

Effects of myocardial protection on hypertrophic rabbit hearts: structural and ultra structural analysis

Efeitos das cardioplegias sangüínea e cristalóide no miocárdio hipertrófico de coelho: avaliação estrutural e ultra-estrutural

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Abstract

Objective: To experimentally compare the structural and ultrastructural changes in isolated hypertrophied rabbits' hearts submitted to cardiac arrest protected using blood and crystalloid cardioplegia solutions.

Method: The study comprised two experimental groups and one control group. In Experimental Group I, cardiac arrest was achieved by the continuous infusion of a tepid blood cardioplegia solution. In Experimental Group II, cardiac arrest was obtained by an intermittent infusion of a cold crystalloid cardioplegia solution. In the Control Group the hearts were submitted to normothermic anoxic arrest for 45 minutes. After the procedures, eight samples of the left ventricle lateral wall were collected and fixed in 10% formaldehyde and 2.5% glutaraldehyde for structural and ultrastructural analysis.

Results: The structural and ultrastructural results demonstrated that the hearts submitted to cardiac arrest protected by continuous tepid blood cardioplegia, Group I, were better preserved and with less accentuated cellular alterations compared to those submitted to cardiac arrest protected using intermittent cold crystalloid cardioplegia and the Control Group.

Conclusion: Continuous tepid blood cardioplegia was more efficient in the preservation of the structural and ultrastructural integrity of the myocardium when compared to intermittent cold crystalloid cardioplegia.

Descriptors: Cardioplegic solutions, pharmacology. Hypertrophy. Cardiac surgical procedures.

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Resumo

Objetivo: Comparar e avaliar experimentalmente as alterações estruturais e ultra-estruturais em corações hipertrofiados isolados de coelhos submetidos à parada protegida pela solução de cardioplegia sangüínea e cardioplegia cristalóide.

Método: O estudo compreendeu um grupo controle e dois grupos experimentais. No grupo I, a parada cardíaca foi obtida pela infusão da solução de cardioplegia sangüínea contínua e tépida. No grupo II, a parada cardíaca foi conseguida pela infusão da solução de cardioplegia cristalóide intermitente e fria. No grupo controle, os corações foram submetidos à parada anóxia normotérmica por 45 minutos. Após experimentos, oito amostras da parede lateral do ventrículo esquerdo foram coletadas e fixadas em formaldeído 10% e glutaraldeído 2,5%

para análises estrutural e ultra-estrutural.

Resultados: Os resultados estruturais e as descrições ultra-estruturais mostraram que os corações submetidos à parada protegida pela cardioplegia sangüínea contínua e tépida (grupo I) estavam mais preservados com alterações celulares menos acentuadas se comparados aos submetidos à parada protegida pela cardioplegia cristalóide intermitente e fria (grupo II) e ao grupo controle.

Conclusão: A cardioplegia sangüínea contínua e tépida (Grupo I) foi mais eficiente na preservação da integridade estrutural e ultra-estrutural do miocárdio, quando comparada à cardioplegia cristalóide intermitente e fria (Grupo II).

Descritores: Soluções cardioplégicas, farmacologia. Hipertrofia. Procedimentos cirúrgicos cardíacos.

INTRODUCTION

Hypertension is the greatest cause of left ventricle hypertrophy [1], affecting around one third of the hypertensive population [2], leading not only to myocardial ischemia, but also to congestive heart failure and indirectly to brain strokes [3]. It is an independent risk factor for cardiovascular events and mortality by any cause [1,2,4].

With myocardial infarction, the heart starts to require more energy to maintain its structures integral, even when arrested. Thus, efficacious myocardial protection is essential to maintain the morphology of the cells [5,6].

The utilization of cardioplegic solutions to protect the myocardium against ischemic damage during on-pump heart surgery started in 1955 with the solution developed by Melrose, however, this caused cellular necrosis due to the high concentration of potassium [7]. From this beginning, new techniques of myocardial protection appeared such as intermittent clamping [8], and crystalloid [9] and blood [9-14] cardioplegia at varying temperatures: hot, normothermic, cold or tepid. Its administration may be intermittent or continuous and either anterograde or retrograde. The richness of recent works in this area shows that the subject continues to evolve, as is the case of

membrane polarized cardioplegia with tetrodotoxin, esmolol and other formulas [15].

