**Effects of the Antitussive Drug Cloperastine on Ventricular Repolarization in Halothane-Anesthetized Guinea Pigs**

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**Abstract.** Cloperastine is an antitussive drug, which can be received as an over-the-counter cold medicine. The chemical structure of cloperastine is quite similar to that of the antihistamine drug diphenhydramine, which is reported to inhibit hERG K⁺ channels and clinically induce long QT syndrome after overdose. To analyze its proarrhythmic potential, we compared effects of cloperastine and diphenhydramine on the hERG K⁺ channels expressed in HEK293 cells. We further assessed their effects on the halothane-anesthetized guinea-pig heart under the monitoring of monophasic action potential (MAP) of the ventricle. Cloperastine inhibited the hERG K⁺ currents in a concentration-dependent manner with an IC₅₀ value of 0.027 μM, whose potency was 100 times greater than that of diphenhydramine (IC₅₀; 2.7 μM). In the anesthetized guinea pigs, cloperastine at a therapeutic dose of 1 mg/kg prolonged the QT interval and MAP duration without affecting PR interval or QRS width. Diphenhydramine at a therapeutic dose of 10 mg/kg prolonged the QT interval and MAP duration together with increase in PR interval and QRS width. The present results suggest that cloperastine may be categorized as a QT-prolonging drug that possibly induces arrhythmia at overdoses like diphenhydramine does.

**Keywords:** cloperastine, antitussive drug, hERG K⁺ channel, QT interval, monophasic action potential

**Introduction**

Drug-induced QT interval prolongation is often associated with the onset of torsades de pointes resulting in a life-threatening ventricular arrhythmia (1, 2). Most of the torsadogenic drugs have been shown to inhibit a rapid component of delayed rectifier K⁺ channels (I_{Kr}) encoded by the human ether-a-go-go-related gene (hERG) (1, 2). Indeed, a histamine H₁-receptor antagonist terfenadine and a gastrointestinal antitussive drug cispamide are known as non-cardiovascular torsadogenic drugs, both of which are now clinically unavailable for patients (3, 4). In 2004, clobutinol, a common over-the-counter antitussive drug, was reported to induce torsades de pointes in a young patient via inhibition of hERG K⁺ channels (5, 6), and then the drug was withdrawn from the worldwide market in 2007 after subsequent clinical investigations. We have also confirmed that clobutinol prolongs the QT interval in our animal model (7). Based on the lesson from such serious cardiac events, we need to make steady effort to avoid drug-induced life-threatening arrhythmias through careful evaluation of suspected older drugs as is the case with new drug candidates, which is recommended by the ICH S7B guideline (8).

Cloperastine is an antitussive drug acting on the cough center without depressing the respiratory center, used in some European countries, Brazil and Japan, which can be received as an over-the-counter cold medicine (9). Importantly, the chemical structure of cloperastine is relatively similar to that of clobutinol (Fig. 1). However, information is limited regarding the cardiovascular effects of cloperastine. To analyze the proarrhythmic potential of cloperastine, in this study, we precisely assessed electrophysiological effects of cloperastine on the hERG K⁺ channels expressed in HEK293 cells and the halothane-anesthetized guinea-pig heart under the monitoring...
of monophasic action potential (MAP) (7, 10). As shown in Fig. 1, the chemical structure of cloperastine extremely resembles that of diphenhydramine. Then, we further compared the electrophysiological effects of cloperastine with those of diphenhydramine, since diphenhydramine has been reported to inhibit hERG K⁺ channels and clinically induce long QT syndrome after overdose (11, 12).

Risks of long QT syndrome resulting from various drugs are summarized in a previous report by Redfern et al. (2), where diphenhydramine is classified as one of the drugs for which there have been isolated reports of torsades de pointes in humans (Category 4).

Materials and Methods

All experiments were approved by the Ethics Committee of Toho University and performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. Twelve Hartley guinea pigs (Kiwa Laboratory Animals, Wakayama) were used in this study.

Electrophysiological recording of expressed hERG K⁺ channel–current

Stable transformants of HEK293 cells expressing hERG were obtained as described in our previous report (13). The cells were plated on collagen-I–coated cell culture dishes 48 to 72 h before electrophysiological recordings. Prior to the recordings, the cells were detached with Accutase (Innovative Cell Technologies, Inc., San Diego, CA, USA) and then re-suspended in the external solution.

