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Characterization of *microminipig* as a laboratory animal for safety pharmacology study by analyzing fluvoxamine-induced cardiovascular and dermatological adverse reactions

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Abstract

Fluvoxamine is a selective serotonin-reuptake inhibitor, of which IC_{50} values for serotonin- and noradrenaline-uptake process were reported to be 3.8 and 620 nmol/L, respectively, also known to directly inhibit cardiac Na^+, Ca^{2+}, and K^+ channels. We characterized *microminipig* as a laboratory animal by analyzing fluvoxamine-induced cardiovascular and dermatological responses under halothane anesthesia. Fluvoxamine maleate was infused in doses of 0.1, 1, and 10 mg/kg over 10 min with a pause of 20 min ($n=4$). The peak plasma concentrations were 35, 320, and 1906 ng/mL, of which free plasma concentrations were estimated as 20, 187, and 1108 nmol/L, respectively. The low and middle doses did not alter any cardiovascular variable. The high dose increased heart rate and mean blood pressure, prolonged QRS width, but shortened QT interval, whereas no significant change was detected in PR interval or QTcF. Moreover, it induced systemic erythema on the skin. Pretreatment of H_1/5-HT_{2A} antagonist cyproheptadine hydrochloride sesquihydrate in a dose of 0.3 mg/kg significantly attenuated the fluvoxamine-induced pressor response; but tended to further enhance sinus automaticity, atrioventricular nodal conduction; and ventricular repolarization in addition to intraventricular conduction delay; whereas it markedly suppressed onset of systemic erythema ($n=4$). In *microminipigs*, cardiovascular adverse effects of the high dose may be manifested as a sum of its inhibitory action on the cardiac ionic channels and its stimulatory effects on serotonergic and adrenergic systems, whereas dermatologic reaction can be induced primarily through H_1/5-HT_{2A} receptor-dependent mechanism. Thus, *microminipigs* may be used for analyzing such multifarious adverse events of clinical serotonergic pharmacotherapy.

Keywords Fluvoxamine · *Microminipig* · Hypertension · Tachycardia · Systemic erythema

Introduction

Fluvoxamine is a selective serotonin-reuptake inhibitor, of which IC_{50} values for serotonin- and noradrenaline-uptake process were reported to be 3.8 and 620 nmol/L, respectively [1]. The drug can also directly inhibit cardiac Na^+, Ca^{2+}, and K^+ channels [2–4], but lacks affinity for muscarine, adrenaline, dopamine, histamine, and serotonin receptors [1]. The clinical incidence of cardiovascular and dermatological adverse events of fluvoxamine was <0.1–5% for palpitation, hypertension, eruption, and itching; and <0.1% for tachycardia, hypotension, orthostatic hypotension, and bradycardia according to the package insert from the manufacturer. Previous safety pharmacology studies have not fully explained the onset mechanisms of such multifarious autonomic dysregulations or dermatological responses as reported in patients [5], although

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hypotension and bradycardia were reproduced in our previous study with the halothane-anesthetized dogs, which were considered to be induced by direct Ca^{2+} and Na^{+} channel inhibition [2].

Microminipigs are extraordinarily small-sized miniature pigs, weighing approximately 7 kg at 6 months of age when they are young mature, which have been optimized for life science research by Fuji Micra Inc. (Shizuoka, Japan) [6]. We have characterized *microminipigs* as an alternative in vivo experimental model for pharmacology and toxicology in comparison with beagle dogs under the halothane anesthesia, and found that the extent of drug-induced cardiovascular responses was greater in *microminipigs* than in dogs [7–12]. These differences have been assumed by smaller effective volume of distribution of a drug, less whole-body fat content, and greater basal sympathetic tone along with its resulting smaller extent of reflex-mediated increase in *microminipigs* than in dogs [7–12]. While the extent of drug responses varies much among a large population of human subjects, the halothane-anesthetized beagle dogs are known to generally have similar sensitivity to healthy human subjects in detecting the drug-induced cardiovascular responses [13]. Thus, *microminipigs* may have potential to mimic a specific population of patients who might be sensitive to adverse events of fluvoxamine which could not be found by dogs.

In this study, we initially administered fluvoxamine alone to *microminipigs* under halothane anesthesia ($n=4$) to clarify how the animals may respond to the drug in comparison with our previous study using the halothane-anesthetized dogs [2]. In order to better analyze the onset mechanisms of fluvoxamine-induced autonomic hyperactivity and dermatological response observed in the initial experiment, we then performed another series of experiment ($n=4$) using H_1/5-HT_{2A} antagonist cyproheptadine [14–18], since selective serotonin-reuptake inhibitors including fluvoxamine can enhance serotonergic tone, not just in the brain but throughout the body [5, 19], and cyproheptadine has been used for the treatment of serotonin syndrome [14–16]. In addition, cyproheptadine is also known to have antimuscarinic effect without blocking adrenoceptors [14–18], which will be important to better understand the current results. Through these analyses, we tried to characterize *microminipig* as a laboratory animal for safety pharmacology study.

