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Ultrastructural study of the myocardium using cardioplegic crystalloid solution with and without procaine in patients undergoing aortic valve replacement

Análise ultra-estrutural do miocárdio usando solução cardioplégica cristalóide com e sem procaína em pacientes submetidos à troca valvar aórtica

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Abstract

Objective: **The aim of this study was to assess whether the presence of procaine in crystalloid cardioplegic solution increases myocardial protection at the ultra structural level.**

Methods: **Eighteen patients that underwent aortic valve replacement in the Hospital de Clínicas de Porto Alegre over a 10-month period were studied. They were randomly allocated into two groups: group A - eight patients receiving cardioplegia without procaine; group B - ten patients receiving cardioplegia with procaine. Myocardial biopsies were performed in three different periods: 1st - before ischemic arrest, 2nd - at the end of ischemic arrest, and 3rd - 15 minutes after reperfusion.**

Results: **The ultra structural analysis comparing the groups in the three moments did not show any statistically significant difference. The mean score in group A at moment I, II and III was 0.1 ± 0.2; 0.4 ± 0.3; 0.4 ± 0.4, and group B 0.2 ± 0.2; 0.4 ± 0.3; 0.7 ± 0.2. Comparative analysis of CK-MB was similar. The spontaneous return to sinus rhythm after aortic declamping in group B occurred in 70% and in group A 12.5% (p=0.024).**

Conclusion: **Both cardioplegic solutions tested were equally effective in myocardial preservation, and we could not demonstrate at the ultrastructural level any benefit when procaine was added. The spontaneous return to sinus rhythm after aortic declamping was significantly greater when procaine was added.**

Descriptors: **Cardioplegic solutions. Procaine. Myocardium/ultrastructure. Heart valve prosthesis. Aortic valve/surgery. Microscopy, electron.**

Resumo

Objetivo: **Avaliar as alterações ultra-estruturais de dois tipos de cardioplegia (com e sem procaína) em corações de pacientes submetidos a troca valvar aórtica eletiva.**

Métodos: **Foram estudados 18 pacientes submetidos a circulação extracorpórea para troca valvular aórtica eletiva, no Hospital de Clínicas de Porto Alegre num período de 10 meses. Cada paciente foi distribuído aleatoriamente em dois grupos: grupo A - oito pacientes que receberam solução cardioplégica sem procaína; grupo B - Dez pacientes que receberam solução cardioplégica com procaína. Em ambos os grupos, o saco pericárdico foi irrigado com solução salina hipotérmica. As biópsias miocárdicas foram realizadas em três momentos: I - antes da parada isquêmica, II- no final do período isquêmico e III-15 minutos após a reperfusão.**

Resultados: **A avaliação ultra-estrutural comparando os grupos nos três momentos não demonstrou diferenças**

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significativas, sendo a média dos escores no grupo A, nos momentos I, II, e III, de 0,1 ± 0,2; 0,4 ± 0,3 e 0,4 ± 0,4. No grupo B, a médio dos escores foi 0,2 ± 0,2; 0,4 ± 0,3 e 0,7 ± 0,2, respectivamente), nos momentos I, II, e III. A curva de CK-MB foi similar entre os dois grupos. O retorno espontâneo do ritmo cardíaco, pós-despinçamento, ocorreu em 70% dos pacientes no grupo B e, em 12,5% no grupo A (p=0,024).

Conclusão: **As duas soluções testadas protegeram o miocárdio de forma eficaz e não foi possível demonstrar, em nível ultra-estrutural, a superioridade da solução contendo procaína. Constatou-se que o retorno ao ritmo espontâneo do coração após o despinçamento aórtico foi significativamente maior no grupo que utilizou procaína adicionada à solução.**

Descritores: **Soluções cardioplégicas. Procaína. Miocárdio/ ultra-estrutura. Próteses valvulares cardíacas. Valva aórtica/ cirurgia. Microscopia eletrônica.**

INTRODUCTION

The use of cardioplegic solutions started in the 1950s, when Melrose et al. [1] used potassium citrate to induce heart arrest. This technique was abandoned in view of the poor outcomes, especially the myocardial necrosis. Later, it was evidenced that the high potassium concentration and the increased osmolality were the variables accountable for the myocardial injury [2]. In 1973, Gay and Ebert [3] re-introduced the use of cardioplegic solution with lower potassium concentrations, achieving functional and metabolic benefits.

