Histological alterations of the human myocardium during minimally invasive direct coronary artery bypass operation

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SUMMARY

To study the ischaemia caused to myocardial cells during the Minimally Invasive Direct Coronary Artery Bypass (MIDCAB) procedure, ten patients underwent surgical revascularization of the anterior descending artery using the MIDCAB technique. During the anastomosis, the left anterior descending (LAD) was snared with two sutures 4-0 prolene. The time of ischaemia was 13-22 min (mean 16.4 min). Three biopsies were taken from the anteroapical part of the left ventricle: a) Prior to the ischaemia b) At the end of the ischaemic period c) 25 minutes after blood flow had been restored.

The degree of cellular and perivascular damage was studied by electron microscopy. Simultaneously, an electrocardiogram (ECG) was performed and the biochemical markers of myocardial ischaemia were measured.

There were no deaths or myocardial infarctions. Slight ischaemic changes were found in all tissue samples before occlusion of the LAD. A semiquantitative analysis showed that a large percentage of myocytes (83.5-90%) in all phases were normal or only slight changes. A few myocytes (3-6%) were severely or irreversibly damaged. The morphometric analysis of mitochondrial oedema revealed no statistically significant differences.

In conclusion, the MIDCAB technique can be applied for the surgical revascularization of the LAD without significant ischaemic changes to the myocardial cells.

Key words: Myocardial ischaemia – Coronary artery bypass

INTRODUCTION

The use of Cardiopulmonary Bypass (CPB) may cause several complications, such as bleeding diathesis, neurological problems, tissue oedema, and renal impairment. The risk of complications increases with concomitant carotid artery disease, diabetes mellitus, renal disease, lung disease and ascending aortic calcification.

Over the past 10 years there has been a steadily increasing interest for beating heart or off-pump coronary artery bypass surgery. Most studies have found benefits in operative mortality, stroke, renal failure, pulmonary complications, bleeding diathesis, and in-hospital length of stay for off-pump as compared with conventional Coronary Artery Bypass Grafting (CABG) (Benetti et al., 1991; Buffalo et al., 1996; Mack et al., 2002; Puskas et al., 2003; Van Dijk et al., 2001).
Previous studies suggest a better preservation of myocardial function with off-pump techniques, such as a decrease in perioperative myocardial enzyme release, and a better preserved left ventricular contraction (Akins et al., 1984; Ascione et al., 1999).

However, to date the effect of off-pump procedures (especially MIDCAB) on myocardial cells, assisted by electron microscopy, has not been studied thoroughly. There is only one preliminary study in this field (Benetti et al., 1986).

In the present study we evaluated the ischaemic changes to myocardial cells observed during the anastomotic period with MIDCAB technique.

**Material and Methods**

Ten patients were studied: eight men and two women, age 41-70 years (mean 62.1 ± 8.4 y.o.). They all had proximal LAD stenosis (80-90%) and underwent surgical revascularization with the MIDCAB technique. An earlier anterior myocardial infarction (>3 months) was reported in three patients. One patient had also undergone Percutaneous Coronary Intervention (PCI) with primary stenting in the previous four months and had developed instent restenosis. The exclusion criteria included: ejection fraction <40%; recent anterior myocardial infarction (<1 month), or the requirement of a concomitant cardiac operation.

The preoperative data are shown in Table 1.

**Table 1. Preoperative and operative data.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>N=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>62.1 ±8.4</td>
</tr>
<tr>
<td>Stable Angina</td>
<td>4</td>
</tr>
<tr>
<td>Unstable Angina</td>
<td>6</td>
</tr>
<tr>
<td>Anterior M.I.</td>
<td>3</td>
</tr>
<tr>
<td>Previous PTCA</td>
<td>1</td>
</tr>
<tr>
<td>Other coronary stenosis &lt;40%</td>
<td></td>
</tr>
<tr>
<td>Cx</td>
<td>2</td>
</tr>
<tr>
<td>RCA</td>
<td>2</td>
</tr>
<tr>
<td>Ejection Fraction</td>
<td></td>
</tr>
<tr>
<td>Good (&gt;50%)</td>
<td>4</td>
</tr>
<tr>
<td>Moderate (40-50%)</td>
<td>6</td>
</tr>
<tr>
<td>Operative</td>
<td></td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>85-127</td>
</tr>
<tr>
<td>Ischaemia time (min)</td>
<td>13-22</td>
</tr>
<tr>
<td>LITA flow (ml/min)</td>
<td>70-220</td>
</tr>
<tr>
<td>LAD diameter (mm)</td>
<td>1.5-2.5</td>
</tr>
</tbody>
</table>

