

Sanguineous normothermic, intermittent cardioplegia, effects on hypertrophic myocardium. Morphometric, metabolic and ultrastructural studies in rabbits hearts

Efeitos da cardioplegia sanguínea normotérmica intermitente, em miocárdio hipertrófico. Estudos morfométricos, metabólicos e ultraestruturais em corações coelhos

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Abstract

Objectives: The present investigation aimed to study the protective effect of intermittent normothermic cardioplegia in rabbit's hypertrophic hearts.

Methods: The parameters chosen were 1) the ratio heart weight / body weight, 2) the myocardial glycogen levels, 3) ultrastructural changes of light and electron microscopy, and 4) mitochondrial respiration.

Results: 1) The experimental model, coarctation of the aorta induced left ventricular hypertrophy; 2) the temporal evolution of the glycogen levels in hypertrophic myocardium demonstrates that there is a significant decrease; 3) It was observed a time-dependent trend of higher oxygen consumption values in the hypertrophic group; 4) there was a significant time-dependent decrease in the respiratory coefficient rate in the hypertrophic group; 5) the stoichiometries values of the ADP: O₂ revealed the downward trend of the values of the

hypertrophic group; 6) It was possible to observe damaged mitochondria from hypertrophic myocardium emphasizing the large heterogeneity of data.

Conclusion: The acquisition of biochemical data, especially the increase in speed of glycogen breakdown, when anatomical changes are not detected, represents an important result even when considering all the difficulties inherent in the process of translating experimental results into clinical practice. With regard to the adopted methods, it is clear that morphometric methods are less specific. Otherwise, the biochemical data allow detecting alterations of glycogen concentrations and mitochondria respiration before the morphometric alterations should be detected

Descriptors: Heart arrest, induced. Hypertrophy, left ventricular. Cardiovascular surgical procedures.

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Abbreviations, Acronyms & Symbols

ADP	Adenosine diphosphate
LVW/BW	Left ventricular and body weight ratio
RCR	Respiratory control ratio

Resumo

Objetivos: O presente estudo teve como objetivo estudar o efeito protetor da cardioplegia normotérmica intermitente em corações hipertróficos de coelhos.

Métodos: Os parâmetros escolhidos foram: 1) relação peso cardíaco/peso corporal; 2) níveis de glicogênio nos músculos cardíacos; 3) alterações ultraestruturais por microscopia óptica e eletrônica; e 4) respiração mitocondrial.

Resultados: 1) O modelo experimental de coarctação da aorta induziu hipertrofia ventricular esquerda; 2) a evolução temporal dos níveis de glicogênio no miocárdio hipertrófico demonstra que há diminuição significativa; 3) observou-se tendência dependente do tempo para

maiores valores do consumo de oxigênio para o grupo hipertrófico; 4) houve diminuição dependente do tempo da taxa de coeficiente respiratório no grupo hipertrófico; 5) os valores estequiométricos da ADP: O₂ revelou a tendência decrescente no grupo hipertrófico; 6) observaram-se lesões mitocondriais do miocárdio hipertrófico, enfatizando a grande heterogeneidade dos dados.

Conclusão: A aquisição de dados bioquímicos, principalmente o aumento na velocidade de quebra do glicogênio, quando mudanças anatômicas não são detectadas, representa um resultado importante, mesmo quando se consideram todas as dificuldades inerentes ao processo translacional de resultados experimentais para a prática clínica. No que diz respeito aos métodos adotados, é evidente que os métodos morfométricos são menos específicos. Os dados bioquímicos permitem a detecção de alterações das concentrações de glicogênio e respiração mitocondrial antes das alterações morfométricas serem detectadas.

Descritores: Parada cardíaca induzida. Hipertrofia ventricular esquerda. Procedimentos cirúrgicos cardiovasculares.

INTRODUCTION

The current success of cardiac surgery was provided, among other relevant factors, by better understanding the myocardium protection. The timeline of this understanding includes: 1) systemic hypothermia [1]; 2) intra-operative cardiac arrest induced by potassium citrate [2]; 3) anoxic arrest of the heart [3]; 4) the use of chemicals that could rapidly determine cardiac arrest [4]; 5) Miscellaneous studies relating to the composition, temperature and mode of drug administration named cardioplegic solution [5-7], and; 6) normothermic cardioplegia [8,9].

In cardiac hypertrophy, whether it is induced in an experimental model or observed clinically, energy metabolism is compromised highlighting that Sink et al. [10] demonstrated that the hypertrophic heart must be stopped immediately emphasizing the need of additives to the cardioplegia solution. It is noteworthy that Cooley et al., in 1972, described the "Stone Heart", i.e., ischemic contracture, more frequent and severe in the hypertrophic heart [11]. This ischemia/reperfusion phenomenon allowed the resumption of the discussion, and final conclusion, about the pivotal role of myocardial protection as a means of avoiding this extremely serious intraoperative complication. Therefore, even after more than 60 years, continued studies of the hypertrophic myocardium energy metabolism under the action of cardioplegia are still necessary to improve surgical procedures in this pathological condition.

