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New Observations Regarding Post-Ischemia/ Reperfusion Myocardial Swelling

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Brief Title: Myocardial Edema Post Reperfusion

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Many of the experimental studies exploring the effect of reperfusion on the pathology of the myocardium were described by the laboratory of Dr. Robert Jennings at Northwestern University in the 1960's and 1970's (1-3). The Jennings' laboratory utilized an anesthetized canine model of mechanical proximal circumflex coronary artery occlusion followed by reperfusion. They observed that coronary artery occlusions of shorter than 20 minutes in duration followed by reperfusion were associated with "reversible" damage – that is the myocardial cells recovered after reperfusion and did not die (3) No myocardial infarction occurs in the setting of a brief episode of 5 to 15 minutes of ischemia, which might be considered the equivalent of an episode of angina. However, other studies did show that a brief episode of ischemia and reperfusion was associated with mild edema of the cardiomyocytes, stunned myocardium, and low reflow (4-9). When the duration of ischemia was prolonged to 20 to 40 minutes and reperfusion was then instituted, myocardial cells within the subendocardium underwent necrosis (3). While areas of cell death appeared in the subendocardium, cells in the midmyocardium and subepicardium were salvaged when reperfusion was instituted between 20-60 minutes after coronary artery occlusion. Reimer and Jennings then showed that as the duration of coronary occlusion was extended from 40 minutes to 3 hours and then 6 hours, the extent of necrosis marched from subendocardium to subepicardium within the ischemic risk zone, which they called the wavefront phenomenon of ischemic cell death (10,11). This observation, confirmed by a number of other laboratories (12), helped pave the way for current and established therapy of acute ST segment elevation myocardial infarction – early and complete reperfusion of the culprit coronary artery. Reperfusion in a timely manner reduces myocardial infarct size compared to not reperfusing an occluded coronary artery.

The ultrastructural and biochemical features of the myocardium subjected to ischemia and reperfusion have been described (1,2). At the end of 40 minutes of ischemia in the subendocardium of the anesthetized canine model, the myocytes already demonstrated an ultrastructural feature that Jennings considered characteristic of irreversibly injured cells: the presence of amorphous dense bodies within the mitochondria (2). Wide I bands suggested relaxation of the sarcomeres. In addition, intermyofibrillar edema, mild subsarcolemmal edema, loss of glycogen granules, mitochondrial edema, nuclear chromatin clumping and margination were common features. Upon reperfusion there was a marked worsening of ultrastructural abnormalities in this region of the heart. The myocytes exhibited evidence of extensive swelling. The sarcolemmal membrane appeared lifted off of the myofilaments with edema fluid below it – so called sarcolemmal blebbing or blistering. The sarcolemmal membrane demonstrated breaks or gaps. Large fluid-filled vacuoles appeared within the cytoplasm. Mitochondria showed additional swelling and separation of the cristae. Besides the amorphous dense bodies which had been present at the end of the period of ischemia, a second type of dense body appeared within the mitochondria –a doughnut -shaped dense body with dark black particles, thought to represent calcium phosphate precipitates. Within seconds of reperfusion calcium overload contributed to the formation of contraction bands with congealing of Z bands and disruption of sarcomere structure. Endothelial cells showed loss of pinocytotic vesicles, diffuse and focal swelling, and clumping of the nuclear chromatin. These ultrastructural findings were corroborated by studies showing that reperfusion was associated with a marked increase in tissue water, and an increase in myocardial sodium and calcium (1). These increases in tissue swelling occurred very rapidly – within seconds to minutes of release of the epicardial coronary artery clamp. The theory is that at the end of the period of ischemia, within the most ischemic subendocardium of the left ventricle,

where collateral flow is lowest in the canine model, some cells are irreversibly injured at the end of ischemia. These cells already have defects in their sarcolemmal membrane, resulting in impaired volume control. When reperfusion occurs these cells are exposed to a sudden influx of fluids and electrolytes; the damage to the sarcolemmal membrane during ischemia allows for the fluids, sodium and calcium to overwhelm the cells leading to what we termed “explosive cell swelling” within seconds to minutes of reperfusion (13). Other features such as opening of the mitochondrial permeability transition pore with reperfusion also may contribute to organelle damage. Reversibly injured cells that were located in the midmyocardium and subendocardium of this model, may also demonstrate some degree of swelling with ischemia/reperfusion, but the degree of cellular edema is considerably less than those cells that die; these cells eventually recover structure and function and are salvaged by the act of reperfusion (12).

