

—Original—

Usefulness of simultaneous and sequential monitoring of glucose level and electrocardiogram in monkeys treated with gatifloxacin under conscious and nonrestricted conditions

Yu YOSHIMATSU, Tomomichi ISHIZAKA, Katsuyoshi CHIBA, and Kazuhiko MORI

Medicinal Safety Research Laboratories, Daiichi Sankyo Co., Ltd., 1-16-13 Kita-Kasai, Edogawa-ku, Tokyo 134-8630, Japan

Abstract: Drug-induced cardiac electrophysiological abnormalities accompanied by hypoglycemia or hyperglycemia increase the risk for life-threatening arrhythmia. To assess the drug-induced cardiotoxic potential associated with extraordinary blood glucose (GLU) levels, the effect of gatifloxacin (GFLX) which was frequently associated with GLU abnormality and QT/QTc prolongations in the clinic on blood GLU and electrocardiogram (ECG) parameters was investigated in cynomolgus monkeys (n=4) given GFLX orally in an ascending dose regimen (10, 30, 60 and 100 mg/kg). Simultaneous and sequential GLU and ECG monitoring with a continuous GLU monitoring system and Holter ECG, respectively, were conducted for 24 h under free-moving conditions. Consequently, GFLX at 30 and 60 mg/kg dose-dependently induced a transient decrease in GLU without any ECG abnormality 2–4 h postdose. Highest dose of 100 mg/kg caused severe hypoglycemia with a mean GLU of <30 mg/dL, accompanied by remarkable QT/QTc prolongations by 20–30% in all animals. In contrast, hyperglycemia without QT/QTc prolongations was noted 24 h after dosing in one animal. A close correlation between GLU and QTc values was observed in animals treated with 100 mg/kg, suggesting that GFLX-induced hypoglycemia enhanced QT/QTc prolongations. Furthermore, the 24-h sequential GLU monitoring data clearly distinguished between GFLX-induced GLU abnormality and physiological GLU changes influenced by feeding throughout the day. In conclusion, the combined assessment of continuous GLU and ECG monitoring is valuable in predicting the drug-induced cardio-electrophysiological risk associated with both GLU and ECG abnormalities.

Key words: free-moving, gatifloxacin, hypoglycemia, monkeys, QT prolongation

Introduction

Glucose (GLU) abnormalities including hypoglycemia and hyperglycemia potentiate the prolongation of monophasic action potential duration and I_{Kr} (the rapid component of the delayed rectifier potassium current) -block-

ing properties of class III antiarrhythmic drugs [13]. Diabetes mellitus lengthens ventricular repolarization and attenuates the repolarization reserve in mammalian heart [23]. Especially, hypoglycemia is frequently associated with QT interval prolongation, which increases the risk for life-threatening arrhythmia [5, 19, 24]. There-

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Address corresponding: Y. Yoshimatsu, Medicinal Safety Research Laboratories, Daiichi Sankyo Co., Ltd., 1-16-13 Kita-Kasai, Edogawa-ku, Tokyo 134-8630, Japan



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fore, a well-designed toxicological testing incorporating both GLU and electrocardiogram (ECG) monitoring is essential to assess the cardiotoxic potential of drug candidates associated with GLU abnormalities before its clinical use. However, limited data are available on the relationship between blood GLU and ECG parameters such as QT interval in animal models.

Gatifloxacin (GFLX), a fluoroquinolone antibacterial agent [8, 12, 14, 27, 31], was well known to be frequently associated with GLU abnormality and QT prolongation in the clinic. For example, oral administration of GFLX induced a high incidence of unexpected life-threatening GLU reactions including hypoglycemia and hyperglycemia in humans [11, 26]. In addition, GFLX is suggested to have the potential for inducing QT/QTc prolongation, which might induce ventricular arrhythmias such as torsades de pointes [1, 3]. Finally, GFLX was withdrawn voluntarily from the U.S. and Japanese markets due to disturbed blood glucose homeostasis.

Blood GLU is regulated by several endogenous substances such as insulin and glucagon, and importantly is altered depending on the feeding or fasting condition. In addition, the response of blood GLU to GFLX is reported to be different between normal and diabetic rats [15]. Thus, it is critical that investigators consider the factors contributing to the variability of GLU levels when interpreting the results of toxicology studies. The utility of the continuous GLU monitoring system (CGMS) for evaluating detailed daily GLU profiles in nonhuman primates has been confirmed [16]. Therefore, we hypothesized that this method can distinguish between drug-induced GLU abnormality and physiological GLU changes influenced by several factors, including feeding throughout the day, in the toxicology testing in a nonhuman primate.

