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European Journal of Pharmacology 466 (2003) 137–146
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Famotidine does not induce long QT syndrome: experimental evidence from in vitro and in vivo test systems

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Received 5 February 2003; received in revised form 26 February 2003; accepted 4 March 2003

Abstract

The effects of famotidine on the cardiac repolarization process were assessed using four different levels of test systems described in the draft stage guideline ICH S7B. A supratherapeutic concentration of famotidine (10^{-5} M), which is >8 times higher than C_{max} obtained after its therapeutic dose, neither inhibited human ether-a-go-go-related gene (HERG) K^+ current expressed in human embryonic kidney 293 (HEK293) cells nor affected any of the action potential parameters of guinea pig papillary muscles. Therapeutic (0.3 mg/kg, i.v.) to supratherapeutic doses (3–10 mg/kg, i.v.) of famotidine did not affect the repolarization process of the halothane-anesthetized canine model, while only supratherapeutic doses exerted the positive chronotropic, inotropic and dromotropic effects without affecting the mean blood pressure. Moreover, supratherapeutic doses of famotidine (1–10 mg/kg, i.v.) neither induced torsades de pointes nor prolonged QT interval in the canine chronic atrioventricular conduction block model. These results suggest that famotidine possesses no cardiovascular effects at a therapeutic dose, while it may exert cardiostimulatory actions after drug overdoses that might potentiate the proarrhythmic potential of co-administered cardiotonic agents by increasing the intracellular Ca^{2+} concentration.

Keywords: Famotidine; Monophasic action potential; Torsades de pointe; HERG; QT interval; Atrioventricular block

1. Introduction

Prolongation of QT interval by a drug, which has been given to patients for a long time, is currently a hot topic of concern for pharmaceutical companies as well as clinicians (Tamargo, 2000; De Ponti et al., 2000, 2001). When QT interval is prolonged by a drug, there is an increased risk of ventricular tachyarrhythmias, including torsades de pointes, particularly when combined with other risk factors, including hypokalemia, structural heart disease and bradyarrhythmias (Tamargo, 2000; De Ponti et al., 2000, 2001). Recently, draft stage guideline ICH S7B for safety pharmacology studies has been announced based upon currently available information to assess the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals (The ICH Steering Committee, 2002).

Famotidine is a highly selective histamine H_2 receptor antagonist (Howden and Tytgat, 1996; Langtry et al., 1989). The drug is indicated for the treatment of duodenal ulcer, gastric ulcer, gastroesophageal reflux disease and Zollinger–Ellison syndrome (Howden and Tytgat, 1996; Langtry et al., 1989). After its introduction for the treatment of acid-related disorders in 1985, estimated 18.8 million patients worldwide have been treated with famotidine (Howden and Tytgat, 1996; Langtry et al., 1989). The excellent tolerability profile of famotidine observed during investigational trials has remained substantially unchanged during post marketing experience (Howden and Tytgat, 1996; Langtry et al., 1989). However, a patient with long QT syndrome that may have been caused by famotidine was reported (Endo et al., 2000). The Ministry of Health, Labour and

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doi:10.1016/S0014-2999(03)01559-0

Welfare in Japan announced an official warning on the proarrhythmic potential of famotidine in September 2001, whereas there is still no evidence showing a causal link between famotidine administration, QT prolongation and onset of torsades de pointes in either human or animals.

In this study, we systematically assessed whether famotidine prolongs the repolarization process of the heart and induces torsades de pointes according to a nonclinical testing strategy described in the draft stage S7B. We first tested the effect of famotidine on the human ether-a-go-go-related gene (HERG) K^+ channel expressed in human embryonic kidney 293 (HEK293) cells by patch clamp technique. Next, the effect of famotidine on the action potential parameters of the isolated guinea pig papillary muscle was examined. Then, we simultaneously assessed the in vivo cardiohemodynamic and electrophysiological effects of famotidine using the halothane-anesthetized, closed-chest canine model to better understand the mutual complex interactions of its multiple effects on the cardiovascular system (Usui et al., 1998; Sugiyama and Hashimoto, 1998; Satoh et al., 1999; Chiba et al, 2000; Sugiyama et al., 2002b). Finally, we utilized the canine chronic complete atrioventricular conduction block model to detect the proarrhythmic property of famotidine (Chiba et al., 2000; Vos et al., 1995, 1998; Sugiyama et al., 2002a,b).

2. Materials and methods

All experiments were performed according to Guidelines for Animal Experiments, University of Yamanashi and Yamanouchi Pharmaceutical.

2.1. Experiment 1: effects of famotidine and dofetilide on K^+ current in the HERG-transfected HEK293 cells

HEK293 cells expressing the HERG K^+ channels were cultured in Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO, USA) containing 10% fetal bovine serum (GIBCO-BRL, Grand Island, NY, USA), 1% penicillin–streptomycin (GIBCO-BRL) and 400 μg/ml geneticin (GIBCO-BRL) in a humidified atmosphere at 37 °C with 95% air and 5% CO_2. The medium was replaced every 2 days. After reaching 70–90% confluency, the cultures were split 1:3.

The cells were placed on the recording chamber in a volume of 2 ml, which was mounted on an inverted microscope (IX70, Olympus, Tokyo, Japan). The chamber was continuously monitored and controlled at 37 °C by a two-channel temperature controller (TC-344B Auto Temperature controller, WARNER Instrument, Hamden, CT, USA). The cells were allowed to settle and adhere to the coverslip on the bottom of the recording chamber, and superfused continuously at a rate of 5 ml/min with pre-warmed external solution, consisting of 137 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose and 10 mM HEPES (pH 7.4, titrated with NaOH). This superfusion rate completely replaces the chamber solution within 1–2 min.

