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Full paper

Characterization of cardiovascular profile of anti-influenza drug peramivir: A reverse-translational study using the isoflurane-anesthetized dog

Ryuichi Kambayashi, Ai Goto, Hiroko Izumi-Nakaseko, Yoshinori Takei, Atsushi Sugiyama *

Department of Pharmacology, Faculty of Medicine, Toho University, 5-21-16 Omori-nishi, Ota-ku, Tokyo 143-8540, Japan

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ABSTRACT

An injectable anti-influenza drug peramivir has been reported to induce QT-interval prolongation in some phase III studies, although its thorough QT/QTc study was negative. We investigated the discrepancy among those clinical studies using isoflurane-anesthetized beagle dogs (n = 4). Peramivir in doses of 1 mg/kg/10 min (sub-therapeutic dose) followed by 10 mg/kg/10 min (clinically-relevant dose) was intravenously administered. Peramivir prolonged QT interval/QTcV and T_{peak}-T_{end}, and tended to delay ventricular repolarization in a reverse-frequency dependent manner, indicating I_{Kr} inhibition in vivo. Meanwhile, peramivir did not alter P-wave duration, PR interval or QRS width, indicating a lack of impact on cardiac conduction via Na^+ or Ca^{2+} channel inhibition in vivo. Peramivir prolonged T_{peak}-T_{end} and tended to prolong terminal repolarization period, which would develop substrates for initiating and maintaining spiral reentry, respectively. Meanwhile, peramivir did not prolong J-T_{peak}c, which could not induce early afterdepolarization, a trigger inducing torsade de pointes. Thus, our results support that clinical dose exposure of peramivir can delay the ventricular repolarization in influenza patients. Peramivir has only a small potential to induce torsade de pointes in patients with the intact hearts, but caution should be paid on its use for patients formerly having the trigger for torsade de pointes.

1. Introduction

Peramivir was approved firstly as an injectable neuraminidase inhibitor for treating influenza virus infection, and has been shown to possess favorable tolerability and safety profile in clinical trials as well as post-marketing surveillance.[1–4] However, in a previous phase III study comparing intravenous peramivir with oral oseltamivir in patients with seasonal influenza virus infection, the most common severe adverse event was found to be QT-interval prolongation, of which incidence was 1.4 % and 2.2 % in patients receiving a single intravenous infusion of peramivir in doses of 300 and 600 mg, respectively, and 2.7 % in those receiving oseltamivir in a dose of 75 mg, p.o. twice a day for 5 days.[5] Also in another clinical trial, peramivir in doses of 300 mg and 600 mg induced QT-interval prolongation with the incidence of 2 % and 1 %, respectively.[6] These findings indicate that peramivir may have some potential to prolong the QT interval, occasionally resulting in the onset of lethal ventricular arrhythmia like torsade de pointes (TdP). However, in a thorough QT/QTc study for peramivir, its single intravenous doses of 600 mg (clinical dose) and 1200 mg (supra-therapeutic dose) over 30 min to healthy subjects (n = 52) were not associated with QTc prolongation or other repolarization abnormalities according to EMA assessment report.[7] Accordingly, since there is an obvious inconsistency in the cardiac safety profile of peramivir between those clinical studies and the thorough QT/QTc study, we tried to study it using a reverse-translational research approach.

In order to precisely characterize the cardiovascular profile of peramivir, we adopted the isoflurane-anesthetized beagle dogs, which allows to estimate the drug-induced electrophysiological responses in healthy human subjects.[8,9] More importantly, we measured the proarrhythmic surrogate markers; early (J-T_{peak}) and late (T_{peak}-T_{end}) ventricular repolarization period as well as terminal repolarization period.[10–12] The J-T_{peak} indicates the net balance between inward currents ($I_{Na,L}$ and $I_{Ca,L}$) and outward ones (I_{Ks} and I_{Kr}) during phase 2 of the action potential. Prolongation of the J-T_{peak} may eventually result in Ca^{2+}overload of the sarcoplasmic reticulum of ventricular cardiomyocytes, which could increase the short-term variability of repolarization, governing “trigger” of premature ventricular contractions.[10] Prolongation of the T_{peak}-T_{end} may reflect the magnitude of I_{Kr} inhibition

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* Corresponding author.
E-mail address: atsushi.sugiyama@med.toho-u.ac.jp (A. Sugiyama).

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and could also predict the global increase of transmural dispersion of ventricular repolarization, controlling "substrate" for the initiation of spiral reentry.[10,13] The terminal repolarization period was obtained by the difference between the duration of monophasic action potential (MAP) and the effective refractory period (VERP) at the same site in the right ventricle, which could reflect the magnitude of local electrical vulnerability, regulating "substrate" for the perpetuation of spiral reentry.[14] Moreover, we compared the current findings of peramivir with those of typical anti-influenza drugs to better characterize the cardiovascular profile of peramivir.[15,16]

2. Materials and methods

Experiments were performed using female beagle dogs weighing approximately 10 kg (n = 4). Animals were obtained through Kitayama Labes Co., Ltd. (Nagano). All experiments were approved by the Toho University Animal Care and User Committee (No. 22-52-470) and performed according to the Guideline for the Care and Use of Laboratory Animals of Toho University.

