Ultrastructural changes in myocardial tissue were studied in 21 patients undergoing elective aorta-coronary bypass operation. The patients were randomized into two groups, with 10 of them receiving continuous retrograde warm and 11 continuous retrograde mild hypothermic blood cardioplegia. Biopsy specimens for electron microscopy were taken from the apical part of the left ventricle before and at the end of the aortic crossclamp period and after reperfusion of the myocardium. The ultrastructural changes were analyzed with use of a semiquantitative scoring system and classified as mild, moderate, or severe. Slight ultrastructural changes were found in both groups even before the aortic crossclamp period. At the end of the aortic crossclamp period the most prominent ultrastructural changes were mitochondrial swelling, damage of capillary endothelium, and clearing of the nucleoplasm or margination of chromatin, but some enlargement in intercalated discs was also discernible. Reperfusion of the myocardium for 15 minutes somewhat further increased the overall score of the ultrastructural changes. Two patients in the warm cardioplegia group had a perioperative myocardial infarction, and this may be one reason for the higher postoperative creatine kinase MB etHux in this patient group. Despite this finding, no major differences in the ultrastructural changes between the two cardioplegia groups could be observed. We conclude that only mild to moderate and principally reversible ultrastructural changes occur in myocardium during continuous retrograde warm and mild hypothermic blood cardioplegia for coronary bypass operation.

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Ultrastructural changes in the myocardium during heart operations have been detected in experimental studies, as well as in human populations. After ischemia of only 10 to 15 minutes, swelling of mitochondria and margination of nuclear chromatin may be observed. Mitochondria are the first cellular components to show the effect and degree of ischemia. Endothelial cells in the microvasculature of the myocardium are also highly vulnerable to ischemic injury. Loss of pinocytotic vesicles and thinning of the endothelium are the first signs of endothelial injury. The “no-reflow” phenomenon, which involves cellular swelling within and around the capillaries, causing occlusion of the lumen, worsening of perfusion, and, finally, ischemic cell damage, was first ascertained in brain capillaries. The same phenomenon has also been observed in the heart.

Adequate protection of the myocardium during heart operations is the major prerequisite for a good outcome. The protective effects of cardioplegic solutions differ, and even very severe cell damage has been described. Myocardial areas distal to complete coronary artery obstructions are poorly protected by antegrade cardioplegia, and retrograde administration of cardioplegic solution through the coronary sinus has emerged as an attractive alternative for these patients. The purpose of this study was to investigate the ultrastructural changes that take place during continuous retrograde warm or mild
hypothermic blood cardioplegia during coronary artery bypass operations.

Methods

Patients. All patients had stable angina pectoris (New York Heart Association [NYHA] class III), three-vessel disease shown by coronary angiography, and normal left ventricular (LV) function as assessed by left ventriculography (Table I). Before operation, the patients signed a consent form approved by the Ethical Committee at the Oulu University Hospital.

Operative and cardioplegic techniques. Twenty-one patients with coronary artery disease undergoing elective coronary artery bypass grafting were randomized into two groups according to the temperature of the cardioplegia solution used. One group (n = 10) was operated on with the use of normothermic blood cardioplegia at a temperature of 37°C and the other (n = 11) with mild hypothermia at a temperature of 28°C.

The blood cardioplegia was diluted in a 7:1 (Dideco D 720 blood cardioplegia delivery set, Dideco S.P.A., Mirandola, Italy) ratio with a solution containing aspartate monohydrate and glutamate monohydrate in 300 ml of water (13 mmol/L each), 250 ml of 5% glucose, 150 ml of tribonate (TRIS bicarbonate; Tribro rat, Kabi, Sweden), 125 ml of citrate-phosphate-dextrose, and 40 ml of KCl (2 mol/L).

