REPERFUSION STRATEGY AFTER REGIONAL ISCHEMIA: COMPARATIVE STUDY OF REPERFUSION CONDITIONS AND COMPOSITIONS: PRESSURE; TEMPERATURE AND ADDITIVES*

Despite favorable effects of controlling reperfusate condition and composition, controlled aortic root reperfusion (CARP) has not been widely used, because of its requirement for complex techniques and equipments. In this study, we investigated the effects of CARP components; pressure, temperature, and additives, with the aim of simplifying this method.

Thirty-two mongrel dogs were used in this experimental study. The proximal left anterior descending (LAD) artery was occluded for 90 minutes while the heart was beating, and then cardiopulmonary bypass (CPB) was initiated, and aorta was cross clamped (XC) and the heart was arrested with warm blood cardioplegia. In Group 1 (n=8), sudden uncontrolled normothermic reperfusion was performed. In Group 2a (n=8), pressure controlled and substrate enriched normothermic blood, and in Group 2b (n=8) pressure controlled unmodified normothermic blood was used. In Group 3 (n=8), pressure controlled unmodified tepid (28°C) blood reperfusion was done. CPB was discontinued after 45 minutes of reperfusion and functional, metabolic, biochemical and histological investigations were performed.

In Group 1, the first cardiac rhythm observed was ventricular fibrillation, but all subjects in Group 2a, 2b and 3 showed spontaneous sinus rhythm (p<0.001). Recovery times were 39±8.5; 11.6±2.07; 11.9±2.2 and 13.9±1.8 minutes for group 1, 2a, 2b and 3, respectively (p<0.0001 for Group 1 vs. other groups; and p=0.051 for Group 2a vs. Group 3). Cardiac output (CO) levels at 30th and 60th minute after weaning from CPB decreased markedly in Group 1 (p<0.0001) and CO levels were similar in Group 2a, 2b and 3. Prolonged lactate production and prolonged oxygen uptake failure were observed in Group 1, compared to Group 2a, 2b and 3. The oxidative stress markers confirmed a significant tissue injury in Group 1. Similar high levels of antioxidant capacity and favorably less TBARS levels were observed in groups 2a, 2b and 3. Light and electron microscopic studies also showed a significant tissue injury in group 1 and a few comparable

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histological changes in group 2a, 2b and 3. It seems that keeping low reperfusion pressure at the initial two minutes is the main effective component of CARP. Substrate enriched warm cardioplegia showed no superiority to warm or tepid unmodified blood reperfusion in myocardial resuscitation of dogs, in which 90 minutes of proximal LAD occlusion was performed.

Key Words: Control, Reperfusion, Injury, Glutamate, Warm, Tepid

With the recent advances in surgical techniques, myocardial protection and better understanding of reperfusion injury mechanisms, an increasing number of patients with acute coronary occlusion are being referred for surgical revascularization. Although restoration of blood flow to ischemic regions is essential, uncontrolled reperfusion may cause expansion of the myocardial damage and may result in myocardial dysfunction. Many studies showed that the fate of jeopardized myocardium is determined by how the reperfusion strategy is managed rather than how quickly the blood supply is restored (1-4).

Thus, controlling reperfusion conditions and/or compositions itself may aid in reducing the myocardial infarct size and ventricular injury. Numerous studies have been conducted using different reperfusion strategies with successful results. However, it includes several elements requiring complex procedures and equipments. In this experimental ischemia-reperfusion study, we investigated the effectiveness of controlled reperfusion components, that is pressure, temperature, and composition of the reperfusate, with the aim of simplifying the method, detecting the sophisticated elements effects still debatable on prevention of the reperfusion injury.

MATERIAL AND METHODS

Thirty-two vaccinated, healthy adult mongrel dogs of either sex, weighing 24 to 25 kg (average weight 24.2 kg) were used as the study subjects. The study design was approved by the Ethic Committee of the Gülhane Military Medicine Academy- Ankara, Turkey. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide For The Care and Use of Laboratory Animals" Prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH) (Publication No 86-23, revised 1985).

All animals were premedicated (0.003 mg/kg Xylazine I.M. and 0.04 mg/kg Atropine sulfate), then anesthetized with Ketamine (5 mg/Kg I.V.) and mechanically ventilated through a cuffed endotracheal tube with a volume-controlled respirator. Anesthesia was maintained with 10 mg/kg thiopental sodium and 2mcg/kg fentanyl as required.

