Antiarrhythmic Profile of a New Class 1 Drug, AHR 10718, on Canine Atrial and Ventricular Arrhythmia Models

Harumi MITSUHASHI and Keitaro HASHIMOTO*

Department of Pharmacology, Yamanashi Medical College,
1110 Tamaho-cho, Nakakoma-gun, Yamanashi 409-38, Japan

Accepted December 25, 1987

Abstract—Antiarrhythmic effects of AHR 10718 were examined using two-stage coronary ligation, digitalis and adrenaline-induced canine ventricular arrhythmias and aconitine-induced canine atrial arrhythmia. The minimum effective plasma concentration for each arrhythmia model was determined for quantitative analysis of the antiarrhythmic effects. AHR 10718 suppressed the above arrhythmias except for adrenaline-induced arrhythmia. The minimum effective plasma concentrations for arrhythmias induced by 24 hr coronary ligation, 48 hr coronary ligation and digitalis were 8.1 ± 0.7 (by 10 mg/kg, i.v.), 2.9 ± 0.9 (by 5 mg/kg, i.v.) and 2.8 ± 0.6 (by 5 mg/kg, i.v.) µg/ml, respectively (mean ± S.D., n=6). The correlation coefficients between the antiarrhythmic effects of AHR 10718 and its plasma concentrations were not high. This pharmacological profile is characteristic of class 1 Na channel blockers, and in particular, it is similar to those of disopyramide, procainamide and SUN 1165 from our previous studies. AHR 10718 is expected to become a clinically useful antiarrhythmic drug.

AHR 10718, (N'(2-(diethylamino)ethyl)-N-(1-methylethyl)-N-(2-(phenylsulfonyl)ethyl) urea, (Z)-butanedioate, is a new antiarrhythmic agent, and in a preliminary report, it was found to be effective orally and parenterally against a wide spectrum of arrhythmias (1). AHR 10718 has been shown to have effects characteristic to class 1 Na channel blocking drugs and also to have a suppressing effect on triggered activity and delayed afterdepolarization (2). These preclinical studies predict effectiveness of this drug in clinical arrhythmias, and so thorough animal studies showing the effectiveness of this drug are needed before clinical studies are started.

In spite of a large number of studies concerning antiarrhythmic agents, there are still difficulties in choosing theoretically the most suitable antiarrhythmic drug in various clinical settings. This may be partly due to the difficulty in elucidating the generation mechanism of arrhythmias for each clinical case, and partly due to lack of enough knowledge correlating the effects of drugs on action potential characteristics or ionic channels to the generation mechanisms of arrhythmias of both men and animals. In an attempt to solve this problem, we have conducting precise drug studies using various experimental arrhythmia models, even though their generation mechanisms are not fully understood. If similarities between animal model arrhythmias and human clinical arrhythmias can be detected, antiarrhythmic drugs might be more suitably chosen under various clinical settings based on their effectiveness on animal experiments. We have already examined many antiarrhythmic agents using canine ventricular arrhythmia models and demonstrated that each class 1 antiarrhythmic drug has its own profile of pharmacological effectiveness (3–6).

In the present study, effects of AHR 10718 were examined using not only the ventricular arrhythmias but also the atrial arrhythmia model, simultaneously determining effective plasma concentrations of the drug in order

* To whom correspondence should be addressed.
to quantitatively compare AHR 10718 with previously examined antiarrhythmic drugs.

Materials and Methods

Production of two-stage coronary ligation-induced arrhythmia: Six beagle dogs, weighing 7–9 kg, were anesthetized initially with 30 mg/kg of thiopental sodium. As reported earlier (3), the chest was opened and the two-stage coronary ligation was performed under halothane anesthesia.

Experiments were done without anesthesia 24 and 48 hr after coronary ligation. The lead II ECG, atrial electrogram from the left atrial appendage, and blood pressure were recorded continuously using telemetry systems (Nihon Kohden and Nishimu). Ten mg/kg and 5 mg/kg AHR 10718 for 24 hr and 48 hr arrhythmias, respectively, were injected through a cannula inserted into the jugular vein. Venous blood samples were taken from the cannula 5 min before and 1, 3, 5, 10, 15, 30 and 60 min after the injection of AHR 10718.

From the ECG and atrial electrogram, total heart rate, number of conducted beats and atrial rate was measured. The total heart rate is the number of all beats counted from the 5 sec strip of lead II ECG, and the conducted beats are those electrical beats with normal shape of P and QRS complex (ST segment often showed changes which were probably due to ischemic change in the ventricle). The atrial rate is the number of sharp atrial deflections counted from the 5 sec strip.

