Effects of Echinochrome on Ventricular Repolarization in Acute Ischemia

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Abstract. *Ischemia-related processes, including electrophysiological alterations, are associated with the generation of reactive oxygen species. Although the cardioprotective action of antioxidants against ischemic injury has been proposed, their electrophysiological effects are not clear. The aim of this study was to investigate the effects of the antioxidant Echinochrome on ischemia-induced alterations of ventricular repolarization in a feline model of coronary occlusion. Experiments were performed on open-chest anesthetized adult cats. Activation-recovery intervals were measured from 64 ventricular epicardial unipolar electrograms recorded simultaneously before and during ligation of the left anterior descending coronary artery. Synthetic Echinochrome was infused five minutes before coronary occlusion in two different doses (1.0 mg/kg and 2.0 mg/kg, i.v.).Pre-occlusion administration of Echinochrome in a dose of 1.0 mg/kg resulted in the reduction of ischemia-induced shortening of activation-recovery intervals and the restricted increase in the repolarization dispersion during 30 minutes of ischemia. Echinochrome in a dose of 2.0 mg/kg demonstrated a more pronounced effect. Ability of the antioxidant Echinochrome to decrease ischemia-induced alterations of ventricular repolarization was demonstrated in a feline model of coronary occlusion. A dose-dependent effect was suggested.*

Keywords: Cardiac Electrophysiology, Repolarization, Ischemia, Antioxidant, Animal Model

1. Introduction

Acute cardiac ischemia causes electrophysiological alterations that may lead to fatal arrhythmias. The pathological increase in reactive oxygen species (ROS) could be a key determinant of ischemia/reperfusion-induced ventricular arrhythmias, as ROS trigger an intercellular calcium increase [1]. Antioxidants can prevent oxidative stress and, therefore, reduce the chance of cardiac arrhythmias [2].

Echinochrome (Ech) is a synthetic formulation of the sea urchin pigment echinochrome A (2,3,5,7,8-pentahydroxy-6-ethyl-1,4-naphthoquinone), which is an antioxidant agent characterized by its iron chelation and free-radical scavenging abilities [3,4]. It was shown its cardioprotective effect associated with the diminution of the infarct zone in ischemia/reperfusion models [5]. However, the electrophysiological mechanisms that underlie the cardioprotective action of the antioxidant are not well understood.

The goal of this study was to investigate the effects of theantioxidant Echinochrome (Ech) on ventricular repolarization in an open-chest feline model of 30-min ischemia.

2. Subject and 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 (US) 2011. The local institutional ethical committee approved the study protocol.

The experiments were performed on anaesthetized (zoletil 15 mg/kg; xylazine 1 mg/kg intramuscularly) adult cats in an open-chest setting under spontaneous sinus rhythm. Detailed protocol of the experiment has been described elsewhere [6].

A 64-electrode epicardial sock placed on the heart ventricles aided in recording the cardiac electrical potentials. Along with the limb lead electrocardiograms, a custom designed system (bandwidth 0.05–1000 Hz; sampling rate 4000 Hz) recorded unipolar electrograms, in reference to Wilson's central terminal.

The data was obtained at baseline state and at the 5, 15, and 30 minutes of the myocardial ischemia induced by the ligation of the left anterior descending coronary artery.

The three groups of animals were studied: the animals (n=7) received Ech in dose 2 mg/kg, the animals (n=5) with Ech application in dose 1 mg/kg, and the control group (n=5).

Synthetic Ech provided by the Pacific Institute of Bioorganic Chemistry of the Far Eastern Branch of the RAS (Vladivostok, Russia) was infused through the catheter inserted into the femoral vein as a 0.2% solution in a 0.1% sodium bicarbonate solution. Ech was administrated 5 min before to occlusion of coronary artery; the control group received a saline solution injection in equivalent volume.