Whole-cell voltage clamp experiments were performed using Port-a-Patch, planar patch clamp system with external perfusion system, temperature control (Nanion Technologies, Munich, Germany). The Port-a-Patch is a semi-automated patch clamp device, which utilizes planar patch clamp chips, made from borosilicate glass, for the patch clamp recordings. The center of the glass chip has an approximately 1 μm-sized aperture, onto which the cell is positioned automatically by suction. The recording chamber was perfused continuously at a flow rate of 1.0 to 2.0 mL·min⁻¹ and the temperature was maintained at 33 – 37°C. The external solution was of the following composition: 140 mM NaCl, 4 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, and 5 mM D-glucose (pH 7.4 with NaOH). The internal solution was of the following composition: 50 mM KCl, 10 mM NaCl, 60 mM K-Fluoride, 10 mM HEPES, and 20 mM EGTA (pH 7.2 with KOH) (14). Glass chips with a resistance of approximately 2 – 3 MΩ were used for recordings.

The hERG K⁺ current was activated from a holding membrane potential of −80 mV by depolarizing voltage clamp pulses. In case of the concentration-dependence experiments and the use-dependence experiments (shown in Figs. 2 and 3B, respectively), the peak amplitude of the tail current on return to −50 mV following a 1500-ms depolarizing pulse to +40 mV was measured. When the current voltage relation was examined (Fig. 3A), the voltage of the test pulse ranged from −80 to +40 mV. In case of the frequency dependence measurement (shown in Fig. 3C), the holding potential was −80 mV and the duration of the depolarizing test pulse and the following pulse to −50 mV was 200 ms. The current was measured before and 3 min after the drug application. Data recording and analysis were performed with EPC10 amplifiers, PatchMaster software (HEKA Electronics, Lambrecht, Germany), and Igor (WaveMetrics, Lake Oswego, OR, USA).

In vivo measurement of monophasic action potentials

Hemodynamic and electrophysiological parameters were measured under halothane-anesthesia as previously described (7). Hartley guinea pigs, weighing about 400 g, were initially anesthetized with sodium thiopental (40 mg/kg, i.p.). After a tracheal cannula was inserted, 1.0% halothane vaporized with 100% oxygen was inhaled with a rodent ventilator (SN-480-7, Shinano, Tokyo). The tidal volume and respiratory rate were set at 10 ml/kg and 60 strokes/min, respectively, and body temperature was maintained at 37°C using a heating pad. The left jugular vein was cannulated for drug administration, and the left carotid artery was also cannulated for measurement of the blood pressure. The surface lead II ECG was obtained from the limb electrodes. Corrected QT interval (QTc) was calculated using Van de Water’s formula [QTc(V) = QT – 0.087(RR – 1000)] (15) and Sakaguchi’s formula
\[ \text{QTc(S)} = \frac{\text{QT}}{(RR/300)^{1/3}} \] (16), where a unit is given in ms. The latter formula has been established for halothane-anesthetized guinea pigs whose standard heart rate is 200 beats/min. A MAP recording/pacing combination catheter (3 F, interelectrode distance of 4 mm, SMC-304; Physio-Tech, Tokyo) was positioned at the right ventricle via the right jugular vein. The signals were amplified with a differential amplifier (DAM 50; World Precision Instruments, Sarasota, FL, USA). The duration of the monophasic action potential signals was measured as an interval, along a line horizontal to the diastolic baseline, from the monophasic action potential upstroke to the desired repolarization level. The interval (ms) at the 90% repolarization level was defined as MAP$_{90}$.

The heart was electrically driven with a stimulator (SEN-3301; Nihon Kohden, Tokyo) and an isolator (SS-104J, Nihon Kohden). The stimulation pulses were rectangular in shape, of 2 V (about twice the threshold voltage), and 3-ms duration. The MAP$_{90}$ was measured during the sinus rhythm (MAP$_{90}$(sinus)) and at a pacing cycle length of 300 ms (MAP$_{90}$(CL300)) and 250 ms (MAP$_{90}$(CL250)). Cardiovascular parameters were continuously monitored with a polygraph system (RM-6000, Nihon Kohden), and analyzed with a real time full automatic analysis system (MP/VAS 3 for Windows ver. 1.0, Physio-Tech).

