Effects of an antioxidant agent on alterations of ventricular repolarization in a coronary artery occlusion-reperfusion experimental model

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BACKGROUND AND OBJECTIVE: Antioxidant agents can prevent oxidative stress and, therefore, cardiac arrhythmias. Electrophysiological mechanisms underlying antiarrhythmic action of antioxidants are not well understood. Echinochrome is an antioxidant and iron-chelating agent of natural origin. Effects of synthetic echinochrome on ischemia- and reperfusion-induced alterations of ventricular repolarization were studied in a coronary artery occlusion-reperfusion model.

METHODS: Cats underwent a brief coronary occlusion-reperfusion sequence (30 min/30 min). To map repolarization, activation-recovery intervals (ARIs) were measured using 30 to 41 left ventricular unipolar extracellular electrograms. Echinochrome was administered intravenously at a dose of 1 mg/kg 5 min before occlusion and/or 5 min before reperfusion.

RESULTS: Echinochrome raised the central aortic pressure and did not affect ARIs in nonischemic myocardium. When echinochrome was administered before reperfusion, restoration of ventricular repolarization during reperfusion was delayed. When echinochrome was administered before both occlusion and reperfusion, the ischemia-induced shortening of ARIs was reduced, and restoration of ARIs during reperfusion was normalized. Ventricular arrhythmias were observed in all of the animals both during occlusion and during reperfusion.

CONCLUSIONS: Echinochrome does not affect repolarization in nonischemic myocardium. The preventive cardioprotective effect of echinochrome is that it reduces ischemia-induced shortening of repolarization duration, diminishes reperfusion-induced increasing of repolarization heterogeneity and raises arterial pressure.

Key Words: *Antioxidant; Cardiac electrophysiology; Experimental model; Ischemia; Reperfusion; Repolarization*

Ischemia- and reperfusion-induced ventricular arrhythmias are caused by alterations of ventricular repolarization because of the influence of reactive oxygen species on ion channels (1). Antioxidants can prevent oxidative stress and, therefore, cardiac arrhythmias. However, electrophysiological mechanisms underlying antiarrhythmic action of antioxidants are not well understood. A combination of an antioxidant and an iron-binding agent is a greater protection against susceptibility to oxidative stress-induced ventricular arrhythmias than the antioxidant alone (2).

Echinochrome (6-ethyl-2,3,5,7,8-pentahydroxy-1,4-naphthoquinone) is an antioxidant and iron-chelating agent of natural origin (3-5). Its cardioprotective and antiarrhythmic effects has been shown (6). Some known physiological effects of echinochrome do not appear to be attributable to its antioxidant and iron-chelating properties. These are normalization of electromechanical coupling in myocardium (7), a cardioprotective effect under calcium disbalance in myocardium (8), and hyperpolarizing action on neurons (9). An effective synthesis of echinochrome was described (10). However, it remains unknown how echinochrome affects cardiac repolarization.

The objective of the present study was an investigation of effects of an antioxidant echinochrome on ischemia- and reperfusion-induced alterations of ventricular repolarization during a brief coronary occlusion-reperfusion sequence in situ. In addition, effects of echinochrome on the central aortic pressure were analyzed. We report our results from using synthetic echinochrome in an open-chest feline model.

METHODS

The work was carried out in accordance with the *Guide for the Care and Use of Laboratory Animals, 8th Edition* published by the National Academies Press (United States) in 2011. The study protocol was approved by the local institutional ethical committee.

Animal preparation

Experiments were performed on 16 adult mongrel cats of both sexes, weighing 2.5 kg to 4.5 kg. Animals were anesthetized with Zoletil 100 (Virbac, France) using a dose of 15 mg/kg intramuscularly and xylazine (Xyla, Interchemie, Holland) using a dose of 1 mg/kg intramuscularly.