In the past two decades, a great variety of cardioplegic solutions have been proposed with different compositions. Some studies recommended the addition of local anesthetics aiming at a better cellular membrane stabilization, with a consequent decreased electrical activity and reperfusion arrhythmias; however, its use remains controversial [4-11].

The ultrastructural myocardial survey is one of the few accurate methods of direct evaluation capable of providing information about the ischemic myocardial alterations through its morphology [12].

The objective of the present study is to verify whether the addition of local anesthetics (procaine) to crystalloid cardioplegic solution increases the myocardial protection at ultrastructural level, as well as studying the electric rhythm of the heart in the intraoperative period.

METHODS

Eighteen consecutive patients, with indication of elective surgery due to aortic valve disease operated on the Hospital das Clínicas de Porto Alegre, were studied during a 10-month period. Patients were randomized into two groups. Group A, the control group, consisted of eight patients receiving cardioplegic solution without procaine, and Group B, consisted of 10 patients receiving cardioplegic solution with procaine.

Inclusion criteria were: a) age > 18 years and < 80 years; b) elective surgery; and c) no myocardial disease associated. Patients with advanced diabetes mellitus with lesion in the target organs, ischemia heart disease, dilated cardiomyopathy, severely impaired ventricular function (EF < 30%), or history of acute myocardial infarction (AMI) were excluded.

The transoperative variables assessed were CPB time, ischemia time, rhythm after aortic unclamping, need of electric cardioversion, use of inotropic drugs, and transient pacemaker use and transient acute myocardial infarction defined as the presence of a new persistent Q wave on the electrocardiogram (ECG), or increased enzymatic CK-MB over three times the baseline value, with echocardiographic evidence of cardiac function impairment.

A 12-lead ECG was performed pre- and postoperatively on the arrival at the Intensive Care Unit (ICU) and once a day, in the morning, during ICU stay. CK-MB serum dosage was attained pre- and postoperatively, at every 6 hours for 42 hours.

The following anesthetic drugs were administered: midazolam (0.5-1.5 mg/kg), fentanyl (10-20 mg/kg), and pancuronium (0.06-0.1 mg/kg), as needed. Cardiopulmonary bypass was established by cannulating the ascending aorta and the right atrium with a 2-stage single cannula. The left ventricle was drained through the right superior pulmonary vein. Membrane oxygenator was used and the surgery was performed under moderate hypothermia (32ºC). After aortic clamping, a transverse aortotomy was performed with infusion of hypothermic cardioplegic solution (4ºC) via antegrade directly into the coronary ostia with separately metal cannulae. Approximately two thirds of the dose were infused into the left coronary artery and the remaining third into the right coronary artery with controlled pressure (80 mmHg). The initial dose was calculated at 300 mL/m2 of body surface area, and half a dose was re-infused at every 25 minutes. The composition of the cardioplegic solution is listed in Table 1. Topic hypothermia with iced saline solution on the pericardial sac was used in all the cases.

The samples to be analyzed under electron microscope were collected at three moments: I – before cardiac arrest, II – at the end of the ischemic period, before aortic unclamping,

and III – 15 minutes after reperfusion. Biopsies were performed with tru-cut-type biopsy needle (Lab. Travenol), at the apex of left ventricle towards the septum. From the material collected, the central portion was immersed in a 2% glutaraldehyde solution in a 0.1 molar phosphate buffer for 90 minutes (pH = 7.4). The samples were washed in a buffer solution and after being fixed 1% osmium tetroxide (osmic acid), they were buffered for 1 hour at room temperature protected from the light, progressively dehydrated in ethanol and included in Epon resin. Ultrafine slices (70 nm) were stretched in copper telae and contrasted with a 2% uraline and lead citrate solution for observation under electron microscope (Philips EM 208S). Five electromicrographies were taken from each moment with a magnification of the areas considered significant ranging from 6300x to 12000x. Material assessment was performed using a semi-quantitative score system described by Kamlot et al. [13], in 1997: 0 = normal; 0.5 = minimum cellular edema; 1 = defined edema (intermyofibrillar and intramitochondrial), margination or building up of early nuclear chromatin clumps, and slight loss of glycogen; 2 = more marked edema, mitochondrial edema, margination or building up of early nuclear chromatin clumps, and loss of glycogen; 3 = severe edema, subsarcomerelamellar vesiculation, contraction bands, amorphous mitochondria, and disrupted sarcolemma membranes; 4 = destruction of the architecture, contraction bandas, disrupted sarcolemma membranes, amorphous mitochondria.