For assessment of instability of the ventricular repolarization, MAP duration (MAP$_{90}$) of 51 consecutive beats under the sinus rhythm was measured before and after the drug administration. Poincaré plots with MAP$_{90}$(n) versus MAP$_{90}$(n + 1) were prepared for the time points of each analysis. The mean orthogonal distance from the diagonal to the points of the Poincaré plot was determined as short-term variability \((= \sum |\text{MAP}_{90}(n+1) - \text{MAP}_{90}(n)| / [50 \times \sqrt{2}])\). On the other hand, the mean distance to the mean of the parameter parallel to the diagonal of the Poincaré plot was determined as long-term variability \((= \sum |\text{MAP}_{90}(n+1) + \text{MAP}_{90}(n) - 2\text{MAP}_{90}($mean$)| / [50 \times \sqrt{2}])\).
Mode of action of cloperastine on the hERG K⁺ current. A: Typical current traces in the absence or presence of 100 nM cloperastine (a) and summarized current–voltage relationships obtained at the end of the depolarizing test pulse (b) and at the tail current peak (c) in the absence (open circles) and presence (closed circles) of the drug. Data are presented as the mean ± S.E.M. from 6 experiments. B: Summarized results for the tonic and use-dependent block of the peak tail current by cloperastine. After continuous stimulation at 0.067 Hz, 100 nM cloperastine was applied and the stimulation was continued after a 3-min pause. The peak amplitude of the tail current in the presence of the drug was expressed as a percentage of that in its absence. Data are presented as the mean ± S.E.M. from 5 experiments. C: Summarized results for the frequency dependence of cloperastine effects on the tail current. The peak amplitude of the tail current was measured under four different stimulation frequencies, 0.067, 0.2, 1, and 2 Hz, in the presence of 100 nM cloperastine and expressed as percentages of the current amplitude in the absence of the drug. Measurement was performed at the 20th stimulation after switching to each frequency. Data are presented as the mean ± S.E.M. from 6 experiments.
These nomenclatures are adopted from heart rate variability investigations using Holter monitoring in humans (17), which have been applied to analyze relationship between instability of the ventricular repolarization and proarrhythmic potential in patients with long QT syndrome and proarrhythmic animal models (18 - 20).

The ECG parameters and MAP$_{90}$ were measured under the sinus rhythm as the mean of the three consecutive recordings. MAP$_{90}$ during the ventricular pacing was obtained at a cycle length of 300 and 250 ms as the mean of those obtained from the three consecutive MAP recordings. After the basal control assessment (C), a dose of 1 mg/kg of diphenhydramine was infused over 10 min. The ECG parameters and MAP$_{90}$ were measured at 5, 10, 15, 20, 25, and 30 min after the start of drug infusion. Next, 10 mg/kg of diphenhydramine was additionally infused over 30 s, and each parameter was measured in the same manner. Also, the effects of cloperastine in doses of 0.1 and 1 mg/kg were assessed in another series of animals.

**Drugs**

Cloperastine hydrochloride (molecular weight = 366.3), diphenhydramine hydrochloride (molecular weight = 291.8), and E-4031 (molecular weight = 474.44) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Clobutinol hydrochloride (molecular weight = 292.24) was bought from Biotrend Chemicals AG (Zurich, Switzerland). In the in vivo study, they were dissolved in saline and intravenously administered at an infusion rate of 2 ml/kg. The following drugs were purchased: thiopental sodium (Mitsubishi Tanabe Pharma, Osaka), halothane (Takeda Chemical Industries, Osaka), and heparin sodium (Ajinomoto Pharmaceuticals, Tokyo). In the in vitro study, 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.

**Statistical analyses**

Data are presented as the mean ± S.E.M. The statistical significances within a parameter were evaluated by one-way repeated-measures analysis of variance (ANOVA) followed by Contrasts for mean values comparison, whereas those of paired data within a parameter were evaluated by the paired t-test. A P-value less than 0.05 was considered significant.