In the present study, to clarify the relationships between drug-induced GLU abnormalities and cardiotoxicity, simultaneous and sequential GLU and surface ECG monitoring with the CGMS and Holter ECG, respectively, were conducted for 24 h in cynomolgus monkeys receiving GFLX under free-moving conditions, which can exclude artifacts such as the need to hold the animals for blood sampling.

Materials and Methods

Drugs

GFLX was purchased from LKT Laboratories Inc. (St.

Paul, MN, USA). GFLX was suspended in 1% methylcellulose solution (Nacalai Tesque Inc., Kyoto, Japan).

Animals

Cynomolgus monkeys (*Macaca fascicularis*) were obtained from Hamri Co., Ltd. (Tokyo, Japan). A total of four monkeys of either sex (n=2/sex) weighing approximately 2.9–5.0 kg at ages 5–7 years were used in this study. The animals were housed individually in a stainless-steel cage (width 594 mm × depth 870 mm × height 1,015 mm) throughout the study period under the following environmental conditions: room temperature, 24°C; relative humidity, 60%; illumination, 150–300 luxes; lighting, 12-h light (7:00 to 19:00) and ventilation, 10–15 air changes/h. The animals were fed 100 g commercial pellet for monkeys (PS; Oriental Yeast Co., Ltd., Tokyo, Japan) once a day in the morning after observation of their clinical signs or 4 h after each dose.

Experimental protocol

The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Daiichi Sankyo Co., Ltd. (Tokyo, Japan). All experimental procedures were performed in accordance with the Guidelines for Animal Experimentation issued by the Japanese Association for Laboratory Animal Science [18]. In rats, hypoglycemia or hyperglycemia was induced through the intravenous administration of GFLX at doses of 25 and 50 mg/kg or a dose of 100 mg/kg, respectively [15]. In clinical trials of GFLX, a transient decrease in blood GLU level was observed in healthy subjects receiving GFLX intravenously at doses of ≥600 mg or in patients with type 2 diabetes receiving GFLX orally at a dose of 400 mg [9, 10]. These human doses correspond to ≥31.0 or 20.7 mg/kg, respectively, in monkeys on the basis of body surface area basis [6]. Based on these data, the highest dose of GFLX in the present study was set at 100 mg/kg which expected to induce blood GLU abnormality. The other dose levels of GFLX were set at 0 (vehicle control) and at 10, 30 and 60 mg/kg to confirm its dose dependency. The animals were orally administered vehicle (0 mg/kg) and four dose levels of GFLX in an ascending dose regimen (10, 30, 60 and 100 mg/kg) at a 1-week interval. All doses were conducted between 10:00 and 10:20, and animals were fed pellet food 4 h after each dose throughout the study. Continuous GLU and ECG monitoring with the CGMS and Holter ECG devices, respectively, were conducted

as previously reported [16, 17]. The time course data of GLU and ECG parameters were obtained at each dose in accordance with the following processes. After 24-h acclimation to wearing primate jacket, the glucose sensor was inserted in the subcutis around the waistline of each animal using introducer needles after the insertion site was shaved and disinfected with 50% isopropanol. The glucose monitor and a Holter monitoring recorder were located in the pocket at the back of the jacket. After 60-min initialization to stabilize the GLU sensor (MiniMed Glucose Sensor: MMT-7002; Medtronic MiniMed Inc., Minneapolis, MN, USA) inserted in subcutaneous tissue, the electronic signals obtained by the GLU sensor (nanoampere readings) responding to subcutaneous GLU concentration were continuously sent into a Holter-style GLU monitor (MiniMed Continuous Glucose Monitor: MMT-7102; Medtronic MiniMed Inc.) connected to the sensor. The GLU monitor sampled the signals at 10-sec intervals and recorded an average signal every 5 min. The data were obtained from the day before each dosing to 24 h after dosing. For the ECG monitoring, the end of the limb electrode was attached to each animal and one end was connected to a Holter monitoring recorder (Digital Quick Corder QR2100; Fukuda M-E Kogyo Co., Ltd., Tokyo, Japan). ECG waveforms were monitored for the same duration as the aforementioned data acquisition period of GLU. The animals kept wearing primate jackets to prevent the animals from removing the sensors and electrodes. The monitoring devices were removed after the completion of 24-h monitoring for each dose.

Monitoring and analysis of GLU with CGMS

The obtained data were analyzed by using an analysis software (Solutions CGMS Sensor Software, version 3.0A; Medtronic MiniMed Inc.). The average of two blood GLU measurements obtained with a blood GLU measuring device (Medisafe Reader GR-101; Terumo Corporation, Tokyo, Japan) was entered immediately into the GLU monitor to perform real-time calibration of the GLU sensor. The GLU values were measured and entered once on the day before dosing; at 1.5 h before dosing; at 1, 2, 4 and 6 h after dosing on the day of dosing; and three times at a 1 h interval on the day after dosing. The data stored in the GLU monitor were downloaded to the analysis software (Solutions CGMS Sensor Software). Continuous GLU levels were calculated automatically from the relation between the input GLU

values and the nanoampere readings from the sensor [28].

