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

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 anesthetized 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(ischemia/reperfusion).

Keywords: Isoflurane, preconditioning, postconditioning, I/R(ischemia/reperfusion), rats heart.

REZUMAT: Izofluranul este un anestezic volatile de tip halogenat utilizat frecvent în practica experimentală și clinică, cu posibilitatea de a proteja cardia în timpul reperfuziei postischemice în cazul înimilor de șobolan în vivo. Un număr de 20 șobolani anestezia și (n=10/lot) au fost supuși unei perioade de ischamie cu durata de 30 min și respectiv de 2 ore de reperfuzie. Postcondiționarea anestezică cu izofluran anestezic (Iso-Post) s-a efectuat doar la unul din loturile de 10 șobolani și 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. Cardioprotecția a fost urmată prin utilizarea a doi indicatori primari: reducerea mărimii infarctului exprimată procentual ca raportul AN/AR (aria necroza /aria la risc ischamică totală) și scăderea incidenței aritmii estimată cu ajutorul scorului aritmogen. De asemenea au fost monitorizate și evaluat și doi indicatori secundari: frecvența cardiacă (FC) și presiunea arterială medie (PAM). Rezultatele noastre obinute pe modelul de I/R(ischamie/reperfuzie) regional în vivo la șobolan, arată că izofluranul utilizat în cazul protocolului de postcondiționare anestezică nu a condus la protecție semnificativă anti-infarct și nici nu a redus semnificativ aritmii induse de I/R.

Cuvinte cheie: Isoflurane, precondiționare, postcondiționare, ischemie/reperfuzie.

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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.

Isoflurane 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 Isoflurane 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 5episode of 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).

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

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (I/R)</th>
<th>ISO-POST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>48,72</td>
<td>44,86</td>
</tr>
<tr>
<td>SEM</td>
<td>0,7715</td>
<td>2,824</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (I/R)</th>
<th>ISO-POST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>46,31</td>
<td>39,17</td>
</tr>
<tr>
<td>SEM</td>
<td>2,342</td>
<td>2,007</td>
</tr>
</tbody>
</table>

Fig. 4. Example of heart in the control group (I / R) after staining with TTC.
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 5 episodes of tachycardia / or fibrillation
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. 5. Example of heart in group Iso-Post after TTC staining.

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
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 compared 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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Stabilization</th>
<th>Preocclusion</th>
<th>Coronary occlusion</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>15’</td>
<td>25’</td>
</tr>
<tr>
<td>I/R</td>
<td>310 ± 14</td>
<td>304 ± 8</td>
<td>308 ± 9</td>
<td>300 ± 11</td>
</tr>
<tr>
<td>ISO-POST</td>
<td>292 ± 22</td>
<td>304 ± 12</td>
<td>304 ± 16</td>
<td>300 ± 18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>292 ± 22</td>
<td>304 ± 12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Cardiac Rate (b/min)</th>
<th>Mean arterial pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/R</td>
<td>129 ± 24</td>
<td>98 ± 9</td>
</tr>
<tr>
<td>ISO-POST</td>
<td>135 ± 23</td>
<td>142 ± 24</td>
</tr>
</tbody>
</table>

References: