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Pharmacokinetics and antiarrhythmic activity of ajmaline in rats subjected to coronary artery occlusion

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1 The pharmacokinetics and the antiarrhythmic action of intravenous ajmaline were investigated in anaesthetized rats subjected to coronary artery occlusion.

2 Ajmaline (0.125–2 mg kg^{-1}, i.v. given just after occlusion) suppressed arrhythmias in a dose-dependent manner, judged by the reduction of premature ventricular complexes. The incidence of malignant arrhythmias (ventricular tachycardia and fibrillation) was preferentially suppressed at the higher doses of ajmaline (1 and 2 mg kg^{-1}).

3 Coronary occlusion induced a change in pharmacokinetics of ajmaline (2 mg kg^{-1}) and its total body blood clearance was significantly decreased from 56.6 ml $min^{-1}kg^{-1}$ in sham-operated rats to 43.1 ml $min^{-1}kg^{-1}$ in rats after coronary occlusion.

4 Ajmaline exhibited a significantly increased negative dromotropic action (increased PQ interval) in rats after coronary occlusion compared with that in sham-operated rats. The difference seems to be due to the pharmacokinetic change since the concentration-effect relationship was similar in the two groups of rats.

5 We suggest that the measurement of drug levels is important in the assessment of antiarrhythmic agents.

Introduction

It is generally accepted that most antiarrhythmic drugs have a relatively low therapeutic index and often produce intolerable side effects. Therefore, a precise understanding of both the pharmacokinetic and pharmacodynamic characteristics of those drugs in cardiac disease is of critical importance for a rational treatment of cardiac arrhythmias.

A model of myocardial infarction in the rat, by coronary artery ligation, was first described by Heimburger (1946) and later modified by Johns & Olson (1954), Kaufman *et al.* (1959) and Selye *et al.* (1960). Since then, a number of investigators have employed this model to study different features of myocardial infarction. This model has been used particularly for the detection and assessment of antiarrhythmic activity of various types of drug, such as β-adrenoceptor blocking agents (Kenedi & Losonci, 1973; Campbell & Parratt, 1983), Class I agents like lidocaine (Clark *et al.*, 1980; Bergey *et al.*, 1982), Org 6001 (Clark *et al.*, 1980; Marshall *et al.*, 1981), mexiletine (Marshall *et al.*, 1981), quinidine, procainamide (Bergey *et al.*, 1982), and calcium antagonists (Fagbemi & Parratt, 1981; Fagbemi *et al.*, 1984; Curtis *et al.*, 1984). However, it is noteworthy that there is little information available concerning the pharmacokinetics of antiarrhythmic drugs in this model.

Accordingly, in the present study, we have investigated both the antiarrhythmic activity and the pharmacokinetics of a drug in rats subject to coronary artery occlusion. As a model drug, we have selected ajmaline, which is a Rauwolfia alkaloid with Class I antiarrhythmic properties (Schmitt & Schmitt, 1960), and of which little is known concerning its antiarrhythmic activity and pharmacokinetics in this rat model. In addition, the negative dromotropic action of ajmaline was studied in order to relate the change in the pharmacokinetics of ajmaline with its phar-

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macodynamic activity against a background of myocardial ischaemia.