Materials and Methods

Experiments were performed by using 8 male *microminipigs*, which were obtained from Fuji Micra Inc.; a set of 4 animals was used for experiment 1, and another set of 4 was done for experiment 2.

Cardiovascular Variables

Microminipigs of 9.4 ± 1.0 months old weighing 10.4 ± 0.4 kg were pre-anesthetized by an intramuscular injection of ketamine (16 mg/kg) /xylazine (1.6 mg/kg) ($n=8$). A 24G cannula was introduced into a superficial auricular vein, through which 1 mg/kg of propofol was intravenously injected for inducing anesthetic condition. After intubation with a 5-mm cuffed endotracheal tube, 1.0% halothane vaporized with 100% oxygen was inhaled with a volume-limited ventilator (SN-480-3; Shinano Manufacturing Co., Ltd., Tokyo, Japan). Tidal volume and respiratory rate were set at 10 mL/kg and 15 breaths/min, respectively. Two sets of clinically available 4F-size catheter sheath (RR-A40G07A; Terumo, Corporation, Tokyo, Japan) were inserted into aorta via the right femoral artery and inferior vena cava via the right femoral vein, respectively. Heparin calcium in a dose of 100 IU/kg was intravenously administered through the catheter sheath placed at the right femoral vein to prevent blood clotting. Aortic pressure was measured through the catheters sheath placed at the right femoral artery. The electrocardiogram was obtained from the A–B lead. The QT interval was corrected with Fridericia's formula (QTcF) [20].

Experimental Protocol

The aortic pressure and electrocardiogram were monitored with a polygraph system (RM-6000, Nihon Kohden, Co., Tokyo, Japan), and analyzed by using a real-time fully automatic data analysis system (WinVAS3 for Windows ver. 1.1R24v; Physio-Tech, Co., Ltd., Tokyo, Japan). Three recordings of consecutive complexes were used to calculate the mean for the cardiovascular variables.

Experiment 1

After the basal assessment, fluvoxamine maleate in a low dose of 0.1 mg/kg was intravenously infused over 10 min, and each variable was assessed at 5, 10, 15, 20, and 30 min after the start of administration ($n=4$). Then, fluvoxamine maleate in a middle dose of 1 mg/kg was intravenously infused over 10 min, and each variable was assessed in the same manner. Finally, fluvoxamine maleate in a high dose of 10 mg/kg was intravenously infused over 10 min, and each variable was assessed 5, 10, 15, 20, 30, 45, and 60 min after the start of administration.

The doses of fluvoxamine maleate were determined based on the previous reports [2], of which human equivalent doses were calculated to be 0.055, 0.55, and 5.5 mg/kg, respectively, based on the equation for dose conversion between

animals and humans [21]. Meanwhile, clinical oral dosage of fluvoxamine maleate is 50–150 mg/day in 2 divided doses; i.e., 25–75 mg in each administration. Since bioavailability is 54.4% [22], the oral doses of 25–75 mg of fluvoxamine maleate will correspond to 0.23–0.68 mg/kg, i.v. when the human body weight is 60 kg, which is around the middle dose in this study.

Experiment 2

After the basal assessment, cyproheptadine hydrochloride sesquihydrate in a dose of 0.3 mg/kg was intravenously infused over 10 min, and each variable was assessed at 5, 10, and 20 min after the start of drug administration ($n=4$) to confirm its effects on the cardiovascular variables. Then, fluvoxamine maleate in the high dose of 10 mg/kg was intravenously infused over 10 min, and each variable was assessed in the same manner to those in experiment 1.

Human equivalent dose of 0.3 mg/kg of cyproheptadine hydrochloride sesquihydrate (molecular weight: 350.88) used in this study can be calculated as 0.166 mg/kg based on the equation for dose conversion between animals and humans [21]. Since the bioavailability is known to be approximately 100% [23], standard oral dose of 4 mg/body of cyproheptadine hydrochloride (molecular weight: 323.86) would correspond to 0.067 mg/kg, i.v. when the human body weight is 60 kg. Thus, the intravenous dose of cyproheptadine hydrochloride sesquihydrate used in this study is > 2 times greater than the clinically recommended oral dose of 4 mg/kg of cyproheptadine hydrochloride when taking the difference of molecular weight into account [16, 17, 21]. We determined the protocol of experiment 2 and the dose of cyproheptadine hydrochloride sesquihydrate based on these previous information along with our preliminary experiments which was described in discussion.