A median sternotomy was performed, the pericardium was incised and suspended. The electrocardiogram was continuously recorded from standard limb leads. Arterial pressure was recorded from a 20-gauge angiocath positioned into the distal ascending aorta. A thermodilution catheter (Abbott E-576 USA) was placed into the pulmonary artery and connected to a cardiac output computer (American Edwards USA). Hemodynamic measurements were performed at baseline and at 30 and 60 minutes after weaning from the cardiopulmonary bypass (CPB). The proximal left anterior descending (LAD) artery adjacent to the first diagonal branch was occluded using a bulldog clamp, imposing a 90-minute period of acute anterior wall ischemia. To suppress ventricular ectopic beats, a total dose of 2 mg/kg of lidocaine was given. No inotropic or other pharmacological agents were given. After 90 minutes of ischemia, the animals were systemically anticoagulated with heparin (3 mg/kg I.V. maintaining an activated clotting time over 400 seconds). An aortic and a two stage venous cannula were inserted into the distal ascending aorta and right atrium. The extracorporeal circuit was primed with 4.5 % hydroxyethyl starch and normal saline solution (1:1). The hematocrit levels during the CPB varied between 28-32 % with this priming solution. Membrane oxygenators (Sorin Biomedica Cardio Saluggia -
Monolyth-Italy) were used in all experiments. During CPB, the mean aortic pressure was maintained at 50-60 mmHg by pumping back all the blood that was received; the systemic flow was kept over 50 ml/kg/min and hydroxyethylstarch was added to reservoir when needed. Acid-base and gas exchange variables as well as blood potassium and lowered calcium levels were monitored and corrected as necessary. An aortic cross clamp (XC) was applied and cardioplegic arrest was maintained after starting CPB in all cases. The cardioplegia (10 ml/kg) solution was prepared by adding Potassium Chloride 30 mEq/L; Diltiazem 10 mg/L and Sodium Bicarbonate 10 mEq/L to the normothermic blood (370C) and infuse into the aortic root needle at a pressure of 100 mmHg and diastolic arrest was achieved without removing the bulldog clamp on the proximal LAD.

Thirty-two animals were divided into four equivalent groups on the basis of reperfusion strategy. Group 1 (Uncontrolled reperfusion group: n=8). In eight cases the XCs were released at 370C and the mean systemic pressure was maintained at 75-100 mmHg. The coronary occluder (bulldog clamp) was removed to allow restoration of blood flow at systemic high pressure. Cases with ventricular fibrillation were converted to sinus rhythm with cardioversion.

Group 2a (enriched hot shot group: n=8). In this group, controlled reperfusion was carried out while the XC on. The coronary occlusion was released and the reperfusion was started with a substrate enriched and potassium added blood solution (table I) infused into the aortic root at a temperature 370C, at a pressure of 20-25 mmHg for the initial two minutes. Thereafter, the infusion pressure was gradually increased (25 mmHg Per minute) to 75 mmHg and normokalemic-substrate enriched solution was infused for the subsequent 20 minutes. The reperusate was prepared in two separate plastic bags, each containing 250 ml of blood solution during reperfusion. For ensuring a continuous infusion, during filling one bag with blood and enriching addition, the other bag was infused with a roller pump. Two sterile tubing lines were utilized for this purpose, one for the delivery of oxygenated blood into the bags (from the arterial line) and one for the cardioplegia delivery into the pump. Throughout the reperfusion stage, the pressure in the aortic root was monitored and the distension of the left ventricle was actively avoided by using left ventricular venting via a vent catheter inserted into the left atrium and then positioned into left ventricle through the mitral valve. After completion of the controlled reperfusion, XC was removed.

Group 2b (simple hot shot group: n=8). In this group, the initial two minutes of reperfusion was also carried out while the XC on. The coronary occlusion was released and reperfusion was started with a potassium added (30 mEq/L) unmodified normothermic blood solution. We kept the aortic root reperfusion pressure at 20-25 mmHg during the first two minutes as in Group 2a and then we released the XC at 75 mmHg systemic pressure.