Most of experimental researches on cardioplegia were made in hearts of normal animals. Thus, specifically, the

present investigation aimed to study the protective effect of intermittent normothermic cardioplegia in rabbits hypertrophic hearts. The parameters chosen were 1) the ratio heart weight/body weight to evaluate the myocardium hypertrophy, 2) the heart muscle glycogen levels, 3) ultrastructural changes by light and electron microscopy, and 4) mitochondrial respiration. As a secondary objective, the study aimed to evaluate the adequacy of the adopted methods.

METHODS

Experimental design

New Zealand rabbits (n=76; 1.7 - 2.5 kg) were anesthetized using pentobarbital sodium (30 mg/kg intravenous). The animals underwent tracheostomy, and they were ventilated using an endotracheal tube (3.0 mm, Rusch, Teleflex Medical, Durham, NC, USA) with 100% O₂ in a pressure-controlled mode (Takaoka 600, K. Takaoka Indústria e Comércio Ltda, São Bernardo do Campo, SP, Brazil). The ear marginal vein was cannulated for volemic reposition with saline solution (NaCl 0.9%, 10 ml/kg/h). The Institutional Animal Care and Use Committee of the Ribeirão Preto Faculty of Medicine, University of São Paulo, Brazil reviewed and approved the procedures for animal handling, which were in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

The study adopted two lines of investigation (biochemical and morphological) for which the animals were randomly divided into two main groups: Group I/

normal (n=36) and Group II/hypertrophy (n=40). The number of rabbits was based on literature data, mainly involving mitochondria respiration. Also, rabbits were elected as experimental animal based on those studies. The samples were collected immediately, 60 and 90 minutes after cardioplegia infusion.

Cardiac hypertrophy was induced by coarctation of the abdominal aorta just distal to the diaphragm, according the technique of Leclercq et al. [12], using a midline laparotomy as access. The aorta was dissected and looped with cotton thread 00 (two zeros), the stenosis of the artery was calibrated with a 2 mm needle (50% stenosis), which was removed after tying the artery. The wall plans were sutured, and the waiting time for the induction of cardiac hypertrophy was 14 days.

On the morning of the fourteenth postoperative day, under anesthesia, all animals were submitted to median sternotomy, identification and fast excision of the heart after sectioning of its vessels (superior and inferior vena cava, aorta artery and pulmonary artery and vein). The whole heart was immediately immersed in a beaker containing 0.9% saline solution and, ice cooler stored to keep the temperature between 0 and 30°C. The hearts were subjected to cardiac arrest by normothermic blood cardioplegia solution (37°C), composed of 40% blood and 60% solution of sodium chloride 0.9% to 50 mEq/L of potassium chloride to induce cardiac arrest and 30 mEq/L for maintenance doses. After cardioplegic arrest, the hearts were subjected to a period of myocardial ischemia of 60 and 90 minutes, kept in a water bath at 37°C, using multidose intermittent antegrade blood cardioplegia every 20 minutes, the proportion of 5 ml/kg body weight. The group with zero time of ischemia was used as the control. The atria were extracted, and the ventricles were cut into small fragments of about 2 mm. During this procedure, repeated washings were done with 0.9% saline solution at a temperature of 0 to 30°C. For each time was carried out biochemical determination of the following parameters: analysis of mitochondrial oxygen consumption and measurement of the glycogen concentration.

Mitochondrial function

The mitochondrial fraction was isolated by the method of Bullock et al. [13] Mitochondrial function was determined by a polarographic method [14], using an OXY 5 polarograph-oxygraph (Gilson Medical Electronics, Inc., W. Beltline Middleton, WI, USA). The respiration medium contained 0.23 M sucrose, 8 mM potassium phosphate, 9.5 mM Tris, pH 7.0, 0.14 mg/ml albumin, and 1 mM EDTA. The mitochondrial fraction was assayed at 1.2 mg/ml protein concentration in the oxygraph chamber. Substrates were added as a mixture of malate, pyruvate, keto-glutarate, and b-hydroxy-butyrate, each at 48 mM

final concentration. State III (activated respiration) was obtained after the addition of 400 nmoles of adenosine diphosphate (ADP). State IV (basal respiration) was measured when all ADP had been converted to ATP, a condition indicated by the return to basal respiratory levels. The ratio of respiration rate after the addition of ADP (state III) to respiration rate during the basal state (state IV) corresponded to the respiratory control ratio (RCR). The parameters of oxidative phosphorylation were calculated according to Chance and Williams [15] and Estabrook and Pullman [16] and were expressed in nanoatoms of oxygen used per mg protein per minute. Mitochondrial protein content was determined by the biuret method [17].

