the prolongation of QT interval in the in situ heart. These electropharmacological profiles of sparfloxacin were in good accordance with our previous studies (Chiba et al., 2000; Satoh et al., 2000), indicating reliability and reproducibility of our experimental system.

It is well known that occurrence of torsades de pointes arrhythmias has been related to an increased heterogeneity of repolarization (dispersion) which can be located between the ventricles (Antzelevitch and Shimizu, 2002; Belardinelli et al., 2003; Van Opstal et al., 2001; Verduyn et al., 1997; Vos et al., 1998). Contrary to our expectation, in this study, no significant change was detected in the interventricular dispersion of repolarization after the sparfloxacin infusion in spite of the dramatic prolongation of the QT interval and MAP duration. It has been proposed that interventricular dispersion of repolarization can become more pronounced during bradycardia, rate changes caused by ectopic beats and/or under pathological conditions such as hypertrophy (Antzelevitch and Shimizu, 2002; Belardinelli et al., 2003; Van Opstal et al., 2001; Verduyn et al., 1997; Vos et al., 1998, 2000), which were absent in the currently used halothane-anesthetized model, having made the interventricular dispersion of repolarization less sensitive marker.

Recently, it was reported that prolongation of phase 3 repolarization could generate early afterdepolarizations by spending too much time in the window voltage for calcium channel reactivation (Hondeghem et al., 2001). The reduction of I_{Kr} will prolong this critical time window so that more calcium ion can enter the cell and lead to early afterdepolarizations (Milberg et al., 2002). In the present study, we measured both MAP and effective refractory period at the same site of the right ventricle and directly compared the drug effects on the repolarization and refractoriness to estimate the duration of the phase 3 repolarization. Sparfloxacin prolonged MAP_{90} and effective refractory period to a similar extent, thus shifting the phase 3 repolarization backward in the cardiac cycle without changing the duration of the electrically vulnerable time window itself (Sugiyama and Hashimoto, 2002); therefore, no early afterdepolarization would have been induced, either.

It is well known that impulses that reach the ventricles during the middle and terminal portions of the T wave can initiate the ventricular tachycardias and fibrillation, since the repolarization is most heterogeneous and Na^+ channels are in different phases of recovery in this phase (Sugiyama and Hashimoto, 2002). In the halothane-anesthetized canine model, the extent of such electrical vulnerability can be estimated by the terminal repolarization period, and drug-induced prolongation and backward shift of the terminal repolarization period have been known to increase the

potential for slow conduction and reentry that allows perpetuation of torsades de pointes (Chiba et al., 2000, 2004; Satoh et al., 1999, 2000; Usui et al., 1998). Thus, sparfloxacin-induced backward parallel shift of phase 3 repolarization by itself may become a proarrhythmic substrate, since it increases the chance of "R on T" phenomenon resulting in the development of torsades de pointes arrhythmias, as previously suggested (Chiba et al., 2000; Kirchhof et al., 1998; Satoh et al., 1999; Sugiyama and Hashimoto, 2002). Similar results were obtained in our previous experiment using the same experimental system with clinically relevant dose of 3 mg/kg of terfenadine (Usui et al., 1998), suggesting that the hypothesis could be extrapolated to other QT prolonging drugs.

There are some study limitations in this study. We randomly placed two MAP catheters, one in the left and the other in the right ventricle, to provide local information about the differences in repolarization between the two ventricles, which might have decreased its sensitivity. Transient beat-by-beat instability of MAP duration was observed at both ventricles in one dog during the fastest pacing rate after the administration of the high dose. However, this aspect was not assessed in this study because of the limited number of animals that can be used for its assessment. Steady-state plasma concentrations were not obtained in this study. Differences in accumulated tissue concentrations after acute i.v. versus chronic oral administration would likely exist.

In conclusion, backward parallel shift of phase 3 repolarization in the cardiac cycle with QT interval prolongation may be more sensitive marker in predicting the potential arrhythmogenic property of sparfloxacin in the halothane-anesthetized dog. Meanwhile, the increment of interventricular dispersion of repolarization or electrical vulnerable period, or demonstration of early afterdepolariozation in MAP might have relatively minor roles in estimating the occurrence of drug-induced torsades de pointes arrhythmias in the "non-remodeled" normal heart, which may also indicate the importance of pathological proarrhythmic models in predicting risks in humans during the nonclinical evaluation of new drugs.

Acknowledgments

This study was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (No. 15590222), and that from Yamanashi Research Center of Clinical Pharmacology.

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