Membrane currents were recorded by the single-pipette, whole-cell, voltage-clamp technique using a patch clamp amplifier (Axopatch 200B, Axon, Union City, CA, USA). Voltage-clamp command pulses were generated by a digital-to-analog converter (Digidata 1322A, Axon) controlled by the pCLAMP software (Version 8, Axon). The pipettes (glass capillary tubes G-1.5, size: 1.5 × 90 mm, Narishige, Tokyo, Japan) having 2–5 MΩ resistance were prepared using a pipette puller (PP-830, Narishige) and microforge (MF-830, Narishige), and filled with the pipette solution consisting of 130 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM EGTA, 5 mM MgATP and 10 mM HEPES (pH 7.2, titrated with KOH). Junction potentials were zeroed before formation of the membrane-pipette seal. After a gigaohm seal was established, the membrane was ruptured by gentle suction for the whole-cell voltage clamp. The cell capacitance and series resistance were electrically compensated by 60–80%. HERG current was activated from a holding potential of −70 mV by a step to 0 mV for 750 ms, followed by a step to −50 mV for 750 ms to record tail current peak amplitude. The current signals were acquired and analyzed by pCLAMP software (Version 8, Axon). Tail currents were recorded before and 10 min after the drug application. The effects of famotidine were assessed at a concentration of 10^{-5} M. In addition, the effects of dofetilide at a concentration of 3×10^{-7} M and a solvent 0.1% dimethylsulfoxide (DMSO) alone were assessed as positive and negative controls, respectively.

2.2. Experiment 2: effects of famotidine on the action potential parameters in the isolated guinea pig papillary muscles

Action potentials were measured using the glass microelectrode method. Male guinea pigs (Crj: Hartley, 4–5 weeks, 297–363 g) were anesthetized with inhalation of ether, and were killed by exsanguination. The heart was immediately excised. A strand of free-running papillary muscle from the right ventricle was carefully dissected and immediately mounted into a 4-ml perfusion chamber. The perfusing Tyrode solution (125 mM NaCl, 4 mM KCl, 25 mM $NaHCO_3$, 1.8 mM NaH_2PO_4, 0.5 mM $MgCl_2$, 2.7 mM $CaCl_2$, 5.5 mM glucose) was oxygenated with a gas mixture of 5% CO_2 and 95% O_2, and kept at 36.4–37.3 °C with a temperature-controlled circulator. The perfusion speed was set at 5 ml/min. The micropipettes, having 20–40 MΩ resistance, were made using a puller (P-87, Sutter, Novato, CA, USA) and filled with 3 M KCl pipette solution. The tissue preparation was electrically driven at 1 Hz through bipolar electrodes using a stimulator (SEN-3301, Nihon Kohden, Tokyo, Japan) and an isolator (SS-403J, Nihon Kohden). The stimulation pulse was square in shape, and of 3–4.5 V amplitude and 1 ms duration. The action potentials were amplified with an amplifying equipment (AVB-11A,

Nihon Kohden). Its differential waveform was obtained with a differential apparatus (SS-1987, Nihon Kohden). The action potentials and the differential waveforms were saved using a computer with software pCLAMP 8 (Axon). Resting membrane potential (RMP), action potential amplitude (APA), maximal upstroke velocity ($\dot{V}_{max}$), and action potential duration at 30% (APD_{30}), 60% (APD_{60}) and 90% (APD_{90}) repolarization levels were quantified using a Clampfit 6.0.5 software (Axon). After confirming that these parameters were stabilized, effects of 10^{-5} M of famotidine were assessed. The duration of incubation of the guinea pig papillary muscles with famotidine before the measurements was taken was 30 min. Using the same experimental protocol as performed in this study, we have confirmed D-sotalol (10–100 μM) can prolong the APD_{90} by about 60% ($n=3$).

2.3. Experiment 3: effects of famotidine on the halothane-anesthetized in vivo canine model

Experiments were carried out using beagle dogs of either sex weighing approximately 10 kg ($n=6$). Dogs were anesthetized initially 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 calcium (100 IU/kg) was intravenously administered.

The surface lead II electrocardiogram (ECG) was obtained from the limb electrodes. The systemic blood pressure was measured at the left femoral artery. A thermodilution catheter (Nihon Kohden, TC-704) was positioned at the right side of the heart through the right femoral vein. The cardiac output was measured by a standard thermodilution method using a cardiac output computer (Nihon Kohden, MFC-1100). The total peripheral vascular resistance (TPR) was calculated using the basic equation: TPR = mean blood pressure/cardiac output. A quad-polar electrode catheter was positioned at the non-coronary cusp of the aortic valve through the right femoral artery to obtain the His bundle electrogram. A pig tail catheter was positioned at the left ventricle through the left femoral artery to measure left ventricular pressure. The maximum upstroke velocity of the left ventricular pressure ($LVdP/dt_{max}$) and the left ventricular end-diastolic pressure (LVEDP) were recorded during sinus rhythm to examine the effects of the drug on the contractility and the preload to the left ventricle, respectively.

