ORIGINAL ARTICLE

Braz J Cardiovasc Surg 2005; 20(4): 416-422

Myocardial metabolism after hypothermic retrograde continuous blood cardioplegia with anterograde warm cardioplegic induction

Metabolismo miocárdico após cardioplegia sangüínea hipotérmica retrógrada contínua com indução anterógrada normotérmica

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RBCCV 44205-780

Abstract

Method: **A metabolic analysis of hypothermic retrograde continuous blood cardioplegia with antegrade warm cardioplegic induction was performed in a prospective study of 15 patients scheduled for elective coronary artery bypass grafting. Arterial and coronary sinus blood samples were simultaneously taken: before establishing cardiopulmonary bypass, after anterograde warm cardioplegic induction, when the aortic clamp was removed and 10, 30 and 60 minutes after reperfusion to analyze the oxygen content and lactate concentration. Four transmural left ventricular biopsy samples were obtained: before aortic clamping, immediately after the initial cardioplegia bolus, immediately before aortic declamping and 30 minutes after reperfusion to analyze the levels of ATP, ADP, AMP and lactate in the myocardium. The CK-MB isoenzyme was analysed in venous blood samples.**

Results: **There were no mortalities in the group. Inotropic support was not necessary in any patients and no peri- or postoperative myocardial infarction was detected. There was a decrease in the arterial-venous extraction of oxygen and lactate in the heart during reperfusion, a partial recovery occurred at 60 minutes of reperfusion. The levels of ATP and the other nucleotides in the myocardium were maintained during aortic clamping, but these levels decreased during the first 30 minutes of reperfusion. The lactate accumulated in the heart muscle during aortic clamping with a decrease occurring during reperfusion.**

Conclusions: **From a metabolic point of view the method could not avoid an anaerobic metabolism during cross-clamping and only after 60 minutes of reperfusion there was a satisfactory metabolic recovery. These alterations are probably a reflection of cellular ischemic injury that occurs during cross-clamping and they seem to be of transitory effect. A better myocardium protection was observed with the addiction of anterograde warm induction cardioplegia.**

Descriptors: **Cardioplegic solutions. Cardiac arrest, induced. Adenosine triphosphate. Lactic acid.**

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Article received in July, 2005
Article accepted in October, 2005

Resumo

***Objetivo*: Determinar as alterações sofridas pelo miocárdio durante a cardioplegia sangüínea hipotérmica retrógrada contínua com a adição da indução cardioplégica anterógrada normotérmica.**

***Método:* Análise metabólica da cardioplegia sangüínea hipotérmica retrógrada contínua com indução anterógrada normotérmica em estudo prospectivo de 15 pacientes consecutivos. Amostras de sangue arterial e do seio coronário foram simultaneamente colhidas para análise do conteúdo de oxigênio e da concentração de lactato. Quatro biópsias miocárdicas foram obtidas para análise dos níveis de ATP, ADP, AMP e lactato no miocárdio. A isoenzima CK-MB foi analisada no sangue venoso.**

***Resultados:* Não houve mortalidade no grupo. Nenhum paciente necessitou de suporte inotrópico na saída de CEC e não foi detectado IAM per ou pós-operatório. Ocorreu diminuição da extração artério-venosa do lactato e do oxigênio pelo coração durante a reperfusão, havendo uma recuperação parcial ao final de 60 minutos de reperfusão. Os níveis miocárdicos de ATP e de seus nucleotídeos foram mantidos durante o pinçamento aórtico, porém houve redução destes nos primeiros 30 minutos de reperfusão. O lactato acumulou-se no músculo cardíaco durante o pinçamento aórtico, havendo redução durante a reperfusão.**

***Conclusões:* Concluímos por uma análise metabólica que o método não conseguiu evitar o metabolismo anaeróbico durante o período de pinçamento aórtico e que somente com 60 minutos de reperfusão foi observado um grau de recuperação metabólica satisfatória. Provavelmente essas alterações são devido à injúria isquêmica celular ocorrida durante o pinçamento aórtico e parecem ter efeito transitório. Observamos melhora da proteção miocárdica com o acréscimo da indução cardioplégica anterógrada normotérmica.**

***Descritores:* Soluções cardioplégicas. Parada cardíaca induzida. Adenosina trifosfato. Ácido láctico.**

INTRODUCTION

In spite of the great development in surgical techniques of off-pump coronary artery bypass grafting, myocardial protection has not lost its importance due to high number of cases in which it is of extreme necessity. Hence, research on myocardial protection continues to be published worldwide. The advantages of sanguineous cardioplegia are well established [1,2] and have been responsible for the reduction of surgical mortality rates with high-risk patients [3]. Several studies have shown better clinical results using continuous sanguineous cardioplegia [4-7] and retrograde perfusion through the coronary sinus [8,9]. The method of retrograde cardioplegia is considered better for patients with significant injury of the left coronary artery trunk and in patients submitted to redo coronary artery bypass grafting (CABG) [10,11]. Clinical works on myocardial protection rarely identified significant differences in the results [12,13], however works using metabolic analysis are important as it is possible to verify small differences in the results.