Group 3 (Tepid reperfusion group: n=8) In this group, animals were cooled (Bıçakçılar heat exchanger-Turkey) to tepid temperature (280C) during the cardioplegic induction. When the oropharyngeal temperature reached to 280C, pump flow was decreased to maintain a systemic pressure of 20-25 mmHg and then,

<table>
<thead>
<tr>
<th>Table I. Enriched blood solution components used in Group 2a.</th>
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<tbody>
<tr>
<td>Blood</td>
</tr>
<tr>
<td>Hematocrit</td>
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<tr>
<td>Hematocrit in perfusate</td>
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<tr>
<td>Temperature</td>
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<tr>
<td>Addition of Potassium</td>
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<tr>
<td>Initial hot shot</td>
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<tr>
<td>Subsequent 20 minutes</td>
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<tr>
<td>Glutamate*</td>
</tr>
<tr>
<td>Aspartate*</td>
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<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Citrate-Phosphate-Dextrose</td>
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<tr>
<td>NaHCO3</td>
</tr>
</tbody>
</table>

*Glutamate-aspartate solution: L-monoosodic-monohydrate glutamate 30mM/100 ml; L-monoosodic-monohydrate aspartate 30mM/100ml (for each 250 ml of reperfusion 10 ml was used) **30% D/W: Dextrose (g) in water
the coronary occluder bulldog clamp and XC were removed. The lowered pump flow continued to maintain the reperfusion pressure at 20-25 mmHg for the initial 2 minutes. Immediately after the 2-minutes low flow state, rewarming was started and at the same time pump flow was gradually increased (25 mmHg per minute) to maintain a systemic pressure of 75 to 100 mmHg.

In all study groups, after releasing the XC, the systemic mean arterial pressure was kept at 100 mmHg by appropriately increasing the pump flow. Total CPB time after reperfusion was standardized to 45 minutes in all groups and CPB was discontinued thereafter. Finally, the hearts maintained in the beating, working state for an additional one hour after weaning from CPB and before final measurements were done.

**Physiologic and biochemical parameters**

Cardiac output (CO) measurements were done before coronary occlusion (baseline) and 30 and 60 minutes after discontinuation of CPB. The arterial blood gases and electrolytes were restored to normal levels and left atrial pressure was kept at 10 mmHg by volume replacements before final measurements were made. The shortest time to restore normal sinus rhythm and optimal contractility after the reperfusion was noted as the "Recovery time".

In order to measure the myocardial metabolism, the oxygen extraction and lactate extraction, blood samples were taken from the aorta or the cardioplegia line and the coronary sinus by needle aspiration. This was done before ischemia (baseline); at the termination of recovery phase; and 30 and 60 minutes after the discontinuation of the CPB. Blood samples were analyzed (Nova-stat profile 9 Nova medical-USA) for PO₂, PCO₂, O₂ saturation, pH, Serum Base Excess, lactate, O₂ content and Hematocrit-Hemoglobin.

Myocardial oxygen extraction = O₂ content of the arterial or reperfusate blood minus O₂ content of coronary sinus blood (ml/dl);

Myocardial lactate extraction = Lactate content of the arterial or reperfusate blood minus Lactate content of the coronary sinus blood (mmol/L) (5). Negative lactate extraction was expressed as lactate production.

After 20 minutes of the reperfusion, the blood samples were taken from the coronary effluent (coronary sinus) and they were stored at 4°C for 120 to 180 minutes during their transport to a specialized laboratory and then they were stored in liquid nitrogen (-170°C) until the study termination. These samples were utilized for further analysis such as thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), myeloperoxidase (MPO) and Glutathion peroxidase (GPX). In TBARS analysis, the Romero's method was utilized (6). In Myeloperoxidase analysis Andrews and Krinsky's method was used (7). In superoxide dismutase analysis, commercially available RANSOD SD126 Kit and in glutathion peroxidase analysis, RANSEL RS504 Kit was used (RANDOX Diamond Road, Crumlin, Co. Antrim United Kingdom BT29 4QY).

Sixty minutes after the discontinuation of the CPB, punch biopsy specimens were taken from the left ventricular face of the apical septum and examined with "light" and "electron" microscope (JEDL Corp. JEM1200-EX Tokyo-Japan). Light microscopic specimens were stained with Hematoxylin-eosin. In electron microscopic studies, the conventional osmium tetroxide fixation method was used.

