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Comparison of the antiarrhythmic and the proarrhythmic effect of almokalant in anaesthetised rabbits

András Farkas, István Leprán *, Julius Gy. Papp

Department of Pharmacology, Albert Szent-Györgyi Medical University, P.O. Box 115 H-6701 Szeged, Hungary

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Abstract

In this study the antiarrhythmic and the proarrhythmic activities of almokalant, a selective class III antiarrhythmic agent, were compared. The antiarrhythmic effect of the drug was tested in pentobarbital-anaesthetised rabbits. Arrhythmia was evoked by occluding and releasing the left circumflex coronary artery. Almokalant in a dose of 250 nmol/kg i.v., significantly decreased the incidence of reperfusion induced ventricular fibrillation (21% vs. 75% in the control group) and increased the proportion of surviving animals during reperfusion (86% vs. 42%). The proarrhythmic effect of almokalant was examined during α_1-adrenoceptor stimulation in chloralose-anaesthetised rabbits. Almokalant (75 nmol/kg per min) triggered torsade de pointes arrhythmias in 8 animals out of 11. The dose of almokalant (mean ± S.E.M.) required to produce this effect was 1181 ± 519 nmol/kg. It is concluded that, although almokalant is an effective antiarrhythmic agent against ischaemia-reperfusion induced arrhythmias, it has marked proarrhythmic activity during α_1-adrenoceptor stimulation. © 1998 Elsevier Science B.V.

Keywords: Almokalant; Antiarrhythmic effect; Reperfusion arrhythmias; Proarrhythmia; Torsade de pointes; (Anaesthetised rabbit)

1. Introduction

After the Cardiac Arrhythmia Suppression Trial (Cardiac Arrhythmia Suppression Trial investigators, 1989) the use of sodium channel blockers in the treatment of patients convalescing from myocardial infarction became limited and the attention was focused on the repolarisation prolonging agents, which exert their effects mostly by blocking potassium channels (Colatsky and Follmer, 1989). These agents prolong the atrial and the ventricular action potential duration and the corresponding effective refractory period and prevent or terminate reentrant ventricular tachycardias leading to ventricular fibrillation (Hondeghem and Snyders, 1990; Katritsis and Camm, 1993; Roden, 1993). The use of these agents in the therapy, however, might also result in severe proarrhythmias.

The typical proarrhythmia observed with the use of drugs delaying ventricular repolarisation is the provocation of polymorphic ventricular tachycardia denoted torsade de pointes (Jackman et al., 1988). Initiation of torsade de pointes is usually associated with a pause or slowing of heart rate and a concomitant increase in QT interval, followed by a series of rapid repetitive polymorphic QRS complexes with characteristic undulating peaks (Roden et al., 1986; Cranefield and Aronson, 1988). Sometimes torsade de pointes can deteriorate into ventricular fibrillation, a mechanism undoubtedly responsible for sudden cardiac death in a number of patients (Bayes de Luna et al., 1989).

Almokalant is a pure class III antiarrhythmic agent, which blocks selectively the rapid component of the delayed rectifier potassium current (I_{Kr}; Wettwer et al., 1992). Clinical studies have already proved that almokalant is an effective antiarrhythmic agent in patients with supraventricular reciprocating tachycardias, atrial flutter and fibrillation and in post-myocardial infarction patients with ventricular extrasystoles (Darpö and Edvardsson, 1995; Crijns et al., 1995; Wiesfeld et al., 1992). Almokalant as well as other selective I_{Kr} blockers can trigger torsade de pointes both in experimental animals (Carlsson et al., 1993a; Verduyn et al., 1995) and in human (Wiesfeld et al., 1993, Darpö et al., 1996).

The aim of the present study was to examine both the antiarrhythmic and the proarrhythmic effect of almokalant, as a representative agent of the pure class III antiarrhythmics, in the same species under well defined in vivo

* Corresponding author. Tel.: +36-62-455-676; fax: +36-62-321-107; e-mail: lepran@phcol.szote.u-szeged.hu

experimental conditions, and to determine the safety of the cardiac action of this agent.

2. Materials and methods

2.1. Animals

The experiments were performed on New Zealand White rabbits from either sex weighing 1.5 to 2.9 kg. The animals were allowed to have tap water and laboratory rabbit chow (Altromin, Gödöllõ, Hungary) ad libitum until the experiment. The animals were handled according to a protocol reviewed and approved by the Ethical Committee for the Protection of Animals in Research of the Albert Szent-Györgyi Medical University, Szeged, Hungary.