Following this acute phase of cell edema, there is likely resorption of fluid from dead cells that degenerate and extrusion of fluid from those cells that have survived the ischemic insult. Inflammatory cells including neutrophils and macrophages enter the debris area of necrotic cells and begin to “clean up the mess.” This inflammatory phenomenon begins within days of the insult and then continues for several weeks as fibroblasts and collagen begin to build a scar to replace the fragile necrotic tissue (14-16).

In the accompanying paper entitled “Myocardial edema after ischemia/reperfusion is not stable and follows a bimodal pattern: Advanced imaging and histologic tissue characterization study,” Fernández-Jiménez et al (17) perform an in-depth study of the time course of edema using cardiac magnetic resonance imaging as well as a direct measure of water content in a porcine model of 40 minutes of coronary artery occlusion followed by reperfusion. They make the important and original observation that there was a rapid and marked increase in tissue water

at 2 hours of reperfusion; at 24 hours the edema is largely resolved; but then there is an increase in water content at day 4 and a further increase at day 7 after reperfusion. Hence myocardial edema followed a bimodal pattern. It is very likely that the first wave of edema observed in the early hours of reperfusion represents in part the explosive cell swelling phenomenon described above. However, examination of the magnetic resonance images in their figures and their transmurality assessment suggests that there is a degree of transmural edema of the left ventricle. This may be due to the fact that the pig model has nearly no collateral flow compared to the dog model, so that irreversible injury may occur across the wall of the heart with a shorter duration of ischemia in the pig model; whereas in the canine model, 40 minutes of ischemia followed by reperfusion causes a subendocardial infarction. It is also possible that transient edema of salvaged tissue is also represented in these images. The second wave of edema that occurs starting day 4 may be due to the expected post-necrotic inflammatory reaction in which neutrophils, mononuclear cells such as macrophages and fibroblasts enter the necrotic area to break down and phagocytize debris and begin repair.

The authors are to be congratulated on defining the time course of myocardial edema over 7 days of reperfusion and making the important observation that the presence of edema is not static, but fluctuates – with an early and dramatic increase in the first few hours, resolution at 24 hours, and then a second wave of edema at 4-7 days. One important and practical aspect of this study is that it suggests that researchers should be careful about assuming that the zone of edema observed on cardiac magnetic resonance imaging can capture a reliable area at risk or ischemic risk zone (18) that was present prior to the infarction, when carrying out studies aimed at testing adjunctive therapies to reduce myocardial infarction size. The zone of edema fluctuates over time and may be influenced by therapy. Any therapy that reduces ischemic necrosis may

reduce the zone of edema in the early hours of reperfusion, thus falsely reducing the size of the ischemic risk zone. Measurement of the zone of edema at 24 hours, a time when the early edema has resolved, will also falsely lower the risk zone. Anti-inflammatory agents have the potential to reduce the zone of edema that occurs at 4-7 days.

Several questions remain to be determined. How long after reperfusion is needed for the edema to resolve? Presumably once the scar is fully formed, the edema should fully and permanently dissipate. The authors studied one duration of ischemia (40 minutes); would these time courses be similar if reperfusion occurred earlier or later than this time period? It would be useful to sample tissue in various regions of the risk zone. What happens to edema within the infarct, within the zone of microvascular occlusion (no-reflow), within salvaged tissue in the risk zone; in the non-infarcted border zone beyond the risk zone; and in the non-infarcted remote zone?

Summary

The fine paper by Fernández-Jiménez (17) adds to our knowledge of the pathophysiology of reperfusion phenomenon in the setting of ST segment elevation myocardial infarction and shows that a noninvasive imaging technique can allow for real time assessment of water content. These investigators have made the important observation that real time imaging detects a true bimodal pattern of tissue edema over the first seven days of reperfusion.