All patients were enlightened regarding the experiment and have signed the informed consent. The study was approved by the Institutional Review Board of the Hospital de Clínicas de Porto Alegre (N°99089).

Analysis of the qualitative variables between groups was performed by the Student's t-test for independent samples, and when needed, the nonparametric Mann-Whitney U test was used. Categorical variables were analyzed using chi-square and Fisher's exact tests. All p values < 0.05 were considered statistically significant.

Table 1. Constituents of cardioplegic solution

Constituents	Group A mEq/l	Group B mEq/l
Na^+	144.0 mEq	144.0 mEq
K^+	20.0 mEq	20.0 mEq
Mg^{++}	32.0 mEq	32.0 mEq
Ca^{++}	4.4 mEq	4.4 mEq
Procaine hydrochloride	—	2.0 mEq
HCO_3^-	10.0 mEq	10.0 mEq

Constituents of 1000 mL solution; pH = 7.4 – 7.6; osmolality 300 - 320 mosm

RESULTS

Of the 18 patients undergoing elective aortic valve replacement, 13 (72.2%) were male and five (27.8%) female. The mean age of Groups A and B was 52 and 55.4, respectively. The distribution of the variables age, gender, weight, and height in each group and the comparisons among them are shown in Table 2. There was no significant difference in the demographic variables between both groups.

In Group A, three patients presented aortic valve stenosis; three, aortic valve insufficiency; and four, double lesion. There was no difference between both groups as to the NYHA functional class, ejection fraction, and preoperative heart rhythm (Table 2).

Table 2. Pre- and intraoperative variables

Variables	Group A n=8	Group B n=10	p
Age (years)	52.0 ± 9.10	55.40 ± 13.40	NS
Gender – male/female	7/1	6/4	NS
Weight (kg)	79.12 ± 13.99	66.70 ± 12.76	NS
Height (meters)	1.73 ± 0.086	1.65 ± 0.072	NS
Diagnosis			
Aortic stenosis	3	4	
Aortic insufficiency	3	2	NS
Stenosis and insufficiency	2	4	
NYHA functional class- I/II/III	1/5/2	1/6/3	NS
Ejection fraction	59.8 ± 11.2	66.5 ± 12.1	
ECG (preoperative)			
Sinusal Rhythm	6	10	
Atrial fibrillation	2	-	NS
Atrioventricular block I	1	3	
IAVR	2	1	
Cardiopulmonary bypass time (min.)	77 ± 16.1	82.3 ± 28.8	NS
Aortic cross-clamping time (min.)	56.6 ± 9.1	61.3 ± 12.1	NS
Cardioplegia Volume (ml)	845 ± 107.2	780.0 ± 152.2	NS

ECG: electrocardiogram; IARV: inespecific alteration of ventricular repolarization; NS: not significant; NYHA: New York Heart Association

When we analyzed the diseases associated, we could observe that there was no statistically significant difference, as follows:

Group A: presence of systemic arterial hypertension in two (25%) patients; chronic obstructive pulmonary disease (COPD) in one (12.5%) patient; and diabetes mellitus in one (12.5%) patient;

Group B: presence of systemic arterial hypertension in three (30%) patients; COPD in two (20%) patients; and diabetes mellitus in two (20%) patients.

There were no intra-hospital deaths in this series.

Group A presented the following measurements: mean aortic cross-clamping time was 56.6 ± 9.1 minutes; CPB time was 77.1 ± 16.1 minutes; and volume of cardioplegic solution infused was 845.0 ± 107.2 mL. Group B presented the following measurements: mean aortic cross-clamping time was 61.3 ± 12.1 minutes; CBP time was 82.3 ± 28.8 minutes; and volume of cardioplegic solution infused was 780.0± 125.2 mL, respectively. A comparison between the averages of each group did not show statistically significant difference. There was no transoperative acute myocardial infarction in this series. Regarding the use of inotropic drugs, two patients of group A and one of group B used in the first 12 hours after surgery. One patient of group A presented electrical activity during an ischemic arrest. Three patients of group A and one patient of group B needed to use a pacemaker in the first 12 hours after surgery. The enzymatic dosages of CK-MB between both groups, represented in Figure 1, did not show significant differences in the eight points compared as well.