A bidirectional steerable monophasic action potential (MAP) recording/pacing combination catheter (EP Technologies, 1675P, Sunnyvale, CA, USA) was positioned at the endocardium of interventricular septum in the right ventricle through the left femoral vein to obtain MAP signals. The signals were amplified with a DC preamplifier (EP Technologies, 300) to measure the duration of the MAP signals as an interval from the MAP upstroke to the desired repolarization level. The interval (ms) at 90% repolarization level was defined as MAP_{90}. The heart was electrically driven using a cardiac stimulator (Nihon Kohden, SEC-3102) with the pacing electrodes of the MAP recording/pacing combination catheter. The stimulation pulses were rectangular in shape, and of 1–2 V (about twice the threshold voltage) and 1 ms duration. The MAP_{90} was measured during the sinus rhythm ($MAP_{90(sinus)}$) and at a shorter pacing cycle length of 400 ms ($MAP_{90(CL400)}$) and 300 ms ($MAP_{90(CL300)}$). The effective refractory period (ERP) of the right ventricle was assessed by the programmed electrical stimulation. The pacing protocol consisted of eight 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 5–10 ms decrements, until refractoriness occurred. The extent of the terminal repolarization phase of the right ventricle (TRP), namely, phase 3 repolarization, was estimated by the difference between the ERP and $MAP_{90(CL400)}$ at the same site using the following equation: $TRP = MAP_{90(CL400)} - ERP$ (Sugiyama and Hashimoto, 1998; Satoh et al., 1999; Chiba et al, 2000; Sugiyama et al., 2002b; Franz, 1994; Kirchhof et al., 1998).

The systemic blood pressure, left ventricular pressure, ECG, His bundle electrogram and MAP signals were monitored with a polygraph system (Nihon Kohden, RM-6000), and analyzed using a real-time full automatic data analysis system (MP/VAS 3 for Macintosh ver 1.0, Physio-Tech, Tokyo, Japan). Each measurement of ECG, MAP as well as atrio-His (AH) and His-ventricular (HV) intervals was the mean of three consecutive recordings. In all cases, the investigator visually confirmed the accuracy of a computer-measured electrophysiological data. QTc was calculated using Bazett's (1920) formula.

The cardiovascular variables were assessed in the following order. The cardiac output was measured twice. The ECG, His bundle electrogram, systemic and left ventricular pressure and MAP signal were recorded under sinus rhythm. In addition, MAP signals were recorded during the ventricular pacing at a cycle length of 400 and 300 ms. Then, ERP was measured at the same site where MAP was recorded. All parameters described above were usually obtained within 1 min. After control assessment, famotidine in a clinically available dose of 0.3 mg/kg was intravenously administered over 10 min and each parameter was assessed 5, 10, 15, 20 and 30 min after the start of the infusion. Then, famotidine in a dose of 3 mg/kg was additionally administered over 10 min and each parameter was recorded in the same manner as that of the clinical dose. Finally, famotidine in a dose of 10 mg/kg was similarly administered over 10 min and parameters were assessed 5, 10, 15, 20, 30, 45 and 60 min after the start of the infusion. Each parameter after the drug administration was compared with the control values, since each variable in this study has been shown to be stable >3 h in the absence of active drug (Sugiyama and Hashimoto, 1998).

2.4. Experiment 4: effects of famotidine and terfenadine on the canine chronic atrioventricular block model

Eight beagle dogs of either sex weighing approximately 10 kg were anesthetized with pentobarbital sodium (30 mg/kg, i.v.). After intubation with a cuffed endotracheal tube, the respiration was controlled using a volume-limited ventilator (Shinano, SN-480-3) with room air. Tidal volume and respiratory rate were set at 20 ml/kg and 15 strokes/min, respectively. The surface lead II ECG was continuously monitored. A quad-polar electrode catheter with a large tip of 4 mm (Cordis-Webster, D7-DL-252, CA, USA) was inserted through the right femoral vein under sterile condition and positioned across the tricuspid valve under the guide of bipolar electrograms from the distal electrode pair. The optimal site for the atrioventricular node ablation, namely, the compact atrioventricular node, was determined on the basis of the intracardiac electrogram, of which a very small His deflection was recorded and atrium/ventricular voltage ratio was >2. The site was usually found at 1–2 cm proximal from the position where the largest His bundle electrogram was recorded. The power source for the atrioventricular node ablation was obtained from an electrosurgical generator (Mera, MS-1500, Tokyo, Japan), which delivers continuous unmodulated radiofrequency energy at a frequency of 500 kHz. After determining the location, the radiofrequency energy of 20 W was delivered for 10 s from the tip electrode to an indifferent patch electrode positioned on the animal's back, which was continued then for 30 s if junctional ectopic complexes were induced. After each application, PR interval was measured to determine whether the atrioventricular node conduction had been affected. The endpoint of this procedure was the development of the complete atrioventricular block with an onset of stable idioventricular escaped rhythm. Proper care was taken for the animals, and the proarrhythmic properties of the drugs were assessed >4 weeks after the onset of the complete atrioventricular block based on the previous reports (Chiba et al, 2000; Vos et al., 1995, 1998; Sugiyama et al., 2002a,b).

Holter recording system (Model 456A, Del Mar Avionics, Irvine, CA, USA) was used to monitor the ECG over 24 h. The ECG was analyzed with a Holter analyzing software (Protrack Model 153, Del Mar Avionics). QTc was calculated using the Bazett's (1920) formula. Two different doses of a drug were assessed in each atrioventricular block dogs without anesthesia. Namely, 2 h after the start of ECG monitoring, famotidine (1 mg/kg, i.v. over 5 min) or terfenadine (3 mg/kg, p.o.) was administered. Two weeks later, effects of 10 times higher doses of the respective drugs were assessed in the same manner.