2.2. Examination of the antiarrhythmic activity

Acute coronary artery ligation and reperfusion were performed according to Thiemermann et al. (1989). Rabbits ($n = 59$) were anaesthetised with pentobarbital sodium (30 mg/kg intravenously into the marginal vein of the right ear). A catheter was introduced into the right carotid artery in order to measure blood pressure (Blood Pressure Monitor BP-1, World Precision Instruments, Berlin, Germany). The catheter was filled with isotonic saline containing heparin (500 I.U./ml), but the animals were not heparinised. Another catheter was introduced into the marginal vein of the left ear for infusion of drugs.

After tracheal cannulation, left thoracotomy was performed and artificial ventilation was immediately started with room air (Harvard rodent ventilator, model 683, Harvard Apparatus, South Natick, MA, USA). Respiratory volume and rate (7 ml/kg/stroke, 40 stroke/min, respectively) were subsequently adjusted to keep the blood gases and pH within a normal range. After pericardiotomy, a loose loop of 4-O atraumatic silk (Ethicon, Edinburgh, UK) was placed around the first branch of the left circumflex coronary artery just under its origin. Both ends of the ligature were led out of the thoracic cavity through a flexible tube.

After 10 min stabilising period saline or almokalant (100 or 250 nmol/kg) was administered intravenously for 10 min in continuous infusion (infusion volume 2 ml) right before the coronary artery occlusion.

After the pretreatment, the loose loop was tightened and fixed by clamping on the silk, and thus local myocardial ischaemia was produced. After 10 min coronary artery occlusion, the ligature was released and 10 min reperfusion followed.

The electrocardiogram (lead I, II, III) was registered during the experiments by a thermographic recorder (ESC 110 4 CH, Multiline KFT, Esztergom, Hungary) using subcutaneous needle electrodes. The length of QT intervals was measured in predetermined intervals. QT interval was defined as the time between the first deviation from the isoelectric line during the PR interval until the end of TU wave. Rate corrected QT interval (QTc) was calculated subsequently by using the equation: $QTc = QT - 0.175(RR\text{-}300)$, where RR is the cycle length (Carlsson et al., 1993a).

Arrhythmias were detected during ischaemia and reperfusion and diagnosed in accordance with the Lambeth conventions as ventricular tachycardia, ventricular fibrillation and other types of arrhythmias including single extrasystoles, bigeminy, salvos and bradycardia (Walker et al., 1988). The onset and duration of arrhythmias were also measured.

At the end of reperfusion, heparin sodium (500 U.I./kg, i.v.) was administered and the animals were killed with an overdose of pentobarbital. The heart was cut out from the chest in order to determine the size of the occluded zone. After retightening the ligation coronary arteries were perfused retrogradely with 20 ml saline and 10 ml of 96% ethanol through the aorta (Leprán et al., 1983). The non-denatured area (occluded zone) was excised and its extent was expressed in percentage of the total weight of ventricles. Generally, in about 75% of the rabbits the main supplying artery of the left ventricle is the left circumflex coronary artery, whereas in the rest of the rabbits (in about 25% of the animals) the left ventricle is supplied mainly by the left anterior descending coronary artery (Toyo-oka et al., 1984). In accordance with this, in our experiments there were 15 rabbits out of 59 (25%), in which the occluded zone was less than 16% of the total weight of ventricles, because the occluded left circumflex coronary artery was poorly developed and the frontal wall and the apex of the heart were supplied by the non-occluded left anterior descending coronary artery. In these animals only ischaemic ECG changes (e.g., ST elevation, QRS distortion) occurred and arrhythmias did not develop. In 2 other animals the whole left circumflex coronary artery was occluded proximally to the origin of the first branch, therefore the occluded zone was almost doubled compared to the highest occluded zone measured after occluding only the first branch (53% and 56% vs. 32%, respectively). In these 2 animals the mean arterial blood pressure fell severely right after coronary artery occlusion and remained in a low level until the occurrence of irreversible ventricular fibrillation in the 6th and 7th min of coronary artery occlusion. Thus the animals, in which the occluded zone was less than 16% or was larger than 32%, i.e., altogether 17 animals were excluded from the final evaluation.