Thus, the current work aims at evaluating structural and ultrastructural alterations occurring after the use of blood and crystalloid cardioplegic solutions in hypertrophic myocardium of rabbits.

METHOD

Nine Norfolk-2000 rabbits variant Botucatu of both genders were utilized with weights ranging from 1850 to 2350 kg, all supplied by the animal house on the Campus of Botucatu – UNESP. The research was approved by the Research Ethics Committee of the Medical School in Botucatu – UNESP.

Conditioning of myocardial hypertrophy

After general anesthesia of the animal using sodium pentobarbital, a crosswise incision was performed in the cervical region, slightly above the sternum. The aortic artery was identified, pericardiotomy was performed and the ascending aorta was isolated and coarctation was provoked using the technique described by Martins et al. [16]. After 5 months with hypertrophic hearts (Figure 1), the animals were euthanized.

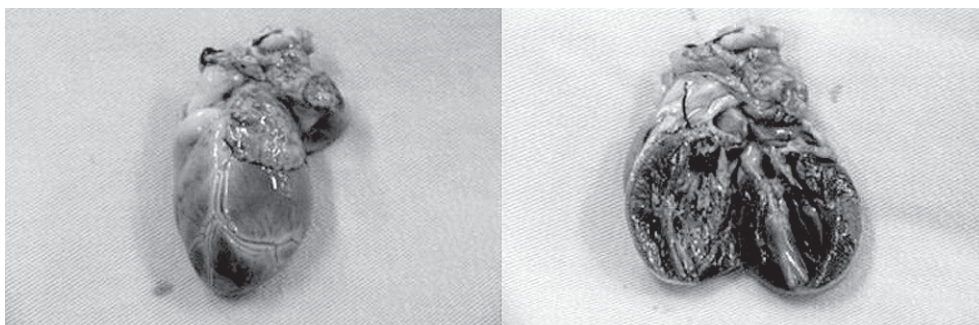


Fig. 1 - Photography of hypertrophic rabbit heart

Group division

The hypertrophic hearts were removed from the rabbits together with their lungs and immediately placed on individual systems composed of a support rabbit (one per heart) that supplied blood to the aortic root (Figure 2) for coronary perfusion over a period of 20 minutes to stabilize the heart. After this period, the animals were divided in three groups:

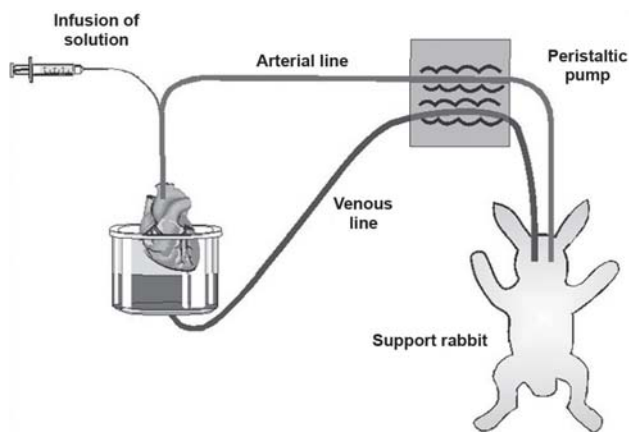


Fig. 2 - Diagram of heart support system

- Experimental Group I (Group 1): three hypertrophic hearts, submitted to myocardial protection, with continuous tepid blood cardioplegia for 45 minutes, starting with an induction “mother” solution (Table 1), for an average time of 1 minute (1 mL/min) for cardiac arrest, followed by a maintenance solution (Table 2) also at 1 mL/min for the remaining time;

Table 1. Components of the “Mother” solution for induction of blood cardioplegia

SALTS	CONCENTRATION
Potassium chloride	75 mEq
Magnesium chloride	40 mEq
Monosodium glutamate	30 mM
Monosodium aspartate	30 mM
Excipient q.s.p.	50 ml

Table 2. Components of “Mother” solution for maintenance-reperfusion of blood cardioplegia

SALTS	CONCENTRATION
Potassium chloride	25 mEq
Magnesium chloride	15 mEq
Monosodium glutamate	15 mM
Monosodium aspartate	15 mM
Excipient q.s.p.	50 ml