2.5. Drugs

Famotidine and dofetilide used in experiments 1 and 2 were both synthesized at Yamanouchi Pharmaceutical. They were dissolved with dimethylsulfoxide (DMSO, Sigma), and the solutions were diluted to $<0.1\%$ DMSO in the respective external solutions based on the manufacturer's extensive laboratory data. The following drugs were purchased: famotidine used in experiments 3 and 4 (Gaster Inj™, Yamanouchi, Tokyo, Japan), terfenadine (Sigma), thiopental sodium (Tanabe, Osaka, Japan), halothane (Takeda, Osaka, Japan), heparine calcium (Mitsui, Tokyo, Japan) and pentobarbital sodium (Tokyo-Kasei, Tokyo, Japan).

2.6. Statistical analysis

Data are presented as the mean ± S.E.M. The statistical significance within a parameter was evaluated by either paired *t*-test or one-way repeated-measures analysis of variance (ANOVA) followed by contrasts for mean values comparison, whereas that between the groups was assessed by one-way factorial ANOVA. A P value <0.05 was considered significant.

3. Results

3.1. Experiment 1: effects of famotidine and dofetilide on K^+ current in the HERG-transfected HEK293 cells

Typical tracings of the effects of 0.1% DMSO, dofetilide and famotidine are depicted in Fig. 1. The tail currents recorded after a 750-ms depolarizing pulse to 0 mV back to −50 mV were hardly affected by 10 min superfusion of 0.1% DMSO (Fig. 1, upper), whereas the current was effectively abolished by dofetilide at a concentration of 3×10^{-7} M (Fig. 1, middle). On the other hand, no inhibitory effect was detected 10 min after the treatment with famotidine at a concentration of 10^{-5} M (Fig. 1, lower). The relative tail currents from four experiments at 10 min after the treatment with DMSO, dofetilide and famotidine were 0.95 ± 0.01, 0.07 ± 0.02 and 0.97 ± 0.01, respectively. In addition, in a limited number of experiments with 10^{-6}–10^{-8} M of famotidine ($n = 1$–2 for each concentration), no significant effect was detected on the HERG current.

3.2. Experiment 2: effects of famotidine on the action potential parameters in the isolated guinea pig papillary muscles

The basal values of RMP, APA, $\dot{V}_{max}$, APD_{30}, APD_{60} and APD_{90} in the isolated guinea pig papillary muscles and the effects of famotidine on these parameters are summarized in Table 1. Famotidine at a concentration of 10^{-5} M did not affect any of these parameters assessed after 30 min of incubation ($n = 5$). In addition, in a limited number of experiments with 10^{-6}–10^{-8} M of famotidine ($n = 1$–2 for each concentration), no significant effect was detected on the action potential parameters, either.

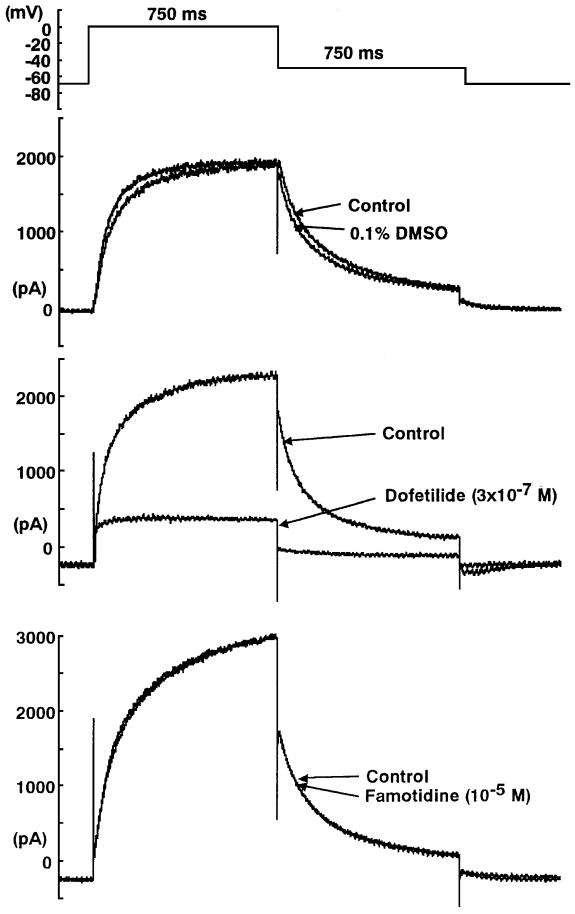

Fig. 1. Typical tracings of the effects of dofetilide, famotidine and their solvent 0.1% DMSO on K^+ currents in HERG-transfected HEK 293 cells. The cells were elicited by a depolarizing pulse of 750 ms to 0 mV from a holding potential of −70 mV, and tail current was recorded during a repolarizing pulse of 750 ms to −50 mV. Effects of DMSO (0.1%; upper panel), dofetilide (3×10^{-7} M; middle panel) or famotidine (10^{-5} M; lower panel) on the tail current were analyzed at 10 min after the drug treatment.