A tracheostomy was performed, and an endotracheal tube was positioned to allow mechanical ventilation. Catheters (internal diameter 1 mm) were inserted into the femoral vein for administration of drugs and saline and for withdrawal of blood samples, and into the aorta (through the left carotid artery) to measure central aortic pressure. Steel needle electrodes were inserted subcutaneously to record the standard bipolar limb lead electrocardiograms. A midsternal thoracotomy was performed, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery was isolated distally (Figure 1), and a silk suture was placed around it to act as a ligature and induce occlusion.

Six flexible multipolar electrodes were inserted by means of a suture needle into the left ventricular wall (Figure 1) to record extracellular electrograms. Each electrode contained eight unipolar recording sites separated by 1 mm. Electrodes were fabricated with isolated copper wires (70 μm diameter), each fastened off with a knot on a vicryl thread (0.8 mm diameter).

Heparin (250 IU/kg) was administered intravenously to prevent coronary artery thrombosis during occlusion. Every 10 min during occlusion, blood samples were withdrawn from the femoral vein to measure the activated partial thromboplastin time. Blood clots did not

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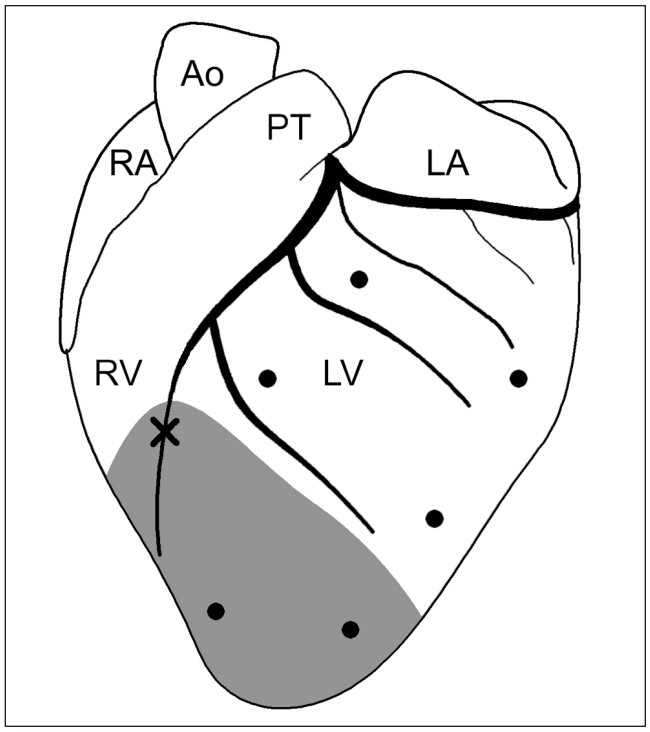

Figure 1) *A scheme of the localization of transmural electrodes (black circles) and occlusion site (cross) in feline hearts. Ao Aorta; LA Left atrium; LV Left ventricle; PT Pulmonary trunk; RA Right atrium (the left atrial appendage is uplifted); RV Right ventricle. The ischemic region is shown by the grey colour*

develop in any of the blood samples, testifying to the adequacy of heparinization and the absence of coronary artery thrombosis.

During the experiment, the core temperature was monitored and maintained constant at 35°C to 37°C. Warm (37°C) saline was applied intermittently to the heart to moisten the epicardium and prevent surface cooling.

On completion of the experiment, the animal was euthanized by extirpation of the heart under general anesthesia.

Animal groups

Three groups of animals were studied. Group E1 (five animals) was given synthetic echinochrome in a dose of 1 mg/kg 5 min before reperfusion (at 25 min of occlusion), and group E2 (five animals) was given synthetic echinochrome in a dose of 1 mg/kg 5 min before occlusion and 5 min before reperfusion. The dosage of echinochrome was chosen on the basis of its ability to protect from myocardial ischemia-reperfusion injury (11,12). Synthetic echinochrome was provided by the G.B. Elyakov Pacific Institute of Bioorganic Chemistry of the Far Eastern Branch of the Russian Academy of Sciences (Vladivostok, Russia). Echinochrome was administered intravenously (through the femoral vein catheter) for 1 min as a 0.2% solution in a 0.1% sodium bicarbonate solution (10 mg of echinochrome was dissolved in 5 mL of a 0.1% sodium bicarbonate solution using ultrasound bath). Group C (six animals) was given an equivalent volume of saline.