Patients of both groups showed significant difference as for the incidence of spontaneous recovery to sinusal rhythm after aortic unclamping as follows: seven (70%) vs. one (12.5%) of groups B and A, respectively. P = 0.024 (Figure 2)

Myocardial ultrastructural aspect in the 18 cases screened did not show significant alterations, both in patients undergoing perfusion with an standard solution (group B) and those with modified solution without procaine (group A). Mitochondria kept their normal spatial structure without significant changes, both in external and internal membrane disposition, in crests disposition and aspect, and in the amount of mitochondrial clumps. There were no significant alterations in mitochondrial matrix in all cases when comparing moment I with moments II and III (Figure 3).

The concentration of glycogen granules in some patients has apparently been reduced at moment III, but this difference was not constant in all cases. The nuclei did not show any king of alteration, keeping the chromatin aspect regularly distributed. T tubules and interspersed discs also showed normal morphology. There were no significant differences as for cellular volume, once intra- and extracellular edema was found to be discreet in some samples.

In all patients, a moderate amount of lipofuscin granules were noticed, especially close to the poles of cardiomyocyte nuclei.

The analysis of the material under electron microscope, according to the proposed score, did not show significant alterations between both groups. In Group A, the mean score at the pre-ischemic phase (I); at the end of ischemic period (II); and at the reperfusion period (III) was 0.1, 0.4, and 0.4, respectively; In group B the mean score at moments I, II, and III were 0.1, 0.4, and 0.7, respectively (Figure 4).

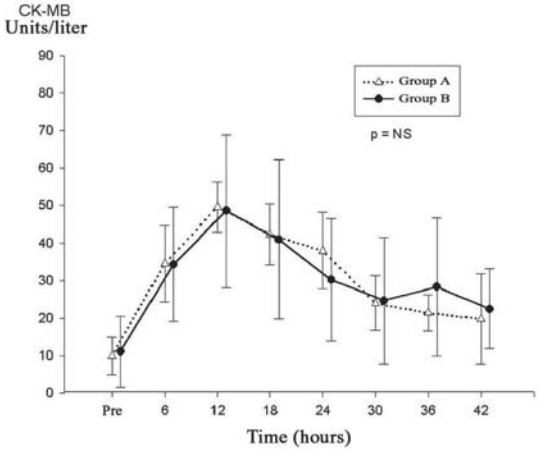

Fig. 1 – Curve of myocardial enzyme release between both groups. Pre- and postoperative specific creatine kinase (CK-MB) isoenzyme serum dosage. Values are presented as mean ± standard deviation (NS = not significant)

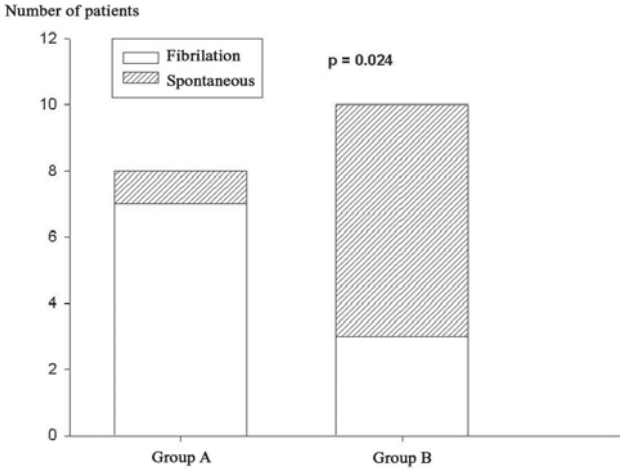

Fig. 2 – Myocardial electrical rhythm after aortic unclamping – p <0.05 is considered statistically significant

