

Acute Myocardial Injury: A Perspective on Lethal Reperfusion Injury

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Abstract

Acute myocardial infarction contributes significantly to mortality in patients with coronary artery disease. Timely reperfusion of an infarct-related artery within a reasonable time-frame after acute coronary occlusion continues to be the most effective intervention in patients to reduce morbidity and mortality. However, restoration of blood flow to reversibly injured cardiocytes (and other cardiac cell types) within the under-perfused region may also provoke additional damage-commonly referred to as lethal reperfusion injury. Debate has gone on, and continues regarding the existence of reperfusion injury and the pathways that are solicited. Consequently, findings from fundamental science studies have facilitated identification of therapeutic targets that may benefit patients with acute coronary syndromes. This review examines evidence from basic science and clinical studies that support the premise of cardiac injury caused by reperfusion. Pathogenesis of post-ischemic cellular injury is discussed along with potential interventions (pharmacologic and non-pharmacologic) currently being used to improve clinical outcomes.

Keywords: Lethal reperfusion injury; Ischemia; Cellular protection; Coronary collateral vessels; Microcirculation; Blood flow; Ischemic conditioning

Introduction

The notion of lethal reperfusion injury in the heart implies that injury ensues to viable myocytes at the time of reperfusion, over and above, the cellular damage normally attributed to the initial ischemic event. The occurrence of reperfusion injury may be inevitable but restoration of blood flow to the infarct-related artery is critical to ensure salvage of reversibly injured myocytes within the area at risk. Debate concerning prevalence of reperfusion injury continues but no true experimental model is presently available to distinguish damage caused by restoration of flow to the perfusion bed of the infarct related artery compared to that present at the end of ischemia. Advocates of the reperfusion injury theory argue that antecedent ischemia in addition to a yet unidentified unfavourable component of reperfusion compromises recovery of cardiac function; however, conclusive evidence for reperfusion-mediated pathology remains to be established [1-4]. Various forms of reperfusion injury can include myocardial and vascular stunning, microvascular injury and no-reflow, arrhythmias, etc. Herein, we briefly consider the evidence regarding the conundrum of reperfusion injury along with interventions that potentially limit its lethal consequences in patients.

For this review, fundamental science and clinical reports were searched using several databases (i.e. PubMed, MEDLINE, Google Scholar); articles concerning reperfusion injury (lethal and otherwise), ischemia-reperfusion injury, apoptosis, microvascular injury, ischemic conditioning and different combinations thereof were consulted. In addition, we referred to data from a number of our own experiments on the subject.

Timely reperfusion of the infarct-related artery continues to be the most effective means to limit development of cellular necrosis. A plethora of pharmacologic and non-pharmacologic interventions

administered at various times before and during ischemia, or at the time of coronary reperfusion provides some relief against ischemic injury [5-7]; however, overall clinical usefulness remains controversial.

Myocardial Ischemia

Diffuse coronary artery disease (i.e. atherosclerosis, arteriosclerosis, etc.) most often results in an acute coronary event (i.e. plaque rupture, thrombosis, platelet emboli, vasoconstriction, etc.) that leads to arterial occlusion; immediate concerns consist of hemodynamic and electrocardiographic effects. Since myocardium is highly dependent on blood flow for delivery of oxygen (almost 70% of blood-borne oxygen is extracted) and nutrients, any interruption in blood flow results in a marked disruption of cardiac function. While myocardial ischemia is a consequence of an imbalance between oxygen supply and demand, failure to remove high-energy phosphate metabolites and carbon dioxide also plays an important role.

Physiopathology of acute myocardial infarction is well established [8-10]. Early experiments documented two kinds of ischemic injury:

- Reversible, where myocytes survived periods of ischemia less than 15 minutes duration.
- Irreversible, with no capacity for myocyte recovery.