The objective of this study is to determine alterations suffered by the myocardium, utilizing continuous retrograde hypothermic sanguineous cardioplegia in addition to normothermic anterograde cardioplegia induction. The aim of this technique is to better preserve myocardium ATP reserves during the asystole induction phase or even to re-establish these reserves if there is a preexistent depletion [14,15].

METHOD

Patients

Fifteen patients electively referred for CABG and who accepted to participate in this study were evaluated. The study was approved by the Medical Ethics Committee of the hospital. Inclusion criteria were obstructive coronary disease involving two or three arteries and an ejection fraction of less than 40%. The exclusion criteria were unstable angina, insulin-dependent diabetes and associated surgeries (endarterectomy, left ventricular aneurysmectomy, valve replacement etc.). All patients were operated on by the same surgeon (CGS).

Operative technique

The anesthesia technique employed was the same for all patients. After median sternotomy and dissection of the left internal thoracic artery, the patients were heparinized, the pericardium was opened and the aorta and right atrium were cannulated using a 22F arterial cannula (DLP®) and a two-stage venous cannula (DLP®), respectively. The heart-lung machine was manufactured by Stöckert® and the membrane oxygenator by Dideco ®. Hemodilution (perfusate of 2000 mL of Ringer lactate solution) and systemic hypothermia, maintaining the rectal temperature between 28-30 ºC, were used.

Myocardial protection

A retrograde cardioplegia catheter (DLP®) was introduced in the coronary sinus through a hole in the right

atrial wall. The pressure of retrograde cardioplegia was continuously monitored and maintained below 50 mmHg. A small needle-like thermometer was introduced in the apex of the left ventricle for continuous monitoring of the myocardial temperature. A special thermal isolator was used between the heart and the diaphragm to protect against possible thermal injury to the phrenic nerve and ice slush was placed on the heart. Oxygenated blood coming from the oxygenator through a Y-shape line was mixed with potassium chlorate solution at a proportion of 4:1 giving a blood potassium concentration of 20 mmol/L and a hematocrit concentration of 22%. The dose of cardioplegic induction was performed with an infusion of 750 mL of anterograde normothermic sanguineous cardioplegia (37 ºC) through the anterograde cardioplegia cannula in the aortic root, followed by an infusion of 500 mL of cold sanguineous cardioplegia at a temperature of 4-6 ºC infused in the coronary sinus, at a velocity of 200-300 mL/min, under a pressure of 50 mmHg. After induction, the concentration of potassium of the cardioplegia was altered to 10 mmol/L (proportion 8:1) and maintained at a temperature of 4-6 ºC and the solution was continuously infused in the coronary sinus at a velocity of 50-75 mL/min under a pressure of 50 mmHg.

When necessary, saline irrigation was used to simplify the distal anastomoses improving the vision of the artery edges, as continuous bleeding through the coronary arteries sometimes hampers visibility. Reheating was initiated during the last distal anastomosis and the proximal anastomoses of the aorta were performed with the help of aortic clamping.

Measurements

Samples of arterial blood (a) and blood from the coronary sinus (cs) were collected for analysis of oxygen and lactate concentration. The samples were simultaneously collected: before establishing the CPB, at the end of normothermic anterograde induction, on opening the aorta and after 10, 30 and 60 minutes of reperfusion (due to technical problems, analysis of the samples collected after cardioplegic induction was performed for only eight patients). With a special pistol (Biopsy-Cut ®), four myocardial biopsies were obtained from the apex of the left ventricle: (1) after establishing the CPB (but before aortic clamping), (2) immediately after the end of cardioplegic induction, (3) before aortic declamping and (4) after 30 minutes of reperfusion. The biopsies were immediately frozen in liquid nitrogen and stored at -80 ºC until analysis. On the day of analysis, the biopsies were cleaned of fat, blood and connective tissue by macroscopic dissection with the temperature (22º C) and humidity (30%) constant and extracted in 0.5 M of perchloride acid. The acid was removed and neutralized using 2 M of $KHCO_3$ and analyzed by enzymatic fluorometry methods to determine the concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), total adenosine nucleotides (TAN) defined as (ATP+ADP+AMP) and lactate. The ATP/ADP ratio was also calculated.