Recordings

The electrocardiogram (leads I, II and III) and central aortic pressure (transducer SP844, 50 μV/V/cmHg, MEMSCAP, France) were continuously monitored on a physiological recorder (Prucka Mac-Lab 2000, GE Medical Systems GmbH, Germany). Forty-eight unipolar electrograms and standard bipolar limb lead electrocardiograms were acquired using a custom-designed computerized multiplexed data acquisition system, allowing simultaneous recording of up to 128 signals with a bandwidth of 0.05 Hz to 1000 Hz at a sampling rate of

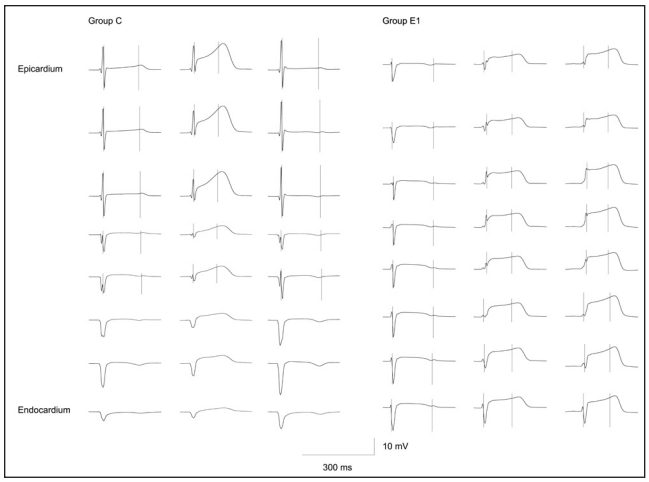

Figure 2) *Ventricular electrograms in cats. Original tracings of unipolar electrograms from a flexible electrode inserted along the epicardial-endocardial axis into the apical left ventricular wall (the ischemic region) at the baseline state (left), 30 min of occlusion (middle) and 30 min of reperfusion (right) in the control (group C) and echinochrome-administered (group E1) cats. Local activation and repolarization times are indicated by upright markers. The tracings without markers were excluded from the analysis because these were unipolar electrograms from recording sites located in the left ventricular cavity*

4000 Hz and an accuracy of 12 bits). Examples of the original graphs of electrograms are shown in Figure 2.

After baseline (preocclusion) electrocardiograms, electrograms and central aortic pressure were recorded under sinus rhythm, coronary occlusion was performed. At the end of the 30 min occlusion, the ligature was released to allow reperfusion for 30 min. Electrocardiograms, electrograms and central aortic pressure were recorded under sinus rhythm at the following time points: 5 min and 30 min of occlusion and 5 min and 30 min of reperfusion.

At the end of the 30 min reperfusion, the left anterior descending coronary artery was religated at the same site, and 1.5 mL of 0.5% Evans blue dye (Reanal, Hungary) was injected into the carotid artery catheter to delineate the in vivo area at risk (Figure 3) and to verify that two of six electrodes were located in this area. Elevation of the ST segment was observed in electrograms recorded in the ischemic region during occlusion (Figure 2). The heart was then cut to determine recording sites located in the left ventricular cavity. Electrograms from these recording sites were excluded from further analysis.

Measurements

Thirty to 41 electrograms were analyzed in each experimental animal. Activation-recovery intervals (ARIs), corrected for heart rate by Bazett's formula, were used for the evaluation of repolarization durations. ARI was defined as the interval between the time of the minimum first derivative of the QRS complex (local activation time) and the maximum first derivative of the T wave (local repolarization time) of unipolar electrograms (13). The computer-assisted measurements of local activation and repolarization times were reviewed and corrected by the experimenter if required. The total ARI dispersion was calculated as the difference between the shortest and the longest corrected ARIs. The boundary gradient was defined as the difference between the averaged corrected ARIs of ischemic and nonischemic regions.