2.1. Surgical preparation

The dogs were initially anesthetized with thiopental sodium (30 mg/kg, i.v.), followed by intubation with a cuffed endotracheal tube. The anesthesia was maintained by isoflurane inhalation (1.5–2.5 %, v/v) vaporized in oxygen with a volume-limited ventilator (SN-480-3; Shinano Manufacturing Co., Ltd., Tokyo). Tidal volume and respiratory rate were set at 20 mL/kg and 15 breaths/min, respectively. The surface lead II electrocardiogram was continuously monitored. Five catheter-sheath sets (Terumo Corporation, Tokyo) were used; two were inserted into the right and left femoral arteries toward the abdominal aorta, respectively, and two were done into the right femoral vein and one was done into the left femoral vein toward the inferior vena cava. Heparin calcium (100 IU/kg) was administered through the right femoral vein to prevent blood clotting.

2.2. Measurement of cardiohemodynamic variables

A pig-tail catheter (Technowood Corporation, Tokyo) was placed at the left ventricle through the right femoral artery to measure the left ventricular pressure, whereas the aortic pressure was measured at a space between the inside of the catheter sheath and outside of the pig-tail catheter through a flush line. The maximum upstroke velocities of the left ventricular pressure ($LVdP/dt_{max}$) and the left ventricular end-diastolic pressure were obtained during sinus rhythm to estimate the isovolumic systolic function and the preload to the left ventricle, respectively. A thermodilution catheter (132F5; Edwards Lifesciences, Irvine, CA, USA) was positioned at right side of the heart through the right femoral vein. The cardiac output was measured using a standard thermodilution method with a cardiac output computer (MFC-1100; Nihon Kohden Corporation, Tokyo) and bolus injection of 5 mL of cold saline. In our previous studies using the same anesthetized canine model as used in this study,[17,18] the repeated volume load did not affect the cardiac output or cardiac contraction for >2 h of the observation period in the absence of pharmacological intervention. The total peripheral vascular resistance was calculated as mean blood pressure/cardiac output.

2.3. Measurement of electrophysiological variables

The P-wave duration, PR interval, QRS width, QT interval, J-T_{peak} and T_{peak}-T_{end} were measured in the lead II electrocardiogram. The QT interval was corrected with Van de Water's formula[19]: $QTcV = QT - 0.087 \times (RR - 1000)$ with RR given in ms, and the J-T_{peak} was done with Johannesen's formula[10]: $J\text{-}T_{peak}c = J\text{-}T_{peak}/RR^{0.58}$ with RR given in seconds.

A standard quad-polar electrodes catheter (Biosense Webster, Inc., Irvine, CA, USA) was positioned at the noncoronary cusp of the aortic valve through the left femoral artery to obtain the His-bundle electrogram. Another electrodes catheter (Biosense Webster, Inc.) was positioned at the sinus nodal area through the right femoral vein to electrically pace and to record the high right atrial electrogram. A MAP recording/pacing combination catheter (1675P; EP Technologies, Inc., Sunnyvale, CA, USA) was positioned at endocardium of the right ventricle through the left femoral vein to obtain MAP signals. The signals were amplified with a DC preamplifier (model 300; EP Technologies, Inc.). The MAP duration (ms) at 90 % repolarization level was defined as MAP_{90}.

The heart was electrically driven with a cardiac stimulator (SEC-3102; Nihon Kohden Corporation) through the pacing electrodes of the catheters placed at the sinus nodal region or at the right ventricle. The stimulation pulses were set rectangular in shape, consisting of 2–2.5 V amplitude (about twice the threshold voltage) and 1 ms duration. The MAP_{90} was measured during sinus rhythm ($MAP_{90(sinus)}$) and ventricular pacing at cycle lengths of 400 ms ($MAP_{90(CL400)}$) and 300 ms ($MAP_{90(CL300)}$). The VERP and AERP were assessed with programed electrical stimulation on the right ventricle and the sinus nodal region, respectively. The pacing protocol consisted of 5 beats of basal stimuli in cycle lengths of 400 ms ($VERP_{(CL400)}$) for VERP, and 400 ms ($AERP_{(CL400)}$), 300 ms ($AERP_{(CL300)}$) and 200 ms ($AERP_{(CL200)}$) for AERP followed by an extra stimulus of various coupling intervals. The coupling interval was shortened in 5-ms decrements until the additional stimulus could no longer elicit a response. The VERP and AERP were defined as the shortest coupling interval that can evoke stimulus-response. The terminal repolarization period of the ventricle was defined as the temporal difference between the $MAP_{90(CL400)}$ and $VERP_{(CL400)}$ at the same site. The atrial selectivity was estimated by the ratio of mean changes in the $AERP_{(CL400)}$ to that in the $VERP_{(CL400)}$.

2.4. Experimental protocol

The high right atrial electrogram, His-bundle electrogram, lead II electrocardiogram, aortic blood pressure, left ventricular pressure and MAP signals were monitored and recorded with a polygraph system (RM-6000; Nihon Kohden Corporation), which were analyzed with a data analysis system (WinVAS3 ver. 1.1R24; Physio-Tech Co., Ltd., Tokyo). Each measurement except for cardiac output, VERP or AERP was adopted for the mean of three recordings of consecutive complexes. The cardiohemodynamic and electrophysiological variables were assessed in the following order at each time point as follows. First, the high right atrial electrogram, His-bundle electrogram, lead II electrocardiogram, aortic blood pressure, left ventricular pressure and MAP signals were recorded under sinus rhythm. Second, the cardiac output was measured 3 times. Third, the MAP signals were recorded during the ventricular pacing at cycle lengths of 400 and 300 ms. Fourth, the VERP was measured at a basic pacing cycle length of 400 ms. Fifth, the AERP was assessed at basic pacing cycle lengths of 400, 300 and 200 ms. After the basal assessment, peramivir in a low dose of 1 mg/kg was intravenously administered over 10 min, and each variable was assessed at 5, 10, 15, 20 and 30 min after the start of administration. Then, the drug in a high dose of 10 mg/kg was infused over 10 min, and each variable was assessed at 5, 10, 15, 20, 30, 45 and 60 min after the start of administration.

