

Myocardial Reperfusion Injury: Etiology, Mechanisms, and Therapies

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Abstract: Reperfusion of ischemic myocardium is required for tissue survival; however, reperfusion elicits pathologic consequences. Myocardial reperfusion injury is a multifarious process that is mediated in part by oxygen free radicals, neutrophil–endothelium interactions, apoptosis, and intracellular calcium overload. The oxygen paradox describes the contradictory need to deliver oxygen to ischemic tissue and the resultant reduction of oxygen to form free radicals that are involved in macromolecule oxidation, membrane disfunction, apoptosis, and damaged calcium sequestering ability, which results in hypercontracture. These cell-damaging crises are amplified by the excessive activation of neutrophils, which promote the formation of proinflammatory mediators, oxygen radicals, and the reduction of endothelial nitric oxide formation, leading to increased neutrophil–endothelium interactions and capillary occlusion. Neutrophil action is twofold, however, because it is required for necrotic debris removal after severe ischemia. The oxygen radicals produced by neutrophils, endothelium, and myocytes may also play a role in activating the apoptotic cascade. Although the role of apoptosis in reperfusion injury is controversial, apoptotic cells are found in infarcted tissue. One of the key mediators may be

increased inner mitochondrial membrane permeability, resulting in reduced ATP formation, release of cytochrome c, and caspase activation, which is key to promotion of apoptosis. Increased mitochondrial membrane permeability occurs during exposure to supraphysiological calcium concentrations. This occurs because of compensatory $\text{Na}^+/\text{Ca}^{2+}$ exchange to remove the excess intracellular sodium resulting from decreased Na^+/K^+ pumping during ischemia and increased Na^+/H^+ exchange following reperfusion. Supraphysiological calcium elicits hypercontracture and cellular damage. The various therapies being developed to diminish myocardial reperfusion injury involve inhibition of the processes described above as well as others. Although single therapies have shown some promise, the complexity of the response to reperfusion has made dramatic improvement elusive. Effective treatment will most likely require multifaceted antagonism of the numerous pathological cascades initiated by reperfusion. **Keywords:** antioxidants, apoptosis, endothelium, hypercontracture, inflammation, ischemia, myocytes, oxidative stress, polymorphonuclear leukocytes, reperfusion injury, sodium–proton exchanger, sodium–calcium exchanger. *JECT* 2004;36:391–411.

INTRODUCTION

Cardiac myocytes—under normal physiologic conditions—exhibit substantial vitality and tolerance to brief periods of ischemia. The coronary blood supply provides these normally aerobic cells with oxygen (O_2) and energy-containing molecules and affords the removal of waste products, such as carbon dioxide (CO_2), hydrogen ion (H^+), and lactate, among others. The myocardial cells, in turn, use energy through three major pathways: glycolysis, glucose oxidation, and fatty acid oxidation. Most of the time (approximately 70%) the heart uses fatty acids under aerobic conditions to generate adenosine triphosphate (ATP)—the primary energy substrate for myocytes (1). However, when stressed by coronary underperfusion of

sufficient length and/or magnitude, myocytes cannot maintain their normal physiologic milieu: with the overloading of their intrinsic cytosolic protective systems, severe injury can occur from low tissue oxygen delivery and resultant myocyte necrosis.

Under such ischemic conditions, the heart alternatively shifts to anaerobic glycolysis for its requisite energy production, which unfortunately is quite inefficient. For example, during prolonged ischemia, ATP levels decrease by 65% at 15 minutes and by 90% at 40 minutes (2). In addition, an ischemic heart catabolizes glucose, producing lactic acid (Figure 1), which causes the pain associated with angina (1) and results in decreased myocardial contractility (3).

Problematically, even if adequate blood flow returns to apparently viable myocardium, a secondary injury—reperfusion—can occur, causing similar (or possibly worse) damage than that caused by ischemia itself. The

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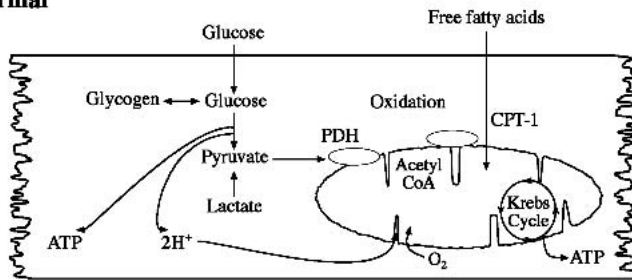
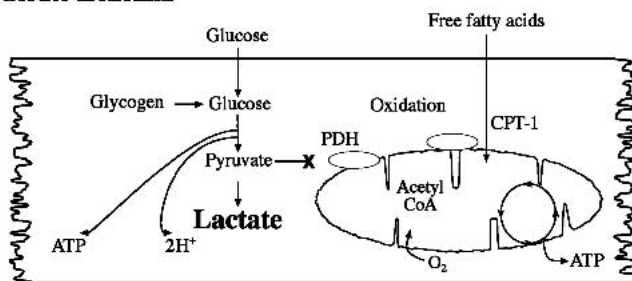
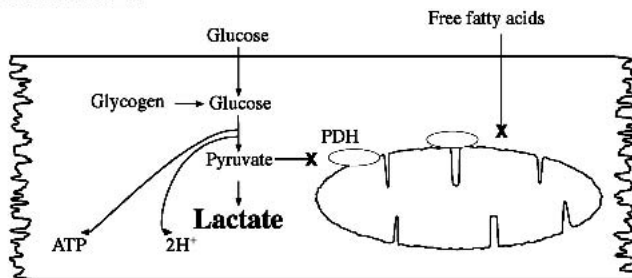
Normal**Moderate Ischemia****Severe Ischemia**

Figure 1. Myocyte ATP production. Normal myocyte energy production (top): Myocardial cells produce energy by aerobic metabolism of both glucose (i.e., glycolysis and Krebs cycle) and fatty acids (i.e., beta-oxidation). In moderate ischemia (middle), the heart alternatively uses anaerobic glycolysis for energy production with some direct fatty acid oxidation. However, this only transiently supplies the needs of the heart and ATP levels are decreased by 65% at 15 minutes and by 90% at 40 minutes. In severe ischemia (bottom), sole anaerobic catabolism of glucose produces excess lactic acid, which potentially causes the pain associated with angina and results in decreased contractility. ATP, adenosine triphosphate; PDH, pyruvic dehydrogenase; CPT-1, carnitine palmitoyltransferase-1. (Adapted from *Am J Cardiol* 1999;83:6G.)

clinical significance of reperfusion injury is substantial: in the western hemisphere, acute coronary occlusion is the leading cause of morbidity and mortality. According to the World Health Organization, it will become the primary cause of death worldwide, possibly by 2020 (4). Thus, it is important to understand myocardial pathophysiology, to improve both survival and quality of life for those afflicted with heart disease, and to develop a platform for the development of future protective strategies.

This review begins by discussing first the known and potential etiologies of myocardial reperfusion injury, including the individual impacts of oxidative stress, neutrophil-endothelium interactions, apoptosis, and myocyte hypercontracture. Next, risk factors for the clinical develop-

ment of reperfusion injury will be reviewed, followed by current and future therapies that have (or are at least expected to have) attenuating effects on each of the aforementioned etiologic agents.

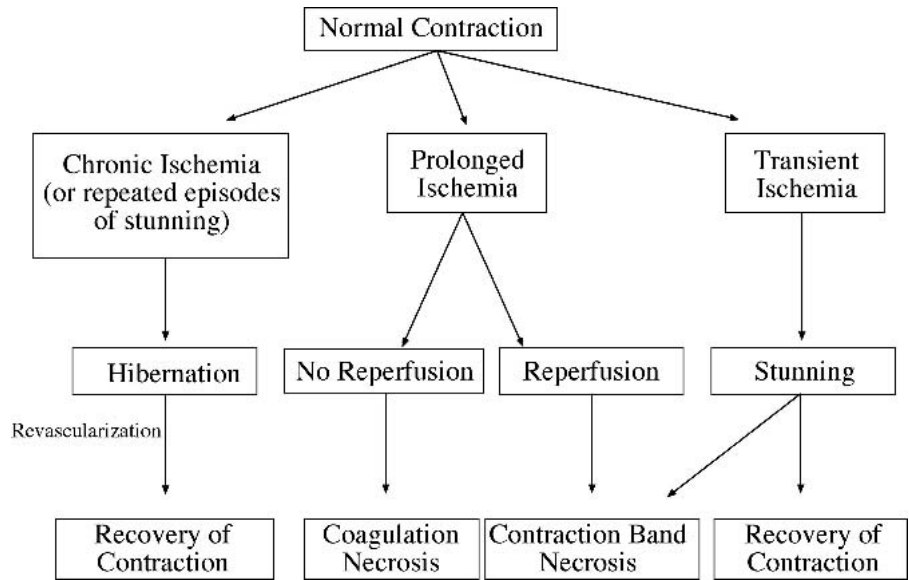
ETIOLOGY OF REPERFUSION INJURY

Numerous conditions may elicit an ischemic myocardium. For example, atherosclerosis of the coronary arteries—coronary artery disease—reduces the effective coronary artery lumen diameter. Blood flow is significantly decreased, and ischemia occurs when >75% of the luminal surface area (i.e., 50% diameter reduction) is lost. Necrosis of the myocardium follows when complete loss of blood flow occurs for >20 minutes. Thus, it is imperative that the ischemic myocardium be reperfused before necrosis occurs, for early myocardial reperfusion improves cardiac contractile function and decreases infarct size (Figure 2) (5). It would seem that reperfusion of viable myocardium would be beneficial for survival of the heart cells; paradoxically, however, the reperfusion of ischemic myocardium is not without its own pathophysiologic consequences. Reperfusion has conflicting effects because it is required to prevent infarction but additionally causes exposure of the myocardium to a variety of potentially deleterious changes to the physiologic milieu that may lead to arrhythmias, myocardial stunning (i.e., reversible contractile dysfunction), or lethal reperfusion injury (6–14). The latter is defined as death of cardiac myocytes that were viable before reperfusion but succumbed to one or more events initiated by reperfusion (10,11,14).