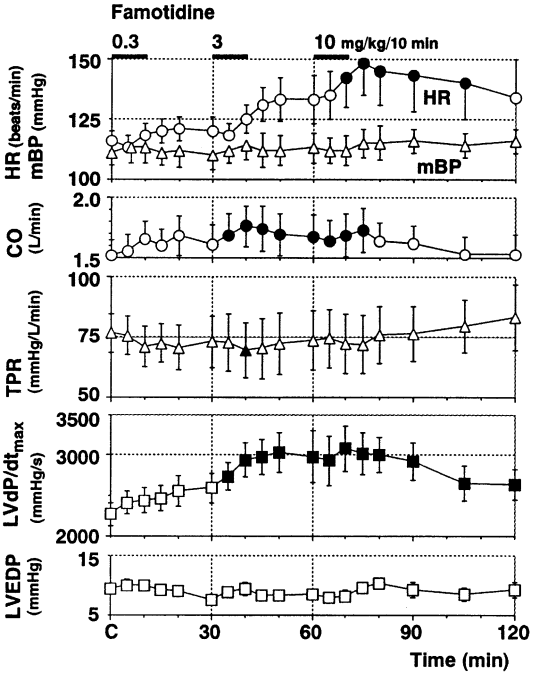

Fig. 2. Time courses of the effect of famotidine on heart rate (circles) and mean blood pressure (mBP; triangles); cardiac output (CO); total peripheral vascular resistance (TPR); maximum upstroke velocity of left ventricular pressure ($LVdP/dt_{max}$); and left ventricular end-diastolic pressure (LVEDP). Data are presented as the mean ± S.E.M. ($n = 6$). Closed symbols represent statistically significant differences from each pre-drug control (C) value by $P < 0.05$.

3.3. Experiment 3: effects of famotidine on the halothane-anesthetized in vivo canine model

3.3.1. Effects on the heart rate and blood pressure

The time courses of changes in the heart rate and mean blood pressure are summarized in Fig. 2 ($n = 6$), and their pre-drug control value (C) were 116 ± 4 beats/min and 111 ± 5 mm Hg, respectively. After the low dose of 0.3 mg/kg as well as the middle dose of 3 mg/kg of famotidine infusion, no significant change was detected in the heart rate or mean blood pressure. After the high dose of 10 mg/kg of famotidine infusion, the heart rate increased and significant change was detected for 10–45 min. Meanwhile, no significant change was detected in the mean blood pressure.

Table 1
Effect of famotidine on action potential parameters in isolated guinea pig papillary muscles

	RMP (mV)	APA (mV)	APD_{30} (ms)	APD_{60} (ms)	APD_{90} (ms)	$\dot{V}_{max}$ (V/s)
Control	−94 ± 1	133 ± 1	98 ± 3	135 ± 2	153 ± 2	240 ± 11
Famotidine	−94 ± 0	133 ± 1	97 ± 3	135 ± 2	152 ± 1	231 ± 11

Action potential parameters were obtained before (Control) and 30 min after the treatment with famotidine in a concentration of 10^{-5} M. Each value represents the mean ± S.E.M. of five experiments. RMP: resting membrane potential; APA: action potential amplitude; APD_{30}, APD_{60} and APD_{90}: action potential duration at 30%, 60% and 90% repolarization level, respectively; and $\dot{V}_{max}$: maximal upstroke velocity of the action potential.

3.3.2. Effects on the cardiac output and TPR

The time courses of changes in the cardiac output and TPR are also summarized in Fig. 2 ($n = 6$), and their pre-drug control values (C) were 1.53 ± 0.13 l/min and 76.6 ± 7.8 mm Hg·min/l, respectively. After the low dose infusion, no significant change was detected in the cardiac output or TPR. After the middle dose infusion, the cardiac output increased and TPR decreased. Significant changes were detected in cardiac output for 5–30 min and in TPR at 10 min. After the high dose infusion, the cardiac output remained being increased, and then returned to the basal level. Significant changes were detected in the cardiac output for 5–15 min, while no significant change was detected in the TPR.

3.3.3. Effects on the $LVdP/dt_{max}$ and LVEDP

The time courses of changes in the $LVdP/dt_{max}$ and LVEDP are also summarized in Fig. 2 ($n = 6$), and their pre-drug control values were 2266 ± 146 mm Hg/s and 9.5 ± 0.7

mm Hg, respectively. After the low dose infusion, no significant change was detected in the $LVdP/dt_{max}$. After the middle dose infusion, the $LVdP/dt_{max}$ increased and significant changes were detected for 5–30 min. After the high dose infusion, $LVdP/dt_{max}$ remained increased but gradually returned to the basal level. Significant changes were detected for 5–60 min. On the other hand, no significant change was detected in the LVEDP during the experimental period.

3.3.4. Effects on the ECG during the sinus rhythm

The time courses of changes in the ECG parameters are summarized in Fig. 3 ($n=6$). PR interval, QRS width and QT interval and QTc at the pre-drug control were 120 ± 4, 68 ± 1, 303 ± 13 ms and 421 ± 16 ms/$s^{1/2}$, respectively. After the low dose infusion, no significant change was detected in any of the parameters. After the middle as well as high dose infusion, the PR interval was shortened, and significant changes were detected from 5 min after the start of the middle dose infusion to the end of the experiment. On the other hand, no significant change was detected in the QRS width, QT interval or QTc during the experimental period. Ventricular arrhythmia was not detected during the observation period of this study.