2.3. Examination of the proarrhythmic activity

The proarrhythmic activity of almokalant was examined in an animal model of acquired long QT syndrome (Carlsson et al., 1990). After sedation with pentobarbital sodium (5 mg/kg, i.v.), male rabbits ($n = 21$) were anaesthetised with α-chloralose (100 mg/kg intravenously into the

Table 1
Heart rate, mean arterial blood pressure and rate corrected QT values before and during ischaemia and reperfusion in anaesthetised rabbits

	n	Basal	Pretreat.	*n*1	Ischaemia (min)					*n*2	Reperfusion (min)				
					1	3	5	7	10		1	3	5	7	10
Control															
HR		288 ± 7.3	288 ± 7.3		284 ± 7.6	281 ± 8.4	281 ± 8.4	275 ± 9.0	276 ± 9.9		273 ± 20.3	280 ± 19.4	282 ± 17.1	281 ± 16.0	282 ± 15.2
MBP	15	86 ± 3.0	86 ± 3.1	12	77 ± 3.4	78 ± 3.4	78 ± 3.3	78 ± 3.5	80 ± 3.6	5	71 ± 11.0	78 ± 6.4	80 ± 4.6	79 ± 4.6	81 ± 4.3
QTc		163 ± 3.3	164 ± 3.6		167 ± 3.8	169 ± 4.3	172 ± 3.5	174 ± 4.7	172 ± 4.2		159 ± 10.0	173 ± 10.5	172 ± 11.0	174 ± 9.2	172 ± 10.6
Alm 100															
HR		278 ± 8.7	282 ± 6.7		274 ± 6.2	270 ± 6.7	268 ± 6.8	269 ± 6.3	263 ± 7.3		276 ± 19.2	254 ± 8.4	255 ± 8.5	260 ± 9.9	261 ± 8.4
MBP	13	89 ± 3.1	90 ± 3.2	13	83 ± 2.7	83 ± 2.6	83 ± 2.8	82 ± 2.6	82 ± 2.8	8	72 ± 6.1	78 ± 3.2	76 ± 4.7	79 ± 3.9	81 ± 3.1
QTc		165 ± 6.4	167 ± 5.9		178 ± 3.7[b]	176 ± 3.3	179 ± 4.1	179 ± 3.5	177 ± 3.4		174 ± 6.3	178 ± 4.9	186 ± 5.1	182 ± 3.3	178 ± 3.4
Alm 250															
HR		280 ± 6.6	270 ± 5.8[a]		264 ± 5.6	265 ± 5.5	264 ± 5.9	265 ± 5.3	266 ± 5.3		265 ± 5.9	263 ± 6.6	264 ± 6.5	269 ± 5.9	271 ± 6.4
MBP	14	88 ± 3.0	88 ± 4.1	14	74 ± 4.5	81 ± 3.2	80 ± 3.3	77 ± 3.7	79 ± 3.2	12	77 ± 4.4	81 ± 3.8	78 ± 3.2	78 ± 3.0	78 ± 3.2
QTc		159 ± 4.2	175 ± 6.5 [a]		184 ± 4.8[b]	182 ± 4.5	184 ± 4.4[b]	181 ± 4.8	183 ± 5.1		182 ± 6.0	180 ± 5.9	179 ± 5.3	178 ± 5.1	178 ± 5.0

Alm 100 and Alm 250, groups of animals pretreated with almokalant in a dose of 100 or 250 nmol/kg; HR, heart rate (min^{-1}); MBP, mean arterial blood pressure (mm Hg); QTc, rate corrected QT interval (ms); *n*, number of animals; Pretreat., values measured after the pretreatment; *n*1, number of animals surviving ischaemia; *n*2, number of animals surviving reperfusion; [a]$P < 0.05$ compared to the basal value; [b]$P < 0.05$ compared to the control group.

marginal vein of the right ear, in 10 ml/kg infusion volume, at a rate of 1 ml/min).

Catheters were introduced into the right carotid artery, the right jugular vein and the marginal vein of the left ear for recording arterial blood pressure and infusion of drugs, respectively. After tracheal cannulation, the animals were mechanically ventilated with room air as described in Section 2.2. Blood pressure and electrocardiogram was registered during the experiments as in the occlusion–reperfusion model. After 10 min stabilising period, continuous phenylephrine infusion at a rate of 15 μg/kg per min was administered into the right jugular vein of the animals for 80 min (in 2 ml infusion volume as a whole). Ten minutes after the beginning of the phenylephrine infusion, simultaneous almokalant infusion was given into the marginal vein of the left ear at a rate of 25 nmol/kg per min or 75 nmol/kg per min for 70 min (also in 2 ml infusion volume as a whole). Isotonic saline (2 ml over a period of 70 min) was administered to the animals in the control group instead of almokalant. During the experiment the onset and duration of arrhythmias were measured. Torsade de pointes was considered to have occurred if five or more closely coupled repetitive extrasystoles with a twisting or torsioning QRS morphology was observed. Heart rate, blood pressure, QT and QTc intervals and the total accumulated dose of almokalant at the first incidence of torsade de pointes or monomorphic ventricular tachycardia were also measured.

