Electrophysiological Effects of an Anti-influenza Drug Oseltamivir on the Guinea-Pig Atrium: Comparison with Those of Pilsicainide

Akira Takahara,*,a Sanae Suzuki,*, Mihoko Hagiwara,*, Shuhei Nozaki,*, and Atsushi Sugiyamab

a Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Toho University; 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan; and b Department of Pharmacology, Faculty of Medicine, Toho University; 5–21–16 Omori-Nishi, Ota-ku, Tokyo 143–8540, Japan.

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We assessed the effects of oseltamivir on the conduction velocity and effective refractory period in the guinea-pig atrium in comparison with those of a class Ic antiarrhythmic drug pilsicainide. The recording and stimulating electrodes were attached on the epicardium close to the sinus nodal region and on the left atrial appendage. Oseltamivir (10–100 µM) as well as pilsicainide (1–10 µM) decreased the atrial conduction velocity in a frequency-dependent manner. Both drugs also increased the effective refractory period in both atria; but the frequency-dependent property of oseltamivir was lacking in the left atrium, and it was less obvious in the right atrium compared with that of pilsicainide. These results suggest that oseltamivir can directly modify the electrophysiological functions in the guinea-pig atrium possibly via combination of Na+ and K+ channel-blocking actions.

Key words oseltamivir; pilsicainide; atrium; conduction velocity; effective refractory period

A neuraminidase inhibitor oseltamivir is one of the most effective drugs against influenza virus infection. In a recent study using the halothane-anesthetized dog, we found a supratherapeutic dose of oseltamivir (30 mg/kg, intravenous (i.v.)) can exert the negative chronotropic, inotropic and dromotropic actions on the heart. More interestingly, the lower dose (3 mg/kg, i.v.), which is still 10 times higher than therapeutic dose range as an anti-influenza drug, increased the P-wave duration without affecting the other electrocardiographic variables. Since the P-wave duration reflects the intra-atrial conduction time, this observation may evoke a new category of drugs showing atrio-selective actions. However, since oseltamivir is known to be metabolized to the active form inhibiting neuraminidase (Ro64-0802), it is unknown whether oseltamivir itself affects the atrial impulse conduction. Our previous study using the isolated guinea-pig atrium electrically driven at 1 Hz demonstrated that 10–100 µM of oseltamivir can decrease the upstroke velocity of the phase 0 depolarization, but prolong the action potential duration, suggesting that the drug may affect ionic channels in the atrium such as Na+ and K+ channels. Using the isolated atrium of the guinea pig, in this study we assessed the effects of oseltamivir on the conduction velocity and effective refractory period at pacing cycle lengths of 150–250 ms in comparison with those of a class Ic antiarrhythmic drug pilsicainide to better understand electrophysiological profiles of oseltamivir in the atrium.

MATERIALS AND METHODS

All experiments were performed according to the Guidelines for Animal Experiments for Toho University. The Hartley guinea pigs of either sex weighing 350–450 g were used in this study.

The isolated atrial preparation consists of the entire right and left atrium of a guinea pig, which was incubated with the Krebs–Henseleit solution of the following composition (in mM): NaCl 118.4, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 24.9, glucose 11.1, gassed with 95% O2/5% CO2 (pH 7.4 at 37°C). Electrophysiological parameters were recorded as described previously. Briefly, the recording and stimulating electrodes were attached on the atrial epicardium close to the sinus nodal region and on the left atrial appendage. Electrograms were amplified with a bioelectric amplifier (AB-621G, Nihon Kohden, Tokyo, Japan) and fed into a computer-based data acquisition system (PowerLab, ADInstruments, Castle Hill, Australia). The spontaneous sino-atrial rate was measured with a heart rate counter (AT-601G, Nihon Kohden) triggered by the electrogram. The preparation was driven at a cycle length of 250, 200 or 150 ms using an electrical stimulator (SEN-7203, Nihon Kohden) and an isolator (SS-104J, Nihon Kohden) with rectangular pulses (about 1.5 times of the diastolic threshold voltage and 3-ms width). The intra-atrial conduction time was measured as the difference between right and left atrial electrograms to calculate the intra-atrial conduction velocity. The pacing cycle lengths were set shorter than those during spontaneous sino-atrial activity which were around 270–300 ms. On the other hand, the effective refractory period of the right and left atrium was assessed by a pacing protocol consisting of ten beats of basal stimuli in a cycle length of 250, 200 or 150 ms followed by an extra stimulus of various coupling intervals. All experiments were performed at 36.5±0.5°C.

Oseltamivir phosphate (molecular weight=410.40) was extracted from Tamiflu® Capsule (Chugai Pharmaceutical, Tokyo, Japan), whereas pilsicainide hydrochloride (molecular weight=317.85) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). The drugs were dissolved in distilled water and small aliquots were added to the organ bath to obtain the desired final concentration. All other chemicals were commercial products of the highest available quality. The statistical significances within a parameter were evaluated by one-way repeated-measures analysis of variance (ANOVA) followed by Dunnett’s test. Differences at p<0.05 were considered significant.

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: akirat@phar.toho-u.ac.jp

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RESULTS

Figure 1 shows typical tracings of effects of oseltamivir on the electrograms obtained from the right atrium (RA) and left atrium (LA), whereas Fig. 2 summarizes the effects of oseltamivir \((n=5)\) and pilsicainide \((n=5)\) on the conduction velocity and effective refractory period.