Production of digitalis-induced arrhythmia: Six mongrel dogs of either sex, weighing 7–14 kg, were anesthetized initially with thiopental sodium. As reported earlier (4), 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 2.5–3.5 μg/kg/min for 18 min. After 3 min of adrenaline infusion and when stable and severe ventricular arrhythmia was produced, 10 mg/kg AHR 10718 was injected into the right femoral vein.

The lead II ECG and blood pressure were continuously recorded. Venous blood samples were taken from the jugular vein 1 min before and 1, 3, 5, 10, 15, 30 and 60 min after AHR 10718 injection.

Production of adrenaline-induced arrhythmia: Six mongrel dogs of either sex, weighing 7–14 kg, were anesthetized initially with thiopental sodium. After the chest was opened through the right 4th intercostal space, 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 2.5–3.5 μg/kg/min for 18 min. After 3 min of adrenaline infusion and when stable and severe ventricular arrhythmia was produced, 10 mg/kg AHR 10718 was injected into the right femoral vein.

The lead II ECG and blood pressure were continuously recorded. Venous blood samples were taken from the jugular vein 1 min before and 1, 3, 5, 10, 15, 30 and 60 min after AHR 10718 injection.

Production of aconitine-induced atrial arrhythmias: Ten mongrel dogs of either sex, weighing 7–15 kg, were anesthetized with 30 mg/kg pentobarbital sodium. After the chest was opened through the right 4th intercostal space, a small piece of filter paper soaked with 1% aconitine was placed on the right atrium (aconitine was dissolved in 0.01 N HCl). The right atrial electrogram was recorded through bipolar electrodes attached to the right atrial appendage, and the lead II ECG was continuously recorded. The blood pressure was recorded from the right femoral artery. In six out of ten dogs, AHR 10718 was administered intravenously 2 min after application of aconitine; and in four other dogs, no drug was given, and they served as the control. Seventeen min after aconitine was applied, the aconitine-soaked filter paper was removed. Since care was taken not to induce ventricular fibrillation, conversion to ventricular fibrillation was not observed during the experimental period. Venous blood samples were taken as described above for the adrenaline arrhythmia.

Plasma AHR 10718 assay: Venous blood samples were centrifuged and the plasma was stored in a freezer at about -25°C before plasma AHR 10718 concentration analysis.

The plasma AHR 10718 assay was carried out using a high-performance liquid chromatograph (HPLC), according to the method described by Wright (personal communica-
A HPLC (Nippon Bunko, BIP-1 Type) equipped with a UV detector (Nippon Bunko, UVIDEC- 100V Type) was used. Conditions were as follows: column, Finepak SILC18, 150×4.6 mm (Nippon Bunko); mobile phase, 10 mM phosphate buffer (pH 3) : acetonitrile (3:7), 1.0 ml/min; detection, UV at 220 nm.

To extract AHR 10718, after 1.75 ml of distilled water and 0.25 ml of 2N-NaOH were added to 0.5 ml of canine plasma and stirred, the mixture was extracted three times with 5 ml of hexane : dichloromethane (1:1), and the organic phase was evaporated to dryness at 55°C under reduced pressure. The residue was first dissolved in 100 μl of 10 mM phosphate buffer (pH 3), which then was diluted to the desired concentration with the same buffer, and 40 μl of the solution was injected into the HPLC system.

There was an excellent linearity (r=0.999) in the range of 50–1000 ng/ml of the calibration curve. The limit of detection was 50 ng/ml.