- Experimental Group II (Group II): three hypertrophic hearts, submitted to myocardial protection with intermittent cold crystalloid cardioplegia solution (Table 3). The time was standardized at 0, 20 and 40 minutes for each heart with infusion of this solution for 2 minutes. Cardiac arrest was achieved at a mean time of 1 minute after starting (Time 0);

Table 3. Components of "ST. THOMAS" crystalloid solution for cardioplegia

SALTS	CONCENTRATION
Sodium	6,0 mEq
Potassium	20,0 mEq
Magnesium	32,0 mEq
Calcium	4,4 mEq
Procaine	2,0 mEq
Excipient q.s.p.	40 ml

- Control Group: three hypertrophic hearts, submitted to anoxic arrest without myocardial protection for 45 minutes. After cardiac arrest with and without protection, all hearts were perfused for 20 minutes in the system that was used for stabilization. Subsequently, the hearts were removed from the system and the left ventricle lateral wall was cut into fragments of about 4 x 4 mm, fixed in 10% formaldehyde for structural analysis and in 2.5% glutaraldehyde for ultrastructural analysis.

RESULTS

Structural evaluation

The results of the structural evaluation of heart fragments from Experimental Groups I and II and the Control Group are shown in Figures 3A-D, 3E-H and 3I-M, respectively. The hematoxylin-eosin staining technique demonstrated better preserved cellular structures in the

experimental groups compared to the Control Group. In Group I, preserved muscle fibers, whole nuclei and very clear nuclear corpuscles were observed as is illustrated in Figures 3A-D. In Group II, fibroblasts (Figure 3E-G), leukocytes (Figure 3H), muscle fibers with slight degeneration and well preserved nuclei were identified as seen in Figure 3E-H. In these groups, several degrees of preservation of heart tissue were also seen, with suspicion of inflammatory process and a slight area of myocardial degeneration (Figures 3A-D and 3E-H). However, preservation was greater in Group I (Figure 3A-D) than in Group II (Figures 3E-H), as demonstrated in Table 4.

In the Control Group, several degrees of heart muscle tissue degeneration with myofibrils presenting a loss of the striation pattern and an intense presence of fibroblasts, leukocytes and degenerated nuclei was seen, as illustrated in Figures 3I-M.

