Antiarrhythmic Plasma Concentrations of Pirmenol on Canine Ventricular Arrhythmias

Keitaro HASHIMOTO, Kozo WATANABE and Atsushi SUGIYAMA
Department of Pharmacology, Yamanashi Medical College, Tamaho-cho, Yamanashi 409-38, Japan
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Abstract—Using two-stage coronary ligation-, digitalis- and adrenaline-induced canine ventricular arrhythmias, antiarrhythmic effects of pirmenol were examined, and the minimum effective plasma concentration for each arrhythmia model was determined. Pirmenol suppressed all the arrhythmias, and the minimum effective plasma concentrations for arrhythmias induced by 24 hr coronary ligation, 48 hr coronary ligation, digitalis and adrenaline were 1.1±0.3 (by 3 mg/kg, i.v.), 1.1±0.3 (by 3 mg/kg, i.v.), 1.1±0.2 (by 3 mg/kg, i.v.) and 2.5±1.5 (by 3 mg/kg, i.v.) μg/ml, respectively (mean±S.D.M., n=6-7). The concentration for adrenaline-induced arrhythmia was significantly higher than those for the other types of arrhythmias. This pharmacological profile is similar to those of mexiletine, tocainide and cibenzoline. Since pirmenol had no deleterious effects on the blood pressure and sinus node activity, its clinical usefulness is expected.

Pirmenol, (±)-cis-(3-(2,6-dimethyl-1-piperidinyl)propyl-alpha-phenyl-2-pyridine-methanol monohydrochloride monohydrate) is a new synthetic antiarrhythmic agent which chemically resembles disopyramide, and it has been reported to be effective in animal and clinical arrhythmias (1-4). Accordingly, it has been reported to be electrophysiologically similar to disopyramide, and it decreases the maximum rate of rise of the action potential (max dV/dt) and increases the duration of action potential. It has been shown to differ from lidocaine, and it resembles procainamide in that pirmenol significantly depresses the membrane responsiveness. Pirmenol does not seem to block the slow Ca2+ current as judged by the absence of an effect to suppress slow action potential characteristics (5, 6).

We have recently reported effects of various antiarrhythmic drugs using canine ventricular arrhythmia models (7-9) and classified antiarrhythmic drugs based on their pharmacological effectiveness (10). Using the same experimental methods, the present canine experiment was designed to examine antiarrhythmic effects of the unique class 1 drug pirmenol qualitatively and quantitatively in comparison with other antiarrhythmic drugs.

Materials and Methods

Production of two-stage coronary ligation-induced arrhythmia: Seven beagle dogs, weighing 7–9 kg, were anesthetized initially with thiopental sodium. As reported earlier (7), the chest was opened and a two-stage coronary ligation was performed under 1.0% halothane anesthesia after intubation under intravenous thiopental sodium, 30 mg/kg. Bipolar atrial electrodes were sutured on the left atrial appendage, and polyethylene canulae were inserted into the left jugular vein and into the left internal carotid artery for injection of drugs and withdrawal of blood samples and for blood pressure recording, respectively.

Experiments were done without anesthesia at 24 and 48 hr after coronary ligation. The lead II ECG, atrial electrogram from the left atrial appendage, and the blood pressure were recorded continuously using telemetry systems (Nihon Kohden and Nishimu). Three mg/kg pirmenol was injected through
a venous cannula over a period of 3 to 5 sec. Rapid bolus injection was used to apply the two compartment model for analysis of the plasma concentration time curves. Venous samples were drawn from the same cannula 5 min before and at 1, 3, 5, 10, 15, 30 and 60 min after injection of pirmenol.

Production of digitalis-induced arrhythmia: Seven mongrel dogs of either sex, weighing 8–15 kg, were anesthetized initially with 30 mg/kg of thiopental sodium. A supplemental dose of 10 mg/kg was sometimes required. As reported earlier (8), 40 µg/kg ouabain was injected intravenously and then followed by an additional 10 µg/kg every 20 min until stable ventricular arrhythmia was produced. Pirmenol, 3 mg/kg, was injected intravenously through a cannula in the right femoral vein over a period of 3 to 5 sec.

