Ultrastructural Evidence of Microvascular Damage and Myocardial Cell Injury After Coronary Artery Occlusion: Which Comes First?

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SUMMARY Both microvascular damage and myocardial cell injury occur after coronary occlusion, but the relationship of these two events is unclear; specifically, it is unknown whether microvascular damage causes myocardial cell injury. Dogs were subjected to coronary occlusion for 20, 40, 60, 90 or 180 minutes, after which subendocardial and subepicardial biopsies were obtained for electron and light microscopy of 1-μ sections. Of 312 biopsies of ischemic myocardium, 181 showed myocardial cell injury with no microvascular damage; 131 showed myocardial cell injury and microvascular damage; but none showed microvascular damage without myocardial cell injury. Although ultrastructural evidence of myocardial cell damage was present in the subendocardium after 20–40 minutes of ischemia, ultrastructural evidence of microvascular damage was not prominent until 60–90 minutes after coronary artery occlusion. Morphologic ultrastructural evidence of microvascular damage lagged behind myocardial cell injury, suggesting that ultrastructural microvascular damage is not a primary cause of ultrastructural myocardial cell injury.

IN THE FIRST FEW HOURS after experimental coronary occlusion, morphologic ultrastructural abnormalities occur not only in the myocardial cells, but also in the microvasculature. Some investigators have postulated that damage to the microvasculature might impede coronary blood flow either during coronary occlusion or upon release of coronary occlusion, thus exacerbating ischemic damage and contributing to myocardial cell injury. With renewed interest in the possibility of emergency coronary artery bypass surgery in patients with acute myocardial infarction, the condition of the microvasculature after coronary occlusion gains new significance. However, the relationship of the extent of myocardial cell injury and the degree of microvascular damage during the early hours of coronary occlusion has not been clarified. Specifically, if microvascular damage were a cause of myocardial cell injury, it should be possible to identify areas of myocardium in which microvascular damage is present before the development of myocardial cell injury. This investigation was undertaken to study the relationship between the ultrastructure of microvascular damage and that of myocardial cell injury within the same anatomic areas over time. Damage to both these structures was assessed in the severely ischemic subendocardial and more mildly ischemic subepicardial myocardium.

Methods

Open-chest mongrel dogs were anesthetized with 10 mg/kg of i.v. sodium thiamylal, intubated and ventilated with room air delivered via a Harvard ventilator. Thoracotomies were performed through the fifth left intercostal space and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery was isolated above the first major diagonal branch 2.0–2.5 cm from the origin of the left main coronary artery. High left anterior descending coronary artery occlusions were performed for 20 minutes in 12 dogs, 40 minutes in nine dogs, 60 minutes in nine dogs, 90 minutes in 15 dogs, and 180 minutes in two dogs. The presence of ischemia was confirmed by the presence of an area of visible epicardial cyanosis. At the end of each occlusion period, transmural biopsies were obtained in vivo from the center of the ischemic region with a specially designed biopsy tool (a modified Tru-Cut Travenol biopsy needle). One or two specimens were also obtained from nonischemic regions that were always supplied by the circumflex coronary system. These specimens were immediately placed in Karnovsky’s fixative and were divided into subendocardial and subepicardial halves. They were fixed for 3 hours and each biopsy (seven to nine per dog) was postfixed in cold 1% osmium, dehydrated in graded alcohols, placed in propylene oxide and then a 1:1 mixture of propylene oxide and Epon 812. The specimens were then embedded in Epon 812. One-micron sections were stained with toluidine blue for light microscopy and thin sections were stained with uranyl acetate and lead, citrate for electron microscopy. Two thick and thin sections from each biopsy were examined without knowledge of their origin, and classified as follows:

a. biopsy showed myocardial cell injury with no microvascular damage;
b. biopsy showed myocardial cell injury plus microvascular damage;

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c. biopsy showed microvascular damage with no myocardial cell injury; or
d. biopsy showed no myocardial cell damage and no microvascular damage.

In addition, morphometric analysis of myocardial cell injury was performed on all 1-μ thick sections by placing a grid with 36 points in the eyepiece of the microscope and examining all sections at magnification × 1000. Both cross sections and longitudinal sections were used. The grid points were spaced such that no more than one point fell on one cell. The grid was placed over two non-overlapping fields per section. Each myocardial cell received a semiquantitative histologic grade of injury according to the following scheme:

0 = normal myocardial cell; 1+ = nuclear chromatin clumping alone or with occasional vacuoles; wide I bands indicative of myocardial relaxation; 2+ = the above plus intermyofibrillar edema and more vacuoles; 3+ = the above plus numerous vacuoles and/or the sarcolemmal membrane lifted off the myofibrils; and 4+ = severe swelling and architectural disruption.

