Short Communication

Simultaneous Assessment of Pharmacokinetics of Pilsicainide Transdermal Patch and Its Electropharmacological Effects on Atria of Chronic Atrioventricular Block Dogs

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Abstract. Pharmacokinetics of pilsicainide transdermal patch and its electropharmacological effects were simultaneously assessed using chronic atrioventricular block dogs. After application of the patch (9.8 mg/kg), pilsicainide was continuously absorbed through the skin with a C_{max} of 0.49 ± 0.13 μg/ml, while its plasma concentration was kept above the clinically reported minimum effective plasma concentration for $2 - 8$ h. Inter-atrial conduction time was significantly prolonged, whereas statistically significant prolongation was not detected in the atrial effective refractory period. Prolongation of the cycle length of atrial fibrillation and anti-fibrillatory action were confirmed. Thus, pilsicainide can be absorbed transdermally to exert long-lasting electropharmacological effects leading to anti–atrial fibrillatory action.

Keywords: pilsicainide, transdermal delivery, atrial fibrillation

Pilsicainide is a class Ic antiarrhythmic drug originally developed in Japan, which has been widely used for patients with atrial as well as ventricular tachyarrhythmias (1). Intravenous administration of 1 mg/kg over 10 min of pilsicainide or $100 - 150$ mg of single oral administration has been reported to affect atrial electrophysiological properties, leading to conversion of atrial fibrillation to sinus rhythm in $45\% - 73\%$ of patients $(2 - 4)$. In our previous animal studies, effects of class I antiarrhythmic drugs including pilsicainide on the atrial and ventricular tachyarrhythmias have been shown to depend on their plasma concentrations (5, 6). In addition, the elimination half-life ($t_{1/2\beta}$) of the plasma concentration of pilsicainide has been known to be significantly shorter than those of other typical class I antiarrhythmic drugs, including aprindine, flecainide, and propafenone (4.6 h vs. $7.2 - 26.5$ h) (7). Thus, a new formulation of pilsicainide that can maintain optimal plasma drug concentration for a longer period may theoretically increase its utility and efficacy as an antiarrhythmic drug. Since we recently developed a novel transdermal patch containing pilsicainide, we simultaneously assessed the pharmacokinetics of the pilsicainide transdermal patch and its electropharmacological profile using the canine chronic atrioventricular block model (8). Previous studies have indicated that this model has a pathophysiology of chronically compensated heart failure together with the increase of sympathetic tone and the stretch activating paradigm of atria and ventricles, leading to fibrosis and conduction abnormalities that may promote the occurrence and maintenance of atrial fibrillation as well as ventricular arrhythmias $(9 - 11)$.

All experiments were carried out according to the Guidelines for Animal Experiments, University of Yamanashi, which are equivalent to those of the US National Institute of Health. Pilsicainide transdermal patches were prepared by spreading the mixture of pilsicainide (Daiichi Sankyo, Tokyo; MW = 272.39), isopropyl myristate (Nof Corporation, Tokyo), and

acrylic-based pressure sensitive adhesive (Jurymer ST-6802; Nihon Junyaku, Tokyo) over the backing membrane (Bando Chemical Industries, Kobe). The optimal weight fraction of pilsicainide, isopropyl myristate, and the adhesive was 7:20:73.

Persistent atrioventricular block was induced in the beagle dogs of either sex weighing about 10 kg as previously described (8 – 10). More than 3 months after the induction of the atrioventricular block, the dogs were anesthetized with pentobarbital sodium (30 mg/kg, i.v.; Tokyo Kasei, Tokyo) and artificially ventilated with 100% oxygen using a respirator. The surface lead II ECG was obtained from the limb electrodes. A heparinized catheter was placed in the left femoral artery to monitor the systemic blood pressure. A standard 6-French quadripolar electrodes catheter (Cordis-Webster, Baldwin Park, CA, USA) was positioned at the top of the right atrium via the right femoral vein to electrically pace the sinus nodal area and record the local electrogram. The spontaneously beating rate of the atrium (=sinoatrial rate) was measured with a heart rate counter triggered by the right atrial electrogram. A second 6-French quadripolar electrodes catheter (Cordis-Webster) was positioned in the esophagus via the os to record the left atrial electrogram. Finally, a third 6-French quadripolar electrodes catheter (Cordis-Webster) was positioned at the inter-atrial septum of the right atrium via the left femoral vein to electrically induce atrial fibrillation.