**Results**

**Effects of cloperastine and diphenhydramine on the hERG K$^+$ currents**

Typical tracings of the effects of cloperastine and diphenhydramine on the K$^+$ current are depicted in Fig. 2A, and effects of diphenhydramine, cloperastine, clobutinol, and E-4031 are summarized in Fig. 2B (n = 5). All drugs inhibited the hERG K$^+$ currents in a concentration-dependent manner; IC$_{50}$ values of cloperastine, diphenhydramine, clobutinol, and E-4031 were 0.027 ± 0.003, 2.7 ± 0.1, 1.8 ± 0.4, and 0.016 ± 0.002 μM, respectively.

To evaluate the voltage-dependence of the inhibitory action of cloperastine, the current-voltage relationship of the hERG K$^+$ current was examined in the absence and presence of 100 nM cloperastine. Typical tracings are shown in Fig. 3Aa, and the summarized current–voltage relationships are shown in Fig. 3Ab and c. Cloperastine inhibited the hERG K$^+$ current at membrane voltages more positive than 0 mV both at the end of the depolarizing pulse and at the tail current peak. On the other hand, cloperastine rather augmented the hERG K$^+$ current at membrane voltages more negative than −40 mV. This caused a hyperpolarizing shift in the membrane voltage for half activation of the channel (V$_{1/2}$; V$_{1/2}$ in the absence and presence of 100 nM cloperastine was −10.9 ± 3.9 and −24.0 ± 3.1 mV, respectively (n = 6).

To evaluate the tonic and use-dependent block by cloperastine, the hERG channel current was evoked after a 3-min pause in the presence of 100 nM cloperastine (Fig. 3B, closed circles). The hERG K$^+$ current tail evoked by the stimulation after the pause was 36.7 ± 2.9% of that before the pause (tonic block). A small progression of inhibition was observed on further stimulation in the presence of the drug (use-dependent block): the hERG K$^+$ current tail evoked by the tenth stimulation after the pause was 28.0 ± 1.1% (n = 5) of that before the pause, which corresponds to 77.2 ± 3.3% of that evoked by the first stimulation after the pause.

To evaluate the frequency-dependence of cloperastine action, the effect of cloperastine was examined under four different stimulation frequencies ranging from 0.067 to 2 Hz, and the results are summarized in Fig. 3C. Under all four stimulation frequencies, the hERG K$^+$ current in the presence of 100 nM cloperastine was about 25% of that in its absence.

**In vivo electrophysiological effects of cloperastine and diphenhydramine**

Typical tracings of the effects of cloperastine on ECG are depicted in Fig. 4, and the time courses of the effects of cloperastine (n = 6) and diphenhydramine (n = 6) on
Fig. 4. Typical tracings of the effects of cloperastine on the electrocardiogram (ECG) and monophasic action potential (MAP) in the halothane-anesthetized guinea pig. A: control, B: 10 min after the start of cloperastine infusion.

Fig. 5. Time courses of the effects of cloperastine (A, n = 6) and diphenhydramine (B, n = 6) on the heart rate (HR) and ECG parameters; PR interval (triangles), QRS width (squares), QT interval (circles), and QTc (inverted triangles: Sakaguchi; triangles: Van de Water). Data are presented as the mean ± S.E.M. The closed symbols represent significant differences from corresponding pre-drug control value (C) of each parameter by \( P < 0.05 \).
the heart rate are summarized in Fig. 5. In the cloperastine group, the pre-drug control value of the heart rate was $201 \pm 7$ beats/min. No significant change was detected in the heart rate after the low dose of 0.1 mg/kg. The high dose of 1 mg/kg decreased the heart rate, and significant changes were detected for 5 – 30 min. In the diphenhydramine group, the pre-drug control value of the heart rate was $200 \pm 4$ beats/min. No significant change was detected in the heart rate after the low dose of 1 mg/kg. The high dose of 10 mg/kg decreased the heart rate for 5 – 10 min.