The endothelial cell injury scoring was made according to the findings of light microscopy and verified with the electron microscopic findings. The scoring was made with 0 to 3 points scale as "0" indicating no visual pathologic change; "1" cytoplasmic dullness and light nuclear chromatin clustering; "2" more pronounced nuclear changes, cellular swelling and intraluminal blebs and fibrin accumulation; "3" loss of endothelial integrity and perivascular hemorrhages (8). The myocyte reperfusion injury scoring was also made with 0 to 3 points scale as "0" indicating no visual pathologic change; "1" indicating seldom contraction bands and minimal myocyte edema; "2" widespread contraction bands, disruption in sarcomers, intracellular and extracellular pronounced edema and tears of sarcolemma; "3" cellular disruption due to sarcomeric rupture or hypercontraction, disruption in Ebner's bands (intercalar discs) (8).

All animals were sacrificed after the
termination of the study.

**Statistical analysis**

Kruskal-Wallis, Fisher Exact and Mann-Whitney U tests were used to detect the differences between the groups when appropriate. The SPSS for Windows (Version 5.0) program package was used for computation in consultation with a specialist. Data are expressed as mean±SD. Differences were considered significant at a probability level of p<0.05.

**RESULTS**

The heart rhythm and the hemodynamic changes during the coronary occlusion-beating stage areas follows.

Following the left anterior descending coronary artery occlusion, akinesis and subsequently systolic bulging were developed in all segments. All animals became hypotensive while the LAD was clamped. Initial and minimal mean blood pressure levels were 100.7±16.4 and 72.4±20.5 mmHg for Group 1; 97.9±19 and 68.5±25.4 mmHg for Group 2a; 96.5±19.4 and 71.3±17.8 for Group 2b; and 98.1±23.6 and 70.2±23.3 for group 3, respectively (p<0.06). Ventricular fibrillation occurred in one subject in Group 1 and two subjects in each of the other three groups, whom required D/C cardioversion towards the end of the 90 minutes of ischemic interval. No subject needed premature establishment of CPB due to hemodynamic deterioration or intractable rhythm disturbance.

**Heart rhythm during reperfusion:** All hearts in Group 1 developed ventricular fibrillation during reperfusion. All of them could be defibrillated successfully and normal sinus rhythm was achieved. All hearts in the groups 2a, 2b and 3 developed spontaneous beats, which resulted in normal sinus rhythm during reperfusion (p<0.001).

**CO measurements:** Almost equivalent sizes of dogs and similar heart rates in all stages of the study allowed us to use CO as a marker of myocardial function parameter. There was no statistically significant difference between four groups in terms of baseline CO levels.