The sinus nodal area was electrically driven using a cardiac stimulator (SEC-3102; Nihon Kohden, Tokyo) (1 – 2 V, 1-ms width, about twice the threshold voltage). The inter-atrial conduction time was measured using the analytical software AcqKnowledge (ver 3.2.6; BIOPAC Systems, Goleta, CA, USA) with an analog/digital converting system (MP-100A, BIOPAC Systems). The effective refractory period was assessed by a programmed electrical stimulation (SEC-3102, Nihon Kohden). Atrial fibrillation was induced by burst pacing of the inter-atrial septum at a cycle length of 60 ms for 10 s using a stimulator (SEN-7203, Nihon Kohden) and an isolation unit (SS-201J, Nihon Kohden) with a rectangular pulse of 60-V amplitude and 10-ms width.

After the control state was assessed, the transdermal patch was applied to the animal's chest wall in a dose of 9.8 mg of pilsicainide/kg (pilsicainide group, n = 4). The cardiovascular variables were assessed at 1, 2, 4, 6, and 8 h. As another series of experiments, time control data were obtained before and at 2, 4, 6, and 8 h under the same protocol in the absence of the transdermal patch to confirm the stability and reliability of the currently used in vivo animal model (control group, n = 4). The systemic blood pressure and ECG were

analyzed using the real time full automatic data analysis system (MP-VAS; Physio-Tech, Tokyo). The blood was withdrawn before and 1, 2, 4, 6, and 8 h after the application of the transdermal patch. The plasma concentration of pilsicainide was assayed using a specific liquid chromatography–tandem mass spectrometry method. In another series of experiments using conscious normal beagle dogs, the blood was withdrawn before and 0.5, 1, 2, 4, 6, and 8 h after the oral administration of pilsicainide (5 mg/kg, n = 3) to measure its plasma concentrations.

Data are each presented as the mean ± S.E.M. The statistical significances of differences in paired data were evaluated by the paired t-test or one-way repeated-measures analysis of variance (ANOVA) followed by Contrasts for mean value comparisons, whereas those in unpaired data were evaluated by the unpaired t-test. A P-value of less than 0.05 was considered significant. No animal died during and after the study protocol. There was no significant difference in the pre-drug control values (C) between the pilsicainide group and the control group. No statistically significant change was detected in any of the cardiovascular variables of the control group during the observation period.
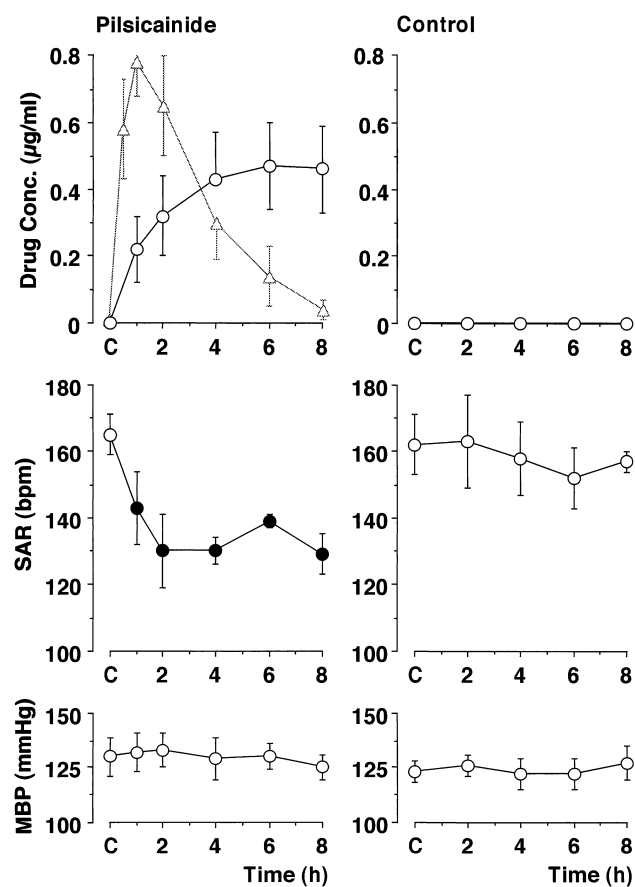
The time courses of change in the plasma pilsicainide concentration, sinoatrial rate, and mean blood pressure are summarized in Fig. 1. After the application of the transdermal patch, the sinoatrial rate decreased for 1 – 8 h.