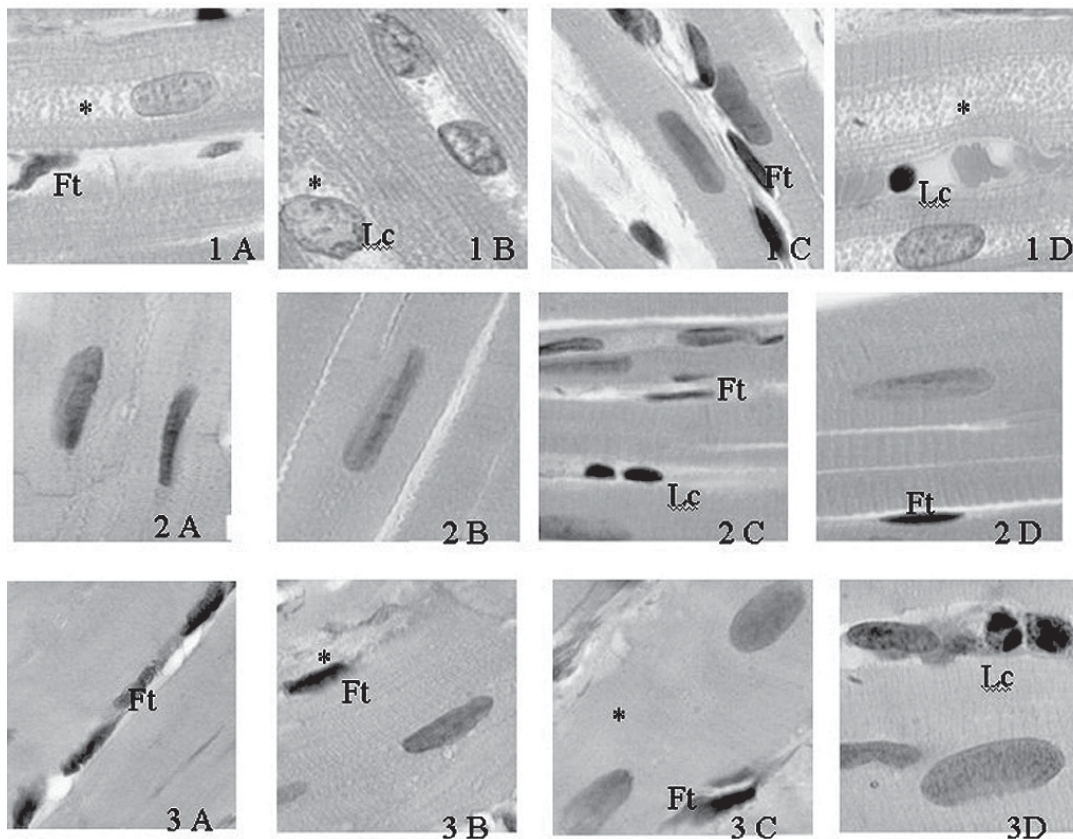


Fig. 3 – Histologic sections of left ventricle lateral wall of hypertrophic rabbit hearts submitted to the heart support system. The samples were stained with hematoxylin-eosin. Submitted to protected cardiac arrest using continuous tepid blood cardioplegia (Experimental Group I) (2A-D); and using intermittent cold crystalloid cardioplegia (Experimental Group II) (3A-D); and without myocardial protection (Control Group) (1A-D).

* = degenerated muscular fiber; Ft = fibroblast; Lc = leukocyte. A: 1080x

Table 4. Degree of anatomopathological alterations of isolated hypertrophic rabbit hearts submitted to cardiac arrest

Groups	PARAMETER		
	Degenerate Fibers	Leukocytes	Fibroblasts
Blood Cardioplegia (Experimental Group I)	-	-	+
Crystalloid Cardioplegia (Experimental Group II)	+	+	++
Control	+++	++	+++

- = minimum; + = mild; ++ = moderate; +++ = intense

Ultrastructural evaluation

The ultrastructural analysis of the Control Group demonstrated heart muscle fibers with signs of cytoplasmatic degeneration (Figure 4A), marginalization of the intercalary disk with cellular retraction (Figure 4B), chromatin compaction in the peripheral area and nuclear folding (Figure 4C). The blood vessels were dilated, with the wall extremely thin with few pinocytosis vesicles (Figure 4D).

Groups I and II presented with distinct ultrastructural alterations.

In Group II (Figure 5A-D), the muscle cells had ultrastructural characteristics similar to Group I, but lipid and glycogen accumulations and dilated T-tubules were not found. However, small areas of necrosis were identified (Figure 5A). The nuclei were well preserved with evident nucleoli (Figures 5A-D), as were the intercalary disks (Figure 5C). The blood vessels were dilated with thicker walls, rich in pinocytosis vesicles (Figure 5D).

In Group I (Figures 6A-F), the cells showed marked ultrastructural preservation with heart muscle fibers showing varying degrees of preservation, nuclei with decondensed chromatin (Figure 6A) and lipid accumulation in the myocyte cytoplasm (Figure 6B). A great concentration of glycogen was observed around the mitochondria, which presented very clear crests (Figure 6C). The intercalary disks were well preserved (Figure 6D), nuclei of heart muscle cells had a

normal format, there was an increase in the nucleolar area and the T-tubules were slightly dilated (Figure 6E). The blood vessel presented a preserved vascular endothelium with a great quantity of pinocytosis vesicles (Figure 6F).