Table II. Measured variables and Kruskal Wallis analysis

<table>
<thead>
<tr>
<th>Cardiac output (ml/min)</th>
<th>Group 1</th>
<th>Group 2a</th>
<th>Group 2b</th>
<th>Group 3</th>
<th>&quot;p&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2147.5±95.7</td>
<td>2156.3±151.2</td>
<td>2145.1±164.6</td>
<td>2178.8±131.1</td>
<td>0.68</td>
</tr>
<tr>
<td>30'Post CPB</td>
<td>1135±111.7</td>
<td>1661±138</td>
<td>1695±115.3</td>
<td>1782±171.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>60'Post CPB</td>
<td>1031±79.9</td>
<td>1897.5±87</td>
<td>1920±110.7</td>
<td>1891.3±128.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Recovery time (min)</td>
<td>39±8.5</td>
<td>11.6±2.1</td>
<td>11.9±2.4</td>
<td>13.9±1.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lactate extraction</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.65±0.26</td>
<td>1.68±0.21</td>
<td>1.69±0.29</td>
<td>1.66±0.23</td>
<td>0.94</td>
</tr>
<tr>
<td>Recovery-termination</td>
<td>-1.90±0.33</td>
<td>-0.61±0.11</td>
<td>-0.66±0.28</td>
<td>-0.58±0.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>30'Post CPB</td>
<td>-0.84±0.26</td>
<td>-0.56±0.37</td>
<td>-0.43±0.26</td>
<td>-0.45±0.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>60'Post CPB</td>
<td>-0.95±0.30</td>
<td>-0.36±0.27</td>
<td>-0.33±0.21</td>
<td>-0.15±0.28</td>
<td>&lt;0.0001</td>
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<tr>
<td>Oxygen extraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>7.10±0.17</td>
<td>7.12±0.19</td>
<td>6.95±0.34</td>
<td>7.03±0.12</td>
<td>0.59</td>
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<tr>
<td>Recovery termination</td>
<td>5.21±0.40</td>
<td>8.60±0.34</td>
<td>8.44±0.56</td>
<td>8.18±0.28</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>30'Post CPB</td>
<td>6.3±0.31</td>
<td>7.68±0.49</td>
<td>7.64±0.36</td>
<td>7.78±0.34</td>
<td>&lt;0.0001</td>
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<tr>
<td>60'Post CPB</td>
<td>6.48±0.28</td>
<td>7.14±0.18</td>
<td>7.06±0.21</td>
<td>7.09±0.23</td>
<td>&lt;0.0001</td>
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<tr>
<td>TBARS</td>
<td>9.02±0.67</td>
<td>6.14±0.54</td>
<td>6.01±0.32</td>
<td>6.13±0.40</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MPO</td>
<td>1615.1±633.5</td>
<td>603.5±90.22</td>
<td>630.9±110.6</td>
<td>599.8±33.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SOD</td>
<td>788±61.6</td>
<td>1280±275.5</td>
<td>1136±257.6</td>
<td>1210±262.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GPX</td>
<td>187.0±53.1</td>
<td>264.4±39.1</td>
<td>293.4±61.8</td>
<td>276.6±57.7</td>
<td>0.008</td>
</tr>
<tr>
<td>Endothelial damage score</td>
<td>2.63±0.52</td>
<td>0.75±0.46</td>
<td>0.63±0.52</td>
<td>0.63±0.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Myocytar damage score</td>
<td>2.25±0.46</td>
<td>1.5±0.53</td>
<td>1.38±0.51</td>
<td>1.5±0.53</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Abbreviations: 30-60'Post CPB: 30/60 minutes after discontinuation of cardiopulmonary bypass. TBARS: Thiobarbituric acid reactive substances. MPO: Myeloperoxidase. SOD: Superoxidizedymutase. GPX: Glutathion peroxydase

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However, CO significantly declined after reperfusion in Group 1 compared to group 2a, 2b and 3 (table II). Despite the recovery observed in Group 2a, 2b and 3, CO levels of Group 1 continued to fall at 30 and 60 minutes after weaning from CPB. Group 3 seemed to have better cardiac output levels among the controlled reperfusion groups after weaning from CPB, but the differences were not significant (at 30th minute Group 2a vs. Group 3: p=0.47, Group 2b vs. Group 3: p=0.25; at 60th minute after weaning from CPB, Group 2a vs. Group 3: p=0.64, Group 2b vs. Group 3: p=0.29). No statistically significant difference was seen between Hot Shot groups in terms of CO levels, either in the 30th or 60th minute (p=0.88 and 0.56 respectively).

Recovery time: Restoration of the sinus rhythm and forceful contractions occurred significantly earlier in Group 2a, Group 2b and Group 3, compared to Group 1 (p<0.001). Hot shot groups showed almost near-significant recovery periods superior to tepid reperfusion group (Group 2a vs. Group 3: p=0.051; Group 2b vs. Group 3: p=0.062). Group 2a and 2b had comparable values (p=0.86).

Lactate extraction: There was no difference between four groups in terms of baseline lactate extraction. At the termination of the recovery period, lactate production and washout were significantly pronounced in Group 1 compared to others groups. At 30th minute after weaning from CPB, Group 1 lactate extraction showed a little recovery compared to other groups, but at 60th minute after weaning from CPB lactate production and washout in Group 1 re-increased. No statistical difference occurred between groups 2a, 2b and 3 at any stage after reperfusion (at the termination of the recovery period: Group 2a vs. 3: p=0.96; Group 2b vs. 3: p=0.74; at 30th minutes after weaning from CPB: Group 2a vs. Group 3: p=0.47, Group 2b vs. Group 3: p=0.91; at 60th minutes after weaning from CPB: Group 2a vs. Group 3: p=0.19; Group 2b vs. Group 3: p=0.26). Group 2a and Group 2b had comparable values at 30th and 60th minutes after weaning from CPB; p=0.36 and p=0.88 respectively.