All patients had preoperative ECGs, biochemical heart indices (CPK, CK-MB, LDH, SGrOT and troponine T), as well as postoperative levels of the above indices on days 1, 2, 3, 5.

Prior to the operation, the patients provided written informed consent, approved by the Ethical Committee of the AHEPA University Hospital.

**Surgical Technique:** MIDCAB was performed through a small left anterior thoracotomy (9-11 cm). The left internal thoracic artery (LITA) was completely harvested and the anastomosis to the LAD was made while the heart was beating under direct vision. We used a mechanical coronary stabilizer (Cardiothoracic Systems Inc., Cupertino, California) and the midportion of the LAD was temporarily occluded with proximal and distal snare s. Heparin, 100 I.U./Kg, was administered prior to the anastomosis. The target Activated Clotting Time (ACT) was 300-350 sec. At the end, protamine was used to reverse the effect of heparin. The ischaemic preconditioning technique was not used (Boonstra et al., 1997; Calafiore et al., 1996).

**Biopsies:** Fine needle biopsies (William Schmidt Inc., Valencia, California) were taken during the following stages:
1. Prior to temporary occlusion of the LAD (1st biopsy)
2. At the end of the anastomosis before LAD reperfusion (end of the ischaemic period 13-22 min, mean 16.4 min) (2nd biopsy)
3. 25 min after the onset of reperfusion (3rd biopsy)

All biopsies were taken from macroscopically normal apical areas of the left ventricle (between the LAD and the diagonal branch).

The tissue samples, taken from the subendocardial layer of the left ventricle, were immediately fixed with 3% glutaraldehyde for two hours, and then postfixed in 2% osmium tetroxide (OsO₄) solution for one hour. Samples were stained with 1% uranyl acetate and lead citrate. dehydration was performed in a graded series of ethanol and the samples were then embedded in Epon fixative. All tissue samples were examined with light microscopy in order to select artifact-free areas for ultra thin sectioning with an ultramicrotome. The ultrastructural study was performed using a transmission electron microscopy (Jeo-2000, FX11, Tokyo, Japan) (Rainio et al., 1995; Schaper et al., 1982; Tasdemir et al., 1993).

The electron photomicrographs were labeled only with film identification number, so that subsequent grading of ultrastructural ischaemic injury could be performed in a blinded fashion. All photomicrographs were studied by two experts.
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Fig. 1. Section from a first biopsy sample. Parts of a myocardial cell showing areas with normal myofilaments and areas with subsarcolemmal oedema. m: mitochondrion; sr: sarcolemma; mf: myofilaments; oe: oedema. x 20,000.

Fig. 2. Section from a second biopsy sample. Electron micrograph showing mitochondria with different degrees of lesion. M: mitochondria with flocculent matrix. The arrowhead (?) shows areas with a disrupted outer membrane; m: swollen mitochondria with disordered cristae. x 60,000.
Fig. 3. Section from a second biopsy sample.Collapsed capillary vessel (c) due to increased perivascular oedema (oe), resulting into the almost complete disappearance of the capillary lumen. en: endothelial cell. x 45,000.

Fig. 4. Section from a third biopsy sample. Increased intracellular oedema (oe) and myofilament degeneration (mf). However, some Z lines (z) are intact. x 15,500.
RESULTS

Clinical Data

Conversion to middle sternotomy was not required in any patient. Operative data (LAD diameter, operation time, ischaemia time, LITA flow) are shown in Table 1.

Exutubation of the patients was performed in the Intensive Care Unit (ICU) during the first six hours after surgery. The ICU stay was 24-36 h and the post-operative length of in-hospital stay was an additional 4-5 days.