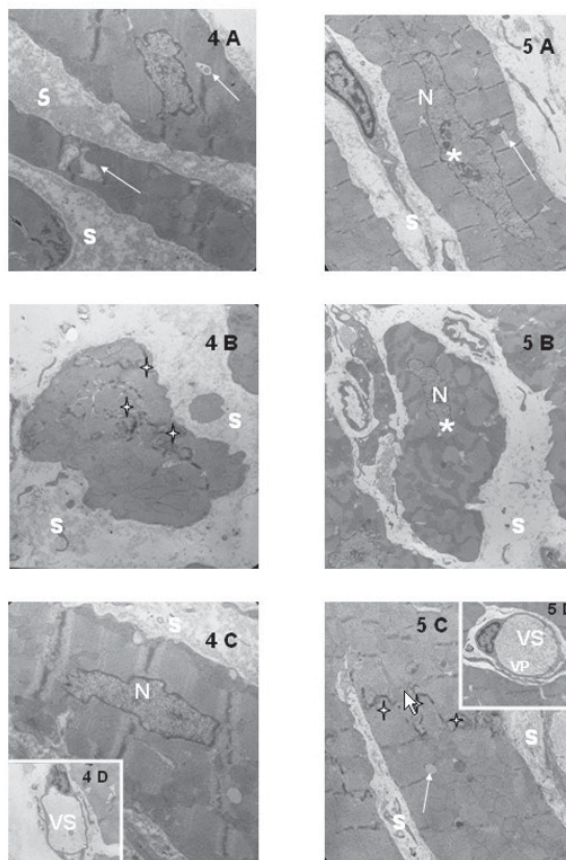


Fig. 4 - Electron micrographs of hypertrophic rabbit myocardium submitted to cardiac arrest by continuous tepid blood cardioplegia (Experimental Group I). Preserved intercalary discs (star), increase in nucleolar area (*), nuclei with decondensed chromatin (N), mitochondrias with evident crests (Mi), abundance of glycogenic (G), accumulation of lipid in the cytoplasm of cardiomyocytes (Li), slightly dilated T-tubules (T), apparent state of myocardial contraction (circle) and blood vessels (BV) with high quantity of pinocytosis vesicles (PB). A: 4A = 4646x; 4B = 10000x; 4C = 21560x; 4D = 46700x; 4E = 7750x; 4F = 12930x

Fig. 5 - Electron micrographs of hypertrophic rabbit myocardium submitted to cardiac arrest by intermittent cold crystalloid cardioplegia (Experimental Group II). Myocardium preserved, small areas of necrosis (arrow), less retraction of the cardiomyocytes, causing a smaller intracellular space (S), preserved intercalary discs (star), nuclei (N) with evident nucleolus (*), blood vessels (BV) with thick walls and large quantity of pinocytosis vesicles (PB). A: 5A = 6000x; 5B = 4646x; 5C = 7750x; 5D = 7750x

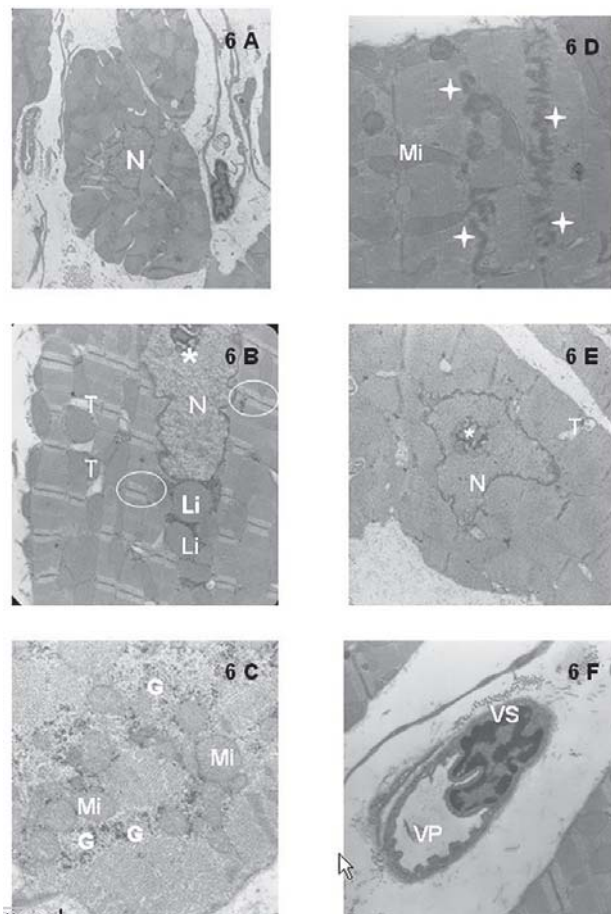


Fig. 6 – Electron micrographs of hypertrophic rabbit myocardium submitted to cardiac arrest without myocardial protection (Control Group). Cardiomyocytes showing signs of cytoplasmatic degeneration (arrow), marginalization of intercalary disc (star), peripheral compaction of chromatin, nuclear folding (N), cellular retraction causing an increase in the intercellular space (S) and slightly dilated blood vessels with extremely thin walls and few pinocytosis vesicles (PB). A: 6A = 7750x; 6B = 7750x, 6C = 10000x; 6D = 7750x

DISCUSSION

Hypertrophic rabbit hearts submitted to cardiac arrest protected by continuous, tepid blood cardioplegia (Group I) had their morphological, structural and ultrastructural characteristics better preserved than those submitted to cardiac arrest protected by intermittent cold crystalloid cardioplegia (Group II) and those in the Control Group.

