

REPERFUSION STRATEGY AFTER REGIONAL ISCHEMIA: SIMULATION OF EMERGENCY REVASCULARIZATION. EFFECTS OF INTEGRATED CARDIOPLEGIA ON MYOCARDIAL RESSUSCITATION

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In this study, we occluded the proximal part of the left anterior descending artery for 90 minutes while the heart was beating. This was followed by cardiopulmonary bypass, aortic cross clamping and 50 minutes delivery of intermittent consecutive antegrade/retrograde (integrated) blood cardioplegia. The aim of this study is to clarify the necessity of pressure controlled reperfusion after integrated cardioplegia.

16 mongrel dogs were divided into two groups. We investigated the effects of integrated blood cardioplegia and sudden uncontrolled reperfusion (Group A; n=8) on myocardial cell metabolism, oxidative stress, and ultrastructure. We compared the results of the same cardioplegia application when it was followed by pressure controlled tepid initial reperfusion (Group B; n=8).

Recovery period was 22,9±1,9 and 14,3±1,7 min (p<0,0001) in Group A and B respectively. Cardiac output levels were significantly lower in Group A compared to Group B (1354±34 vs. 1600±52 ml/min respectively; p=0,0001) at 30 min after weaning from CPB. At 60 min after weaning from CPB, both groups showed impairment in cardiac output levels (1489±127 vs. 1849±124 ml/min respectively; p=0,001). Lactate and oxygen extraction measurements showed continuation of lactate production and delayed oxidative metabolism restoration in Group A. Oxidative stress studies showed significant lipid peroxidation in Group A and the defense mechanism was able to produce scavengers. In histologic studies made by light and electron microscopy, we mainly observed marked tissue edema in Group A and almost totally preserved cell and organelle integrity in both groups.

This study shows that integrated cardioplegia may resuscitate the vulnerable myocardium in a dog model in which the left anterior descending artery was occluded proximally. This resuscitation is not complete and pressure controlled

reperfusion during initial two minutes is still required as an adjunct procedure to resuscitation. This may result in lesser tissue edema and most importantly lesser myocardial stunning.

Key words: *Emergency, Revascularization, Control, Stunning, Edema*

Emergency coronary revascularization has been shown to be life saving for myocardial cells jeopardized by ischemia and some reperfusion techniques directed to the metabolic and functional recovery may cause early return of contractility after revascularization.

In our previous experimental study, a model of "ischemia and reperfusion" was provided by 90 minutes of proximal LAD occlusion. We comparatively investigated the effects of reperfusion conditions (pressure and temperature) and compositions (substrate enriched and unmodified) on myocardial resuscitation. We showed that pressure controlled reperfusion groups in which substrate enriched warm, unmodified warm, or unmodified tepid blood reperfusions were used, did not differ significantly in terms of metabolic, functional and ultrastructural results. The control group in which the reperfusion was started with high pressure showed significant myocardial damage. We concluded that ensuring gentle reperfusion at the initial two minutes of reperfusion is the main effective component of controlled reperfusion.

In this study, we investigated the clinical practice of urgent myocardial revascularization of acute evolving myocardial infarction. Time interval from the beginning of ischemia until revascularization was standardized and the effect of cardioplegia for the resuscitation of jeopardized myocardium was investigated. The aim of this experimental study was to understand the negligibility of pressure-controlled reperfusion after administration of integrated cardioplegia for resuscitation of jeopardized myocardium.

MATERIAL AND METHOD

Sixteen vaccinated, healthy adult mongrel

dogs of either sex, weighting 23 to 25 kg (average 24 kg) were used and divided into two groups, each including 8 dogs. The study was approved by the Ethic Committee of 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), anesthetized with Ketamine (5 mg/Kg I.V.) and mechanically ventilated through a cuffed endotracheal tube with a volume-controlled ventilator. Anesthesia was maintained with 10 mg/kg thiopental sodium and 2mcg/kg fentanyl, as required. All of the premedications, induction and maintenance of anesthesia was performed as described in the previous study.

