Journal of Experimental Medical & Surgical Research
Cercetări Experimentale & Medico-Chirurgicale
Year XIX Nr.3 / 2013 Pag. 75 - 79

JOURNAL of Experimental Medical & Surgical RESEARCH

THE CARDIOPROTECTION MODEL IN RATS INDUCED BY ISCHEMIA-REPERFUSION IN VIVO BY POSTCONDITIONING WITH ISOFLURANE ANESTHESIA

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SUMMARY:
Isoflurane is a halogenated anesthetic volatile type commonly used in experimental and clinical practice, with possible cardioprotective in postconditioning and preconditioning of pharmacological ischaemia. This paper aims to compare the effects of isoflurane during reperfusion in postischemic postconditioning in rat hearts in vivo. A total of 20 anaesthetized rats (n = 10/lot) was subjected to ischemia for a period over 30 min and 2 hours of reperfusion , respectively . Postconditioning with isoflurane anesthesia (iso -post) has been carried out only on one of the groups consisting of 10 rats, and the single administration of isoflurane (2.1 %) 3 minutes prior to reperfusion up to 2 minutes after reperfusion, with a total episode administration of reperfusion for 5 minutes. Cardioprotective was monitored using two primary indicators: reduction of infarct size expressed as a percentage as the ratio AN / AR (necrosis area / risk area) and decreased incidence of arrhythmias arrhythmogenic estimated by score arrhythmogenic . Were also monitored and evaluated and two secondary indicators: heart rate (HR) and mean arterial pressure (MAP). Our results obtained on the model of I / R (ischaemia / reperfusion)regional in vivo at rat shows that anesthesia with Isofluran on postconditioning protocol did not lead to significant anti -infarct protection and not was reduced nor significantly arrhythmias that were induced by I/ R(ischaemia/reperfusion).
Keywords: *Isoflurane, preconditioning, postconditioning, I/R(ischemia/reperfusion), rats heart.*

REZUMAT: *Izofluranul este un anestezic volatil de tip halogenat utilizat frecvent in practica experimentală si clinică, cu posibilă cardioprotecţie, atât în precondiţionare cât şi în postcondiţionare farmacologică ischemică. Prezenta lucrare şi-a propus să compare efectele izofluranului de postcondiţionare în timpul reperfuziei postischemice în cazul inimilor de şobolani in vivo. Un număr de 20 şobolani anestezia şi (n=10/lot) au fost supuşi unei perioade de ischemie cu durata de 30 min si respectiv de 2 ore de reperfuzie. Postcondiţionarea anestezică cu izofluran (Iso-Post) s-a efectuat doar la unul din loturile de 10 şobolani si a constat în administrarea unică de izofluran (2,1%) cu 3 minute înainte de reperfuzie până la 2 minute după reperfuzie, totalizând un episod de administrare la reperfuzie cu durata de 5 minute. Cardioprotecţia a fost urmărită prin utilizarea a doi indicatori primari: reducerea mărimii infarctului exprimată procentual ca raportul AN/AR (aria necroza /aria la risc ischemica totala) şi scăderea incidenţei aritmiilor estimată cu ajutorul scorului aritmogen. De asemenea au fost monitorizaţi si evalua şi doi indicatori secundari: frecvenţa cardiacă (FC) şi presiunea arteriala medie (PAM). Rezultatele noastre ob inute pe modelul de I/R (ischemie/reperfuzie) regională in vivo la şobolan, arată că Izofluranul utilizat în cazul protocolului de postcondi ionare anestezică nu a condus la protec ie semnificativă anti-infarct si nici nu a redus semnificativ statistic aritmiile induse de I/R.*
Cuvinte cheie: *Isoflurane, precondiţionare, postcondiţionare, ischemie/reperfuzie.*

Received for publication: 11.06.2013
Revised: 21.07.2013

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OBJECTIVES

Standardization of the experimental model of ischemia / reperfusion regional in vivo at rat hearts - similar reproduce human clinical conditions in acute coronary syndromes.

Isofluran an Halogen-volatile anesthetic is widely used in the clinic and are known for the tissue protective properties in terms of their administration protocols as pre - respectively, in postconditioning at various animal models of ischemia / the experimental reperfusion, as well as in humans. The purpose of this study was to test Isofluran in vivo if had the effect on postconditioning . Protocol postconditioning studied was the one who, according to the literature, the maximum was induced on cardioprotection in isolated administration.