Monitoring and analysis of ECG with Holter ECG

ECG signals were recorded continuously from 1 h before dosing to 24 h after dosing by the surface ECG (NASA and CC5 leads) obtained with a Holter's electrocardiograph attached to the animals. The ECG components (PR interval, QRS width and QT interval) were analyzed with an ECG analysis software (HS1000VL; Fukuda M-E Kogyo Co., Ltd.) based on the stable ECG waveform obtained around 0.5 h before dosing and at 1, 2, 4, 6, 12 and 24 h after dosing. At each scheduled measurement time, 10 consecutive ECG waves were analyzed. The rate-correlated QT interval was calculated with Bazett's formula [$QTcB = QT / (60 / \text{heart rate})^{1/2}$] [2]. Bazett's formula was employed because it was used by the Japan Pharmaceutical Manufacturers Association for database construction of *in vivo* QT assays in cynomolgus monkeys [21] and was reported to be relatively adequate for *in vivo* QT assay for this species [25].

Toxicokinetics

Blood (0.4 ml) was drawn from the femoral vein under conscious condition before dosing, 1, 2, 4, 6 and 24 h after dosing and placed into blood sampling tubes containing lithium heparin (MICROTAINER; Nippon Becton Dickinson and Co., Ltd., Franklin Lakes, NJ, USA). This blood sampling procedure did not affect plasma glucose levels, but did ECG measurement. Therefore, blood sampling under the restraint of the animals was conducted after ECG measurements. The samples were centrifuged at 10,000 rpm and 4°C for 5 min (Microfuge 22R; Beckman Coulter Inc., San Diego, CA, USA) to obtain plasma. Plasma GFLX concentration was measured by using liquid chromatography/mass spectrometry/mass spectrometry, and the C_{\max} ($\mu\text{g/ml}$), t_{\max} (h) and $AUC_{0-24\text{h}}$ ($\mu\text{g}\cdot\text{h/ml}$) were calculated.

Statistical analysis

Quantitative data are expressed as the mean and SE of four animals obtained by using a calculation software (Microsoft Office Excel 2003; Microsoft Corporation, Redmond, WA, USA). For statistical analysis among multiple groups, the parameters were statistically analyzed by using Dunnett's multiple comparison test. The relationship among GLU, QTc and GFLX concentrations