Plasma Drug Concentration

A volume of 3 mL of blood was withdrawn from the right femoral artery just before the assessment of cardiovascular variable, at 10, 15, and 30 min after the start of the low- and middle-dose administration, and at 10, 15, 30, and 60 min after the start of the high-dose administration in experiment 1. The blood samples were centrifuged at 1500 *g* for 30 min at 4 °C. The plasma was stored at − 80 °C until the drug concentration was measured. The plasma concentration was determined as follows. The 10 µL of H_2O or standard solution and 20 µL of 60% (v/v) perchloric acid were added to the 100 µL of plasma samples. After vortex mixing, the sample solution was centrifuged at 20,000×*g* for 5 min at 4 °C. The supernatant in a volume of 40 µL was injected onto an analytical C_{18} reversed-phase column (150 mm × 4.6 mm, 3 µm, CAPCELL PAK C18, UG120; Shiseido Co., Ltd., Tokyo, Japan) maintained at 40 °C. The mobile phase in the high-performance liquid chromatography (HPLC) system (Prominence /LC solution, Shimadzu Co., Kyoto, Japan) was an aqueous solution containing 50% (v/v) of acetonitrile in 1/15 mol/L of phosphate buffer (pH 6.4) at a flow rate of 0.8 mL/min. The elution profiles of fluvoxamine were monitored with a UV detector at a wavelength of 254 nm.

Drugs

Fluvoxamine maleate for in vivo experiment was extracted from a commercially available tablet (Luvox®, AbbVie GK, Tokyo, Japan) with distilled water in a concentration of 10 mg/mL, which was diluted with saline to concentrations of 1 and 0.1 mg/mL, whereas that for plasma concentration assay was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Ketamine (Ketalar®, Daiichi Sankyo Co., Ltd., Tokyo, Japan), xylazine (Celactal®, Bayer Healthcare Co., Tokyo, Japan), propofol (Frensenius Kabi Co., Ltd., Tokyo, Japan), halothane (Fluothane®, Takeda Pharmaceutical Co., Ltd., Osaka, Japan), heparin calcium (Caprocin®, Sawai Pharmaceutical Co., Ltd., Osaka, Japan), and cyproheptadine hydrochloride sesquihydrate (Tokyo Chemical Industry Co., Ltd.) were purchased.

Statistical Analysis

Data are presented as mean ± SEM. ($n=4$ for each experiment). The statistical significances within a parameter in experiments 1 and 2 were evaluated by one-way, repeated-measures analysis of variance (ANOVA) followed by Contrasts as a post-hoc test for mean values comparison, whereas those between the groups in experiment 2 were assessed by two-way, repeated-measures ANOVA or unpaired *t* test. A p value <0.05 was considered to be statistically significant.

Results

No animal exhibited lethal ventricular arrhythmias or cardiohemodynamic collapse, leading to the animal's death during the experimental periods.

Experiment 1: Analysis of Fluvoxamine-Induced Cardiovascular and Dermatological Responses

Plasma Drug Concentration

The time course of the plasma concentration of fluvoxamine is depicted in Fig. 1. The peak plasma concentrations of fluvoxamine after the start of 0.1, 1, and 10 mg/kg infusion were 35 ± 8 ng/mL (0.11 µmol/L), 320 ± 105 ng/mL (1.00 µmol/L), and 1,906 ± 998 ng/mL (5.99 µmol/L),

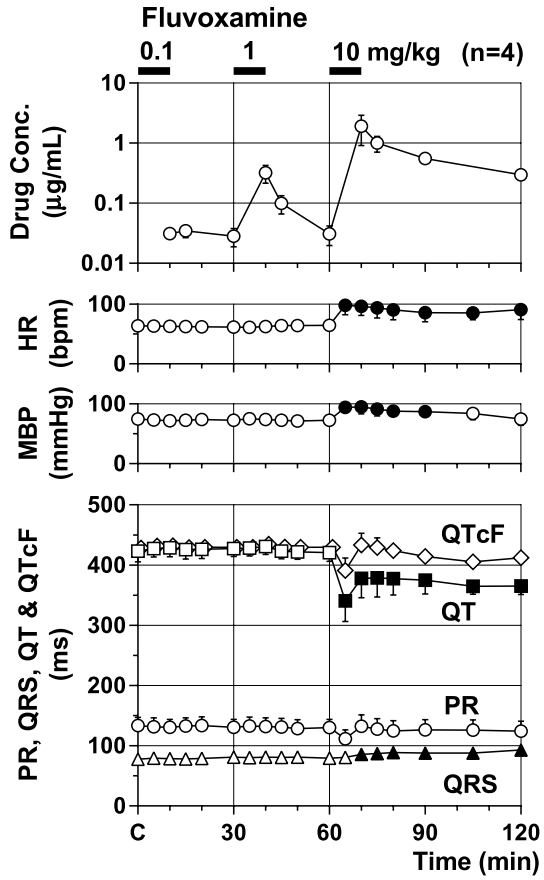

Fig. 1 Time courses of the plasma drug concentration (Drug Conc.), the heart rate (HR), mean blood pressure (MBP), PR interval (PR), QRS width (QRS), QT interval (QT), and QT corrected by Frideri-cia's formula (QTcF). Data are presented as mean ± SEM. ($n = 4$). Closed symbols represent significant differences from each control value (C) by $p < 0.05$

respectively. The plasma concentrations apparently decreased in distribution and elimination phases after the administration of the middle and high doses, whereas that peaked at 15 min after the start of administration of the low dose, which was 5 min after the completion of the infusion.