THE STUDY MATERIAL

He was represented by 20 adult Sprague -Dawley rats weighing 370-450 g, divided into 2 groups (n = 10 rats / group , first lot=control-lot and second-lot =postconditioning with isoflurane (ISO-POST lot) who received food and water ad libitum , solid food is suppressed by 12 hours prior to the experiment. All experiments were performed in accordance with the Guide for the Care and Use of Animals (U.S. National Institutes of Health No. 85-23 , 1996) and the Committee of Ethics and Conduct and Pharmacy " Victor Babes " Timisoara , which approved methodology working on the basis of a draft drawn up in advance.

EXPERIMENTAL PROTOCOL

Anesthesia was obtained by intraperitoneal administration of a mixture of ketamine (80 mg / kg) with xylazine (5 mg / kg) . Both groups of 10 rats were subjected to ischemia lasting period of 30 min , respectively , for 2 h reperfusion . Anesthesia postconditioning was carried out only one of the groups consisting of 10 rats , and the single administration of isoflurane (2.1 %) 3 minutes prior to reperfusion up to 2 minutes after reperfusion , with a total administration reperfusion episode lasting 5 minutes. The experimental design is schematized in Fig . 1 .

STUDY RESULTS AND DISCUSSION.

Cardioprotection was monitored by using 2 primary indicators :

A) reduced infarct size expressed as a percentage as the ratio AN / AR and

B) reduce the incidence of arrhythmias estimated by arrhythmogenic score .

Analysis of infarct area was made with image processing software (Image J software) that allows individual selection and calculation of areas ischemic areas (AR) and of necrotic areas (AN) by computerized planimetry . By multiplying each of these sections analyzed aryl weight and summing the results obtained were the final dimensions AR , AN for each heart .

Analysis was performed according to a score arrhythmogenic after Curtis and Walker modified , as follows : 0 - no arrhythmias ; 1 - between 1- 30 ventricular extrasystoles ,

2 - more than 30 ventricular extrasystoles ; 3 - less than 3 episodes of tachycardia and / or fibrillation ventricular , 4 - 3 to 5episoade tachycardia and / or fibrillation and 5 - more than 5 episodes of tachycardia and / or fibrillation .

Cardioprotection as secondary indicators of hemodynamic variables were used : (i) heart rate (HR) and (ii) mean arterial pressure (MAP) . Hemodynamic Data (peripheral derivation DII and peripheral channel pressure) were continuously acquired throughout the duration of the experiment and the interpretation , is measured at certain points in time during ischemia and respectively reperfusion .

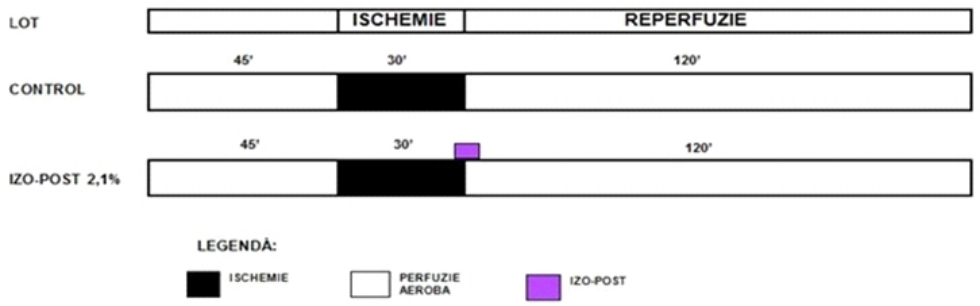


Fig. 1. The experimental study of the effects of the anesthetic isoflurane postconditioning.

Primary indicators of cardioprotection:

A. The size of experimental myocardial

In the Fig. 2 and 3 show the effects of isoflurane administration in the group of rats subjected postconditionarii anesthesia (Iso-Post), effects were compared with control-lot subjected to ischemia / reperfusion (I / R)n in the absence of protective interventions.

Expression of the area at risk (AR) as a percentage from left ventricle (AR /% of LV area) do not show differences between groups, which attest to the reproducibility of the experimental model, the fact that the placement of the wire was performed at the same level of coronary artery occlusion is quasi-identical (see picture below).