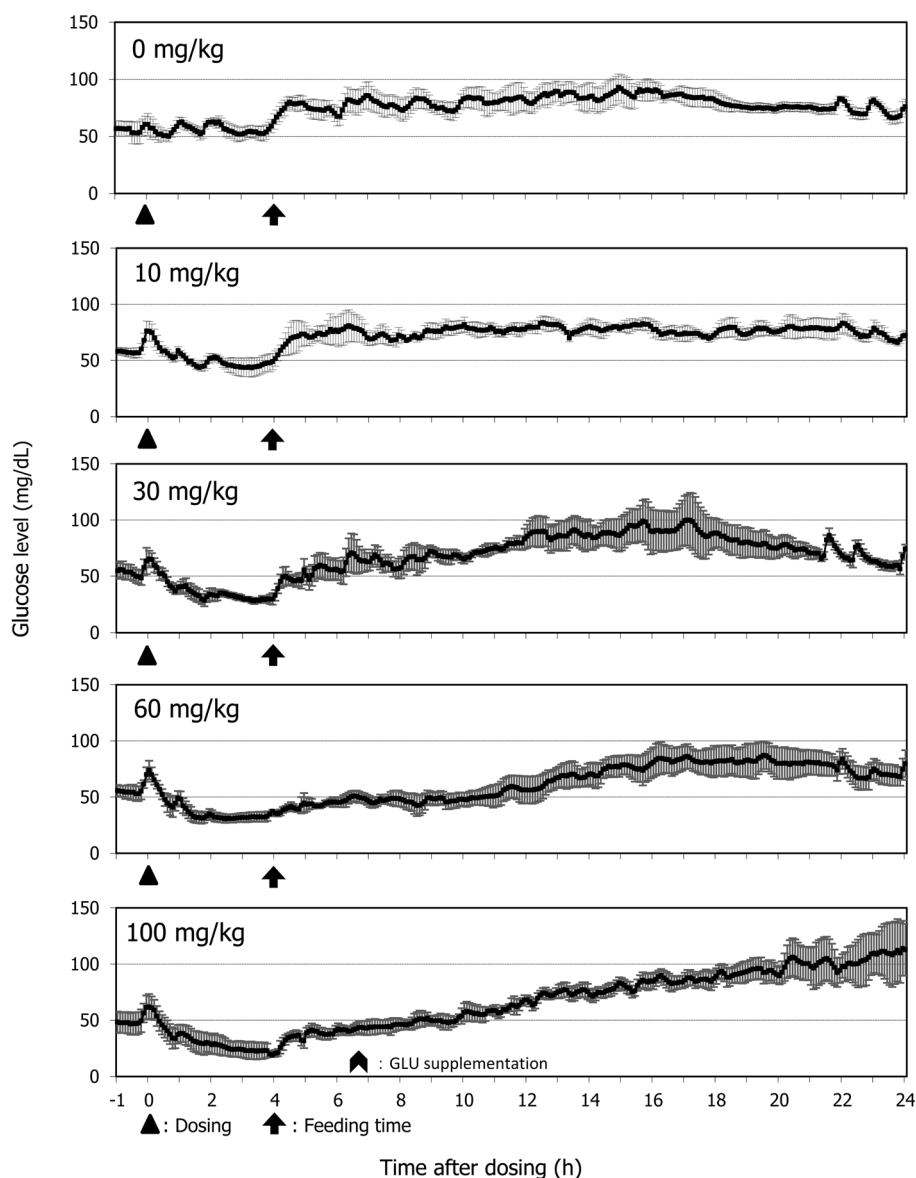


Fig. 1. Tracing of the 24-h continuous monitoring data for plasma glucose levels in monkeys. Animals were treated with a single oral dose of the vehicle (0 mg/kg) or gatifloxacin at 10, 30, 60 or 100 mg/kg. Data are presented as mean \pm SE (n=4) for each group. GLU: glucose.

was evaluated statistically through Pearson's correlation coefficient analysis. These statistical analyses were performed with SAS System release 8.2 (SAS Institute Japan Ltd., Tokyo, Japan). A P value $< 5\%$ was considered statistically significant.

Results

Effects on GLU levels

No statistically significant difference in the respective

predose control GLU values was noted among all the dosing days. Tracing of the 24-h continuous monitoring data for plasma GLU levels in each group is shown in Fig. 1. GLU was significantly decreased at 4 h in animals treated with GFLX at 30 mg/kg compared with that in the vehicle-treated animals (Fig. 2). Similar changes were observed in animals treated with GFLX at 60 mg/kg; however, this was not statistically significant (Fig. 2). Then, GLU returned to approximately predose values after feeding and these hypoglycemic effects disappeared

Table 1. Plasma concentrations and toxicokinetic parameters of gatifloxacin after a single oral dosing to monkeys

Dose (mg/kg)	Quantitative value ($\mu\text{g/mL}$)					TK parameter		
	Time after dosing (h)					C_{max} (mg/mL)	t_{max} (hr)	$\text{AUC}_{0-24\text{h}}$ (mg·hr/mL)
	1	2	4	6	24			
10	2.90 \pm 0.21	3.71 \pm 0.20	2.81 \pm 0.16	1.75 \pm 0.15	BLQ	3.71 \pm 0.20	2.00 \pm 0.00	31.55 \pm 1.60
30	4.56 \pm 2.35	7.82 \pm 3.34	8.72 \pm 1.90	7.43 \pm 0.43	0.84 \pm 0.08	11.00 \pm 1.70	3.50 \pm 1.00	110.70 \pm 12.80
60	6.79 \pm 5.10	13.75 \pm 12.30	14.62 \pm 10.30	11.02 \pm 7.20	1.27 \pm 0.40	16.64 \pm 12.31	2.75 \pm 1.50	175.43 \pm 116.65
100	15.10 \pm 9.00	25.44 \pm 15.40	33.38 \pm 13.70	29.18 \pm 7.00	4.02 \pm 1.30	34.63 \pm 11.58	4.50 \pm 1.00	448.00 \pm 123.03

Data are the mean \pm SE (n=4) for each group. BLQ: below the lower limit of quantification ($<0.5 \mu\text{g/ml}$).