2.4. Drugs

The following drugs were used: almokalant (Astra Hässle, Mölndal, Sweden), phenylephrine (L-Phenylephrine HCl, Koch-Light Laboratories, Colnbrook-Bucks, England), heparin-sodium (Richter Gedeon RT, Budapest, Hungary), pentobarbital-sodium (Nembutal, Phylaxia-Sanofi, Budapest, Hungary), α-chloralose (Fluka Chemie, Buchs, Switzerland). Almokalant was prepared as a concentrated stock solution (100 mmol/ml) by Astra Hässle. The stock solution was diluted further with isotonic saline. All other drugs were dissolved in isotonic saline. Each dose was prepared on the day of the experiment and all doses in the text refer to bases of the compounds.

2.5. Statistical evaluation

The percentage incidence of arrhythmias was calculated and compared by using Fisher's exact probability test. Continuous data were expressed as mean ± standard error of the mean (S.E.M.). Means from the same sample were compared with Student's paired-samples *t*-test. Means from independent samples were compared with one way analysis of variance and if significant, multiple comparisons were performed with modified *t*-test according to the 'Least Significant Difference' method to assess which group was significantly different. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. The antiarrhythmic activity of almokalant

Almokalant in a lower dose (100 nmol/kg) did not have any effect on the heart rate, the mean arterial blood pressure and the QTc intervals, whereas pretreatment with

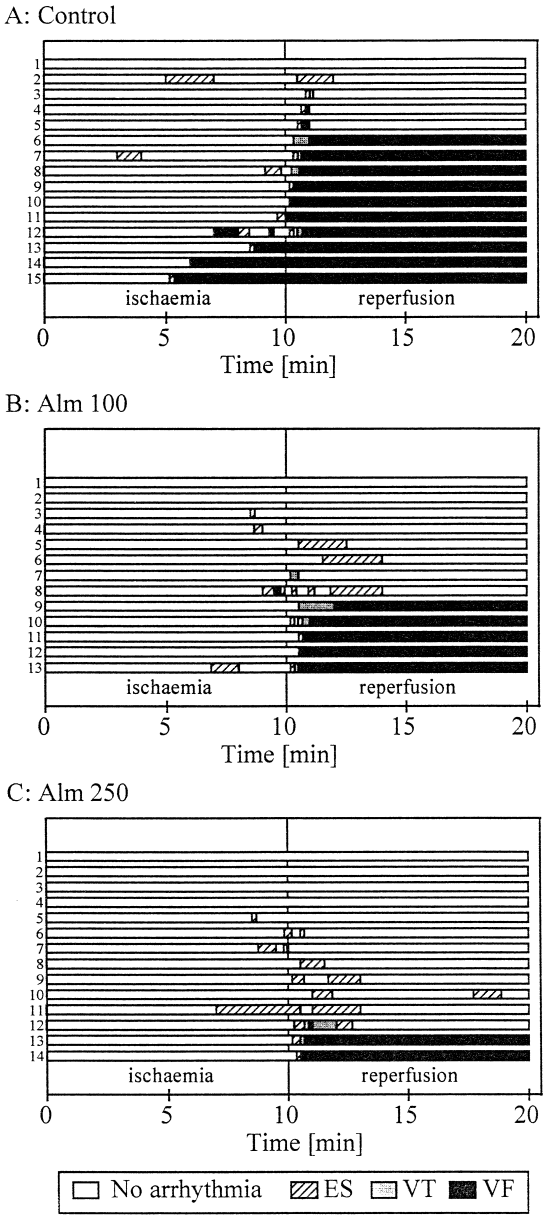

Fig. 1. The arrhythmia map of the control group (A) and groups of animals pretreated with almokalant in a dose of 100 or 250 nmol/kg (B and C, respectively). Each number of the ordinates refers to a separate animal and every row shows the arrhythmias of the given animal during the 10-min ischaemia and the 10-min reperfusion. In every group the animals are listed in order of severity of their arrhythmias. No arrhythmia, periods without arrhythmia; ES, extrasystoles, salvos, and/or bigeminy; VT, monomorphic ventricular tachycardia; VF, ventricular fibrillation.