Measurements of myocardial glycogen

Glycogen was extracted with 30% KOH from 500 mg of the left ventricle myocardium. After centrifugation at 800g for 10 min, 1 mL of supernatant solution was transferred to a tube incubated on ice and mixed with 2 mL fluid with anthrone. The mixture was boiled for 10 min and then cooled immediately on ice, followed by incubation at room temperature for 10 min. The absorbance was read at 620 nm by a spectrophotometer and, the values were expressed in % of humid weigh [18].

Morphologic study

A cross-sectional slice of the left ventricle chamber was obtained after the cardioplegic arrest was immersed and fixed in Bouin solution for 24 hours, dehydrated in ethanol and included in Paraplast. Cuts (5 µm) were stained with hematoxylin-eosin, Mallory's trichrome and PicroSirius Red with the aid of a digitizing tablet (MINI-MOP - Kontron Electronics). Measurements were taken directly on the blades and the values are expressed in millimeters (final images 800 Xs).

For transmission electron microscopy, a group of blocks was selected and processed for ultrathin sections. The areas of cytoplasm, where mitochondria predominated, were photographed under a microscope Philips EM 208 (Transmission Electron Microscopy) at original magnification of 20.000X. The negatives were double enlarged and copied yielding the final images at 40.000X. These amplifications were analyzed for the determination of mitochondrial diameters (maximum and minimum) through the use of the tablet (MINI-MOP - Kontron Electronics), and the results were expressed in micrometers as the correction was made for the magnification factor.

Statistical analysis

For statistical analysis of the body weight and to assess the ventricular induction of myocardial hypertrophy was used the t-test to compare values with similar and different variances for significance of 5%.

For the biochemical parameters (glycogen and mitochondrial respiration), statistical comparisons were carried out between control and hypertrophic myocardium groups, and among times of ischemia. Initially, tests were normal (Shapiro and Wilk) not finding suitable conditions for the application of parametric statistics. It was decided then to apply the Kruskal-Wallis test on each group separately (normal versus hypertrophic) in order to study the effect of ischemia time on biochemical variables, adopting the significance level of 5%.

The Mann-Whitney test was carried out in each subgroup of time (0, 60 and 90). For multiple comparisons among the subgroups of time (0, 60 and 90), for each biochemical variable mentioned above, it was used the method of Dunn.

RESULTS

Induction of experimental cardiac hypertrophy

In Figure 1A, are represented the values of body weight of all animals studied during cardioplegic arrest times of 0, 60 and 90 min. It is observed a similarity between Groups I and II (significance of 5%).

In Figure 1B are represented the values of ventricular myocardium weight with the statistical t-test showing a significant increase (significance of 5%) in the hypertrophied heart at all ischemic times.

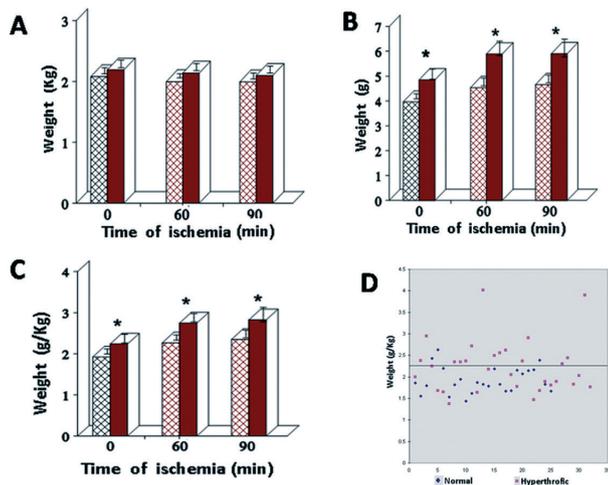


Fig. 1 - A - body weight (kg), B - left ventricle weight (g), C - left ventricle weight LVW/body weight (BW) ratio (g/kg); D - values of the ratio LVW/BW (g/kg) characterizing the data dispersion. Normal myocardium (column textured) and hypertrophic (full column) underwent infusion of cardioplegia at ischemia times of 0 min, 60 min and 90 min. The columns represent the mean values and the bars above the columns the standard error of the mean. In the figure D, values in the x-axis correspond to increasing numbers of samples and the line which bisects the graph corresponds to the value LVW/BW of 2.3 g/kg. The asterisk means significant difference between groups, with $P < 0.05$

Figure 1C shows the values of the left ventricular and body weight ratio (LVW/BW in g/kg) with the statistical t-test showing a significant increase (significance of 5%) in the hypertrophied heart at all ischemic times.