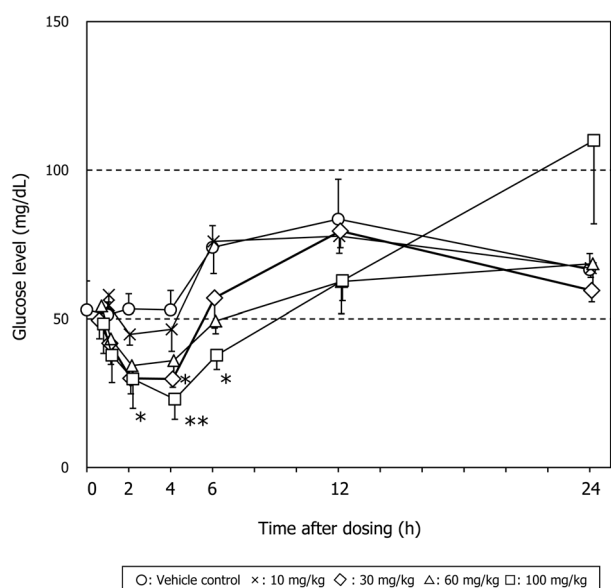


Fig. 2. Effect of gatifloxacin on plasma glucose levels in monkeys. Data are presented as mean \pm SE (n=4) for each group. * $P<0.05$, ** $P<0.01$: significantly different from the vehicle control group.

by 6 h postdosing (Fig. 2). In animals treated with GFLX at 100 mg/kg, GLU was further decreased from 2 to 4 h. GLU tended to recover but was still significantly lower at 6 h after dosing. Three of the four animals (animal no. 1, 2 and 4) receiving GFLX at 100 mg/kg, which showed continuous hypoglycemia, were supplemented with pelleted food (50 g for animal no. 1, 2) and 50% GLU solution (1 ml/kg for animal no. 4) at 6.5–7 h after dosing in addition to scheduled feeding to avoid the moribund condition associated with severe hypoglycemia (Fig. 3). Following the supplementation, GLU gradually returned to the predose values by 12 h after dosing and then was increased around 24 h after dosing (Fig. 3). These values at 24 h post dose were approximately 2-times higher than time-matched vehicle treated ones. Among four animals treated with GFLX at 100 mg/kg, one animal showed

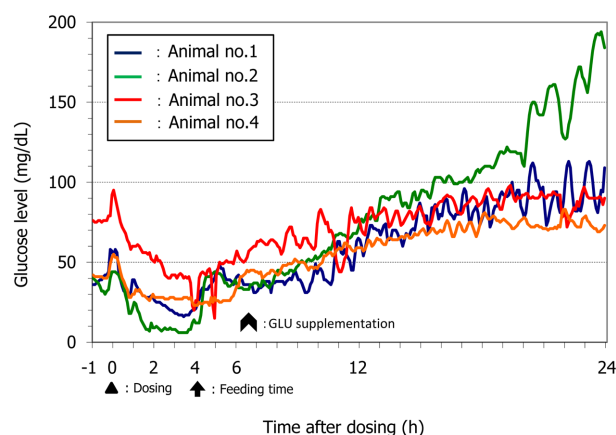


Fig. 3. Tracing of the 24-hr individual plasma glucose monitoring data in monkeys. Animals were treated with a single oral administration of gatifloxacin at 100 mg/kg. GLU: glucose.

overt hyperglycemia (Fig. 3). Thus, the 24-h continuous GLU monitoring data clearly distinguished between GFLX-induced GLU abnormality and physiological GLU changes influenced by several factors including feeding (Fig. 1).

Effects on ECG parameters

No statistically significant difference in the respective predose control ECG values was noted among all the dosing days (Fig. 4). No significant changes were detected in any ECG parameter in animals treated with GFLX at ≤ 60 mg/kg compared with those of the time-matched vehicle-treated control animals. Significant prolongation of the PR and QT/QTc intervals was detected in animals treated with 100 mg/kg GFLX at 4 and/or 6 h after dosing. No significant changes were detected in the QRS width at any dose.

Plasma drug concentrations

The C_{max} and $\text{AUC}_{0-24\text{h}}$ of GFLX were generally increased with escalating doses (Table 1). The C_{max} at 30

