Sensitivity and Specificity of Ischemia Modified Albumin in Wistar Rats

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Abstract

Background: Acute myocardial infarction (AMI) is a condition determined by an acute ischemic process resulting in myocardial necrosis. Cardiac markers in reversible ischemia currently have limited sensitivity.

Objective: To check the sensitivity of ischemia modified albumin (IMA) as a cardiac marker.

Methods: Experimental study held at the Animal Experimentation Laboratory of Universidade Regional Integrada (URI), Erechim, RS, from 2011 to 2013. After myocardial ischemic induction in Wistar-Tecpar rats aged about 60-90 days through administration of isoproterenol hydrochloride, the IMA content was evaluated at different times.

Results: The IMA values remained reduced during the three first hours after ischemic induction by isoproterenol hydrochloride.

Conclusion: In this study, ischemia modified albumin was considered a sensitive marker, particularly in the first three hours of ischemia.

Keywords: Albumins; Myocardial ischemia; Myocardial infarction

Introduction

Cardiovascular diseases account for approximately 32% of deaths in the general population, being the first cause of mortality in Brazil¹. Acute myocardial infarction (AMI) is the most common consequence of most heart diseases and represents a serious epidemic problem due to its progression².

AMI is defined as a focus of necrosis resulting from poor tissue perfusion, with signs and symptoms resulting from cardiac cell death³. The presence of necrotic areas results from the sharp reduction in oxygen supply or increased demand or the combination of both mechanisms⁴⁻⁶.

Acute myocardial infarction is confirmed when there is evidence of myocardial necrosis in a clinical setting of ischemia with an increase in myocardial necrosis markers above the 99th percentile of the upper reference limit combined with symptoms suggestive of myocardial ischemia, development of new Q waves on electrocardiogram (ECG), new significant changes in the ST-segment, T wave, or new left branch block (LBB), evidence of viable myocardial loss or new segmental abnormality of ventricular contractility or identification of intracoronary thrombus on angiography or necropsy⁷.

IMA has been reported as a marker capable of reflecting the myocardial ischemic condition. The amino-terminal
portion of plasma albumin normally has an affinity with ionic heavy metals such as cobalt and copper. The N-terminal portion is modified during exposure to the ischemic condition due to the production of free radicals and reactive oxygen species, resulting in the generation of IMA with low affinity with heavy metals.

On the other hand, troponins are highly used as myocardial injury markers, since they have a complex that regulates the actin-myosin interaction in skeletal and cardiac striated muscles and, therefore, plays a role in the electromechanical linkage of these muscles, and is not found in smooth muscles. Its dosage enables the differentiation of the skeletal or myocardial origin of muscle injuries, as well as detection of the minimal myocardial injury observed in certain clinical or experimental situations. The initial release of cardiac troponin in the cell cytosol followed by slower dispersion of cardiac myofilaments in degradation are the two mechanisms responsible for this long duration kinetic profile.

Among the models that enable research on infarction, AMI induced by isoproterenol hydrochloride in rats causes changes in hematological, biochemical, oxidative stress markers and histopathological parameters. In addition to these indications, literature data shows that isoproterenol hydrochloride causes a significant increase in serum concentrations of myocardial injury markers such as TGO, TGP, CPK, CKMB, LDH and troponin. This study aimed to assess the sensitivity of the cardiac marker modified albumin in Wistar rats after induction of experimental myocardial infarction by isoproterenol hydrochloride and analyze its histological changes.

Methods

Experimental study developed at the Animal Experimentation Laboratory of Universidade Regional Integrada do Alto Uruguai e das Missões (URI) - Erechim, RS, Brazil, from 2011 to 2013. For the study, Wistar-Tecpar rats aged 60 days and weighing 300-450 grams were used. The rats were provided by the Animal House of the University.

The study was approved by the Committee on Animal Use of URI/Erechim under protocol number 030/PIA/11. The procedures related to this study have strictly followed the rules established by the Guide for the Care and Use of Laboratory Animals and the Ethical Principles on Animal Experimentation of Colégio Brasileiro de Experimentação Animal (COBEA).

The animals were kept in cages, each of which with four animals under room temperature (22±4°C) with photoperiod of 12 hours/light and 12 hours/dark, fed with standard rodent chow and water ad libitum. The animals were randomly divided into three groups with 8 animals in each group: C (control); ISO 30’ (application of isoproterenol hydrochloride in 30 minutes); and ISO 6h (application of isoproterenol hydrochloride in 6 hours).

The induction of acute myocardial infarction was performed in the ISO 30’ and ISO 6h groups by subcutaneous administration of hydrochloride isoproterenol at a dose of 40 mg/kg/body weight diluted in 2 ml of saline solution based on the study of Lobo Filho et al. The control group received only the vehicle (saline solution) subcutaneously in 2 ml volume. Once the time required for the application of isoproterenol hydrochloride has passed, that is, 30 minutes and 6 hours, respectively, the animals were anesthetized with 0.20 mL/kg/IM Zoletil® 50 to collect blood through abdominal aorta puncture for analysis of IMA. Cardiac apex was collected for histological analysis.

