SUMMARY

Background: This study is performed to compare crystalloid, blood and carnitine blood cardioplegias (CP) in elective coronary artery bypass grafting.

Methods: Forty-five patients were randomized to three technique of myocardial protection (15 patients in each): Group I cold crystalloid CP, Group II tepid blood CP, Group III tepid blood CP with 5mM/L of carnitine. Blood samples taken from coronary sinus and arterial blood were collected in preoperative and postoperative period (6, 12, 24, and 48 postoperative hours) for enzyme measurements. Also hemodynamic parameters were measured at same time periods.

Results: All the patients involved completed the study. Hemodynamic parameters in all groups were similar. Also neither preoperative nor postoperative myocardial infarction was observed in any group. CK-MB and c Tn-I levels were elevated in all three groups after cross clamping until 6th hour and 12th hour for LDH all three groups. In all enzyme measurements, especially in cTn-I, decline in enzyme levels started earlier in BCCP group than other two groups.

Conclusions: Although there was no significant difference between three groups in hospital mortality and morbidity, blood with carnitine provides better myocardial protection and recovery from ischemia than do crystalloid cardioplegia and blood cardioplegia.

Key Words: Coronary Artery Bypass Grafting, Cardioplegia, Carnitine, Ischemia-Reperfusion Injury

ÖZET

Koroner Bypassa Kamnitinın Kan Kardioplejesinde Kullanımı

Amaç: Bu çalışma kristaloid, kan ve karnitin kardioplejilerinin elektif koroner baypas cerrahisinde miyokard korunması üzerine etkilerini araştırmak üzere planlandı.


Tartışma: Her ne kadar üç grup arasında hastane morbidadesi ve mortalitesi açısından anlamlı fark yoksa da, karnitin kardioplejisi kristaloid ve tek başına kan kardioplejisine nazaran daha iyi miyokard koruması sağlamakla beraber reperfüzyon hassasi en aza indirmektedir.

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Received: July 08, 2002 Accepted: Nov 07, 2002
Despite the modern principles of myocardial protection used in cardiac surgery, functional as well as biochemical signs of ischemia-reperfusion injury can be seen after release of the aortic cross-clamp (1). Reperfusion injury of the myocardium may compromise postoperative cardiac function (1). It can be diagnosed as increased release of SGOT, LDH, CPK, CPK-MB, and Troponin-I and also with ECG changes (2-6).

L-Carnitine is a naturally occurring amino acid that is the requisite carrier of long-chain fatty acids across the mitochondrial membrane where they undergo beta-oxidation (7). Oxidation of long-chain fatty acids is by far most important aerobic source of adenosine triphosphate in the mammalian heart, and adequate myocardial levels of L-Carnitine are essential for normal energy production (8). Multiple clinical and experimental studies have documented that a carnitine deficiency characterized by decreased myocardial carnitine levels can be associated with DM (9,10), infarcted myocardium (11,12), and dilated cardiomyopathy (13,14).

In this study we compared three different cardioplegic solutions used in elective coronary artery bypass grafting (CABG). The effects of these cardioplegics on myocardium were evaluated with measurement of cardiac enzyme levels and hemodynamic parameters.

MATERIAL AND METHODS:

Patient Data

Forty-five patients underwent CABG, with the same surgical technique and same surgical team were prospectively randomized to receive one of three different strategies of myocardial preservation; Group I (15 patients) received antegrade crystalloid cardioplegia (CCP), Group II (15 patients) received antegrade blood cardioplegia (BCP), and Group III (15 patients) received antegrade blood cardioplegia with carnitine (BCCP). Patients with an ejection fraction less than %30, those undergoing reoperation, or those with concomitant heart valve disease were not included. All randomized patients completed the study. Informed consent was taken from all participating patients, which was approved by the Ethics Committee of Ankara University Faculty of Medicine, Turkey.