When each point falling on a myocardial cell was assigned a numerical value of 0–4, a distribution of severity of ischemic damage per section was determined. A mean ischemic score was determined for each biopsy by calculating the average severity (0–4) of myocardial cell injury.

Mean ischemic scores derived from this grading system have been shown to correlate with the degree of ischemia as assessed by regional myocardial blood flow and the rise in intramural Pco2;10 also, this grading system has been useful in showing early reduction of ischemic damage by pharmacologic interventions.11

In addition, the degree of damage to the microvasculature within the grid field was recorded. The de-
gree of myocardial cell damage and concomitant microvascular damage seen on electron micrographs of corresponding sections was also recorded.

Results

Both myocardial cells and the microvasculature from the nonischemic myocardium appeared normal (fig. 1). After coronary occlusion the degree of damage in myocardial cells became progressively worse. The damage was evident primarily in the subendocardial half of the myocardium 20–90 minutes after the start of coronary occlusion. At 20 minutes the alterations consisted of nuclear chromatin clumping, wide I bands, loss of glycogen, and, in some cells, intermyofibrillar edema and early mitochondrial swelling as manifested by loss of mitochondrial matrix density and separation of cristae (fig. 2). By 40 minutes interstitial edema and mitochondrial swelling were more severe and some mitochondria contained amorphous dense matrix bodies (fig. 3). At 60–180 minutes intracellular edema was more prominent, mitochondrial dense bodies were numerous and breaks in the sarcolemmal membrane were present (figs. 4–6).

Ultrastructural endothelial damage was not seen until after 60 minutes of ischemia, was most prominent in the subendocardium and consisted of a loss of: (1) pinocytotic vesicles, (2) localized areas of endothelial swelling, which have been referred to as endothelial blebs, (3) endothelial gaps, (4) occasional foci of hemorrhage, and (5) intra- and extravascular fibrin deposition (fig. 6). At 60 minutes endothelial changes were present in approximately 20% of vessels and by 90–180 minutes these changes were present in approximately 40% of vessels, with another 20% showing loss of pinocytotic vesicles alone.

Red blood cell stasis was occasionally present after 40 minutes of coronary occlusion; it was present in some vessels that otherwise appeared normal at 60
FIGURE 3. Ischemic myocardium after 40 minutes of coronary occlusion. The nucleus (n) shows chromatin clumping and margination. Intermyofibrillar edema (e) is marked. Mitochondria (m) are swollen, with loss of matrix density and separation of cristae. Some mitochondria contain amorphous matrix densities (arrow), which some authors have associated with irreversible myocardial injury. Glycogen is absent. Again, the capillary (c) appears intact. Magnification \( \times 11,250 \).

The comparison of myocardial cell grade and concomitant microvascular damage is shown in table 1. When myocardial cells were normal (grade 0) or showed 1+ changes, microvascular damage was lacking. When myocardial cells demonstrated 2+ changes, the endothelial cells usually appeared normal, with occasional localized zones of swelling in a very small percentage of capillaries. Endothelial damage was more prominent when myocardial cells showed grade 3+ and 4+ damage. Microvascular damage remained confined within and never progressed outside areas minutes and in damaged vessels, and was prominent in otherwise normal-appearing vessels and abnormal vessels at 90 minutes of coronary occlusion.

Of 312 biopsies from ischemic myocardium, 181 biopsies showed myocardial cell injury with no microvascular damage and 131 biopsies showed myocardial cell injury plus microvascular damage; no biopsies showed microvascular damage without myocardial cell injury. Of 90 biopsies from non-ischemic sites, all showed normal myocardial cells and normal microvasculature.
that already showed myocardial cell damage. Hence, ultrastructural microvascular damage tended to lag behind myocardial cell damage.

Figure 7 shows the mean ischemic score for myocardial cells. The mean ischemic score increased as a function of the duration of ischemia, and the subendocardial ischemic zone always showed more severe damage at any given time interval than the subepicardial region. The microvascular damage as it relates to cell grades is plotted on the right. Again, 20–40 minutes after the start of ischemia, significant myocardial cell damage is present, with little or no microvascular damage.