After median sternotomy, the pericardium was incised and suspended. The proximal left anterior descending (LAD) artery adjacent to the first diagonal branch was occluded using a bulldog clamp to create 90-minutes of acute anterior wall ischemia. Variables were monitored and cardiac output measurements were done.

After 90 minutes of ischemia, systemic anticoagulation was done with heparin (3 mg/kg I.V, so as to keep activated clotting time over 400 seconds). An aortic cannula inserted into the distal ascending aorta and two single vena cava cannulas through right atrium. A membrane oxygenator and a roller pump were used for cardiopulmonary bypass (CPB) as described in the previous study. Priming solution was prepared with 4,5 % hydroxyethylstarch and normal saline (1:1).

An aortic cross clamp (XC) was applied and warm blood cardioplegic arrest was achieved within 5 minutes following initiation of CPB in all cases. Cardioplegic (10 ml/kg) solution was prepared by adding Potassium Chloride 30 mEq/L; Diltiazem 10 mg/L and Sodium Bicarbonate 10 mEq/L to normothermic blood (37°C) and infused via aortic root needle at a pressure of 100 mmHg and diastolic arrest was

achieved without removing the bulldog clamp from the proximal LAD.

After initiation of CPB and application of XC, all animals were cooled to 28°C (nasopharyngeal temperature). Maintenance of diastolic cardiac arrest was achieved by isothermic (tepid) blood cardioplegia. Tepid blood cardioplegia components included 10 mEq/L Potassium, 4 mg/L Diltiazem and 10 mEq/L Sodium Bicarbonate; the total dose of cardioplegia including the induction cardioplegia was 50 mg/kg in all subjects.

Vena Cava snares were pulled down, right atrium was opened and a transatrial retrograde coronary sinus cardioplegia catheter (Baxter RC2014-USA) was inserted into the coronary sinus under direct vision. A 5/0 polypropylene purse string was made to the coronary sinus entrance in order to secure the proper position of the catheter. Retrograde blood cardioplegia was delivered via the coronary sinus at 35 mmHg pressure and consecutively antegrade cardioplegia was administered with a pressure of 75 mmHg, each at a dose of 250 ml. Tepid blood cardioplegia was prepared in a plastic bag filled with oxygenated blood obtained from the arterial line, infused with a roller pump and delivery pressure was monitored.

After the induction or maintenance of antegrade cardioplegia, no cardioplegia was given for 5-6 minutes providing clinically similar antegrade/retrograde "consecutive and intermittent" cardioplegia. XC period was standardized as 50 minutes for all experimental animals. In both groups, the bulldog clamp on the LAD was not released during cardioplegia administration in order to simulate a coronary artery bypass grafting operation. Thus, cardioplegia was not administered directly to the area at risk of reperfusion injury.

After the termination of 50 minutes of XC and cardioplegic myocardial arrest, two groups were reperfused in two different manners:

Group A (Reperfusion Without Pressure Control):

Bulldog clamp and XC were released simultaneously at a systemic blood pressure of 75-mmHg. After two minutes of initial reperfusion at tepid temperature, rewarming was started. Pump flow was increased to

obtain a mean blood pressure of at least 100 mmHg. Initial and subsequent rhythm, and duration of recovery period were noted after 45 minutes of reperfusion; CPB was discontinued and the venous cannulas were removed. Then heart was allowed to beat for 60 minutes. In this period the left atrial pressure was kept at 10 mmHg. At the end of the 60 minutes, final measurements were made and biopsy specimens were taken.

Group B (Pressure Controlled Reperfusion)

The pump flow was diminished, circulating blood was drained into the reservoir, XC and LAD occluder bulldog clamp was released simultaneously when the systemic arterial blood pressure reached to 20-25 mmHg. After the first 2 minutes of low-pressure tepid blood reperfusion, rewarming started. Pump flow was increased gradually (25 mmHg per minute) until 100 mmHg of mean blood pressure. Initial and subsequent heart rhythm, and duration of recovery period were noted after 45 minutes of the reperfusion, CPB was discontinued and atrial cannulas were removed, the heart was allowed to beat for 60 minutes, the remainder of the study was terminated as described for Group A.

