Preconditioning abolishment by midazolam in isolated hearts of rats

Abolição do precondicionamento pelo midazolam em corações isolados de ratos

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ABSTRACT

Purpose: To study the effects of benzodiazepine midazolam in the coronary flow (Cflo), cardiac frequency (CF) and myocardial contractility in isolated hearts of rats subjected to ischemic preconditioning (IPC).

Methods: 30 Wistar rats were used, undistinguished by gender. After anesthesia with ethyl ether, the hearts were put into perfusion (Krebs-Henseleit solution, 95% O2 and 5% CO2, 37°C, 110-120mmHg), in disposable Langendorff type system. Five groups of six animals were constituted: GI- Control; GII- Ischemia; GIII- IPC; GIV- Ischemia + 100mcg of midazolam; GV- IPC + 100mcg of midazolam. After stabilization (t0), and on times t5, t10, t15, t20 and t25, CF, Cflo, systolic pressure (SP) and diastolic pressure (DP) and dP/dt were recorded. DP was maintained at 5 ± 2 mmHg. The statistical method ANOVA and Tukey Test were employed for p < 0.05.

Results: No significant variations have occurred between Cflo and CF. On Pd/td, differences have occurred (p<0.05) between groups I and II (respectively 94.7±23.0 and 62.3±12.1%). The preconditioning (GIII), improved significantly the results in the group II (respectively 62.3±12.1 and 87.1±12.4 %). The decrease in dP/dt in group II was not prevented by midazolam (GIV) (62.3±12,1 and 60.5±15.8 %). In group III, dP/dt was 87.1±12.4%, whereas in group V, only 55.5±17.2% (p<0.05)

Conclusion: Midazolam, when administered before the ischemia, was unable to prevent the ischemic deterioration of the myocardium. When administered before the preconditioning, it has abolished its protective effect.

Key words: Ischemic Preconditioning. Heart. Midazolam. Rats.

Introduction

The first demonstration of the potential to reduce the extension of myocardial injury and heart attack in the coronary occlusion was made by Murry et al.¹ using canine models. These researchers stated that the occlusion of the left circumflex coronary artery of the animals for 40 minutes resulted in heart attack in the ischemic area. Using four cycles of ischemia for 5 minutes, alternating with reperfusion, the heart attack area was significantly reduced, reaching to the conclusion that this procedure, named ischemic preconditioning (IPC), allows higher resistance to subsequent extended ischemia.
Yellon et al.3 demonstrated that the protection through IPC also occurs in the human myocardium, which raised a great interest regarding this study.

In cardiology, benzodiazepines, including midazolam, are used as anxiolytics and sleep inducers. They are also used as pre-anesthetic medication, before surgeries and diagnosis and therapeutic procedures that have repercussion in the myocardial perfusion and feasibility.

Midazolam acts in the central nervous system similarly to other benzodiazepines, and potentiates the effects of the Gamma-Amino Butyric acid (GABA), the main inhibition mediator of the CNS3.

Hernández4, Pontes5, and Medeiros et al.6 demonstrated, in isolated hearts of rats, inhibition of the myocardial contractility after diazepam, midazolam and propofol infusion, and the depressive myocardial effect of midazolam was confirmed by Hekerdemian et al.7 and Ozturk et al.8.

The present study has the purpose to analyze the influence of midazolam in the ischemic preconditioning induced in isolated hearts of rats.

Methods

The ethical principles of animal testing were followed, which have been established by the Brazilian School of Animal Testing (Colégio Brasileiro de Experimentação Animal - COBEA)9. Isolated hearts of 30 Wistar albino rats were used, undistinguished by gender, with weight ranging from 210 and 340mg, an average of 269mg. The weight of the hearts ranged from 0.74g and 1.95g, an average of 1.29g. After the procedures of inhaled anesthesia with ethyl ether, systemic heparin infusion and thoracotomy, the hearts were isolated and immediately put into perfusion with Krebs-Henseleit solution, balanced with 95% O2 and 5% CO2, and maintained at 5±2 mmHg except during the periods of ischemia, when it was maintained at zero. The effects on the myocardial contractility were measured by calculating the values of the first ventricular pressure temporal derivative (dP/dt). Data obtained in the experiment were analyzed by the ANOVA statistical method, combined with the Tukey Test, with significance level of 95% (p<0.05), and the control values (t0) and the ones obtained with 20 minutes of myocardial reperfusion(t20) were considered for comparison.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>GI</th>
<th>GII</th>
<th>GIII</th>
<th>GIV</th>
<th>GV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cflo</td>
<td>83.8±6.7</td>
<td>83.0±22.3</td>
<td>79.7±18.5</td>
<td>69.0±7.6</td>
<td>72.5±19.7</td>
</tr>
<tr>
<td>CF</td>
<td>92.5±12.2</td>
<td>88.8±13.9</td>
<td>101.5±19.7</td>
<td>85.2±10.8</td>
<td>83.2±13.3</td>
</tr>
<tr>
<td>SP</td>
<td>97.3±5.8</td>
<td>58.0±18.0*</td>
<td>83.0±8.9*</td>
<td>74.3±9.9*</td>
<td>69.2±15.1*</td>
</tr>
<tr>
<td>dP/dt</td>
<td>94.7±23.0</td>
<td>62.3±12.1*</td>
<td>87.1±12.4*</td>
<td>60.5±15.8*</td>
<td>55.5±17.2*</td>
</tr>
</tbody>
</table>

Cflo – Coronary flow, CF – Cardiac frequency, SP – systolic pressure, dP/dt – Pressure/time temporal derivate, *p<0.05.
Jennings and Reimer\textsuperscript{10} showed that even though the reperfusion is essential to protect the ischemic myocardium, it doesn’t prevent the death of cardiomyocytes, especially if the ischemia is prolonged.