Figure 1D demonstrates the dispersion of the ratio values of ventricular weight/body weight in normal and hypertrophic groups, but corresponding to 0 min ischemia. This analysis aims to characterize the eventual presence of cardiac hypertrophy in the control group, when it is supposed that the intracellular edema factor was absent, making sure that the values reflected the myocardium hypertrophy in this study group.

Glycogen and mitochondrial function

Figure 2 represents the glycogen levels found in normal and hypertrophic myocardium of rabbits when subjected to infusion of normothermic blood cardioplegia. Statistical analysis was performed between samples; the Mann-Whitney analysis of normal and hypertrophic group revealed no significant differences (significance of 5%). However, the Kruskal-Wallis test that analyzes the temporal evolution of the glycogen levels in hypertrophic myocardium demonstrates that there is a significant decrease (significance of 5%) at 90 minutes compared to time 0 (zero), whereas normal myocardium did not differ in any time (significance of 5%).

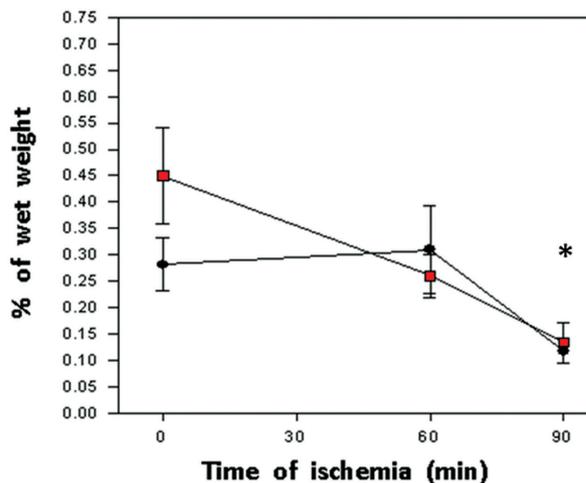


Fig. 2 - Shows the levels of glycogen in normal hearts (black circles) and hypertrophic hearts (red squares) of rabbits submitted to cardioplegic arrest for 60 and 90 minutes and its respective control group. Normothermic blood cardioplegia was used, and the glycogen concentration values are expressed in % of wet weight. The symbols represent the average values with the corresponding standard deviation. Group I, normal heart (control/n = 12; time 60 min/n = 13 and time 90 min/n = 11); Group II, hypertrophic heart (control/n = 19; time 60 min/n = 12, and; time 90 min/n = 12). The asterisk refers to a significant difference ($P < 0.05$) between time 0 and 90 min of the hypertrophic group

Figure 3A represents the values of the state III mitochondrial oxygen consumption, demonstrating the effect of ischemia time on the group of normal and hypertrophic myocardium. The analysis between groups by Mann-Whitney, at 90 min, showed a trend of higher values for the hypertrophic group. The Kruskal-Wallis test found no difference among samples obtained at different times (significance of 5%).

Figure 3B represents the values of the state IV mitochondrial oxygen consumption, at times 0, 60 and 90 min, found in normal and hypertrophic myocardium after infusion of normothermic cardioplegic solution. The Mann-Whitney test shows a significant increase in values at 90 min (significance of 5%) of the hypertrophic group when compared to normal. The Kruskal-Wallis test did not show differences among the results (significance of 5%).

The values of the respiratory control ratio (RCR) observed

in Figure 3C, were measured in normal and hypertrophic myocardium after infusion of cardioplegic solution at 0, 60 and 90 min. The Mann-Whitney test found no significant differences between groups (significance of 5%). However, the analysis between the different times observed by the Kruskal-Wallis test, showed a significant decrease in the RCR (significance of 5%), in the hypertrophic group, at 90 min of ischemia when compared with time zero.

Figure 3D represents the stoichiometric values of the ADP:O₂ found in normal and hypertrophic myocardium after normothermic cardioplegic arrest. The Mann-Whitney test revealed a downward trend of the values of the hypertrophic group when compared with normal myocardium. For the hypertrophic group, the Kruskal-Wallis test showed differences between them with a significant decrease ($P < 0.05$) values for ADP:O₂.