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References

1. Whalen DA, Jr., Hamilton DG, Ganote CE, Jennings RB. Effect of a transient period of ischemia on myocardial cells. I. Effects on cell volume regulation. Am J of Pathol 1974; 74: 381-397.
2. Kloner RA, Ganote CE, Whalen DA, Jr , Jennings RB. Effect of a transient period of ischemia on myocardial cells. II. Fine structure during the first few minutes of reflow. Am J of Pathol 1974; 74: 399-422.
3. Jennings RB, Ganote CE, Reimer KA. Ischemic tissue injury. Am J Pathol 1975; 81: 179-198.
4. Kloner RA, Jennings RB. Consequences of brief ischemia: Stunning, preconditioning, and their clinical implications. Part 1. Circulation 2001; 104: 2981-9
5. Kloner RA, Jennings RB. Consequences of brief ischemia : stunning, preconditioning, and their clinical implications. Part 2. Circulation 2001; 104: 3158-67.
6. Kloner RA, Ellis SG, Carlson NV, Braunwald E. Coronary reperfusion for the treatment of acute myocardial infarction: post ischemic ventricular dysfunction. Cardiology 1983; 70: 233-46.
7. Heyndrickx GR , Baig H, Nellens P, Leusen I, Fishbein MC, Vatner SF. Depression of regional myocardial blood flow and wall thickening after brief coronary occlusions. Am J Physiol 1978; 234: H653-659.
8. Kloner RA, Ellis SG, Lange R, Braunwald E. Studies of experimental coronary artery reperfusion. Effects on infarct size, myocardial function, biochemistry, ultrastructure, and microvascular damage. Circulation 1983; 68: (2 Pt 2): I 8-15.
9. DeBoer LW, Ingwall JS, Kloner RA, Braunwald E. Prolonged derangements of canine myocardial purine metabolism after a brief coronary artery occlusion not associated with anatomic evidence of necrosis. Proc Natl Acad Sci USA 1980; 77: 5471-5.

10. Reimer KA, Lowe JE, Rasmussen MM, Jennings RB. The wavefront phenomenon of ischemic cell death 1.) Myocardial infarct size vs duration of coronary occlusion in dogs. Circulation 1977; 56: 786-94.
11. Reimer KA, Jennings RB. The "wavefront phenomenon" of myocardial ischemic cell death II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. Lab Invest 1979; 40: 633-44.
12. Ellis SG, Henschke CI, Sandor T, Wynne J, Braunwald E, Kloner RA. Time course of functional and biochemical recovery of myocardium salvaged by reperfusion. J Am Coll Cardiol 1983; 1: 1047-1055.
13. Jennings RB, Ganote CE, Kloner RA, Whalen DA, Jr, Hamilton DG. Explosive swelling of myocardial cells irreversibly injured by transient ischemia. Recent Adv Stud Cardiac Struct Metab. 1975; 6: 405-413.
14. Roberts CS, Maclean D, Maroko P, Kloner RA. Relation of early mononuclear and polymorphonuclear cell infiltration to late scar thickness after experimentally induced myocardial infarction in the rat. Basic Res Cardiol 1985; 80: 202-9.
15. Roberts CS, Schoen FJ, Kloner RA. Effect of coronary reperfusion on myocardial hemorrhage and infarct healing. Am J Cardiol 1983; 52: 610-614.
16. Parisi AF, Nieminen M, O'Boyle E, Moynihan PF, Khuri SF, Kloner RA, Folland ED, Schoen FJ. Enhanced detection of the evolution of tissue changes after acute myocardial infarction using color-encoded two-dimensional echocardiography. Circulation 1982; 66: 764-70.
17. Fernández-Jiménez R et al. (JACC please fill in complete reference as it should appear) Myocardial edema after ischemia/reperfusion is not stable and follows a bimodal pattern: advanced imaging and histological tissue characterization study. J Am Coll Cardiol 2014.

18. Aletrus AH, Tilak GS, Natanzon A, Hsu LY, Gonzalez FM, Hoyt RT Jr, Arai AE. Retrospective determination of the area at risk for reperfused acute myocardial infarction with T2- weighted cardiac magnetic resonance imaging: histopathological and displacement encoding with stimulated echoes (DENSE) functional validations. Circulation 2006: 113: 1865-70.

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