Analysis of ventricular arrhythmias

An arrhythmia score was given as follows: 0, no arrhythmias; 1, monomorphic ventricular extrasystoles <30/min; 2, single or coupled monomorphic ventricular extrasystoles >30/min; 3, polymorphic ventricular extrasystoles; 4, coupled polymorphic ventricular extrasystoles; 5, unstable ventricular tachycardia (<30 s); 6, stable ventricular

TABLE 1
Activation-recovery intervals (ARIs), boundary gradient and total dispersion of ARIs during coronary artery occlusion-reperfusion in cats

		Group E1, n=5		Group C, n=6		Group E2, n=5	
Time point		Nonischemic region	Ischemic region	Nonischemic region	Ischemic region	Nonischemic region	Ischemic region
ARIs							
Baseline		234±51	239±29	232±29	221±33	245±35	229±38
Occlusion	5 min	220±60	157±34*†	224±24	175±31*†	238±33	194±32*†‡§
	30 min	215±60	136±46*†¶	238±28	163±32*†¶	240±32	180±27*†‡§¶
Reperfusion	5 min	222±45	177±62*†**	234±27	192±58†**	242±29	171±31*†**
	30 min	220±41	175±61*†‡**	240±26	238±46*¶**	249±30	221±36‡§¶**
Boundary gradient of ARIs							
Baseline		12±8		11±12		6±6	
Occlusion	5 min	51±26*		56±15*		35±20‡	
	30 min	62±9*		74±25*		54±27*	
Reperfusion	5 min	59±9*		57±36*		72±30*	
	30 min	68±18*‡		24±20**		54±26*‡	
Total dispersion of ARIs							
Baseline		73±38		66±12		78±23	
Occlusion	5 min	101±22		111±20*		85±10‡	
	30 min	123±10*		126±32*		112±20*	
Reperfusion	5 min	115±14		113±47*		120±18*	
	30 min	137±26*‡		87±41**		97±8*‡§	

*Data are presented in ms as the mean ± SD. Group C Control; Group E1 Given synthetic echinochrome in a dose of 1 mg/kg 5 min before reperfusion (at 25 min of occlusion); Group E2 Given synthetic echinochrome in a dose of 1 mg/kg 5 min before occlusion and 5 min before reperfusion. *P<0.05 occlusion and reperfusion versus baseline; †P<0.05 ischemic versus nonischemic region in the same period; ‡P<0.05 Group E1 or group E2 versus group C; §P<0.05 Group E2 versus group E1; ¶P<0.05 30 min of occlusion/reperfusion versus 5 min of occlusion/reperfusion for the corresponding region; **P<0.05 reperfusion versus 30 min of occlusion for the corresponding region*

tachycardia (>30 s); and 7, ventricular fibrillation. Arrhythmias were assessed at 5 min intervals throughout occlusion and reperfusion. For each interval, arrhythmias of the greatest grade were taken into account. Scores for each animal were summed for occlusion and reperfusion. The mean arrhythmia score for each group was then calculated for occlusion and reperfusion.

Statistical analysis

All data are presented as the mean ± SD. Comparisons between groups were carried out using the Mann-Whitney test. Analyses of differences in arrhythmia incidence between groups were carried out using the χ^2 test. Comparisons between time points were carried out by the Wilcoxon test. A value of P<0.05 was considered to be statistically significant.

RESULTS

At baseline, there was no difference in ARIs among the animal groups (Table 1). In each group, there was no difference in ARIs between the regions affected and unaffected by the subsequent ischemia (Table 1). Preocclusion infusion of echinochrome (group E2) did not affect repolarization during the following 5 min.

