

Original article

Electropharmacological characterization of aciclovir in the halothane-anesthetized dogs:
a proposal of evaluation method for cardiovascular safety pharmacology of anti-virus
drugs

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Running title: Cardiovascular effects of aciclovir

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Abstract

Given limited information regarding the pathophysiology underlying aciclovir-associated, clinically-observed cardiovascular adverse events including chest pain, tachycardia, bradycardia, palpitation, arrhythmia, hypertension and hypotension, we investigated its electropharmacological effects using the halothane-anesthetized beagle dogs. Aciclovir in doses of 2 and 20 mg/kg was sequentially infused over 10 min with an interval of 20 min (n=4), which would achieve sub-therapeutic to supra-therapeutic levels of plasma concentrations. Aciclovir decreased the total peripheral vascular resistance along with the blood pressure in a dose-related manner, which increased the heart rate, ventricular contraction and atrioventricular nodal conduction speed probably via a reflex-mediated increase of sympathetic tone. No significant change was detected in the intra-atrial or intra-ventricular conduction, indicating that aciclovir may not inhibit atrial or ventricular I_{Na} . Aciclovir prolonged the repolarization period in a dose-related as well as in a reverse frequency-dependent manners, indicating that aciclovir may inhibit I_{Kr} , which was supported by the T_{peak} - T_{end} prolongation. Aciclovir transiently prolonged the J- T_{peak} c possibly through a reflex-mediated increase of sympathetic tone, indicating an increase of net inward current in the early repolarization phase. Thus, aciclovir may directly inhibit I_{Kr} , and also have the potential to indirectly induce Ca^{2+} overload leading to early afterdepolarization. These in vivo electropharmacological profile of aciclovir would partly explain the onset mechanism of clinical adverse events.

Keywords: Aciclovir, Anti-virus drug, Cardiovascular adverse events, I_{Kr} inhibition, QT prolongation

Introduction

Various off-target cardiovascular effects of anti-virus drugs have been reported [1-6]. For example, oseltamivir decreased the blood pressure, and suppressed the sinus automaticity, ventricular contraction and atrioventricular as well as intraventricular conduction in addition to the repolarization delay [1]. Meanwhile, amantadine increased the blood pressure, enhanced the ventricular contraction and atrioventricular conduction, but suppressed the intraventricular conduction in addition to the repolarization delay [2]. Moreover, vidarabine has been shown to improve the left ventricular ejection fraction and survival rate of experimentally-induced congestive heart failure model of mice [3], but hardly to affect the repolarization process in dogs [4]. Notably, both oseltamivir and vidarabine have been suggested to be able to suppress atrial fibrillation [5,6].

Aciclovir is an acyclic guanosine derivative with clinical activity against the infections of herpes simplex virus and varicella zoster virus [7]. It has been described in a case report [8] as well as in an interview form from the manufacturer that aciclovir may be associated with cardiovascular adverse events including chest pain, tachycardia, bradycardia, palpitation, arrhythmia, hypertension and hypotension. However, information is still limited regarding the causal link between the administration of aciclovir and these cardiovascular adverse events. In order to better understand the pathophysiology underlying those adverse events, we precisely evaluated electropharmacological effects of aciclovir using the halothane-anesthetized beagle dogs which were demonstrated to be able to mimic the drug-induced, cardio-mechanical and electrophysiological responses observed in human subjects [1,2,4,9].

We adopted the experimental protocol previously used for amantadine and oseltamivir [1,2], since it is one of the most established methods for safety pharmacological evaluation of drugs [9]. The results of aciclovir were compared with those of amantadine and oseltamivir in those studies [1,2] to better analyze the aciclovir-associated, clinically-observed cardiovascular adverse events.

Materials and methods

Experiments were performed in female beagle dogs weighing approximately 10 kg (n=4). Animals were obtained through Kitayama Labes Co., Ltd. (Nagano, Japan).

General anesthesia and surgical preparation

The dogs were initially anesthetized with thiopental sodium (30 mg/kg, i.v.). After intubation with a cuffed endotracheal tube, anesthesia was maintained by inhalation of halothane (1% v/v) vaporized in oxygen with a volume-limited ventilator (SN-480-3; Shinano Manufacturing Co., Ltd., Tokyo, Japan). Tidal volume and respiratory rate were set at 20 mL/kg and 15 breaths/min, respectively. Four clinically available catheter-sheath sets (FAST-CATHTM 406119; St. Jude Medical Daig Division, Inc., MN, USA) were used; two were inserted into the right and left femoral arteries toward abdominal aorta, and the other two were done into the right and left femoral veins toward inferior vena cava, respectively. Heparin calcium (100 IU/kg) was administered to prevent the blood clotting through a flush line of the catheter sheath placed at the right femoral vein.

Cardiohemodynamic variables

A pig-tail catheter was placed at the left ventricle through the right femoral artery to measure the left ventricular pressure, whereas aortic pressure was measured at a space between the inside of the catheter sheath and the outside of the pig-tail catheter through a flush line. Left ventricular pressure at a time point of peak of R wave on electrocardiogram was defined as the left ventricular end-diastolic pressure [10]. The maximum upstroke velocity of the left ventricular pressure (LVdP/dt_{max}) and the left ventricular end-diastolic pressure were obtained during sinus rhythm to estimate the contractility and the preload to the left ventricle, respectively. A thermodilution catheter (TC-504NH; Nihon Kohden Corporation, Tokyo, Japan) was positioned at the right side of the heart through the right femoral vein. The cardiac output was measured by using a standard thermodilution method with a cardiac output computer (MFC-1100, Nihon Kohden Corporation). The total peripheral vascular resistance was calculated with the basic equation: total peripheral vascular resistance=mean blood pressure/cardiac output.