The first demonstration of the likelihood to reduce the number of cardiac cells dead after the IAM was made by Murry et al.\textsuperscript{1}. These researchers stated that the occlusion of the left circumflex coronary artery of dogs for 40 minutes resulted in heart attack of 29.4 ± 4.4% of the ischemic area. If, before the occlusion, the animals were subjected to four cycles of 5 minutes of ischemia, alternating with 5 minutes of reperfusion, the area attacked would be reduced to 7.3 ± 2.1%, and the authors named this protective effect as “ischemic preconditioning”, which gained worldwide interest due to its applicability potential, especially after Yellon et al.\textsuperscript{2} has show that this also occurs in the human myocardium.

Even though the IPC mechanism is not entirely clarified, it is known that immediately after the ischemia, the organism increases the production of several chemical mediators that trigger the cardio protection process\textsuperscript{11}. Amongst these mediators, the most important ones seem to be aceticholyme\textsuperscript{12}, angiotensin II\textsuperscript{13}, and mainly adenosine\textsuperscript{14}. The connection of these substances to their receptors in the external surface of the sarcolem starts the next phase that occurs in the cytosol. In this second phase, signalization routes are activated with several stages, in which participate several substances reactive to oxygen\textsuperscript{15}, phospholipase, diacylglycerol (DAG) and inositol\textsuperscript{16}. Finally, these routes seem to converge mainly to activate the protein kinase (PKC) enzyme, which interacts with mitochondrial\textsuperscript{17,18} structures, resulting in alteration of the cellular metabolism and under cardio protection.

Currently, numerous researches on IPC have the purpose to clarify its biochemical mechanism, pursuing the development of drugs that induce the safe and effective cardiac preconditioning.

Benzodiazepines, even though widely used as pre-anesthetic or sedatives in procedures that can cause myocardial ischemia, have effects on the IPC that are not well known. However, works by several researchers have evidenced negative inotropic effects of these substances on the heart, including for midazolam. In 1991, Hernández\textsuperscript{2} observed the depressive effect of diazepam over rats’ myocardium. Shekerdemian et al.\textsuperscript{18} reported the reduction of 24.1% in cardiac debt in human hearts perfused with midazolam during heart surgery. In the same year, Nonaka et al.\textsuperscript{19}, observed the negative inotropic effect of diazepam and midazolam in a culture of rats’ cardiomyocytes. Ozturk et al.\textsuperscript{5} observed that midazolam, in doses ranging from 10mcg and 100mcg, have caused myocardium depression in rabbits. Medeiros et al.\textsuperscript{6}, studying the effects of diazepam, midazolam and propofol in isolated hearts of rats, reported that benzodiazepines have caused depression in myocardial contractility.

The present investigation confirms the data initially reported\textsuperscript{19}, showing that the statistical comparison of dP/dt values in the groups ischemia (GII) and IPC (GIII) confirm the protective effects of the IPC in the ischemic myocardium. It could also be observed that midazolam, when associated to the IPC procedure (GV), caused a significant reduction in myocardial contractility (dP/dt), if compared to the same procedure without using the medication (GIII).

Even though the statistical analysis didn’t show significant differences in CF and Cflo in the groups studied, it could be stated that the biggest percentile reductions of these variables, in time, have occurred in those subjected to the ischemia (GII) and in those treated with midazolam (GIV e GV).

Regarding SP, significant statistical differences were observed between the control (GI) and ischemia (GII) groups, showing the harmful effects of the ischemia on the heart. Significant statistical differences could also be observed in this variable between the ischemia (GII) and IPC (GIII) groups, confirming the protective effect of the IPC in the myocardium. The administration of midazolam (GV) has not prevented the functional depression determined by the ischemia without IPC (GII) and has blocked, in group IV, the beneficial effect dependent of the preconditioning without drugs, observed in group III.

The choice for anesthesia with F.A. ether constitutes a special aspect in the method employed, and, in the recent past, in other surveys made by our Institution, the acquisition and use of ether without the specification “For Analysis” has resulted in functional damages to the isolated hearts under study. The use of F.A. ether enables a stable cardiac performance in the control group and full effect of the preconditioning in the group studied without midazolam.

![FIGURE 1 - Percentile variation of dP/dt in studied groups](image-url)
The limitations of the present study include the biologic difficulty to correlate animal testing data with similar situations in humans, the number of experiments carried out so far and also not analyzing the morphology variations, especially in the ultramicroscopic level, to differentiate between tissue injury and functional limitation. However, the results obtained confirm in rats the recent publication of Rivo et al.\textsuperscript{20} describing similar effects of midazolam abolishing the preconditioning in rabbit hearts.

\textbf{Conclusion}

Midazolam has not interfered significantly in the cardiac frequency and coronary flow in the groups analyzed. It has not protected the myocardium of the investigated group with induced ischemia and, when associated to IPC, has prevented the protection of myocardial contractility (dP/dt) if compared to the same procedure without the medication.

\textbf{References}


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