Operative Technique

A standard median sternotomy incision was used in all patients and no minimally invasive technique was used. Then left internal mammary artery and saphenous vein were prepared. Canulation for the cardiopulmonary bypass was carried out in the usual fashion; arterial canulation to ascending aorta and venous canulation with a two-stage canulla to right atrial appendage were performed. All the patients cooled to 32 ºC and also topical cooling with ice slush was also applied. A cardioplegia (CP) delivery canulla with separate vent line (DLP Medtronic, Grand Rapids, MI) was inserted into the ascending aorta. Coronary sinus catheter with autoinflating silicone cuff (DLP Medtronic) was positioned by transtatrial-closed technique. After cross clamping of the aorta, CP was delivered through the aortic root. As an initial bolus, a 10 to 15mL/kg CP was infused to aortic root with a pressure of 75 mmHg. Additionally a 400ml of CP was given in every 20 minutes during cross clamping period in addition to infusion of a 50-100ml of CP after each vein graft distal anastomosis. If the heart starts to beat also an additional dose of 400ml of CP was given. The re-warming was started during performing the last anastomosis. After completion of all distal anastomoses, the aortic cross-clamp was removed. Proximal anastomoses were performed while partial occluding clamp was applied. In none of the patients retrograde CP was given.

Preparation of Cardioplegic Solution

1) Crystalloid Cardioplegia (CCP):

Plegisol (Abbot CP solution) at 4°C was used, in the following composition 100cc: 643mg sodium Chloride, magnesium Chloride 325,3mg, and potassium chloride 17,6mg (K+ concentration of 16±1 mEq/l).
2) Blood Cardioplegia (BCP):

Blood CP was administered with Dideco D 514 delivery set, which mixes and cools a hyperkalemic crystalloid concentration with oxygenated blood in a 1:3 dilution. To achieve a final potassium concentration similar to that of the crystalloid solution additional potassium was added.

3) Blood and Carnitine Cardioplegia (BCCP):

5mM/L carnitine was added to the BCP. The administration of the cardioplegia was same.

None of the patients in three groups received a terminal “hot shot” of warm cardioplegia.

Enzyme Measurements

Samples for LDH, CK-MB, and Troponin-I were taken from coronary sinus during the operation, and from peripheral venous line postoperatively. Blood samples were taken just before cross-clamping, 5 and 10 minutes after cross clamp release, 10 minutes after cardiopulmonary bypass, and postoperatively in 6th, 12th, 24th, and 48th hours.

Techniques for enzyme measurements:

CK-MB assay:

Photometric determination of the activity of creatine kinase MB–isoenzyme (CK-MB) (N-acetylcysteine-activated) on an immunological basis was used.

Troponin-I assay:

Serum cardiac Troponin-I (cTn-I) concentrations were measured using the Stratus cardiac Troponin-I assay (Dade International Inc. Miami, FL, USA). This is a two-site immunoassay, which uses two monoclonal antibodies that are specific for the cardiac isotype of TnI. Values greater than or equal to 0.6 ng/ml were considered positive.

LDH assay:

LDH concentrations were measured with Olympus AU-600 model autoanalyser.

Hemodynamic Assessment

Hemodynamic parameters including heart rate (HR), mean arterial blood pressure (MAP), central venous pressure (CVP), mean pulmonary artery pressure (mPAP), pulmonary capillary wedge pressure (PCWP), cardiac output (CO), cardiac index (CI) were recorded before CPB, and 1,6,12,24, and 48 hours after CPB. CO was measured in triplicate by using the thermodilution technique.

Statistical Analysis

All data were entered on the SPSS statistical analysis program for Windows. The data are presented as mean ± one standard deviation. Significance was assumed if the p value was less than 0.05. One way and multiple analysis of variation (ANOVA) F-tests were used to compare the three groups.

RESULTS:

There were no deaths in all groups, and all the patients involved, completed the study. Mean age was 59.4±5, 2 in CCP group, 56.3±6, 2 in BCP group 60.0±5, 5 in BCCP group. Most of the patients in three groups were NYHA Class I or II. There were no significant differences in preoperative data between these three groups (Table 1).

The average bypass time was 111.7±25, 103.9±29 and 107.2±30 minutes in CCP, BCP and BCCP groups whereas cross-clamp times were 44.3±10, 38.4±9 and 41.9±14, with no statistical difference between three in both. There wasn’t a significant difference in mean myocardial core temperature immediately proceeding aortic declamping in all groups. LIMA and saphenous vein were used as grafts. LIMA was bypassed to LAD in all patients. Sequential grafts were performed in 8 patients (4 in CCP, 2 in BCP and 2 in BCCP). The number of distal anastomoses was similar in all groups with no significant difference. Operative data is summarized in (Table-2).