2.5. Drugs

The clinical dose of peramivir is 300 mg/day for adult patients, which is intravenously infused over ≥15 min.[1] The maximum dose of 600 mg/day is allowed to be administered to patients in whom the symptoms may be exacerbated.[1] Given that the body weight of a patient is 60 kg, their therapeutic dose could be calculated as 5–10 mg/kg/day. In addition, its usual dose for pediatric patients is 10 mg/kg/day with a

maximum dose of 600 mg/day, which is administered in the same manner as that for adult patients.[2] Thus, in the current in vivo study, we selected the doses of 1 mg/kg and 10 mg/kg of peramivir, reflecting the subtherapeutic dose and clinically-recommended dose, respectively. The injectable solution of peramivir hydrate (RAPIACTA® for Intravenous Drip Infusion, 10 mg/mL as peramivir, Shionogi & Co., Ltd., Osaka) was used, and diluted with saline to obtain 1 mg/mL solution. The diluted solution and the original solution were infused at a rate of 1 mL/kg/min to the dogs to perform the administration of 1 and 10 mg/kg/10 min of peramivir, respectively. The other drugs used were thiopental sodium (Ravonal®, Mitsubishi-Tanabe Pharma Co., Osaka), isoflurane (Isoflurane inhalation solution [Pfizer], Mylan Seiyaku Ltd., Tokyo) and heparin calcium (Caprocin®, Sawai Pharmaceutical Co., Ltd., Osaka).

2.6. Statistical analysis

Data are presented as mean ± S.E.M. Differences within a parameter were evaluated with one-way, repeated-measures analysis of variance (ANOVA) followed by Fisher's LSD test which was employed as a post-hoc test for mean values comparison. Differences between parameters at each time point were assessed by paired *t*-test or one-way, repeated-measures ANOVA followed by Fisher's LSD test. The statistical analysis was performed using GraphPad Prism 8 (ver. 8.43; GraphPad Software, LLC, La Jolla, CA, USA). A p-value <0.05 was considered to be significant.

3. Results

No animals exerted any lethal ventricular arrhythmia or hemodynamic collapse leading to their death during the experimental period.

3.1. Effects on the cardiohemodynamic variables

Typical tracings of the aortic pressure and left ventricular pressure are shown in Fig. 1, whereas the time courses of the cardiohemodynamic variables are summarized in Fig. 2. The pre-treatment basal control values of the heart rate, mean blood pressure, cardiac output, total peripheral vascular resistance, $LVdP/dt_{max}$ and the left ventricular end-diastolic pressure were 114 ± 7 bpm, 105 ± 8 mmHg, 2.65 ± 0.56 L/min, 45 ± 8 mmHg min/L, 2496 ± 103 mmHg/s and 9 ± 2 mmHg, respectively. The low dose increased the cardiac output at 30 min, but it decreased the total peripheral vascular resistance at 15 and 30 min after the start of administration, whereas no significant change was detected in the other variables. The high dose increased the cardiac output, but decreased the total peripheral vascular resistance for 5–60 min after the start of administration, whereas no significant change was detected in the other variables.

3.2. Effects on the electrocardiographic variables

Typical tracings of the electrocardiogram are shown in Fig. 1, whereas the time courses of the electrocardiographic variables are summarized in Fig. 3. The pre-treatment control values (C) of the P-wave duration, PR interval, QRS width, QT interval, QTcV, J-T_{peak}c and T_{peak}-T_{end} were 53 ± 3 ms, 94 ± 6 ms, 54 ± 2 ms, 284 ± 10 ms, 325 ± 9, 188 ± 16 and 100 ± 6 ms, respectively. The low dose prolonged the QT interval and QTcV for 10–30 min after the start of infusion, whereas no significant change was detected in the other variables. The high dose prolonged the QT interval as well as QTcV for 5–60 min and the T_{peak}-T_{end} for 10–30 min and at 60 min after the start of administration, whereas no significant change was detected in the other variables.

3.3. Effects on the electrophysiological variables

Typical tracings of the His-bundle electrogram and MAP signals are shown in Fig. 1, whereas the time courses of the electrophysiological variables are summarized in Figs. 4 and 5. The pre-treatment control values (C) of the AH interval, HV interval, $MAP_{90(sinus)}$, $MAP_{90(CL400)}$, $MAP_{90(CL300)}$, $VERP_{(CL400)}$ and terminal repolarization period were 74 ± 6 ms, 28 ± 0 ms, 245 ± 7 ms, 235 ± 4 ms, 215 ± 7 ms, 213 ± 8 ms and 22 ± 6 ms, whereas those of $AERP_{(CL400)}$, $AERP_{(CL300)}$ and $AERP_{(CL200)}$

