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BRIEF METHODS

Identification of Necrotic Tissue by Phase–Contrast Microscopy at an Early Stage of Acute Myocardial Infarction

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In conventional light microscopy, the identification of necrotic tissue at an early stage (less than 10 hours after coronary occlusion) of acute myocardial infarction is limited by the lack of contrast between necrotic and non-necrotic myocytes. In the present study, use of phase-contrast microscopy for analysis of hematoxylin eosin stained sections of microembolized myocardium increased the contrast between necrotic and non–necrotic fibers considerably. As verified by electron microscopy, phase–contrast microscopy truly identified irreversibly injured myocardium. Therefore, use of phase–contrast microscopy may improve the detection of early myocardial infarction.

Myocardium that has been ischemic for days or weeks is clearly detected as necrotic by standard histological analysis. The criteria of necrosis include dissolution of myocyte nuclei (karyolysis) and myocyte cells, infiltration of leukocytes, and increased fibroblasts and collagen fibers (Mallory et al, 1939). In contrast to a fully developed myocardial infarct, the histological diagnosis of acute myocardial necrosis remains difficult. The classic criteria of early necrosis, ie, hypereosinophilia, contraction bands, thinning and waviness of fibers, as well as the increase in the intercellular space (Bouchardy and Majno, 1974; Fishbein et al, 1978), can be so subtle that even large necrotic foci are not clearly detected. Therefore, different techniques, based on fluorescence microscopy, immunofluorescence microscopy, or electron microscopy, have been established to identify early myocardial necrosis (Hayakawa et al, 1984; Schaper, 1990; Sybers et al, 1972). These methods can identify necrotic fibers in an early stage of acute myocardial infarction but are time-consuming and require long–term experience and technical resources, which are not easily available.

To overcome these problems, we evaluated the potential of phase–contrast microscopy to detect early myocardial necrosis. To our best knowledge, this is the first report emphasizing the advantages of phase–contrast microscopy for identifying early myocardial infarction.

Two different animal models of myocardial infarction were used. In the first model, patchy necroses were induced in 6 anesthetized dogs by injection of microspheres (42 μm diameter; 3000 per ml/min inflow) into the left circumflex coronary artery. Eight hours later myocardial samples were taken from control and microembolized areas. In the second model, myocardial infarcts were induced in 6 anesthetized pigs by reducing coronary blood flow by 40–50% over 1½ hours followed by 4 hours of reperfusion. Tissue samples were taken from control and ischemic areas.

In a first step, tissue samples, taken from both models, were fixed in formaldehyde (4%) and embedded in paraffin. From the total of 24 tissue samples, 99 sections with a thickness of 7 μm were stained with hematoxylin eosin. Sections with patchy necrosis and with confluent infarcts were analyzed by light microscopy (DMLB, Leica, Bensheim, Germany; at magnifications of ×40, ×100, and ×200), with and without phase–contrast. The analyzed area of each section was 55 ± 19 (SD) mm^2, ranging from 25 to 114 mm^2.

In a second step, results of phase–contrast microscopy were compared with those of electron microscopy. Clearly adjacent samples for light– and electron microscopy were taken from the control area, the center, and the border of a confluent myocardial infarct (pig model, infarct area was macroscopically identified by TTC staining). The samples for light microscopy were treated, as described above. Samples for electron microscopy were fixed in glutaraldehyde (4%), postfixed in osmium tetroxide (2%), dehydrated in a graded series of alcohols, and embedded in epon. Ultrathin sections were stained with uranyl acetate and lead citrate and were analyzed using electron microscopy (EM 900, Zeiss, Oberkochem, Germany). Electron microscopic comparison of phase–contrast to microscopic results from tissue samples with patchy necrosis (dog model) was not performed, because the necrotic areas could not be macroscopically identified in TTC staining.

In conventional bright–light microscopy (Fig. 1, left panels) necrotic foci are subtle and hard to distinguish from surrounding viable myocardium. In phase–contrast microscopy necrotic foci are more prominent (Fig. 1, right panels). Additionally, necrotic foci, viewed with phase-contrast microscopy, have a clear border to the surrounding viable myocardium.

Electron microscopy (Fig. 2, left panel) confirmed that necrotic tissue, as identified by phase–contrast microscopy, is indeed irreversibly injured. Reversibly injured myocardium (middle panel) is similar in contrast as control myocardium (right panel) in phase–contrast microscopy.

Phase–contrast microscopy enhances the contrast between necrotic and non–necrotic myocardium. The enhanced contrast improves the detection of even small necrotic foci in otherwise viable myocardium, and the clear border of necrotic foci also facilitates histological quantification of necrosis. Electron microscopy reveals information about particular cellular structures, such as mitochondria, nucleus, and sarcomeres of ischemic cell injury (Schaper, 1990). Phase–contrast microscopy is inferior in this respect; however, it requires less technical resources and is applicable to most light microscopes. Additionally, no special tissue preparations or staining procedures are needed. Even the infusion of marker substances, as required for some fluorescent (Sybers et al, 1972) and immunofluorescent (Kajstura et al, 1996) methods, is not necessary.

Therefore, phase-contrast microscopy is a helpful microscopic technique for the identification and quantification of necrotic tissue in an early stage of myocardial infarction. Analysis of autopsy material revealed that this procedure is also applicable to human tissue.