The CK-MB isoenzyme was analyzed in venous blood samples taken before CPB and 1, 3, 6, 9, 12 and 24 hours after aortic declamping. A method of mass spectrometry (IMx STAT CK-MB, Abbot laboratories, Abbot Park, IL 60064, USA) that is highly sensitive was utilized to determine the MB fraction. The invasive pressures of the radial artery and right and left atria were used for postoperative hemodynamics monitoring.

Statistical analysis

The student t-test was used for statistical analysis. Significant differences were defined as the probability of p-value < 0.05 for each test. The values are presented as means and standard deviations.

RESULTS

Clinical results

Two patients were eliminated from the study due to displacement of the catheter from the coronary sinus. The ages of the patients, genders, ejection fractions (EF), number of peripheral anastomoses, time of aortic clamping and of CPB and time to obtain asystole are illustrated in Table 1. Thirty per cent of the patients (four patients) had histories of prior AMI. No deaths occurred in the group. No patient needed inotropic support. There was no perioperative or postoperative AMI (elevation of enzymatic levels followed by the appearance of a Q-wave in at least two derivations). One patient was reoperated due to instability of the sternum but afterwards evolved well. Six (46%) patients presented with atrial fibrillation during the hospital stay.

Table 1. Patient data
(Mean ± Standard deviation)

Nº of patients	13
Men/Women	11/2
Age (years)	63 ± 7.7
Preoperative EF (%)	71 ± 12.5
Distal anastomoses (Nº)	3.6 ± 0.7
Time of CPB (min)	100 ± 17.5
Time of aortic clamping (min)	53 ± 13.9
Time to obtain asystole (seconds)	52 ± 30

Metabolic results

Lactate metabolism

Sequential measurements of the sanguineous myocardial lactate (arterial and of the coronary sinus) collected before CPB, at the end of normothermic cardioplegic induction, on opening the aorta and 10, 30 and 60 minutes after the initiation of reperfusion are shown in Figure 1 (negative values indicate lactate production). Before CPB the difference (a-cs) of lactate was +0.04 mmol/L. At the end of normothermic cardioplegic induction this value increased to +0.09 mmol/L, a statistically non-significant increase. At the start of reperfusion, there was a change in lactate release giving a difference of -0.22 mmol/L which progressively increased to -0.79 mmol/L at 10 minutes of reperfusion and at 30 minutes of reperfusion this difference was -0.25 mmol/L (all differences were statistically significant). Only after 60 minutes of reperfusion, the lactate arterial-venous difference had returned to the initial levels (+ 0.03 mmol/L).

Oxygen metabolism

Calculations of the arterial-coronary sinus (a-cs) sequential blood oxygen differences performed before CPB and on finishing normothermic cardioplegic induction, on opening the aorta and after 10, 30 and 60 minutes of reperfusion are displayed in Figure 2. The a-cs oxygen saturation difference before CPB was 108 mL/L. At the end of normothermic cardioplegic induction this value fell to only 10 mL/L. When the aorta was declamped, the difference was only 18 mL/L. This low oxygen difference increased to 84 mL/L after 60 minutes of reperfusion; still lower than the initial value (all the measurements compared to the control are statistically significant).

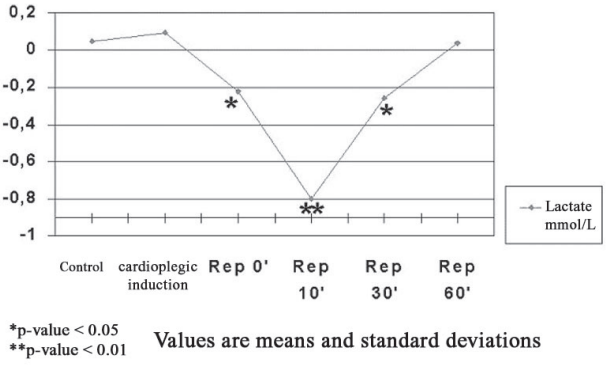

Fig. 1 – A-V lactate difference sampled in the radial artery and the coronary sinus simultaneously

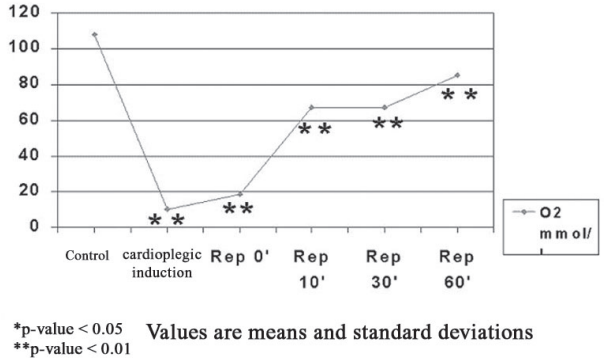

Fig. 2 - A-V difference of the oxygen content sampled in the radial artery and the coronary sinus simultaneously

Table 2. Values of ATP, ADP, AMP, TAN, ATP/ADP seen in the myocardium (biopsies).