The lead II ECG, atrial electrogram from catheter tip electrodes inserted from the right external jugular vein into the right atrium, and the blood pressure were continuously recorded through a catheter in the right femoral artery. Venous blood samples were drawn from the jugular vein 5 min before and at 1, 3, 5, 10, 30 and 60 min after pirmenol injection.

Production of adrenaline-induced arrhythmia: Seven mongrel dogs of either sex, weighing 7–15 kg, were anesthetized initially with thiopental sodium. As reported earlier (9), after intubation, 1.0% halothane, vaporized with 100% oxygen, was administered with a volume-limited ventilator. Adrenaline was infused through the left femoral vein at a rate of 2.5 µg/kg/min for 18 min. At 3 min after the start of adrenaline infusion, 3 mg/kg pirmenol was injected into the right femoral vein within seconds.

The lead II ECG, atrial electrogram from catheter tip electrodes in the right atrium and blood pressure were continuously recorded. Venous samples were drawn from the jugular vein 1 min before and at 1, 3, 5, 10 and 15 min after injection.

Plasma pirmenol assay: A sensitive and specific determination of pirmenol in plasma was performed at the Nomura Research Institute of Life Science, Kanagawa, Japan using a high performance liquid chromatographic method, which was fundamentally similar to that of Johnson and Pachla (11). The limit of quantification was 10 ng/ml.

Evaluation of the antiarrhythmic effects: The severity of arrhythmia was expressed by the arrhythmic ratio: number of ventricular ectopic beats divided by the total heart rate. Ventricular beats were judged by the different shape of the ventricular complex from the normal QRS complex. For the three arrhythmia models, the arrhythmic ratios before drug injection was almost 1, and there were no spontaneous improvements in these ratios. As reported earlier (7–9), the minimum effective plasma concentration of pirmenol was determined as follows: The last minute of statistically significant decrease (P<0.05) in the arrhythmic ratio compared with that at 0 time was determined. Then the corresponding plasma concentration was calculated from the experimentally derived plasma concentration-time equations, and this was regarded as the minimum effective plasma concentration.

Results

Effects of pirmenol on two-stage coronary ligation-induced arrhythmia: After 1–2 days of coronary ligation, beagle dogs showed ventricular tachycardia, as shown in the ECG A of Fig. 1 and also by the values of the arrhythmic ratios of almost 1, 5 min and just before injection (Fig. 2). The preliminary experiments using 1–5 mg/kg pirmenol, i.v., showed that at a dose of 1 mg/kg, pirmenol had no or only a weak antiarrhythmic effect, while at a dose of 5 mg/kg, it induced vomiting; therefore, a 3 mg/kg dose was used. This dose suppressed the arrhythmia at 24 hr after coronary ligation soon after its injection, and the decrease in the arrhythmic ratio lasted continuously up to 20 min as shown in Fig. 2. The total heart rate, atrial rate and blood pressure did not change significantly, but the blood pressure and atrial rate tended to increase simultaneously with the occurrence of some CNS excitation, (for example, induction of irritable or tremor like movements). As shown in Fig. 3, arrhythmia was still present at 48 hr after ligation, and the plasma concentration of pirmenol was zero, although pirmenol at the dose of 3 mg/kg had been administered 24 hr before. The
same 3 mg/kg dose of pirmenol was injected to the same dog and it showed a stronger and longer lasting antiarrhythmic effect up to 55 min, as judged by the statistically significant decrease in the arrhythmic ratio. The plasma concentration-time curve fitted well with that predicted by the two-compartment open model. The parameters of the curve of the 24 hr experiment were analyzed using the non-linear regression program “MULTI” (12) and an NEC PC-9801 Vm computer (Tokyo, Japan). The curve was expressed as concentration \( C(t) = A e^{-\alpha t} + B e^{-\beta t} \), and the parameters are: \( A = 5.95 \pm 10.03 \mu g/ml \), \( \alpha = 0.40 \pm 0.27 \) min, \( B = 1.50 \pm 0.54 \mu g/ml \) and \( \beta = 0.014 \pm 0.004 \) (n=6). Parameters for the 48 hr experiments were: \( A = 1.63 \pm 0.47 \mu g/ml \), \( \alpha = 0.60 \pm 0.58 \) min, \( B = 1.88 \pm 0.59 \mu g/ml \) and \( \beta = 0.010 \pm 0.003 \) (n=7). The minimum antiarrhythmic plasma concentrations for the canine coronary ligation-induced arrhythmias after 24 and 48 hr were calculated to be 1.1±0.3 \( \mu g/ml \) (at 20 min) and 1.1±0.3 \( \mu g/ml \) (at 55 min), respectively.