There were no deaths and no myocardial infarctions, based on electrocardiographic, CK-MB or TrTrelease criteria (Birdi et al., 1977; Hodakowski et al., 1996; Mair et al., 1992; Stamou et al., 2000).

None of the patients required blood transfusions, or inotropic support, and no renal impairment or neurologic deficits were noted.

Ultrastructural Changes

The general condition of the myocardial cells was assessed by study of at least 20 myocytes and 10 endothelial cells for each phase in each patient. A study of 40-60 mitochondria for each phase and of 6-8 samples for each phase in each patient was required. All lesions were of focal nature, and were surrounded by normal tissue.

Phase A

Most cardiac fibres were apparently normal and were interconnected with normal intercalated discs. The nuclei showed a smooth perimeter with a homogeneous distribution of chromatin. The mitochondria were oval and had a thick mitochondrial substance with intact cristae and external membranes. There was a normal arrangement of myofilaments. The same normal appearance was observed in the endothelial cells.

The absence of glycogen granules from most samples was striking. However, there were some limited areas where the cardiac fibres showed subsarcolemmal oedema, as well as moderate alterations of the mitochondria (swollen, crystal disorganization) and the same changes were also observed in endothelial cells (Fig. 1). These alterations were expected even at the 1st biopsy, because all patients had coronary artery disease and CCS II-III symptoms (repeated episodes of angina, repeated ischaemia - reperfusion sequences) (Rainio et al., 1995). Some of the ultrastructural changes might have been due to chronic «hibernation» of the myocardium (Rainio et al., 1995; Vanoverschelde et al., 1993).

Phase B

No serious alterations were observed. Normal cardiac fibres predominated over pathological fibres, and there were some fibres that showed intracellular oedema, especially between the myofilaments and under the sarcolemma. The size and shape of the mitochondria were different; their matrix was granular or amorphous with evident disruption of internal cristae, whereas some of the mitochondria showed breakage of their outer membrane (Fig. 2).

Some sarcomeres were disrupted and featured rupture of the myofilaments. The nuclei and the intercalated discs were normal. There were no glycogen granules. The endothelial cells did not show any decrease in pinocytic vesicles or rupture of their basal membrane. However, there were several collapsed capillaries due to increased perivascular oedema, which resulted in almost complete disappearance of the capillary lumen (Fig. 3).

Phase C

The lesions of the cardiac fibres in this group were identical to the lesions of the previous group: many mitochondria were normal while others were swollen with granular or electron-lucent matrix (vacuolar matrix), and some others had crystal disorganization or rupture of their outer membrane. There was intracellular oedema under the sarcolemma and between myofilaments (some of them were disrupted) (Fig. 4). The nuclei as well as the intercalated discs were normal. The endothelial cells showed no significant changes, while an abundance of pinocytic vesicles and myelinoid vesicles was only found in one sample. No perivascular oedema or collapsed capillary vessels were observed.

Semiquantitative analysis

Perivascular and intracellular oedema, endothelial cells, intercalated discs, mitochondria, nuclei, and myofilaments were studied in all tissue biopsies.

A semiquantitative analysis was performed, assessing the severity of ischaemic injury in each phase on a scale of I (normal) to V (lethal) (Axford-Gatley and Wilson, 1988; Axford-Gatley et al., 1990; Lindal et al., 1988; Rainio et al., 1995; Schapper et al., 1975).

The results of the semiquantitative analysis are depicted in Table 2. In all phases of the
study, a large percentage of myocytes (83.5-90%) were normal or had only slight changes. Only a few myocytes (3-6%) were severely or irreversibly damaged (Axford-Gatley et al., 1990; Schapper et al., 1975).

Table 2. Results of semiquantitative analysis Ultrastructural changes in myocytes from the left ventricular subendocardium.