During coronary occlusion, ARIs in the nonischemic region remained unchanged, while ARIs in the ischemic region shortened progressively in all the groups (Table 1). This resulted in a significant increase in the boundary gradient and total dispersion of ARIs (Table 1). Group E2 showed the least shortening of ARIs in the ischemic region compared with two other groups (Table 1). Pre-reperfusion infusion of echinochrome (group E1) did not affect repolarization during the last 5 min of occlusion. There were no differences in the total ARI dispersion and the boundary ARI gradient between the groups at 30 min of occlusion (Table 1).

During reperfusion, ARIs in the nonischemic region remained unchanged, while ARIs in the ischemic region lengthened in all the animal groups (Table 1). At 30 min of reperfusion, ARIs in the

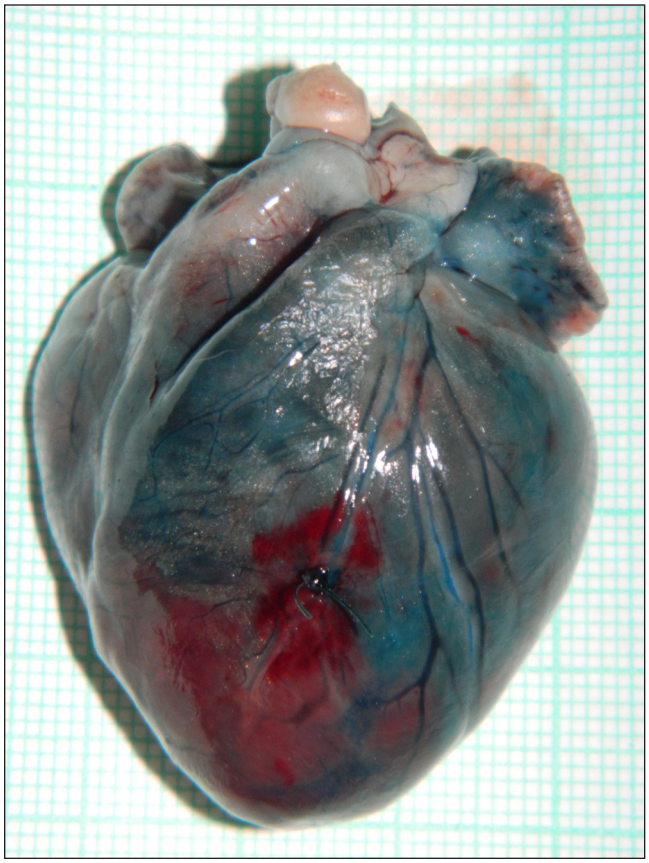

Figure 3) *Assessment of area at risk in feline hearts (perfusion with Evans blue). The nonperfused (ischemic) region is not dyed*

TABLE 2
Comparison of the animal groups in relation to types of ventricular arrhythmias during coronary artery occlusion-reperfusion

	Occlusion			Reperfusion		
Type of arrhythmias	Group C (n=6)	Group E1 (n=5)	Group E2 (n=5)	Group C (n=6)	Group E1 (n=5)	Group E2 (n=5)
No arrhythmias	0	1	0	1	2	0
Monomorphic ventricular extrasystoles (<30/min)	5	4	5	2	0	2
Single or coupled monomorphic ventricular extrasystoles (>30/min)	2	1	2	2	1	1
Polymorphic ventricular extrasystoles	0	1	2	0	0	0
Coupled polymorphic ventricular extrasystoles	1	1	0	0	0	1
Unstable ventricular tachycardia (<30 s)	1	1	0	2	2	1
Stable ventricular tachycardia (>30 s)	0	0	0	1	1	0
Ventricular fibrillation	0	0	0	0	0	1

Data presented as n. Group C Control; Group E1 Given synthetic echinochrome in a dose of 1 mg/kg 5 min before reperfusion (at 25 min of occlusion); Group E2 Given synthetic echinochrome in a dose of 1 mg/kg 5 min before occlusion and 5 min before reperfusion

TABLE 3
Comparison of the animal groups in relation to the occurrence of ventricular arrhythmias during coronary artery occlusion–reperfusion