After aortic crossclamping warm high-potassium cardioplegic solution was administered into the aortic root for 2 minutes at a flow rate of 200 ml/min, and as soon as the heart was arrested the delivery of cardioplegia was switched to a retrograde method and continued with low-potassium cardioplegia in a randomized manner (37°C or 28°C) via a self-inflating coronary sinus cannula (catalog number Rc-014T, Research Medical, Inc., Valencia, Calif.) was done before induction of cardiac arrest and aortic crossclamping. The second biopsy was done before aortic declamping and the third after 15 minutes of reperfusion during cardiopulmonary bypass. All the biopsy samples were taken from the apical parts of the left ventricular wall, which was macroscopically normal. For technical reasons, the first biopsy sample was not available for analysis in two patients in the mild hypothermic group, the second biopsy sample from two patients in the warm cardioplegic group, and the third biopsy sample from one patient in both groups. The tissue plug specimens were immediately fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.4, for 1 hour and then postfixed in 1% OsO$_4$ in 0.1 mol/L phosphate buffer, pH 7.4, for 1 hour, dehydrated in acetone, and embedded in Epon LX 112 fixative. Semithin sections stained with toluidine blue were prepared from all tissue samples. The sections were cut with a Reichert Ultracut E-ultramicrotome (Leica, Inc., Buffalo, N.Y.) and examined under a Philips 410 LS transmission electron microscope (Philips Electronic Instruments, Inc., Mahwah, N.J.), with use of an acceleration voltage of 60 kV.

Scoring. The intercellular junctions (intercalated discs) intracellular and extracellular edema, mitochondria, capillaries, nuclei and myofibrils were analyzed separately in each biopsy specimen by a semiquantitative method with scoring from 0 (unchanged) to 3 (severe alterations). The electron microscopist was blinded as to the sequence of the specimens and the group to which the patients belonged. A total score of all ultrastructural changes less than 5 was defined as mild damage, scores ranging from 5 to 10 as moderate, and scores exceeding 10 as severe ultrastructural damage.

Statistical analysis. Statistical analysis was done with use of the SPSS statistical package program (SPSS, Inc., Chicago, Ill.). Unpaired Student’s test and Fisher’s exact test were used to compare the clinical characteristics and
Fig. 1. Postoperative CK-MB effluxes in warm (closed circles) and mild hypothermic (open circles) cardioplegia groups.

Table II. Summary of stenoses of coronary arteries

<table>
<thead>
<tr>
<th>Cardioplegia</th>
<th>LMCA</th>
<th>LAD</th>
<th>CX</th>
<th>RCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%-50% &gt;50%</td>
<td>0%-80% &gt;80%</td>
<td>0%-80% &gt;80%</td>
<td>0%-80% &gt;80%</td>
</tr>
<tr>
<td>Warm</td>
<td>7     3</td>
<td>3   7</td>
<td>7   3</td>
<td>2   8</td>
</tr>
<tr>
<td>Hypothermic</td>
<td>8     3</td>
<td>9   5</td>
<td>6   3</td>
<td>8   8</td>
</tr>
<tr>
<td>Total</td>
<td>15    6</td>
<td>16</td>
<td>15</td>
<td>16</td>
</tr>
</tbody>
</table>

There are no statistical differences between the patient groups. LMCA, Left main coronary artery; LAD, left anterior descending artery; CX, circumflex artery; RCA, right coronary artery.

Results

Clinical data. Clinical data (number of patients, age, NYHA classification, ejection fraction) and operative data (crossclamp time, cardiopulmonary bypass time, ischemia time, number of distal anastomoses) were comparable between the two patient groups (Table I). Coronary stenoses were analyzed separately, and in this respect the groups were also comparable (Table II). There were no significant differences in the preoperative and postoperative values of the cardiac index or the calculated left and right stroke work indexes (not shown). The postoperative CK-MB efflux in both groups is shown in Fig. 1 and this was significantly \( p = 0.005 \) higher in the warm cardioplegia group. Two patients in the warm cardioplegia group had CK-MB levels greater than 60 IU/L, but no new Q waves were observed.