Electrophysiological variables

The lead II electrocardiogram was obtained from the limb electrodes. The

P-wave duration, PR interval, QRS width and QT interval were measured, and QT interval was corrected with Van de Water's formula: $QT_c = QT - 0.087 \times (RR - 1,000)$ with RR given in ms [11]. The $J-T_{peak}$ and $T_{peak}-T_{end}$ were measured as previously described [2]. When the end of T-wave was obscure, we used the monophasic action potential (MAP) signal as a guide to estimate the end. The $J-T_{peak}$ was corrected for the heart rate with a coefficient as previously described: $J-T_{peak}^c = J-T_{peak} / RR^{0.58}$ with RR given in seconds [12]. Correction was not performed on the $T_{peak}-T_{end}$, since previous QT/QTc studies have shown that the $T_{peak}-T_{end}$ exhibited minimal heart rate dependency at the resting heart rate [12].

A standard 6-French, quad-polar electrodes catheter (Cordis-Webster Inc., Baldwin Park, CA, USA) was positioned at the non-coronary cusp of the aortic valve through the left femoral artery to obtain the His bundle electrogram. A bi-directional steerable MAP recording/pacing combination catheter (1675P; EP Technologies, Inc., Sunnyvale, CA, USA) was positioned at the endocardium of the interventricular septum in 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 duration of the MAP signals was measured as an interval, along a horizontal line corresponding to the diastolic baseline, 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 with a cardiac stimulator (SEC-3102; Nihon Kohden Corporation) via the pacing electrodes of the combination catheter placed in the right ventricle. The stimulation pulses were rectangular in shape, 2-2.5 V (about twice the threshold voltage) and 1 ms duration. The MAP_{90} of the ventricle was measured during sinus rhythm ($MAP_{90(sinus)}$) and at a pacing cycle length of 400 ms ($MAP_{90(CL400)}$) and 300 ms ($MAP_{90(CL300)}$). The ventricular effective refractory period (VERP) was assessed with the programmed electrical stimulation. The pacing protocol consisted of 5 beats of basal stimuli in a cycle length of 400 ms followed by an extra stimulus of various coupling intervals. Starting in the late diastole, the coupling interval was shortened in 5-ms decrements until the additional stimulus could no longer elicit a response. The VERP was defined as the shortest coupling interval that could produce a response. The terminal repolarization period of the ventricle, reflecting phase-3 repolarization of the action potential, was calculated by the difference between the $MAP_{90(CL400)}$ and VERP; terminal repolarization period = $MAP_{90(CL400)} - VERP$, at the same site to estimate the extent of electrical vulnerability of the ventricular muscle [9,13].

Experimental protocol

The aortic pressure, left ventricular pressure, electrocardiogram, His bundle electrogram and MAP signals were monitored with a polygraph system (RM-6000, Nihon Kohden Corporation) and analyzed by using a real-time fully automatic data analysis system (Win VAS 3 for Windows ver. 1.1R24v; Physio-Tech Co., Ltd., Tokyo, Japan). Three recordings of consecutive complexes were used to calculate the mean for the electrocardiogram indices, MAP duration as well as atrio-His (AH) and His-ventricular (HV) intervals. The cardiovascular variables were assessed in the following order. The electrocardiogram, His bundle electrogram, aortic pressure, left ventricular pressure and MAP signals were recorded under sinus rhythm. Then, the cardiac output was measured 3 times. Next, MAP signals were recorded during the ventricular pacing at a cycle length of 400 and 300 ms. Finally, VERP was measured. All parameters described above were usually obtained within 2 min at each time point. After the basal assessment, aciclovir in a low dose of 2 mg/kg was intravenously infused through the catheter sheath placed at the left femoral vein over 10 min, and each variable was recorded at 5, 10, 15, 20 and 30 min after the start of administration (n=4). Then, aciclovir in a high dose of 20 mg/kg was infused in the same manner, and each variable was recorded at 5, 10, 15, 20, 30, 45 and 60 min after the start of administration (n=4).

Drugs

Aciclovir (Zovirax[®] for I.V. Infusion 250, GlaxoSmithKline K.K., Tokyo, Japan) was diluted with saline in a concentration of 2 and 20 mg/mL. The other drugs used were thiopental sodium (Ravonal[®] 0.5 g for Injection, Mitsubishi-Tanabe Pharma Co., Osaka, Japan), halothane (Fluothane[®], Takeda Pharmaceutical Co., Ltd., Osaka, Japan) and heparin calcium (Caprocin[®], Sawai Pharmaceutical Co., Ltd., Osaka, Japan).

Statistical analysis

Data are presented as mean±SEM. Differences within a parameter were evaluated with one-way, repeated-measures analysis of variance (ANOVA) followed by Contrasts as a post-hoc test for mean values comparison, whereas those between the extents of the prolongation in the MAP_{90(sinus)}, MAP_{90(CL400)} and MAP_{90(CL300)} were assessed by two-way, repeated-measures ANOVA. A *p* value <0.05 was considered to be significant.

Results

No animals experienced any lethal ventricular arrhythmias or hemodynamic collapse leading to death during the experiments.

Effects on cardiohemodynamic variables

Typical tracings of the aortic and left ventricular pressures are depicted in Fig. 1, and the time courses of changes in the heart rate, mean blood pressure, cardiac output, total peripheral vascular resistance, $LVdP/dt_{max}$ and left ventricular end-diastolic pressure are summarized in Fig. 2 (n=4). Their pre-drug control values (C) were 103 ± 7 beats/min, 108 ± 4 mmHg, 2.06 ± 0.32 L/min, 57 ± 10 mmHg/L/min, $2,030 \pm 195$ mmHg/s and 11 ± 1 mmHg, respectively. The low dose increased the cardiac output for 10-20 min and the $LVdP/dt_{max}$ at 15 min, but decreased the total peripheral vascular resistance at 10 and 20 min; whereas no significant change was detected in the other variables. The high dose increased the heart rate for 10-15 min but decreased it for 45-60 min. It also increased the cardiac output for 5-30 min and the $LVdP/dt_{max}$ for 5-60 min, but decreased the mean blood pressure at 10 and 30 min, the total peripheral vascular resistance for 5-30 min and the left ventricular end-diastolic pressure for 5-60 min.