Conditions that reduce coronary blood flow may lead to an ischemic myocardium. Possible scenarios inducing reperfusion injury include coronary thrombus formation, followed by thrombolytic therapy (urokinase, streptokinase, or tissue plasminogen activator). If the obstruction is relieved and coronary blood flow is restored before necrosis, reperfusion injury may occur. Other instances where ischemia is followed by reperfusion include: (1) coronary artery vasospasm followed by coronary artery dilation; (2) arteriosclerotic coronary arteries opened via percutaneous transluminal coronary angioplasty and stenting; and (3) surgical revascularization via coronary artery bypass grafting. Global myocardial ischemia can result from application of an aortic cross clamp during cardiopulmonary bypass (CPB). Upon cross clamp removal, the heart is reperfused rapidly. Global myocardial ischemia also occurs in donor hearts removed for transplantation. These hearts remain ischemic until the coronary blood flow is reestablished in the organ recipients.

Until recently, the scientific community believed that cardiac myocytes were unable to undergo mitotic proliferation because dead myocytes are replaced with noncontractile fibroblasts that become fibrous scar tissue (15).

Figure 2. Varying effects of ischemia on contraction. A reduction in coronary blood flow that results in ischemia occurs when more than 75% of the coronary luminal surface area (i.e., 50% diameter reduction) is lost. Repeated episodes of sublethal ischemia can promote a process called “hibernation” associated with a change to a fetal myocardial phenotype. The complete loss of blood flow for more than 20 minutes can lead to either coagulative or contraction band myocardial necrosis, depending on the absence or presence of calcium during the moment of cell death. Early reperfusion after ischemia can result in transient myocardial stunning (i.e., reversible contractile dysfunction). (Adapted from *Am J Cardiol* 1999; 83:4G.)



When a substantial number of cardiac myocytes die, the workload for the remaining cells is increased. The compensatory response to this increased workload is hypertrophy, wherein the myocytes can double in size (16,17). The increased workload imposed upon the myocardium can ultimately lead to depressed cardiac function of previously uninvolved areas of the heart. Infarcted myocardium also can predispose the heart to irritability, which can increase the incidence of arrhythmias or sudden cardiac death. The original belief that myocytes are unable to undergo mitotic division is now under scrutiny (17). Beltrami and colleagues (18) have published evidence that myocytes do indeed undergo mitotic proliferation after myocardial infarction (MI). They hypothesize that myocyte proliferation may replace damaged myocardium, and that mitosis in healthy myocardium implies that myocytes are replaced throughout the life of the human (18).

MECHANISMS OF MYOCARDIAL REPERFUSION INJURY

The major mechanisms of myocardial reperfusion injury are oxidative stress (predominantly oxygen-free radicals), neutrophil–endothelium interactions, apoptosis, and hypercontracture (i.e., myocyte Ca²⁺ overloading) (Figure 3). It is unlikely that any single pathophysiologic mechanism of myocardial reperfusion injury causes lethal myocardial injury. When multiple pathologic events occur simultaneously, injury is additive and the probability of irreversible myocardial injury occurring increases significantly.

OXIDATIVE STRESS

The best-delineated mechanism of reperfusion injury is

oxidative stress resulting from the formation of oxygen free radicals. A free radical is an atom or molecule that can exist independently with one or more unpaired electrons in its outer electron shell. Because of this unpaired electron, the molecule is unstable and highly reactive and swiftly combines with other atoms to stabilize its electron imbalance. Cells that encounter the reactive oxygen species (ROS) undergo oxidative damage as the ROS becomes more stable at the expense of nearby molecules in contact. It has been estimated that 5% of oxygen (O₂) used by tissues is ultimately transformed to ROS (9). Usually O₂ is able to accept four electrons, but an additional electron yields the superoxide anion (O₂^{•-}). Two extra electrons produce hydrogen peroxide (H₂O₂). Three additional electrons results in a hydroxyl radical (*OH) and, finally, four additional electrons lead to the production of water (19). Sources of ROS elicited by early reperfusion include activated neutrophils, variations within the mitochondrial electron chain, reactions catalyzed by the enzyme xanthine oxidase (XO), autooxidation of catecholamines, the activation of the arachidonic cascade, cyclooxygenase, and lipoxygenase (10,14,19–21). Myocytes possess endogenous antioxidant mechanisms that neutralize ROS, but after reperfusion many scavenging molecules are “washed out” of the areas in which ROS protection is critically needed (9–13,20,22,23). ROS can cause reduced contractility and lipid peroxidation of cellular membranes that can eventually progress to myocyte structural damage (24,25). ROS has a direct role in apoptosis and is involved in a wide range of pathological conditions, such as ischemic-reperfusion (I/R) injury, aging, and neurodegenerative diseases (24,26).

Current evidence suggests that the sources of ROS are cardiac myocytes, neutrophils, and endothelial cells (20,27). Although most electrons from the citric acid cycle

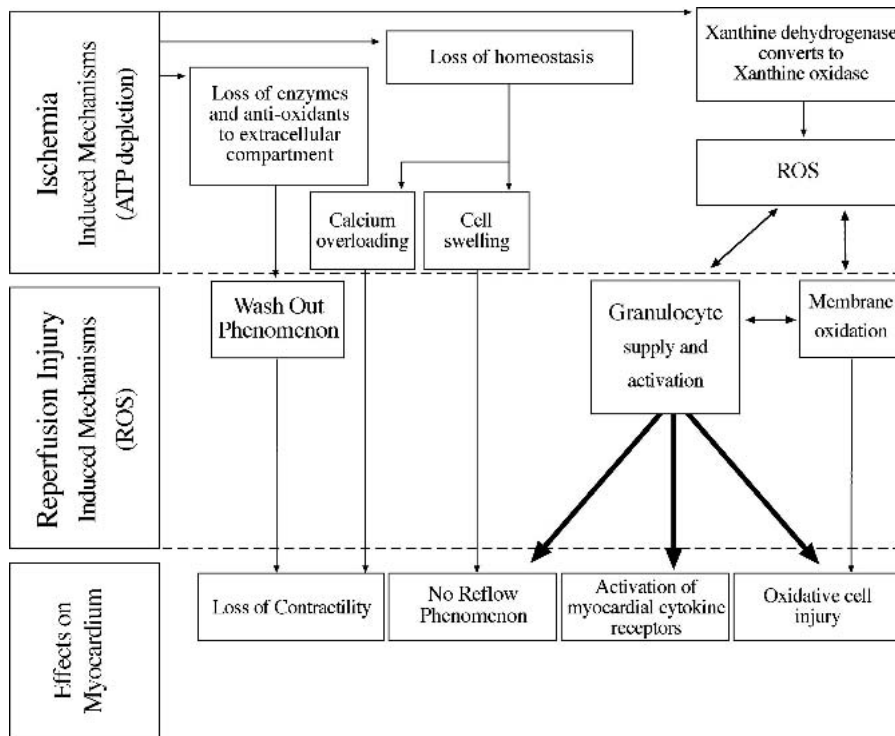


Figure 3. Ischemia and reperfusion processes effect on myocardium. ATP depletion during ischemia can increase susceptibility to oxidative stress through the production of ROS and disrupt the cell's homeostatic mechanisms. Although myocytes possess endogenous antioxidant mechanisms that neutralize ROS, during reperfusion many scavenging molecules are "washed out" of the areas where ROS protection is critically needed. These damaging influences alter neutrophil-endothelium interactions, promote apoptosis, and lead to hypercontracture. ROS = reactive oxygen species. (Adapted from Resuscitation 2000;45:38.)

enter the mitochondrial electron transport chain and reduce molecular oxygen to water, there is evidence of "leakage" of single electrons onto molecular oxygen to form $O_2^{\bullet-}$ via ubiquinone at the level of complex I and II (28,29). Under physiologic conditions, small quantities of ROS are formed during mitochondrial respiration, but they can be detoxified by endogenous scavenging mechanisms (24,30). ROS production may promote mitochondrial DNA damage and impair mitochondrial function (24,30). The high susceptibility of mitochondrial DNA to mutation and oxidative damage is likely a reflection of this localized production of ROS by electron transport oxidation (24). Increased levels of mitochondrial ROS have been demonstrated during reperfusion using electron spin resonance (ESR) spectroscopy and spin trapping (31).

ROS mechanisms and reactions have been well studied (Table 1). Common ROS involved in reperfusion injury include superoxide anions ($O_2^{\bullet-}$), hydroxyl radicals ($\bullet OH$), and singlet oxygen (1O_2) (26). In the Haber-Weiss reaction, O_2 reacts spontaneously with hydrogen peroxide (H_2O_2) forming two hydroxyl radicals ($\bullet OH$) and molecular oxygen (O_2) (10,22,24). In the Fenton reaction (also known as the iron-catalyzed Haber-Weiss reaction), iron (II) and H_2O_2 produce $\bullet OH$ (10,24). Peroxynitrite ($ONOO^-$), another ROS involved with myocardial reperfusion injury, has drawn recent research attention (10,20,23). The formation of $ONOO^-$ occurs when $O_2^{\bullet-}$ interacts with nitric oxide (NO), an endothelium-derived relaxant factor (10,20,23,24). The reaction can continue

Table 1. Reactions that produce free radicals

Superoxide production	$O_2 + e^- \rightarrow O_2^{\bullet-}$
Hydrogen peroxide production (33,50,116)	$2O_2^{\bullet-} + 2H^+ \rightarrow O_2 + H_2O_2$
Haber-Weiss reaction (10,22,24)	$O_2^{\bullet-} + H_2O_2 \rightarrow O_2 + 2\bullet OH$
Fenton reaction (10,24)	$Fe^{2+} + H_2O_2 \rightarrow \bullet OH + OH^- + Fe^{3+}$
Peroxynitrite production (10,20,23,24)	$O_2^{\bullet-} + NO \rightarrow (ONOO^-)$
Peroxynitrous acid production (10,20,23,24)	$ONOO^- + H^+ \rightarrow ONOOH$
Breakdown of peroxynitrous acid (10)	$ONOOH \rightarrow \bullet OH + NO_2$
NO_2^* & CO_3^* production (20,32)	$ONOO^- + CO_2 \rightarrow NO_2^* + CO_3^*$

when $ONOO^-$ becomes protonated to yield peroxynitrous acid ($ONOOH$). The spontaneous breakdown of $ONOOH$ can yield nitrogen dioxide (NO_2) and $\bullet OH$ products (10). The formation of $ONOO^-$ neutralizes the deleterious $O_2^{\bullet-}$ radical and prevents the formation of $\bullet OH$, but NO, which has cardioprotective effects, is consumed in the $ONOO^-$ producing reaction and allows peroxynitrite to inflict myocyte damage (20,21,24). Peroxynitrite also may react with CO_2 to produce increasingly toxic ROS, such as NO_2^* and CO_3^* (20,32). The role of NO in free radical formation is not definitively defined and may depend on the level and timing of a rise in NO levels after reperfusion. In isolated rat heart models, peroxynitrite is formed during reperfusion and may contribute to post-ischemic myocardial dysfunction (10).