	Control	Card. Ind.	Before rep	30´ of rep	Valid Nº
ATP	22.59±5.1	20.17±5.9*	21.37±3.0	18.15±5.3**	13
ADP	2.48±0.29	2.75±0.64	3.30±0.89*	2.57±0.47	13
AMP	0.12±0.60	0.24±0.19	0.28±0.15*	0.17±0.14**	13
TAN	25.19±5.24	23.16±6.14*	24.96±3.21	20.90±5.31**	13
ATP/ADP	9.08±1.95	7.39±2.38**	6.86±1.91*	7.10±2.59*	13

*ATP = Adenosine Triphosphate, ADP = Adenosine Diphosphate, AMP = Adenosine Monophosphate, TAN = total of adenosine nucleotides, ATP/ADP = relation between ATP and ADP. The values are mg/g of dry muscle and presented as means ± standard deviations (*p-value < 0.05 and **p-value < 0.01 in relation to the control).*

Myocardial biopsies

The first myocardial biopsy performed before aortic clamping, shows that the ATP in the heart muscle was 22.6 mg/g of dry muscle. The two biopsies performed during aortic clamping, immediately after cardioplegic induction and before declamping, showed levels of 20.2 and 21.4 mg/g, respectively. After thirty minutes of reperfusion there was a reduction to 18.1 mg/g. The ATP/ADP ratio, which is considered a cellular metabolic function marker, was also analysed. The results are illustrated in Table 2.

The control lactate level in the myocardium before CPB was 13.20 mg/g of dry muscle. The two biopsies attained during aortic clamping gave values of 16.14 mg/g immediately after cardioplegic induction and 23.90 mg/g before aortic declamping (p-value < 0.01). After 30 minutes of reperfusion, the level of lactate in the muscle had diminished to 19.67 mg/g, which is still statistically significant when compared with the control biopsy (p-value < 0.01). The results are shown in Figure 3.

CK-MB enzyme serum levels

The peak CK-MB enzyme serum level occurred six hours after aortic declamping. The results are illustrated in Table 3 and Figure 4.

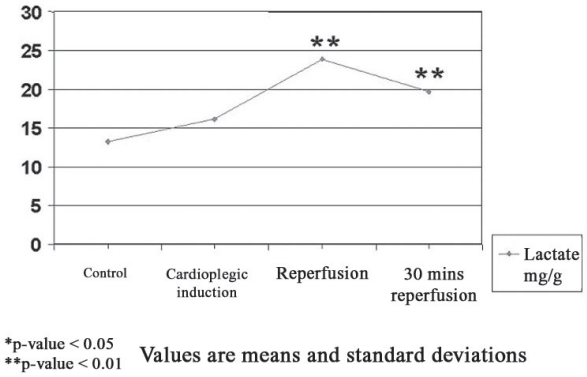

Fig. 3 – Lactate levels identified in the myocardium biopsies

Table 3. CK-MB serum enzyme Levels (means ± standard deviations)

Time (hours)	Pre-CPB	1	3	6	9	12	24
CK- MB (mg/l)	1.9±0.7	38±11	58±25	61±21	54±24	39±29	31±24

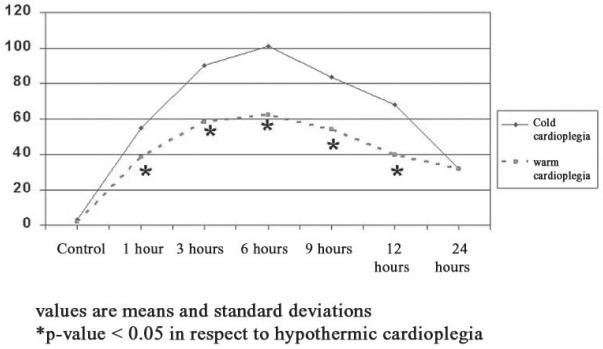

Fig. 4 – CK-MB enzyme levels on venous blood. Differences between normothermic and hypothermic cardioplegia

COMMENTS

From the clinical point of view, the results obtained in the study were good. There was a reduction in the time to obtain asystole with the start of normothermic induction in comparison to a previous work [16]. This can considerably reduce the ATP loss during the period to obtain the end of electromechanical activity. The metabolic results were very similar to a previous work [16] using the same cardioplegic method, but without anterograde normothermic cardioplegic induction. A high incidence of postoperative atrial fibrillation (46%) was observed.