Oxygen extraction: There was no difference between four groups in baseline oxygen extraction values. However, oxygen extraction values significantly deteriorated in group 1 at the termination of the recovery period and it persisted at 30 and 60 minutes after weaning from CPB. Oxygen extraction values showed a significant difference among the hot shot groups and tepid reperfusion group at the termination of the recovery period (Group 2a vs. Group 3: p=0.021; Group 2b vs. Group 3: p=0.016). After weaning from the CPB, these differences disappeared (at 30th minute Group 2a vs. Group 3: p=0.72, Group 2b vs. Group 3: p=0.58; at 60th minute Group 2a vs. Group 3: p=0.79, Group 2b vs. Group 3: p=0.92) Group 2a and 2b had comparable values at 30th and 60th minutes after weaning from CPB (p=0.86 and p=0.89 respectively).

Analysis of oxidative stress: Uncontrolled reperfusion resulted in significantly higher TBARS and MPO levels in Group 1 (see table II).

Hot shot and tepid reperfusion groups showed comparable values of TBARS (Group 2a vs. Group 3: p=0.79, Group 2b vs. Group 3: p=0.96). Group 2a and 2b didn’t show significant difference (p=0.54).

Hot shot and tepid reperfusion groups didn’t show any difference in MPO levels (Group 2a vs. Group 3: p=0.72, Group 2b vs. Group 3: p=0.58). Group 2a and 2b had comparable levels of MPO (p=0.56).

The antioxidant reserve capacity including SOD and GPX levels, showed marked alterations in all groups. Group 1 showed approximately 30% lower SOD levels compared to other groups. Hot shot and tepid reperfusion groups had also comparable values (Group 2a vs. Group 3: p=0.44; Group 2b vs. Group 3: p=0.21). Group 2a and 2b didn’t show any significant difference (p=0.29).

GPX levels in group 1 were significantly lower compared to other groups. Hot Shot and Tepid Reperfusion groups showed comparable values of GPX levels (Group 2a vs. Group 3: p=0.96 Group 2b vs. Group 3: p=0.56). Group 2a and Group 2b also did not have statistically different GPX values (p=0.61).

Ultrastructure: Group 1 specimens showed widespread and significantly pronounced endothelial damage compared to Group 2a, 2b
and 3. Hot Shot groups and Tepid Reperfusion Group showed comparable values, Group 2a vs. Group 3: p=0.72, Group 2b and Group 3 had identical values indicating no difference in controlled pressure reperfusion groups (Group 2 and 3).

Myocyte damage score was also significantly higher in Group 1 than in other groups, but the damage was not as widespread and intensive as in endothelial damage. Myocyte damage didn't differ between Hot Shot Groups and Tepid Reperfusion Group. Group 2a had identical scores with Group 3; Group 2b vs. Group 3: p=0.87, and Group 2a and 2b had comparable values (p=0.87).

In table II the studied parameters and statistical differences between the groups are presented. In figures 1 to 3 histological study examples are presented.

**DISCUSSION**

Controlling the reperfusion conditions is aimed to prevent the reperfusion injury and resuscitate the cells that exhausted almost all their energy charges. Modifying the reperfusate composition, pressure,

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**Figure 1a.** A Group I model; no-reflow phenomenon is presented. Note the interstitial hemorrhage and contraction bands. Endothelial damage and perivascular edema result in red cells stasis and "stringing" into the capillary bed. **Figure 1b** shows a specimen taken from Group III. Note the normal endothelial and myocyte morphology and clearly visible striae.

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**Figure 2a.** A red blood cell (RBC) in the capillary contacting to endothelial cell. Note the "convoluted" appearance of endothelium, bleb formation and foamy appearance in cytoplasm. Note the extensive edema (E) between endothelial cell and myocyte. In **Figure 2b**, an example from Group 3 is presented. Note the absence of convoluted appearance, and presence of only few dwarf blebs and vacuoles in cytoplasm. Edema (E) formation is markedly reduced compared to Group 1.
temperature, and initializing reperfusion in a decompressed and arrested myocardium were reported to minimize reperfusion injury and resuscitate the jeopardized myocardium by diminishing energy consumption and rapidly replenishing the energy stores (1-4).