The oxygen paradox hypothesis is based upon the basis

that oxygen can inflict injury to the ischemic myocardium during reperfusion (10). Upon reperfusion, myocytes go from a hypoxic state to normal oxygen tension very rapidly, thus initiating the sequential reduction of O_2 resulting in ROS production (10,12,20,28). ROS such as $O_2^{\cdot-}$ and $\cdot OH$ cause the oxidation of membrane phospholipids, proteins, and DNA, resulting in myocardial cell damage and dysfunction (24,30). Peroxidation of phospholipids alters membrane properties, which in turn can affect ion channels, receptors, and the actions of enzymes that interact with membranes. In addition, peroxidation in the sarcolemma affects the Ca^{2+} -pump ATPase and Na^+ - K^+ ATPase functions (33). ROS have been reported to depress the Ca^{2+} sequestering ability of the sarcoplasmic reticulum (SR) Ca^{2+} -pump, leaving the myocyte unable to remove cytoplasmic Ca^{2+} (33), thereby increasing the odds of developing hypercontracture. Altering the permeability and electrolyte balance of cardiac myocyte membranes is not without significant consequences, however, for the changes in membrane permeability that occur during cardiac action potentials are essential to the normal functioning of this cell type. ROS also elicit the release of histamine from mast cells that reside in arterioles contributing to vasoactive and inflammatory effects (25).

It also has been shown that increased ROS production during early reperfusion often coincides with the incidence of tachyarrhythmias in isolated rat hearts. In a study by Ravingerova et al., during the first minutes of reperfusion, the presence of cerium precipitate was indicative of an increase in H_2O_2 production (7). Maximal H_2O_2 concentrations were found in myocyte mitochondria and endothelial cells.

NEUTROPHIL-ENDOTHELIUM INTERACTIONS

During myocardial ischemia-reperfusion, neutrophils are activated by inflammatory molecules released by cardiac myocytes, endothelial cells, and mast cells, resulting in a neutrophil attack against itself (34,35). Neutrophils are activated by complement (C5a) and cytokines such as interleukins (IL), IL-6, IL-8, tissue necrosis factor- α (TNF- α), neutrophil-activating peptide, and platelet activating factor (35). Leukocyte involvement, specifically neutrophils, has been known to be involved in reperfusion injury for years. In 1824, Dutrochet first described that leukocytes can adhere to the vascular wall and can emigrate into the surrounding tissue (36). Polymorphonuclear leukocytes are a particular type of phagocytic leukocyte that is responsible for attacking and destroying bacteria, viruses, and other potentially harmful foreign agents. Neutrophils are the first cells to arrive at an infection site and contain cytotoxic granules that kill pathogens. Pathophysiologically, neutrophils induce tissue injury that can lead to organ dysfunction and organ failure (37). Neutrophils con-

tribute to myocardial reperfusion injury by the production of ROS, endothelial dysfunction (34,38,39), capillary plugging, and direct myocyte injury (Figure 4) (39,40). Neutrophils also cause coronary vascular constriction, which can decrease cardiac performance (41). Through a process called "no-reflow," aggregating neutrophils can occlude microvessels and increase capillary blood flow resistance, causing microcirculatory ischemia and even tissue infarction (42). No-reflow is defined as severe microvascular dysfunction that limits perfusion during reperfusion (12,13).

The vascular endothelium is a simple squamous cell membrane lining the entire human vascular tree. More than a trillion endothelial cells line the inside of the cardiovascular system, and the area of this monolayer membrane is 1000 m^2 , or an area slightly larger than the infield of a major league baseball diamond (43). The endothelium forms a barrier between blood and tissue and because of this can measure, integrate, and transduce blood-borne signals and functions like a "sensory organ" (44). The endothelial barrier prohibits large solutes and macromolecules from moving between the vessel and the interstitial space. The endothelium is inherently involved with neutrophil activities, for it inhibits neutrophil adherence via NO and adenosine but also is involved with recruiting neutrophils (details of the complex mechanisms of neutrophil-endothelium binding is beyond the scope of this review) (35). Altered endothelial cells have been found in diseases/conditions, such as adult respiratory distress syndrome, atherosclerotic vascular disease, stroke, brain trauma, pulmonary and systemic hypertension, renal failure, Alzheimer's disease, cancer, and certain snake bites (43,44).

A key predisposing factor for neutrophil-induced reperfusion injury is endothelial dysfunction (45). Endothelial

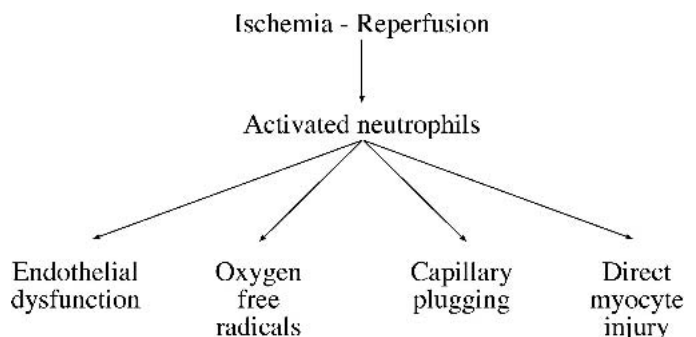


Figure 4. Activated neutrophils play multiple roles in the mechanism of reperfusion injury. When neutrophils are in an activated state and no pathogen is present, the neutrophil can inflict damage on cells that it comes into contact with through ROS, and direct myocyte injury. Through a process called "no-reflow," aggregating neutrophils can cause microcirculatory ischemia and worsening tissue infarction. Neutrophils also cause coronary endothelial dysfunction and vasoconstriction, which can decrease cardiac performance.

dysfunction occurs in the first 2.5 to 5 minutes of reperfusion, with the major physiologic consequence being decreased release of NO (38,45). Effects of endothelial dysfunction include platelet and neutrophil activation, vasoconstriction, increased ROS production, and increased protein and fluid extravasation (12). The endothelium also contributes to cardiovascular homeostasis by maintaining the fluidity of blood by producing anticoagulants and by adjusting vessel diameter in a variable hemodynamic environment (12,46). Vasoactivity is achieved by the appropriate balance of molecules that cause vasodilation (e.g., NO, prostacyclin, and adenosine) or vasoconstriction (e.g., endothelin and angiotensin II) (12,13). Diminished endothelial release of NO permits increased neutrophil-endothelium interactions. NO has many beneficial properties, including scavenging O_2^{*-} to yield $ONOO^-$, inhibiting neutrophil O_2^{*-} release and mast cell release of histamine, reducing neutrophil activation and adhesion, inhibiting platelet aggregation and adhesion, and vasodilation (25).

The specific interactions between endothelium and circulating leukocytes are an essential physiologic defense mechanism against infection, but during myocardial ischemia-reperfusion, excessive neutrophil activation caused by ROS burst and sequestration result in significant tissue damage (36,47). In particular, neutrophils inflict cellular damage and gain access into the extravascular compartments by releasing proteases (collagenase, elastase, heparinase, gelatinase), ROS (O_2^{*-} , H_2O_2), hypochlorous acid, arachidonic metabolites, cytokines (IL-8), anaphylatoxins, and other cytotoxic or proinflammatory mediators, which exacerbate tissue injury (10,34,35,38,48). Neutrophil azurophilic granules release myeloperoxidase (MPO), an enzyme that converts H_2O_2 to hypochlorous acid, which is 50 times more potent than H_2O_2 in killing bacteria (34). MPO, although found in monocytes, is predominantly located in neutrophils. Because of this, MPO activity often is used as an index of neutrophil accumulation (34,49). Collagenase and elastase are capable of breaking down essentially all components of the endothelial inner membrane as well as junctional proteins that preserve endothelial barrier function (48). Neutrophil and endothelial cell interactions contribute directly to ROS production via converting the enzyme, xanthine dehydrogenase (XD), into xanthine oxidase (XO). XO is located in coronary endothelial cells but not human cardiac myocytes (50,51). Activation of elastase converts XD to XO via a calcium-dependent mechanism (20,50,52,53). Upon reperfusion, XO can react with O_2 (acting as an electron acceptor) and the substrate xanthine (a breakdown product of ATP) to yield O_2^{*-} (19,50,52,53). By the Fenton reaction, O_2^{*-} reduces Fe^{3+} to Fe^{2+} , in which, Fe^{2+} can further react with H_2O_2 produced by activated neutrophils yielding $*OH$ (52). $*OH$

seems to be the radical most directly responsible for endothelial cell toxicity (52).

Endothelial injuries have significant consequences involving vascular homeostasis and myocardial reperfusion injury. Damaged endothelium will have blunted production of homeostatic vasoactive compounds, including adenosine, NO, prostacyclin, angiotensin II, endothelin, and endothelium-derived constricting factor. A decrease in these molecules could have a deleterious effect on cardiac output, pressure regulation, or hemostasis of the coronary vasculature. These effects on the myocardium could be amplified in times of stress (i.e., ischemic/reperfused myocardium). In addition, injury to endothelial cells increases permeability leading to fluid shifts into the extravascular spaces and perivascular edema. A healthy endothelium prohibits the aggregation of platelets and decreases the neutrophil extravasation to the heart that is undergoing reperfusion.