Methods

Experimental protocol

Cardiac arrhythmias were induced in anaesthetized rats by the method of Clark *et al.* (1980). Male Wistar rats (310–390 g) were anaesthetized with sodium pentobarbitone, 60 mg kg^{-1} administered as required. Systemic arterial blood pressure was recorded from the femoral artery by means of a capacitance transducer (Toyo Baldwin MPU-0.5–290-0-III). A catheter was placed in the femoral vein for administration of drugs, and the trachea was cannulated to allow artificial ventilation. The electrocardiogram (ECG) was recorded with bipolar standard limb leads. Arterial blood pressure and ECG were monitored continuously by use of a polygraph (San-ei Model 366) and a recorder (San-ei Model 8K21-L). Body temperature was maintained with appropriate heating lamps. The animals were ventilated with room air with a stroke of 15 ml kg^{-1} and a rate of 54 strokes min^{-1} (Harvard Rodent Respirator Model 683) to maintain normal Po_2, Pco_2 and pH parameters. The chest was opened by a left thoracotomy at the fourth intercostal space, and after opening the pericardium the heart was exteriorized by gentle pressure on the chest wall. A 6/0 braided silk suture attached to a 10 mm micro-pointed reverse cutting needle (Nesco ER1006s) was placed under the left coronary artery near its origin and the heart was then replaced in the chest cavity. Any animal in which this procedure itself produced arrhythmia or sustained fall in mean arterial blood pressure (MABP) to less than 70 mm Hg before occlusion was discarded from the study at this point. After a stabilizing period of 15 min, acute myocardial ischaemia (AMI) was induced by permanent occlusion of the artery. Sham-operated control rats (SHAM) were subjected to the same procedure except for tying the ligature.

Ajmaline (Gilurytmal, Nippon Chemiphar, 25 mg ml^{-1}) was diluted with isotonic saline, and 2 mg kg^{-1} was administered intravenously as a bolus injection at 1 ml kg^{-1} after the equilibration period (SHAM) or just after coronary artery occlusion (AMI). ECG and arterial blood pressure were monitored up to 120 min after ajmaline injections. Blood samples (0.15 ml) for the assay of the blood ajmaline were collected at 2, 5, 10, 30, 60, 90 and 120 min, and at 120 min an extra 5 ml of blood was taken for the determination of marker enzyme activities in plasma.

For the dose-response study for ajmaline antiarrhythmic actions, 0, 0.125, 0.5 and 1.0 mg kg^{-1} of ajmaline were also administered for AMI rats and the same procedures as above were performed for a minimum period of 30 min after coronary artery occlusion.

ECG analysis

From the ECG recordings run at a paper speed of 10 mm s^{-1}, only those beats of apparent sinus origin were considered to be normal sinus beats and all other ventricular complexes were classified as premature ventricular complexes (PVCs). The severity of arrhythmias was assessed by noting the mortality, the incidence and duration of ventricular fibrillation (VF) and ventricular tachycardia (VT, defined as any run of seven or more consecutive PVCs) and by counting the total number of PVCs for 30 min post occlusion.

Moreover, PQ and RR intervals were measured from high speed (250 mm s^{-1}) ECG recordings, and PQ interval changes measured before and after the injection of ajmaline.

Determination of marker enzyme activities in plasma

The blood obtained at 120 min after drug injection was immediately heparinized and centrifuged. The plasma activities of lactate dehydrogenase (LDH), glutamate-oxaloacetate transaminase (GOT) and creatine phosphokinase (CPK) were assayed by an automated assay system (JEOR JCA-SIM 6R).

Ajmaline assay

A specific reverse phase high performance liquid chromatographic (h.p.l.c.) method described previously (Hori *et al.*, 1984) was used to quantitate ajmaline in blood. The assay involved extraction of 0.1 ml sample and 1.0 ml glycine buffer (pH 10.0, 0.1 M, saturated with sodium chloride) with 5 ml of diethylether, and re-extraction of the organic phase with 0.2 ml of 0.85% phosphoric acid solution. A 50 µl sample of the water phase was subjected to h.p.l.c. The fluorescence spectromonitor was operated at an excitation wavelength of 295 nm and an emission wavelength of 375 nm.

Pharmacokinetic analysis

The disposition of ajmaline after intravenous administration was analysed by a two-compartment open model according to the following equation: $Cb(t) = Ae^{-\alpha t} + Be^{-\beta t}$, where Cb (t) is the whole blood concentration at time t, A and B are the extrapolated intercepts on time zero, and α and β are the rapid and slow hybrid disposition rate constant, respectively (Gibaldi & Perrier, 1982). Pharmacokinetic parameters for individual animals were determined by non-linear least squares regression analysis (Yamaoka *et al.*, 1981) using a digital computer. In addition, the

steady state distribution volume (Vd_{ss}) and total body blood clearance (Cl_t) were calculated.