Determination of the minimum effective plasma concentration: The severity of arrhythmia was expressed by the arrhythmic ratio: the 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 all three ventricular arrhythmias except aconitine-induced atrial arrhythmias, the arrhythmic ratios before drug injection were almost 1, and there were no spontaneous improvements in these ratios. As reported earlier (3, 4), the minimum effective plasma concentration of AHR 10718 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 AHR 10718 on two-stage coronary ligation-induced arrhythmia: After 24 to 48 hr of coronary ligation, beagle dogs showed multifocal ventricular tachycardia, as indicated by the −5 and 0 time values in Fig. 1. While the 48 hr arrhythmia spontaneously became less severe, the 24 hr arrhythmia was so severe that there were almost no conducted beats at the start of the experiment. Doses of AHR 10718, 5 and 10 mg/kg i.v., were examined in the preliminary experiments; and since a 5 mg/kg dose suppressed the 48 hr arrhythmia but did not suppress the 24 hr arrhythmia, 10 and 5 mg/kg doses were chosen for the 24 hr and 48 hr arrhythmias, respectively. As shown in Fig. 1, 10 mg/kg AHR 10718, i.v., significantly increased the number of conducted beats and decreased the arrhythmic ratio for up to 12 min, but there were no significant changes in the total heart rate, atrial rate, and the blood pressure of the dog with 24 hr arrhythmia. Although vomiting was induced within 2 min after administration of 10 mg/kg AHR 10718, no other central nervous system side effect was produced at this dose. The same dogs were used the next day for the study of AHR 10718 effects on the 48 hr arrhythmia. As shown in Fig. 2, this arrhythmia was less severe and the plasma concentration of AHR 10718, just before the 48 hr experiments, was zero, even though 10 mg/kg AHR 10718 had been administered 24 hr before. A lower dose of AHR 10718, 5 mg/kg, i.v., showed significant antiarrhythmic effects in 48 hr arrhythmias. There was a significant decrease in arrhythmic ratio with longer duration of action up to 30 min. As shown in Fig. 2, there were no significant changes in the total heart rate and blood pressure, but the atrial rate was increased. There was no central nervous system side effect such as vomiting. The plasma concentration time curve fitted well with that predicted by the two compartment open model as shown in Fig. 3. The parameters of the AHR 10718 concentration-time equation, expressed as concentration=Ae^{−αt} +Be^{−βt}, of the 24 hr experiment were: A=129.0±72.8 μg/ml, alpha=1.00±0.65/min, B=11.8±2.6 μg/ml, and beta=0.031±0.016/min (n=6), where A is the concentration at 0 time of the distribution curve and B is that of the elimination curve, and alpha is the time constant of the former curve and beta is that of the latter. The parameters for the 48 hr experi-
ments were: A=73.7±49.4 μg/ml, alpha=1.47±0.83/min, B=8.2±1.9 μg/ml, and beta=0.035±0.016/min (n=6). The minimum antiarrhythmic plasma concentrations for the canine 24 hr and 48 hr coronary ligation-induced arrhythmias were calculated as 8.1±0.7 μg/ml (at 12 min) and 2.9±0.9 μg/ml (at 30 min), respectively.

Effects of AHR 10718 on digitalis-induced arrhythmia: After injection of a total dose of 70–80 μg/kg ouabain, almost all the beats became of ventricular origin. As shown in Fig. 4, 5 mg/kg AHR 10718, i.v., significantly (P<0.05) decreased the total heart rate and the arrhythmic ratio and increased the number of conducted beats. This antiarrhythmic effect lasted up to 10 min. The blood pressure was at first transiently decreased, and after recovery, it again gradually decreased, but these changes were not statistically signifi-

![Graph showing the effects of AHR 10718 on arrhythmia](image-url)
The plasma concentration-time curves fitted well with that predicted by the two-compartment open model as shown in Fig. 3. The parameters of the AHR 10718 concentration-time equation were: A=16.7±8.5 μg/ml, alpha=0.55±0.28/min, B=2.7±1.4 μg/ml, and beta=0.028±0.019/min (mean±S.D., n=7). The minimum effective plasma concentration was calculated like that at 10 min, 2.8±0.6 μg/ml.

Effects of AHR 10718 on adrenaline-induced arrhythmia: As reported previously (4), adrenaline infusion for 3 min at a rate of 2.5–3.5 μg/kg/min induced tachycardia with almost all the beats consisting of ventricular ectopic beats. AHR 10718 at the doses of 5, 10 and 15 mg/kg were examined in the preliminary study; and since 5 and 10 mg/kg doses did not suppress the arrhythmia and a 15 mg/kg dose induced ventricular fibrillation, a 10 mg/kg dose, which seemed to be a sufficiently high dose without inducing ventricular fibrillation, was used for the present experiment. As shown in Fig. 5, a dose of 10 mg/kg AHR 10718 was ineffective on adrenaline arrhythmia, although it showed a
slight decrease in the number of ventricular ectopic beats. The plasma concentration-time curve of AHR 10718 fitted well with that predicted by the one compartment open model theory as shown in Fig. 3. The parameters of the concentration-time equation, expressed as concentration = \( Ae^{-\alpha t} \), were: \( A = 50.4 \pm 22.1 \mu g/ml \), and \( \alpha = 0.20 \pm 0.11/\min \) (n=6), where \( A \) is the concentration at 0 time and \( \alpha \) is the time constant. The maximum concentration of AHR 10718 was 44.1 \( \pm 13.5 \mu g/ml \).