The role of neutrophil in myocardial ischemia was first established by histological studies that showed a direct correlation between the ischemia time and infarct size and the extent of neutrophil accumulation within the myocardial tissue (10). The neutrophil is directed sites of inflammation by chemotactic factors, which include complement fragment C5a, C3a, IL-8, and transforming growth factor- α (10). The major source of the chemotactic agents is the neutrophils themselves, which operate in an autocrine-like feedback manner (10). It also has been found that activated endothelial cells and myocardial tissue can release chemotactic factors.

Leukocytes exposed to ischemic tissue may re-enter the systemic circulation in an activated state upon reperfusion (48). For example, prolonged visceral ischemia from aortic cross clamping not only results in mortality from cardiac failure but also from multiple organ failure (48). These results suggest that myocardial ischemia induces not only leukocyte activation and adhesion molecule expression in the myocardium but also predisposes distant vascular sites to increased vascular injury (48).

In aspects concerning myocardial reperfusion injury, neutrophils usually are labeled as injurious cells that damage viable myocardium, whereas any salutary benefits of neutrophil activation often are overlooked. In a study by Youker et al., the data presented show that neutrophils do indeed exacerbate the injury of initial reperfusion but that neutrophils also are involved with myocardial healing (54). The augmented leukocyte influx and phagocytotic activity during myocardial reperfusion has been associated with accelerated clearance of necrotic debris (54). Other studies have shown that stimulated or transmigrated neutrophils have decreased apoptosis and, thus, may remain in tissues for much longer times (54). It has become clear that the inflammatory process is critical in the repair of myocardial injury (54). The contradictory effects of neu-

trophils may explain the inconsistent results that have been found when attempting to reduce reperfusion injury using anti-neutrophil strategies.

Neutrophil accumulation was noted as the major difference in studies comparing myocardium that was permanently ischemic versus transient ischemic myocardium that was reperfused after ischemia (55). Increased neutrophil accumulation in ischemic/reperfused myocardium indicates that different mechanisms are involved in the cell death pathway in permanent ischemia versus ischemia followed by reperfusion (55). Although the factors that induce apoptosis in the ischemia/reperfused myocardium are not fully understood, proinflammatory mediators such as ROS and cytokines released from activated neutrophils may trigger apoptosis (55).

APOPTOSIS

Apoptosis is another mechanism of myocardial injury associated with ischemia–reperfusion. Apoptosis (Greek, meaning leaves or flowers falling off trees) is genetically induced cell death or so-called “cellular suicide” (4,56–64). The control of cell number in multicellular organisms is maintained through an intricate balance between cell proliferation and cell death (60). Even though a significant amount of information exists concerning cell proliferation in many species, less is understood about the processes that control cell death (60), even though recent evidence suggests that the processes involved in cell life and death are intimately linked (57). For example, multicellular organisms eliminate damaged, redundant, or pathogenic cells by apoptotic mechanisms (61). Furthermore, apoptosis is essential for proper embryonic organ formation; control of cell death helps to establish the morphology of the organ during development (60). Apoptosis is a significant contributor of cell death in chronic processes like neurodegenerative diseases, graft versus host disease, autoimmune disorders, diabetes, cancer, and acute insults, such as sepsis and ischemia–reperfusion injury, (62,63,65,66). Current knowledge of apoptosis has led to the suggestion that ischemia–reperfusion mediates apoptosis by or in combination with: (1) upregulation of Bax (proapoptotic protein); (2) downregulation of Bcl-2 (anti-apoptotic protein); (3) activation of Fas or TNF- α receptors; (4) activation of p53 and c-Jun kinase pathways; and (5) neutrophil and/or macrophage activation and infiltration (67).

Apoptosis and necrosis differ significantly at the cellular level (Table 2). Interestingly, the amount of ATP available to a cell that suffers a fatal insult will determine whether the cellular demise is apoptotic or necrotic (56,59,63–69). Accidental or pathologic death termed necrosis, results from ischemia, toxins, viruses, or complement attack. Necrosis can be identified by organelle destruction, cell lysis and associated inflammation (15,60).

Table 2. Necrosis versus apoptosis (15,56,58,59,63–69)

Necrosis	Apoptosis
Accidental	Programmed death cascade
Always pathologic	Pathological or physiological
ATP not required	ATP required
Poorly regulated	Tightly regulated
Plasma membrane destroyed early	Plasma membrane almost intact until late
Cellular content leakage	No cellular content leakage
Inflammation	Minute or no inflammation
Mitochondrial swelling	No mitochondrial swelling
Oncosis (swelling of entire cell membrane)	Cell shrinkage
No changes in nuclear morphology	Changes in nuclear morphology, such as endonucleolysis (DNA fragmentation pattern), karyorrhexis (nuclear fragmentation), pyknosis (chromatin condensation)
No selective protein degradation via caspases	Selective protein degradation via caspases

Features of myocyte necrosis include cell swelling, plasma membrane breakdown, a lack of DNA mediated cell death and caspase protein degradation, swelling and disruption of the SR or mitochondria, and granular densities in the matrix of the mitochondria (15,59). As a result of sarcolemmal rupture, calcium overload and other lethal electrolyte imbalances, rapid cellular disintegration occurs (27).

During apoptosis, cell death occurs in the absence of cell membrane rupture with minimal or no inflammation (59). Apoptosis has DNA involvement and caspases degrade proteins in the apoptotic cascade. Ultrastructural manifestations of apoptosis include pyknosis (chromatin condensation), endonucleolysis (DNA fragmentation), karyorrhexis (nuclear fragmentation), cell shrinkage, condensation of the cytoplasm, and mild convolution of the nuclear and cellular outlines (58,59,62,65). Apoptotic cells experience double-stranded breaks in DNA whereas necrotic cells do not (64). In contrast to necrosis, nuclear fragmentation occurs during apoptosis and the cell surface develops pediculated protuberances or “blebbing” and separate membrane-bounded apoptotic bodies, which are phagocytosed by adjacent cells (15,59,60). The surface exposure of phosphatidylserine residues (normally on the inner membrane) allows for the recognition and elimination of apoptotic cells by their healthy neighbors (15,59). The exposure of this residue has been exploited in a nuclear imaging strategy using radiolabeled annexin V, which binds phosphatidylserine, and therefore noninvasively identifies cells in the early stages of apoptosis.

Regulation of apoptosis is a highly controlled and complex process that is just beginning to be understood. Apaf-1 (apoptosis-activating factor) is a conserved mammalian molecule that has been found to play a major role in the control of apoptosis (70). Apaf-1 binds to cyto-

chrome c along with ATP to form the apoptosome, which cleaves procaspase 9 into the active caspase 9 protein (Figure 5) (61,70). In oxidative phosphorylation, cytochrome c is an electron carrier that transfers electrons between complexes III and IV. When cytochrome c is released from the mitochondria, the result is a disruption in electron transport, decreased production of ATP, and increased ROS production (55,61). Caspases are the family of endogenous cysteine proteases that activate apoptotic cell death (71,72). Caspases allow cellular destruction to occur by the inactivation of apoptotic inhibitors, the destruction of cellular structures, and the deregulation of protein activity (ie, protein loses or gains function) (62). Caspases open permeability transition (PT) pores, which allow cytochrome c to exit the mitochondria (Figure 6) (55). Caspases are maintained in a proinactive state because activation of proteolysis is an irreversible process (62). Although Apaf-1 is important for apoptosis to occur, recent evidence shows that it is not necessary for activation of the apoptotic cascade. Apoptosis can occur via the

activation of death receptors, tissue necrosis factor- α (TNF- α) and Fas, which cleave procaspase 8 into caspase 8 (61,73). Apaf-1 and caspase-independent apoptosis activation can occur by Apoptosis Inducing Factor (AIF) release from the mitochondria (74).

Many genes were reported to be linked with the regulation of programmed cell death under physiological and pathological conditions, but the Bcl-2 family has been suggested as a major, controlling point in the pathway of apoptotic cell death (55,60,61,65,75). The Bcl-2 proteins are believed to control apoptosis by allowing or inhibiting Apaf-1 from binding with cytochrome c, and by controlling the release of cytochrome c from the mitochondria (76). Its family members consist of Bcl-2, Bcl-x, Bcl-w, Bid, Boo (main inhibitors of apoptosis), and Bax, Bak, Bik, and Bim (main accelerators of apoptosis) (55). Bak and Bax possess a channel forming ability that is similar to some bacterial toxins (55). The ratio of Bcl-2 to Bax proteins has been suggested to determine survival or death after an apoptotic stimulus (55,75). Bcl-2 protein expression is increased in ischemic myocytes, while Bax protein expression remains unchanged (75).

It is hypothesized that the current involvement of the mitochondria in apoptosis is remnant of bacteria's ability to kill opposing prokaryotes before bacteria became the mitochondria of eukaryotic cells as assumed in the endosymbiotic theory (56,77). Mitochondria possess proapoptotic molecules and are plentiful in myocardium, which allows mitochondria to play a pivotal role in processes after ischemia-reperfusion exposure (78). The mitochondrial permeability transition (MPT) is the physiologic process in which the selectively permeable inner mitochondrial membrane becomes nonselectively permeable to small solutes (up to 1500 Daltons) (68), resulting in uncoupling, swelling, and loss of intramitochondrial low molecular weight molecules (79). MPT ultimately leads to apoptotic or necrotic cell death (80). MPT facilitates the release of cytochrome c, which initiates caspase activation (78). MPT occurs when the mitochondria is exposed to supraphysiological calcium concentrations, and sensitivity to $[Ca^{2+}]$ increases when exposed to oxidative stress, adenine nucleotide depletion, increased phosphate concentrations, low membrane potential, and agents like carboxyatractyloside (79). If a mitochondrial membrane hole is not repaired in an appropriate manner, disruption of the mitochondrial transmembrane potential occurs and, thus ATP production will cease (59,77).