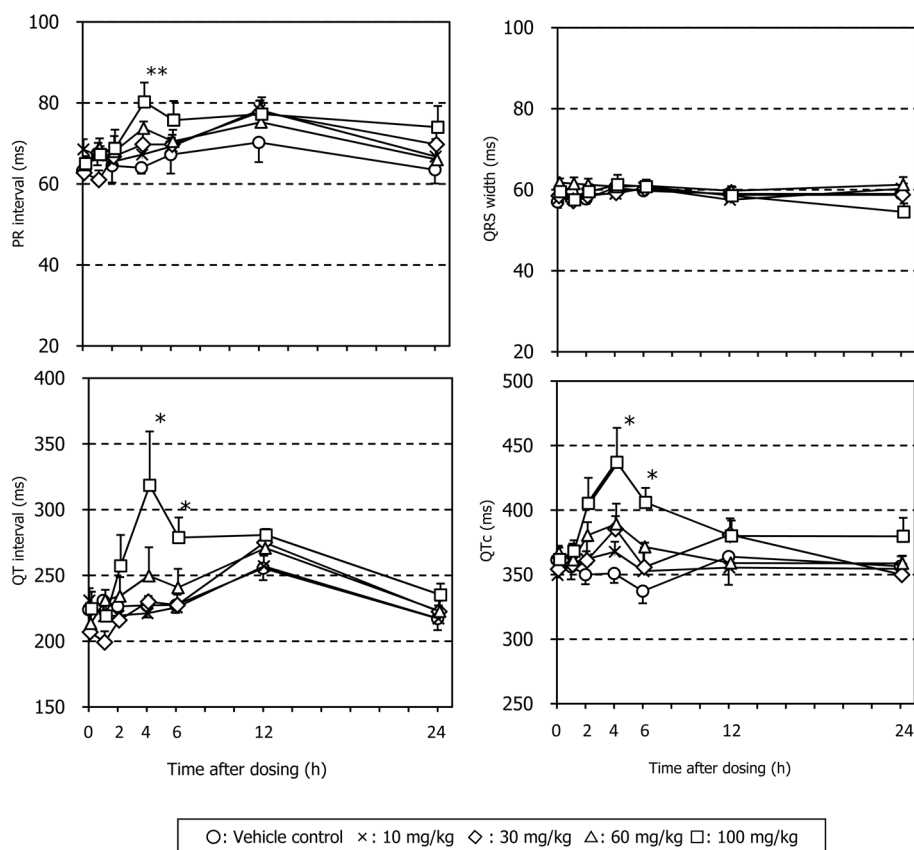


Fig. 4. Effect of gatifloxacin on electrocardiogram in monkeys. Animals were treated with a single oral dose of the vehicle (0 mg/kg) or gatifloxacin at 10, 30, 60 or 100 mg/kg. Data are presented as mean \pm SE (n=4) for each group. * P <0.05, ** P <0.01: significantly different from the vehicle control group.

and 100 mg/kg GFLX was $11.0 \pm 3.5 \mu\text{g/ml}$ ($29.4 \mu\text{mol/L}$) and $34.6 \pm 11.6 \mu\text{g/ml}$ ($92.2 \mu\text{mol/L}$), respectively (Table 1).

Relationship among plasma gatifloxacin concentrations, plasma glucose levels and QTc values

A statistically significant correlation among plasma gatifloxacin concentrations, plasma glucose levels, and QTc values (Fig. 5). Correlation coefficient value between plasma GFLX and GLU ($R=-0.5983$, $n=96$) was lower than that between plasma GFLX concentrations and QTc values ($R=0.7360$, $n=96$). A close correlation between GLU and QTc values was also observed in animals treated with 100 mg/kg GFLX ($R=0.6489$, $n=27$).

Clinical signs

Neither death nor moribundity was observed throughout the study period. No treatment-related symptoms

were seen in any animal treated with GFLX at 10 or 30 mg/kg. Following the treatment with GFLX at 60 mg/kg, transient hypoactivity with pallor of conjunctiva and oral mucosa was observed in one of the four animals around 2 h after dosing. Vomiting was also observed in three animals at the same dose level. Following the treatment with GFLX at 100 mg/kg, transient but severe symptoms, such as partial eyelid closure in three animals, hypoactivity with bilaterally dilated pupils in one animal, and trembling in one animal, were found around 2–4 h after dosing.

Discussion

In the present study, we monitored GLU and ECG parameters simultaneously and sequentially for 24 h in monkeys given GFLX to confirm the relationships between GLU abnormalities and electrocardiographic changes. GFLX at ≥ 30 mg/kg dose-dependently de-