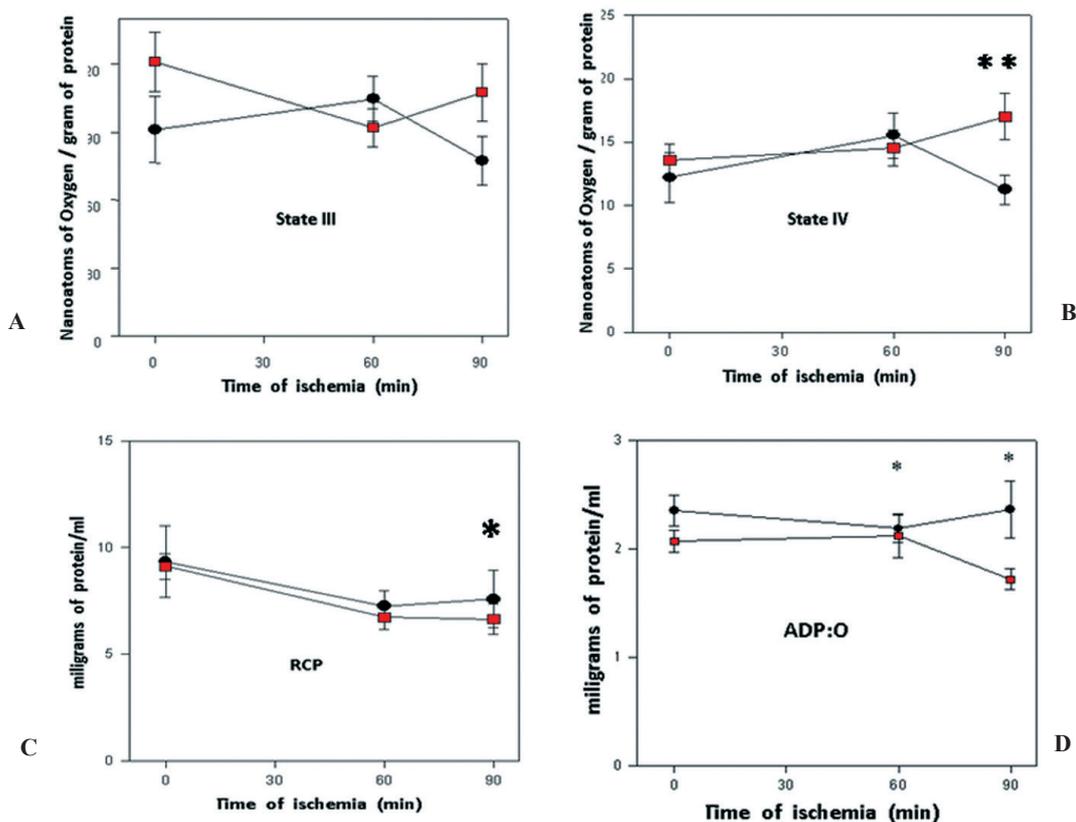


Fig. 3 - Analysis of mitochondrial respiration: A - State III, B - State IV, C - Respiratory Control Ratio (RCR), and D - mitochondrial oxygen consumption (ADP:O₂). Comparison between normal hearts (black circles) and hypertrophic hearts (red squares) of rabbits submitted to the infusion of normothermic blood cardioplegic solution with cardiac arrest of 60 and 90 minutes and the control group. The mitochondria (1 mg protein/ml) were tested at 30°C using Alpha-ketoglutarate as respiratory substrates. Breathing was activated with 400 nanomoles of ADP. Values are expressed in oxygen nanoatoms O₂/mg protein.min. The symbols represent the average values with the corresponding standard deviation. Group normal heart (control n = 12, n = 60 min n = 13, 90 min n = 12); Group hypertrophic heart (control/n = 16; 60 min/n = 12; 90 min/n = 12). A - No significant differences; B - The asterisks (**) refer to the significant increase ($P < 0.05$) in the hypertrophic group compared with the normal at 90 min, C - The asterisk refers to a significant difference ($P < 0.05$) between the time 0 and 90 min of the hypertrophic group, D - the asterisk refers to a significant difference ($P < 0.05$) between 60 and 90 min and 0 and 90 min of the hypertrophic group