The role of apoptosis in reperfusion injury has recently been addressed in both rat and rabbit models, where reperfusion was shown to accelerate the occurrence of apoptotic cell death in cardiomyocytes (64). ROS have been implicated in tissue injury during reperfusion and oxidative stress induced-apoptosis in isolated neonatal rat cardiomyocytes. However, reperfusion reduces the number

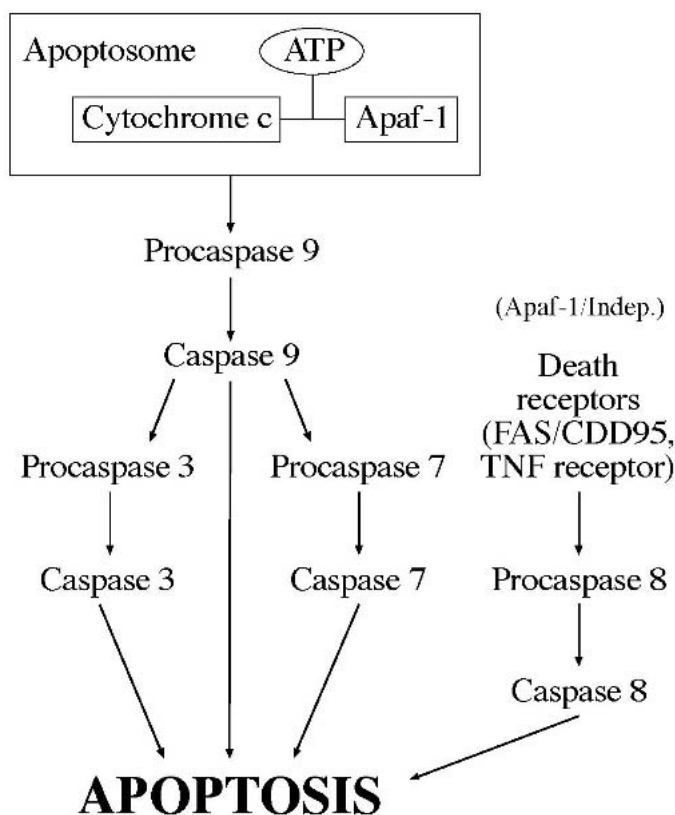


Figure 5. Apaf-1 dependent and independent apoptotic cascades. Apaf, apoptosis activating factor-1. Apaf-1 binds to cytochrome c along with ATP to form the apoptosome, which cleaves procaspase 9 into the active caspase 9 protein. Caspases are the family of endogenous cysteine proteases that activate apoptotic cell death by: the inactivation of apoptotic inhibitors, the destruction of cellular structures, and the deregulation of proteins by the separation of regulatory and catalytic domains. Apoptosis can also occur *via* the activation of death receptors (TNF receptor 1, FAS/CD95), which cleave procaspase 8 into caspase 8.

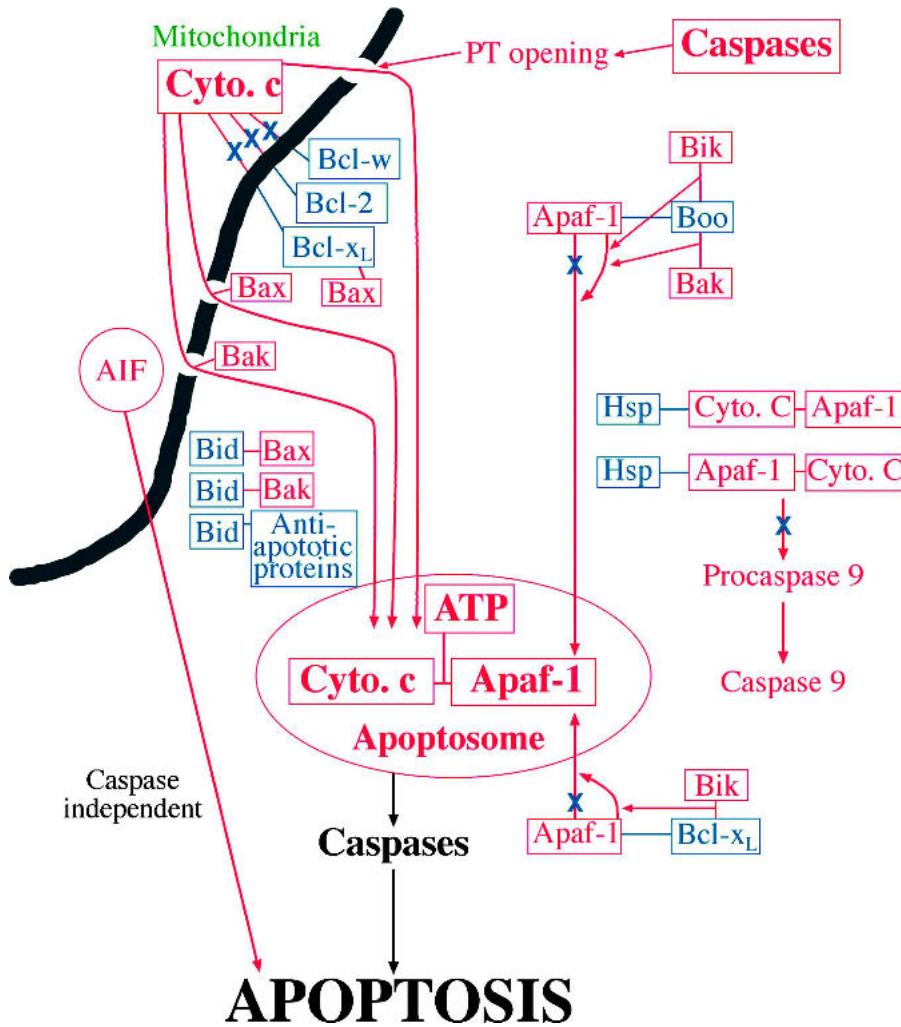


Figure 6. Regulatory model of apoptotic cascade. (Red) pro-apoptotic, (Blue) anti-apoptotic. Cytochrome c is normally used for electron-chain transport. When released from the mitochondria, it disrupts electron transport, decreases production of ATP, and increases ROS generation. Caspases are able to open permeability transition (PT) pores, which allow cytochrome c to exit the mitochondria. Although the apoptosome (i.e., Apaf-1 bound to cytochrome c and ATP) is an important initiator of apoptosis, release of apoptosis-inducing factor (AIF) from the mitochondria is an additional recently recognized mechanism. The Bcl-2 proteins control apoptosis by inhibiting Apaf-1 from binding with cytochrome c, and by controlling the release of cytochrome c from the mitochondria (76). Bax, Bak, Bik, and Bim are accelerators of apoptosis. The ratio of Bcl-2 to Bax proteins determines the survival or death after an apoptotic stimulus.

of apoptotic cells in the rat ischemic myocardium (64). This contradiction illustrates the dual role of reperfusion in myocardial salvage and injury. Fliiss and Gattinger suggest that although coronary reperfusion lowers the overall number of myocytes undergoing apoptosis in rats, it accelerates myocyte apoptosis in nonsalvageable myocardium (81,82).

What initiates apoptosis has been a subject of debate, but recent research has provided a hypothesis. In a study by Freude and colleagues (58), it was reported that apoptotic cell death is initiated by ischemia but reperfusion is necessary for the finalization of the apoptotic cascade. In a study by Zhao et al. (55), results demonstrated that myocyte apoptosis is primarily activated by ischemia followed by reperfusion, but not by ischemia alone. One hypothesis is that the ischemic insult activates the apoptotic cellular cascade, and the initiation of the apoptotic cascade is delayed until ATP is liberated by aerobic means due to reperfusion (55).

The significance of apoptosis in the pathogenesis of ischemic injury is still controversial (64). Research by

Freude et al. (58) suggests that 90 minutes of global warm ischemia followed by reperfusion primarily results in myocardial necrosis and that apoptosis is of minor importance. Contradictory results obtained by Condorelli et al. (83) and Chen et al. (84) demonstrate that apoptosis does play a significant role in the response to ischemia. Mice that overexpressed cardiospecific caspase 3 had increased apoptotic activity and when exposed to ischemia displayed increased infarctions and poor cardiac contractility (83). Mice that overexpressed Bcl-2 had reduced infarction and improved cardiac function when exposed to ischemia (84). For decades, MI was believed to be the final result of prolonged ischemia, but there is increasing data that suggests myocytes irreversibly injured during MI are comprised of both necrotic and apoptotic cells (64). In a study by Saraste and colleagues (85), human myocardial samples were studied from patients that died of acute MI, and apoptotic myocytes were identified predominantly in border zones of histologically recent infarction. Myocyte apoptosis has been identified in both the experimental and clinical arenas after ischemia-reperfusion injury (86,87).

MYOCYTE HYPERCONTRACTURE

A fourth mechanism of myocardial reperfusion injury is hypercontracture (irreversible cell shortening) of cardiac myocytes induced by calcium ion overload and disruption of ionic homeostasis. Ca^{2+} myocyte overloading causes functional diseases including arrhythmias and lethal reperfusion injury (88,89). Heart tissue from several vertebrate species has been documented to demonstrate a phenomenon deemed "calcium paradox", in which reperfusion with a Ca^{2+} containing solution after an interval of Ca^{2+} -free perfusion results in tissue damage, depletion of ATP, Ca^{2+} overload of the sarcoplasm, and disruption of the cell membrane (90–93). In particular, myocyte perfusion with a Ca^{2+} -free solution yields decreased extracellular Ca^{2+} levels, so that extracellular Na^+ is able to enter the myocyte via L-type Ca^{2+} channels, which are permeable to monovalent cations (90,93). This increase of intracellular Na^+ is partly controlled by increased Na^+/K^+ pump activity. Debate exists over the role Na^+ plays in the Ca^{2+} paradox, Na^+ overloading may be the foundation of the Ca^{2+} paradox or it might simply aggravate the damage imposed by reintroduction of Ca^{2+} (90). The mass of Ca^{2+} released by the SR is influenced by at least three factors: (1) the SR Ca^{2+} level; (2) the rate of change of myoplasmic-free Ca^{2+} ; and (3) the mass of Ca^{2+} available to trigger the release (94,95).