3.3.5. Effects on the AH and HV intervals and MAP_{90} during the sinus rhythm

The time courses of the His bundle electrogram and MAP parameters are summarized in Fig. 3 ($n=6$). The AH and HV intervals and $MAP_{90(sinus)}$ at the pre-drug control were 88 ± 3, 30 ± 2 and 242 ± 7 ms, respectively. After the low dose infusion, no significant change was detected in any of the parameters measured. After the middle as well as high dose infusion, the AH interval was shortened, and significant change was detected from 5 min after the middle dose infusion to the end of the experiment. On the other hand, no significant change was detected in the HV interval and $MAP_{90(sinus)}$ during the experimental period.

3.3.6. Effects on the MAP_{90}, ERP and TRP during the ventricular pacing

The time courses of changes in the $MAP_{90(CL300)}$, $MAP_{90(CL400)}$, ERP and TRP are summarized in Fig. 3 ($n=6$), and their pre-drug control values were 221 ± 7, 239 ± 8, 217 ± 6 and 22 ± 5 ms, respectively. No significant change was detected in any of these parameters during the experimental period.

3.4. Experiment 4: effects of famotidine and terfenadine on the canine chronic atrioventricular block model

Typical tracings of the effects of famotidine and terfenadine on the ECG are depicted in Fig. 4 (left), and the number of animals showing torsades de pointes is summarized in Fig. 4 (right). The QT interval and QTc (R-R interval; ventricular rate) at pre-drug control were 300 ± 12 ms and 235 ± 15 ms/$s^{1/2}$ (1.6 ± 0.1 s; 37 ± 2 beats/min) in the low dose of 1 mg/kg of famotidine group ($n=4$), and 325 ± 17 ms and 248 ± 17 ms/$s^{1/2}$ (1.7 ± 0.1 s; 35 ± 2 beats/min) in the high dose of 10 mg/kg of famotidine group ($n=4$), respectively. Meanwhile, those were 333 ± 12 ms and 266 ± 3 ms/$s^{1/2}$ (1.6 ± 0.1 s; 39 ± 2 beats/min) in the low dose of 3 mg/kg of terfenadine group ($n=4$), and 351 ± 15 ms and 255 ± 10 ms/$s^{1/2}$ (1.9 ± 0.1 s; 32 ± 1 beats/min) in the high dose of 30 mg/kg of terfenadine group ($n=4$),

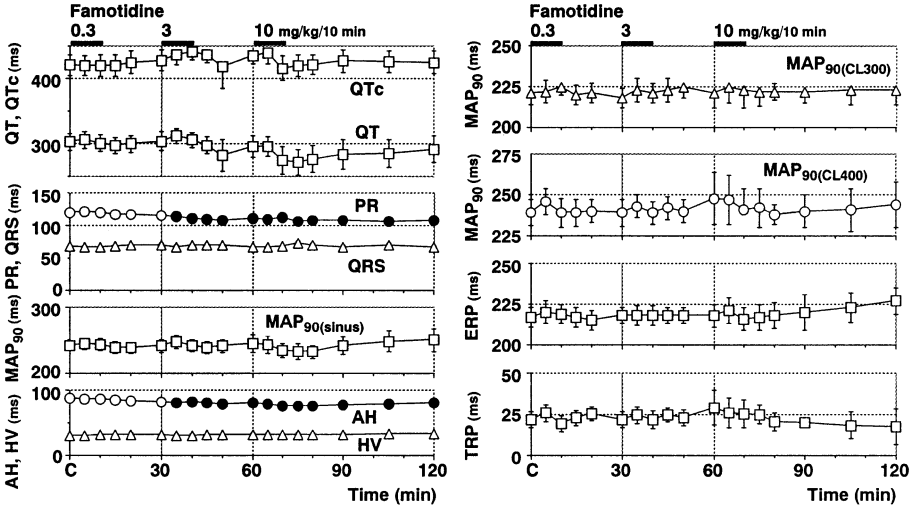

Fig. 3. Time courses of the effect of famotidine on QT interval (squares), QTc (squares); PR interval (circles), QRS width (triangles); duration of monophasic action potential at 90% repolarization level (MAP_{90}) during sinus rhythm ($MAP_{90(sinus)}$; squares); and atrio-His interval (AH; circles), His-ventricular interval (HV; triangles) (left panel). Time courses of the effect of famotidine on MAP_{90} during the ventricular pacing at a cycle length of 300 ms ($MAP_{90(CL300)}$); MAP_{90} during the ventricular pacing at a cycle length of 400 ms ($MAP_{90(CL400)}$); effective refractory period of the right ventricle (ERP); and terminal repolarization period (TRP) (right panel). Data are presented as the mean ± S.E.M. ($n=6$). Closed symbols represent statistically significant differences from each pre-drug control (C) value by $P<0.05$.