Addition of depleted Krebs cycle elements such as glutamate, aspartate, and various number of enzymes or chelates such as ROS scavengers (SOD, allopurinol, deferroxamine etc.) into the reperfusate, or lowering the calcium levels or leukocyte content of the reperfusate have been shown experimentally to reduce the reperfusion damage. These techniques and additives require "completely" different surgical strategies in order to control the reperfusate.

We designed this experimental study to discover the "negligible components of the controlled reperfusion" in order to simplify the method without altering the reliability.

During hypoxia, anaerobic metabolism produces metabolites, which cause a fall in the vascular tone and produce reactive hyperemia at the initial phases of reperfusion (3,9,10).

Sawatari et al (9), investigated coronary flow and resistance with low reperfusion pressure (20 mmHg) and high reperfusion pressure (>75 mmHg) after 2 hours of hypothermic global ischemia in neonatal lambs. The high pressure reperfusion resulted in a burst of initial coronary flow that subsequently fell below the levels of low-pressure reperfusion group at the 15th minute. After reperfusion, myocardial oxygen consumption was significantly lower in the high-pressure reperfusion group than the low-pressure reperfusion group (9). Although this hyperemic vascular response in the ischemic and peri-ischemic area lasts for 30 to 120 minutes (11), it is reported that keeping a low perfusion pressure in the initial 2 minutes can prevent the overflow and subsequent tissue edema and endothelial dysfunction that might result in coronary no-reflow phenomenon and myocardial dysfunction (10,12). In our experimental model, all hearts were arrested with cardioplegia during the coronary occlusion. This allowed us to initialize the reperfusion in a standstill heart, so energy consumption was prevented and energy stores were replenished rapidly.

In Group 1, the first rhythm after institution of the reperfusion was ventricular fibrillation. In other groups, the initial rhythm was always spontaneous sinus rhythm. This finding proves that ensuring a gentle reperfusion in the initial 2 minutes is a strong predictor of initial heart rhythm. Clinical, biochemical and histological studies showed that the initial heart rhythm also shows strong correlation with reperfusion injury. Ventricular arrhythmias may be a manifestation of inadequate protection and cellular damage of myocardium and/or the
conduction system (13). In Group 2a and 2b, reperfusion at the initial 2 minutes was performed while the XC on. The main difference between these two groups was the use of either "enriched" or "unmodified" blood reperfusionate. Obtained clinical, biochemical and histological results suggest that addition of glucose, aspartate-glutamate and citrate into the blood reperfusionate during the initial 2 minutes of reperfusion and the following 20 minutes provides no superiority to the reperfusion with unmodified blood, in 90 minutes of ischemia-reperfusion dog model.

Group 3 animals were cooled to 28°C to obtain a secure 2-minute hypotensive period while removing the XC. This hypothermia provides a secure time for the lowering of whole body perfusion, without brain ischemia and without acidosis due to low pump flow. With the entry of unmodified 28°C blood into the coronary arteries, a slow regular spontaneous rhythm began (10 to 15 beats per minute). Interestingly, despite the hypothermia, ventricular fibrillation was not seen; we think that this could be attributed to diltiazem and resistance of the dog hearts to ventricular fibrillation (14). We used diltiazem to maintain the "stand-still" situation during the low-potassium cardioplegia, it’s previously reported that diltiazem does not show a protective effect against cellular calcium influx and reperfusion injury (15). After 2 minutes of a hypotensive period (20-25 mmHg), rewarmed was started and pump flow was gradually increased. The heart was rewarmed rapidly and subsequently spontaneous sinus rhythm developed. The recovery period in the Tepid Reperfusion Group was slightly longer than Hot Shot Groups because of the delayed rewarmin. In all animals the perfusion pressure was kept at 100 mmHg during CPB to obtain a good functional recovery (16). In human subjects, it’s not necessary to keep the pressure as high as this for obtaining better myocardial recovery.