In reversibly injured myocytes early restoration of blood flow results in complete recovery of cellular function with no discernible sequelae. On the other hand, irreversible myocyte injury (i.e. necrosis) produces marked cellular ultrastructural changes such as cell swelling, denaturation of intracellular proteins, membrane disruption, presence of contraction bands and mitochondrial calcification, etc. due to metabolic failure and rapid depletion of high-energy stores [11-13]. Other modes of cellular injury and death (i.e. apoptosis, autophagy, oncosis) also merit attention. Conditions that control the transition from one status to the other have been widely debated [14,15]. Recently, Jennings suggested, "myocytes are irreversibly injured when they fail to survive after restoration of the environment to normal" [3]. Furthermore, loss of structural integrity accompanied by replacement

with scar tissue is an important consideration. Timing of cell injury and location across the myocardial wall are also important criteria; for the most part, potentially salvageable myocytes are localised in the midmyocardial and epicardial layers. This observation led to the realization that ischemia-induced myocyte injury follows a transmural gradient across the ventricular wall following acute coronary occlusion [16,17]. Earlier studies also hypothesized that distinct states of flow reduction and ischemic injury affect myocyte vulnerability to damage [18,19]. These conditions are important as successful reperfusion therapy necessitates that rapid restoration of blood flow must be achieved to impede development of a transmural infarct. Failure to restore blood flow can be caused by microvascular dysfunction; it is important to remember that significant damage also occurs to the coronary vasculature during ischemia that can influence functional recovery of different cellular components of the myocardial architecture. In addition, while myocytes comprise more than 80% of ventricular mass, other cell types integral to cardiac function (i.e. intra- and extra-cardiac sympathetic/parasympathetic neurons) are also negatively affected by ischemia; their injury threshold is not well established [20].

Cardiocytes and cardiac neurons conceivably share common survival pathways but this remains to be proven [21]. Interestingly, viable nerves that course over an infarcted region tend to remain so since oxygen and energy needs can be fulfilled via blood from extra cardiac sources [22]. Nonetheless, successful restoration of blood flow within the infarct-related artery and to ischemic myocardium is essential to limit untoward effects on cardiac performance.

Microcirculation and collateral vessels in ischemia

In the heart, reductions in oxygen delivery are generally countered by increases in blood flow or increased capillary density; this was initially described by Krogh [23] who documented that under resting conditions only a small number of coronary capillary vessels were open. He put forward that increases in oxygen demand could promote recruitment of additional capillary vessels; the extent of this response was dependent on factors such as topography of the vascular network [24,25], vascular tone [26,27], and mechanical properties of red blood cells. [28] In addition, disruptions in oxygen supply and demand stimulated growth of coronary collateral vessels that can augment myocardial perfusion and thereby prevent further myocardial necrosis within the ischemic zone. The coronary collateral circulation is a recognized alternative source of blood supply to jeopardized myocardium [29,30]. Growth of collateral vessels is highly regulated over an extended period [31,32]; this was confirmed in dogs subject to repetitive, brief coronary occlusions [33]. The notion of recruitable, newly formed coronary collateral vessels at onset of ischemia to preserve myocyte viability is not completely accurate; it would seem more reasonable that pre-existing arterioles/capillaries are recruited under ischemic conditions. A host of factors (i.e. intramyocardial tissue pressure, coronary perfusion pressure, location, etc.) are involved in recruitment of existent collateral vessels within the ventricular wall [34]. Restoration of coronary perfusion pressures combined with reduced extravascular tissue pressures could explain, in part, the existence of patchy infarcts that are often observed in reperfused hearts.

Opening of coronary collateral channels in humans during coronary occlusion and during ischemic preconditioning has been reported [35-37]. More specifically, opening of coronary collateral vessels in conditioned myocardium has been associated with a marked reduction

of ST segment elevation during repeated coronary occlusions. On the other hand, Tomai and co-workers could not confirm these findings during coronary angioplasty in patients with STEMI [38]. The inability to show a positive relation between myocardial tissue viability and improved blood flow at the level of the microcirculation post-ischemia may be due to the lack of sensitivity of currently employed techniques to measure spatial distribution of blood flow within the deeper myocardial layers. Animal studies to evaluate recruitment of coronary collateral circulation are sparse; one study in rabbits documents a trend to improved micro vessel blood flow produced by ischemic conditioning [39]. However, it is not clear that ischemic preconditioning preserves coronary vasodilator reserve [40,41].