Time courses of change in the inter-atrial conduction time and atrial effective refractory period are summarized in Fig. 2. After the application of the transdermal patch, the inter-atrial conduction time was significantly prolonged for 1 – 8 h at a pacing cycle length of 400 ms and for 4 – 8 h at a pacing cycle length of 300 and 200 ms. The atrial effective refractory period at each basic pacing cycle length tended to be prolonged, which did not achieve statistical significance.

Typical tracings of electrograms of the right and left atria, ECG, and blood pressure during and after the burst pacing and the effects of the transdermal patch on the duration and cycle length of atrial fibrillation are shown in Fig. 3. After the application of the transdermal patch, the duration decreased and the cycle length was prolonged for 2 – 8 h.
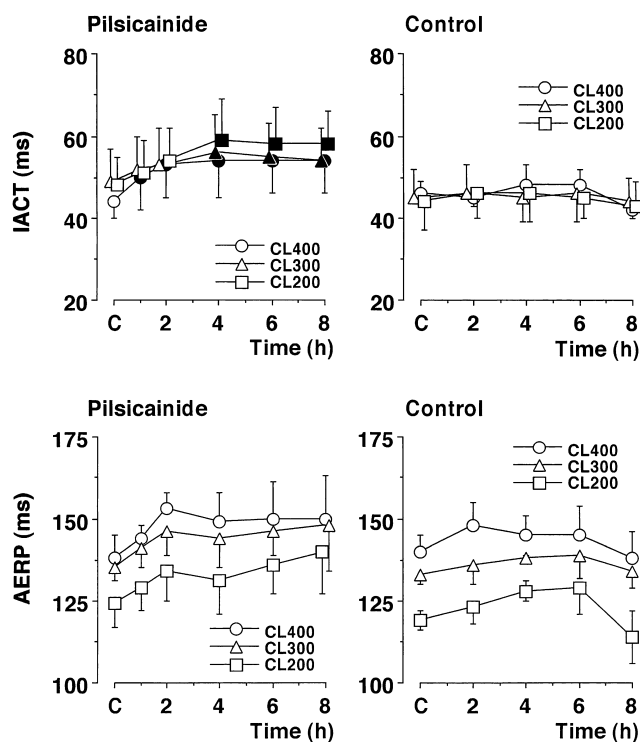
All animals survived the current study protocol, indicating that the pilsicainide transdermal patch may lack risks for inducing cardiohemodynamic collapse or torsades de pointes at the current dose since the model has been demonstrated to be highly sensitive in detecting the drug-induced lethal ventricular arrhythmias such as torsades de pointes (9, 10, 12).

Transdermal delivery is generally difficult for most of

Fig. 1. Time courses of changes in the plasma concentration of pilsicainide (Drug Conc.) (circles: transdermal patch, triangles: p.o. administration), sinoatrial rate (SAR), and mean blood pressure (MBP). After the transdermal administration, pilsicainide was detected in the plasma, with average values for C_{max}, AUC_{0-8h}, and T_{max} as follows: $0.49 \pm 0.13\,\mu g/ml$, $2.97 \pm 0.88\,\mu g \cdot h/ml$, and $6.0 \pm 1.4\,h$, respectively (n = 4). Average values for C_{max}, AUC_{0-8h} and T_{max} after oral administration of pilsicainide (5 mg/kg) were $0.78 \pm 0.10\,\mu g/ml$, $2.79 \pm 0.76\,\mu g \cdot h/ml$, and $1.0 \pm 0.0\,h$, respectively (n = 3). Data are each presented as the mean ± S.E.M. The closed symbols represent the significant differences from the pre-drug control value (C).


Fig. 2. Time courses of changes in the inter-atrial conduction time (IACT) and atrial effective refractory period (AERP) during a pacing cycle length of 400, 300, and 200 ms (CL400, CL300, and CL200, respectively). The IACT was defined as the difference between right and left atrial electrograms (in ms). The AERP was assessed by a pacing protocol that consisted of 5 beats of basal stimuli followed by an additional stimulus at various coupling intervals, which was defined as the shortest coupling interval (in ms) that still produced an electrical response. Data are each presented as the mean ± S.E.M. (n = 4 for each group). The closed symbols represent the significant differences from the pre-drug control value (C).

the drugs except for those with the following physico-chemical properties: molecular weight of <500 Dalton, lipophilicity indicated by logP = 1 – 3, and melting point of <200°C (13). The molecular weight, partition coefficient, and melting point of pilsicainide are 272.39 Daltons, −1.16 (octanol/water, pH 6.6), and 94°C, respectively, which supports the possibility of trans-dermal absorption of pilsicainide, leading to the development of its transdermal patch. As shown in this study, pilsicainide in the transdermal patch can be continuously absorbed through the skin. Moreover, its C_{max} value ($0.49 \pm 0.13\,\mu g/ml$) was close to the effective plasma concentrations for canine ventricular arrhythmia models and for patients with atrial fibrillation (2, 5, 7),

while the plasma concentration was kept above the clinically reported minimum effective plasma concentration of $0.25\,\mu g/ml$ for 2 – 8 h (7). Thus, the pilsicainide transdermal patch may be a useful formulation for maintaining its therapeutic plasma concentration.