Effects on electrocardiographic variables

Typical tracings of the effects of aciclovir on the electrocardiogram are depicted in Fig. 1, and the time courses of changes in the electrocardiographic variables are summarized in Fig. 3 (n=4). The pre-drug basal control values (C) of the PR interval, QRS width, QT interval, QTc, P-wave duration, $J-T_{peakc}$ and $T_{peak}-T_{end}$ were 106 ± 2 ms, 61 ± 1 ms, 250 ± 10 ms, 285 ± 7 , 58 ± 2 ms, 243 ± 8 and 72 ± 8 ms, respectively. The low dose prolonged the QT interval, QTc and $T_{peak}-T_{end}$ for 10-30 min, but shortened the PR interval for 10-15 min; whereas no significant change was observed in the other variables. The high dose prolonged the QT interval, QTc, $T_{peak}-T_{end}$ for 5-60 min and the $J-T_{peakc}$ for 5-15 min, but shortened the PR interval for 5-30 min; whereas no significant change was detected in the QRS width or P-wave duration.

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

Typical tracings of the His bundle electrogram and MAP are depicted in Fig. 1, and the time courses of changes in the AH and HV intervals, and $MAP_{90(sinus)}$ are summarized in Fig. 4 (n=4). Their pre-drug control values (C) were 81 ± 3 ms, 28 ± 2 ms

and 232 ± 9 ms, respectively. The low dose prolonged the $MAP_{90(\text{sinus})}$ at 30 min, whereas no significant change was detected in the other variables. The high dose prolonged the $MAP_{90(\text{sinus})}$ at 5 min and for 20-60 min, but shortened the AH interval for 5-30 min; whereas no significant change was observed in the HV interval.

Effects on MAP_{90} , VERP and terminal repolarization period during the electrical pacing

The time courses of changes in the $MAP_{90(\text{CL400})}$, $MAP_{90(\text{CL300})}$, VERP and terminal repolarization period are summarized in Fig. 4 (n=4). Their pre-drug control values (C) were 227 ± 5 ms, 209 ± 4 ms, 195 ± 5 ms and 32 ± 4 ms, respectively. The low dose hardly altered any of these variables. The high dose prolonged the $MAP_{90(\text{CL400})}$ and VERP for 30-60 min, whereas no significant change was detected in the $MAP_{90(\text{CL300})}$ or terminal repolarization period. In addition, the extent of prolongation in the $MAP_{90(\text{sinus})}$, $MAP_{90(\text{CL400})}$ and $MAP_{90(\text{CL300})}$ from their respective pre-drug control values (C) were calculated, which was more prominent at slower ventricular rate for 30-60 min after the high dose, indicating the reverse frequency-dependent property of aciclovir on the repolarization delay (Table 1).

Discussion

Our findings suggest that aciclovir has potential to directly inhibit I_{Kr} and to indirectly induce Ca^{2+} overload in the ventricular muscles.

Rationale for the drug doses used in this study

The clinically recommended intravenous dose of aciclovir for treating herpes encephalitis was 5-10 mg/kg for 1 h every 8 h, whereas the C_{max} after its intravenous administration in doses of 5 and 10 mg/kg for 1 h to healthy human subjects was 8.1 and 14.8 $\mu\text{g/mL}$, respectively according to the interview form from the manufacturer. Based on our previous experiments using the same halothane-anesthetized dogs as used in this study [9], the peak plasma concentration after the intravenous administration of 1 mg/kg over 10 min of a drug could be roughly estimated to be 1 $\mu\text{g/mL}$. Thus, the doses of 2 and 20 mg/kg of aciclovir in this study would provide approximately 2 and 20 $\mu\text{g/mL}$, respectively, suggesting that currently used doses of aciclovir would achieve sub-therapeutic to supra-therapeutic levels of plasma concentrations. In addition, it has been reported that the clearance, volume of distribution and half-life were 3.48-5.83 mL/min/kg, 0.97-1.17 L/kg and 2.2-3.6 h in dogs, and that those were 4.71 mL/min/kg, 0.69 L/kg and 2.4 h for human subjects, respectively [14,15]. This pharmacokinetic information would help to extrapolate currently obtained pharmacodynamic results in the dogs to human subjects.

Cardiohemodynamic effects

The low dose of aciclovir slightly but significantly decreased the total peripheral vascular resistance, mildly enhancing the reflex-mediated sympathetic tone, which may have increased the ventricular contraction and cardiac output along with modest increase of the heart rate that kept the mean blood pressure with a trivial decrease of -1 mmHg at 10 min. Meanwhile, the high dose further decreased the total peripheral vascular resistance, more potently enhancing the reflex-mediated sympathetic tone, which further increased the ventricular contraction and cardiac output along with significant increase of the heart rate that also made the mean blood pressure with a small but significant decrease of -4 mmHg at 10 and 30 min. Since it is known that the relationship between the drug concentration and its cardiohemodynamic responses confirmed in the halothane-anesthetized dogs can well mimic that in human subjects [9], currently observed cardiohemodynamic effects could be in part extrapolated to patients treated with clinically recommended intravenous dose of aciclovir. It should be also

noted that the high dose decreased the heart rate for 45-60 min after the administration, which could be explained by its I_{Kr} inhibitory action as discussed below. In addition, the high dose decreased the left ventricular end-diastolic pressure, which can be secondarily caused by the increased cardiac output and/or may suggest the presence of a direct venodilator effect of aciclovir.