Lactate metabolism

Similar to our earlier study [16], the lactate arterial-coronary sinus difference before CPB showed a normal concentration of lactate. The myocardium, in normal aerobic conditions, uses lactate in the production of energy (ATP) and releases it during anaerobic metabolism. The a-cs difference of lactate at the end of normothermic cardioplegic induction was positive, suggesting the utilization of the lactate as an energy source. But the O_2 arterial-venous difference in this period was insignificant, creating doubts about how the myocardium can utilize lactate as an energy source in practically anaerobic conditions. After aortic declamping there was a release of lactate. This can result from both an abnormal production at that time or an earlier accumulation during aortic clamping or even both hypotheses, indicating some degree of anaerobic metabolism at the start of reperfusion. However, the continuous

retrograde hypothermic sanguineous cardioplegia seems not to have released enough oxygen to cells, probably due to the low temperature [17] or due to regions of the heart that were not satisfactorily perfused by the retrograde cardioplegia. The progressive accumulation of lactate in the myocardium during aortic clamping identified in biopsies, reinforces the idea of an anaerobic metabolism, at least in the region where the biopsies were performed. Lactate production continued to increase during the first ten minutes of reperfusion. This anaerobic metabolism in the presence of what probably is normal oxygen release can indicate the presence of temporary myocardial cell dysfunction [18]. After only 60 minutes of reperfusion the lactate production was reduced.

0_2 concentrations

The oxygen myocardial concentrations were very low after normothermic cardioplegic induction and after aortic declamping. This concentration increased progressively during reperfusion, although after 60 minutes of reperfusion it was still significantly lower than the pre-CPB control. This may reinforce the hypothesis that there is a reduction in the cellular capacity of using the supplied O_2. This cellular metabolic dysfunction, of at least one group of cells, is probably at the mitochondrial plane [4, l8-20],

ATP reserves

In relation to the adenine nucleotide levels there was a preservation of these during aortic clamping, probably due to low consumption during asystole [21]. But, even so, there is an increase in the ADP and AMP levels because of the breakdown of the ATP. These metabolic parameters also indicate that there was an increase in the production of ATP 30 minutes after the start of reperfusion, even though the levels remained low. However, in spite of starting metabolic recovery, ATP aerobic production is not enough to maintain the ATP levels. This inadequate cellular metabolism during reperfusion might be due to mitochondrial alterations with transitory incapacity to maintain a normal aerobic metabolism. Also it is possible that depletion of adenine nucleotides with consequent loss of ATP precursors had delayed the regeneration of myocardial ATP [14, 19, 22-25].

CK-MB

Postoperative serum CK-MB levels observed in this group of patients (Figure 4) may indicate a temporarily compromise in cellular function. The cellular membrane is very sensitive to ischemia and after prolonged periods of anoxia even relatively big molecules such as enzymes can cross the cellular membrane. But, comparing the postoperative serum CK-MB curve of this group with the group of our earlier work [12] (Figure 4), in which normothermic anterograde induction was not utilized, an important reduction in the postoperative serum CK-MB levels was seen in this work. However, it seems that myocardial protection by continuous retrograde hypothermic sanguineous cardioplegia was improved with the addition of normothermic anterograde induction.

CONCLUSIONS

The method could not avoid anaerobic myocardial metabolism during aortic clamping, even though continuous sanguineous cardioplegia was used. Alterations in the myocardial cellular metabolism during the first hour of reperfusion were observed. Alterations in the cellular metabolism are probably transitory, as almost complete recovery occurs 60 minutes after the start of reperfusion.

Compared to the earlier work [16], the addition of normothermic induction reduced the time to obtain asystole (Table 1) and there was a significant reduction in the postoperative CK-MB serum levels (Figure 4).

The clinical results obtained were good and this was considered a safe and simple method. Further metabolic studies using different temperatures of cardioplegia and the use of ATP precursors are suggested.

ACKNOWLEGEMENTS

The authors wish to thank the nurses Laila Östersjö, Jenny Larsson, Catharina Hjelm and Jane Strand, for their dedication to the patients of this study, as well as, the anesthesiologists, perfusionists and nurses of the intensive care unit.

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