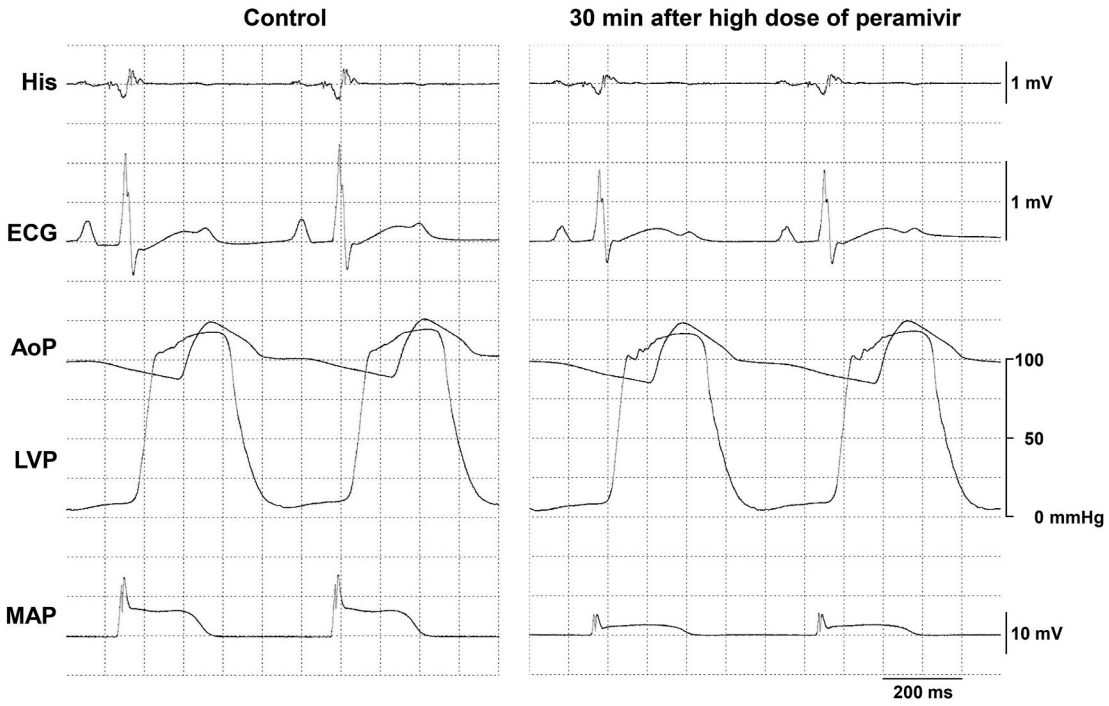

Fig. 1. Typical tracings showing the His-bundle electrogram (His), lead II electrocardiogram (ECG), aortic blood pressure (AoP), left ventricular pressure (LVP) and MAP signals (MAP) during sinus rhythm at pre-drug basal control (Control, left) and 30 min after the start of infusion of 10 mg/kg of peramivir (30 min after high dose of peramivir, right).

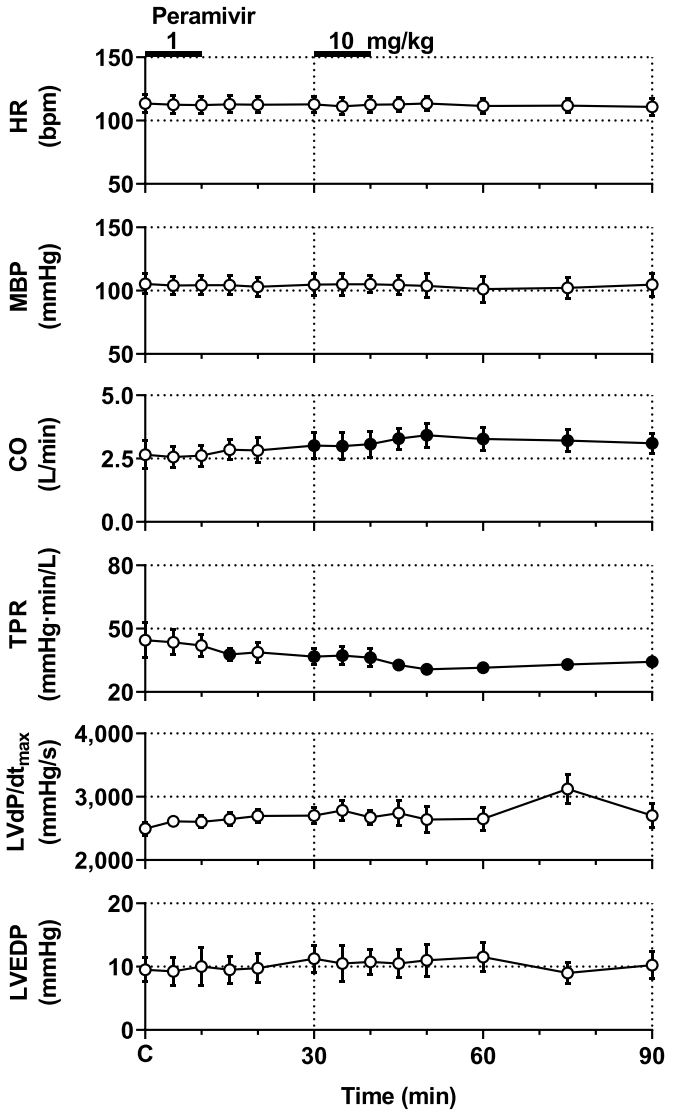

Fig. 2. Time courses of changes in the heart rate (HR), mean blood pressure (MBP), cardiac output (CO), total peripheral vascular resistance (TPR), maximum upstroke velocity of the left ventricular pressure ($LVdP/dt_{max}$) and left ventricular end-diastolic pressure (LVEDP) after the administration of peramivir. Data are presented as mean ± S.E.M. (n = 4). Closed symbols represent significant differences from each basal control value (C) by $p < 0.05$.