Direct comparison of the three groups revealed the enzyme production was significantly greater in CCP.

cTn-I was detectable in three groups before cross clamping. cTn-I levels sharply increased
after declamping. No statistical differences were present between three groups until weaning of cardiopulmonary bypass (CPB) (p>0.05), but crystalloid group had the highest levels. cTn-I levels peaked after weaning of CPB in BCCP group whereas it was highest 6 hours after the operation in other groups. In all time periods after weaning off CPB we found significant differences between three different cardioplegia groups (p<0.05). At the 48th hour only the difference between CCP and BCP was significant (Figure 1).

Like Troponin-I results, no statistical difference was present in CK-MB levels before declamping in three groups (p>0.05). After removal of the clamp a sharp increase in all groups was observed. CK-MB levels peaked at the 6th hour and started to decrease in all groups. In all time periods after 6th hour, there were significant differences between three groups (p<0.05). Lowest values were recorded in BCCP group whereas CCP group has the highest values. Decrement in cTn-I levels was sharper than it was in other two groups (Figure 2).

### Table 1- Preoperative data of the patients.

<table>
<thead>
<tr>
<th></th>
<th>CCP GROUP (15)</th>
<th>BCP GROUP (15)</th>
<th>BCCP GROUP (15)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>59.4±5.2</td>
<td>56.3±6.2</td>
<td>60.0±5.5</td>
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<tr>
<td>Sex (no. of men)</td>
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<td>8</td>
<td>10</td>
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<tr>
<td>Body surface area (m²)</td>
<td>1.87±0.11</td>
<td>1.89±0.16</td>
<td>1.88±0.13</td>
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<td>Ejection fraction</td>
<td>0.59±0.10</td>
<td>0.57±0.9</td>
<td>0.58±0.9</td>
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<td>Preop cardiovascular intervention</td>
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<td>4</td>
<td>5</td>
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<tr>
<td>Hypertension</td>
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<td>8</td>
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</tr>
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<td>NYHA class I</td>
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### Table 2- Operative data

<table>
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<th>BCP GROUP</th>
<th>BCCP GROUP</th>
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</thead>
<tbody>
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<td>Number of anastomosis</td>
<td>3.1±0.8</td>
<td>2.8±1.0</td>
<td>3.0±0.9</td>
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<td>Cross-clamp time (min)</td>
<td>67.7±13.1</td>
<td>70.1±10.9</td>
<td>65.7±11.5</td>
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<td>CPB time (min)</td>
<td>93.9±17.6</td>
<td>87.6±16.0</td>
<td>85.6±14.3</td>
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<td>Temperature of CP (°C)</td>
<td>5.7±1.3</td>
<td>32.7±0.9</td>
<td>31.8±0.6</td>
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<td>Temperature of myocar. (during cross clamp) (°C)</td>
<td>12.8±1.7</td>
<td>33.4±2.7</td>
<td>31.9±3.1</td>
</tr>
<tr>
<td>Myocardial Temp after de-clamping (°C)</td>
<td>36.4±1.0</td>
<td>35.8±0.7</td>
<td>36.9±0.5</td>
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<tr>
<td>Spontaneous defibrillation</td>
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<td>7</td>
<td>8</td>
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<tr>
<td>Number of conduction disturbances</td>
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<td>2</td>
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</table>
Figure 1: Cardiac Troponin-I levels in CCP, BCP and BCCP groups.

Figure 2: CK-MB levels in CCP, BCP and BCCP groups.
Figure 3: LDH levels in CCP, BCP and BCCP groups.

Figure 4: Comparison of cardiac index (CI) in CCP, BCP and BCCP groups.
After declamping LDH levels in all groups started to increase and peaked at the 12th postoperative hour. In all time periods BCCP group has the lowest values and CCP has the highest. The curve after 12th hour was similar of the LDH graphic. A sharp decrease in BCCP group, and more horizontal decrease in other two groups, which were insignificantly different between themselves but the difference between them, and BCCP group were significant (p<0.05) (Figure 3).

Hemodynamic measurements were not significantly different between three groups (Figure 4). But especially in the early postoperative period cardiac index (postoperative 6th and 12th hours) was higher in cTn-I group than other two groups. There were similar results in PVR and SVR measurements.