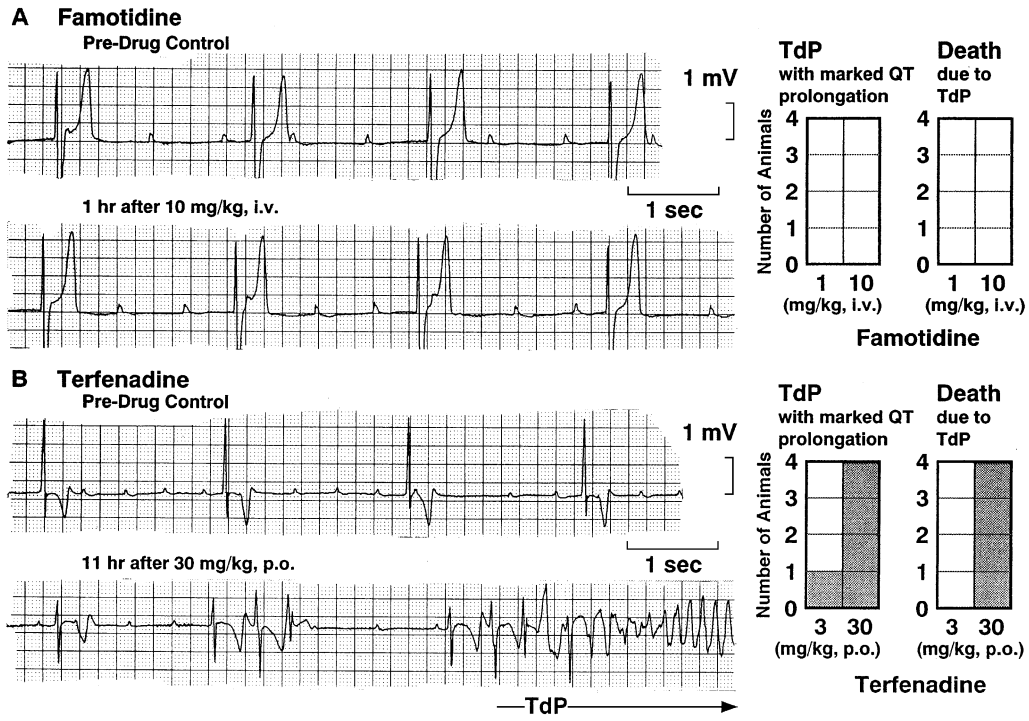

Fig. 4. Typical tracings of ECG demonstrating absence of torsadogenic action of famotidine (A) and presence of such effects of terfenadine (B) (left), and summary of the number of animals complicating torsades de pointes (TdP) (right). Typical episode of TdP was recorded 11 h after oral administration of 30 mg/kg of terfenadine, which degenerated into ventricular fibrillation (lower panel). Long–short initiating sequence was obvious in the preceding ECG. Meanwhile, neither TdP nor QT prolongation was detected after the intravenous administration of 10 mg/kg of famotidine (upper panel).

respectively. No significant difference was detected in these basal control values among these four groups.

After the administration of the low dose as well as high dose of famotidine, neither QT interval prolongation nor onset of torsades de pointes was observed. On the other hand, 1 h after the administration of the low dose of terfenadine, torsades de pointes was detected in one animal out of four, which lasted for 2.2 s and was spontaneously terminated. The QT interval and QTc were prolonged to 401 ms and 309 ms/s$^{1/2}$, respectively, just before the onset of torsades de pointes in this animal. After the administration of the high dose of 30 mg/kg of terfenadine, two to three episodes of torsades de pointes was detected in each animal, and the latest torsades de pointes degenerated into the ventricular fibrillation, leading to death. The initial torsades de pointes was observed at 6.6 ± 2.7 h after the drug administration, whereas the latest torsades de pointes leading to the animal's death was induced at 10.8 ± 2.9 h. Onset of torsades de pointes was closely related to the R on T phenomenon. The QT interval and QTc were prolonged to 409 ± 24 ms and 291 ± 17 ms/s$^{1/2}$, respectively, just before the onset of torsades de pointes.

4. Discussion

Given the lack of study of the effects of famotidine on the cardiac repolarization process, we assessed them using in vitro and in vivo test systems according to the draft stage ICH Consensus Guideline S7B (The ICH Steering Committee, 2002). Namely, ionic current assay and action potential duration assay were used to assess the in vitro electrophysiological effects of famotidine, whereas the halothane-anesthetized canine model and the chronic atrioventricular block model of dogs were used to assess the in vivo electropharmacological and proarrhythmic effects of famotidine. Such studies may be important especially at the time when the availability of this drug has changed from prescription-only to over-the-counter in many countries.

4.1. Concentration and dose of famotidine in this study

It has been reported that a 50% anti-secretatory effect (IC_{50}) of famotidine is associated with a plasma concentration of 0.013 mg/l (Langtry et al., 1989), and that single oral doses of the 20 and 40 mg tablets in man produce peak concentrations of 0.040–0.060 and 0.075–0.100 mg/l, respectively (Langtry et al., 1989). In addition, intravenous administration of a clinically recommended dose of 20 mg of famotidine can raise a peak plasma concentration of about 0.5 mg/l (=1.5 μM) in healthy male volunteers (Yeh et al., 1987), whereas protein binding is relatively low at 16% (Langtry et al., 1989). Thus, currently used 10 μM (=3.4 mg/l) of famotidine in vitro can be considered to reflect a supratherapeutic concentration that might be encountered during drug overdose. Meanwhile, in vivo famotidine doses

in this study (0.3–10 mg/kg) can be considered to provide clinically effective plasma concentration to supratherapeutic ones.

4.2. In vitro electrophysiological study

The proarrhythmic potential of famotidine was qualitatively estimated using both the ionic current assay and action potential duration assay. Inhibitory effect of famotidine on HERG K^+ current expressed in HEK293 cells was assessed in comparison with that of dofetilide, a typical I_{Kr} channel blocker (Gwilt et al., 1991; Satoh et al., 1999). We did not observe any inhibitory effect of famotidine on HERG K^+ current, whereas dofetilide inhibited it >90%, suggesting that famotidine does not block I_{Kr}. Famotidine in a supratherapeutic concentration also hardly affected any of the action potential parameters of guinea pig papillary muscles, indicating that famotidine lacks direct electrophysiological effects on the in vitro heart.