Statistics

Data are expressed as mean ± s.e.mean. Statistical significance of difference between mean values was calculated using a non-paired *t* test provided that the variances of groups were similar. If this was not the case, Mann-Whitney's U-test was applied. Multiple comparison was performed using Scheffé-type test following Kruskal-Wallis analysis. Paired *t* test was applied for the corresponding values. Difference between regression lines was tested by analysis of covariance. Difference of incidences was compared by Fisher's exact test. *P* values of less than 0.05 (two-tailed) were considered to be significantly different.

Results

Antiarrhythmic effect of ajmaline

In control (dose zero) rats, coronary ligation resulted in the occurrence of arrhythmias which involved single PVC, bigeminal rhythm, VT and VF. The onset of arrhythmias was generally 5 min post occlusion and these arrhythmias usually continued until 15 min after occlusion. A unique feature of this model is that VF usually reverted spontaneously to sinus rhythm.

Table 1 summarizes the antiarrhythmic activity of ajmaline against occlusion-induced arrhythmias as a function of dose. There was a dose-related reduction in the number of PVCs ($P<0.01$) and the duration of VF ($P<0.05$), but the reduction in the duration of VT was not statistically significant ($P<0.1$, Kruskal-Wallis test). Moreover, the highest dose (2 mg kg^{-1}) of ajmaline had a marked protective effect against these ischaemic arrhythmias; the number of PVCs and the incidence of VT and VF were all significantly reduced compared to the non-treated AMI rats. However, the incidence of PVCs was not suppressed even with the highest dose of ajmaline.

Plasma marker enzyme activities

Three kinds of enzyme activities in plasma were measured at 2 h after the injection of ajmaline (2 mg kg^{-1}) in both AMI and SHAM rats in order to assess the myocardial damage induced by coronary artery occlusion. As shown in Table 2, the activities of LDH and GOT in AMI plasma were significantly higher than those in SHAM plasma. Ajmaline did not prevent the occlusion-induced myocardial damage, since the activities of LDH, GOT and CPK in plasma of non-treated AMI rats were 2344 ± 212, 435 ± 43 and 1708 ± 210 iu l^{-1} ($n = 4$), respectively and were not significantly different from those of ajmaline-treated rats.

Haemodynamic effect of ajmaline

Following coronary artery occlusion, the RR interval did not change significantly from the pre-occlusion values (151 ± 6 ms, $n = 4$) in non-treated rats. MABP fell transiently on occlusion but it recovered to pre-occlusion values (114 ± 12 mm Hg, $n = 4$) by 10 min after occlusion.

MABP was decreased by the intravenous injection of ajmaline (2 mg kg^{-1}) in both AMI and SHAM rats, and its reduction at 2 min after dosing was 46 ± 5 mm Hg ($n = 6$, $P<0.005$) and 24 ± 6 mm Hg ($n = 4$, $P<0.05$), respectively. The RR interval was increased by ajmaline and maximal prolongation was found at 2 min after injection (mean increase, 21 ± 3 ms in AMI ($n = 6$, $P<0.005$) and 25 ± 6 ms in SHAM ($n = 4$, $P<0.05$)). These responses were transient and by 10 to 30 min both MABP and RR intervals had returned to pretreatment levels.

