Effect of Terfenadine and KW-4679, a Novel Antiallergic Compound, on Action Potential of Guinea Pig Ventricular Myocytes

Yoshimitsu Kato, Tatsuya Mori, Kenji Ohmori and Michio Ichimura*

Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411, Japan

Received November 14, 1995   Accepted December 24, 1995

ABSTRACT—It has been reported that terfenadine caused torsade de pointes ventricular arrhythmias. The prolongation of action potential duration (APD) in ventricles is considered to be one of the mechanisms of this adverse effect. We examined the effect of antiallergic drugs, terfenadine and KW-4679 ((Z)-11-[3-(dimethylamino)propylidene]-6,11-dihydropyridine-$b,e$-oxepin-2-acetic acid hydrochloride), on action potentials in isolated guinea pig ventricular myocytes. Terfenadine (30 nM–1 μM) increased APD in a concentration-dependent manner. On the other hand, KW-4679 (0.1 μM–100 μM) exerted no significant effects on action potential parameters. These results present no evidence that KW-4679 has the possibility to cause ventricular arrhythmias.

Keywords: Antiallergic drug, Action potential, Ventricular myocyte

Second-generation antihistamines such as terfenadine and astemizole are clinically used in antiallergic therapy. Recently, however, serious ventricular arrhythmia has been reported in patients taking terfenadine or astemizole (1–4). These proarrhythmic effects of terfenadine and astemizole have been seen only in patients with overdose, liver disease, or concomitant administration of a medication that interferes with hepatic cytochrome P-450 enzymatic metabolism such as ketoconazole and erythromycin. These drugs may act by blocking the delayed rectifier potassium current (IK), leading to the increase in action potential duration (APD) (5).

KW-4679 ((Z)-11-[3-(dimethylamino)propylidene]-6,11-dihydropyridine-$b,e$-oxepin-2-acetic acid hydrochloride) is a novel antiallergic compound that also possesses antihistamine activity, like terfenadine (6). The ED$_{50}$ value of KW-4679 in the passive cutaneous anaphylaxis (PCA) test in rats was 0.049 mg/kg, p.o., and the ID$_{50}$ value to inhibit anaphylactic bronchoconstriction in guinea pigs was 0.030 mg/kg, p.o. (6). On the other hand, it was reported that the ED$_{50}$ value of terfenadine in the PCA test was about 5 mg/kg, p.o., and the ID$_{50}$ value to inhibit antigen-induced bronchoconstriction was between 1.0 and 2.0 mg/kg, p.o. (7). Namely, the antiallergic potency of KW-4679 is 30–100-fold higher than that of terfenadine in animal models.

If KW-4679 is to be developed for use in humans, it is necessary to investigate whether KW-4679 may increase APD. In the present study, we examined the effect of terfenadine and KW-4679 on action potentials of isolated guinea pig ventricular myocytes.

The following experimental procedure was employed: Terfenadine was purchased from Sigma (St. Louis, MO, USA), and KW-4679 was synthesized at the chemical synthesis division of our laboratories. All other chemicals were commercially available products of the highest grade of quality. Hartley guinea pigs weighing 400–600 g (Japan SLC, Hamamatsu) were heparinized (1,000 U/kg, i.p.) and anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Ventricular myocytes were isolated as described previously (8). The isolated hearts were perfused via the aorta with Tyrode’s solution (gassed with 100% O$_2$ and warmed to 36°C) of the following composition: 143 mM NaCl, 4 mM KCl, 0.5 mM MgCl$_2$, 1.8 mM CaCl$_2$, 0.33 mM Na$_2$H$_2$PO$_4$, 5.5 mM glucose and 5 mM HEPES (adjusted to pH 7.3 with NaOH). The heart was perfused with Tyrode’s solution for 5 min. Then the heart was perfused with nominally Ca$^{2+}$-free Tyrode’s solution for 15 min and successively the same solution containing 0.5 mg/ml collagenase (Yakult, Tokyo) for about 10 min. Thereafter, the collagenase was washed out by the KB solution (cell storage solution by Isenberg and Klockner) of the following composition (mM concentration): 70 mM glutamic acid, 15 mM taurine, 30 mM KCl, 10 mM

*To whom correspondence should be addressed.
KH₂PO₄, 0.5 mM MgCl₂, 11 mM glucose, 10 mM HEPES and 0.5 mM EGTA (adjusted to pH 7.3 with KOH). The isolated ventricular cells were stored in KB solution until used.