**Effects of pirmenol on digitalis-induced arrhythmia:** After the intravenous injection of a total dose of 70–90 \( \mu g/kg \) ouabain, almost all the beats were of ventricular origin, as shown in the -5 and 0 time values of arrhythmic ratio (Fig. 4). Two doses of pirmenol, 1 and 3 mg/kg, were examined in the preliminary experiments. Pirmenol at a dose of 1 mg/kg showed only a weak antiarrhythmic effect; therefore, the dose of 3 mg/kg was chosen for this study. As shown in Fig. 4, pirmenol at a dose of 3 mg/kg decreased the total heart rate and increased the number of conducted beats. The arrhythmic ratio decreased for 8 min. The plasma pirmenol concentration-time curves for each experiment

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Fig. 1. Antiarrhythmic effects of 3 mg/kg pirmenol, i.v., on 24 hr two-stage coronary ligation-induced arrhythmia. BP=blood pressure, HR=heart rate.
fitted well with that predicted by the two-compartment open model. The parameters of the curves were: A=1.43±1.07 μg/ml, α=0.16±0.08/min, B=0.82±0.36 μg/ml and β=0.011±0.008/min (n=7). The calculated minimum antiarrhythmic plasma concentration of pirmenol for canine digitalis-induced arrhythmia (at 8 min) was 1.1±0.2 μg/ml.

Effects of pirmenol on adrenaline-induced arrhythmia: Adrenaline infusion at rates of 2.5–3.5 μg/kg/min induced multifocal ventricular tachycardia of nearly 250/min and consisted almost entirely of ventricular ectopic beats. Pirmenol at doses of 1–6 mg/kg were examined in the preliminary study. Pirmenol at the lowest dose of 1 mg/kg had no antiarrhythmic effect, and pirmenol at a dose of 6 mg/kg induced sinus arrest and a slow idioventricular rhythm and severe hypotension at plasma concentrations of 5.0 and 9.8 μg/ml; therefore, a dose of 3 mg/kg was chosen in the present study. As shown in Fig. 5, ventricular ectopic beats decreased only transiently at 1 min after pirmenol injection, and arrhythmia reappeared. The plasma concentration-time curves fitted well with that predicted by the two-compartment open model theory. The parameters of the

Fig. 2. Summary of the effect of pirmenol on 24 hr coronary ligation arrhythmia. Pirmenol at 3 mg/kg, i.v., decreased the arrhythmic ratio and increased the number of conducted beats. Vertical bars represent standard deviation. *P<0.05, **P<0.01.
curves were: \( A = 3.47 \pm 3.32 \text{ mg/ml} \), \( \alpha = 0.92 \pm 0.54/\text{min} \), \( B = 1.22 \pm 0.59 \text{ mg/ml} \) and \( \beta = 0.026 \pm 0.019/\text{min} \) (n=7). The minimum anti-arrhythmic pirmenol plasma concentration for canine halothane-adrenaline induced arrhythmia (at 1 min) was 2.5\( \pm \)1.5 \( \mu \)g/ml. This concentration was significantly higher than other concentrations obtained using other arrhythmia models (P<0.05).