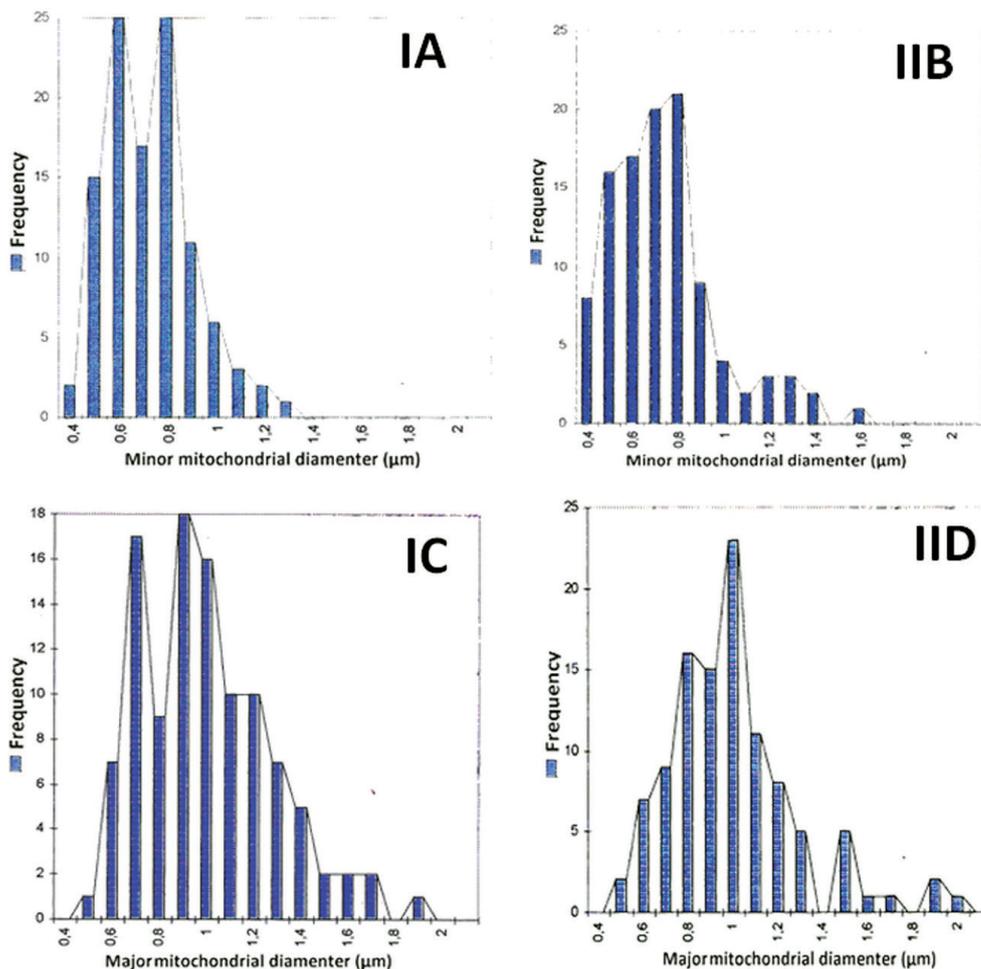


Fig. 4 - The graphs represent the minor (Group IA and Group IIB) and major (Group IC and IID) mitochondrial diameters analyzed by electron microscopy, increased 40.000x. One can observe a large heterogeneity of data, why were divided by its frequency. There was a trend to higher mitochondrial diameter in Group II, and the data of minor diameters are most similar in its distribution. However, in Group II and its respective subgroup of 60 min of ischemia, there was a marked increase in both mitochondrial diameters, demonstrating intracellular edema with cardioplegic arrest

The graphs of Figure 4 represent the minor (A and B) and major (C and D) mitochondrial diameters analyzed by electron microscopy, increased 40.000x. One can observe a large heterogeneity of data, when divided by its frequency. Comparing data from Groups I and II and their respective subgroups of cardioplegic arrest in time zero, there was a higher trend in Group II of increasing the mitochondrial diameter. The data of minor mitochondrial diameters are most similar in its distribution. However, in Group II and its respective subgroup of 60 min of ischemia, there was a marked increase in both mitochondrial diameters, demonstrating intracellular edema with cardioplegic arrest.

Figure 5 presents light (A, B) and electron (B, C) micrographs of transverse sections of the myocardium

of rabbits submitted to cardioplegic cardiac arrest. A - micrograph (block No. 11647b) belonging to the subgroup 0 minutes in Group I, shows myocardial fibers involving the interstitium where it is observed blood vessels and collagen (800X); B - micrograph (block No. 11597g) belonging to 0 minutes subgroup in Group II reveals a significant increase in the transverse diameter of myocardial fibers occupying the space of interstitium (800X); C - micrograph (block No 11647h) belonging to the subgroup 0 minutes in group I reveal various aspects of normal mitochondria (40.000X), and; D - Micrograph (block No 11692f) belonging to the subgroup 0 minutes in Group II shows mitochondria with increased diameter and loss of their morphological characteristics (40.000X).