Physiologic and biochemical parameters

Cardiac output measurements were done before coronary occlusion (baseline) and 30 and 60 minutes after discontinuation of CPB. Arterial blood gases, pH and electrolytes returned to normal levels and left atrial pressure was kept at 10 mmHg by volume replacements before final measurements were made. The shortest time to the restoration of sinus rhythm and optimal contractility after the reperfusion was noted as "Recovery time". In order to measure myocardial metabolism, oxygen extraction and lactate extraction, blood samples were taken simultaneously from aorta and coronary sinus by needle aspiration. This was done before ischemia (baseline); at the termination of recovery period; and 30 and 60 minutes after discontinuation of CPB. Blood sample analyses and calculations of lactate-oxygen extractions were done as described in our previous study.

After 20 minutes of the reperfusion, blood samples were taken from coronary effluent (coronary sinus) and stored at 4°C during 120

to 180 minutes until they were transported to a specialized laboratory and then stored in liquid nitrogen (-170°C) until the termination of the study. These samples were used for further analysis such as investigations for thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), myeloperoxidase (MPO) and glutathione peroxidase (GPX). For TBARS analysis, Romero's method was used (2). For Myeloperoxidase analysis Andrews and Krinsky's method was utilized (3). For superoxide dismutase analysis, commercially available RANSOD SD126 Kit and in Glutathione peroxidase analysis RANSEL RS504 Kit was used (RANDOX Diamond Road, Crumlin, Co. Antrim United Kingdom BT29 4QY).

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

Endothelial and myocyte damage scoring was made according to the light microscopy findings and verified with electron microscopy findings. Scoring was done according to 0 - 3

points scale as described in our previous study (4).

Statistical analysis

Fisher Exact and Mann-Whitney U tests were used to detect differences between groups, where appropriate. SPSS for Windows (Version 5.0) program package was used for computation. Data were expressed as mean±SD. Differences were considered significant when p<0,05.

RESULTS

All animals became hypotensive while LAD was clamped. Initial and minimal mean blood pressure levels were 96,3±12,6 and 64,4±16,5 mmHg for Group A (p<0,001), and 99,2±14 and 63,5±15,4 mmHg for Group B (p<0,001), respectively. This fall in systemic pressure was not significant between the groups (p>0,8). None of the subjects required premature CPB establishment due to hemodynamic deterioration. One subject in Group A developed ventricular fibrillation during ischemic beating heart period, that was converted to sinus rhythm with DC cardioversion.

During the early reperfusion period, in Group A; ventricular fibrillation occurred in 6 animals, remaining 2 animals showed spontaneous sinus rhythm; in group B all

Table I. Measurements and p values.

| | Group A | Group B | p |
|----------------------------------|------------|------------|--------|
| Cardiac output | | | |
| Base line | 2156±154 | 2144±91 | 0,79 |
| 30th min. after weaning from CPB | 1354±34 | 1600±52 | 0,0001 |
| 60th min. after weaning from CPB | 1489±127 | 1849±124 | 0,001 |
| Lactate extraction | | | |
| Base line | 1,69±0,15 | 1,75±0,16 | 0,51 |
| Recovery Period Termination | -0,96±0,24 | -0,70±0,13 | 0,03 |
| 30th min. after weaning from CPB | -0,84±0,32 | -0,36±0,21 | 0,001 |
| 60th min. after weaning from CPB | -0,78±0,23 | -0,24±0,12 | 0,0001 |
| Oxygen extraction | | | |
| Baseline | 7,07±0,15 | 7,00±0,19 | 0,88 |
| Recovery Period Termination | 7,35±0,19 | 8,13±0,13 | 0,0001 |
| 30th min. after weaning from CPB | 7,86±0,29 | 7,75±0,27 | 0,44 |
| 60th min. after weaning from CPB | 7,60±0,23 | 7,23±0,10 | 0,001 |
| OXYDATIV STRESS | | | |
| TBARS | 7,18±0,43 | 6,22±0,56 | 0,003 |
| MPO | 659±126 | 612±91 | 0,13 |
| SOD | 1403±212 | 950±52 | 0,0001 |
| GPX | 341±87 | 313±29 | 0,87 |
| HISTOLOGIC STUDY | | | |
| Endothelial damage score | 1,5±0,76 | 0,88±0,35 | 0,13 |
| Myocyte damage score | 1,75±0,71 | 1,5±0,53 | 0,5 |