Relationship between pirmenol plasma concentration and its antiarrhythmic effect: The plasma concentration data and the corresponding values of the arrhythmic ratio obtained from the 24 hr coronary ligation arrhythmia experiment are plotted in Fig. 6. There was a correlation of \( r = -0.59 \), but the data plotted in the figure were scattered and complete disappearance of arrhythmia, arrhythmic ratio of 0, occurred at concentrations ranging from about 0.7 to 4 \( \mu \)g/ml. In all four experiments, only the regression lines and the coefficients are shown in Fig. 7. The negative correlation coefficients (r) were not high (0.35–0.59).

Discussion

The present experiment using the three canine ventricular arrhythmia models con-
Fig. 4. Summary of the effects of 3 mg/kg pirmenol, i.v., on digitalis-induced arrhythmia. Pirmenol decreased the total heart rate and arrhythmic ratio and increased the number of conducted beats. *P<0.05, **P<0.01.

firmed previous reports that pirmenol is effective in animal experimental arrhythmias (1-4). Using 24 hr Harris arrhythmia, Mertz and Steffe (2) reported that bolus intravenous injections or high rate continuous intravenous infusion of pirmenol were effective; and in the high rate infusion study, pirmenol at plasma concentrations of 0.5, 0.8 and 2.1 µg/ml produced 52, 80 and 95% conversion of ventricular tachycardia to normal sinus beats, respectively. Our experiment using 3 mg/kg pirmenol showed that the minimum effective concentration was 1.1 µg/ml, and this is very close to the effective concentration stated above. At this concentration, the maximal decrease of the average arrhythmic
Fig. 5. Summary of the effects of 3 mg/kg pirmenol, i.v., on halothane-adrenaline arrhythmia. Pirmenol showed only a transient antiarrhythmic effect. *P<0.05, **P<0.01.

ratio to almost 0 increased to about 0.35 (20 min after injection). This concentration, though neglecting the plasma protein binding of pirmenol, 83–90% in human plasma (13), is close to the in vitro threshold concentration of pirmenol, $10^{-6}$ to $1.5 \times 10^{-6}$ M (0.4–0.6 µg/ml), which decreased the max dV/dt of canine Purkinje fiber action potential and its automaticity (5, 6). This indicates that the blockage of Na$^+$ channels is the mechanism of the antiarrhythmic effects of pirmenol. In addition, our study also determined the minimum effective plasma concentrations of pirmenol on digitalis- and adrenaline-induced arrhythmias; This kind of data has not been reported in the other studies (1–4). Different from the results reported by Steffe et al. (1) that pirmenol was effective both on digitalis and epinephrine-induced arrhythmias, we found only a transient antiarrhythmic effect on halothane-adrenaline arrhythmia with nearly 3 times higher concentration than that for coronary ligation and digitalis arrhythmias. The difference might be due to the method of producing the adrenaline arrhythmia. We produced the arrhythmia by adrenaline infusion under halothane anesthesia, while Steffe et al. produced it by a bolus adrenaline injection in 4th day Harris dogs in the conscious state. Compared with the
antiarrhythmic profile of other drugs reported in our previous studies using the same canine arrhythmia models and determining the minimum effective plasma concentrations of antiarrhythmic drugs (Table 1) (14), pirmenol shows the same antiarrhythmic profile as mexiletine, tocainide and cibenzoline, where these four drugs were effective on three canine ventricular arrhythmias, but least effective on adrenaline-induced arrhythmia.

This may indicate that pirmenol does not preferentially block Ca\(^{2+}\) channels, and this is reflected in the absence of severe hypotensive effects and sinus depression. Similar results using isolated cardiac fiber were reported by Reder et al. (5) and Dukes et al. (6) that pirmenol may have no depressing effect on Ca\(^{2+}\) channels.