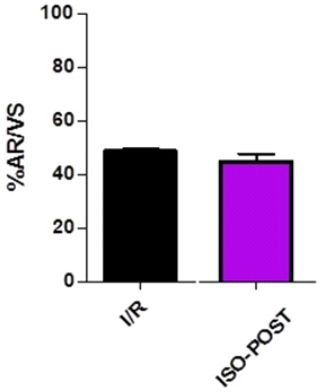


Fig. 2. Area at risk (AR) in groups conditioned with isoflurane

For both batches, analyzing experimental data (Table 1) showed low values of standard error mean (SEM) obtained for risk areas studied, indicating a high homogenity in the studied groups confirming reproducibility in terms of experimental conditions.

Table 1. Average percentage values of areas at risk (AR)

Group	Control (I/R)	ISO-POST
Media	48,72	44,86
SEM	0,7715	2,824

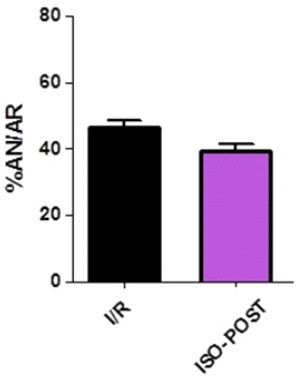


Fig. 3. Infarcted area in control group comparative with isoflurane anesthesia group.

In the Fig. 3 is observed that the postconditioning with isoflurane (Iso Post) was not associated with the cardioprotective size infarcted area (AR + AN) although slightly lower showed no significant difference compared to control group.

Table 2. Mean percentage values of areas of necrosis (AN)

Group	Control (I/R)	ISO-POST
Media	46,31	39,17
SEM	2,342	2,007

In the case of areas of necrosis (AN), on the one hand it can be observed a significant reduction of the percentage value suggesting that this strategy medium applied to exert a measurable biological and secondly to obtain low values of average standard errors for each of the groups studied (Table 2) is a further confirmation of the reproducibility of the experimental method used and the fact that it was used a sufficient number of animals to achieve statistical significance.

The figures 4-5 are representative examples of staining with TTC (2,3,5 trifeniltetrazol Chloride = TTC) for the groups under study.

B. Episodes of ventricular arrhythmias

Analysis was performed according to a score arrhythmogenic note 1 to 5 where:

Fig. 4. Example of heart in the control group (I / R) after staining with TTC.

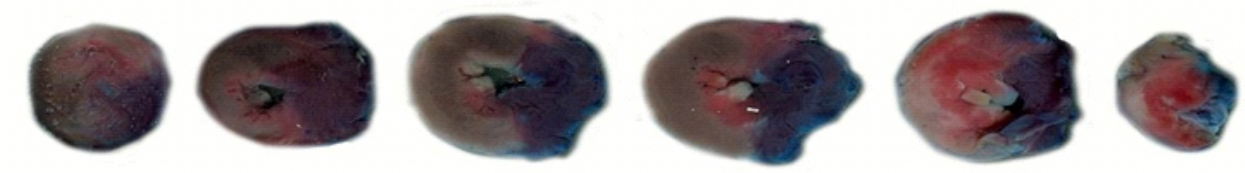
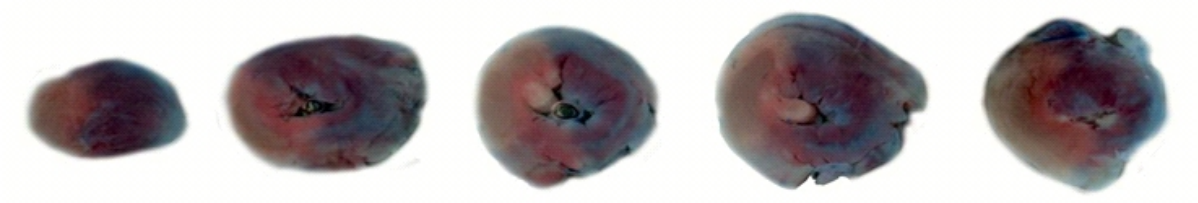


Fig. 5. Example of heart in group Iso-Post after TTC staining.



- 0 - no arrhythmias;
- 1 - ventricular extrasystoles from 1 to 30;
- 2 - more than 30 ventricular extrasystoles;
- 3 - less than 3 episodes of tachycardia and / or fibrillation;

  4 - 3 to in 5 episodes of tachycardia / or fibrillatio
- 5 - more than 5 episodes of tachycardia and / or fibrillation.