Three initial causes of immediate reperfusion injury have been described by the literature: re-establishment of ATP production via aerobic metabolism, rapid increase of tissue pH, and rapid normalization of tissue osmolality (27,96). Hypercontracture is made possible by re-energi-

zation of the ischemic cell in which destructive contractile forces are generated because of Ca^{2+} overload and increased cytoskeletal fragility (27). During ischemia, reduced oxygen availability decreases the amount of ATP that results from aerobic reactions; thus, the heart uses anaerobic metabolism. Secondary to glucose fermentation, myocytes become increasingly acidic due to the increased production of lactate and H^+ (97). When ATP levels decrease, cells lose function of the Na^+/K^+ ATPase and Na^+ levels increase (92,98,99). In an effort to neutralize the decreased intracellular pH, cells activate their sodium ion-hydrogen ion exchanger (NHE) (88,98). This exchanger is used by the majority of eukaryotic cells to maintain ionic homeostasis and to protect cardiac myocytes from intracellular acidosis (98). The NHE is an integral plasma membrane protein that electrochemically exchanges extracellular Na^+ for intracellular H^+ with 1:1 stoichiometry (100). If the intracellular $[\text{Na}^+]$ is excessive, osmolar disturbances such as cellular edema and swelling may occur. The myocyte will correct the osmolality by pumping Na^+ outward and moving Ca^{2+} inward to decrease the osmolar load of the cell with the use of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCE), which exacerbates the hypercalcemic intracellular state of the myocyte (Figure 7). The Ca^{2+} -ATPase pump will pump excess Ca^{2+} into the SR. If the SR overfills with excess Ca^{2+} , a vicious cycle of Ca^{2+} uptake and release will result that uses excessive ATP without achieving calcium ion homeostasis (93). Cells in which these pumps have been damaged during an ischemic period are unable to recover and cell death is likely (27). The intracellular accumulation of Ca^{2+} continues in such cells as a diminished Na^+ gradient favors Ca^{2+} entry

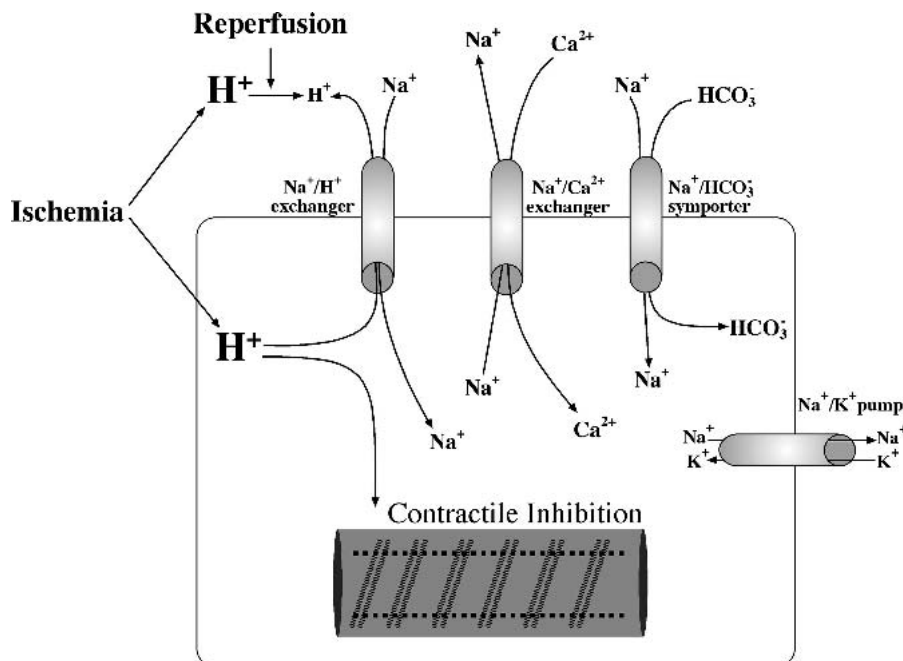


Figure 7. Ionic mechanisms of the myocyte. Myocytes become increasingly acidic by the increased production of lactate and H^+ during ischemia. To neutralize the decreased intracellular pH, the cells activate their sodium ion-hydrogen ion exchanger (NHE). With ATP depletion, the Na^+/K^+ ATPase pump is ineffective and intracellular Na^+ levels are increased. Pumping Na^+ outward and moving Ca^{2+} inward to decrease the osmolar load of the cell with the use of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCE) causes a hypercalcemic state and inhibition of contraction. (Adapted from *Circ Res* 1994;74:797.)

through a $\text{Na}^+/\text{Ca}^{2+}$ exchanger operating in reverse mode (27).

With the oxygen paradox, it has been acknowledged that if the myocardium is deprived of oxygen and then reperfused, injuries to the sarcolemma and myofibrillar hypercontracture will develop at the time of reoxygenation from resumption of energy production (27,101). Histological analysis clearly demonstrates that when reperfusion is performed early enough to produce some myocardial salvage, infarcts are composed exclusively of contraction band necrosis (27). Contraction band necrosis reflects hypercontracture of myocytes, which is associated with sarcolemmal disruption and cell death, and data demonstrate that this occurs within the first minutes of reperfusion (27).

The assumption that a quick correction of acidosis is beneficial was questioned recently by Piper and associates, who suggest that acidosis formed during ischemia can be indeed beneficial in injury reduction when reperfusion occurs (27). Greater understanding of the pH -regulating transsarcolemmal transport process has led to the belief that inhibition of proton elimination from the acidotic myocyte can be advantageous, since low intracellular pH prohibits the myofibrillar machinery from contracting. Prolongation of myocyte acidosis in the initial state of reperfusion in isolated myocytes produces a protective effect for the myocytes (102,103). If acidosis could inhibit myofibrillar contractility until the myocardium was prepared to resume contraction, less myocardial injury might result. Acidosis is also beneficial for the inhibitory action on MPT (78) and cytosolic Ca^{2+} oscillations that result in arrhythmias (102). Contradictory to long held beliefs, prolonged intracellular acidosis may actually protect myocardium from reoxygenation-induced mechanical injury by allowing myocytes adequate time for re-establishing normal cellular ionic balance during the first stage of energy recovery (27).

Elevated Na^+ concentrations in the cell can cause significant consequences independent of hypercontracture. Increased intracellular Na^+ concentrations will lead to myocardial cellular edema. In the ischemic myocardium, the small volume of water available in the non-perfused tissue limits cellular uptake of water. Upon reperfusion, when water becomes available again, Na^+ overloading can result from the NHE effort to correct acidosis. The NHE involvement in cell volume regulation also has been demonstrated for other types of cells (27). Myocardial edema is not as detrimental physiologically as other etiologies (i.e., hypercontracture), but together they can lead to sarcolemmal fragility and render the cell more susceptible to damage via osmotic stress (27).

Unfortunately, hypercontracture is not an isolated event. If a myocyte has experienced Ca^{2+} overload and hypercontracture, the cell can communicate with neigh-

boring cells by gap junctions. The adjacent noninjured myocytes can be potentially exposed to increased Ca^{2+} concentrations and hypercontracture by being in proximity to myocytes injured from hypercontracture-mediated injury. In addition, exchange of large mechanical forces between a hypercontracting cell and its neighbors through the intercalated disks can cause mechanical disruption. Removal of Ca^{2+} from the extracellular fluid causes damage to the intercalated disk junctions (94). When such fibers are returned to a normal environment, the sarcolemma develops 'holes' in the bilayer and with continued reperfusion, the fibers contract strongly, pulling apart from one another, destroying the architecture (94). Decreased ATP levels, intracellular enzymes, myoglobin, energy stores, creatine phosphate, and the inhibition of sarcolemmal ATPase activity all result from the physical damage that occurs when hypercalcemia occurs in myocytes (94).

RISK FACTORS FOR MYOCARDIAL REPERFUSION INJURY

Cardiac risk factors such as diabetes, hypercholesterolemia, and hypertension have been implicated with an increased incidence of myocardial reperfusion injury, but the exact mechanisms are not clear (12,104). Major problems exist in ascertaining how risk factors affect ischemia-reperfusion injury. Most animal models are nonpathologic, lacking cardiac associated co-morbidities. Hypercholesterolemia (which can cause vascular wall injuries) predisposes the endothelium to an increased insult when reperfusion occurs. It is also associated with decreased endothelial NO production, which can result in reduced vascular reactivity and impaired ability of the endothelium to prevent neutrophil-endothelial adhesion (104). Diabetes mellitus has also been found to increase reperfusion-induced damage by deviations in neutrophil adhesion, yielding endothelial and myocyte dysfunction and changes in ROS susceptibility (12,104,105). Hypertriglyceridemia and hypertension in rats increase the severity of injury imposed upon the myocardium via ROS (106). Hyperthyroidism is associated with decreased antioxidant activity and increased incidence of ROS-induced ischemia-reperfusion injury (107). Smoking, stress, and infection have been found to increase circulating neutrophil counts and increased incidence of ischemia-reperfusion injury (28,105). Not surprisingly, age has been identified as an ischemia-reperfusion injury risk factor. In a study with both young and old rat hearts, older hearts experienced greater injury resulting in increased mortality, area of infarction, contraction band necrosis, mitochondrial damage, and DNA fragmentation (108). Organs procured from older donors have been shown to be at increased risk for reperfusion injury after transplantation.

CURRENT AND POTENTIAL THERAPIES

Methods to combat myocardial reperfusion injury are not adequately developed. An agent that aids in the reduction of myocardial reperfusion injury might only address one aspect of the pathological condition. It is important that a potential course of treatment involve a variety of inhibitory mechanisms that are appropriately timed to ensure a complete reperfusion injury treatment. Many positive basic science benchmark studies have resulted in little or no clinical success. Explanations for this deviation often include variations in species, timing, and dosage.