Electrophysiological effects

There is no report describing the in vitro effects of aciclovir on the cardiac ionic currents including I_{Kr} , I_{Ks} etc. or the in vitro/ex vivo action potentials. Aciclovir enhanced the atrioventricular nodal conduction in a dose-related manner, which could be explained by the reflex-mediated increase of sympathetic tone. No significant change was detected in the intra-atrial or intra-ventricular conduction, suggesting that even a supra-therapeutic concentration of aciclovir may not inhibit atrial or ventricular I_{Na} . Meanwhile, aciclovir delayed the repolarization in a dose-related as well as in a reverse frequency-dependent manners, which suggests that aciclovir could inhibit I_{Kr} . This hypothesis can be confirmed by the currently observed prolonging effect of aciclovir on the $T_{peak}-T_{end}$, of which prolongation largely depends on the I_{Kr} inhibition [12]. It could be also supported by the time course of the $T_{peak}-T_{end}$, in which the extent of prolongation was transiently attenuated for 10-15 min after the high dose administration along with the increase of heart rate, reflecting the reverse frequency-dependent I_{Kr} inhibition by aciclovir. In addition, the high dose significantly prolonged the $J-T_{peakc}$ for 5-15 min, indicating increase of net inward current in the early repolarization phase, which may be explained by the increase of inward I_{Ca} induced by the reflex-mediated increase of sympathetic tone, that favors initiation of arrhythmia through an occurrence of early afterdepolarization [12,13,16].

Comparison of the effects of anti-virus drugs on the electrophysiological variables

In order to better characterize the electrophysiological effects of aciclovir, we compared them with those of amantadine and oseltamivir using our previous studies [1,2]. Each effect was compared when the drug-induced QT prolongation was the greatest as shown in Table 1. Each drug delayed the ventricular repolarization (QT, QTc and MAP_{90}) along with the prolongation of late repolarization period ($T_{peak}-T_{end}$), whereas the effects on the early repolarization period ($J-T_{peakc}$) varied among the drugs. It should be noted that when the extent of QT prolongation was the greatest, aciclovir slightly shortened the early repolarization period unlike amantadine and oseltamivir, indicating that aciclovir will not increase net inward current in this period. Meanwhile,

aciclovir as well as amantadine prolonged the repolarization phase in a reverse frequency-dependent manner, whereas oseltamivir showed a frequency-dependent prolongation of repolarization. While the formers may reflect their I_{Kr} -inhibitory property, the latter might be associated with I_{Ks} suppression. The terminal repolarization period has been used as a reliable marker for estimating a potential risk for re-entrant arrhythmias [9,13]. Aciclovir as well as oseltamivir hardly altered this marker, whereas amantadine tended to prolong it, indicating that aciclovir and oseltamivir may have smaller risk for re-entrant arrhythmias than amantadine.

Conclusions

Aciclovir decreased the total peripheral vascular resistance along with the reduction of blood pressure, which may enhance the sinus automaticity, ventricular contraction and atrioventricular conduction via indirect sympathomimetic mechanism, partly explaining the clinically observed adverse events. Importantly, the electropharmacological analyses on aciclovir-induced repolarization delay suggest that aciclovir may directly inhibit I_{Kr} , and also have potential to induce Ca^{2+} overload leading to early afterdepolarization, although it may have small risk toward re-entrant arrhythmia. Thus, these electropharmacological profile of aciclovir along with amantadine and oseltamivir can become a reliable guide to systematically analyze unmet cardiovascular adverse events of anti-virus drugs.

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Compliance with Ethical Standards

Conflicts of interest: The authors declare no conflicts of interest.

Ethical Approval: All experiments were approved by the Toho University Animal Care and User Committee (No. 18-51-395 and No. 19-52-395) and performed according to the Guideline for the Care and Use of Laboratory Animals of Toho University.

References

- 1 Kitahara K, Nakamura Y, Tsuneoka Y, Adachi-Akahane S, Tanaka H, Yamazaki H, et al. Cardiohemodynamic and electrophysiological effects of anti-influenza drug oseltamivir in vivo and in vitro. *Cardiovasc Toxicol.* 2013;13:234-43.
- 2 Cao X, Nakamura Y, Wada T, Izumi-Nakaseko H, Ando K, Sugiyama A. Electropharmacological effects of amantadine on cardiovascular system assessed with $J-T_{\text{peak}}$ and $T_{\text{peak}}-T_{\text{end}}$ analysis in the halothane-anesthetized beagle dogs. *J Toxicol Sci.* 2016;41:439-47.
- 3 Iwatsubo K, Bravo C, Uechi M, Baljinnayam E, Nakamura T, Umemura M, et al. Prevention of heart failure in mice by an antiviral agent that inhibits type 5 cardiac adenylyl cyclase. *Am J Physiol Heart Circ Physiol.* 2012;302:2622-8.
- 4 Wada T, Nakamura Y, Cao X, Ohara H, Izumi-Nakaseko H, Ando K, et al. Antiviral drug vidarabine possessing cardiac type 5 adenylyl cyclase inhibitory property did not affect cardiohemodynamic or electrophysiological variables in the halothane-anesthetized dogs. *J Toxicol Sci.* 2016;41:115-22.
- 5 Frommeyer G, Mittelstedt A, Wolfes J, Ellermann C, Kochhäuser S, Leitz P, et al. The anti-influenza drug oseltamivir reduces atrial fibrillation in an experimental whole-heart model. *Naunyn Schmiedebergs Arch Pharmacol.* 2017;390:1155-61.
- 6 Suita K, Fujita T, Cai W, Hidaka Y, Jin H, Prajapati R, et al. Vidarabine, an anti-herpesvirus agent, prevents catecholamine-induced arrhythmias without adverse effect on heart function in mice. *Pflugers Arch.* 2018;470:923-35.
- 7 Safrin S. Antiviral agents. In: Katzung BG, editor. *Basic & Clinical Pharmacology* 14th edition. New York: McGraw Hill Education; 2018. pp. 863-94.
- 8 Wolters H, Mayer A, Gerhardt U, Hohage H. [Hypotension in aciclovir therapy] in German. *Praxis (Bern 1994).* 1998;87:1614-8.
- 9 Sugiyama A. Sensitive and reliable proarrhythmia in vivo animal models for predicting drug-induced torsades de pointes in patients with remodelled hearts. *Br J Pharmacol.* 2008;154:1528-37.
- 10 Nagueh SF, Sun H, Kopelen HA, Middleton KJ, Khoury DS. Hemodynamic determinants of the mitral annulus diastolic velocities by tissue Doppler. *J Am Coll Cardiol.* 2001;37:278-85.
- 11 Van de Water A, Verheyen J, Xhonneux R, Reneman RS. An improved method to correct the QT interval of the electrocardiogram for changes in heart rate. *J*