The data presented below are the occurrence of arrhythmias caused by the 30 minutes of ischemia test (index ischemia). Fig.6.

The figures 7-10 are shown examples of arrhythmias recorded during the experiment studied groups.

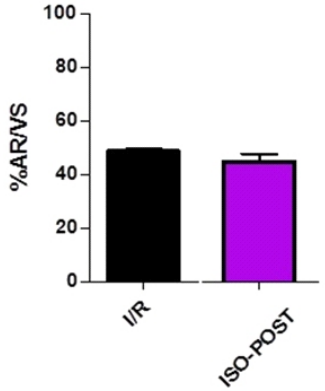


Fig. 6. The incidence of arrhythmias induced by ischemic postconditioning with isoflurane.

Fig. 7. Extrasystoles systematized in control group

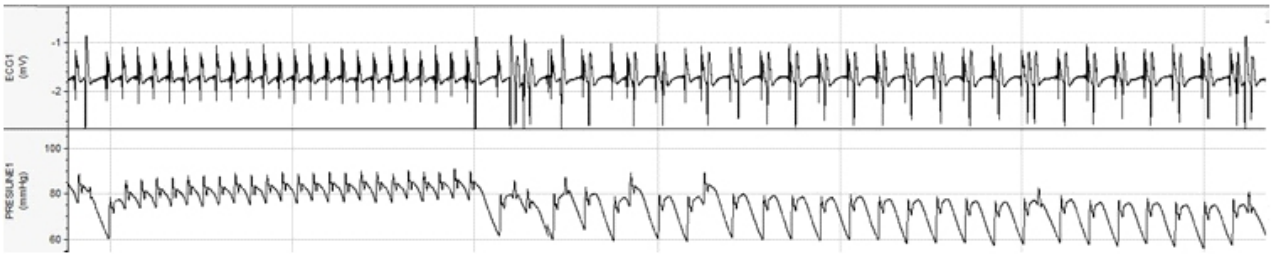


Fig. 8. Example of ventricular tachycardia in control group

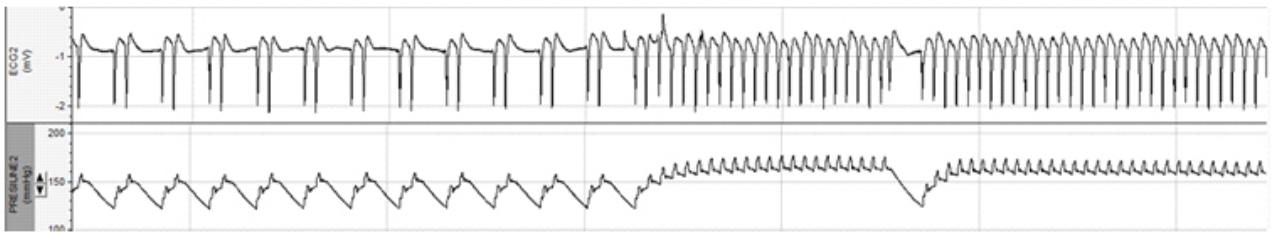


Fig. 9. Example of systematic extrasystoles (bigeminy) in group Iso-POST

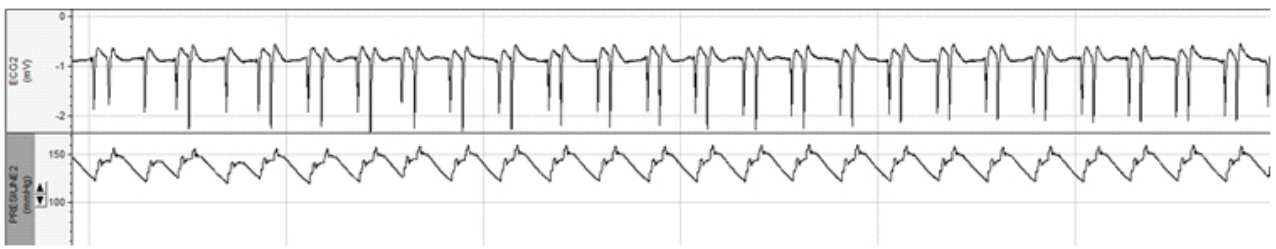
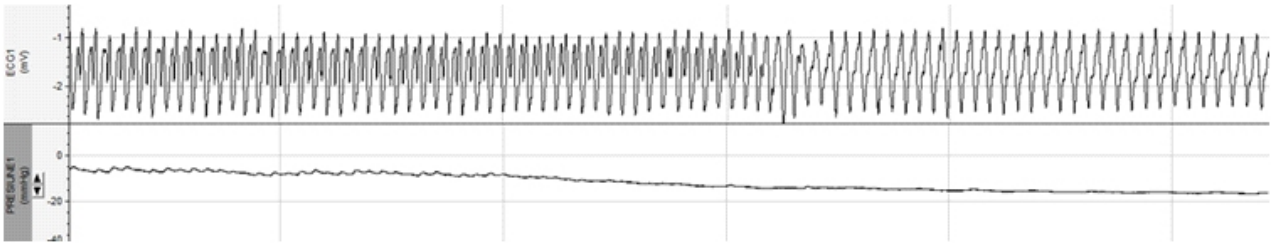


Fig. 10. Example of ventricular tachycardia installed during reperfusion in group Iso-POST



Secondary indicators of cardioprotection:

As we mentioned to standardize experimental model, the two secondary indicators, heart rate (FC) and mean arterial pressure (MAP) were continuously monitored during the experiments, the data being collected but only at certain times according to a predetermined protocol. The average values of hemodynamic parameters at predetermined points of time are presented in Table 3.

As shown in the table 3, there were no significant differences in the recovery of functional parameters reperfusion in the group treated with isoflurane at a certain time of reperfusion comparativ with control group. However the IZO-POST group is highlighted slight reduction, but not statistically on significant arrhythmias, induced by I/R.

CONCLUSIONS.

Isoflurane used for anesthesia postconditioning protocol did not result in significant anti-infarction myocardial protection, nor reduced arrhythmias induced by I / R (ischaemia / reperfusion).