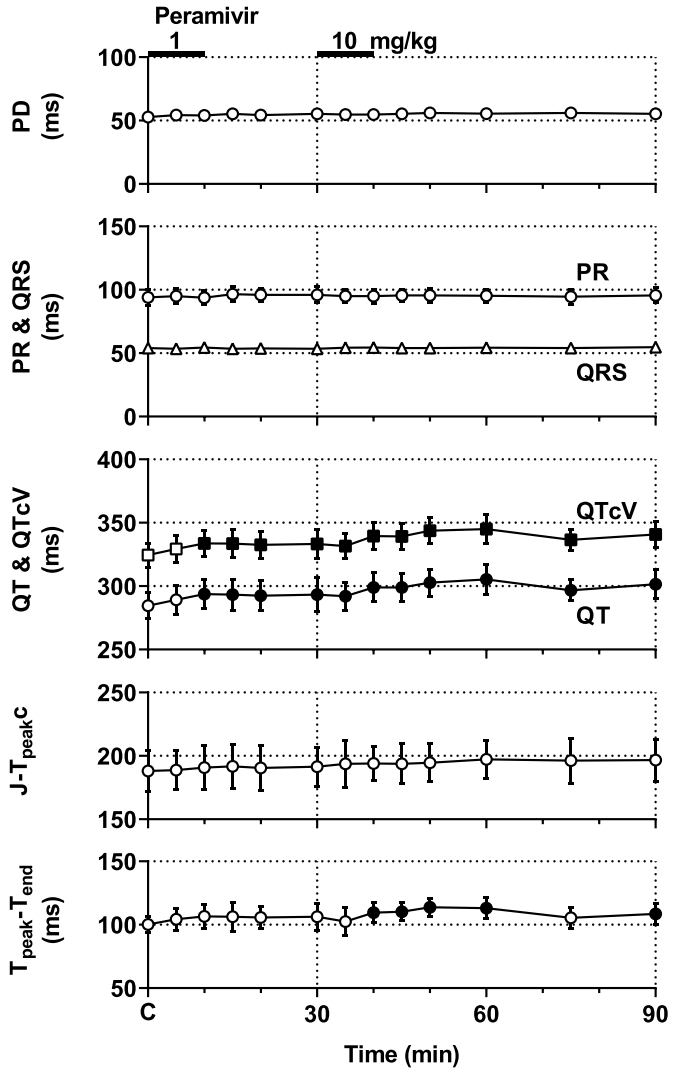

Fig. 3. Time courses of changes in the P-wave duration (PD), PR interval (PR), QRS width (QRS), QT interval (QT), QT interval corrected with Van de Water's formula (QTcV),[19] J-T_{peak} corrected with Johannesen's formula (J-T_{peak}c)[10] and T_{peak}-T_{end} after the administration of peramivir. Data are presented as mean ± S.E.M. (n = 4). Closed symbols represent significant differences from each basal control value (C) by $p < 0.05$.

were 164 ± 4 ms, 158 ± 5 ms and 150 ± 5 ms, respectively. The low dose prolonged the $AERP_{(CL400)}$ for 15–30 min after the start of administration, whereas no significant change was detected in the other variables (Figs. 4 and 5). The high dose prolonged the $MAP_{90(CL400)}$, $MAP_{90(CL300)}$, $VERP_{(CL400)}$ and $AERP_{(CL400)}$ for 5–60 min, and the $AERP_{(CL300)}$ for 10–60 min after the start of administration, whereas no significant change was detected in the other variables.

The extents of prolongation in the $MAP_{90(CL400)}$ and $MAP_{90(CL300)}$ were +25 ± 11 ms and +22 ± 11 ms, respectively at 30 min after the high dose administration when the prolongation of QT interval was the greatest, indicating that peramivir tended to prolong the ventricular repolarization in a reverse-frequency-dependent manner, although it did not achieve a statistical significance. The extents of prolongation in the $AERP_{(CL400)}$, $AERP_{(CL300)}$ and $AERP_{(CL200)}$ were +13 ± 1 ms, +6 ± 3 ms and +3 ± 1 ms at 20 min after the low dose administration, and +15 ± 5 ms, +13 ± 5 ms and +6 ± 2 ms at 60 min after the high dose administration, respectively when the prolongation of $AERP_{(CL400)}$ was the greatest. The increment of $AERP_{(CL400)}$ was greater than those of $AERP_{(CL300)}$ and $AERP_{(CL200)}$ after the low dose infusion, indicating its reverse-frequency-dependent prolongation, whereas no significant difference was detected among those extents after the high dose infusion. Atrial selectivity was calculated as a ratio of the increment of $AERP_{(CL400)}$ to that of $VERP_{(CL400)}$ when the magnitude of increment of $AERP_{(CL400)}$ was the greatest, which was 1.3 at 60 min after the high dose administration as shown in Table 1.