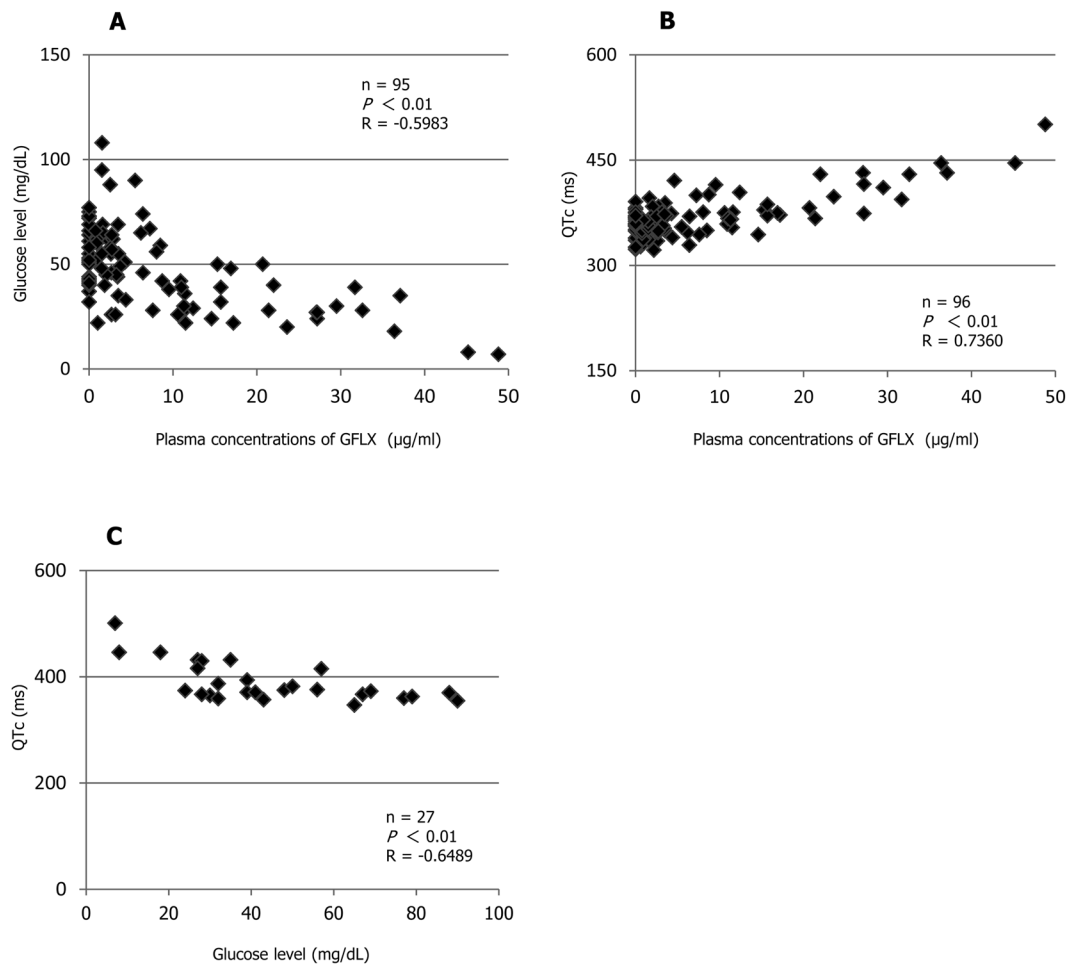


Fig. 5. Relationship among plasma gatifloxacin concentrations, plasma glucose levels, and QTc values in monkeys. Animals were treated with a single oral administration of the vehicle (0 mg/kg) or gatifloxacin at 10, 30, 60 or 100 mg/kg. A: Correlation between plasma gatifloxacin concentrations and plasma glucose levels by using data in all groups. B: Correlation between plasma gatifloxacin concentrations and QTc values by using data in all groups. C: Correlation between plasma glucose levels and QTc values by using data in the 100 mg/kg group. GFLX: gatifloxacin.

creased the GLU concentrations. A statistically significant correlation between plasma GFLX and GLU values was observed in the present study; however, the coefficient value appeared to be rather low compared with that between plasma GFLX concentrations and QTc values mentioned below, presumably owing to confounding factors associated with GLU homeostasis such feeding or GLU rescue in the 100 mg/kg group together with interindividual variation. However, the 24-h continuous GLU monitoring data clearly distinguished between GFLX-induced GLU abnormality and physiological GLU changes influenced by several factors including feeding and/or intentional GLU rescue. It is critical for investigators to consider both animal welfare and the

interpretation of results in the toxicological testing. Toxicokinetic data identified that GFLX at 30 mg/kg, with its concentration being around 10 $\mu\text{g/ml}$ (26.7 $\mu\text{mol/L}$), produced hypoglycemia in monkeys. Similar hypoglycemia was observed in rats after a single intravenous administration of GFLX at doses of ≥ 25 mg/kg [15]. Ishiwata *et al.* (2006) also reported that serum GFLX concentration in rats given 50 mg/kg was 8.6 $\mu\text{g/ml}$ at 1 h after dosing [15]. Furthermore, the peak plasma concentration of GFLX in healthy subjects receiving an intravenous infusion of GFLX at 600 mg/kg or in patients with type 2 diabetes receiving a single oral dose of GFLX at 400 mg, which showed a transient decrease in GLU, was reported to be 6.0 $\mu\text{g/ml}$ [9] or around 4.0

$\mu\text{g/ml}$ [10, 32], respectively. Although the response of the serum GLU concentration to GFLX was different in normal and diabetic conditions [15], the sensitivity of monkeys to GFLX with regard to the effect on blood GLU homeostasis was suggested to be comparable to that in rats or humans, based on our result and those of previous publications [9, 10, 15, 32].