Consequences of Reperfusion of the Infarct-Related Artery

Lethal reperfusion injury

Lethal reperfusion injury refers principally to myocyte death related to reperfusion rather than the preceding ischemia [42]. Restoration of arterial blood flow to the infarct-related artery can unequivocally reduce the extent of ischemic myocyte damage; however, questions remain regarding whether further damage occurs in already compromised myocytes within, or adjacent to, the ischemic area. Myocardial contractile stunning is considered a clear example of functional reperfusion injury and was first described in canine studies by Heyndrickx and colleagues [43]; they showed that reversibly injured myocardium did not contract as efficiently after reperfusion of the infarct-related artery. Use of oxygen-free radical scavengers at onset of reperfusion positively influenced post-ischemic contractile dysfunction and afforded significant myocyte protection [44,45]. An impressive number of studies have examined the phenomenon of severe myocyte injury stimulated by reoxygenation (i.e. oxygen paradox); however, it is not clear whether the oxygen paradox manifests injury produced during the period of oxygen depletion [46].

No-reflow phenomenon

No-reflow describes the inability to restore blood flow to tissues even after reopening of the infarct-related artery [47], this phenomenon is not exclusive to the heart and has also been described in other major organs [48,49]. In the heart, the area of no-reflow is principally confined to the anatomic risk zone (i.e. underperfused region). Causes of no-reflow comprise ultrastructural alterations (i.e. cell swelling, membrane gaps, etc.) within the microvasculature that worsen despite restoration of patency in the infarct-related vessel [50,51]. No-reflow is not considered to be a primary contributor to cell necrosis [52]; anatomic studies indicate that irreversible myocyte injury occurs before microvascular damage [53]. Reopening of an infarct-related artery exacerbates this phenomenon and is characterized by loss of vascular hyperemia (i.e. blood flow reserve) and blood flow over time within the ischemic region [54, 55]. Reduced vasodilator responses in larger conductance vessels (i.e. epicardial arteries) with extended durations of ischemia also contribute to impaired post-reperfusion blood flow [56-58]. Finally, no-reflow likely affects post-ischemic myocyte healing by limiting accessibility of inflammatory cells to the ischemic zone, and contributes to infarct expansion, ventricular dilatation and remodeling [59]. Involvement of neutrophils, and other inflammatory cell types, in lethal reperfusion injury has previously been examined by Vinten-Johansen [60].

During an acute ischemic event, the need to restore coronary patency is imperative not only to limit damage at the level of myocytes but also of the coronary microcirculation. Different interventions, either pharmacologic or not, have been studied; for example, primary Percutaneous Coronary Interventions (PCI) are used to reopen the infarct-related artery but they also provoke the release of micro-particulate debris and platelet aggregates and thereby cause further injury downstream within the microvasculature [61,62]. Micro embolization during early reperfusion might be involved in development of injury, but it can also be a confounder to impede salvage of ischemic myocardium [63-65]. Recently, Skyschally and colleagues demonstrated a significant increase in infarct size in a porcine model of ischemia-reperfusion when microspheres were injected into the infarct-related artery immediately upon reperfusion [65]. They intimated that additional damage from microembolization occurs within a 'border zone' found within the anatomic risk area following arterial occlusion [66]. The degree and potential importance of injury due to micro-emboli (comprising debris from plaque fissure or rupture) in humans after PCI is probably underestimated [67]; this is probably also the case for release of soluble vasoactive substances post-PCI from injured vascular endothelium [68,69].

At the level of the microvasculature ultrastructural changes (i.e. cell swelling, membrane gaps, etc.) can contribute to myocyte damage [47,70]; however, the degree of damage varies across the ventricular wall. Microvascular damage could contribute to irreversible myocyte injury since blood flow, even after restoration of patency to the infarct-related artery, is impeded to reversibly injured myocytes. Understanding different factors that control post-ischemic coronary capillary blood flow across the myocardial wall also remains an important challenge. It is clear that impairment of the microcirculation or larger vessels immediately upstream of the microcirculation, in various pathologies (i.e. hypertension, cardiomyopathy, myocardial infarction) plays a crucial role in cellular viability and eventual recovery of ventricular performance.