Effects on the Cardiovascular Variables

Representative tracings showing the effects of fluvoxamine on the electrocardiogram and aortic pressure are depicted in Fig. 2. Time courses of changes in the heart rate, mean blood pressure, PR interval, QRS width, QT interval, and QTcF are summarized in Fig. 1 ($n = 4$), of which pre-drug control values (C) were 64 ± 7 bpm, 75 ± 4 mmHg, 132 ± 14 ms, 84 ± 4 ms, 431 ± 19 ms, and 433 ± 10, respectively. No significant change was detected in any of these variables after the administration of the low or middle dose of fluvoxamine. The high dose of fluvoxamine increased the heart rate and mean blood pressure for 5–60 min and for 5–30 min, respectively; shortened the QT interval for 5–60 min; but prolonged the QRS width for 10–60 min; whereas no significant change was detected in the PR interval or QTcF.

Effects on the Skin Appearance

Erythema without edema was observed after the intravenous infusion of the high dose (Fig. 3a). The reaction began with reddening of the chest wall and gradually involved the whole skin. Erythema reached a maximum by 15 min after the start of the infusion, and then gradually diminished from 30 min. Since the erythema completely disappeared by 60 min after the administration of the high dose, we could not evaluate the signs of pain with that erythema after the recovery from the anesthesia. Similar dermatological response was observed in each animal.

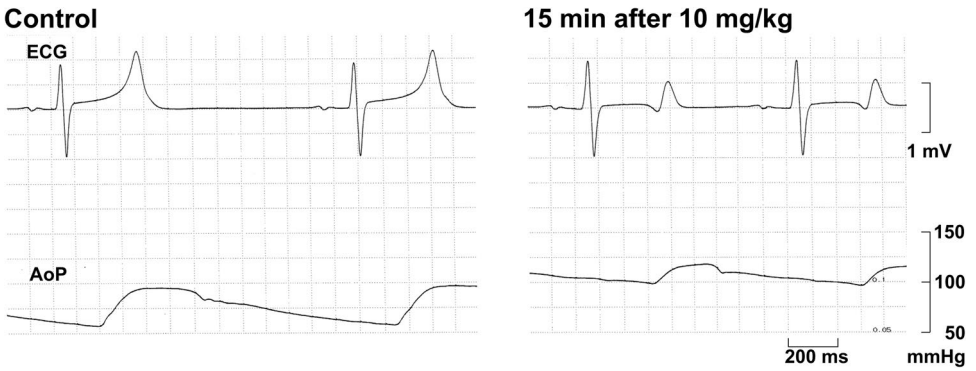

Fig. 2 Typical traces showing the A–B lead electrocardiogram (ECG) and aortic pressure (AoP) at pre-drug control (Control) (left panel), and 15 min after the start of intravenous administration of 10 mg/kg of fluvoxamine (15 min after 10 mg/kg) (right panel). Note that the prolongation of QRS width along with the T-wave morphological change and the increase of aortic pressure were induced after the administration of fluvoxamine