4.3. In vivo electrophysiological study

MAP in addition to surface ECG was recorded to assess the effects of famotidine on the repolarization process (Franz, 1994; Kirchhof et al., 1998). The heart rate was maintained at 150 and 200 beats/min in addition to the sinus rhythm by cardiac pacing at each time point to avoid the influence of heart rate variation. We have reported that drugs which have been reported to exert torsadogenic action in clinical practice prolong the MAP duration of the canine halothane-anesthetized model, when the maximum daily dose of the drug was given over 10 min (Usui et al., 1998; Sugiyama and Hashimoto, 1998; Satoh et al., 1999; Chiba et al, 2000; Sugiyama et al., 2002b). However, >10 times higher of maximum daily dose of famotidine did not affect the repolarization phase in this study, suggesting lack of potential risk of QT prolongation in vivo.

Numerous studies of in vivo models of torsades de pointes have been published, including conscious hypokalemic dogs, canine model with cesium, rabbit model with class III agents, canine model with myocardial infarction and canine model with chronic atrioventricular block (Eckardt et al., 1998). There is a high variability in occurrence of torsades de pointes in these models (Eckardt et al., 1998); however, the chronic atrioventricular block dog has been shown to be highly sensitive in detecting the drug-induced torsades de pointes (Chiba et al., 2000; Sugiyama et al., 2002a,b; Volders et al., 1999; Vos et al., 1995, 1998). The higher sensitivity of this model is explained by pre-drug existence of diminished K^+ current as well as structural remodeling, resulting in the enhanced drug-induced QT prolongation (Sugiyama et al., 2002a; Volders et al., 1999; Vos et al., 1995, 1998). In the present study, a nonspecific I_{Kr} channel blocker terfenadine (DuBuske, 1999; Salata et al., 1995; Usui et al., 1998) in submaximum to supratherapeutic doses prolonged the repolarization phase followed by the onset of torsades de pointes. On the other hand, such proarrhythmic effects of terfenadine were not detected in a previous study with the anesthetized rabbit model during α_1-adrenoceptor stimulation (Lu et al., 2000). Thus, these results confirm that our model is suitable for predicting the occurrence of non-cardiovascular drug-induced torsades de pointes. In such canine chronic atrioventricular block model, famotidine did not induce torsades de pointes, suggesting lack of torsadogenic potential in vivo.

4.4. Cardiohemodynamic effects

After the administration of therapeutic dose of famotidine, no significant change was detected in any of the cardiovascular parameters. However, additional administration of middle dose of famotidine exerted the positive inotropic and dromotropic effects with a transient decrease of TPR, leading to an increase of CO. Additional administration of high dose of famotidine induced a positive chronotropic effect in addition to those observed after the middle dose. No significant change was detected in the mean blood pressure, LVEDP or intraventricular conduction during the whole experimental period. These findings of famotidine have not been described elsewhere. Histamine is known to cause a positive chronotropic, inotropic and dromotropic actions through excitation of histamine H_2 receptors in man and animals species (Guo et al., 1984; Levi et al., 1975; Motomura and Hashimoto, 1989), whereas famotidine has been shown to be a specific histamine H_2 receptor antagonist without agonist or antagonist effects on histamine H_1, muscarinic, nicotinic, α- or β-adrenoceptors (Howden and Tytgat, 1996; Langtry et al., 1989). Based on these previous knowledge, one can speculate that in the absence of effective concentrations of histamine, high dose of famotidine may partially stimulate the histamine H_2 receptors of the cardiovascular system, leading to the stimulation of adenylate cyclase, which may exert the cardiostimulatory responses; namely, the positive chronotropic, inotropic and dromotropic effects. Other explanation may be a central effect of famotidine that may have modulated the autonomic tone on the cardiovascular system to exert the cardiostimulatory responses, since famotidine penetrates the normal blood–brain barrier (Langtry et al., 1989).

4.5. Clinical implication

Although the Ministry of Health, Labour and Welfare in Japan announced an official warning on the proarrhythmic potential of famotidine, the total number of the cases is only three (Endo et al., 2000). More importantly, these patients were critically ill and received other drugs including diuretics, antiarrhythmics and cardiotonic agents, thus, the concomitant pathophysiological conditions that may also affect QT interval might have been ignored. As a huge

number of patients have been treated with famotidine (Howden and Tytgat, 1996; Langtry et al., 1989), it seems impossible to establish a causal relationship of the cases to the occurrence of QT prolongation. In addition, famotidine does not notably bind to cytochrome *P*-450 or gastric alcohol dehydrogenase, therefore it may cause no significant drug interactions (Howden and Tytgat, 1996; Langtry et al., 1989). These previous knowledge and the present findings strongly suggest that famotidine hardly induces acquired long QT syndrome except for some patients with a silent carrier of the long QT syndrome. It would be more possible that a sympathetic-mediated mechanism and/or direct cardiostimulatory effects of famotidine at high concentrations may potentiate the proarrhythmic potential of cardiotonic agents by increasing the intracellular Ca^{2+} concentration.

4.6. Conclusions

The present preclinical studies suggest that famotidine singly at therapeutic doses in man is not likely to induce disturbances of ventricular repolarization or torsades de pointes related to the acquired long QT syndrome. The testing strategy described in this paper will provide pragmatical guidelines for assessing the potential for drug-induced long QT syndrome.

Acknowledgements

The authors thank Dr. Hiroshi Yoshida and Dr. Kentaro Ando for technical assistance.

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