Table 1 Effect of ajmaline on occlusion-induced arrythmias (0–30 min) in the anaesthetized rats

Ajmaline (mg kg^{-1})	n	*Number of PVCs* (complexes)	*Duration of VT* (s)	*Duration of VF* (s)	*Mortality*
0	12	1139 ± 231 (12/12)	62 ± 16 (12/12)	24 ± 10 (7/12)	0/12
0.125	6	657 ± 177 (6/6)	34 ± 17 (5/6)	19 ± 9 (4/6)	1/6
0.5	6	451 ± 193 (6/6)	22 ± 13 (4/6)	8 ± 7 (2/6)	0/6
1.0	6	332 ± 164 (5/6)	19 ± 10 (4/6)	0 ± 0 (0/6)*	0/6
2.0	6	121 ± 49** (5/6)	10 ± 6 (3/6)*	0 ± 0 (0/6)*	0/6

n = number of experiments, PVCs = premature ventricular complexes, VT = ventricular tachycardia and VF = ventricular fibrillation. Values are expressed as means ± s.e.mean for the survivals. The incidence of each type of arrhythmia is given in parentheses.
*($P<0.05$) and **($P<0.025$) denote significant difference from the control group (dose zero).

Table 2 Comparison of plasma enzyme activities between coronary artery occluded (AMI) and non-occluded (SHAM) rats

	SHAM ($n = 4$)	*AMI* ($n = 6$)
LDH ($iu\,l^{-1}$)	854 ± 60	2290 ± 349*
GOT($iu\,l^{-1}$)	130 ± 12	366 ± 36***
CPK ($iu\,l^{-1}$)	757 ± 82	1426 ± 274

Ajmaline ($2\,mg\,kg^{-1}$, i.v.) was injected immediately after coronary artery occlusion (AMI) or corresponding time (SHAM) and plasma samples for the determination of enzyme activities were obtained at 120 min after the drug administration. LDH = lactate dehydrogenase, GOT = glutamate-oxaloacetate transminase and CPK = creatine phosphokinase. Values are expressed as mean ± s.e.mean for n animals.
*$P < 0.05$ and ***$P < 0.005$.

Table 3 Pharmacokinetic parameters of ajmaline at a intravenous dose of $2\,mg\,kg^{-1}$ in coronary occluded (AMI) and non-occluded (SHAM) rats

		SHAM ($n = 4$)	*AMI* ($n = 6$)
A	($\mu g\,ml^{-1}$)	1.79 ± 0.18	1.75 ± 0.20
B	($\mu g\,ml^{-1}$)	0.458 ± 0.017	0.524 ± 0.040
α	(min^{-1})	0.229 ± 0.018	0.228 ± 0.023
β	(min^{-1})	0.0166 ± 0.0010	0.0145 ± 0.0012
Cl_t	($ml\,min^{-1}\,kg^{-1}$)	56.6 ± 1.9	43.1 ± 2.2***
Vd_{ss}	($l\,kg^{-1}$)	2.73 ± 0.06	2.56 ± 0.15

Parameters were calculated from the two-compartment open model by non-linear iterative least squares method using a digital computer. A and B are the extrapolated intercepts on time zero, α and β are the rapid and slow disposition rate constant respectively, Cl_t is the total body blood clearance and Vd_{ss} is the steady state distribution volume. Values are expressed as mean ± s.e.mean for n animals.
***$P < 0.005$.

Pharmacokinetics of ajmaline

Figure 1 shows the blood concentrations of ajmaline following bolus intravenous injection ($2\,mg\,kg^{-1}$) in AMI and SHAM rats. The blood concentrations of ajmaline in AMI rats were significantly higher than those in SHAM rats between 10 and 120 min after injection. The drug concentration data were fitted to the two compartment open model and the estimated pharmacokinetic parameters are listed in Table 3. A significant decrease in the total body blood clearance of ajmaline was found in AMI rats compared to SHAM rats.