REDUCTION/INHIBITION OF ROS

ROS-induced myocardial reperfusion injury is of great concern during treatment; however, it is likely not the main cause of lethal myocardial reperfusion injury. Equal numbers of studies demonstrate improvement or no improvement of infarct size when antioxidant therapy is applied during reperfusion (27). These inconsistent results are thought to occur due to differences in animal species, co-morbidities, variations of collateral blood flow, ischemia duration, method of drug delivery, drug administration timing, and end-points tested (21,109). Antioxidants are believed to work via the following mechanisms such as: (1) inhibiting ROS formation, (2) scavenging ROS, (3) reducing apoptosis via the upregulation of the Bcl-2 (anti-death) gene, (4) augment endogenous antioxidant production, and (5) dissipate the production of ROS by binding metal ions (110). The human body possesses endogenous antioxidant defense molecules (Table 3) that will afford protection unless the antioxidant defense mechanism itself is damaged by ischemia-reperfusion, or if the defenses are overwhelmed with oxidative stress, leaving the cell vulnerable to damage by the ROS.

Vitamin E, a known antioxidant, is a homogenous group of potent lipid-soluble, chain-breaking antioxidants that prevent the propagation of free radical reactions (107,111,112). In 1996, the Cambridge Heart Antioxidant

Study (CHAOS) reported in more than 2000 patients with angiographically proven coronary atherosclerosis that vitamin E supplementation (400–800 IU/day) for approximately 2 years significantly reduced the incidence of cardiovascular death and nonfatal MI by 77% (111). Decreases in lipid peroxidation of low-density lipoproteins (LDL) may be responsible for this positive result (111). It has been concluded that vitamin E, like every redox-active compound, may exert either anti- and pro-oxidant effects depending on the reaction partners present (111). In a study by Lassnigg et al., cardiac surgery patients were given parenteral vitamin E emulsion to reach plasma vitamin E normalization but that did not affect biochemical markers and clinical outcome (112). More recently, a meta-analysis by Vivekananthan et al. looked at the effects of vitamin E and/or β carotene on long-term cardiovascular mortality and morbidity. Meta-analysis of vitamin E trials involved 81,788 patients and β carotene trials involved 138,113 patients in all-cause mortality analyses (113). The data revealed that vitamin E did not decrease mortality compared to control treatment, nor did it significantly reduce the risk of cardiovascular death (113). The use of β carotene resulted in a minute but considerable increase in all-cause mortality and a small increase in cardiovascular death (113). The authors concluded that the data do not support regular use of vitamin E and that the use of β carotene should cease based upon the increased risks (113). A synergistic effect between vitamin E and selenium has demonstrated biomembrane protection from ROS. Both compounds have similar responses to ROS, and can substitute for each other in certain circumstances (111). Vitamin E readily reduces alkyl peroxy radicals of unsaturated lipids, thereby generating hydroperoxides that are reduced by the selenoperoxides (in particular, phospholipid hydroperoxide glutathione peroxidase) (111).

As mentioned earlier, mitochondria are particularly predisposed to injury from oxidative stress because there is a continual obligate loss of electrons from the respiratory chain (80). In previous studies, the addition of antioxidants to the mitochondria was found to be beneficial, but the actual amount of the antioxidant compound that transverses the membrane was less than desired. In a study by Smith and colleagues, a moiety of vitamin E was coupled to the lipophilic triphenylphosphonium cation, which led to an approximately 80-fold increase of vitamin E in the mitochondria's inner membrane (80). 2-[2-(triphenylphosphonio)ethyl]-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol bromide was the antioxidant compound used in this experiment, and it did not alter any of the functions or processes of the mitochondria and only increased the concentration of antioxidant within the mitochondria.

Ascorbic acid (vitamin C), a precursor of vitamin E,

Table 3. Major endogenous antioxidants and antioxidant action

Name	Action
Catalase (9,22,33,50,117)	$H_2O_2 \rightarrow 2H_2O + O_2$
Superoxide dismutase (8,9,22,117)	Catalyzes $O_2^{\cdot-}$ dismutation to H_2O_2
Glutathione peroxidase (GPx) (8,9,22,117)	$H_2O_2 + 2GSH$ (reduced glutathione) $\rightarrow 2H_2O + GSSG$ (oxidized glutathione)
Glutathione (22,33)	Cellular reductant
Vitamin C (ascorbic acid) (22,33)	Antioxidant and cofactor for vitamin E
Vitamin E (α -tocopherol) (22,111)	Break lipid peroxidation and LDL reaction
β -carotene (pro-vitamin A) (33)	Inhibits oxidation of LDL
Coenzyme Q10 (ubiquinone) (33)	Redox active electron carrier

prevents ROS formation and allows vitamin E regeneration. A study by Dusinovic and colleagues revealed that acute MI patients on thrombolytic therapy exhibited lower levels of plasma vitamin E compared with healthy volunteers but there was no significant difference in levels of vitamin C (114). A study by Dingchao and associates demonstrated protective effects from high dose ascorbic acid (250 mg/kg) in 85 patients that were on CPB (115). These patients exhibited a higher postoperative cardiac index and decreased hospital stay (115).

Because of their role in the catalytic activity and spatial conformation of the antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), several trace elements—copper, zinc, manganese, and selenium—might play an important part in the antioxidant defense capacity of cardiac tissue (17). Results from animal experiments have shown that the dietary intake of such trace elements can diminish the degree of reperfusion damage (17). In most mammalian species, SOD and GPx appear to be the most active antioxidant enzymes in the myocardium (8,22). Selenium is a cofactor necessary for GPx formation, and selenium deficiencies can result in depressed levels of GPx, which allows for greater injury from I/R (8).

There are various endogenous enzymes or molecules that are produced to decrease ROS load. SOD facilitates the reaction of the superoxide radical into O_2 and H_2O_2 as seen in the reaction of $2O_2^{*-} + 2H^+ \rightarrow O_2 + H_2O_2$ (50,33,116). SOD increases the dismutate reaction rate by a factor of 10^9 , when compared with uncatalyzed reaction rates (50). Fast ROS eliminating reactions are essential to minimize the amount of damage that ROS contacting cells will incur. SOD eliminates one ROS and produces another lethal molecule, H_2O_2 . Catalase facilitates the breakdown reaction of H_2O_2 : $2H_2O_2 \rightarrow 2H_2O + O_2$ and creates water and molecular oxygen (50,33,117). SOD and catalase together scavenge O_2^{*-} and $*OH$ and remove H_2O_2 (117). A study by Galang and colleagues demonstrated that perfusion with a combination of SOD and catalase before ischemia–reperfusion could reduce apoptosis, DNA fragmentation, and myocardial reperfusion injury by reducing ROS (26).

Other ROS scavengers (Table 4) include glutathione, fatty acid-binding protein, ONO-3144, indapamide, dimethyl sulfoxide, phytic acid, and EGB 671. Glutathione scavenges O_2^{*-} and protects the sulfhydryl group of proteins (118) and is a cellular reductant (gets reduced so other proteins do not) (33). Dimercaptopropanol also provides protection by keeping major sulfhydryl group of proteins in a reduced state (119). Cysteine functions to increase glutathione (50). Emoxipine is known to enhance glutathione reductase and glutathione peroxidase (120). Fatty acid-binding protein scavenges O_2^{*-} , $*OH$, and $*OCl$ (121). Indapamide scavenges free radical intermedi-

Table 4. Compounds and their antioxidant actions

Name	Action
Allopurinol (22,33,131)	Inhibitor of xanthine oxidase, scavenges $*OH$ and $*OCl$
Oxypurinol (131)	Inhibitor of xanthine oxidase, scavenges $*OH$ and $*OCl$
Fatty acid-binding protein (22,121)	Scavenges O_2^{*-} , $*OH$, and OCl
Desferrioxamine (126)	Chelates iron
Indapamide (22,122)	Scavenges free radical intermediates
Phytic acid (125)	Chelates iron and scavenges iron
Emoxipine (120)	Enhances glutathione reductase and glutathione peroxidase
Dimethyl sulfoxide (22)	Scavenges O_2^{*-} and $*OH$
Proanthocyanidin (130)	Scavenges $*OH$ and peroxy radicals
Propofol (78,134)	Inhibits non-specific pores of MPT?
Cyclosporin A (66,78,92,135)	Inhibits MPT
Mannitol	Scavenges $*OH$
Ibuprofen (132,133)	Chelates iron and inhibits LDL oxidation
Lidocaine (35)	Inhibits the production and release of O_2^{*-} from activated neutrophils

ates (22,122). Dimethyl sulfoxide and EGB 671 scavenge the reactive oxygen species of O_2^{*-} and $*OH$ (123,124). Phytic acid is known to scavenge $*OH$ and chelates iron (125). Desferrioxamine also chelates iron (126). β -carotene (pro-vitamin A) inhibits the oxidation of LDLs (33). It appears there is a multitude of antioxidants working to reduce or eliminate the ROS that is present when an oxidative stress is introduced within the cellular environment. Little is known about the interactions between ROS and genetic expression of SR proteins. It is believed that exogenous SOD and catalase protect SR function in the short-term by the prevention of lipid peroxidation, and in the long-term by the prevention of alterations in SR protein gene expression (127). Metallothionein, a transitional metal-binding protein that is important in the detoxification of heavy metals, also inhibits ROS, such as H_2O_2 , O_2^{*-} , OH^{*-} (128). In vivo studies with metallothionein-overexpressing transgenic mice have displayed a dramatic resistance to stress conditions including those of ischemia–reperfusion injury (128).

Interestingly, antioxidants can be found where not expected, for example in wine. Epidemiological data has shown researchers that red wine consumption has been linked to the prevention of coronary artery disease (129). This correlation between red wine consumption and increased cardioprotective properties against ischemic reperfusion injury has been referred to as the French Paradox (130). In a study by Sato and colleagues, the antioxidant effect of proanthocyanidin, which is found in the seeds of red grapes, has shown that proanthocyanidin is a potent scavenger of hydroxyl and peroxy radicals and decreased lipid peroxidation in reperfused myocardium (130).