Both the heart muscle cells and cells of adjacent tissues, fibroblasts and endothelial cells maintained morphological patterns close to normal. The absence of edema, the presence in electrodense mitochondria rich in decondensed crests,

nuclei with heterochromatin associated to the nuclear envelope, accumulation of lipids and glycogen demonstrated the protective effect. The fibroblasts and endothelial cells also presented cellular integrity. The low potassium concentration associated to magnesium in blood cardioplegia (Group I), permitted arrest of the heartbeats and a reduction in energy consumption during the induction period. Moreover, with continuous perfusion of oxygen supplied by the blood of the donor rabbit at 37°C, ischemia was avoided and the aortic clamping could be considered a period of heart reserve maintenance rather than a period of ischemic damage [13].

Myocardial protection using a solution of continuous tepid blood cardioplegia as described by Braile, reduces ischemic and functional damage, as seen both by the small increase in serum troponins and the small elevation of lactate and better functional preservation, as it provides good protection to the myocardium with maintenance of the basic metabolism continuously supplying the substrates that maintain all the “oxidative machine” functioning. The addition of glutamate and aspartate reduces energy deficits, leading to anatomical and functional recovery of the myocardium, which otherwise would be irreversibly damaged and suffer necrosis. The introduction of blood cardioplegia demonstrated that the myocardium even with cardiac arrest and under hypothermic conditions maintains cellular activity and consumes energy. Furthermore, its requirements are best supplied with oxygen from the administration of solutions, with the most appropriate being a blood perfusate to infuse the cardioplegic agents. The blood perfusate supplies oxygen to tissues, removes carbon dioxide, has natural buffering systems at ideal concentrations to control pH, satisfactory colloid-osmotic pressures, ideal concentrations of several important ions for cellular function, nutritive substrates and naturally removes harmful free radicals [17].

The lipids stored in cardiomyocytes are mainly triglycerides, that is, fatty acid esters and glycerol, common in cells and that depend on the degradation of fatty acids for their energy supply. The oxidative metabolism in mitochondrias is maintained not only by the pyruvate produced from the glycolysis of sugars in cytosol, but also from fatty acids. Pyruvate and fatty acids are selectively transported from the cytosol to the mitochondrial matrix, where they are broken down in acetylic groups producing acetyl-coenzyme A. This, in turn, is broken down in the citric acid cycle, producing high-energy electrons that enter into the respiratory chain, depending on the presence of oxygen [18]. An analysis of just glycogen demonstrated that Group I presented with a larger quantity, which can be understood as providing better myocardial protection.

At the moment that the aorta is clamped, the heart metabolism becomes anaerobic with the utilization of

glycogen and glucose stored for the production of energy. In the myocytes, cellular glycogen is consumed within a period from 20 to 30 minutes of anoxia [19]. In this study, the period of anoxic arrest was 45 minutes and the glycogen reserves in cells of Group II and the Control Group were depleted. Group I was preserved due to the continuous supply of oxygen and substrates provided by this method of myocardial protection.

In Group II, the potassium and magnesium of the intermittent cold crystalloid cardioplegia solution functioned as induction agents of diastolic arrest: the sodium maintains the solution slightly hypertonic in relation to the interstitial liquid by controlling osmolarity, with the aim of preventing myocardial edema; sodium bicarbonate adjusts the pH to acceptable levels in hypothermia. The main objective, in these cases, is to induce rapid cardiac arrest, without depletion of ATP reserves. Hypothermia is the most important factor in myocardial protection. Hypothermic cardioplegia attenuates the ischemia effects on the myocardium and prevents the loss of high-energy phosphate during aortic clamping. However, in specific circumstances, such as with ventricular hypertrophy severe heart failure, significant myocardial ischemia and cardiogenic shock, the myocardium can present a significant metabolic deficit with lower production of high-energy phosphates [20]. The cold which, on one side reduces the energy consumption in the heart, on the other reduces its production, impairing calcium pump function, increasing its concentration in cytosol, which increases tension in the ventricular walls, leading to an increase in energy consumption. The beating heart in hypothermia consumes more energy than in normothermia. Apart from the calcium pump, other pumps and enzymatic and buffering systems are also blocked by the cold [21].