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Lethal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase A</td>
<td>143 (71.5%)</td>
<td>37 (18.5%)</td>
<td>14 (7%)</td>
<td>4 (2%)</td>
<td>2 (1%)</td>
<td>200</td>
</tr>
<tr>
<td>Phase B</td>
<td>136 (68%)</td>
<td>31 (15.5%)</td>
<td>21 (10.5%)</td>
<td>8 (4%)</td>
<td>4 (2%)</td>
<td>200</td>
</tr>
<tr>
<td>Phase C</td>
<td>140 (70%)</td>
<td>35 (17.5%)</td>
<td>14 (7%)</td>
<td>7 (3.5%)</td>
<td>4 (2%)</td>
<td>200</td>
</tr>
<tr>
<td>Total</td>
<td>419</td>
<td>103</td>
<td>49</td>
<td>19</td>
<td>10</td>
<td>600</td>
</tr>
</tbody>
</table>

Sample size: 20 myocytes for each phase of each patient

Morphometric analysis

For each phase in each patient the tissue samples were photographed using electron microscopy under the same magnification (x 12,000). All photographs (about 250) were scanned and all data were transferred to a computer. These images were processed with the AUTOCAD R14 software from Autodesk, USA. This program offers the possibility of computing the area enclosed by some curve in an image if sufficient perimeter points (points on the curve) are specified by the user. The precision of this area computation depends on the choice and number of the characteristic perimeter points lying on the curve enclosing the image.

In the present study we specified about 30-60 characteristic perimeter points for each image (mitochondrion). The AUTOCAD program gives the required value of the area in abstract drawing units (internal to the program) and not in square inches or centimeters. This allows direct comparison between the area values of the mitochondria in the photos processed, since the same resolution was used for all photos, fixed at x 12,000 in the electron microscopy and a fixed resolution during the scanning process.

Using the graphic AutoCAD R14 program all the non-irreversibly damaged mitochondria were studied (the existence of a double outer membrane, cristae moderately disordered, without flocculent densities) and the area of each mitochondrion was estimated as a percentage of the internal drawing units. In total, the surface of 1697 mitochondria was estimated.

Statistical Analysis

The statistical analysis was accomplished using the SPSS 12.0 statistical package for Windows 2000. The means and the standard deviations were estimated, as well as the 95% confidence interval of the mean for the surface of the mitochondrion in each phase of the study. The mean surface of the mitochondrion in each of the three phases was used for each patient. Analysis of variance - ANOVA - was applied to check the hypothesis tests.

The results of the statistical evaluation of the mitochondrial surfaces in the three different phases are shown in Table 3. No statistically significant differences were observed upon application of one-way ANOVA (F ratio=0.4728, p=0.6295).

Table 3. Statistical evaluation results. Average rates of the mitochondria surfaces.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>St. Dev.</th>
<th>95% Conf. for Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase A</td>
<td>0.0093</td>
<td>0.0025</td>
<td>0.0072 - 0.0114</td>
</tr>
<tr>
<td>Phase B</td>
<td>0.0096</td>
<td>0.0030</td>
<td>0.0071 - 0.0121</td>
</tr>
<tr>
<td>Phase C</td>
<td>0.0104</td>
<td>0.0015</td>
<td>0.0082 - 0.0125</td>
</tr>
<tr>
<td>Total</td>
<td>0.0098</td>
<td>0.0023</td>
<td>0.0088 - 0.0107</td>
</tr>
</tbody>
</table>

The values of the mitochondrial areas are in abstract drawing units (internal in the AutoCAD program).

Using the 95% confidence intervals for the means, it was apparent that there were no statistically significant differences in any of the comparisons (Phase A to Phase B, Phase A to Phase C, Phase B to Phase C).

Discussion

The MIDCAB technique is the less invasive method for surgical revascularization (avoidance of CPB, sternotomy or manipulations in the ascending aorta). With this technique reperfusion of the LAD is possible without significant movement of the heart, resulting in minimal disturbance of coronary flow (Borst and Grundeman, 1999). The MIDCAB technique is mostly applied in proximal LAD disease, and by achieving successful revascularization, blood flow can be restored to a significant part of the myocardium (40-45%).

In the present study, 10 patients with proximal LAD disease and recurrent attacks of angina (repeated ischaemia/reperfusion attacks) were revascularized using the MIDCAB technique. The ischaemic preconditioning technique was not used.

During the anastomotic period, the LAD was snared proximally and distally using two sutures of 4-0 prolene. The biopsy samples taken before the anastomosis were compared with those taken during the time of ischaemia (13-22 min, mean: 16.4 min) and reperfusion.
(25 min after release of the snares and reestablishment of blood flow). All biopsy samples showed some degree of cellular damage.