- Pharmacol Methods. 1989;22:207-17.
- 12 Johannesen L, Vicente J, Mason JW, Sanabria C, Waite-Labott K, Hong M, et al. Differentiating drug-induced multichannel block on the electrocardiogram: randomized study of dofetilide, quinidine, ranolazine, and verapamil. *Clin Pharmacol Ther.* 2014;96:549-58.
 - 13 Sugiyama A, Hashimoto K. Effects of a typical I_{Kr} channel blocker sematilide on the relationship between ventricular repolarization, refractoriness and onset of torsades de pointes. *Jpn J Pharmacol.* 2002;88:414-21.
 14. Krasny HC, de Miranda P, Blum MR, Elion GB. Pharmacokinetics and bioavailability of acyclovir in the dog. *J Pharmacol Exp Ther.* 1981;216:281-8.
 15. Nicholas HG, Holfors MB. Pharmacokinetics & pharmacodynamics: rational dosing & the time course of drug action. In Katzung BG, editor. *Basic & clinical pharmacology* 14th ed. New York: McGraw-Hill Education; 2018. pp. 41-55.
 - 16 Clauss S, Bleyer C, Schüttler D, Tomsits P, Renner S, Klymiuk N, et al. Animal models of arrhythmia: classic electrophysiology to genetically modified large animals. *Nat Rev Cardiol.* 2019;16:457-75.

Figure legends

Fig. 1

Typical tracings showing the His bundle electrogram (His), lead II electrocardiogram (ECG), aortic pressure (AoP), left ventricular pressure (LVP) and monophasic action potential (MAP) during sinus rhythm at pre-drug basal control (Control, left) and 10 min after the start of 20 mg/kg of aciclovir infusion (10 min after 20 mg/kg aciclovir, right).

Fig. 2

Time courses of 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 aciclovir. Data are presented as mean±SEM (n=4). Closed symbols represent statistically significant differences from each control value (C) by $p < 0.05$.

Fig. 3

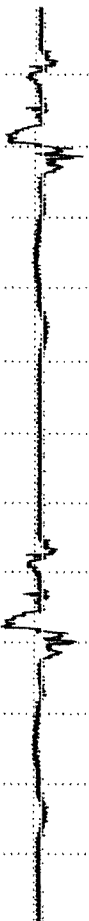
Time courses of the PR interval (PR), QRS width (QRS), QT interval (QT), corrected QT interval (QTc), P-wave duration (P duration), J-T_{peak}c and T_{peak}-T_{end} after the administration of aciclovir. Data are presented as mean±SEM (n=4). Closed symbols represent statistically significant differences from each control value (C) by $p < 0.05$.

Fig. 4

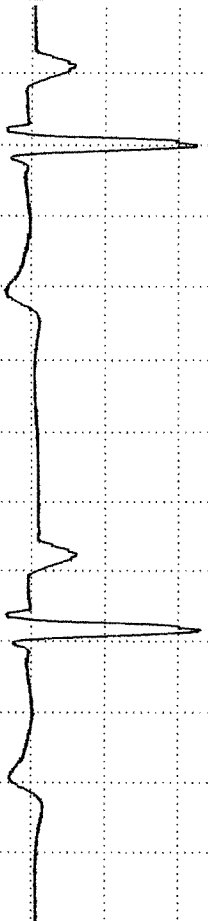
Time courses of the atrio-His (AH) and His-ventricular (HV) intervals; monophasic action potential duration at 90% repolarization level (MAP₉₀) during sinus rhythm (MAP_{90(sinus)}), at a pacing cycle length of 400 ms (MAP_{90(CL400)}) and 300 ms (MAP_{90(CL300)}); ventricular effective refractory period (VERP); and terminal repolarization period (TRP) after the administration of aciclovir. Data are presented as mean±SEM (n=4). Closed symbols represent statistically significant differences from each control value (C) by $p < 0.05$.