Conservation of the integrity of nucleoli in the two experimental situations may be explained by the fact that they contain the mechanisms necessary to synthesize ribosomes. During proliferation and cellular regeneration many ribosomes are required to synthesize proteic masses for cytoplasmatic expansion. Thus, during the reaction of cells to injury, ribosomes produce defense and repair enzymes for cell survival [22,23]. However, the marked presence of nucleoli in all cells analysed in Group I and Group II is due to the activation of nucleolar metabolic mechanisms that lead to cell protection.

In the Control Group, morphological alterations resulting from anoxic arrest indicated partial oncosis of the myocardium. In total ischemia, tissue oncosis can be extensive, with cells seen at light microscopy stained with hematoxylin-eosin, as a red mass with intense acidophilia, due to the loss of cytoplasmatic nucleic acids with or without the nucleus. In regards to partial oncosis, the mechanical function of the heart may be recovered due to the presence of cells without irreversible damage. Cells that suffered less

damage were capable of surviving the changes in their structure and function, adapting or neutralizing the physiological stress to which they had been submitted.

Ischemia causes vacuolization of the mitochondria and the rupture of lysosomes, provoking extravasation of their enzymes to the cytoplasm. The enzymes, in turn, digest cellular components, neutralizing the pH or making it alkaline. Finally, the dead cells can be substituted as a form of myelinic figures [24]

There is a direct relation between the period of ischemia and reduction of myocardial ATP. The quantity of ATP at the end of the ischemic period also has a direct relationship with the degree of functional recovery of the myocardium. Lima-Oliveira et al. [13] demonstrated experimentally that low-volume blood cardioplegia was efficient to maintain the myocardial cells, fibroblasts and endothelial cells.

Reperfusion is a critical phase for the myocardium, as the heart must recover the deficits and produce electromechanical work with great energy consumption, exactly in the phase in which it needs the energy most [25,26]. Prolonged ischemia makes the reperfusion process difficult, due to the excess of red blood cells in capillaries narrowed due to endothelial edema that is, plugging by leukocytes. These release proteolytic enzymes and free radicals, increasing capillary permeability and interstitial edema. However, the leukocytes are important mediators in re-oxygenation and also act on ischemic and reperfusion injuries [27]. The structural results in the Control Group and Group II demonstrated a higher concentration of leukocytes, a quantity easily explained by the absence of oxygen in their protection methods. Group I presented with a lower concentration of leukocytes due to the fact that the myocardial protection supplied oxygen and substrates continuously, thereby minimizing the injury of this mediator to re-oxygenation.

According to the myocardial protection method or the myocardial conditions, undetectable damage may occur or damage reversed by reperfusion or even permanent myocardial damage caused by reperfusion. Hearts that have great energy deficits and are ischemic, hypertrophic, dilated, cyanotic or immature must be considered special. [28]

Although reperfusion is considered a critical phase to the myocardium, as the heart must recover the deficits and produce electromechanical work with great energy consumption, our results demonstrated that, if the myocardial protection technique is adequate and supplies the metabolic requirements of the tissue, it is possible to eliminate damage to muscle fibers. The addition of glutamate and aspartate amino acids to the cardioplegic solution improved myocardial metabolic efficiency, restoring energy deficits, leading to anatomical and functional recovery of the heart muscle that otherwise would be condemned to irreversible damage and oncosis.

On using continuous tepid blood cardioplegia, the ischemic phenomenon and deficits of substrates and oxygen do not occur. Thus, the reperfusion phenomenon with its serious consequences is minimized.

CONCLUSIONS

Continuous tepid blood cardioplegia (Group I) proved to be more efficient in the preservation of the structural and ultrastructural integrity of the myocardium of isolated hypertrophic rabbits heart when compared to intermittent cold crystalloid cardioplegia (Group II).

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