	Ventricular extrasystoles				Ventricular tachycardia/fibrillation			
	Occlusion		Reperfusion		Occlusion		Reperfusion	
Group	0–15 min	16–30 min	0–5 min	6–30 min	0–15 min	16–30 min	0–5 min	6–30 min
Group C (n=6)	5	4	2	2	0	0	3	0
Group E1 (n=5)	4	2	1	0	0	1	2	0
Group E2 (n=5)	3	5	4	1	0	0	2	0

Data presented as n. Group C Control; Group E1 Given synthetic echinochrome in a dose of 1 mg/kg 5 min before reperfusion (at 25 min of occlusion); Group E2 Given synthetic echinochrome in a dose of 1 mg/kg 5 min before occlusion and 5 min before reperfusion

TABLE 4
Comparison of the experimental groups in relation to arrhythmia scores* in a feline model of coronary artery occlusion-reperfusion

Period	Group C (n=6)	Group E1 (n=5)	Group E2 (n=5)
Occlusion	4.7±2.9	6.6±7.1	4.4±1.7
Reperfusion	4.2±2.6	3.6±4.6	4.2±4.1

*Group C Control; Group E1 Given synthetic echinochrome in a dose of 1 mg/kg 5 min before reperfusion (at 25 min of occlusion); Group E2 Given synthetic echinochrome in a dose of 1 mg/kg 5 min before occlusion and 5 min before reperfusion. *0, no arrhythmias; 1, monomorphic ventricular extrasystoles <30/min; 2, single or coupled monomorphic ventricular extrasystoles >30/min; 3, polymorphic ventricular extrasystoles; 4, coupled polymorphic ventricular extrasystoles; 5, unstable ventricular tachycardia (<30 s); 6, stable ventricular tachycardia (>30 s); and 7, ventricular fibrillation*

ischemic region were slightly greater than those at baseline in group C and were not different from those at baseline in group E2, but were significantly less than those at baseline in group E1. ARIs in the ischemic region at 30 min of reperfusion in group E1 were significantly shorter than those in group C and group E2 (Table 1). The boundary ARI gradient and the total ARI dispersion were not restored to the baseline values by the 30 min reperfusion in the echinochrome-administered groups, in contrast with group C (Table 1). However, the total ARI dispersion at 30 min of reperfusion was significantly closer to the baseline value in group E2 as compared with group E1 (Table 1).

Heart rate in group E1 remained unchanged during the occlusion-reperfusion period: 176±18 beats/min, 182±21 beats/min and 180±18 beats/min at baseline, 30 min of occlusion and 30 min of reperfusion, respectively. In group C, heart rate at baseline, 30 min of occlusion and 30 min of reperfusion was 155±17 beats/min, 163±18 beats/min and 154±12 beats/min (P<0.05 versus occlusion), respectively. In group E2, heart rate at baseline, 30 min of occlusion and 30 min of reperfusion was 140±17 beats/min, 147±19 beats/min, and 161±14 beats/min (P<0.05 versus baseline), respectively. There were the following differences in heart rate: between group C and group E1 at 30 min of reperfusion (P<0.05); and between group E1 and group E2 at baseline (P<0.05) and at 30 min of occlusion (P<0.05).

All the animal groups showed ventricular arrhythmias both during occlusion and reperfusion (Table 2). Arrhythmias were observed in almost all the animals both during occlusion and during reperfusion. There were no ventricular arrhythmias during occlusion in only one cat in group E1 and no reperfusion-induced ventricular arrhythmias in two cats in group E2 and one cat in group C. There were no significant differences in the incidence of ventricular extrasystoles and ventricular tachycardia among the animal groups during occlusion (Table 3). In all the groups, reperfusion-induced ventricular tachycardia (and spontaneously reversible fibrillation lasted for 35 s in one cat in group E2) developed mainly within the initial 5 min of reperfusion, while reperfusion-induced ventricular extrasystoles were observed throughout reperfusion (Table 3). There was no significant difference in arrhythmia scores among the animal groups (Table 4).