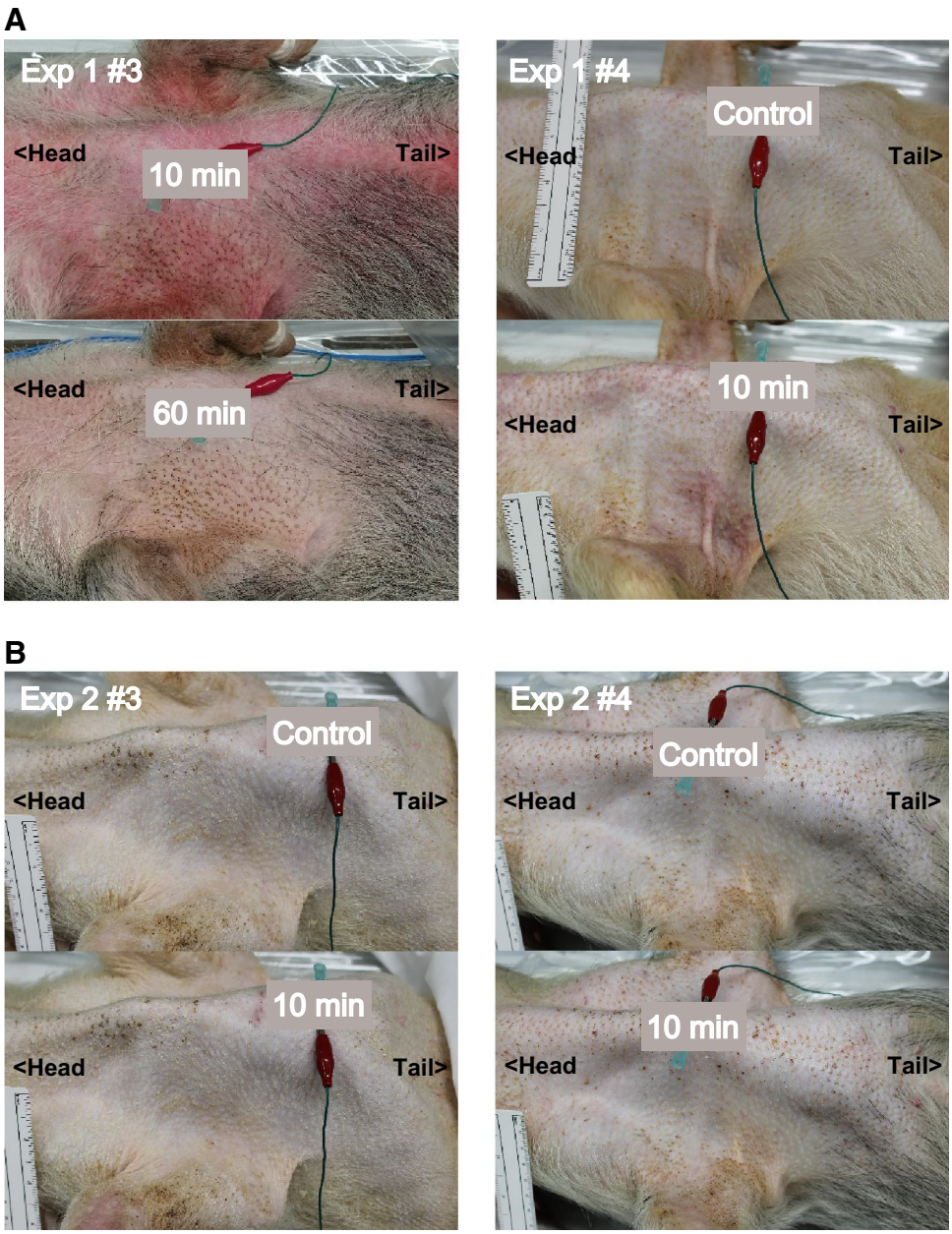

Fig. 3 Dermatological responses induced by fluvoxamine in the absence (**a**) and presence (**b**) of cyproheptadine (0.3 mg/kg, i.v.) pretreatment. Photos of the chest wall of *microminipigs* were depicted at 10 min and 60 min after the intravenous administration of 10 mg/kg of fluvoxamine (Exp 1 #3, the most severe case), and before (Control) and 10 min after the intravenous administration of 10 mg/kg of fluvoxamine (Exp 1 #4, Exp 2 #3 and Exp 2 #4). Note that diffuse skin flush was observed after the fluvoxamine administration only in the absence of cyproheptadine pretreatment (**a**)

Experiment 2: Effects of Cyproheptadine on the Fluvoxamine-Induced Responses

Time courses of changes in the heart rate, mean blood pressure, PR interval, QRS width, QT interval, and QTcF are summarized in Fig. 4 ($n=4$).

Effects of Cyproheptadine Pretreatment on the Cardiovascular Variables

Pre-drug control values (Pre) in the heart rate, mean blood pressure, PR interval, QRS width, QT interval, and QTcF before cyproheptadine administration were 60 ± 3 bpm, 74 ± 2 mmHg, 126 ± 6 ms, 69 ± 5 ms, 410 ± 24 ms, and 408 ± 20, respectively ($n=4$) (Fig. 4). Cyproheptadine hydrochloride sesquihydrate in a dose of 0.3 mg/kg slightly but significantly increased the mean blood pressure at 5 and 10 min after the start of infusion, of which increment was 6 and 5 mmHg, respectively, whereas no significant change was detected in the other variables.

Effects of Fluvoxamine in the Presence of Cyproheptadine

Pre-drug control values (C) in the heart rate, mean blood pressure, PR interval, QRS width, QT interval, and QTc before the administration of a high dose of 10 mg/kg of

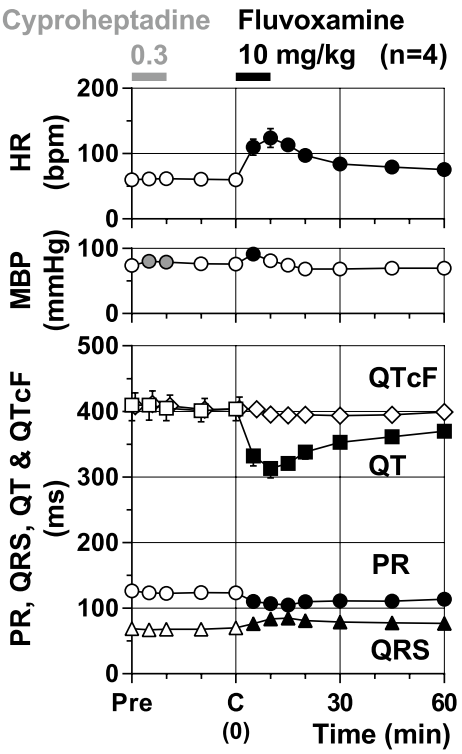

Fig. 4 Time courses of the heart rate (HR), mean blood pressure (MBP), PR interval (PR), QRS width (QRS), QT interval (QT), and QT corrected by Fridericia's formula (QTcF). Data are presented as mean ± SEM. ($n = 4$). Gray symbols represent significant differences from each control value before the administration of cyproheptadine (Pre) by $p < 0.05$, whereas black symbols indicate those from each control value before that of 10 mg/kg of fluvoxamine (C) by $p < 0.05$

fluvoxamine maleate were 60 ± 2 bpm, 76 ± 3 mmHg, 124 ± 6 ms, 70 ± 5 ms, 404 ± 18 ms, and 403 ± 18, respectively (Fig. 4). The high dose of fluvoxamine increased the heart rate for 5–60 min and the mean blood pressure at 5 min; shortened the PR interval and QT interval for 5–60 min; but prolonged the QRS width for 5–60 min; whereas no significant change was detected in the QTcF as summarized in Table 1.