The preanastomotic biopsy samples showed slight endothelial and mitochondrial damage, most likely due to recurrent attacks of angina (CSS class II-III symptoms). Changes such as cellular oedema, increased collagen levels and lipofuscin accumulation might have been the result of chronic hypoperfusion - «hibernation» of the myocardium (Rainio et al., 1995; Schaper et al., 1975; Vanoverschelde et al., 1993).

The biopsy samples taken during the ischaemia time showed that the normal cardiac fibres far exceeded the number of pathological fibres. There was some degree of intracellular oedema, which was also apparent in a few mitochondria. The mitochondria also had crystal disorganization, a flocculent matrix or external membrane rupture. There were several collapsed capillary vessels due to perivascular oedema but there was no decrease in their pinocytotic vesicles.

The lesions of the 3rd biopsy samples were almost identical, but without perivascular oedema or collapsed vessels.

Endothelial cells are more vulnerable than myocytes to ischaemic injury and ensuing reperfusion (a loss of pinocytotic vesicles and a narrowing of the endothelium). Myocardial oedema is a well-known consequence of ischaemic damage and is due to an increased permeability of capillaries, venules and cellular membranes. This may lead to interstitial oedema and fluid accumulation within the cytoplasm and mitochondria (Lindal et al., 1988; Rainio et al., 1995; Schaper et al., 1992).

Mitochondrial changes preceded changes in the nucleoplasm (Rainio et al., 1995; Schaper et al., 1975). Changes in mitochondria are the most accurate ultrastructural indicator of ischaemic injury and mitochondrial swelling is one aspect that can be quantitated by morphometry (Axford-Gatley et al., 1990; Jennings and Gunote, 1976; Regitz, 1985).

The semiquantitative analysis showed that myocardial changes were mild or moderate, and there were no significant changes in the three consecutive phases. A large percent of the myocytes (83.5-90%) in all phases of the study were normal or had only slight changes. Only a few myocytes (3-6%) were severely or irreversibly damaged (Axford-Gatley et al., 1990; Schaper et al., 1975). The criteria for irreversible cellular damage include: empty mitochondria with loss of parts of their membrane, a clearing of the nucleoplasm with chromatin clamping, a loss of the cell membrane, large vacuoles, and cell debris in the extracellular space. These changes are often confined to single cells, whereas neighbouring cells appear much less damaged. Irreversibility exists when the above criteria are present in more than half of the cells viewed (Jennings and Gunote, 1976; Schaper et al., 1975). It is not clear whether semiquantitative analyses have more or less potential accuracy than morphometric analyses as a measure of ischaemic injury (Axford-Gatley et al., 1990; Lindal et al., 1988; Rainio et al., 1995).

Consequently, we studied mitochondrial oedema in all incompletely destroyed mitochondria. The area of 1697 mitochondria was estimated and the mean area was calculated for each phase in each patient.

Morphometric study of mitochondrial oedema in the three consecutive phases, and subsequent analysis revealed no statistical significant differences. As a result the ischaemic changes can be said to have been slight and transient.

In his preliminary study, Benetti et al. (1986) took myocardial biopsies from four patients suffering from LAD disease who were surgically revascularized using a single vein graft. Two biopsy samples were taken: the first after the initiation of ischaemia and the second six min. after the reestablishment of blood flow. There were no histological changes in the biopsy samples.

From the findings obtained in our study, we conclude that the findings of Benetti et al. (1986) in his preliminary work on a very limited sample of four patients are valid and stand up to statistical analysis. We wish to emphasize that there were no histological changes in the biopsy samples and that our ultrastructural findings are in accordance with our biochemical results (CK-MB, Tr-T).

In conclusion, our study has shown that when the MIDCAB technique is applied for the surgical revascularization of the LAD, there are no significant ischaemic changes to myocardial cells.

**Study limitations**

1. Non randomized study.
2. A complete morphometric study for all organelles of myocardial cells was extremely difficult, and hence our study only focused on the mitochondrial changes.
ACKNOWLEDGEMENTS

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REFERENCES