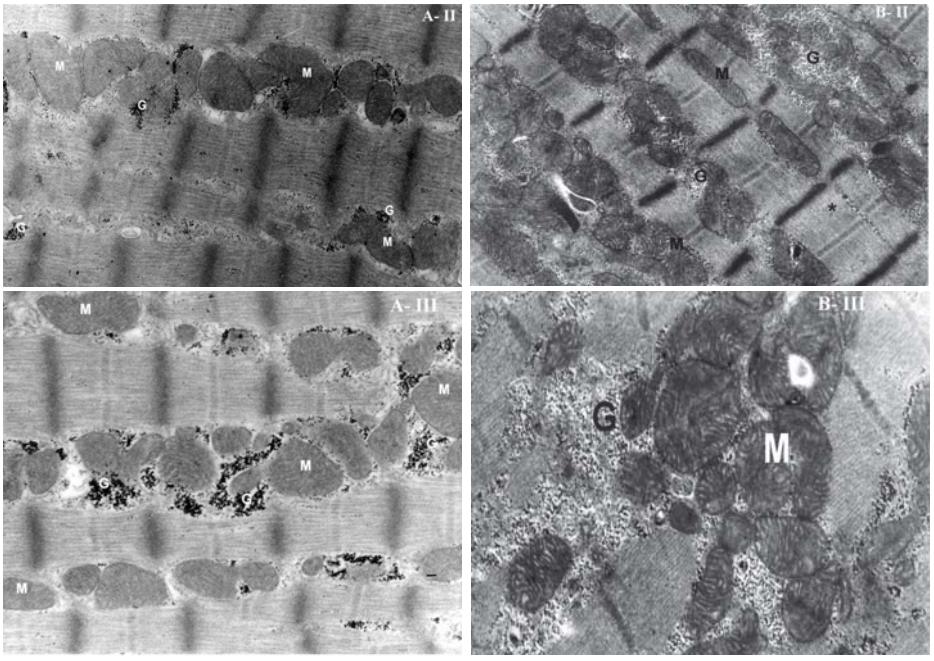

*Fig. 3 – Electromicrography of cardiac muscle - M-mitochondria; G-glycogen and * Sarcomere. A-II: Electromicrography at the endo of ischemic period (moment II – group A) showing the mitochondria of normal aspect with a subtle reduction in the amount of glycogen. Magnification 6300x. A-III: Electromicrography athe the end of reperfusion period (moment III – group A). The aspect is identical to the previous one. Magnification 6300x. B-II: Electromicrography at the end of the ischemic period (moment II – group B), highlighting the normal aspects of the mitochondria, plenty of glycogen, and miofibrillae with normal aspect. Magnification 14400x. B-III: Electromicrography at the end of the reperfusion period (momento III – group B), showing plenty of glycogen, normal aspect mitochondria with marked and compact crests, without intramitochondrial edema. Magnification 24000x*

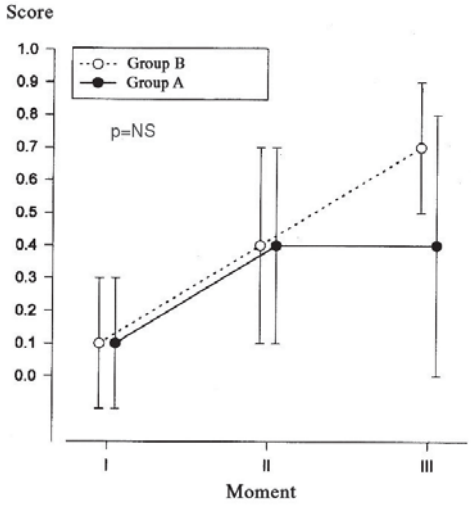

Fig. 4 – Electron microscopy scores of groups A and B. I: preischemia; II: at the end of the ischemic period, and III: Reperfusion. Values are presented as mean ± standard deviation. NS = not significant

DISCUSSION

The study of myocardial protection methods during cardiac surgeries is one of the most published themes, both clinically and experimentally, since the beginning of the cardiac surgery.

The ischemic phase of the transoperative period triggers a series of cellular events which starts slowly and becomes increasingly noxious. The reperfusion process, critical to the re-establishment of normal cellular functions, paradoxically, can enlarge the lesion secondary to the ischemic process.