The IMA reference values were those of the control group. Troponin I was used as standard marker from the serum samples collected as previously described.

The apical regions of the hearts were fixed with 5% buffered formalin for 24 hours. To perform the histological procedure, the apexes were used after remaining in 70% alcohol for 1 hour; in 80% alcohol for 1 hour; in 90% alcohol for 1 hour; in 100% alcohol for 12 hours; in Xylol alcohol for 30 minutes; in xylene for 30 minutes; in xylene II for 30 minutes; in Xylol paraffin for 30 minutes; in paraffin I for 1 hour; in paraffin II for 1 hour; and were later embedded in paraffin. Four-micrometer cuts were stained with hematoxylin-eosin (HE) and then photographed. The histological images were taken using the software Image Pro Plus 6.0 (Media Cybernetics, USA).
In the histological analysis, polymorphonuclear neutrophils were counted in a particular random area of interest.

The modified albumin data were statistically analyzed by ANOVA followed by Tukey test in the software GraphPad Prism 5 DEMO, with reference to the values found in the control group, that is, number 0. The histological data were analyzed by one-way ANOVA and post-hoc Bonferroni test using the software PASW Statistics 18 (IBM, USA). The data were considered significant when \( p < 0.05 \).

### Results

IMA contents in the ISO 30’ group, that is, after 30 minutes of ischemic induction by isoproterenol hydrochloride, presented a statistically significant decrease in its content (625.0±49.15 u/L; \( p < 0.01 \)) compared to the control group (747.125±29.97 u/L). The IMA content in the ISO 6h group presented no statistically significant difference compared to the control group, averaging 726.16±48.60 u/L (Figure 1).

The values of troponin I (gold standard biomarker) presented by the ISO 6h group were statistically significant (\( p < 0.01 \)), averaging 4.22±1.35 ug/L compared to the control group of 0.01 ug/L. The values for troponin I presented by the ISO 30’ group presented no statistically significant difference (\( p < 0.01 \)) compared to the control group: 0.79±0.15 ug/L (Figure 2).

To incorporate and destroy different types of pathogens, that is, to protect from invaders, our body presents as a first line of defense the polymorphonuclear leukocytes (PMNLs), especially neutrophils. These cells, due to their highly specialized functions, are known as professional phagocytes\(^{15}\). The occurrence of polymorphonuclear leukocytes (Figure 3) was then analyzed.
Discussion

Epidemiological data related to AMI indicate that about 45-60% of deaths occur in the first hours from the disease onset and approximately 80% in the first 24 hours. These figures raise concerns and the need for an accurate diagnosis and rapid action in order to prevent and minimize the cardiac injury time\(^1\).

The prognosis of patients with AMI will depend on various factors, including the time the patient takes to reach the medical service, the time to obtain diagnosis and the magnitude of cardiac muscle involvement\(^16\). To confirm the diagnosis of AMI and stratify the risks, it is essential to carry out certain tests, such as ECG and determination of serum markers of myocardial injury\(^3\).

In patients presenting symptoms of AMI, biochemical markers are useful to confirm diagnosis. In addition, they provide important prognostic information, since there is a direct association between high serum markers and the risk of cardiac events in the short and medium term. The results of necrosis markers should be available within 60 minutes from the collection. If the central laboratory does not achieve this goal, point of care technologies should be considered\(^7\).

The main limitation of conventional troponins is their low sensitivity when the patient has an onset time shorter than 6 hours. Considering this factor, high-sensitivity troponins (Trop-US) are being introduced, making it possible to detect lower levels of troponin in less time after the onset of ischemia. Trop-US has a detection power 10-100 times higher than conventional troponins. However, the impact of Trop-US in AMI detection is unclear and there is controversy about the cutoff value in the risk stratification\(^7\).

After acute myocardial infarction, there are changes in ventricular architecture involving both the infarcted and the non-infarcted areas. These morphological changes are consequences of genetic, cellular and molecular cardiac abnormalities clinically detected by changes in ventricular composition, mass, volume and geometry\(^17\). This data can be confirmed in this study, since the number of polymorphonuclear cells had increased gradually over time after the induction of myocardial infarction by isoproterenol hydrochloride, that is, the longer the time of ischemia, the greater the number of polymorphonuclear cells and cell destruction.

Previous studies involving patients in hospital care with chest pain in less than three hours observed 82% sensitivity of IMA, 45% for electrocardiogram and 20% for troponin, showing that IMA has greater sensitivity in less time of ischemia than the troponins, which are now used as the gold standard in the diagnosis of AMI\(^18\), corroborating the findings of this study.

Additional studies are suggested, since this one is limited due to the absence of comparison to other biochemical markers and methods to quantify the damaged heart area.

Conclusion

In this study, IMA showed high sensitivity as a cardiac marker at the onset of ischemia.

Potential Conflicts of Interest
This study has no relevant conflicts of interest.

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Academic Association
This study is not associated with any graduate programs.
References