The analysis of the recorded epicardial electrograms included the definition of the activation–recovery intervals (ARIs), which were corrected for heart rate by Bazett's formula and used for the evaluation of local repolarization duration. ARI was defined as the interval between the activation time (AT) and the end of the repolarization time (RT), determined as dV/dt min during the QRS complex and dV/dt max during the ST-T complex, respectively [7]. The time measurements were done with respect to the QRS onset in the I limb lead. Dispersion of repolarization was calculated as the difference between the ARI max in the non-ischemic area and ARI min at the ischemic zone.

The data are expressed as Me (25%; 75%). Statistical examination used the Friedman test, followed by the Newman–Keuls test for paired comparisons. The Mann-Whitney test aided in the comparison between groups. A chi-squared test assessed the difference in the occurrence of the ventricular arrhythmias. The differences were considered significant when $p<0.05$.

3. Results

The administration of Ech prior to the coronary occlusion did not change the local durations and distribution of the ARIs on the epicardial surface of the ventricles. There were no differences in baseline distribution of the ARIs among all groups. Acute coronary occlusion resulted in the shortening of the ARIs in the ischemic area ($p<0.05$), whereas the ARIs from non-ischemic area were invariable at ischemia during the exposure. A significant increase of dispersion of the repolarization ($p<0.05$) was demonstrated in both control and treated by Ech animals in response to acute ischemia.

The shortening of ARIs in the ischemic zone was less in animal treated by Ech (2 mg/kg) compared to control group at 15 min and 30 min of ischemia ($p<0.05$, Tabl.1). Therefore, the increase of repolarization dispersion in Ech group (2 mg/kg) was limited ($p<0.05$) in comparison with the control animals at 5 min (Δ 24 (17;34) vs Δ 53 (37;71) ms), at 15 min (Δ 37 (18;38) vs Δ 61 (59;81) ms), and at 30 min (Δ 40 (15;44) vs Δ 63 (63;65) ms). Ech in concentration of 2 mg/kg demonstrated more pronounced effect to decrease the ischemia-induced ARIs shortening (in 1.6 $p<0.05$), and the increase of repolarization dispersion at 30 min ischemia was less (in 1.7 $p<0.05$) versus the 1 mg/kg concentration of Ech.

The incidence of ischemia-induced ventricular arrhythmia did not differ between subjects treated with Ech and control animals. During ischemia, the ventricular arrhythmias, such as polymorphic and monomorphic extrasystoles as well as coupled extrasystoles, began after 10 min of coronary occlusion, and occurred under a period of exposure.

Table 1. Intensity of ischemic injury (**Δ** ARIs) during coronary occlusion (*Me* (25%;75%), ms).

ARIs shortening from baseline state (**Δ**)in ischemic area	Control(n=5)	Echinochrome 1 mg\kg(n=5)	Echinochrome 2 mg\kg (n=7)
5 min ischemia	46(38;57)	33(31;56)	17(14;35)
15 min ischemia	69(56;71)	38(19;58)	27(19;33)$^{\#}$
30 min ischemia	69(51;87)	43(36;70)	25(12;40)$^{\#*}$

$^{\#}$p<0.05 compared to control group
* p<0.05 compared to Ech 1 mg/kg

4. Discussion

The main finding of the present study is the ability of Ech to reduce ischemia-induced shortening of repolarization, and therefore to avoid the increase of repolarization dispersion which can provide the potential substrate for ventricular arrhythmias.

The role of oxidative stress in ischemic tissues has been described [8]. It has been suggested that ROS affect the ion channels [1] and contribute to the genesis of arrhythmias associated with ischemia/reperfusion.

Synthetic Echinochrome was used as an antioxidant and an iron-binding agent [3, 4], seeing that it could have a greater protective effect on oxidative stress-induced ventricular arrhythmias than the antioxidant alone [9].