Effects of AHR 10718 on aconitine-induced arrhythmias: As can be seen in the atrial rate of control dogs in Fig. 6, aconitine application induced atrial fibrillation lasting more than 15 min. Application of aconitine on the right atrium induced atrial fibrillation, increasing the atrial rate to 744\( \pm 239 \) beats/min within a min, and this effect lasted as long as 17 min or more. Since the preliminary experiments using 2 and 5 mg/kg indicated that both doses showed an antiarrhythmic effect, i.e., abolition of atrial fibrillation, and 5 mg/kg showed an antiarrhythmic effect lasting more than 15 min, a 2 mg/kg dose was used in the present experiment. Two min after aconitine was applied, 2 mg/kg AHR 10718 was administered intravenously. This dose of AHR 10718 converted atrial fibrillation into atrial tachycardia. The blood pressure just before application of aconitine was 111\( \pm 25 \) mmHg and that at the time of AHR 10718 injection was 105\( \pm 29 \) mmHg, and there were no statistically significant changes after the injection.

Although 1:1 atrioventricular conduction was observed immediately after the administration of AHR 10718, it did not persist long and as the rate of atrial tachycardia increased, not all the atrial excitation conducted to the ventricle, but atrial fibrillation did not reappear within 15 min. The plasma concentration-time curve of AHR 10718 fitted well with that predicted by the one compartment open model theory as shown in Fig. 3. The parameters of the concentration-time equation were \( A = 14.5 \pm 8.9 \mu g/ml \) and \( \alpha = 0.53 \pm 0.08/\min \) (n=6). The maximum concentration of AHR 10718 was 8.4\( \pm 4.4 \) \( \mu g/ml \) at 1 min, which was decreased to 0.7\( \pm 0.2 \) \( \mu g/ml \) after 15 min.

Relationship between AHR 10718 plasma concentration and its antiarrhythmic effect: All the plasma concentration data and the corresponding values of the arrhythmic ratio obtained from ventricular arrhythmia experiments were plotted as shown in Fig. 7. A statistically significant correlation was only observed in 24 hr coronary ligation and digitalis arrhythmias, but the negative correlation coefficients (r) were low, 0.46 and 0.40, respectively.

Difference among AHR 10718 plasma concentration-time curves of 5 different arrhythmia models: In order to examine the difference among the plasma concentration-time curves obtained under 5 different experimental conditions, 24 hr coronary ligation, 48 hr coronary ligation, digitalis, adrenaline and aconitine arrhythmias, the curves are normalized to make the 0 time plasma concentration to a unit of 1.0 and plotted in Fig. 3. The three curves of the 24 and 48 hr coronary ligation and aconitine arrhythmia experiments were almost superimposable.

Discussion

Antiarrhythmic effects of AHR 10718: The present results indicate that AHR 10718 is effective on coronary ligation- and digitalis-induced ventricular arrhythmias, and also on
aconitine-induced atrial arrhythmia, which confirmed previous reports that the drug is effective against various kinds of arrhythmias (1, 2).

As far as the effectiveness of AHR 10718 on ventricular arrhythmias is concerned, similar patterns of effectiveness on ventricular arrhythmias were observed with disopyramide, procainamide and SUN 1165 in our previous results (3–5, 8). These drugs were effective on coronary ligation- and digitalis-induced ventricular arrhythmias, but were ineffective against adrenaline-induced arrhythmias. Comparing the minimum effective plasma concentrations of AHR 10718 for these arrhythmias, the drug suppressed digitalis arrhythmia with a lower plasma concentration than the coronary ligation arrhythmia. The minimum effective plasma concentration for the latter arrhythmia, 8.1 µg/ml, was approximately three times higher than the former, 2.8 µg/ml, and a similar

**Fig. 4.** Summary of the effects of 5 mg/kg i.v. AHR 10718, on digitalis-induced arrhythmia. AHR 10718 showed an antiarrhythmic effect and also decreased the total heart rate. *P<0.05. **P<0.01.
quantitative relation between these two kinds of arrhythmias was also observed in our previous experiments with procainamide, disopyramide and SUN 1165 (5). Antiarrhythmic effects of those drugs which suppress digitalis arrhythmia were suggested to be attributed to their Na channel blocking effects in our previous studies (5-7). An electrophysiological study of AHR 10718 by Damiano et al. showed that the drug decreased the max dV/dt of canine Purkinje fibers at concentrations ≥ 5x10^{-6} M (2), about 2 μg/ml, which corresponded approximately to our plasma concentrations of the drug in the present study. This coincidence between effective concentrations in vitro and our effective plasma concentrations confirms the previous findings that the antiarrhythmic effects of AHR 10718 are most likely attributable to its Na channel blocking effects.

