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Editorial

Ultrastructural findings in hearts with regional ischaemia

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The connection between coronary artery occlusion and myocardial infarction was first recognized by Herrick (1912) and Obrastzow and Straschesko (1910). In the development of myocardial necrosis, the myocytes reach a point where they are unable to regain normal function and structure upon reperfusion, but the period endured by the myocytes depends on a number of factors such as the degree of blood flow reduction, cardiac work load, temperature, and the species under study. Irreversible cell injury probably first develops in the central part of the hypoperfused myocardium. The reduction in blood flow appears to be more severe here than at the periphery, where the myocytes may benefit from collateral blood flow. As shown in the dog, coronary occlusion provokes a more pronounced reduction of blood flow in the subendocardium than in the subepicardial parts of the ventricular wall. Thus, lateral as well as transmural extension of the infarct has been reported (Homans et al. 1985; Reimer and Jennings 1979).

During the last three decades, irreversible ischaemic cell injury has been studied thoroughly. An extensive cytoplasmic oedema, sarcolemmal fragmentation, nuclear chromatin clumping and margination, nuclear membrane fragmentation. accumulation of dense bodies in the mitochondria combined with mitochondrial swelling and cristeolysis signify irreversible cell injury (Greve et al. 1989; Kloner et al. 1983). Such signs of irreversible cell injury have been observed 10–20 min after coronary occlusion in vivo (Greve et al. 1990; Spinale et al. 1989).

Morphological signs of reversible cellular damage have been studied less intensively and the order and significance of each step preceding cell death is far from fully comprehended. These injuries, which are less obvious than irreversible injuries, probably indicate changes in pH, metabolism, and electrolyte distribution. After 10 min of occlusion of the left anterior descending coronary artery (LAD) in cat hearts, we have observed moderate cytoplasmic oedema, accumulation of lipid droplets, and swelling of the mitochondria without any signs of cristeolysis or accumulation of mitochondrial dense bodies (Greve et al. 1990). Some of these cells have an isolated disruption of the sarcolemma, which is occasionally accompanied by restricted cytoplasmic oedema beneath the hole. The superjacent basal lamina usually remains intact. Thus, morphologically sarcolemmal damage seems to take place in two steps: small isolated disruptions and extensive fragmentation. The sarcolemma is probably weakened during ischaemia. The stress added by the fixation, dehydration, and embedding procedures may provoke rupture of the fragile sarcolemma and be responsible for the isolated disruptions observed. Membrane fragmentation, however, probably occurs in vivo. Ischaemically induced cell swelling imposes an additional burden on the sarcolemma, but the swelling is probably not able to rupture the intact membrane per se (Jennings et al. 1986; Steenbergen et al. 1985). However, an extensive cellular oedema may well cause rupture of an already weakened membrane. Prolonged depletion of high energy phosphates in ischaemia may induce disintegration of the cytoskeleton and contribute to the damage of the superjacent sarcolemma (Ganote and Vander Heide 1987; Steenbergen et al. 1985, 1987). However, Jennings et al. (1989) have shown that ATP depletion does not necessarily cause prompt sarcolemmal disruption. Furthermore, during ischaemia sarcolemma as well as other cell membranes lose phospholipids by calcium activation of membrane lipases (Katz and Messineo 1981; Post et al. 1988).

Before the infarct has reached its final size, it is surrounded by a hypoperfused, but still non-necrotic zone. The entire myocardium originally supplied by the occluded artery is at risk of developing necrosis if it is not reperfused within a limited time, unless it receives blood by alternative routes. The necrotic zone, which starts centrally, may grow during ischaemia and occupy an increasing fraction of the hypoperfused zone. The necrotic wavefront is, therefore, preceded by reversible ischaemic injuries. Cox et al. (1968) reported a morphologically distinct border zone surrounding myocardial infarcts, but the evidence for the existence of this zone has been controversial (Hearse and Yellon 1981).