We then compared the effect of fluvoxamine in the presence or absence of cyproheptadine (Table 1). Although *microminipigs* were already treated with the low and middle doses of fluvoxamine before the administration of the high dose in experiment 1, we detected no difference between the pre-drug control values in experiment 2 and those at 30 min after the start of the middle-dose infusion in experiment 1. The effects of fluvoxamine in experiment 1 were reanalyzed by comparing the values at 30 min after the start of the middle-dose infusion with each of those after the administration of the high dose. The high dose of fluvoxamine increased the mean blood pressure for 5–30 min, whereas no significant change was detected in the other variables as shown in Table 1. Significant difference between the presence and absence of cyproheptadine pretreatment was detected only in the fluvoxamine-induced changes in the time courses of the mean blood pressure.

Onset of erythema was significantly suppressed by the pretreatment with cyproheptadine as shown in Fig. 3b, although erythema was locally and modestly observed after the intravenous infusion of the high dose, which completely disappeared by 30 min after its start of administration. The same profile of dermatological response was confirmed in each of the 4 animals, indicating its high reproducibility.

Discussion

We tried to characterize *microminipig* as a laboratory animal by analyzing fluvoxamine-induced cardiovascular and dermatological adverse events under halothane anesthesia. Toxic dose of 10 mg/kg, i.v. of fluvoxamine increased the heart rate and mean blood pressure, prolonged the QRS width, but shortened the QT interval, whereas it induced systemic erythema on the skin in *microminipig*. While tachycardia, hypertension, and erythema have been reported in patients as adverse events of serotonin-reuptake inhibitors, such effects have not been reproduced in laboratory animals [2, 5].

Pharmacokinetic Profile of Fluvoxamine in *Microminipig*

The clinically recommended maximum daily p.o. dose of fluvoxamine described in the package insert from the manufacturer has been 150 mg/body in Japan. In a previous phase I study, a single oral administration of 25, 50, 100, and 200 mg of fluvoxamine provided the C_{max} values of 9, 17, 44, and 92 ng/mL, respectively [22]. Thus, the doses of the drug assessed in this study can be considered to provide therapeutic to toxic levels of plasma drug concentrations. Since lack of species difference in the protein binding ratio of fluvoxamine has been suggested [2] and the plasma protein binding ratio of fluvoxamine is known to be 81% in humans and 82% in rats [24], free peak concentrations were estimated as 20, 187, and 1,108 nmol/L, respectively.

In our previous study using the halothane-anesthetized canine model [2], fluvoxamine maleate in intravenous doses of 0.1, 1, 10 mg/kg provided peak plasma concentrations of 55, 289, and 3720 ng/mL, which was 1.57, 0.90, and 1.95 times greater in the beagle dogs than in *microminipigs*, respectively. This observation was markedly different from what we had expected based on our previous study [7]; namely, the effective volume of drug distribution of *microminipigs* is smaller than that of beagle dogs, providing higher plasma drug concentrations in *microminipigs*. It has been known in humans that fluvoxamine is metabolized

Table 1 Comparison of the fluvoxamine-induced cardiovascular responses between the presence and absence of cyproheptadine pretreatment

Time	Cont	5 min	10 min	15 min	20 min	30 min	45 min	60 min
ΔHR (bpm)								
C + F	0 ± 0	+ 50 ± 11**	+ 64 ± 12**	+ 53 ± 8**	+ 37 ± 7**	+ 24 ± 5**	+ 19 ± 5*	+ 16 ± 5*
F	0 ± 0	+ 34 ± 12	+ 32 ± 13	+ 29 ± 14	+ 26 ± 13	+ 22 ± 12	+ 21 ± 7	+ 27 ± 14
ΔMBP (mmHg)								
C + F†	0 ± 0	+ 16 ± 6**	+ 5 ± 4	− 1 ± 2	− 7 ± 4	− 7 ± 5	− 6 ± 5	− 6 ± 4
F	0 ± 0	+ 22 ± 3**	+ 23 ± 6**	+ 19 ± 6**	+ 15 ± 4*	+ 15 ± 3*	+ 12 ± 5	+ 2 ± 8
ΔPR (ms)								
C + F	0 ± 0	− 13 ± 4**	− 17 ± 4**	− 19 ± 3**	− 14 ± 5**	− 13 ± 6**	− 13 ± 4**	− 10 ± 6**
F	0 ± 0	− 18 ± 4	+ 2 ± 9	− 2 ± 7	− 6 ± 6	− 4 ± 4	− 4 ± 5	− 6 ± 7
ΔQRS (ms)								
C + F	0 ± 0	+ 6 ± 2*	+ 14 ± 4**	+ 14 ± 3**	+ 11 ± 1**	+ 9 ± 1**	+ 7 ± 2**	+ 7 ± 2**
F	0 ± 0	+ 1 ± 1	+ 6 ± 2	+ 7 ± 2	+ 9 ± 3	+ 8 ± 2	+ 8 ± 3	+ 13 ± 10
ΔQT (ms)								
C + F	0 ± 0	− 71 ± 18**	− 91 ± 21**	− 83 ± 18**	− 66 ± 18**	− 51 ± 22**	− 42 ± 21**	− 34 ± 22*
F	0 ± 0	− 80 ± 21	− 43 ± 25	− 42 ± 25	− 43 ± 22	− 46 ± 18	− 56 ± 6	− 56 ± 8
ΔQTcF								
C + F	0 ± 0	0 ± 12	− 8 ± 15	− 9 ± 16	− 9 ± 15	− 10 ± 19	− 8 ± 18	− 5 ± 19
F	0 ± 0	− 38 ± 17	+ 4 ± 17	0 ± 15	− 5 ± 12	− 15 ± 11	− 23 ± 11	− 17 ± 13