Table 2
Effect of almokalant on the survival rate and incidence of arrhythmias during ischaemia and reperfusion in anaesthetised rabbits

	n	Survived (%)	Incidence of arrhythmias (%)			
			None	VF	VT	Other
Ischaemia						
Control	15	80	47	27	0	47
Alm 100	13	100	69	8	0	31
Alm 250	14	100	71	0	0	29
Reperfusion						
Control	12	42	8	75	42	58
Alm 100	13	62	31	39	31	46
Alm 250	14	86[a]	43	21[a]	14	57

Alm 100 and Alm 250, groups of animals pretreated with almokalant in a dose of 100 or 250 nmol/kg; *n*, number of animals; VF, ventricular fibrillation; VT, monomorphic ventricular tachycardia; Other, extrasystoles, salvos, and/or bigeminy; [a]$P < 0.05$ compared to the control group.

a dose of 250 nmol/kg decreased the heart rate modestly, while did not have significant effect on the mean arterial blood pressure (Table 1). The higher dose of almokalant prolonged the QTc intervals significantly, compared to the basal values (Table 1). None of the pretreatments induced any arrhythmia before the coronary artery ligation.

In the control group the heart rate (during sinus rhythm) did not change significantly in the course of coronary artery occlusion and reperfusion, while the mean arterial blood pressure fell right after the coronary artery ligation and remained at a lower level till the end of reperfusion (Table 1). In both groups pretreated with almokalant the heart rate and blood pressure response was not significantly different from those in the control group during coronary artery occlusion and reperfusion (Table 1). The longer QTc intervals were present throughout the ischaemia and reperfusion in the group of animals pretreated with almokalant in a dose of 250 nmol/kg per min (Table 1).

Arrhythmia maps of the three groups show the arrhythmias of each animal during the 10 min ischaemia and the 10 min reperfusion (Fig. 1), and the incidence of arrhythmias is shown in Table 2. During the 10 min occlusion period 3 animals died in the control group due to irreversible ventricular fibrillation, whereas all of the animals pretreated with almokalant survived occlusion. Ventricular fibrillation did not appear at all during occlusion in the group of animals pretreated with the higher dose of almokalant (Table 2).

In the control group the incidence of ventricular fibrillation was very high and the survival rate was low during reperfusion. In contrast, in the group of animals pretreated with almokalant in a dose of 250 nmol/kg the incidence of ventricular fibrillation was significantly lower and the survival rate was significantly higher during reperfusion than in the control group (Table 2).

3.2. The proarrhythmic activity of almokalant:

In the first 10 min, when only phenylephrine was administered to every animal, the heart rate decreased and the mean arterial blood pressure increased significantly in all three groups (Table 3). In both almokalant treated groups the heart rate values were statistically not different from those in the control group. In contrast, the blood pressure was significantly lower in the 80th min in the group of animals treated with almokalant infusion at a rate of 25 nmol/kg per min and from the 20th min in the group of animals treated with almokalant infusion at a rate of 75 nmol/kg per min, as compared to the control group (Table 3).

There was a significant QTc prolongation in all three groups in the first 10 min, when only phenylephrine was infused to the animals (Fig. 2). Almokalant infusion produced a dose related further prolongation of the QTc interval compared to the control group (Fig. 2).

Table 3
Effect of almokalant on the heart rate and the blood pressure in anaesthetised rabbits during continuous phenylephrine infusion

	n	Basal	Phe	Phe + drug						
			10 min	20 min	30 min	40 min	50 min	60 min	70 min	80 min
Control										
HR	10	284 ± 10.8	217 ± 7.5[a]	192 ± 10.2	195 ± 9.0	192 ± 10.8	190 ± 12.1	192 ± 12.1	188 ± 14.0	184 ± 14.7
MBP		87 ± 4.3	120 ± 2.8[a]	117 ± 3.0	118 ± 2.3	118 ± 2.3	116 ± 2.3	116 ± 3.1	118 ± 3.3	117 ± 2.6
Alm 25										
HR	10	289 ± 11.4	219 ± 16.4[a]	209 ± 15.7	213 ± 16.1	203 ± 13.6	211 ± 11.4	218 ± 10.2	212 ± 13.3	220 ± 13.7
MBP		98 ± 3.4	117 ± 3.2[a]	117 ± 2.9	114 ± 2.6	109 ± 3.6	106 ± 4.6	105 ± 4.7	106 ± 4.4	80 ± 6.0[b]
Alm 75										
HR	11	289 ± 10.0	204 ± 15.1[a]	186 ± 14.0	182 ± 12.4	193 ± 15.6	197 ± 14.2	196 ± 15.9	198 ± 12.8	222 ± 14.6
MBP		93 ± 3.6	108 ± 3.4[a]	98 ± 6.4[b]	94 ± 4.7[b]	92 ± 4.1[b]	87 ± 5.7[b]	87 ± 6.3[b]	82 ± 6.4[b]	77 ± 3.1[b]

Alm 25 and Alm 75, groups of animals treated with almokalant infusion in a rate of 25 or 75 nmol/kg per min; HR, heart rate (min^{-1}); MBP, mean arterial blood pressure (mmHg); *n*, number of animals; Phe, phenylephrine infusion in a rate of 15 mg/kg per min; [a]$P < 0.05$ compared to the basal value; [b]$P < 0.05$ compared to the control group.