Enhanced insulin secretion was suggested to be one of the most likely causes of hypoglycemia induced by GFLX [7]. In fact, GFLX-induced hypoglycemia in rats was accompanied by an increase in insulin secretion depending on the dose of GFLX [15]. An *in vitro* study provided mechanical evidence suggesting that GFLX inhibited pancreatic β -cell ATP-sensitive potassium (K_{ATP}) channel currents, which are known to regulate insulin secretion, in MIN6m9 cells and enhanced insulin secretion in murine pancreatic islets [29]. The IC_{50} value of GFLX-induced K_{ATP} inhibition was $42.4 \mu\text{mol/L}$ (approximately $16 \mu\text{g/ml}$) [29], and this was comparable to the plasma GFLX concentrations around the time when GLU began to decrease after administration (approximately $11 \mu\text{g/ml}$). In addition to the hypoglycemic potential, GFLX was also reported to induce hyperglycemia [11]. In rats, a single intravenous administration of GFLX at 100 mg/kg induced transient hyperglycemia accompanied by increased insulin secretion [15]. Many endogenous compounds including catecholamines such as epinephrine, glucagon, and glucocorticoids were suggested to be associated with GLU elevation [15, 30]. On the other hand, reduced insulin secretion was considered to be one of the causes of hyperglycemia induced by GFLX [34]. It has been reported that oral administration of GFLX at 400 mg/day for 3 days caused hypoglycemia first, followed by hyperglycemia in a patient with diabetic nephropathy [33]. In the present monkey study, GFLX at 100 mg/kg produced a transient hypoglycemia, followed by gradual hyperglycemia at around 24 h post-dose, by which time plasma GFLX had been largely eliminated from the circulation. Thus, the mechanism underlying GFLX-induced hypoglycemia and hyperglycemia in monkeys in addition to the occurrence of diabetic nephropathy remains to be clarified. Therefore, further investigation including blood chemistry as well as histopathological changes in the heart and pancreas should be necessary to support the currently observed effects of GFLX.

In ECG monitoring, GFLX at 100 mg/kg induced prolongations of the QT/QTc and PR intervals. A close

correlation between plasma GFLX concentrations and QTc values was observed. This implied that QT/QTc values under physiological conditions were not affected by feeding, unlike GLU values in the free-moving monkeys. Furthermore, toxicokinetic data indicated that GFLX at concentrations $>30 \mu\text{g/ml}$ could produce QT/QTc prolongation in monkeys. The human ether-a-go-go-related gene (*hERG*) encodes the rapid component of the delayed rectifier potassium current (I_{Kr}), which is one of the important currents in the cardiac repolarization phase. Blockade of *hERG* currents is known to be the main mechanism of drug-induced QT/QTc prolongation. The IC_{50} value of GFLX-induced *hERG* inhibition was reported to be $130 \mu\text{mol/L}$ (approximately $50 \mu\text{g/ml}$) [20], which is somewhat higher than the plasma GFLX concentrations showing QT/QTc prolongation (approximately $30 \mu\text{g/ml}$). Interestingly, the I_{Kr} -blocking action of class III antiarrhythmic drugs was suggested to enhance the prolongation of cardiac action potential duration under hypoglycemic condition in Langendorff-retroperfused guinea pig hearts [13]. In addition, QT/QTc prolongation accompanied by hypoglycemia was observed in patients with diabetes type I or II treated with sulfonylurea [24], insulin [19], or other medicines [22]. In the present study, a close correlation between GLU and QTc values was also observed in animals treated with 100 mg/kg GFLX. Collectively, GFLX-induced hypoglycemia was suggested to enhance its inhibitory action on *hERG* currents, resulting in QT/QTc prolongation in monkeys. Potentiation of the cardiac monophasic action potential duration prolongation by class III antiarrhythmic drugs was also enhanced under hyperglycemia conditions [13]. However, in this monkey case, hyperglycemia was noted 24 h after dosing without QT/QTc prolongation.

In clinical observations, transient but severe symptoms, such as partial eyelid closure in three animals, hypoactivity with bilaterally dilated pupils in one animal, and trembling in one animal given GFLX at 100 mg/kg . Hypoglycemia is well documented to be associated with neurogenic and neuroglycopenic symptoms in humans [4]. Therefore, abnormal clinical signs due to GFLX were considered to be related to hypoglycemia.

In conclusion, combined assessment of continuous blood GLU and ECG monitoring in free-moving monkeys is a valuable method for predicting the cardioelectrophysiological risk in humans for pharmaceuticals associated with both GLU and ECG abnormalities.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

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