Ultrastructural changes. The most typical feature in the first biopsy specimens taken before aortic crossclamping was increased intracellular edema, but slight mitochondrial and capillary endothelial changes could also be observed in both study groups (Table III). At the end of the aortic crossclamp period ultrastructural changes became more prominent and they were noticed in all the analyzed cell structures. Enlargement of disc interspaces (intercalated disc), mitochondrial swelling, clearing of the nucleoplasm or margination of chromatin, and intracellular edema were the most conspicuous features. The ultrastructural changes of intercalated discs, mitochondria, and nuclei were time-dependent and increased on reperfusion; however, warm
Table III. Semiquantitative scoring of the ultrastructural changes in warm and mild hypothermic cardioplegia groups

<table>
<thead>
<tr>
<th></th>
<th>Warm</th>
<th>Hypothermic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Intercalated disc</td>
<td>0 ± 0</td>
<td>0.11 ± 0.11</td>
</tr>
<tr>
<td>Edema (NS)</td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Mitochondria (p = 0.001)</td>
<td>0.5 ± 0.2</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Capillaries (NS)</td>
<td>0.7 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Nuclei (p = 0.065)</td>
<td>0.4 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Myofibrils (NS)</td>
<td>0.6 ± 0.3</td>
<td>0.8 ± 0.2</td>
</tr>
</tbody>
</table>

The biopsy samples were taken before aortic crossclamp (I), before aortic declamp (II), and at the end of cardiopulmonary bypass (III). The p values in parentheses refer to time-dependent changes (repeated measures of analysis of variance); there were no statistically significant differences in ultrastructural changes between the two cardioplegia groups. NS, Not significant.

Table IV. Number of patients in different classes of ultrastructural changes

<table>
<thead>
<tr>
<th>Ultrastructural changes</th>
<th>Warm (10)</th>
<th>Mild hypothermic (11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Slight</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Moderate</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Severe</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

One biopsy specimen could not be obtained for analysis at each stage (stages I through III as described for Table III) in both groups. There were no statistical differences in the distribution of ultrastructural changes in different classes between the two study groups.

and mild hypothermic cardioplegia groups did not differ from each other. Lipofuscin and myelin figures were also seen in both groups.

The ultrastructural changes were analyzed by a semiquantitative method and classified as mild (score <5), moderate (5 to 10), or severe (>10). In the second biopsy specimens taken before aortic declamping slight ultrastructural changes were detected in five patients in both groups. Moderate ultrastructural changes were found in four patients in the warm cardioplegia group and in five patients in the mild hypothermic cardioplegia group. Severe irreversible damage was not observed in any of the samples (Table IV). In slight ultrastructural changes the basement membrane of the endothelium was well preserved, and the number of pinocytotic vesicles was normal, as were the mitochondrial cristae. Lipid-containing intramitochondrial vacuoles were found in the specimens of two patients, one in both groups. The size and shape of the nuclei were normal. In the biopsy specimens classified as having moderate ultrastructural changes, the size and shape of the mitochondria varied, and the mitochondrial matrix contained amorphous material, such as rod-like inclusion bodies. Crystalline inclusions and electron-dense particles were also observed (Figs. 2 and 3). After reperfusion intracellular edema was estimated to be somewhat more pronounced (although this was not significant) in the mild hypothermic group, and it formed blebs in the sarcoplasmatic reticulum (Fig. 4). The basement membrane of the endothelium was disrupted and endothelial swelling was remarkable (Fig. 5). Two specimens of patients in the mild hypothermic group showed a homogeneous fluidlike content in the capillary lumen and intracytoplasmic vacuoles (Fig. 6).

Signs of the no-reflow phenomenon were seen in five patients of the warm group and in six patients of the mild hypothermic group, and these signs were evident in both the second and third sets of biopsy specimens (Fig. 7).

Collapsing of the endothelium and increased extracapillary edema were typical findings, but the endothelial basement membrane remained intact.