animals had spontaneous sinus rhythm ($p < 0.001$). Recovery period for Group A was 22.9 ± 1.9 min, and it was 14.3 ± 1.7 min for Group B ($p < 0.0001$). Study parameters and "p" values are presented in table I.

The baseline cardiac output values of both groups were similar. However in Group A, there was a significant decrease in cardiac output after reperfusion. At 30th and 60th minutes after weaning from CPB, both groups showed impairment in cardiac output levels, but values were significantly lower in Group A, compared to Group B.

Lactate extraction seemed to improve faster in Group B until 60th minutes after weaning from CPB. In Group A, a gradual improvement was also observed, but it was slower than Group B.

Oxygen extraction values showed that Group A had a failure to uptake and utilize oxygen in

a delayed recovery period. It seemed that this metabolic disturbances was improved at 30th minutes after weaning from CPB and slightly continued thereafter.

Oxidative stress parameters in Group A showed that significant lipid peroxidation occurred in Group A. Slightly increased MPO and GPX levels didn't differ significantly among the two groups. SOD levels were found to be increased significantly in Group A compared to Group B.

Endothelial cell and myocyte damage scores were slightly higher in Group A, but the difference between the two groups was not statistically significant. In histologic studies we observed marked edema in Group A, however mitochondrial and organelle structures were almost completely normal in both groups. Figures 1 to 3 shows examples from histologic studies.

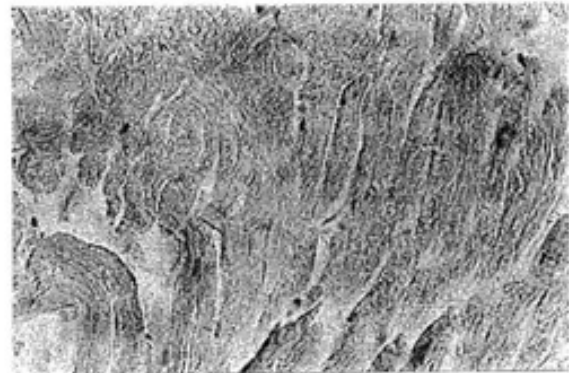
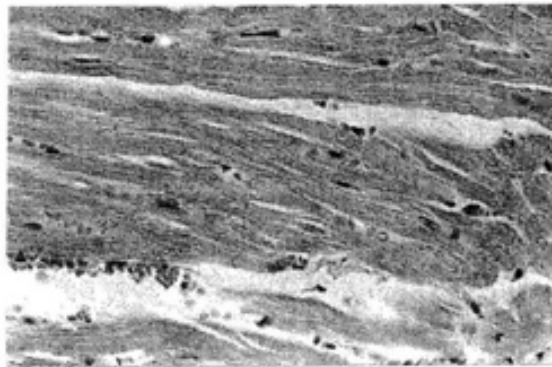


Figure 1. a. A specimen from 'Group A'. Note the marked interstitial edema, mild degree of contraction band formation and blood cell adherence/accumulation in capillary endothelium (arrow), without development of any no-reflow phenomenon (H&E x40). **b.** A specimen from 'Group B'. Lesser edema is observed. Note clearly visible stria and absence of contraction bands.