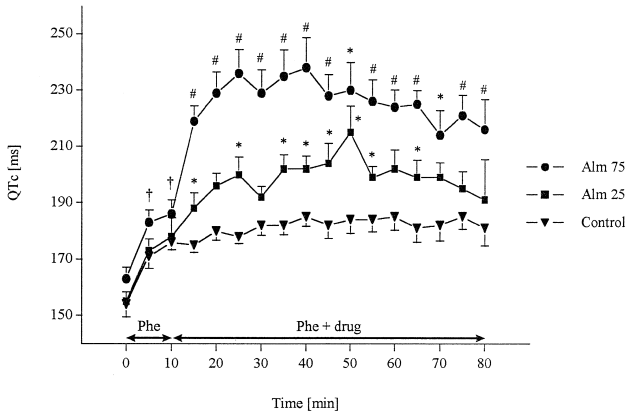

Fig. 2. The effect of almokalant on the rate corrected QT intervals during phenylephrine infusion. Alm 25 and Alm 75, groups of animals treated with almokalant infusion in a rate of 25 or 75 nmol/kg per min; QTc, rate corrected QT interval (ms); Phe, phenylephrine infusion in a rate of 15 mg/kg per min; $^{\dagger}P < 0.05$ compared to the basal value in all three groups; $* P < 0.05$ compared to the control group; $^{\#}P < 0.05$ compared to the control and the Alm 25 group.

In each group every animal survived the first 10 min, when only phenylephrine was administered, but only 26% (8 out of 31) of them had no any arrhythmia. Sporadic extrasystoles, bigeminy and salvos appeared in 55% (17 out of 31) of the animals. The incidence of bradycardia was 39% (12 out of 31). Torsade de pointes or monomorphic ventricular tachycardia or ventricular fibrillation did not occur in this period.

In the control group, after the first 10 min, when saline was administered simultaneously with phenylephrine, only sporadic extrasystoles, bigeminy and salvos occurred and none of the animals died, whereas in the groups of animals treated with almokalant simultaneously with phenylephrine even torsade de pointes, monomorphic ventricular tachycardia and ventricular fibrillation appeared (Table 4). The incidence of torsade de pointes was significantly higher in the group of animals treated with almokalant infusion at a rate of 75 nmol/kg per min. Torsade de pointes usually ended spontaneously after several seconds. Sometimes monomorphic ventricular tachycardia and torsade de pointes were attached for a short period or transformed into each other continuously for a longer time. There was one animal treated with almokalant at lower infusion rate in which only monomorphic ventricular tachycardia developed as a malignant ventricular arrhythmia and torsade de pointes did not. One animal died in both almokalant treated groups due to deterioration of torsade de pointes into irreversible ventricular fibrillation (Table 4).

Table 4
The survival rate and incidence of arrhythmias in anaesthetised rabbits treated with almokalant during continuous phenylephrine infusion

	n	Survived (%)	Incidence of arrhythmias (%)					
			None	VF	VT	TdP	Bradycardia	Other
Control	10	100	10	0	0	0	90	60
Alm 25	10	90	10	10	10	20	40	80
Alm 75	11	91	0	18	18	73[a]	73	91

Alm 25 and Alm 75, groups of animals treated with almokalant infusion in a rate of 25 or 75 nmol/kg per min; *n*, number of animals; VF, ventricular fibrillation; VT, monomorphic ventricular tachycardia; TdP, torsade de pointes; Bradycardia, heart rate $< 200\ \text{min}^{-1}$; Other, extrasystoles, salvos, and/or bigeminy; $^{a}P < 0.05$ compared to the Alm 25 and control group.

After 35.3 ± 17.6 min infusion of almokalant at a rate of 25 nmol/kg per min, i.e., a dose of 883 ± 440 nmol/kg (min. = 125; max. = 1650 nmol/kg) the drug induced torsade de pointes or monomorphic ventricular tachycardia in 3 animals out of 10. In the group of animals treated with almokalant infusion at a rate of 75 nmol/kg per min infusion of 1181 ± 519 nmol/kg (min. = 75; max. = 4500 nmol/kg) drug over a period of 15.8 ± 6.9 min produced torsade de pointes or monomorphic ventricular tachycardia in 8 out of 11 animals.