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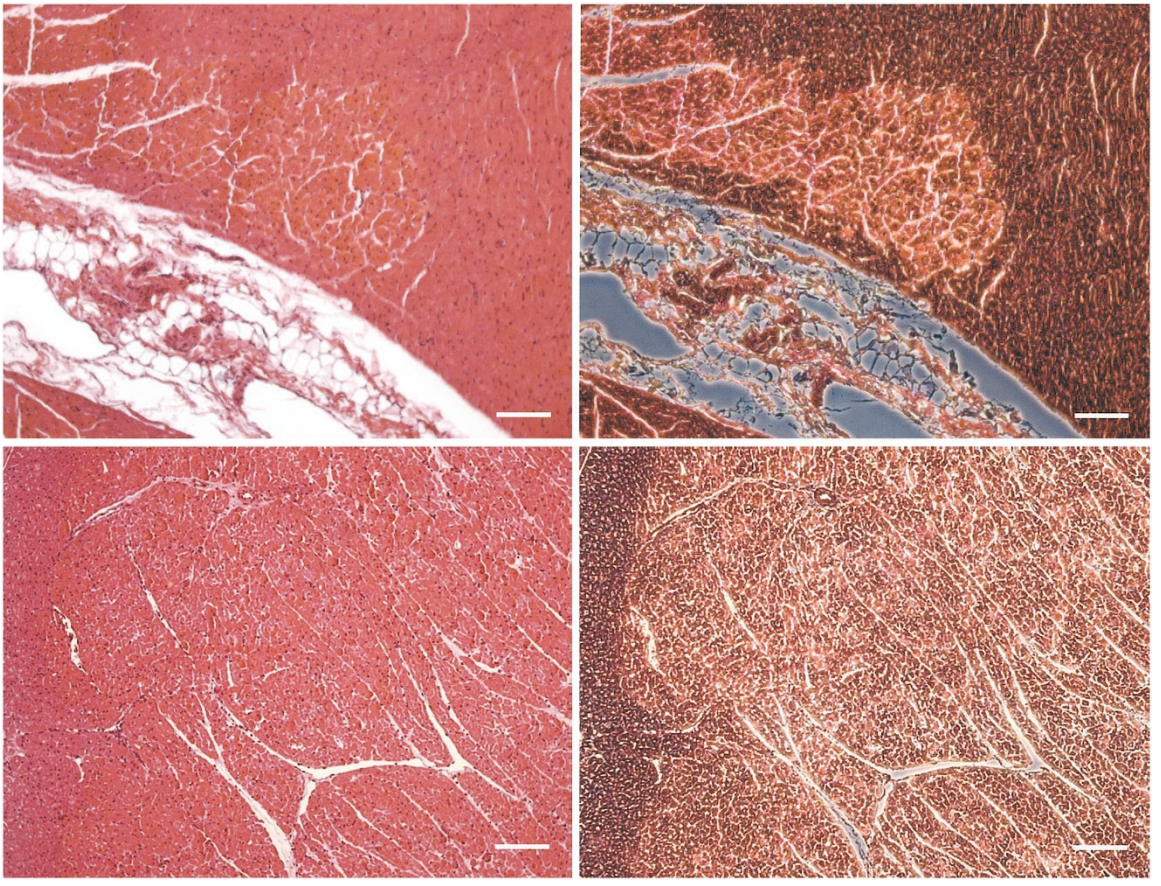

Figure 1.

Light microscopy of myocardial infarction. In conventional bright-light microscopy (left panels) the necrotic foci are hard to distinguish. Phase-contrast microscopy (right panels) enhances the contrast between the necrotic myocytes and non-necrotic myocytes and, therefore, facilitates the histological identification of myocardial infarction. Upper panels: canine heart; lower panels: swine heart. *Bar*, 100 μm.

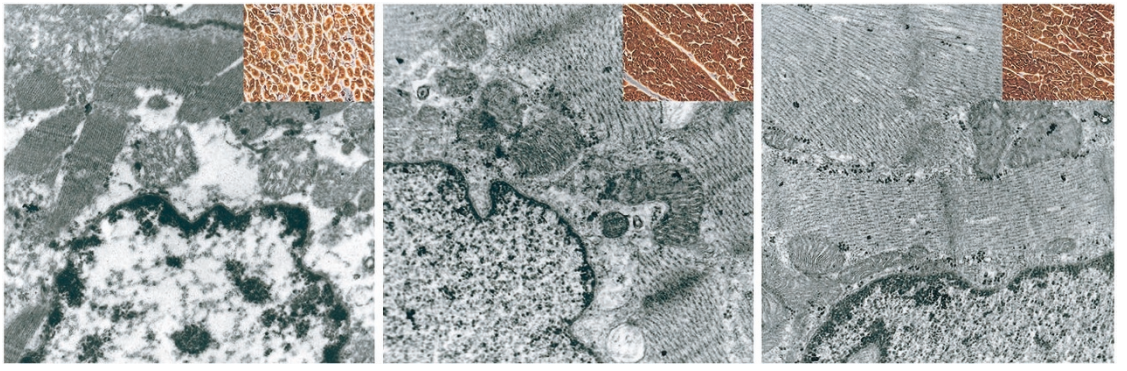

Figure 2.

Electron microscopy of myocardial tissue. Left panel displays irreversibly injured myocardium from the infarcted area. The chromatin of the nucleus is clumped and marginated. The mitochondria are swollen, partly disrupted, and contain flocculent densities. In addition, contraction bands are prominent. The middle panel displays myocardium taken from the border zone of the infarct. There is a moderate sarcomere relaxation and swelling of mitochondria. Normal nuclei and mitochondria from control myocardium are displayed in the right panel. The right upper corner of each picture indicates the phase-contrast microscopy images of tissue samples taken from the same area as the tissue samples taken for the electron microscopy. Notice that only in irreversibly injured myocardium the myocardial fibers are brighter than in healthy myocardium. Magnification, $\times$13,000 (left panel); $\times$22,000 (middle and right panel).

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