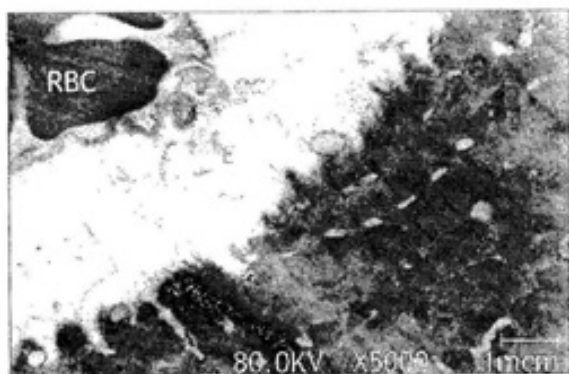


Figure 2. a. A specimen from 'Group A'. A red blood cell (RBC) in the capillary lumen adjacent to the endothelial cell is seen. Note marked perivascular edema (E) and convoluted appearance of endothelium. **b.** A specimen of 'Group B'. A red blood cell can be seen adjacent to the convoluted endothelial cells. Convolution of the endothelial cell was not different in Group A, compared to group B. However perivascular edema was significantly lesser in Group B.

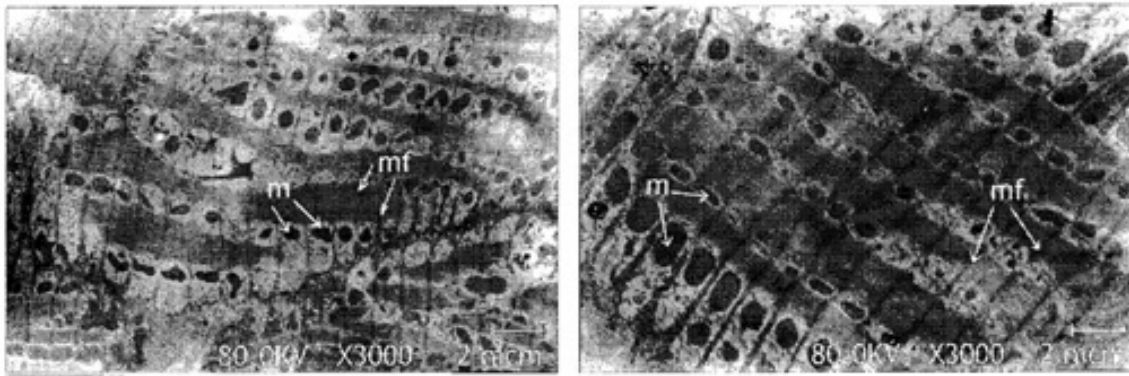


Figure 3.a. A sample from Group A. Marked intracellular edema was the main histologic change. Mitochondria (m) and myofibrillar arrangements (mf) are totally preserved.
b. A sample from group B. There is edema also in sarcoplasmic reticulum around mitochondria, but in reduced intensity. Mitochondrial and myofibrillar arrangements are also preserved.

DISCUSSION

The effect of cardioplegia on cellular resuscitation and reperfusion injury was not well investigated in ischemia-reperfusion model previously. This important component of revascularization surgery may itself completely resuscitate the myocardium and render controlled aortic root reperfusion "unnecessary".

Resuscitation directed to the myocardial area supplied by the occluded coronary artery should be initiated as early as possible. Prolonging the time to reperfusion may increase ischemia and cause further damage to the viable myocytes neighboring the infarcted area. Oxygenated blood cardioplegia is a worldwide preferred cardiac protection technique because of the superior effects of oncotic, rheologic and antioxidant properties of the blood itself (5). Hypothermia is an adjunct strategy aimed to lower myocardial oxygen demand and the rate of cardioplegia, thus providing myocardial hypoxia tolerance when needed, such as when cardioplegia is needed to be stopped for obtaining a dry operative field (6).

Also the important cellular protective effects of hypothermia in emergency revascularization cases are reduction in the rate and strength of the process leading to myocardial cell death and promotion of electromechanical quiescence (7). But, profound hypothermia itself may cause dysfunction of the unaffected areas (8) and

summation of uncontrolled reperfusion injury and unsuitable cardioplegia-reperfusion couple may cause contractility loss and may lead to death. The objective of cardioprotective approaches during cardiac operations is complete avoidance of myocardial stunning and necrosis (9).