4. Discussion

In this study the antiarrhythmic and the proarrhythmic effects of almokalant have been examined in rabbits. Although, useful arrhythmia and also proarrhythmia models have been developed for several other species, including the dog (Vos et al., 1995), rabbit was preferred by us. The anatomy of the coronary arteries of the rabbit is rather variable, but there is no collateral circulation in the rabbit's heart unlike in dog's heart (Maxwell et al., 1987), and thus, myocardial infarction in the rabbit can mimic better human myocardial infarction. Though it needs skill to perform coronary artery occlusion in rabbits, the proarrhythmia model, unlike in the dog (Vos et al., 1995), is quite simple in this species, and does not need difficult surgical techniques.

The present study demonstrated that intravenous almokalant prevent reperfusion induced ventricular fibrillation. Other blockers of the delayed rectifying potassium current (I_K), such as D-sotalol, dofetilide, sematilide, E-4031 and UK66,914 vary in their effectiveness against reperfusion arrhythmias. For example, Pasnani and Ferrier (1994) found D-sotalol to be ineffective against ischaemia and reperfusion induced arrhythmias in isolated guinea pig right ventricular free wall preparations. In contrast, UK66,914 possessed marked antiarrhythmic effect on reperfusion arrhythmias in isolated rabbit hearts (Rees and Curtis, 1993). It was published recently that D-sotalol, E-4031 and MS-551 (a non-selective potassium channel blocker) are effective against reperfusion arrhythmias and arrhythmias induced by programmed electrical stimulation, whereas dofetilide and sematilide prevent only arrhythmias

induced by programmed electrical stimulation but did not suppress reperfusion arrhythmias in anaesthetised dogs (Chen et al., 1996; Xue et al., 1996).

Reperfusion induced arrhythmias may be mediated both via reentry mechanism (Coronel et al., 1992) and triggered activities (Pogwizd and Corr, 1992; Hayashi et al., 1996). Selective prolongation of repolarisation (class III effect) is one possibility to prevent and terminate reentrant arrhythmias, but it has no effect on arrhythmias induced by triggered activities. Abrahamsson et al. (1993) showed that almokalant prolongs the action potential duration in a dose dependent manner both in isolated Purkinje and in ventricular muscle cells of the rabbit by recording transmembrane action potentials. Duker et al. (1992) found that almokalant (1.0 mmol/kg, i.v.) significantly prolonged the epicardial monophasic action potential duration and the atrial and ventricular effective refractory period but it had no effect on the atrial and ventricular conduction in anaesthetised dogs. In this study almokalant pretreatment (in a dose of 250 nmol/kg) significantly prolonged QTc interval, i.e., ventricular repolarisation. Thus, the possible mechanism by which almokalant prevented reperfusion arrhythmias is the lengthening of action potential duration and the refractory period of myocardial fibres (achieved by selective blockade of I_{Kr}) in the reentrant circuit to such an extent that the propagating reentrant impulse no longer finds excitable myocardium but blocks in refractory tissue. The effectiveness of I_K block as a mechanism for prevention of both ischaemia and reperfusion induced arrhythmias was demonstrated also by Rees and Curtis (1993).

In contrast to its effectiveness on reperfusion induced ventricular fibrillation almokalant was found to elicit torsade de pointes in the Carlsson et al. (1990). The torsadogenic potential of several selective class III agents, i.e., clofilium, sematilide, almokalant, D-sotalol, ibutilide, E-4031 and dofetilide, has been tested during α_1-adrenoceptor stimulation (Carlsson et al., 1990, 1993a; Buchanan et al., 1993). All of the examined class III agents induced torsade de pointes in this model. The underlying mechanisms of torsade de pointes are not yet known fully but appearance of early afterdepolarisations and enhanced dispersion of ventricular repolarisation are supposed to be the main electrophysiologic prerequisites for initiation of torsade de pointes especially in the presence of predisposing factors like low heart rate, electrolyte abnormalities (hypokalemia and hypomagnesemia), lengthening of repolarisation, depressed left ventricular function and/or life threatening arrhythmias in the case history (Hohnloser and Singh, 1995).