The width of the border zone is probably related to the extent of collateral circulation as well as to the variables under study. In cat hearts subjected to 3 h of

LAD occlusion, there is a sharp interface between normally perfused and ischaemic myocardium which may be visualized both by fluorescein staining and by regional blood flow measurements with radiolabelled microspheres (Greve et al. 1989). Ultrastructural studies of serial sections collected across this interface have revealed a sudden increase in number of myocytes with extensive fragmentation of the sarcolemma, accompanied by chromatin clumping and margination, and massive cytoplasmic oedema (Greve et al. 1989). We refer to these areas of sudden change as the morphological border line and it appears to be less than 330 μm wide. Thus, there is an abrupt change in the morphology across the border line from cells mainly appearing normal to mainly necrotic ones.

On the normal side of the border line, there is a 1.5- to 2.0-mm-wide border zone characterized by moderate cytoplasmic oedema. Mitochondrial swelling and lipid droplet accumulation were greater in the border zone than in the central ischaemic zone (Greve et al. 1989; Jodalen et al. 1985). A few cells in the border zone have isolated small breaks in the sarcolemma, whereas more extensive fragmentation of the sarcolemma is rare (Greve et al. 1989). During mild ischaemia, it has been suggested that mitochondria may have sufficient oxygen to oxidize some lactate and fatty acids. Thus, the cells may function by a mixture of aerobic and anaerobic metabolism (Jennings and Ganote 1976). The reality of this in the border zone is, however, still not established. Cells with focal disruptions and even some cells with an intact sarcolemma have moderate cytoplasmic oedema after 3 h of LAD occlusion in the cat model. Both ionic changes and accumulation of metabolic waste products alter the osmotic pressure in the cell interior and in the extracellular space, and may cause the cytoplasmic oedema. The mitochondria are more swollen both on the normal and the ischaemic sides of the border line than in the central ischaemic zone, but disorganization of the cristae and amorphous matrix densities are uncommon in mitochondria in the border zone. Thus, the mitochondrial swelling and the moderate cytoplasmic oedema probably reflects early or less severe cell injury in ischaemia.

Accumulation of lipid droplets in ischaemia is a well-known phenomenon (Jodalen et al. 1985; Liedtke 1988), and its characteristic pattern across the border zone is striking (Greve et al. 1989). In the cat model, the maximal fractional volume of lipid droplets is observed less than 330 μm from the border line on its normal side. The accumulation of lipid droplets also extends into the tissue on the ischaemic side of the border line. The accumulation of lipid droplets in the border zone exceeds the amount found in the central ischaemic zone, and may indicate different metabolism in the border and central ischaemic zones, or that the myocytes survive for a longer period at the periphery of the ischaemic zone. Accumulation of lipid droplets has been assumed to result from increased synthesis of triglycerides from plasma non-esterified fatty acids, redistribution of intracellular lipids, as well as a reduced lipolysis of endogenous triglycerides within the ischaemic tissue (Katz and Messineo 1981; Liedtke 1988).

Myocytes in the non-ischaemic part of cat hearts exhibit ultrastructural changes 10 min after LAD occlusion, such as a slight cytoplasmic oedema and minimal swelling of mitochondria (Greve et al. 1988, 1990) which may indicate increased work load on these cells. Loss of functioning left ventricular tissue initiates hypertrophy of the non-ischaemic part of the heart, starting within 3 days after the onset of infarction (Anversa et al. 1985). Apart from the cytoplasmic oedema observed in the non-ischaemic myocardium, there is probably no significant cell growth here during the acute phase of myocardial infarction. However, the early biological mechanisms initiating hypertrophy of the non-ischaemic myocardium should be studied more intently in future.

Experimental studies have shown that irreversible ischaemic cell injury occurs after 20 min, whereas in clinical practice patients may benefit from revascularization of the ischaemic myocardium for up to 4 h or more after the onset of ischaemia, probably due to some collateral circulation (Koshal et al. 1988; O'Neill et al. 1987). Further characterization of the development of ischaemic injury is therefore necessary, especially the order and significance of each step preceding the point of no return both in the central ischaemic and border zones. Such studies would require sensitive methods capable of detecting minute changes. Reperfusion is necessary to verify the reversibility of the changes. Development of collateral circulation in man requires careful interpretation of experimental studies in a clinical context.

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