After reperfusion, Group 1 subjects showed a marked continuation of anaerobic metabolism. This was verified by the prolonged recovery time, and the lactate and oxygen extraction values. Pressure controlled reperfusion, either in Hot Shot Group or in Tepid Reperfusion Group, showed a rapid metabolic recovery after a brief duration and lactate extraction, oxygen extraction levels approached to baseline levels at the 60th minute after weaning from CPB. Oxygen utilization in reparative and energy production processes showed that early oxygen extraction rises at the termination of the recovery period in pressure controlled reperfusion groups. In contrast, in Group 1, the problem in oxidative metabolism seen in oxygen extraction at the termination of the recovery worsened after 30 minutes to 60 minutes after weaning from CPB. Histological studies showed that restricted blood flow by the no-reflow phenomenon could be the explanation of this metabolic defect.

On the other hand, the Tepid reperfusion group, showed good functional, metabolic and histological recovery after 90 minutes of ischemia-reperfusion model, as well as Hot Shot Groups. Metabolic recovery more rapidly occurred in the Hot Shot Groups; at the termination of the recovery period, the oxygen extraction was more pronounced than Tepid Reperfusion Group, but this difference turned against the Hot shot Group at 30th minute after weaning from CPB. This finding proves that cooling-rewarming may cause a delay in oxygen utilization but it completely resolves after 30 to 60 minutes of reperfusion.

Elevated TBARS levels indicate cellular reperfusion injury in Group 1. Also MPO levels indicate endothelial injury due to the potential role of leukocytes in capillary and parenchymal reperfusion damage (17). SOD levels were significantly lower in Group 1, also the same was true for GPX levels. The decreased endogenous antioxidant reserves concomitant with the elevations in TBARS and MPO indicate that the greatest tissue injury results in Uncontrolled Reperfusion Group. In our study, the reperfusion temperature (normothermic or tepid) didn’t show any effect on the oxidative stress and the related tissue injury.

Use of “blood” as the myocardial reperfusionate provides increased oxygen transport due to red cells, buffering of acidosis, ROS scavenging
and diminishing myocardial edema with the aid of protein contents. Thus the blood components are extremely important natural warriors against reperfusion injury (18). In our study, we were unable to show any beneficial effect of additives in the reperfusate. Group 2a and 2b showed almost equivalent results related to reperfusion injury. Although there are various studies showing the benefits of adding substrates to the reperfusate, this has not gained widespread support. Frierson and colleagues compared "Buckberg’s controlled reperfusion" and "simple (uncontrolled) reperfusion" on dogs after 100 to 180 minutes of proximal LAD occlusion. After one week, they found that left ventricular wall motion scores and infarcted areas were identical in both two groups (19). Edwards and colleagues clinically demonstrated that substrate enriched warm reperfusion provides no superiority to simple unmodified reperfusion (20). Although our reperfusate composition or Edwards and colleagues’ cardioplegia composition are not completely identical with Buckberg’s cardioplegia, we also couldn’t show any clear beneficial role of additives on early myocardial functional and metabolic recovery. Because of the majority of studies comparing the effect of temperature of the reperfusate were done at low temperature (deep hypothermia to normothermia), the benefits of tepid reperfusion couldn’t not be explained by English literature. Elvadity (21), and Hayashida and colleagues (5) proved advantageous effects of tepid cardioplegia on myocardial metabolism and functional recovery. Elvadity and colleagues showed that myocardial metabolism and recovery with tepid blood cardioplegia is superior to cold crystalloid or cold blood cardioplegia. Hayashida and colleagues, showed that tepid combination blood cardioplegia reduced metabolic demands but permitted immediate recovery of cardiac function. In this study, we observed similar myocardial oxygen consumptions in tepid and warm blood cardioplegia, pointing to the identical rate of mitochondrial oxidative phosphorylation. On the other hand, tepid blood cardioplegia provides lower anaerobic metabolism (lactate extraction) than warm blood cardioplegia, due to the decreased oxygen demand of myocardium. Our findings showed that lactate production is lowered with tepid reperfusion but this was not significant. In conclusion, the study shows that substrate enriched pressure controlled warm blood reperfusion didn’t show any superiority to pressure controlled reperfusion with unmodified tepid or warm blood reperfusion, in a 90 minutes ischemia-reperfusion model. The mainly effective component of controlled reperfusion seems to be the pressure control of the reperfusate.

REFERENCES

6- Higuera V, Raya A, Rodrigo JM, Serra MA, Roma J, Romero FJ. Interferon decreases serum lipid peroxidation products of hepatitis C patients. Free
Radical Biology and Medecine 1994;16:131-133