Clinical studies reported that trough plasma pirmenol concentrations of 1.6 \(\mu\)g or higher suppressed the ventricular premature complex (15). This concentration is close to our canine minimum effective plasma concentrations for coronary ligation and digitalis-induced arrhythmias, indicating that there might not exist a species difference in the concentration of pirmenol to suppress ventricular arrhythmia. In our study, the correlation of the plasma concentration of pirmenol and its antiarrhythmic effect was statistically significant, although the negative correlation coefficients in Fig. 7 is not high. Although pirmenol showed an antiarrhythmic effect even at fairly low concentrations, simple collection of data of the assayed plasma concentration and the corresponding value of the arrhythmic ratio resulted in an intercept value of less than 1 on the arrhythmic ratio axis, although the control preinjection value of the average arrhythmic ratio was almost 1 in both coronary ligation and digitalis induced arrhythmias. Nevertheless the present study confirmed again that the absolute values of the plasma concentration of an antiarrhythmic drug for individual animals or clinical cases are subject to wide variation, and thus less helpful in predicting the antiarrhythmic effects, but with sequential determination, drug plasma concentration data may become useful in predicting changes in the antiarrhythmic effect and detecting the toxicity of drugs (14, 16).

Since pirmenol is effective on various arrhythmia models and has no cardiovascular and central nervous system side effects at its antiarrhythmic concentrations, it may become a useful antiarrhythmic drug clinically as has already been shown in studies using a limited number of patients (17–20).

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Table 1. Antiarrhythmic and electrophysiological profiles of class 1 antiarrhythmic drugs on canine ventricular arrhythmia models (From references 10 and 14)

| Drugs          | Canine minimum effective plasma concentration (µg/ml) | Canine Na-channel blocking conc. (µg/ml) | Action potential duration | Canine Ca-channel block | Human antiarrhythmic plasma conc. (µg/ml) |
|----------------|------------------------------------------------------|-----------------------------------------|---------------------------|-------------------------|------------------------------------------|
|                | Dig Arrhy  | Cor Arrhy  | Adr Arrhy | kinetics |                        |                          |                                          |
| Pirmenol       | 1.1*       | 1.1*       | 2.5       | 0.4–4    | Lengthen               | No                        | 1.6–                                    |
| Phenytoin      | 12.1       | 9.8       | 11.3      | 5–20     | Shorten                | Yes                       | 10–18                                   |
| E-0747         | 1.4        | 1.6        | 1.8       | 1        | Slow                   | Shorten                   | 0.5–2                                   |
| Mexiletine (B) | 1.8*       | 1.9*       | 3.7       | 2        | Fast                   | Shorten                   | 0.5–2                                   |
| Tocainide (B)  | 6.2*       | 11.4**     | 23.7      | 60       | Fast                   | Shorten                   | 3–6                                     |
| Cibenzolone    | 0.6**      | 1.9*       | 3.5*      | 0.5–1    | Slow                   | (—)                       | Yes                                     | 0.3–                                    |
| AN-132         | 0.8**      | 3.4*       | 9.1       | 5        | Slow?                  |                           |                                         |
| Aprindine      | 0.8*       | 1.6        | 1.0*      | 0.5      | Slow                   | Shorten                   | 0.25–1.25                               |
| Propafenone    | 1.8*       | 3.5        | 0.6**     | 0.25–2   | Slow?                  | Shorten                   | Yes (Dir & Ind)                        | 0.5–1                                   |
| Disopyramide (A) | 1.7*    | 5.3        | (—/W)     | 5        | Slow                   | Lengthen                  | Yes                                     | 3–5                                     |
| Procainamide (A) | 10.1*  | 26.4       | (—/W)     | 30       | Slow                   | Lengthen                  | No (Increase)                          | 3–14                                    |
| SUN 1165       | 0.9*       | 2.5        | (—/W)     | 3        | Slow                   | Shorten                   |                                         |
| AHR 10718      | 2.8*       | 8.1        | (—/W)     | 2        | Shorten                |                           |                                         |
| Lidocaine (B)  | 3.5        | (—)        | (—)       | 10       | Fast                   | Shorten                   | No                                      | 2–5                                     |

*Concentration significantly lower than concentration of the same drug without asterisk. **Concentration significantly lower than concentration of the same drug with or without asterisk. Dig Arrhy=digitalis induced arrhythmia, Cor Arrhy=coronary ligation induced arrhythmia, Adr Arrhy=adrenaline induced arrhythmia, Dir=direct effect, Ind=indirect effect via beta adrenergic receptor, (—)=no effect. (—/W)=no effect or worsening. conc.=concentration.
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