Table 3. Mean ± SEM absolute hemodynamic parameters.

Group	Stabilization	Pre-oclusion	Coronary oclusion		Reperfusion			
			15′	25′				15′
Cardiac Rate (b/min)								
I/R	310 ± 14	304 ± 8	308 ± 9	300 ± 11	I/R	310 ± 14	304 ± 8	308 ± 9
ISO-POST	292 ± 22	304 ± 12	304 ± 16	300 ± 18	ISO-POST	292 ± 22	304 ± 12	304 ± 16
Mean arterial pressure (mmHg)								
I/R	129± 24	98± 9	74± 23	79± 14	I/R	129± 24	98± 9	74± 23
ISO-POST	135± 23	142± 24	126±18	116± 19	ISO-POST	135± 23	142± 24	126±18

References:

1. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986; 74: 1124-1136.
2. Obal D, Saskia Dettwiler, W Schlack et al. The influence of mitochondrial K ATP channels in the cardioprotection of preconditioning and postconditioning by sevoflurane in the rat in vivo. Anesth Analg 2005; 101:1252-1260.
3. Stadnicka A, Marinovic J, Bosnjak ZL et al. Volatile anesthetic-induced cardiac preconditioning. J Anesth 2007; 21: 212-219.
4. Weber N, Schlack W. Inhalational Anaesthetics and Cardioprotection. In Schutttler J. and Schwilden H. (eds.). Handbook of Experimental Pharmacology 182, Springer-Verlag Berlin Heidelberg 2008; 187-207.
5. Yellon DM, DJ Hausenloy. Preconditioning and postconditioning: new strategies for cardioprotection. Diabetes Obes Metab 2008;10(6): 451 – 9.
6. Tanaka K, Ludwig LM, Kersten JR, et al. Mechanisms of cardioprotection by volatile anaesthetics. Anaesthesiology 2004;100:707-721.
7. Pagel P. Postconditioning by volatile anesthetics: salvaging ischemic myocardium at reperfusion by activation of prosurvival signaling. J Cardiothorac Vasc Anesth 2008; 22(5):753-765.
8. V. Ordodi, G. Gheorghiu, C. Henția, D. Săndesc, Danina Muntean et.al. In vivo studies of cardioprotection induced by volatile anaesthetics - experimental model in rodents. Bulletin USAMV-CN, 65(1): 401-405 (2008) .