Control

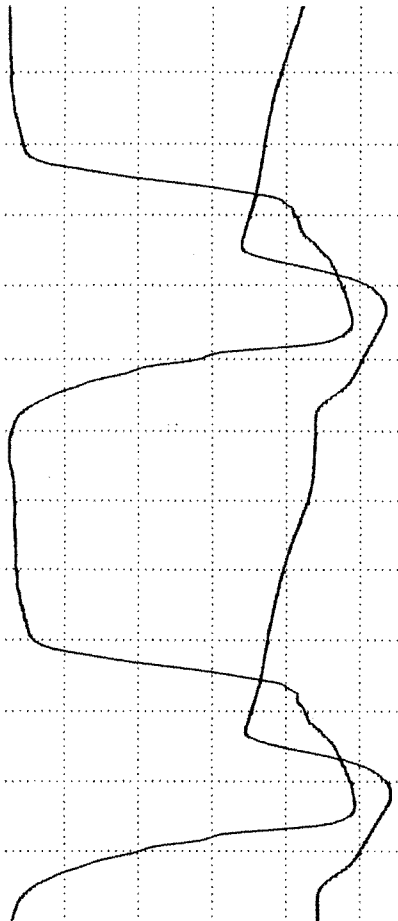
His



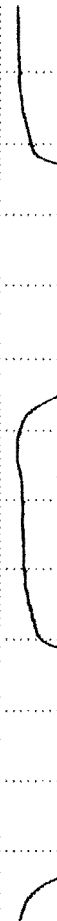
ECG



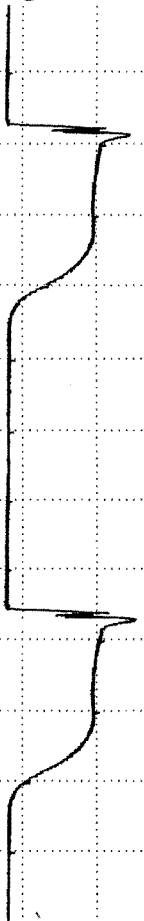
AoP



LVP



MAP

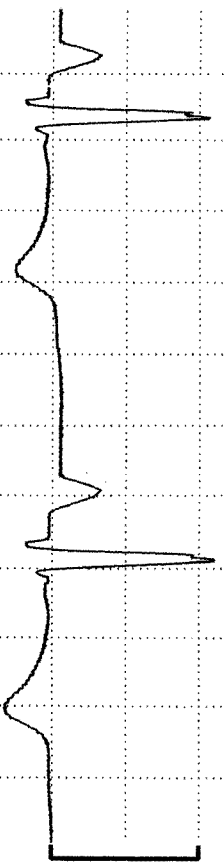


10 min after 20 mg/kg aciclovir

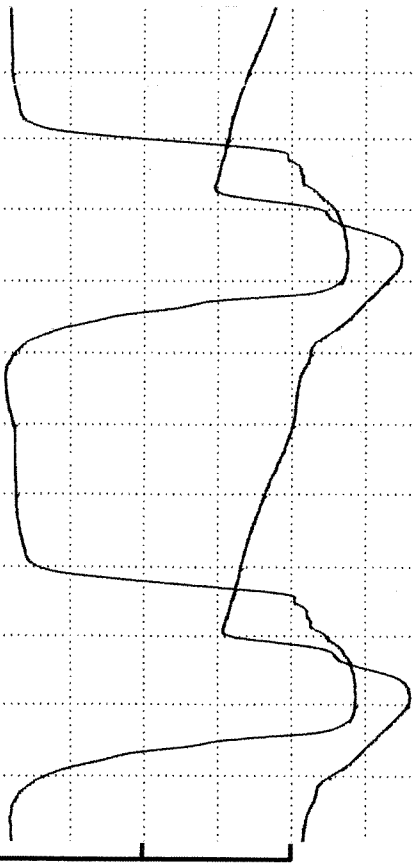
His



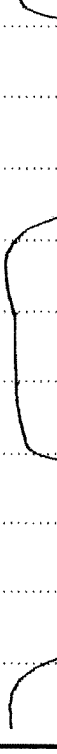
ECG



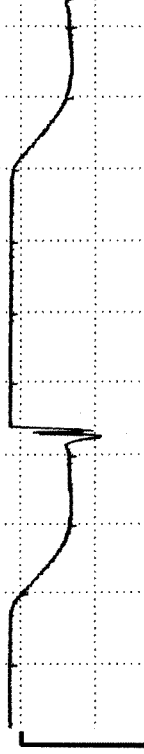
AoP



LVP



MAP



1 mV

1 mV

100

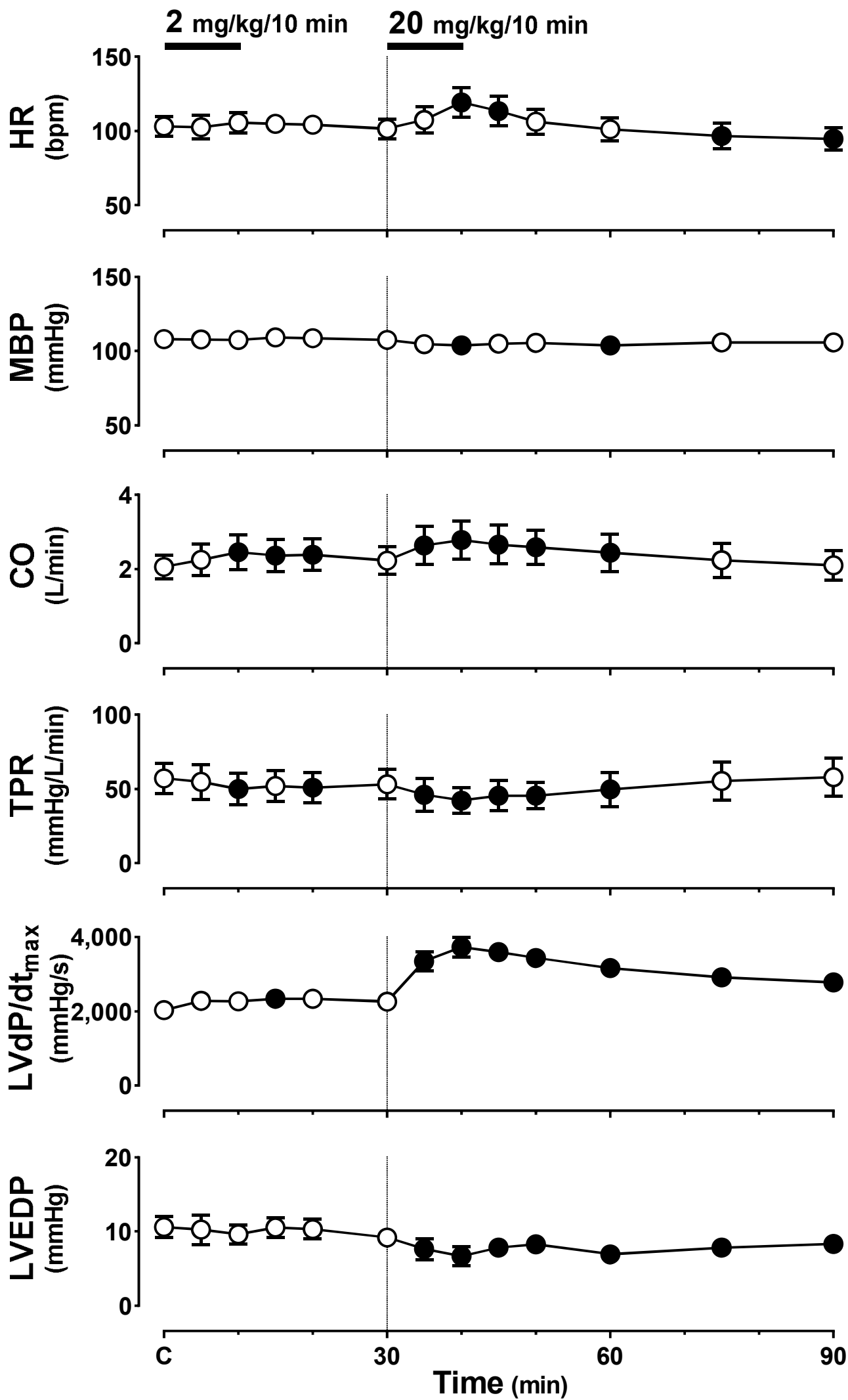
50

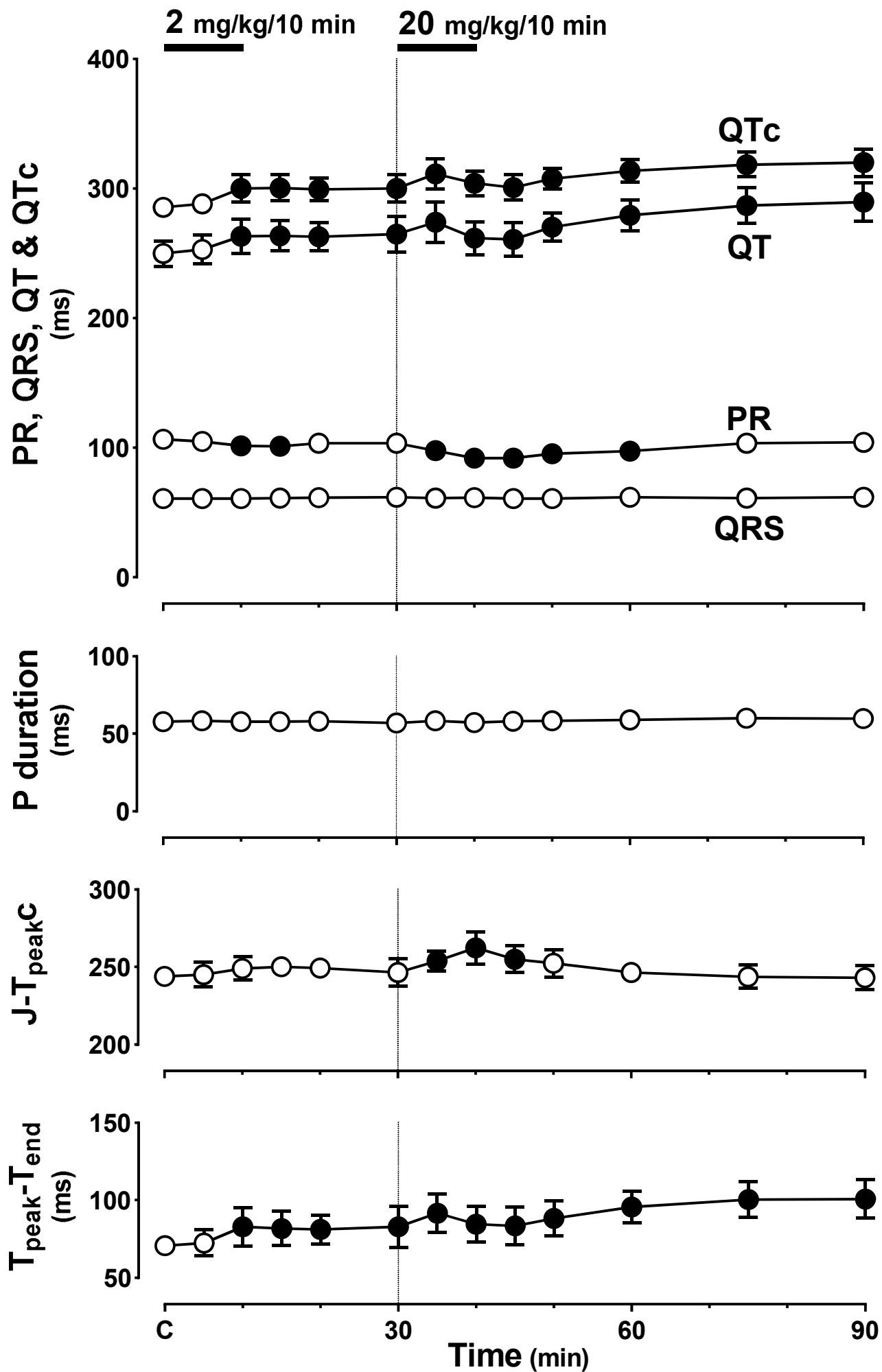
0

20 mV

mmHg

200 ms





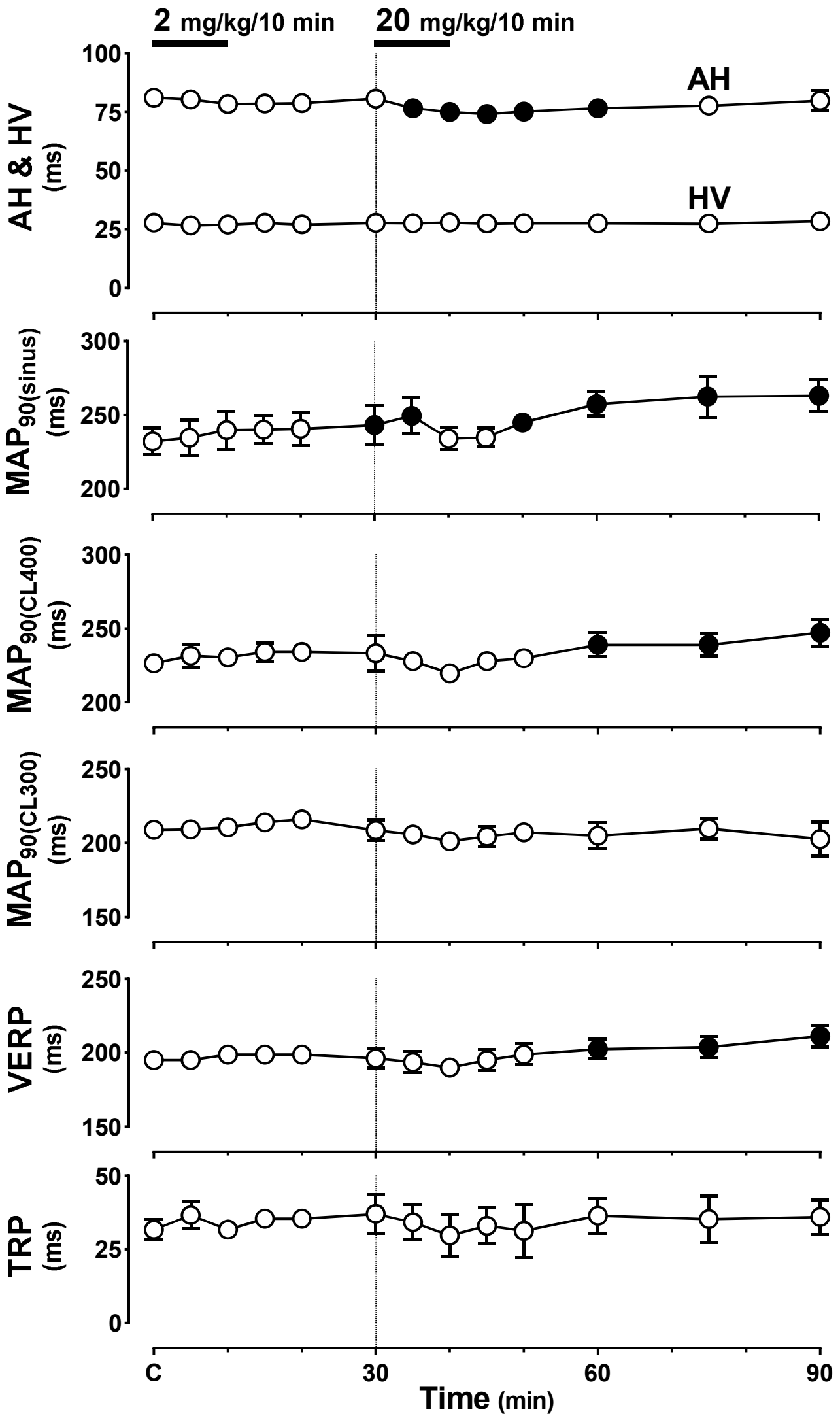


Table 1. Comparison of electropharmacological effects of anti-virus drugs

Drugs		Aciclovir	Amantadine	Oseltamivir
(Time after administration)		(60 min)	(10 min)	(10 min)
Dose	mg/kg, i.v.	20	10	30