Since the more sustained plasma drug concentration attained by the pilsicainide transdermal patch may affect its electropharmacological profile, we assessed it together with its pharmacokinetics. The sinoatrial rate decreased 1 – 8 h after application of the pilsicainide transdermal patch, which is in accordance with the previous observation after intravenous pilsicainide administration in anesthetized dogs and may reflect Na^+-channel inhibition (14). The inter-atrial conduction time was significantly prolonged 2 – 8 h after application of the pilsicainide transdermal patch, also reflecting Na^+-channel inhibition (14), whereas statistically significant prolongation was not detected in the atrial effective refractory period. The observation that pilsicainide
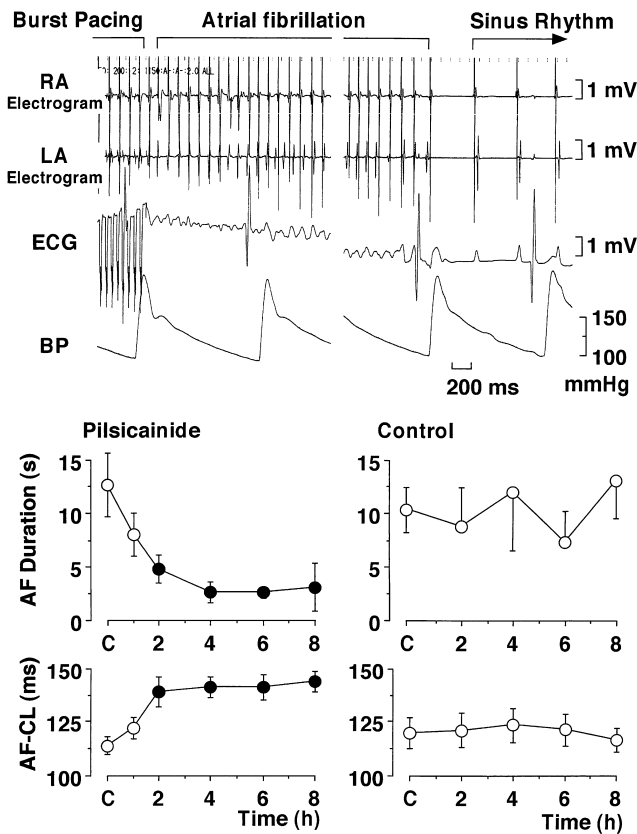
Fig. 3. Burst pacing-induced atrial fibrillation in the chronic atrioventricular block dog. Typical tracings of electrograms of right (RA) and left (LA) atria, ECG, and blood pressure (BP) during and after the burst pacing are depicted in the upper figures, whereas time courses of changes in the duration (AF Duration) and cycle length (AF-CL) of the atrial fibrillation are shown in the lower figures. Atrial fibrillation was defined as a period of rapid irregular atrial rhythm resulting in an irregular baseline of the ECG. The duration of atrial fibrillation was measured after its induction, and the cycle length was determined using the left atrial electrograms. The burst pacing was repeated 10 times at each time point. When the atrial fibrillation was maintained for >30 s, it was terminated electrically. In this case, the duration of atrial fibrillation was calculated as 30 s. Data are each presented as the mean ± S.E.M (n = 4 for each group). The closed symbols represent the significant differences from the pre-drug control value (C).

affected the conduction velocity more potently than the effective refractory period was essentially in accordance with a previous clinical study (15). Prolongation of the cycle length of atrial fibrillation and anti-fibrillatory action appeared 2 h after the application of the pilsicainide transdermal patch and lasted for ≥6 h. Taken together, the pilsicainide transdermal patch can exert long-lasted electropharmacological effects leading to anti–atrial fibrillatory action in vivo.

In conclusion, pilsicainide in the transdermal patch can be absorbed through the skin and shows long-lasting anti–atrial fibrillatory action on the pathologically remodeled canine atria. Application of pilsicainide transdermal patch at bedtime may become a useful prophylaxis for an atrial fibrillation attack that occurs early in the morning.

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