The time courses of the changes (Δ) in the cardiovascular responses of fluvoxamine (10 mg/kg, i.v.) between presence (C + F; $n = 4$) and absence (F; $n = 4$) of cyproheptadine (0.3 mg/kg, i.v.) pretreatment

HR: heart rate, MBP: mean blood pressure, PR: PR interval, QRS: QRS width, QT: QT interval, QTcF: QT interval corrected by Fridericia's formula, Cont: basal control values of cardiovascular variables at 5–10 min before the start of 10 mg/kg, i.v. of fluvoxamine administration

*$p < 0.05$, **$p < 0.01$ v.s. respective pre-fluvoxamine (10 mg/kg, i.v.) basal control value

†$p < 0.05$ C + F group v.s. F group

into fluvoxamine alcohol by CYP2D6 [25]. CYP2D activity of liver microsomes is > 10 times greater in *microminipigs* than in dogs as well as in human subjects [26], which may partly explain current observation, supposing that fluvoxamine would be also metabolized by CYP2D in *microminipigs* and dogs like in humans.

Cardiohemodynamic Effects of Fluvoxamine

The high dose of fluvoxamine increased the heart rate and mean blood pressure in *microminipigs* in contrast to the observation in our previous study with the halothane-anesthetized beagle dogs [2], in which the heart rate and mean blood pressure were significantly decreased by the same dose of fluvoxamine. Although fluvoxamine may have potential to induce bradycardic and vasodilator effects through its direct Ca^{2+} and Na^{+} channel inhibition, it could also enhance serotonergic tone via its suppressive action on the serotonin transporter with IC_{50} value of 3.8 nmol/L [1], which might have overcome the direct inhibitory effects, resulting in the onset of tachycardia and hypertension in the case of *microminipig*. As fluvoxamine lacks affinity for cholinergic receptors [1], the drug-induced hypertension by itself may have induced reflex-mediated increase of vagal tone, which might attenuate the extent of the tachycardia. In addition, the toxic dose of fluvoxamine might have some potential to directly enhance adrenergic tone in addition to serotonergic one, since the IC_{50} value for noradrenaline-uptake process was 620 nmol/L [1].

In order to better understand the pathophysiology underlying the fluvoxamine-induced cardiohemodynamic responses observed in *microminipigs*, we assessed the effects of fluvoxamine on the aortic pressure and heart rate after the systemic administration of cyproheptadine in experiment 2. The pressor effect of fluvoxamine was significantly suppressed, whereas the tachycardia was tended to be further enhanced as shown in Fig. 4 and Table 1. The former could be explained by the 5-HT_{2A}-receptor blocking action of cyproheptadine. Meanwhile, the latter might be associated with both the blockade of reflex-mediated increase of vagal tone by the antimuscarinic action of cyproheptadine [18] and the fluvoxamine-induced increase of adrenergic tone, although the fluvoxamine by itself can directly suppress the sinus nodal automaticity through cardiac ionic channel inhibition [2].

Electrophysiological Effects of Fluvoxamine

The high dose of fluvoxamine prolonged the QRS width, but shortened the QT interval, whereas the PR interval tended to be shortened, which did not achieve statistical significance. The same dose of fluvoxamine prolonged the PR interval, QRS width and QT interval in the halothane-anesthetized beagle dogs [2]. The change in the QRS width observed in *microminipigs* (+13 ms) was qualitatively similar but its extent was greater compared with that in beagle dogs (+3 ms), indicating that the ventricular Na^+ channels were more potently inhibited by fluvoxamine in *microminipigs* than in dogs [27], although the inhibition might have been partly counteracted by the increase of serotonergic as well as adrenergic tone in the case of *microminipigs*. The modest shortening of the PR interval may be explained by the enhancement of serotonergic as well as adrenergic tone, which might have been partly counteracted by the hypertension-induced, reflex-mediated increase of vagal tone in addition to the direct Na^+ and Ca^{2+} channel inhibition. The shortening of the QT interval could be largely a rate-dependent response, since the QTcF was hardly altered after the high dose, indicating that inhibition of the inward and outward currents could be well-balanced in the presence of increased serotonergic as well as adrenergic tone in *microminipigs* unlike in dogs [2].