Previous experimental studies pointed to the cytoprotective and anti-ischemic effects of Ech in animal model of coronary artery occlusion-reperfusion [10]. The clinical investigations confirm that treatment with Ech prevents infarct expansion induced by reperfusion [11]. These studies primarily examined other points of cardiac injury. The electrophysiological effects of Ech in feline model of coronary occlusion were demonstrated in the present study. The pre-occlusion infusion of Ech reduced the ischemia-induced shortening of ARIs. This effect could be due to the inhibition of an ionic current through the ATP-regulated potassium channels (KATP channels); however, further research is required to confirm this assumption.

All of the animal groups demonstrated ventricular arrhythmias during coronary occlusion. This was consistent with the significantly increased ARI dispersion at coronary occlusion in all the animal groups despite to the restriction in increasing repolarization dispersion in animals treated by Ech. It is possible that the restriction of the increase in the repolarization dispersion, which was induced by Ech, was insufficient to prevent the ventricular arrhythmias during ischemia. On the other hand, other key factors providing trigger activity or abnormal automaticity outside the sinoatrial node must be taken into account.

5. Conclusions

The study demonstrated the ability of the antioxidant Echinochrome to decrease ischemia-induced alterations of ventricular repolarization in a feline model of coronary occlusion. The findings suggest a dose-dependent effect of Echinochrome.

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References

[1] Carmeliet E. Cardiac ionic currents and acute ischemia: from channels to arrhythmias. *Physiological Reviews,* 79: 917-1017, 1999.

[2] Karahaliou A, Katsouras C, Koulouras V, Nikas D, Niokou D, Papadopoulos G, Nakos G, Sideris D, Michalis L. Ventricular arrhythmias and antioxidative medication: experimental study. *Hellenic Journal of Cardiology*, 49: 320-328, 2008.

[3] Lebedev AV, Ivanova MV, Levitsky DO. Echinochrome, a naturally occurring iron chelator and free radical scavenger in artificial and natural membrane systems. *Life Sciences*, 76: 863-875, 2005.

[4] Lebedev AV, Ivanova MV, Levitsky DO. Iron chelators and free radical scavengers in naturally occurring polyhydroxylated 1,4-naphthoquinones. *Hemoglobin*, 32: 165-179, 2008.

[5] Shvilkin AV, Serebriakov LI, Tskitishvili OV, Sadretdinov SM, Kol'tsova EA, Maksimov OB, Mishchenko NP, Novikov VL, Levitskiĭ DO, RudaMIa. Effect of echinochrom on experimental myocardial reperfusion injury. *Kardiologiia*, 31: 79-81, 1991.

[6] Sedova KA, Bernikova OG, Goshka SL, Pokhilo ND, Atopkina LN, Shmakov DN, Kharin SN. Effects of an antioxidant agent on alterations of ventricular repolarization in a coronary artery occlusion-reperfusion experimental model. *Experimental & Clinical Cardiology,* 1-6, 2013.

[7] Millar CK, Kralios FA, Lux RL. Correlation between refractory periods and activation-recovery intervals from electrograms: effects of rate and adrenergic interventions. *Circulation*, 72: 1372-1379, 1985.

[8] Xing D, Chaudhary AK, Miller FJ Jr, Martins JB. Free radical scavenger specifically prevents ischemic focal ventricular tachycardia. *Heart Rhythm*, 6: 530–536. 2009.

[9] Lebedev AV, Levitskaya EL, Tikhonova EV, Ivanova MV. Antioxidant properties, autooxidation, and mutagenic activity of echinochromea compared with its etherified derivative. *Biochemistry,* 66:885-893, 2001.

[10] Mischenko NP, Fedoreev SA, Zapara TA, Ratushnyak AS. Effects of histochrom and emoxypin on biophysical properties of electroexcitable cells. *Bulletin of Experimental Biology and Medicine,* 1479: 196-200, 2009.

[11] Buĭmov GA, Maksimov IV, Perchatkin VA, Repin AN, Afanas'ev SA, Markov VA, Karpov RS. Effect of the bioantioxidant histochrome on myocardial injury in reperfusion therapy on patients with myocardial infarction. *Terapevticheskii Arkhiv,* 74:12-16, 2002.