Discussion

Specific microvascular ultrastructural abnormalities have been described during the early stages of coronary artery occlusion, and have been associated with the “no-reflow phenomenon” (inability to reperfuse a previously ischemic region). These microvascular changes include loss of pinocytotic vesicles, paleness of the endothelium, local-
Table 1. Myocardial Cell Grade and Concomitant Microvascular Damage

<table>
<thead>
<tr>
<th>Myocardial cell grade</th>
<th>Concomitant microvascular abnormality</th>
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<tr>
<td>0—Normal</td>
<td>Normal</td>
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<tr>
<td>1—Nuclear chromatin clumping alone or with occasional vacuoles; wide I bands</td>
<td>Normal</td>
</tr>
<tr>
<td>2—Criteria for grade 1 plus intermyofibrillar edema and more vacuoles; swollen mitochondria</td>
<td>Endothelium usually normal; occasionally swollen (&lt;2%); occasional red blood cell stasis</td>
</tr>
<tr>
<td>3—Criteria for grade 2 plus numerous vacuoles and sarcolemmal membrane lifted off myofibrils</td>
<td>Endothelium often normal; or decreased pinocytotic vesicles, swelling, blebs, gaps; red blood cell stasis</td>
</tr>
<tr>
<td>4—Severe swelling and architectural disruption</td>
<td>Endothelial blebs, gaps; foci of hemorrhage</td>
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ized areas of endothelial swelling as manifested by intraluminal membrane-bound protrusions or blebs, endothelial gaps, and, less frequently, intravascular or extravascular fibrin deposition, platelet plugs and foci of hemorrhage. Areas of red blood cell stasis are often observed, but their significance is uncertain. This phenomenon could be secondary to low collateral blood flow in the ischemic zone or represent increased local blood viscosity.

Some investigators have postulated that microvascular damage may contribute to irreversible ischemic injury of the myocardial cells by impeding collateral blood flow either during the coronary occlusion or upon reflow. If this is so, one might expect to find areas in which ultrastructural microvascular damage occurred before the development of ultrastructural myocardial cell damage. In the present study, we did not see such areas; rather, morphologic damage to myocardial cells occurred before the development of ultrastructural evidence of microvascular damage. This was true of both subendocardial and subepicardial layers of myocardium at 20, 40, 60, 90 and 180 minutes after coronary occlusion. Ultrastructural damage of the myocardial cells was clearly visible by 20–40 minutes of ischemia in the subendocardium, while the microvasculature did not begin to show ultrastructural damage in the same regions until 60–90 minutes of ischemia.

These results suggest that microvascular damage is not a primary cause of the development of irreversible ischemic myocardial cell damage; the results are in agreement with a study by Reimer et al., who showed that infarcts produced by transient ischemia varying from 40 minutes to 6 hours followed by 4 days of reperfusion had areas of hemorrhage which, by gross inspection and histology, were always confined within and never extended outside areas of myocardial cell necrosis. In the present study, ultrastructural evidence of microvascular damage was similarly confined to areas where the myocardial cells already appeared severely damaged, and never extended outside of these areas. Conversely, areas with mild and severe myocardial cell damage but without microvascular damage were present. Presumably, areas with reversible myocardial cell injury and an intact microvasculature should be available for reperfusion and hence myocardial salvage. Recent studies in our laboratory and also in Jennings's and Reimer's laboratory have shown that reperfusion 20 minutes to 3 hours after coronary artery occlusion results in

FIGURE 5. Ischemic myocardium after 180 minutes of coronary occlusion. There is striking intracellular edema with swollen mitochondria and intramitochondrial amorphous dense bodies (arrows). Glycogen is absent. There are breaks (arrowheads) in the sarcolemmal membrane. The capillary endothelium (en) shows a decrease in pinocytotic vesicles but is otherwise intact. Other capillaries at 180 minutes did show damage. Magnification × 13,500.

FIGURE 7. Mean ischemic score for myocardial cells and the concomitant microvascular damage and duration of occlusion for both subendocardial and subepicardial myocardium. The subendocardium always showed more severe damage at any given time interval than the subepicardium. Between 20–60 minutes of ischemia, significant myocardial cell damage was present, with little or no microvascular damage. RBC = red blood cell.
Figure 6. Ischemic myocardium after 180 minutes of ischemia. The myocardial cell is edematous and the myofilaments are in disarray. Mitochondria (m) are swollen and the cristae are disrupted. Glycogen is absent. Sarcolemmal breaks (arrow head) are numerous. The capillary shows swollen endothelium (arrow), intraluminal blebs (b) and loss of pinocytotic vesicles; the endothelial nucleus (n) shows clearing of central chromatin with chromatin margination. Magnification × 20,000.
significant myocardial salvage in the outer myocardial wall, where myocardial cells are reversibly injured and the microvasculature is usually intact.12,16

The present study was not a functional study and hence does not rule out the possibility that functional microvascular abnormalities might precede ultrastructural abnormalities. Certainly, functional abnormalities of myocardial cells consisting of abnormal contraction occur within seconds after coronary occlusion — well before morphologic abnormalities occur. Altered permeability could occur in endothelial cells before the development of morphologic evidence of ultrastructural damage. However, a recent study by Fishbein et al.17 showed that when colloidal carbon-black was used as a marker of increased capillary permeability, vessels with increased permeability were always confined well within areas of histologic necrosis and never extended beyond them.

Acknowledgment

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