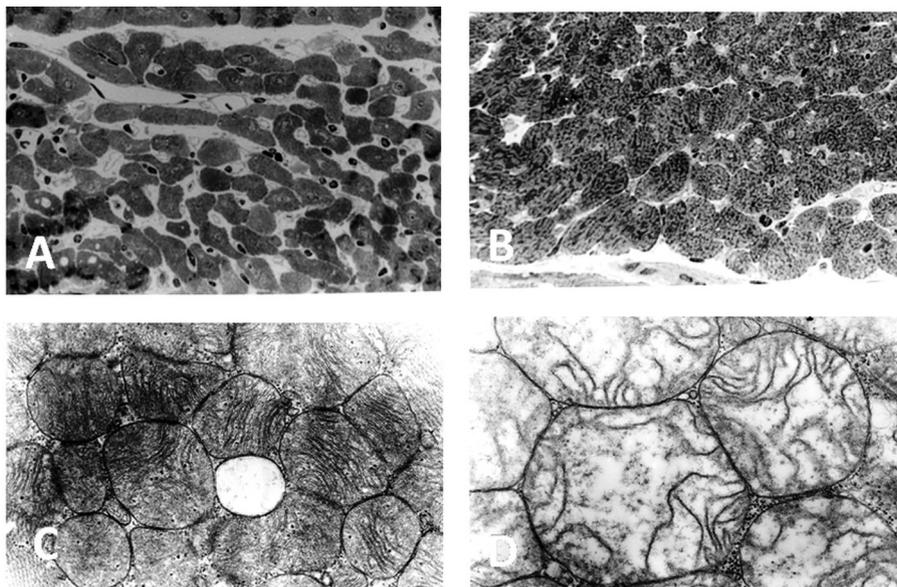


Fig. 5 - Light (A, B) and electron (C, D) micrographs of transverse sections of the myocardium of rabbits submitted to cardioplegic cardiac arrest. A - micrograph of a specimen belonging to the subgroup 0 minutes in Group I, shows myocardial fibers sectioned transversely, and in the involved interstitium is observed blood vessels and collagen (800X); B - micrograph of a specimen belonging to 0 minutes subgroup in Group II reveals a significant increase in the transverse diameter of myocardial fibers occupying the space of interstitium (800X), C - micrograph belonging to the subgroup 0 minutes in group I reveals various aspects of normal mitochondria (40.000X), D - Micrograph belonging to the subgroup 0 minutes in Group II shows mitochondria with increased diameter and loss of their morphological characteristics (40.000X)

DISCUSSION

Myocardial hypertrophy is considered the most efficient event, among the compensatory mechanisms of heart diseases, when the muscle is exposed to overload depending on extramyocardial disease. Several mechanical and neurohormonal factors act as myocardial growth factors and change the pattern of protein synthesis, resulting in a ventricular remodeling. The several mechanisms in response to the decrease of cardiac performance, initially adaptive, became developmentally pernicious [19].

The basic data of the present investigation were: 1) the experimental model, coarctation of the aorta induced left ventricular hypertrophy; 2) the temporal evolution of the glycogen levels in hypertrophic myocardium demonstrates that there is a significant decrease; 3) It was observed a time-dependent trend of higher oxygen consumption values for the hypertrophic group; 3) there was a significant time-dependent decrease in the respiratory coefficient rate in the hypertrophic group; 4) the stoichiometric values of the ADP: O₂ revealed the downward trend of the values of the hypertrophic group; 5) It was possible to observe damaged mitochondria from hypertrophic myocardium emphasizing the large heterogeneity of data.

Experimental model

Concerning the experimental model, coarctation of

the aorta induced left ventricular hypertrophy. Indeed, there has been a bandage of the aorta in order to cause a pressure overload in the proximal portion. The sustained high pressure trigger a complex process whose ultimate expression was the increased thickness of the myocardium without enlargement of the ventricular cavity as a consequence of adaptive morphological response [20]. The data, confirming the appropriateness of the methodology are shown in Figure 1A markedly and significant weight of the left ventricle of rabbits in the experimental hypertrophy protocol can be observed. To rule out the possible influence of the number of experiments the dispersion values of the ratio ventricular weight/body weight in normal and hypertrophic groups, were considered at 0 min ischemia. This analysis aims to characterize the presence of cardiac hypertrophy in the control group, when the intracellular edema factor is absent and thus ensure greater safety margin for the values of biochemical analysis related to the cellular metabolism of the hypertrophic myocardium. In the present study, we found in the control group average of 2.2 for the ratio LVW/BW, which is quite similar to the threshold value of 2.3 for LVW/BW, based on studies of Hatt et al. [21] in rabbit's hypertrophic heart induced by aortic regurgitation. It is logical to assume that increased values of LVW/BW are associated to earlier left ventricle failure. In the same study, the average LVW/BW for animals with questionable myocardial failure was 3.0,

and 3.9 for obvious failure. In this initial understanding of myocardial hypertrophy, it is reasonable to distinguish two types of cardiac hypertrophy: a physiological normal or increased contractility, and, a pathologically reduced contractile function [22].