Arterial pressure did not differ among the animal groups at baseline (Table 5). In group C, the systolic, diastolic and mean pressures tended to decrease during occlusion and tended to rise to baseline values during reperfusion, and there were no significant changes in the pulse pressure. Group E1 showed the similar dynamics of arterial pressure during occlusion. Pre-reperfusion infusion of echinochrome (group E1) resulted in the rapid rise in the systolic, diastolic and mean pressures. Then, during reperfusion, the systolic, diastolic and mean pressures slightly fell and did not differ from the baseline values. The pulse pressure in group E1 remained unchanged during occlusion and reperfusion. In group E2, the rapid rise in the systolic, diastolic and mean pressures occurred after each infusion of echinochrome with the further decrease to baseline values; the significant increase in the pulse pressure was only after the second (pre-reperfusion) infusion of echinochrome.

DISCUSSION

Electrophysiological mechanisms underlying antiarrhythmic action of antioxidants are not well understood. As has been reported previously, a combination of an antioxidant and an iron-binding agent is a greater protection against susceptibility to oxidative stress-induced ventricular arrhythmias than the antioxidant alone (2). The purpose of the

TABLE 5
The effect of echinochrome on arterial pressure (mmHg) during coronary artery occlusion-reperfusion in cats

			Occlusion			Reperfusion	
Group	**Baseline**	**Baseline + E**	**5 min**	**25 min**	**30 min + E**	**5 min**	**30 min**
Systolic pressure							
Group C	103±14	–	96±11	91±9	–	96±15	104±18
Group E1	94±15	–	88±9	82±16	101±18*	92±16†‡	93±9†
Group E2	97±21	110±20*	93±14‡	95±10	106±14*	107±32†	90±7†‡
Diastolic pressure							
Group C	79±16	–	72±12	68±10	–	75±12	75±17
Group E1	74±12	–	67±13	64±17	79±18*	72±15‡	70±14
Group E2	76±21	86±19*	75±14	75±8	82±10*	77±16	71±7‡
Mean pressure							
Group C	87±15	–	80±12	77±9	–	82±13	85±16
Group E1	80±13	–	74±16	70±16	86±18*	79±15‡	77±12
Group E2	83±21	94±20*	81±15‡	81±9	90±11*	89±24	78±7‡
Pulse pressure							
Group C	24±2	–	23±5	27±5	–	21±4	28±8
Group E1	22±6	–	23±8	18±6	22±4	20±5	23±6
Group E2	21±1	24±4	18±2	20±3	24±5*	30±15*	20±3

*Data presented as mean ± SD. E Echinochrome; Group C Control, n=6; Group E1 Given synthetic echinochrome in a dose of 1 mg/kg 5 min before reperfusion (at 25 min of occlusion), n=5; Group E2 Given synthetic echinochrome in a dose of 1 mg/kg 5 min before occlusion and 5 min before reperfusion, n=5. *P<0.05 versus the state before administration of echinochrome; †P<0.05 versus occlusion at 25 min; ‡P<0.05 versus the state with infusion of echinochrome (at 30 min of occlusion)*

present study was to investigate effects of echinochrome on ischemia- and reperfusion-induced alterations of ventricular repolarization in an open-chest feline model of a brief coronary occlusion-reperfusion sequence. Synthetic echinochrome was used as an antioxidant and an iron-binding agent (3-5). Previously, evidence for cardioprotective effects of echinochrome from myocardial ischemia-reperfusion injury has been reported in acute canine and chronic rabbit models of coronary artery occlusion-reperfusion (11,12). Clinical investigations confirm that treatment with echinochrome prevents infarct expansion induced by reperfusion (14).

The major findings of the present study are as follows: echinochrome does not affect repolarization of nonischemic myocardium; echinochrome reduces ischemia-induced shortening of repolarization; and the positive effect of echinochrome on arterial pressure appears quickly.