4. Discussion

We assessed the cardiovascular profile of peramivir. The clinical dose exposure of peramivir exerted electrophysiological actions on the ventricle as well as atrium in addition to vasodilator action. The clinical free C_{max} after the intravenous administration of 600 mg of peramivir over 15 min could be estimated as 66.4 μg/mL (202 μmol/L) supposing the linear relationship is conserved between the intravenous dose and its C_{max} in a previous phase I study for peramivir.[21] Therefore, a similar or higher peak plasma concentration could be attained after the high-dose infusion (10 mg/kg/10 min i.v.) in the current study. Those current findings along with the previous knowledge were precisely analyzed as

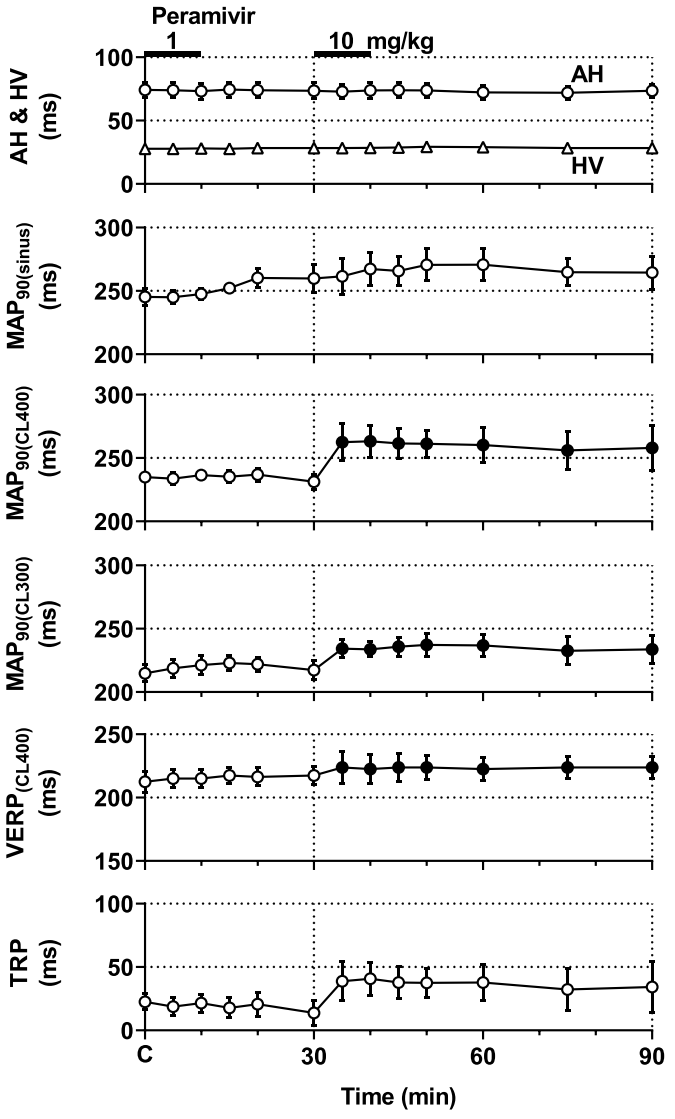

Fig. 4. Time courses of changes in the atrio-His (AH) and His-ventricular intervals (HV); monophasic action potential duration at 90 % repolarization level during sinus rhythm ($MAP_{90(sinus)}$), and ventricular pacing at cycle lengths of 400 ms ($MAP_{90(CL400)}$) and 300 ms ($MAP_{90(CL300)}$); ventricular effective refractory period at a basic pacing cycle length of 400 ms ($VERP_{(CL400)}$); and terminal repolarization period (TRP) after the administration of peramivir. Data are presented as mean ± S.E.M. (n = 4). Closed symbols represent significant differences from each basal control value (C) by $p < 0.05$.

discussed below.

4.1. Cardiohemodynamic effects

Peramivir decreased the total peripheral vascular resistance in a dose-related manner, indicating its vasodilator effect in vivo. In the EMA assessment report,[7] binding inhibition of 25.5 % was found for human α_1-adrenoceptors by 10 μmol/L of peramivir, suggesting that the vasodilator effect in this study could be associated with its α_1-adrenoceptor blockade. Meanwhile, peramivir tended to slightly elevate the $LVdP/dt_{max}$, and increased the cardiac output, which kept the mean blood pressure unaltered, suggesting the occurrence of vasodilator action-induced reflex-mediated increase of sympathetic tone. However, peramivir did not exert the positive chronotropic action. Since peramivir could inhibit K^+ current including I_{Kr} as discussed below, it might delay the repolarization of the sinus node,[22] which may have counteracted the impact of increased sympathetic tone. Thus, peramivir-induced vasodilator response could be within the range of autonomic regulation.

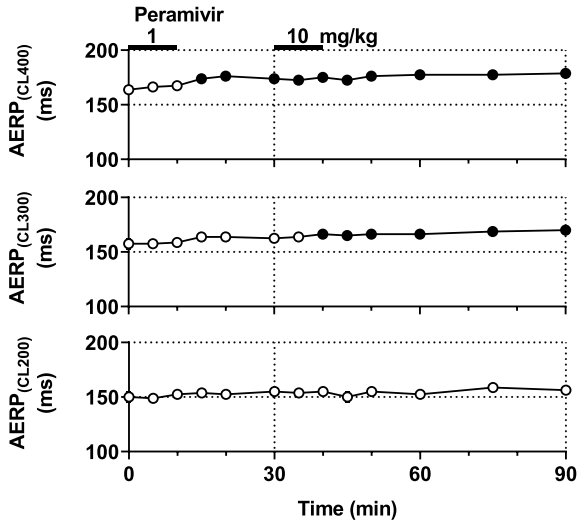

Fig. 5. Time courses of changes in the atrial effective refractory period at basic pacing cycle lengths of 400 ms ($AERP_{(CL400)}$), 300 ms ($AERP_{(CL300)}$) and 200 ms ($AERP_{(CL200)}$) after the administration of peramivir. Data are presented as mean ± S.E.M. (n = 4). Closed symbols represent significant differences from each basal control value (C) by $p < 0.05$.