As for the adrenaline-induced arrhythmia, AHR 10718 was ineffective against this arrhythmia. Since adrenaline arrhythmia is caused by increased transmembrane influx of calcium produced by adrenaline (4, 9), we assume that AHR 10718 does not have a depressant effect on this Ca current. This speculation is supported by the electrophysiological observation by Damiano et al. that the drug did not suppress the slow inward current (2). In addition, other experimental studies indicated that AHR 10718 at 10 mg/kg, i.v., showed only weak antiadrenergic effects in dogs (10). Therefore, it is expected that this drug may not be effective against arrhythmias related to increased sympathetic tone.

In order to study effects of drugs on atrial arrhythmia, aconitine has frequently been used as the arrhythmogenic stimulus with several application methods (11-16). In the present study, we used a simple technique, the local application of aconitine to a restricted area of the right atrium. AHR 10718 also suppressed this arrhythmia immediately after ad-

---

**Fig. 5.** Summary of the effects of 10 mg/kg AHR 10718, i.v., on adrenaline-induced arrhythmia. In spite of high plasma concentrations, AHR 10718 had no antiarrhythmic effect.

**Fig. 6.** Summary of the effects of 2 mg/kg AHR 10718, i.v., on aconitine-induced atrial arrhythmia. Atrial fibrillation turned to tachycardia soon after injection, and the effect lasted at least 15 min after injection. *P<0.05, **P<0.01.
administration, and the effect lasted for the experimental observation period of 15 min. The generation mechanism of aconitine-induced arrhythmia is reported to be enhanced automaticity owing to the increase in sodium inward current in the myocardial membrane (13, 14). Alternatively, several studies have also indicated the involvement of the enhanced parasympathetic nervous system in aconitine-induced arrhythmia, and there is a report that this arrhythmia model was especially useful to find drugs having anticholinergic action (12). However, unlike disopyramide, AHR 10718 was reported to have no anticholinergic action at doses of 1 to 10 mg/kg, i.v. (10), and yet it suppressed aconitine-induced arrhythmia in the present experiment. Proakis et al. (1) used acetylcholine-induced arrhythmia as an atrial arrhythmia model and showed that AHR 10718 was three times more potent than quinidine in suppressing this arrhythmia. There is no doubt from the present effective plasma concentration data that the antiarrhythmic activity of AHR 10718 against aconitine-induced arrhythmia is explained by the blocking of the Na current, suppressing automaticity of the atrial muscle fibers.

Pharmacokinetic characteristics: As our previous studies have shown, the correlation between the plasma concentrations of antiarrhythmic drugs and their antiarrhythmic effect is rather poor, but the negative correlation coefficients of AHR 10718, ranging from −0.40 to −0.46, are lower than other drugs we tested: for example, cibenzoline, mexiletine and aprindine (6, 7, 17, 18). This may be due to the short period of observation where tissue and plasma drug concentration may not reach equilibrium, especially soon after intravenous injection; however, the relationship may become better in the clinical situation. Also from our previous studies, canine and human minimum effective plasma concentration range may be around 3 to 8 μg/ml.

Fig. 7. Correlation between plasma AHR 10718 concentrations and its antiarrhythmic effects. Open dots represent calculated average minimum effective plasma concentrations.
Figure 3 shows that the elimination phase of the concentration-time curves of the 24 hr, 48 hr coronary ligation and digitalis arrhythmias were very similar. It may indicate that the elimination mechanism of AHR 10718 is independent of the different hemodynamic conditions of each experimental arrhythmia model, while the distribution phase is influenced by the hemodynamic differences among the arrhythmia models we used (18).

Clinical usefulness: Though animal studies can not predict the precise clinical usefulness of antiarrhythmic drugs, the previous electrophysiological studies and the present result indicate that AHR 10718 may be effective against a wide spectrum of arrhythmias generated by various forms of automaticity and conduction disturbances, and thus this drug is worthy of further experimental and clinical studies.

Acknowledgments: The authors thank Mochida Pharmaceutical Co., Ltd. for the gift of AHR 10718. We also thank Mr. J. Sendoda for his technical assistance and Misses M. Yamada and M. Maruyama for preparing the manuscript.

References
18 Hashimoto, K.: Test of pharmacokinetic principles in dogs with experimental ventricular arrhythmias. In Clinical Pharmacology and Pharmacokinetics of Cardiovascular and Renal Drugs, Edited by Sadavongvivat, C. and Iwai, S., p. 59–70, International Center for Medical Research, Kobe University School of Medicine, Kobe (1987)