In this study, we established an experimental ischemia-reperfusion model that previously had resulted in marked reperfusion damage unless the initial reperfusion pressure was not controlled. We simulated the intermittent cardioplegia infusion in this model and we investigated the effects of cardioplegia on resuscitation of ischemically damaged myocardium. Induction of blood cardioplegia was performed in normothermic conditions in order to benefit from the faster aerobic ATP production, from channeling of gained energy to reparative processes and from the nullifying of the adverse effects of XC (10). The maintenance of blood cardioplegia was performed under tepid temperature condition. Myocardial recovery period may be longer with the "tepid" temperature, but our previous study findings support that tepid blood reperfusion results in as good metabolic improvement as warm ischemia-reperfusion in injured myocardium with a lower anaerobic metabolism. We preferred to make a coronary revascularization simulation under tepid temperature conditions because of its simplicity and as it does not require any additional heat exchanger or pump and as it provides the initial two minutes of simplified

"pressure controlled aortic root reperfusion" with a simple release of XC as described in our previous study.

In order to carry the blood cardioplegia to the area supplied by an occluded coronary artery, retrograde coronary sinus route is a rational alternative, especially for an emergency revascularization case. But retrograde cardioplegia alone is not enough for entire cardiac protection, because in left ventricle one third, and in right ventricle more than half of cardioplegia drains directly into the right atrium without filling the capillaries by the way of Thebesian vein shunts (11).

Combining antegrade/retrograde cardioplegia is recommended for obtaining a regular flow distribution even in the areas that are supplied by the occluded coronary artery (11). Metabolic studies show that different myocardial regions are perfused by these two different routes of delivery (6). For all those reasons we preferred the intermittent, consecutive antegrade/retrograde tepid blood cardioplegia technique, which is currently the preferable technique in our clinic for emergency revascularizations and for cardiac operations requiring long duration of XC. This cardioplegia delivery system is not completely identical with Buckberg's "integrated" cardioplegia (6), but this system can also be called "integrated" because the complementary perfusion affects two delivery roots.

In Group A the recovery period was longer and cardiac output levels were significantly lower after reperfusion, compared to Group B. Also lactate extraction was higher in Group A, both after recovery and after weaning from CPB. This finding may suggest that reestablishment of oxidative metabolism is delayed in Group A and more rapidly restored in Group B. Higher oxygen extraction levels in Group B after reperfusion supports this suggestion. Higher oxygen extraction after recovery period means faster oxygen use for metabolic demands and cell repair (12).

Group A subjects had 15% higher TBARS levels. In this group, antioxidant system marker "SOD" levels were 33% higher. The most acceptable neutrophil activation and endothelial damage parameter "MPO" (13) and other antioxidant system marker "GPX"

(14) were not significantly increased.

Increased SOD and GPX levels show that anti-oxidant reserve of the myocardium was able to produce radical oxygen species (ROS) scavengers. This equilibrium between ROS and ROS scavengers provided cellular protection of the myocardium, indicating cardioplegia technique was good enough for cell protection and resuscitation.

Endothelial and myocyte injury score was mildly higher in group A than Group B, but this was not statistically significant. In this study we observed that the Initial High Pressure Reperfusion Group (Group A) showed a positive but delayed progress in metabolic and functional recovery. By this finding we can state that reperfusion without pressure control after integrated cardioplegia may result in a "reversible" metabolic and contractile dysfunction which is called "stunning and/or hibernating" due to lipid peroxidation mediated tissue edema.

In order for these findings to be clinically more relevant, further studies with longer ischemic models, different cardioplegia delivery techniques (such as delivery from the grafts and simultaneous antegrade/retrograde cardioplegia), and longer reperfusion periods are required.

For emergency revascularization, integrated cardioplegia, which is the combination of pressure controlled reperfusion within the initial two minutes with simple release of XC at 20-25 mmHg arterial pressure is a valuable technique for the prevention of free oxygen radical production and related myocardial stunning.

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