Transmembrane potentials were recorded using conventional glass microelectrodes as described previously (5). Microelectrodes were filled with 3 M KCl (tip resistance 30–60 MΩ) and were connected to the head-stage of a microelectrode amplifier (MEZ-7200; Nihon Kohden, Tokyo) with high input impedance and capacity neutralization. Cells were superfused with Tyrode’s solution at 37°C. Action potentials were evoked by passing brief current pulses (5-msec duration, about 1.2 times threshold) through the recording electrode using an active bridge circuit. The output of a microelectrode amplifier monitored through a dual beam cathode ray oscilloscope was fed into an AD converter attached to a computer (PC9801 DX; NEC, Tokyo) for analyses. Cells were stimulated at a frequency of 1 Hz during the experiments. Drugs were applied cumulatively from low to high concentration. Terfenadine was dissolved in dimethyl sulfoxide (DMSO), while KW-4679 was dissolved in distilled water. Then they were diluted in Tyrode’s solution before the experiments. The final concentration of DMSO was less than 0.01%, and this concentration of DMSO had no effects on the measured action potential parameters.

 Significant difference between means of data was evaluated by Student’s paired t-test. A P value less than 0.05
was considered statistically significant.

Table 1. Effects of terfenadine on action potential parameters of isolated guinea pig ventricular myocytes

|                  | Control       | Terfenadine |
|------------------|---------------|-------------|
|                  | 0.03 µM      | 0.1 µM     | 0.3 µM     | 1 µM         |
| **RP (mV)**      | −84.5± 0.8   | −84.3± 1.0 | −84.1± 0.9 | −84.0± 1.0   | −83.5± 1.3   |
| **APA (mV)**     | 125.2± 2.6   | 125.1± 3.0 | 124.1± 3.0 | 123.1± 3.5   | 108.7± 2.7** |
| **APD_{90} (msec)** | 237.0± 25.7 | 261.9± 33.9| 272.4± 34.0*| 270.9± 36.6 *| 347.5± 59.5*|
| **APD_{60} (msec)** | 259.5± 27.1 | 291.1± 36.8*| 303.6± 36.6*| 308.7± 40.5* | 406.5± 60.8**|

RP, resting potential; APA, action potential amplitude; APD_{90} and APD_{60}, action potential duration at 90% and 50% of repolarization, respectively. Data are means±S.E.M., n=6. *P<0.05 **P<0.01 vs control.

Figure 1 shows typical traces of the effect of 1 µM terfenadine and 1 µM KW-4679 on the action potential. Tables 1 and 2 summarize the effects of terfenadine and KW-4679 on action potential parameters, respectively. Terfenadine at the concentration range of 30 nM to 1 µM increased APD_{90} (APD at 90% of repolarization) in a concentration-dependent manner. Moreover, terfenadine at 1 µM significantly increased APD_{90} (APD at 50% of repolarization) and decreased the action potential amplitude (APA) (Fig. 1 and Table 1). These effects of terfenadine could be partially canceled by washing with drug-free Tyrode's solution. Terfenadine had no effect on the resting potential (RP). On the other hand, KW-4679 at concentrations from 0.1 µM to 100 µM had no significant effects on action potential parameters (APD, APA and RP) (Fig. 1 and Table 2).

Table 2. Effect of KW-4679 on action potential parameters of isolated guinea pig ventricular myocytes

|                  | Control       | KW-4679          |
|------------------|---------------|------------------|
|                  | 0.1 µM        | 1 µM             | 10 µM            | 100 µM         |
| **RP (mV)**      | −84.9± 1.4    | −84.5± 1.4       | −84.8± 1.6       | −84.5± 1.4     | −84.4± 1.4     |
| **APA (mV)**     | 121.6± 1.8    | 121.4± 1.7       | 121.5± 1.7       | 121.2± 1.8     | 121.4± 1.9     |
| **APD_{90} (msec)** | 243.5± 16.3  | 250.1± 18.6      | 246.7± 20.8      | 245.4± 23.9    | 256.1± 21.9    |
| **APD_{60} (msec)** | 272.2± 15.7  | 278.3± 18.5      | 274.8± 20.9      | 274.4± 24.5    | 292.6± 21.7    |

Definitions are as in Table 1. Data are means±S.E.M., n=9.

However, other mechanisms are also considered to be correlated with the induction of the ventricular arrhythmias when terfenadine is used.

With 1 µM terfenadine, a decrease in APA was observed. This change in APA suggests that terfenadine has an inhibitory effect on sodium current. Inhibition of the maximum rate of rise (V_{max}) of action potential by terfenadine was also reported in canine Purkinje fibers (13) and guinea pig ventricular muscles (14). Reduction of V_{max}, which means the inhibitory effect on sodium current, results in a decrease of conduction velocity and prolongs the time for propagation of the activation wave throughout the ventricle, and thereby produces asynchrony in the process of activation among various regions of the ventricle. Furthermore, displacement of [3H]-nitrendipine binding by terfenadine and related compounds was reported, implying the interference of terfenadine with voltage-dependent calcium channels (15). Effects of terfenadine on these channels might also contribute to the induction of ventricular arrhythmias.