Glycogen metabolism

Considering hypertrophy as a disease process, not just a physiological response, and its risk factor role for clinical complications commonly present in cardiac surgery, the biochemical approach of myocardial protection is mandatory. Thus, the present study in rabbits used intermittent antegrade blood cardioplegia which, admittedly, provides protection to the myocardium reduces the energy expenditure and decreases the rate of glycogenolysis. The normothermia experimental option experiment was adopted, based on studies performed in our laboratory showing a larger decrease glycogen levels in normothermic condition, compared with hypothermia [23]. In the present study, there was no difference in glycogen concentration (time zero x 90 minutes) within the control group samples. Surely, this observation is due to the influence of the variability of sample values in an experimental situation in which there was an increase of glycogen breakdown. However, the decrease in the concentrations of the samples occurred at lower speed when compared to the drop rate observed in hypertrophic hearts. The results of the muscle glycogen content showing high variability are already referred in the scientific literature [24]. The statistical analysis did not establish a difference in the levels of myocardium glycogen after ninety minutes of ischemia. The variability factor, as already mentioned in the preceding paragraph, must have contributed to the lack of difference. One data to be highlighted in this research is that it was possible to observe significant differences among the values of muscle glycogen in hypertrophic hearts, considering the values obtained after ninety minutes of cardioplegia infusions. As simplest interpretation of these results, one should consider that glycogenolysis is established more rapidly in the muscle subjected to conditions which favor hypertrophy. In order to complete the conceptualization of the study, it is necessary to emphasize that metabolic adaptation to anaerobic condition is made by the degree of the glycogenolytic pathway activation. This may explain the differences among positive results observed in normal hearts, and negative results observed in hearts subjected to conditions that promote hypertrophy.

Mitochondrial respiration

The first detail to be discussed concerning the preparation of mitochondria is the control samples that presented RCR close to 10, which is a valuable indicator

of the quality. The values of the state III mitochondrial oxygen consumption, which denotes the effect of ischemia time, showed at 90 min, a trend of higher values for the hypertrophic group, but, analyzing the temporal evolution it was not observed differences among the results. The values of the state IV showed a significant increase of the hypertrophic group when compared to normal, at 90 minutes, without differences in the temporal evolution. The RCR did not show significant differences between normal and hypertrophic group. However, the analysis of the evolution time line showed significant RCR decrease in the hypertrophic group, at 90 min of ischemia, when compared with time zero. The stoichiometric values of the ADP: O₂ found in the normal and hypertrophic myocardium after normothermic cardioplegic arrest revealed the downward trend of the values of the hypertrophic group with the time line statistical analysis revealing decreased values. Observing the respiratory values, all together, the only possible conclusion is that the hypertrophic myocardium is more susceptible to changes elicited by prolonged ischemia.

Morphometric analysis

Electron microscopy revealed many aspects of normal myocardial fibers, while the hypertrophic hearts showed significantly increased transverse diameters occupying the interstitial space. The same occurred in relation to the diameters of the mitochondria showed an increase in their diameters and loss of their morphological characteristics (Figures 4 and 5). However, one can observe the large heterogeneity of data, when were divided by frequency.

Demonstrating intracellular edema with cardioplegic arrest, in a study of ultrastructural analysis of hypertrophic rabbits myocardium, Goldstein et al. [25] showed a range from 7 to 142 days for clinically manifestations be confirmed anatomically. Furthermore, these authors reported that the pleomorphism variations make it difficult, or almost impossible, to estimate the number or size of normal mitochondria. Perhaps this fact explains the difficulty in finding clearest results in relation to the values of mitochondrial diameter during the period studied. However, the trend of population shift to higher values is clearly shown in frequency histograms.

Concluding remarks

The acquisition of biochemical data, especially the increase in speed of glycogen breakdown, when anatomical changes are not detected, represents an important result even when considering all the difficulties inherent in the process of translating experimental results into clinical practice. Regarding the temperature, the route of cardiac arrest and delivery of cardioplegic solution is still at the surgeon discretion, including his practice and

expertise, always seeking for the best. At last, regarding hypertrophied heart protection, experimental studies have shown the superiority of tepid blood cardioplegia in relation to hypothermic crystalloid solution. Cardioplegic strategies to protect the hypertrophic heart during cardiac surgery is, surely, the most controversial subject concerning cardioprotection and an eternal challenge since all tested cardioplegic techniques confer suboptimal myocardial protection. Therefore, we should always have in mind that the state-of-the-art was not achieved yet [26-30].

Limitations of the study

The investigation showed that normothermic sanguineous, intermittent cardioplegia protects the hypertrophic myocardium against the deleterious effects of ischemia followed by reperfusion. But, no comparative studies with other techniques of myocardial protection. Therefore, any attempt to clinical correlation would be speculative.

As the induction of ventricular hypertrophy was performed by an aortic coarctation for 14 days, it is likely to consider the physiological type because this is an adaptation to a pressure overload. This doubt about the type of hypertrophy can be considered a possible limitation of the study. At least two well-conducted studies by Brazilian authors proved the adequacy of myocardial hypertrophy induced by the technique of aortic coarctation [31,32].

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