Neither preoperative nor postoperative myocardial infarction was observed. Also no acquired left bundle branch was observed in either group. 2 patients in CCP, and 1 in BCCP group had an acquired right bundle branch block. Three patients in CCP and BBCCP group, and 2 patients in BCP group experienced atrial fibrillation in the early postoperative period (in first 12 hours). All the patients easily recovered with amiodarone treatment except one in CCP group needed cardioversion because of hemodynamic instability. There were no significant differences in arrhythmia incidence between three groups.

**DISCUSSION:**

The optimal cardioplastic solutions for myocardial protection and the route of administrating them have been discussed controversially for a long time. Antegrade cold crystalloid cardioplegia is the simplest and first used method of myocardial protection. Crystalloid cardioplegics preserve ventricular function, prevent depletion of high-energy substrates, and maintain ultrastructural integrity (15). Then blood CP was used. The superiorities of blood cardioplegia include a) oxygen delivery, b) the buffering capacity of blood, c) capillary flow distribution where red cells are essential, d) prevention of free radical generation and chain-breaking of their effects, e) maintenance of oncotic pressure and f) restriction of hemodilution (16-18).

Nemoto et al. have successfully demonstrated the effects of carnitine on cardiac functions in experimental isolated adult mammalian hearts (19), but there are few studies like these, done in humans.

CPK-MB, and LDH are released after myocardial injury, but more specific marker of cardiac injury is cTn-I with a wider diagnostic window as compared to CK-MB, and LDH. It has been demonstrated that cTn-I, unlike CK-MB, and LDH, is not influenced by peripheral muscular disease and is usually unchanged after noncardiac operation (20-22).

In this study, cTn-I, CPK-MB, and LDH levels were measured to evaluate the myocardial protection between different groups in selected time intervals. Levels of all these three enzymes elevated after declamping because of ischemia. CK-MB and cTn-I levels were high till 6th hour, whereas LDH levels started to decrease after 12th hour. This shows that LDH is a less specific cardiac ischemia marker than other two enzymes. Moreover recovery in cTn-I levels was earlier than CK-MB levels.

Codd et al (5) reported that infarct size and CK-MB was significantly greater in patients given crystalloid cardioplegia than in those given blood cardioplegia. There was a similar conclusion in the study of Elwatidy et al (23), which showed tepid blood cardioplegia had superiority in both metabolic and functional recovery. In this study also it is shown that crystalloid cardioplegia had a high incidence of postoperative arrhythmia especially ventricular arrhythmia.

Clinical outcome was favorable in three groups with no hospital deaths or perioperative infarction on electrocardiographic monitoring. These results clearly reflect the adequacy of myocardial protection by all three cardioplastic
solutions in elective myocardial revascularization.

In our study CK-MB and c Tn-I levels were elevated in all three groups after cross clamping until 6th hour and 12th hour in LDH group. In all enzyme measurements, especially in cTn-I, decline in enzyme levels started earlier in BCCP group than other two groups. This shows us that the myocardium protected by BCCP started to recover before other two groups. Probably insignificant increase in cardiac index in the early postoperative period (in the 6th and 12th postoperative hours) was also due to fast recovery with carnitine cardioplegia.

As it is known that carnitine is necessary for the transport of activated long-chain fatty acid esters cross the mitochondrial membrane and stored to be used in Krebs cycle and oxidative phosphorylation. Restoration of normal cardiac metabolism is predicated on maintenance of adequate cellular levels of this substance. Conversion from anaerobic glycolysis to aerobic is one of the earliest reactions after reperfusion. Carnitine given in cardioplegia during ischemia supports substrates to the aerobic glycolysis, which is the main mechanism for ATP production for the cell. Of course increased amount of ATP production will fasten recovery of metabolic functions of the myocyte, which will cause less reperfusion injury.

Also BCP group has significant superiority in recovery to CCP group. This is due to known benefits of BCP. Especially natural buffering capacity of blood which is important in buffering, and blood as an antioxidant that prevents free radical production are the most important factors that cause rapid recovery in BCP than in CCP group.

Although there was no significant statistical difference between three groups in hospital mortality and morbidity, blood with carnitine provides better myocardial protection and recovery from ischemia than do crystalloid cardioplegia and blood cardioplegia.
REFERENCES

12. Shug AL: Changes in tissue levels of carnitine during myocardial ischemia and anoxia: Arch Biophys Biophys 1978;82:3-13