Myocyte injury and death

Loss of myocytes due to ischemia is not uniform across the myocardial wall; different modes of cellular injury: 1) Apoptosis: cell death Type I; 2) Autophagy: cell death Type II; 3) Oncosis: spontaneous cell death; and 4) Necrosis are described [71,72]. In apoptosis, the integrity of the plasma membrane remains intact until the late stages of cell death; however, in necrosis plasma membranes rupture along with swelling of organelles and loss of intracellular content occurs early in the process [15,73]. Morphological characteristics of apoptosis include cell rounding, pyknosis, karyorrhexis and membrane blebbing. Intrinsic (mitochondrial) and extrinsic (death receptor) pathways are believed to mediate apoptosis [74]. Though essential during cardiac development, apoptosis is associated with ischemic heart disease, reperfusion injury, cardiomyopathy and heart failure [75]; the contribution of apoptosis to reperfusion injury remains the subject of ongoing debate [76,77]. Intracellular signalling pathways, along with morphologic, biochemical and molecular pathways responsible for regulation of apoptosis in cardiocytes have recently been discussed [14,15,78]. Ohno and co-workers postulated that myocyte damage during ischemia-reperfusion progressed from a reversible oncosis state to irreversible oncosis with or without DNA fragmentation [79]. Microscopic features of oncosis is well demonstrated for in situ evolving myocardial infarcts; this is not the case for apoptotic necrosis [80,81]. On the other hand, autophagy allows the cell deprived of essential nutrients and growth factors to

survive; however, extended periods of ischemia (during which all available substrates for survival are depleted) ultimately result in cell death. Autophagy was first described in 1962 but not within the context of ischemia-reperfusion injury or cardiac tissues [82]; three different types (i.e. macro-, micro-autophagy, chaperone-mediated autophagy) have been identified [83]. Of note, the process of autophagy is mediated by a host of triggers and stimulators, sensors and genetic regulators that have been linked to cellular injury and necrosis in the pathogenesis of myocardial infarction. The contribution of autophagy to myocyte death following ischemia-reperfusion remains to be established; however, it probably plays a role in ventricular remodeling following restoration of perfusion to the ischemic tissue bed. Since myocytes are terminally differentiated and do not divide, salvage remains the ultimate goal in order to ensure restoration of contractile function. Interestingly, some studies report that a small percentage of myocytes can be renewed depending on age and that fewer than 50% of cardiomyocytes are exchanged during a normal life span [84-88]; their potential contribution to overall cardiac performance is not known.

Piot and co-workers documented a close relation between infarct development and DNA fragmentation suggesting that necrosis and apoptosis pathways were interrelated [89,90]. More recently, Buja and Weerasinghe proposed a hybrid model whereby apoptotic and oncotoc pathways could both be activated in cardiocytes during ischemia [91]. This hypothesis is based on the intracellular availability of ATP; lethal injury would progress along an apoptotic pathway depending on residual ATP levels within the affected myocytes, along an oncotoc pathway when ATP levels were exhausted and finally via activation of multiple intracellular death pathways. They also indicated that in contrast to classical apoptosis, lethally injured myocytes did not undergo rapid fragmentation and removal but developed secondary changes that triggered inflammation pathways. In our laboratory (unreported findings), we were unable to document significant DNA fragmentation or poly-[ADP-ribose]-polymerase (PARP) cleavage in rabbits subject to up to 120 minutes acute coronary occlusion followed by up to 96 hours reperfusion. Under normal conditions, PARP initiates energy consuming DNA repair by catalyzing ADP-ribosylation of nuclear proteins that results in a rapid depletion of intracellular NAD⁺ and high-energy phosphate stores. Our findings suggested that apoptosis was not an important contributor to ischemic cell death; however, others have reported significant apoptosis in rabbits subject to 30 in coronary occlusion followed by 4 hours reperfusion [92,93]. It is possible that myocytes not yet rendered irreversibly injured within the ischemic zone might be open targets for either apoptotic or oncotoc pathways even after restoration of blood flow to the ischemic zone.