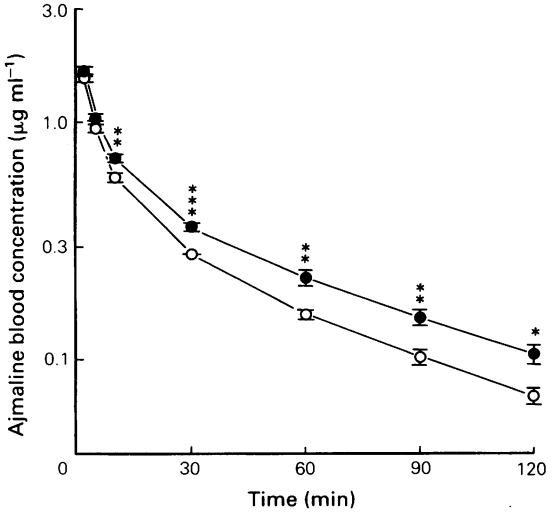

Figure 1 Time course of ajmaline concentration in whole blood in AMI (●, $n = 6$) and SHAM (○, $n = 4$) rats following injection of ajmaline $2\,mg\,kg^{-1}$ i.v. Ajmaline was injected immediately after coronary artery occlusion (AMI) or corresponding time (SHAM). The vertical bar represents s.e.mean.
*$P < 0.05$, **$P < 0.025$ and ***$P < 0.005$.

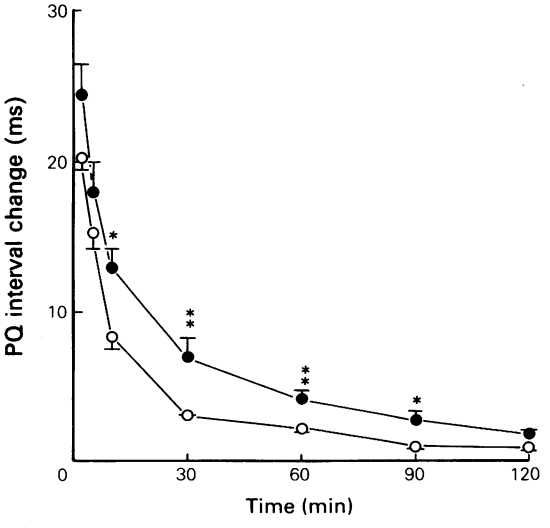

Figure 2 Time course of PQ interval changes in AMI (●, $n = 6$) and SHAM (○, $n = 4$) rats following injection of ajmaline $2\,mg\,kg^{-1}$ i.v. The vertical bar represents s.e.mean.
*$P < 0.05$ and **$P < 0.025$.

Negative dromotropic effect of ajmaline on PQ interval

In non-treated AMI rats, the PQ intervals remained constant for 120 min after occlusion. The pre-occlusion value of PQ interval was 39.7 ± 0.9 ms ($n = 4$) and the mean coefficient of intra-individual variation over 120 min was 1.3%.

Following injection of ajmaline (2 mg kg^{-1}), both AMI and SHAM rats showed rapid prolongation of the PQ interval from the pre-dose level. Figure 2 compares the time course of PQ internal changes in AMI and SHAM rats. AMI rats showed significantly longer prolongation of PQ intervals than SHAM rats, which corresponded to the difference in the blood concentration of ajmaline between the two groups of rats. When the changes in PQ intervals were plotted against the blood concentration of ajmaline at the corresponding time, a linear correlation was found in both AMI ($Y = 14.8X + 1.1$, $r = 0.992$, $P < 0.005$) and SHAM ($Y = 13.7X - 0.1$, $r = 0.989$, $P < 0.005$) rats, and there was no significant difference in the slope of regression line between the two groups.

Discussion

Ajmaline has been found effective against experimental and clinical atrial and ventricular arrhythmias (Arora & Madan, 1956; Dick & McCawley, 1963; Bazika *et al.*, 1966; Bojorges *et al.*, 1975; Obayashi *et al.*, 1976; Wellens *et al.*, 1980; Sethi *et al.*, 1984). In the present study we have shown that ajmaline protects against ischaemia-induced arrhythmias in a dose-related manner in anaesthetized rats. Following coronary artery ligation there were fewer PVCs and more importantly, reduced incidence of VT and VF in those rats treated with ajmaline (2 mg kg^{-1}). These results confirm previous observation in anaesthetized dogs in which ajmaline (2 mg kg^{-1}) was shown to be effective against coronary occlusion-induced arrhythmias (Obayashi *et al.*, 1976).