The process of myocardial injury during ischemia and reperfusion is complex, and it is not quite understood yet. It seems well-established that the cellular membrane has an important influence on its ultrastructure. This leads to significant ionic and structural changes in other cellular organelles as well [14-16].

The immunoinflammatory system also seems to be involved in such alterations in the reperfusion period by means of arachidonic acid products [17-19]. Ventricular fibrillation after the return of coronary circulation is a common event, and it is not often recognized as a problem. However, we know that the myocardium is highly vulnerable in this period, always keeping in mind that the oxygen consumption in normothermia is higher and that such a state can trigger subendocardial ischemia and, eventually, postoperative myocardial depression.

Local anesthetics are being added to cardioplegic

solutions for quite a long time. Procaine exerts its effects by blocking the sodium inflow through the cell membrane [20], and by inhibiting the enzyme phospholypase A activation [21], which has destructive properties to the cell membrane. These mechanisms can be involved with the arrhythmic action of this drug and become a myocardial protection factor in the reperfusion and ischemia period.

In our study, in control group A, the majority of the patients presented ventricular fibrillation after aortic unclamping, requiring electrical cardioversion. In group B, 70% of the patients has a spontaneous return to heart rhythm (p=0.024); this result is opposed to the literature data [6,7,20].

In the assessment of the myocardial protection degree offered by the varied types of cardioplegic solutions, the quantification of the damage is done indirectly. Several authors have shown that the electron microscopy is a mean of direct assessment, becoming a highly-sensitive method to detect early cell damage in the myocytes [22-26].

In our study, the comparison of the electromicrographies of the 18 patients screened did not evidence significant morphological differences between both groups in different moments of time when the biopsies were performed (Moment I – p = 0.876; Moment II – p = 0.584, and Moment III – p = 0.194) based on the assessment scores of lesion evaluation proposed by Kamlot et al. [13] who although has performed an experimental study, also did not find differences between both study groups.

Particularly, the mitochondria that were the target of special attention always showed undamaged external and internal membranes, besides the amount of matrix granules in the mitochondria in different moments in both groups of patients.

The contraction bands of myofibrilla bundles found in some sections alone, without other compatible ultrastructural alterations were not considered, once we could interpret them as a manifestation of ischemic damage, an alteration resulting from the traumatic action of the sample collection, as well demonstrated by Adomian et al. [27], in 1978, in an animal study.

The nuclei remained with their normal structure, with the chromatin regularly distributed. There was no significant cellular edema or leukocyte infiltration in the reperfusion period. The decrease of glycogen granules in moment III could mean a consumption of this element during the ischemic period. This hypothesis does not apply to all the patients, once some of them did not present difference in the content of glycogen granules in the three moments. Another possibility could be the different glycogen distribution depending on both the portion of myofibrilla and the sectioning orientation. In any case, the difference, if any, was relatively discreet and did not show statistical significance.

Studies involving humans addressing the myocardial ultrastructural analysis after cardioplegia are not frequent in the literature, there being a preponderance of experimental studies in animals. The method of ultrastructural analysis is highly sensitive, showing early cellular alterations even before a significant cellular lesion occurs. Structural phenomena detected displayed steady and small magnitudes and were not capable of demonstrating significant injuries to the myocardium (most all the time only a slight reduction in the amount of glycogen or a mild edema). Observing the boxplot of the ultrastructural analysis result, we have a false impression of a significant difference between both groups in moments II and III. We emphasize that in order to the curves to become clearer between both groups, a maximum value (1) was placed in Y-axis, once the ultrastructural alterations found were minimum, having in mind that the Kamlot's score for myocardial injury assessment ranges from 0 to 4. We believe that the possibility of a beta error is reduced, because the p values calculated for each moment were far away of the significance level.

Our clinical and ultrastructural results showed that the crystalloid, hypothermic, and hyperkalemic cardioplegic solution with procaine offered an adequate protection to the group of patients studied. The adequate protection, easy administration, volume control, electrolytic balance, transoperative technical aspect, and decreased arrhythmias, besides the absence of noxious effects to the myocardium are the greatest appeals justifying its use.

CONCLUSION

Both cardioplegic solutions used in the study showed good myocardial protection. There was no significant difference with the use of procaine in an ultrastructural level.

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