Table 1
Effects of anti-influenza drugs on the proarrhythmic and anti-atrial fibrillatory surrogate markers.

Drugs	Peramivir	Oseltamivir	Amantadine hydrochloride
Doses	10 mg/kg, i.v.	30 mg/kg, i.v.	10 mg/kg, i.v.
Proarrhythmic surrogate markers			
Elapsed time (min)	30	10	10
ΔQTcV	+20*	+20*	+42*
ΔJ-T_{peak}c	+9	+4	+10
ΔT_{peak}-T_{end} (ms)	+13*	+12	+33*
ΔTRP (ms)	+15	−4	+14
Anti-atrial fibrillatory surrogate markers			
Elapsed time (min)	60	10	60
Δ$AERP_{(CL400)}$ (ms)	+15*	+81*	NA
Δ$VERP_{(CL400)}$ (ms)	+11*	+4	+16
Atrial selectivity	1.3	20.3	NA

The values represent the changes from each pre-drug basal control value (Δ) for the proarrhythmic (upper) and anti-atrial fibrillatory (lower) surrogate markers when the magnitude of increments of QTcV and $AERP_{(CL400)}$ was the greatest, respectively. Atrial selectivity was calculated as a ratio of Δ$AERP_{(CL400)}$ to Δ$VERP_{(CL400)}$. The results of oseltamivir and amantadine hydrochloride were obtained from our previous reports.[15,16,20] *$p < 0.05$ v.s. pre-drug basal control value (C). QTcV: QT interval corrected with Van de Water's formula[19]; J-T_{peak}c: J-T_{peak} corrected with Johannesen's formula[10]; TRP: terminal repolarization period; AERP: atrial effective refractory period; VERP: ventricular effective refractory period; CL: basic pacing cycle length; and NA: not available.

4.2. Electrophysiological effects

Peramivir altered neither the P-wave duration, PR interval and QRS width, nor the AH interval and HV interval, indicating that it will not impact the intra-atrial, atrioventricular or intra-ventricular conduction. These results indicate that the therapeutic dose of peramivir may not inhibit Na^+ or Ca^{2+} channel in vivo. On the other hand, peramivir prolonged the T_{peak}-T_{end} besides the QT interval/QTcV prolongation in a dose-related manner. Moreover, peramivir prolonged the MAP_{90} with a

tendency for a reverse-frequency dependent profile which is typically observed for I_{Kr} blockers.[23] These findings indicate that the ventricular repolarization delay may be associated with I_{Kr} inhibition, although peramivir in a concentration of 300 μmol/L was reported to hardly inhibit I_{Kr} expressed in HEK293 cells according to the information from manufacturer (Shionogi & Co., Ltd.). Thus, one can speculate that other molecular mechanisms including I_{K1} inhibition would also play a role in peramivir-induced ventricular repolarization delay in vivo.

4.3. Proarrhythmic effects on the ventricle

Peramivir prolonged the T_{peak}-T_{end} (Fig. 3) and also tended to prolong the terminal repolarization period (Fig. 4), which will increase the transmural dispersion of repolarization and the local electrical vulnerability of ventricles, respectively, leading to the formation of substrates for initiating and maintaining the spiral reentry. Meanwhile, peramivir did not prolong the J-T_{peak}c, which may indicate a lack of increase in net inward current during plateau phase of action potential, suggesting that peramivir will not induce myocardial Ca^{2+} overload leading to the onset of early afterdepolarization playing as a trigger for TdP. Since peramivir will not provide the trigger despite developing the substrates, its potential to develop TdP will be small.

To better characterize the proarrhythmic risk of peramivir, its effects on the proarrhythmic surrogate markers were compared with those of anti-influenza drugs amantadine and oseltamivir when the magnitude of increment of QTcV was the greatest, which was obtained from our previous studies using the halothane-anesthetized dogs (Table 1).[15,16] The extent of prolongation of J-T_{peak}c was in the order of amantadine $\geq$ peramivir $>$ oseltamivir, indicating that the risk of peramivir to induce Ca^{2+} overload may be greater among anti-influenza drugs. The magnitude of prolongation of T_{peak}-T_{end} was in the order of amantadine $\gg$ peramivir $\geq$ oseltamivir, suggesting that the risk of peramivir for increasing the transmural dispersion of repolarization will be smaller. The increment of terminal repolarization period was in the order of peramivir $\geq$ amantadine, whereas oseltamivir shortened it, suggesting that the risk of peramivir to increase the local electrical vulnerability will be greater. Accordingly, the integrated proarrhythmic risk analysis based on the findings of three surrogate markers indicates that the risk of peramivir may be greater than that of oseltamivir, whereas it would be similar to or less than that of amantadine.