The rat is now frequently used for ischaemia and infarction studies and is considered to be suitable for the qualitative and quantitative evaluation of drugs active against iscahemia-induced arrhythmias (Winslow, 1984; Curtis *et al.*, 1984). Changes in drug disposition due to cardiac dysfunction may affect the intensity and the duration of drug effect. Therefore we have investigated the pharmacokinetics as well as antiarrhythmic activity of ajmaline in the present study. Significantly higher blood concentrations of ajmaline were found in AMI rats compared to SHAM rats and a pharmacokinetic analysis has revealed a reduction in the total body blood clearance of ajmaline in AMI rats. After intravenous administration of ajmaline at a dose of 50 mg in man, the serum concentration declines in a few minutes to undetectable levels (Kleinsorge & Gaida, 1962; Saetre *et al.*, 1974), and less than 4% of dose is excreted in the urine as intact drug (Kleinsorge & Gaida, 1961). These reports suggest the rapid elimination by an extrarenal mechanism in man. In the present study, ajmaline showed a rapid elimination from the blood with mean half-lives of 3 and 42 min for α- and β-phase respectively and the total body blood clearance exceeded 50 ml min^{-1} in SHAM rats. Liver perfusion studies have shown the avid metabolism of ajmaline in rat liver (unpublished data). Thus, it seems likely that the elimination process of ajmaline is not capacity-limited but blood flow rate-limited (Wilkinson & Shand, 1975), and the principal cause for the change in the total body blood clearance of ajmaline may be the reduced cardiac output or the diminished hepatic blood flow produced by coronary artery occlusion.

Experiments in animals have shown that the antiarrhythmic effects of ajmaline are due to prolongation of the refractory period and the slowing of conduction in both the atrial and the ventricular conduction system (Schmitt & Schmitt, 1960; Bojorges *et al.*, 1975). Concerning coronary occlusion-induced arrhythmias in the rat, Winslow (1984) suggested that the majority of PVCs are due to re-entry through the AV node and that drugs which slow AV conduction might therefore be expected to exert an antiarrhythmic effect. Thus, the measurement of changes in PQ intervals as an electrophysiological response to ajmaline may provide additional information about the antiarrhythmic effect of ajmaline in the ischaemic rat. Anatomically, the septal artery of the rat arises from the right or left coronary and passes deep to run in the intraventricular septum (Halpern, 1957). As to the geometry of myocardial infarcts, Spadaro *et al.* (1980) reported that the occlusion of the left coronary artery results in an infarct that does not include the intraventricular wall. In the present study, the PQ intervals were not affected by the coronary artery occlusion and remained constant in non-treated AMI rats. Thus, the changes in PQ intervals produced by ajmaline can be considered to reflect an effect on the atrial conducting system including AV node. We found a good correlation between the blood concentration of ajmaline and the prolongation of PQ interval in both AMI and SHAM rats, which suggests that the slowing effect of ajmaline on the cardiac conducting system depends on the drug concentration in the blood. As shown in Figure 2, AMI rats showed significantly longer prolongation of PQ intervals than SHAM rats. However, the sensitivity of the conducting system to ajmaline seems to be not affected by coronary occlusion, since there was no significant difference in the slope of the regression line between the blood concentration and the increase in PQ intervals. Hence, the greater effect of ajmaline on PQ interval in AMI rats simply reflects the increased

blood concentration of ajmaline caused by the decreased drug clearance due to coronary occlusion.

Coronary artery occlusion in rats as a model of ischaemia-induced arrhythmias has several advantages including its simplicity, reproducibility and low cost in terms of time, money and amount of compound required. Integrated analysis of the pharmacokinetics and pharmacodynamics of the drug in this model provides useful information necessary for formulating a rational treatment of cardiac arrhythmias.

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