QT	ms (%)	+40±7 (+16±3)	+46±13 (+18±5)	+25±11 (+10±4)
QTc	ms (%)	+35±6 (+12±2)	+42±12 (+14±4)	+20±9 (+7±3)
J-T _{peak} ^c	ms (%)	-3±5 (0±3)	+10±10 (+6±6)	+4±3 (+2±1)
T _{peak} -T _{end}	ms (%)	+30±7 (+42±7)	+33±13 (+40±19)	+12±7 (+19±10)
MAP _{90(sinus)}	ms (%)	+31±2 (+13±1)	+56±12 (+24±6)	+25±7 (+10±3)
MAP _{90(CL400)}	ms (%)	+21±5 (+9±2) ^a	+21±21 (+10±9) ^a	+33±5 (+14±3) ^a
MAP _{90(CL300)}	ms (%)	-6±12 (-3±6) ^{b,c}	+19±13 (+9±6) ^c	+34±2 (+16±1) ^c
VERP _(CL400)	ms (%)	+16±2 (+8±1)	+8±10 (+4±5)	+38±7 (+17±3)
TRP	ms (%)	+4±3 (+12±9)	+14±16 (+60±61)	-4±8 (-1±51)

Each value represents absolute change (% change) from its corresponding pre-drug control value (n=4) when the magnitude of QTc prolongation was the greatest. Cycle lengths during sinus rhythm were 645±50, 651±101 and 621±45 ms in the aciclovir, amantadine and oseltamivir-administered animals, respectively (n=4 for each treatment). Data of amantadine and oseltamivir were obtained from our previous studies [1,2]. MAP₉₀: monophasic action potential duration at 90% repolarization level; MAP_{90(sinus)}: MAP₉₀ during sinus rhythm; MAP_{90(CL400)}: MAP₉₀ at a pacing cycle length of 400 ms; MAP_{90(CL300)}: MAP₉₀ at a pacing cycle length of 300 ms; VERP_(CL400): ventricular effective refractory period at a basic pacing cycle length of 400 ms; and TRP: terminal repolarization period. ^a*p* <0.05, MAP_{90(sinus)} vs. MAP_{90(CL400)}; ^b*p* <0.05, MAP_{90(CL400)} vs. MAP_{90(CL300)}; and ^c*p* <0.05, MAP_{90(sinus)} vs. MAP_{90(CL300)}.