Vascular apoptosis might also be an important component of reperfusion injury but has not received a great deal of attention for various reasons (primarily due to lack of methods to confirm its presence). Apoptosis is associated with various cardiovascular risk factors, hypertension and hypercholesterolemia and may thereby be indirectly associated with physiopathology of syndromes that develop from coronary disease (i.e. infarction, heart failure, etc.) [94]. Finding apoptosis in coronary vessels after restoration of patency to the infarct-related artery is tricky and this has led to the impression that it does not contribute significantly to coronary pathogenesis. However, in animal studies apoptosis has been documented in neointima and vascular smooth muscle after balloon injury or stent placement, which suggests that it may be an important factor in restenosis [95,96]. During ischemia a plethora of endogenous compounds that activate, or

play a role in, cellular protective (anti-apoptotic) or destructive (pro-apoptotic) pathways are released from different cell types. Whether apoptosis is important in the physiopathology of post-ischemic myocardial injury is debatable but the question does merit some consideration since reinstatement of blood flow is a primary requirement for recovery of cardiac contractile function.

Myocardial Protection Strategies

Post-ischemic myocardial injury continues to be an important factor for patient mortality and morbidity. Consequently, limitation of infarct size after an acute coronary event is crucial to improve clinical outcomes in these patients. Development of pharmacologic, as well as non-pharmacologic interventions designed to delay loss of cardiocytes, vascular endothelial cells and smooth muscle, and neurons has been an ongoing challenge for almost a half-century. Though progress from basic science and clinical studies, particularly with respect to understanding mechanisms involved in cell injury and death, has been made significant work still needs to be done.

Timing of any reperfusion intervention is crucial; the original concept that 'time is muscle' has been adapted to include the microvasculature and microvascular perfusion. Establishing when lethal ischemic injury commences in humans is essentially impracticable (regardless of improvements in imaging techniques) but it stands to reason that early, rather than late, reperfusion therapy increases the potential to salvage myocardium [42,97,98]. However, in patients with small infarcts and STEMI (ST segment elevation myocardial infarction) myocardial salvage may be more difficult to demonstrate since the benefits of treatment may be small. Additionally, establishing when and if, reperfusion injury actually exists needs to be confirmed. The failure to reduce infarct size in humans after successful reperfusion of the infarct-related artery suggests that reperfusion injury might not occur or that it might not be preventable. Ndrepepa et al. recently proposed that preventive therapies against ischemic injury fail because they cannot prevent cell death within a zone of irreversible ischemic injury even though they may postpone timing of cell death [99]. Gersh and colleagues proposed that a 'golden window of opportunity' to limit myocardial injury existed within the initial 2-3 hours after onset of cardiac symptoms; the duration of this critical period is affected by the presence of functional coronary collateral vessels, myocardial oxygen demands, etc. [100]. At present, the potential contribution of late reperfusion therapy to mortality reduction is debatable.

Challenges remain apropos translation of cytoprotection strategies from experimental to clinical practice. Hundreds of interventions have been performed in experimental studies with a view to limit ischemic injury but to date, interventions intended to improve patient outcomes have been disappointing. This may be due to various factors including, among others, poorly designed pre-clinical studies prior to embarking on clinical trials, inadequate pharmacological studies, complexities of the human in vivo model, and inadequate design of clinical studies to test cell protection therapies [101-104]. A short summary of emerging treatments is provided here; however, we recommend the reader consult the wealth of scientific publications on this subject already available in the literature.

Pharmacologic approach

Since possible reperfusion injury occurs when blood flow to the infarct-related artery is restored, it would seem logical to initiate

pharmacotherapy at, or just before, reperfusion. Which endogenous mechanisms, intracellular signalling pathways, or end-effectors of protection, etc. should be targeted? Important endogenous protective mechanisms include adenosine production, opening of ATP-sensitive potassium channels (KATP), release of nitric oxide, etc. [105,106]. Ischemia induces release of autotoxins that activate complex intracellular signalling pathways some of which interconnect at the level of the mitochondria. For instance, several mitochondrial pathways involve the mitochondrial Permeability Transition Pore (mPTP) that is an important pathway for cell death [107-110]. In addition, mitochondrial, and sarcolemmal KATP channels are also potential cellular protection targets and continue to be evaluated in clinical studies [101,111]. Adenosine receptors (A1 and A2 subtypes) as well as protein kinase C receptors also continue to be targeted [112-115]; mitogen activated protein kinase blockers have also been evaluated [116,117]. Pharmacologic treatments aimed at the Reperfusion Injury Salvage Kinase (RISK) and Survivor Activating Factor Enhancement (SAFE) pathways that activate pro-survival pathways possibly by inhibiting opening of mPTP at the level of the mitochondria are being studied [111,118-121]. In fact, preliminary findings supporting the concept of reperfusion injury have been provided using the mPTP inhibitor, cyclosporine; administration prior to reperfusion by percutaneous coronary intervention (PCI) markedly reduced infarct size in patients [122]. Another cytoprotective kinase, 5' adenosine monophosphate-activated protein kinase (AMPK) is activated by ischemia; it regulates glucose and fatty acid metabolism by stimulating glycolysis, glucose uptake and fatty acid oxidation [5,123,124]. Nitric oxide may decrease reperfusion injury by stabilizing endothelial function and thereby maintaining blood flow, and by preventing activation of inflammation [125], it might also exert direct beneficial effects on myocyte survival (independent of endothelial cells) via opening of KATP channels [126]. These cardioprotective effects probably depend on nitric oxide bioavailability; however, in excess nitric oxide can also exert marked deleterious effects.