4.4. Electrophysiological effects on the atrium

The increment of $AERP_{(CL400)}$ was greater than those of $AERP_{(CL300)}$ and $AERP_{(CL200)}$ after the low dose infusion, indicating its reverse frequency-dependent prolongation. A similar trend was observed among those after the high-dose infusion. These results suggest that peramivir-induced AERP prolongation may be associated with the I_{Kr} inhibition in addition to alternative mechanisms as discussed for the ventricular repolarization delay.[23]

Since we have demonstrated that neuraminidase inhibitor oseltamivir has electrophysiological effects on both the atrium and ventricle, exerting potent anti-atrial fibrillatory action on the in vivo arrhythmia model of dogs,[20,24] we explored such potential of peramivir. AERP is known to be a surrogate marker to predict anti-atrial fibrillatory action.[20,24] Since the cycle length of atrial fibrillation is known to be 85–190 ms,[25,26] $AERP_{(CL200)}$ is considered to be able to better predict the anti-atrial fibrillatory effect than $AERP_{(CL400)}$ or $AERP_{(CL300)}$. However, peramivir hardly prolonged the $AERP_{(CL200)}$, suggesting that its anti-atrial fibrillatory efficacy will be limited.

To better characterize the atrial electrophysiological properties of peramivir, its effects on $AERP_{(CL400)}$ and atrial selectivity over the ventricle were compared with those of oseltamivir (Table 1) when the magnitude of increment of $AERP_{(CL400)}$ was the greatest.[20] The extent of prolongation of $AERP_{(CL400)}$ was >5 times smaller for peramivir than for oseltamivir, suggesting that anti-atrial fibrillatory efficacy could not be expected for peramivir. Moreover, the atrial selectivity was >15 times lower for peramivir than for oseltamivir, making it difficult to increase the dose of peramivir for prolonging the AERP without modulating the VERP.

4.5. Clinical implication and study limitations

We discussed why the thorough QT/QTc study failed to detect peramivir-induced QT-interval prolongation as follows. First, some patients who have a decreased ventricular repolarization reserve could be included in the phase III studies, whereas subjects having such factors are commonly excluded from the thorough QT/QTc study. Second, in some patients with influenza virus infection, hyperthermia would induce dehydration, leading to a decrease in systemically circulating plasma volume[27] followed by reductions of renal blood flow and glomerular filtration rate.[28] The reduced plasma volume may increase the peak plasma concentration of peramivir. In addition, since approximately 90 % of intravenously administered peramivir was excreted into the urine in unchanged form,[21] the decreased renal blood flow and glomerular filtration rate could maintain higher plasma concentration of peramivir. These speculations along with our current findings could partly explain why peramivir prolonged the QTc only in patients with influenza virus infection.[5–7]

While thorough QT/QTc study is useful for judging the presence or absence of drug-induced QT-interval prolongation in human subjects, QT-interval prolongation by itself cannot necessarily predict the onset of TdP.[10,11] To overcome this limitation, several surrogate markers have been proposed and are now being discussed in ICH E14/S7B Q&A stage 2.[29] In this study, clinically-relevant dose of peramivir delayed the ventricular repolarization; moreover, it could develop the substrates for initiating and maintaining spiral reentry in the isoflurane-anesthetized dog, implicating that peramivir would exert similar electrophysiological impacts on the hearts of patients. Moreover, in a previous study using perfused canine ventricular wedge preparations, I_{Kr} blocker E−4031 significantly prolonged the action potential duration of M cell in a temperature-dependent manner, which was not observed in epicardial or endocardial tissue.[30] Thus, in patients with influenza virus infection-induced hyperthermia, I_{Kr} inhibition potentially induced by peramivir might further increase the transmural dispersion of repolarization as a substrate for TdP. Meanwhile, peramivir did not provide the trigger for TdP. Thus, caution should be paid on the use of peramivir for patients who formerly have the trigger for the onset of TdP, including congenital long QT syndrome, chronic heart failure, concomitant use of drugs enhancing the QT-interval prolongation and/or electrolyte disturbance.[31] In addition, since peramivir slightly but significantly prolonged the AERP and VERP, it could increase the threshold for electrical pacing and defibrillation like some antiarrhythmic drugs, which may induce functional failure of implanted pacemaker and/or cardioverter-defibrillator.[32,33]

There are several limitations in this study. First, we compared current findings of peramivir obtained under the isoflurane anesthesia with those of oseltamivir and amantadine done under the halothane anesthesia (Table 1).[15,16,20] In our previous comparative studies using the same group of beagle dogs,[8,9] drug-induced cardiovascular responses were qualitatively and quantitatively similar between the isoflurane and halothane anesthesia, indirectly providing the rationale for currently performed direct comparisons of the effects of those three drugs on the proarrhythmic and anti-atrial fibrillatory surrogate markers. Second, the plasma concentration after the infusion of peramivir was not measured in this study, since we intravenously administered its clinically relevant dose. Third, the in vitro molecular mechanisms of electrophysiological effects of peramivir for the ventricular late repolarization delay were not fully assessed, which need to be elucidated.

5. Conclusions

The clinical dose exposure of peramivir will delay the ventricular repolarization in patients, which may not produce the trigger for inducing TdP but can develop its substrates. While peramivir has only a small potential to induce TdP in the intact heart, caution should be paid on its use for patients formerly having the trigger for the onset of TdP. Since peramivir can prolong the atrial and ventricular electrical refractoriness, it may have some potential to induce functional failure of an implanted pacemaker and/or cardioverter-defibrillator.

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Declaration of competing interest

The authors declare no conflicts of interest.

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