The time courses of the effects of cloperastine and diphenhydramine on the ECG parameters are summarized in Fig. 5. In the cloperastine group, pre-drug control values of the PR interval, QRS width, QT interval, QTc(V), and QTc(S) were $60 \pm 1$, $46 \pm 1$, $200 \pm 6$, $261 \pm 6$, and $200 \pm 5$ ms, respectively. After the low dose, no significant change was detected in the ECG parameters. The high dose further increased the QT interval, QTc(V), and QTc(S), and significant changes were detected for 5 – 30 min. Meanwhile, no significant change was detected in the PR interval or QRS width. In the diphenhydramine group, pre-drug control values of the PR interval, QRS width, QT interval, QTc(V), and QTc(S) were $59 \pm 3$, $46 \pm 1$, $195 \pm 8$, $256 \pm 7$, and $195 \pm 7$ ms, respectively. After the low dose, no significant change was detected in the ECG parameters. The high dose increased the PR interval for 10 – 15 min; QRS width at 5 min; QT interval and QTc(V) for 5 – 10 and 20 – 30 min; and QTc(S) at 5 min and for 20 – 30 min.

Typical tracings of the effects of cloperastine on the monophasic action potential are depicted in Fig. 4. The time courses of the effects of cloperastine and diphenhydramine on the MAP90(sinus), MAP90(CL300), and MAP90(CL250) are summarized in Fig. 6. In the cloperastine group, pre-drug control values of the MAP90(sinus), MAP90(CL300), and MAP90(CL250) were $182 \pm 6$, $179 \pm 4$, and $166 \pm 4$ ms, respectively. After the low dose, no significant change was detected in the MAP90(sinus), MAP90(CL300), or MAP90(CL250). The high dose increased these parameters for 5 – 30 min. In the diphenhydramine group, pre-drug control values of the MAP90(sinus), MAP90(CL300), and MAP90(CL250) were $165 \pm 6$, $169 \pm 6$, and $158 \pm 4$ ms, respectively. The low dose increased the MAP90(sinus) at 10 and 30 min. Meanwhile, no significant change was detected in the MAP90(CL300) or MAP90(CL250). The high dose further increased the MAP90(sinus) for 5 – 30 min. It also increased the MAP90(CL300) at 5 min and for 20 – 30 min and MAP90(CL250) at 30 min.

Typical results of the effects of cloperastine and diphenhydramine on the Poincaré plots of the MAP90 are shown in Fig. 7. Table 1 summarizes the short-term and long-term variability of MAP90 before and after the drug administrations. Diphenhydramine significantly increased the short-term variability and long-term variability.

![Fig. 6](image-url)  
Fig. 6. Time courses of the effects of cloperastine (A, n = 6) and diphenhydramine (B, n = 6) on the duration of monophasic action potential at a level of 90% repolarization during the sinus rhythm (MAP90(sinus), circles) and that during the ventricular pacing at a cycle length of 300 ms (MAP90(CL300), square) and 250 ms (MAP90(CL250), inverted triangles). Data are presented as the mean ± S.E.M. The closed symbols represent significant differences from the corresponding pre-drug control value (C) of each parameter by $P < 0.05$. 
Discussion

In this study, cloperastine as well as clobutinol and diphenhydramine inhibited the hERG K⁺ currents in a concentration-dependent manner. Some chemical moieties have been suggested to be responsible for inhibiting the I_{Kr}, such as presence of three or four atoms between the phenyl ring and the basic amine (21), which may be applicable to cloperastine as well as clobutinol and diphenhydramine (Fig. 1). The IC₅₀ values of diphenhydramine, clobutinol, and E-4031 (a representative inhibitor of hERG K⁺ channels) were comparable to those in the previous studies (6, 12, 22, 23). Of note, the potency of cloperastine (IC₅₀; 0.027 μM) was comparable to that of E-4031 (IC₅₀; 0.016 μM), suggesting that cloperastine is highly suspected of causing drug-induced QT-interval prolongation. The inhibitory effect of cloperastine on the hERG K⁺ current was prominent at depolarized membrane potentials (Fig. 3A), resulting in a shift of the activation voltage to the negative direction. A similar shift was reported with several other antiarrhythmic agents acting on the hERG K⁺ current, and this was called ‘facilitation’ (24). Such a phenomenon was also observed with chlorpheniramine, an antihistamine with similar chemical structure to cloperastine (25). It is postulated that hERG-channel blockers possessing this facilitating activity may have lower arrhythmogenic risk. Both tonic and use-dependent block was observed with cloperastine (Fig. 3B), and the use-dependent block as well as the frequency dependence of inhibition observed with cloperastine was relatively small (Fig. 3C).