Non-pharmacologic approach

While primary PCI is the benchmark and preferred intervention for reperfusion of the infarct-related artery, it may not always be accessible to patients due, in part, to geographic location of interventional hospitals, etc. Prolonged treatment delays during transfer of patients between non-interventional and interventional hospitals considerably increase mortality risk since 'time is muscle'. As a result, there is an increasing utilisation of combined pharmacological treatment with mechanical reperfusion, also known as 'facilitated PCI' to establish early reperfusion [127]. Prognosis (i.e. in-hospital outcome and survival) in patients with STEMI improved markedly when they arrived at hospital with already open, compared to closed, infarct-related vessels [128-130]. Further investigations are ongoing to evaluate benefits and safety when PCI is combined with a host of pharmacologic compounds compared to PCI alone; results will help establish guidelines to improve clinical outcomes.

An additional complication of PCI after successful opening of a major coronary vessel is the release from the ruptured atherosclerotic lesion of particulate debris that inevitably occludes downstream microcirculation to provoke cellular damage [131]; soluble vasoactive compounds are also released from vascular endothelium following PCI that could cause additional injury [61,62,98,132]. Mechanical thrombectomy systems (i.e. passive aspiration, active mechanical catheters, etc.) that help to prevent distal embolization of

atherothrombotic debris during PCI and limiting injury have been quite successful [133-137].

Activation of endogenous cellular protective pathways during ischemia-reperfusion has also received widespread attention; more than 30 years ago, Murry and colleagues described 'ischemic preconditioning' [138]. Briefly, repeated cycles of ischemia of short duration followed by reperfusion prior to a prolonged coronary occlusion markedly limited cellular injury (i.e. infarct size, arrhythmias, endothelial dysfunction, etc.). Ongoing studies are looking at potential mechanisms responsible for cytoprotection (i.e. multiple receptor dependent and independent triggers, mediators and end-effectors [139]); a detailed examination of these studies is beyond the scope of this review. Several thousand studies reported since have documented the cytoprotective efficacy of conditioning strategies (pre, per, post, remote, etc.) in a host of experimental models [121,140-142]. Low-pressure reperfusion (variant of post-conditioning) at the time of reperfusion also merits consideration for post-ischemic myocyte protection [143]. However, cytoprotection potential of these interventions in humans remains controversial [144,145].

Conclusion

Although reperfusion strategies clearly improve patient outcomes, no clear consensus is available regarding the existence of reperfusion injury. Several examples of reperfusion injury including myocardial and vascular stunning, no-reflow and reperfusion arrhythmias have been discussed in a large number of experimental and clinical studies yet clear demonstration of its existence is not yet forthcoming. The physiopathology of ischemic injury is multifactorial and complicated (more so, in the presence of comorbidities); however, progress is being made and a considerable variety of therapeutic interventions (pharmacologic and non-pharmacologic) continue to be investigated. Understanding mechanisms that contribute to cellular injury and death remains a worthy challenge. However, numerous questions still need to be resolved to determine timing and best practices to improve clinical outcomes. For the moment, early restoration of patency to the infarct-related artery remains the only intervention capable of attenuating infarct development and potentially salvaging reversibly injured myocardium. Ultimately, determining whether reperfusion injury exists may be irrelevant if timing of therapeutic interventions cannot be instituted early enough to reduce or delay development of cellular damage.

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