There was no effect of echinochrome on ARIs in the nonischemic region, and the preocclusion infusion of echinochrome reduced the ischemia-induced shortening of ARIs. One might assume that echinochrome inhibits ionic current through the ATP-regulated potassium channels (K_{ATP} channels); however, appropriate research is required to confirm this assumption.

Ventricular repolarization in the control cats was restored to the baseline state by the 30 min reperfusion. In contrast, restoration of ventricular repolarization was delayed within the 30 min reperfusion in cats treated with the pre-reperfusion infusion of echinochrome. This delay testified that a normalization of ionic currents was decelerated. This might be attributable to the activation both of potassium (other than the K_{ATP} current) and calcium currents by echinochrome (9) and to the dependence of potassium currents on intracellular calcium (15).

Increased ventricular repolarization heterogeneity can provide the potential substrate for ventricular arrhythmias. An index of repolarization heterogeneity is spatial dispersion of repolarization (16,17). In the study reported here, all the animal groups showed both occlusion and reperfusion ventricular arrhythmias. This was in consistent agreement with the increased total ARI dispersion at occlusion and reperfusion in all the animal groups. However, the twofold increased total ARI dispersion at 30 min of reperfusion in the echinochrome-administered cats (group E1) testified maintenance of a greater remaining risk of ventricular arrhythmias compared with two other groups. In spite of this fact, there was no difference in reperfusion-induced arrhythmia incidence and scores between the animal groups. Our observations were in consistent agreement with no effect of echinochrome on the incidence of cardiac rhythm disorders reported for dogs by other researchers (12). However, the decreased number, frequency, duration and severity of reperfusion arrhythmic episodes have been observed by application of echinochrome before myocardial reperfusion in patients (7,14). The discrepancies between clinical and experimental study results may be due to species differences. It is possible that there is a certain threshold of the antiarrhythmic activity of antioxidant agents, whereas the probability of reperfusion-induced arrhythmias in dogs and cats is less than in humans because of differences in the coronary collateral circulation. Residual flow to ischemic myocardium during coronary artery occlusion in dogs and cats is significantly greater than that in humans (18,19). As a result, reperfusion-induced oxidative stress and, therefore, a probability of reperfusion-induced arrhythmias appear to be less.

Infusion of echinochrome resulted in the rise in arterial pressure. This rise could be attributable to an increase in cardiac output and/or vascular resistance. The assumption that an increase in stroke volume (the inotropic effect) could occur after echinochrome administration, was in consistent agreement with other findings. Increasing ventricular contractility could be associated with a normalization of the pump function of the heart, because echinochrome activates the calcium current (9) and promotes stabilization of intracellular calcium content (8,20) through maintenance of the activity of cardiomyocyte membrane receptors and ryanodine receptors of the sarcoplasmic reticulum (20). The echinochrome-induced increase in arterial pressure reported for our study were in consistent agreement with the positive inotropic effect of echinochrome on isolated human right atrial strips (7). However, an increase in vascular resistance could not be excluded because echinochrome could affect calcium homeostasis in vascular muscle cells as it occurs in cardiac myocytes.

The present study has limitations. Lipid peroxidation products were not measured. Therefore, we had no data on whether the infusions of echinochrome caused decreasing generation of reactive oxygen species within the myocardium. However, it was shown previously that echinochrome, being a very lipophilic compound (5) with a high free radical scavenging activity (3-5), decreases lipid peroxidation (21,22). Also, no histological examination was performed, and we do not have data on infarct size. However, the dosage used causes a significant reduction of infarct size in experimental animals (11,12).

Neither cardiac output nor vascular resistance was measured. Both these parameters are needed to suggest the mechanism of the rise in arterial pressure after infusion of echinochrome.

In summary, echinochrome does not affect repolarization in non-ischemic myocardium, but reduces ischemia-induced shortening of repolarization and diminishes reperfusion-induced increasing of repolarization heterogeneity. Infusion of echinochrome has a rapid positive effect on arterial pressure.

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