As KW-4679 has 30- to 100-fold higher potency in anti-allergic activity than terfenadine in animal experiments, KW-4679 is expected to have clinical effects at lower concentrations compared with terfenadine. It was reported that after an overdose associated with QT prolongation, the serum level of terfenadine might be approximately 0.1
With 0.1 μM KW-4679, no significant effects on action potential parameters were observed. Moreover, even up to 100 μM, KW-4679 exerted no significant effects on action potential parameters. Accordingly, KW-4679 is unlikely to cause any side effects on the heart like those reported in patients taking terfenadine or astemizole.

In conclusion, terfenadine at concentrations from 30 nM to 1 μM significantly increased APD₉₀ in a concentration-dependent manner. On the other hand, KW-4679 at the concentration range of 0.1 μM to 100 μM exerted no effects on APD and other action potential parameters. These results present no evidence that KW-4679 has the possibility to provoke torsade de pointes ventricular arrhythmias.

REFERENCES

1. Davies AJ, Harindra V, McEwan A and Ghose RR: Cardioxic effect with convulsions in terfenadine overdose. Br Med J 298, 325 (1989)
2. Monahan BP, Ferguson CL, Killeavy ES, Lloyd BK, Troy J and Cantilena LR: Torsades de pointes occurring in association with terfenadine use. JAMA 264, 2788–2790 (1990)
3. Hoppu K, Tikanoja T, Tapanainen P, Remes M, Saarenpaa-Heikilla O and Kouvalainen K: Accidental astemizole overdose in young children. Lancet 338, 538–539 (1991)
4. Leor J, Harman M, Rabinowitz B and Mozes B: Giant U waves and associated ventricular tachycardia complicating astemizole overdose. Am J Med 91, 94–97 (1991)
5. Salata JJ, Jurkiewicz NK, Wallace AA, Stupienski RF, Guinosso PJ and Lynch JJ: Cardiac electrophysiological actions of the histamine H₁ receptor antagonists astemizole and terfenadine compared with chlorpheniramine and pyrilamine. Circ Res 76, 110–119 (1995)
6. Ohshima E, Otaki S, Sato H, Kumazawa T, Obse H, Ishii A, Ishii H, Ohnori K and Hirayama N: Synthesis and anti-allergic activity of 11-(aminooalkylidene)-6,11-dihydrobibenz-[6,e]oxepin derivatives. J Med Chem 35, 2074–2084 (1992)
7. Akagi M, Mio M, Miyoshi K and Tasaka K: Antiallergic effects of terfenadine on immediate type hypersensitivity reaction. Immunopharmacol Immunotoxicol 9, 257–279 (1987)
8. Takahashi S, Kato Y, Adachi M, Agata N, Tanaka H and Shigenobu K: Effects of cyclopiazonic acid on rat myocardium: inhibition of calcium uptake into sarcoplasmic reticulum. J Pharmacol Exp Ther 272, 1095–1100 (1995)
9. Pinney SP, Koller BS, Franz MR and Woosley RL: Terfenadine increases the QT interval in isolated guinea pig heart. J Cardiovasc Pharmacol 25, 30–34 (1995)
10. Rampe D, Wible B, Brown AR and Dage RC: Effects of terfenadine and its metabolites on a delayed rectifier K channel cloned from human heart. Mol Pharmacol 44, 1240–1245 (1993)
11. Woosley RL, Chen Y, Frieman JP and Gillis RA: Mechanism of the cardiotoxic actions of terfenadine. JAMA 269, 1532–1536 (1993)
12. Crumb WJ, Wible B, Arnold DJ, Payne JP and Brown AM: Blockade of multiple human cardiac potassium currents by the antihistamine terfenadine: possible mechanism for terfenadine-associated cardiotoxicity. Mol Pharmacol 47, 181–190 (1995)
13. Lang D, Wang C and Wenger T: Terfenadine alters action potentials in isolated canine Purkinje fibers more than acrivastine. J Cardiovasc Pharmacol 22, 432–442 (1993)
14. Tanaka H, Masumuya H, Kato Y and Shigenobu K: Inhibitory effects of terfenadine on the rising phase of action potentials and sinus rates in isolated guinea pig myocardium. Gen Pharmacol (in press)
15. Zhang M, Caldirola P and Timmerman H: Calcium antagonism and structure-affinity relationships of terfenadine, a histamine H₁ antagonist, and some related compounds. J Pharm Pharmacol 45, 63–66 (1993)