After the application of 10 \(\mu M\) of oseltamivir, the conduction velocity decreased only at the shortest pacing cycle length of 150 ms, whereas it was further decreased at each of three pacing cycle lengths after the application of 100 \(\mu M\) (Fig. 2Aa). The high concentration changed the parameters by \(-0.10\pm0.05\), \(-0.13\pm0.04\) and \(-0.14\pm0.01\) m/s at pacing cycle lengths of 250, 200 and 150 ms, respectively. After the application of 1 \(\mu M\) of pilsicainide, no significant change was detected in the conduction velocity, whereas it decreased at each of three pacing cycle lengths after the application of 10 \(\mu M\) (Fig. 2Ba). The high concentration changed the parameters by \(-0.18\pm0.04\), \(-0.23\pm0.03\) and \(-0.28\pm0.04\) m/s at pacing cycle lengths of 250, 200 and 150 ms, respectively.

After the application of 10 \(\mu M\) of oseltamivir, no significant change was detected in the effective refractory period of the right or left atrium, whereas those increased in the right and left atrium at each of three pacing cycle lengths after the application of 100 \(\mu M\) of oseltamivir (Fig. 2Ab). The high concentration changed the parameters in the right atrium by +15\pm6, +16\pm5 and +21\pm6 ms and those in the left atrium by +17\pm5, +17\pm5 and +17\pm4 ms at pacing cycle lengths of 250, 200 and 150 ms, respectively, indicating lack of frequency-dependent prolongation of the effective refractory period in

Fig. 1. Typical Tracings of Effects of Oseltamivir on the Electrograms Obtained from the Right Atrium (RA) and Left Atrium (LA)

The preparation was electrically driven at a pacing cycle length of 200 ms. S: electrical stimulation.

Fig. 2. Electrophysiological Actions of Oseltamivir \((n=5)\) and Pilsicainide \((n=5)\) on the Atrium

A: Effects of oseltamivir (10 and 100\(\mu M\)) on the atrial conduction velocity and effective refractory period in the right and left atrium. B: Effects of pilsicainide (1 and 10\(\mu M\)) on the atrial conduction velocity and effective refractory period in the right and left atrium. All parameters were obtained before and 30 min after the application of each drug. Data are means\(\pm S.E.M\). *p<0.05, compared with the respective control values.
the left atrium. After the application of 1 µM of pilsicainide, no significant change was detected in the effective refractory period of the right or left atrium, whereas those increased in the right and left atrium at each of three pacing cycle lengths after the application of 10 µM of pilsicainide (Fig. 2Bb). The high concentration changed the parameters in the right atrium by +18±3, +21±3 and +28±5 ms and those in the left atrium by +23±4, +30±5 and +39±7 ms at pacing cycle lengths of 250, 200 and 150 ms, respectively.

**DISCUSSION**

In our previous study using the halothane-anesthetized canine model, the peak plasma concentrations of oseltamivir after intravenous administration of 3 and 30 mg/kg over 10 min were 10.6 µg/mL (34 µM) and 117.5 µg/mL (376 µM), respectively, the former of which only prolonged the P-wave duration without affecting the other electrocardiogram variables. In this study, 10–100 µM of oseltamivir decreased the conduction velocity but increased the effective refractory period, indicating that currently observed in vitro results may reflect the previous in vivo observations of the atrial effects of oseltamivir.

Our previous study also showed that 10–100 µM of oseltamivir decreased the upstroke velocity of the phase 0 depolarization but prolonged the action potential duration in the guinea-pig atrium in vitro at a pacing cycle length of 1000 ms, supporting that the drug may inhibit atrial Na⁺ and K⁺ channels. Experiments are now ongoing to confirm the direct effects of oseltamivir on various ionic channels. In a limited number of experiment using HEK293 cells expressing Nav1.5, oseltamivir at 300 µM attenuated veratridine-induced changes in fluorescence intensity of membrane potential sensitive dye by 23.5% (n=4). In the HEK293 cells coexpressing m4 muscarinic receptor and Kir3.1/Kir3.4, the drug at 30 µM suppressed thallium flux response to carbachol by 48.4% (n=3). Furthermore, oseltamivir at 100 µM has been reported to suppress the human ether-a-go-go-related gene (hERG) current by 37.5%. Taken together, one can speculate that such multi-ion channel-blocking properties of oseltamivir might have induced the currently observed decrease of atrial conduction velocity and increase of atrial effective refractory period.

Previous clinical studies have demonstrated that class I antiarrhythmic drugs have ‘frequency-dependent effects’ on the atrial conduction and effective refractory period, and that most of the class III antiarrhythmic drugs have ‘reverse frequency-dependent effects’ on the atrial effective refractory period. In this study, oseltamivir as well as pilsicainide decreased the atrial conduction velocity in a frequency-dependent manner, confirming that oseltamivir can inhibit atrial Na⁺ channels similarly to class Ic antiarrhythmic drugs. Also, 10 µM of pilsicainide increased the atrial effective refractory period in a frequency-dependent manner as reported previously. Meanwhile, 100 µM of oseltamivir increased the effective refractory period in both atria, but frequency-dependent property was lacking in the left atrium and less obvious in the right atrium. These observations suggest that in the atrium the extent of prolongation in the effective refractory period induced by frequency-dependent Na⁺ channel-blocking property of oseltamivir may be counterbalanced by its reverse frequency-dependent K⁺ channel-blocking property; and that the difference in the electrophysiological responses to oseltamivir between the right and left atrium may depend on their constitutive heterogeneity as reported previously in the mouse atrium. Thus, the currently observed unique electrophysiological actions of oseltamivir on the atrium can provide important information for its clinical utility and/or limitations for influenza-infected patients with atrial fibrillation.

In conclusion, oseltamivir can directly modify the electrophysiological functions in the guinea-pig atrium possibly via combination of Na⁺ and K⁺ channel-blocking actions.

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**REFERENCES AND NOTE**