In our experiments the antiarrhythmic dose of almokalant (250 nmol/kg) was administered at a slow infusion rate for 10 min or at high infusion rate for 3.33 min. The high infusion rate of a class III agent increases markedly the incidence of torsade de pointes, probably by increasing the dispersion of repolarisation (Carlsson et al., 1993a). In our hands almokalant infusion at a higher rate prolonged QTc interval more than the infusion of almokalant at lower rate, and this marked QTc prolongation coincided with high incidence of torsade de pointes. These findings are in discordance with the results of Carlsson et al. (1993a), because they found no significant difference between the corresponding QTc values of the high and low infusion rate group, despite the former having a higher propensity to cause torsade de pointes. The data about the importance of QTc lengthening are quite contradictory and the question whether there is a relationship between any critical QTc prolongation and proarrhythmias is not yet answered. Buchanan et al. (1993) also found no correlation between the proarrhythmic potential of class III agents and the degree of QTc (or QT) interval prolongation in their experiments with the same model of acquired long QT syndrome. These findings are in agreement with others' conclusion that the incidence of torsade de pointes is not quantitatively related to the degree of QTc prolongation caused by repolarisation delaying agents (Soffer et al., 1982; Lazzara, 1993). Thus, the predictive value of the QTc interval prolongation for developing proarrhythmia needs further examination.

Class III agents typically produce torsade de pointes. However, in a clinical study ibutilide and sotalol induced not only torsade de pointes but also monomorphic ventricular tachycardia in patients with atrial fibrillation or flutter (Kowey et al., 1996). Darpö et al. (1996) reported a case in which an almokalant treated patient with WPW syndrome developed torsade de pointes after a pacing induced pause, and this tachycardia degenerated into ventricular fibrillation that required immediate defibrillation. In our experiments almokalant infusion (during α_1-adrenoceptor stimulation) produced monomorphic ventricular tachycardia and irreversible ventricular fibrillation as well as torsade de pointes. Carlsson et al. (1990, 1993a) reported only on premature ventricular complexes and torsade de pointes induced by class III agents in rabbits. Maybe this discrepancy is attributable to the fact that the latter authors terminated their experiments at the time of the first appearance of torsade de pointes. In our study two animals developed monomorphic ventricular tachycardia prior to the first torsade de pointes and one developed monomorphic ventricular tachycardia without the occurrence of torsade de pointes. Likewise, Buchanan et al. (1993) observed frequently the development of wide complex tachycardia, which was not pause dependent like torsade de pointes following administration of class III agents.

Carlsson et al. (1993b, 1996) and Hallman and Carlsson (1995) found that both pretreatment and acute intervention with lidocaine, nisoldipine or flecainide prevent torsade de pointes induced by almokalant infusion. In our experiments in the high infusion rate group, in 3 out of 8 animals with torsade, the short repetitive sequences of torsade de pointes occurred within 2 min period and in one additional animal within 6 min period. After these short attacks no more ventricular tachycardia, but just premature ventricu-

lar complexes developed in these animals, though the infusion of almokalant was not terminated and no suppressive agent was administered. These observations suggest that acute intervention with antiarrhythmic agents in order to suppress torsade de pointes induced by a class III agent in this model may give false positive results, and pretreatment should be preferred to investigate any intervention for influencing the torsadogenic potential of antiarrhythmic agents.

As a result of our study the margin of safety of almokalant was estimated by comparing the proarrhythmic dose (during α_1-adrenoceptor stimulation) of almokalant to the antiarrhythmic dose, which was effective against ischaemia-reperfusion induced arrhythmias. The proarrhythmic dose of almokalant was about 4–6 times higher than the antiarrhythmic dose under the conditions of this study. We do not want to overemphasise this finding, because it may not be valid in clinical setting, but it could be useful for comparison of new antiarrhythmic agents under similar experimental conditions. Though different anaesthetic agents were used in the two applied experimental models in our study, we think that the differences of the results were not attributable to the use of different anaesthesia. Recently, Bril et al. (1996) suggested that intravenous pentobarbital sodium can be used instead of α-chloralose without altering the proarrhythmic response to repolarisation prolonging agents in Carlsson's torsade model.

In conclusion, our study has provided evidence that almokalant, a selective class III antiarrhythmic agent, is effective against reperfusion arrhythmias, though it has a marked proarrhythmic effect during α_1-adrenoceptor stimulation in anaesthetised rabbits. We demonstrated experimentally that a class III drug is able to produce not only torsade de pointes as a malignant proarrhythmia, but also monomorphic ventricular tachycardia and ventricular fibrillation. The proarrhythmic response to almokalant is, in fact, quite complex in the applied rabbit model of acquired long QT syndrome. Furthermore, our study demonstrated that the combination of the 'coronary artery occlusion–reperfusion model' and the 'acquired long QT syndrome model' in rabbits is suitable to assess and compare experimentally the antiarrhythmic efficacy and the proarrhythmic activity of repolarisation delaying agents.

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