Then, we further assessed its electrophysiological effects on the in vivo heart using the halothane-anesthetized animal model. The anesthetic halothane has been demonstrated to be more desirable to detect drug-induced QT interval prolongation via reduction of repolarization reserve (26, 27). In this in vivo study, we assessed cloperastine at doses of 0.1 and 1 mg/kg, i.v. Its antitussive effects have been observed at 1 mg/kg, i.v. in previous studies with guinea pigs (9), whereas a clinically recommended dose of cloperastine is 60 mg per os daily (Table 2). Thus, the current study is considered to be carried out at sub-therapeutic to therapeutic dose ranges. As shown in the results, cloperastine in a dose of 1 mg/kg significantly prolonged the QT interval and MAP duration together with bradycardia, whereas no obvious effects were observed in the PR interval or QRS width (Fig. 5), which implies that the drug inhibited cardiac K⁺ channels but...
not Na\(^+\) or Ca\(^{2+}\) channels in the in vivo condition. The MAP duration was also prolonged during the constant ventricular pacing (Fig. 6). These electrophysiological characteristics of cloperastine in vivo were essentially in accordance with those of E-4031 in our previous study using the same animal model (7). These results may suggest that the prolongation of ventricular repolarization by cloperastine in this animal model is closely associated with its ability to inhibit hERG K\(^+\) currents.

To estimate the extent of cardiac risk of cloperastine, we assessed effects of diphenhydramine, a chemically related drug to cloperastine as shown in Fig. 1, on the ventricular repolarization in halothane-anesthetized guinea pigs. Clinically, diphenhydramine has been reported to induce lethal arrhythmias including torsades de pointes after administration of an overdose (11, 28 – 30). In this study, diphenhydramine significantly prolonged the QT interval and MAP duration together with bradycardia in a dose of 10 mg/kg, which were essentially in accordance with a previous study using guinea pigs (31). In addition, the high dose of diphenhydramine prolonged the PR interval and QRS width (Fig. 5), which implies that the drug inhibited cardiac Na\(^+\) and Ca\(^{2+}\) channels as well as K\(^+\) channels in the in vivo condition. Relationship between effective and QT-interval prolonging doses in guinea pigs are summarized in Table 2. Experimental studies have shown that the effective dose of diphenhydramine in an asthma-like symptom model was 10 mg/kg (32) and that antitussive effects of cloperastine was 1 mg/kg (9), suggesting that both cloperastine and diphenhydramine can prolong the ventricular repolarization period at experimentally effective doses. Therefore, the extent of risks for long QT syndrome caused by cloperastine will be estimated to be similar to that by diphenhydramine.

Although cloperastine inhibited the hERG K\(^+\) channels 100 times more potently than diphenhydramine, the in vivo effects of cloperastine on the QT interval was about 10 times stronger than that of diphenhydramine (Table 2). A previous study of drug distribution in guinea pigs has shown that the tissue level of diphenhydramine in the heart was 4 times higher than that in plasma (33), whereas the tissue level of \(^{14}\)C-labeled cloperastine in the heart was 2/3 of the plasma level in rats (34). The pharmacokinetic information may partly explain the reason why cloperastine requires higher doses to prolong the QT interval in the in vivo condition in spite of the potent effects on the hERG K\(^+\) channels. This may also be consistent with the reports that diphenhydramine more often causes torsades de pointes than cloperastine in clinical use. Other possible mechanisms such as suppression of cardiac ion channels other than hERG K\(^+\) currents may be included, which could mask QT-prolonging effects of drugs associated with hERG K\(^+\)–channel inhibition. Indeed, it is known that drugs with a potent inhibitory action on the hERG K\(^+\) channels, such as the L-type Ca\(^{2+}\) channel–blocker verapamil (IC\(_{50}\); 0.094 μM), do not always prolong the QT interval (35). However, it is unlikely that hERG-K\(^+\) channel–blocking doses of cloperastine obviously inhibit cardiac Ca\(^{2+}\) or Na\(^+\) channels in this in vivo study, since the drug did not affect the PR interval or QRS width of the ECG in contrast to diphenhydramine. In addition, the facilitating activity of hERG-channel blockers possibly alters the relationship between in vitro and in vivo efficacies (24). Based on the current information, we can consider that the pharmacokinetic profile of cloperastine that hardly distributes to the heart is explained as a primary reason for its in vivo efficacy of QT-prolonging action. When electrophysiological and/or pharmacokinetic properties of cloperastine, diphenhydramine, clobutinol, and E-4031 are further clarified, additional explanations will be possible.