The mean ischemic times were 5.2 ± 2.8 (5.3%) minutes and 8.4 ± 8.3 (8.3%) minutes in the warm and mild hypothermic groups, respectively (difference not significant). The ischemic times did not correlate with the score values of ultrastructural changes in either of the study groups.

The ultrastructural changes in the third biopsy samples taken after release of the crossclamp were somewhat more prominent than those in the second biopsy samples taken at the end of aortic crossclamping.

The score values in patients who received aprotinin during the operation did not differ from the values in patients who did not receive aprotinin.

Discussion

In this study 21 patients with coronary artery disease undergoing elective coronary artery bypass grafting were randomized into two groups on the
basis of the temperature of the retrograde blood cardioplegia solution. The ultrastructural changes that occurred in the myocardium during the operations were subsequently analyzed. The cell damage during aortic crossclamping (short-time ischemia) and reperfusion (15 minutes after aortic declamping) was compared to that observed in biopsy samples taken before aortic crossclamping. All the biopsy samples were taken from macroscopically normal left anterior myocardium.

It has been shown earlier that the results of semiquantitative analysis correlate well with those of morphometric analysis, making it a useful non-parametric method for studying ischemic cell damage. Mitochondria have been suggested to be better preserved during warm retrograde than cold antegrade cardioplegia, but no differences have been found between blood antegrade or intermittent cardioplegia. We found the most distinct ultrastructural changes in the mitochondria of myocytes and the endothelium. In some cases mitochondria showed a honeycomb appearance and zigzag profiles of cristae, but they were not totally destroyed. One patient in the mild hypothermic group showed the development of intramitochondrial Jennings bodies, which are granular densities of calcium phosphate caused by permanent ischemia. Crystaline inclusions and rodlike bodies were also found in one patient, but they can be regarded as a nonspecific feature of cell damage.

It has been suggested that low protein-denatur-
Fig. 4. Transmission electron micrograph showing subsarcolemmal edema forming blebs (arrowheads) (original magnification ×21,300).

Fig. 5. Transmission electron micrograph showing swelling of endothelial cell (e) and disruption of basement membrane (arrowhead) (original magnification ×28,000).
Fig. 6. Transmission electron micrograph showing degeneration of endothelial cell (e) with intracytoplasmic myelin vacuole (m). Capillary lumen (L) is filled with homogeneous fluid (original magnification ×28,000).

Fig. 7. Transmission electron micrograph showing collapsed capillary (c) with increased extracellular edema (e) (original magnification ×14,200).
non is associated with reperfusion injury in open heart operations. On the other hand, it may well be that some of the changes, such as cellular edema, increased collagen, and lipofuscin accumulation might have been a result of chronic "hibernation" of the myocardium, which is known to be associated with certain ultrastructural changes. 12-14

Aprotinin is a protease inhibitor that might protect the myocardium from the adverse effects of ischemia by suppressing the release of lysosomal enzymes. The mitochondria and myofibrils of isolated canine hearts given aprotinin are better preserved than those without aprotinin but, on the other hand, aprotinin may impair the functional recovery of the left ventricle. 15 Both of our cardioplegia groups included three patients who received aprotinin during the operation, but no differences in their postoperative recoveries could be detected, nor were their mitochondrial changes different from those of the patients not given aprotinin.

Significant differences of CK effluxes after the operations were noticed in favor of the mild hypothermic group. Two patients from the normothermic cardioplegia group had a perioperative myocardial infarct, which increased the CK-MB value, and this may be one reason for the observed difference. There was no perioperative or 30-day mortality in this series. It was interesting to note that the two patients with perioperative myocardial infarctions also showed the most severe myofibrillar changes in the biopsy specimens.

We conclude that continuous retrograde blood cardioplegia protects myocardium sufficiently to prevent extensive cellular damage, inasmuch as only slight to moderate ultrastructural changes were observed during coronary bypass operations of considerable length. No significant differences in the myocardial ultrastructure could be detected in the normothermic or mild hypothermic cardioplegia groups.

REFERENCES