In order to better explain the hypothesis underlying the fluvoxamine-induced electrophysiological responses, we assessed the effects of fluvoxamine on the electrocardiogram after the systemic administration of cyproheptadine in experiment 2. The prolongation of the QRS width as well as the shortening of the PR interval and QT interval was tended to be further enhanced in experiment 2 as shown in Fig. 4 and Table 1. The former could be explained by the inhibition of the fluvoxamine-induced increase of the serotonergic tone. Meanwhile, the further shortening of the PR interval might be induced by the antimuscarinic actions of cyproheptadine [18] along with the fluvoxamine-induced increase of adrenergic tone, although the fluvoxamine by itself can directly suppress the atrioventricular nodal conduction through Ca^{2+} channel inhibition. The shortening of the QT interval could be primarily a rate-dependent response, since the extent of fluvoxamine-induced changes in the QTcF was much smaller in the presence of cyproheptadine, indicating that inhibition of the inward and outward currents could be also well-balanced in this condition.

Erythema

Systemic erythema was observed with high reproducibility in the absence of the pretreatment of cyproheptadine as depicted in Fig. 3. Such dermatological reaction was not observed in our previous study with the halothane-anesthetized beagle dogs or other studies [2]. Since such fluvoxamine-induced reaction was dramatically diminished by the pretreatment with cyproheptadine, it indicates that H_1/5-HT_{2A} receptors could be closely related to the onset of the systemic erythema. Thus, *microminipig* may become an effective way to predict the onset of serotonergic tone-related systemic erythema by new chemical entities.

Summary of the Preliminary Experiments and Study Limitations

Since the background data of cyproheptadine for the cardiovascular effects as well as H_1/5-HT_{2A} receptor blocking action was limited [14–18], we assessed it in a limited number of preliminary experiments using the same study condition as those in experiments 1 and 2. Cyproheptadine hydrochloride sesquihydrate in a dose of 0.1 mg/kg did not exert any modulatory effects on the high dose of fluvoxamine-induced dermatological responses ($n=2$). Cyproheptadine hydrochloride sesquihydrate in a dose of 1 mg/kg by itself induced severe bradycardia leading to the cardiohemodynamic collapse, which needed emergency care for treating the pathological condition ($n=1$). Thus, we selected 0.3 mg/kg of cyproheptadine hydrochloride sesquihydrate based on these preliminary experiments along with the previous information [14–18]. Moreover, we assessed the cardiovascular and dermatological responses of the high dose of 10 mg/kg of fluvoxamine alone, and confirmed that the similar dermatological responses to those observed in experiment 1 were induced ($n=1$). Using these information, we prepared the experimental protocol of experiment 2; however, the dose and/or blocking action of cyproheptadine to inhibit the 5-HT receptor-dependent pharmacological/toxicological actions of fluvoxamine need to be further elucidated.

There are some more limitations. First, several serotonin agonists would need to be used for better characterizing *microminipig*, although it may not be feasible to reproduce the pharmacological/toxicological effects of a selective serotonin-reuptake inhibitor by a systemic administration of serotonin agonists in vivo because of their pharmacokinetic differences. Second, we used halothane anesthesia to better analyze the cardiovascular effects of fluvoxamine, which made it difficult to evaluate the effects of fluvoxamine on the central nervous system while dermatological effects can be evaluated at the same time. Although the halothane anesthesia by itself may interfere with the heart in addition to the central nervous system [28, 29], each cardiovascular variable is reported to be stable for >2 h once the animals are put under the general anesthesia in the absence of pharmacological treatment [7–13]. Third, although fluvoxamine maleate for in vivo experiment was extracted from a commercially available tablet, we did not measure concentrations of the dosing formulation. The solubility of fluvoxamine to the

water is 14 mg/mL, and the highest theoretical concentration was 10 mg/mL, suggesting that fluvoxamine was fully dissolved to the water; thus, the concentration of the dosing formulations could be almost identical to the theoretical concentration.

Conclusions

In *microminipigs*, cardiovascular adverse effects of the toxic dose of fluvoxamine may be manifested as a sum of its inhibitory action on the cardiac ionic channels and its stimulatory effects on the serotonergic as well as adrenergic systems along with the reflex-mediated vagal tone, whereas dermatologic reaction can be induced primarily through the H_1/5-HT_{2A} receptor-dependent mechanism. Thus, *microminipigs* may be used for analyzing such multifarious adverse events of serotonergic pharmacotherapy as reported in human subjects.

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Compliance with Ethical Standards

Conflict of interest The authors indicated no potential conflict of interest.

Ethical Approval All experiments were approved by the Toho University Animal Care and User Committee (No. 15-52-275, 16-53-275, 17-54-275, 18-51-394) and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Toho University.

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