The short-term variability of the ventricular repolarization period is a well-established predictive marker for proarrrhythmia, and increment of the short-term variability is recognized to show greater beat-to-beat variability of ventricular repolarization, reflecting premonitory phenomenon of early afterdepolarization (18). Notably, the parameter differentiates the extent of torsadogenic potential of QT-prolonging drugs even with multi ion channel–blocking action, such as amiodarone, bepridil, and terfenadine (19, 36). Recently, the short-term vari-

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<th>Drug</th>
<th>hERG inhibition in HEK293 cell IC(_{50}) (μM)</th>
<th>QT prolongation in guinea pig (mg/kg, i.v.)</th>
<th>Effective dose in guinea pig (mg/kg, i.v.)</th>
<th>Clinical daily dose (mg, oral)</th>
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<td>60</td>
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<td>Diphenhydramine</td>
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<td>10(^{20})</td>
<td>150</td>
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<tr>
<td>Clobutinol</td>
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<td>1(^{7})</td>
<td>40(^{60})</td>
<td>240</td>
</tr>
<tr>
<td>E-4031</td>
<td>0.016</td>
<td>0.01(^{7})</td>
<td>–</td>
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Numbers expressed as superscripts show the cited references.
ability has been applied in clinical research, showing that patients with drug-induced or congenital long-QT syndrome have greater values of QTc and short-term variability compared with asymptomatic patients (19, 37). Furthermore, its usefulness was also confirmed in patients with non-ischemic heart failure (38). In this study, cloperastine slightly increased the short-term variability of the MAP duration; however it did not reach statistical significance. On the other hand, diphenhydramine significantly increased the short-term variability from the low dose of 1 mg/kg, which may reflect the clinical reports that diphenhydramine induced torsades de pointes arrhythmia (11, 12). Further investigations of the different cellular actions of the two drugs on the ventricular repolarization phase may lead to better understanding of the mechanisms of drug-induced long QT syndrome. These results suggest that cloperastine in contrast to diphenhydramine may not affect instability of ventricular repolarization at sub-therapeutic to therapeutic dose ranges.

Cloperastine has shown potential advantages over opioid antitussives, which frequently cause sedation and respiratory depression. In clinical studies, cloperastine has been shown to be well tolerated and effective in the treatment of cough due to various etiologies in adults as well as child patients (39). There were no reports of side effects, such as dry mouth, constipation, intestinal problems, and arrhythmia, and there were no statistically significant changes in blood pressure, heart rate, or any of the other biohumoral parameters studied (9). On the other hand, another antitussive drug clobutinol was reported to induce torsades de pointes in a young patient (5). In this animal model, the dose of clobutinol that prolonged QT interval was same as that of cloperastine (1 mg/kg) (7), whereas the clinical daily dose of clobutinol is fourfold more than that of cloperastine, as summarized in Table 2. Although cloperastine is believed to be safe based on the long-term clinical usage or experience, the present results suggest that caution has to be paid to administration of cloperastine overdose to patients with diagnosed or suspected long QT syndrome. Furthermore, we have to pay attention to patients with respiratory tract infection during the co-administration of cloperastine and an antibiotic drug that is suspicious of causing QT-interval prolongation.

In conclusion, the proarrhythmic risk of cloperastine can be estimated to be similar to that of diphenhydramine in the halothane-anesthetized guinea-pig model. The present results suggest that cloperastine may be categorized as a QT-prolonging drug that possibly induces arrhythmia after overdose, like diphenhydramine does. Thus, an increase in dose of cloperastine or co-administration of cloperastine and suspicious drugs that prolong the QT interval should be clinically judged with careful considerations.

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