Antioxidant properties, mainly free radical scavenging, also are found in commonly used clinical agents: mannitol, allopurinol, ibuprofen, and propofol. Mannitol is an os-

motoc oncotic that is used as a diuretic, and has also been found to scavenge hydroxyl radicals. Allopurinol is used to treat gout, and is an inhibitor of xanthine oxidase and scavenges $\cdot\text{OH}$ and $\cdot\text{OCl}$ (33,131). Ibuprofen is a common nonsteroidal anti-inflammatory drug that is used for the relief of pain and has weak ROS scavenging properties, but can inhibit LDL oxidation in a dose-dependent manner by chelating iron (132,133). This is important, for the oxidation of LDL by ROS facilitates atherogenic properties (132). Propofol is an intravenous anesthetic that has recently been found to possess antioxidant properties that are an additional benefit when used during open-heart surgery, although the exact protective mechanism is not currently understood. A possible mechanism is inhibition of the opening of the non-specific pores that are involved in the MPT (78,134). The MPT injures the myocardium due to mitochondrial uncoupling, which yields the breakdown of ATP instead of the production of ATP (59,134). Cellular demise is eminent when the mitochondrion is catabolizing ATP rather than producing it. It has been proposed that the MPT may function by deciding ultimately whether a cell lives or dies and by which type of death mechanism, necrosis or apoptosis (134). In addition, the cyclic immunosuppressive peptide, cyclosporin A in nanomolar concentrations can inhibit the MPT (66,78, 92,135) and release of cytochrome c (66).

REDUCTION OF NEUTROPHIL ACTIVITY

Another way to decrease the incidence of myocardial ischemia–reperfusion injury would be to minimize neutrophil activity and neutrophil–endothelium interactions. Endothelial cell death mediated by activated neutrophils requires endothelial products (e.g., H_2O_2 , elastase) and neutrophil products (e.g., $\text{O}_2^{\cdot-}$, Fe), thus, strategies that reduce neutrophil numbers or serum iron have been shown to reduce reperfusion injury. Keeping the ROS sensitive endothelium healthy would allow it to continue to produce agents that inhibit neutrophil and platelet accumulation (34). Although neutrophils directly injure myocytes, a systemic anti-neutrophil strategy might delay the recovery process. In past studies, high-dose steroid (i.e., methylprednisone) treatment for patients with acute MI only exacerbated the injury attributed to reduced healing from lack of necrotic tissue removal, which resulted in aneurysmal formation and rupture (35,136). Another possible treatment is the use of monoclonal antibodies that inhibit the adhesion of neutrophils to ischemic myocardium. The use of monoclonal antibodies decreases the infarct size by reducing the number of neutrophils capable of adhering to ischemic myocardium (137). Leukocyte-depleting filters (LDFs) have been developed to reduce complications associated with activated leukocytes. Leukocyte filtration can occur systemically during CPB, dur-

ing cardioplegia administration, and with red blood cell administration. In a double-blinded study by Matheis et al., LDFs were randomly assigned use versus a standard arterial blood filter. They found that patients in the LDF group required less catecholamines, and troponin T plasma levels (a maker of cardiac injury) were significantly lower (138). In another study, Hayashi and colleagues found that leukocyte-depleted cardioplegia was superior in patient populations that had aortic cross clamps greater than 120 minutes (40). Benefits of leukocyte-depleted cardioplegia included an increased incidence of spontaneous defibrillation following aortic cross clamp removal and higher levels of plasma nitrate and nitrite in coronary sinus effluent, which is indicative of decreased endothelium dysfunction (40). A similar study by Palatianos et al. (139) demonstrated that neutrophil-filtered cardioplegia resulted in a decreased need for inotropic support, reduced incidences of reperfusion ventricular fibrillation, and reduction of postoperative creatine kinase myocardial bands and cardiac troponin I levels. Ibuprofen administration resulted in reduced neutrophil accumulation in the myocardium and provided protection for myocardium at risk (20). Ibuprofen decreased MI size in dogs exposed to 60 minutes of coronary occlusion followed by 24 hours of reperfusion (20). Lidocaine, a fast Na^+ channel inhibitor, inhibits activated neutrophils from producing and releasing $\text{O}_2^{\cdot-}$ (35).

NO directly affects neutrophils and the endothelium with regards to ischemia–reperfusion injury (34). NO inhibits neutrophil degranulation, $\text{O}_2^{\cdot-}$ release and adherence to coronary artery endothelium (23,25,34). NO also reduces platelet activation, aggregation and adhesion and decreases mast cell activation and histamine release (23,25,34). Exogenous NO has been used for its cardioactive benefits (i.e., decreased pulmonary vascular resistance via cAMP-mediated vasodilation) without much knowledge of the protective effects on the endothelium. NO is a molecule that is primarily released from endothelial cells (17,140–143), myocytes, neutrophils and mast cells (23,25). The amount of NO produced also was found to moderate fluid accumulation of rats on CPB (143). The addition of L-arginine (precursor to NO) was found to increase NO production and *N*-nitro-L-arginine methyl ester (L-NAME), an L-arginine analog was found to decrease NO production. This study exemplified nitric oxide's ability to control capillary permeability and provide protection from edema (143). L-arginine's protective myocardial effect (i.e., decreased I/R injury) via decreased neutrophil accumulation is currently being investigated (25,105). Pre-ischemic, exogenous L-arginine supplementation improves functional recovery and increases cyclic guanosine monophosphate (cGMP) concentrations, which decrease the sensitivity of cardiac myofilaments to Ca^{2+} during reperfusion (144). Urodilatin, a member of the natriuretic

peptide family, increases cGMP concentrations and exerts protective effects through the stimulation of guanylyl cyclase (144). NO also produces undesirable effects on cardiac myocytes such as the production of peroxynitrate (10,17,23,25) and the desensitization of the myofilament to Ca^{2+} (131). In a study by Masini et al., it was shown that the use of NOS inhibitors caused increased reperfusion injury. NOS inhibitors L-NMMA (L-NG-monomethyl-arginine) and L-NAME lead to increased lipid peroxidation and calcium overload; the role of endogenous NO is believed to be protective against contractile dysfunction (25). NO may have protective properties, but its involvement in ROS production is not yet clear.

The possible benefits of reducing reperfusion injury associated with NO production must be weighed against the production of peroxynitrite metabolites. Peroxynitrite has been linked to direct cellular injury of myocytes at high concentrations; however, at low concentrations (≤ 3 mM) it decreased both neutrophil-endothelial interactions and neutrophil accumulation in myocytes that experienced I/R (21,23). Hearts that were perfused with crystalloid cardioplegia had a higher incidence of increased myocardial injury compared to hearts perfused with blood cardioplegia (21,23). One proposed mechanism is that ONOO⁻ had only myocytes to react with in crystalloid cardioplegia versus a variety of molecules in blood such as albumin, and other proteins that had glutathione and cysteine groups found in blood cardioplegia. In blood-perfused hearts, the deleterious ONOO⁻ is detoxified by glutathione and uric acid to reduce ONOO⁻ levels and convert ONOO⁻ to the protective NO molecule (21,23).

A breakdown product of ATP, adenosine is evident after large amounts of ATP have been degraded. Adenosine is broken down into xanthine, hypoxanthine, and inosine (33,92). Adenosine is regarded to be a potentially powerful cardioprotective agent, and has been found to elicit protective effects when given as a pretreatment against ischemia and reperfusion (39). Decreased neutrophil adherence to the vascular endothelium, and decreased endothelial dysfunction has been attributed to adenosine administration (34,39,42). There are a variety of adenosine receptors that produce cardioprotective effects. Adenosine 1 (A_1) receptors exert their effects pre- and post-ischemia via decreasing metabolic demand and by the activation of the ATP-sensitive K^+ (K_{ATP}) channels (145). A_2 receptors provide protection mainly during reperfusion by reducing neutrophil function and by the reduction of neutrophil adherence to the vascular endothelium (145). A recent study by Jordan et al. showed that A_3 receptor activation might also provide I/R myocardial protection via decreased neutrophil adherence to the vascular endothelium without concurrent vasodilatory activity (145). Research with canine models has demonstrated a significant decrease in infarct size with adenosine admin-

istration (146,147). In another canine model, multidose adenosine administration was superior to a single dose with regards to reduced neutrophil-endothelium adherence, infarct size, and maintained regional coronary blood flow (42). This study demonstrated that adenosine therapy needs to address the late reperfusion phase (>24 hours) as well as the early reperfusion phase (<6 hours) to reduce reperfusion injury (42). Although clinical trials have confirmed experimental studies, the protective effect has, in general, not been as impressive (148,149).

A better understanding of endothelial cell function could possibly lead to the modulation of genes responsible for ischemia-reperfusion injury. It is known that the endothelium is involved in the direction of neutrophils to a site of injury, although endothelial transcription and ultimate protein expression are believed to be different for reperfusion injury versus sepsis (150). If the difference is at the genetic level, it may be possible to inhibit the transcription of proteins that signal reperfusion injury without affecting the production of agents that signal sepsis, so selective inhibition would not compromise host immunity. A heightened level of understanding of the processes involved will be the key to inhibiting myocardial reperfusion injury that is mediated by neutrophils and by neutrophil-endothelium interactions. Other neutrophil activity decreasing agents include perfluorochemicals and protease inhibitors. Perfluorochemicals are known for their oxygen carrying capacity, but have also been linked to decreased adherence of neutrophils to anoxic endothelial cells (105,151). Another approach under study is inhibition of the neutrophil produced proteases (105). Any potential mechanism that decreases the activity of neutrophils and incidence of neutrophil-endothelium adherence will potentially decrease neutrophil induced myocardial damage.

REDUCTION IN APOPTOSIS

Apoptosis research has made great progress in understanding the complex mechanisms involved by identifying major genes that are integral to the apoptotic pathway, but it is difficult to effectively inhibit a process that we do not completely understand. The presence of antioxidant enzymes during reperfusion was shown to attenuate apoptotic activation, and showed a direct relationship between ROS and apoptosis (26). Apoptotic involvement in ischemia-reperfusion injury has been demonstrated by various studies. Mice who exhibited cardiac specific caspase 3 overexpression had increased apoptotic activity, and when exposed to ischemia, experienced more infarctions and poor cardiac function. Caspase inhibitors and counter-regulatory growth factors are inhibitors of apoptosis and have been found to decrease infarct size when given early during reperfusion (Figure 8) (4). The inhibition of caspase is difficult to achieve because the inhibiting non-

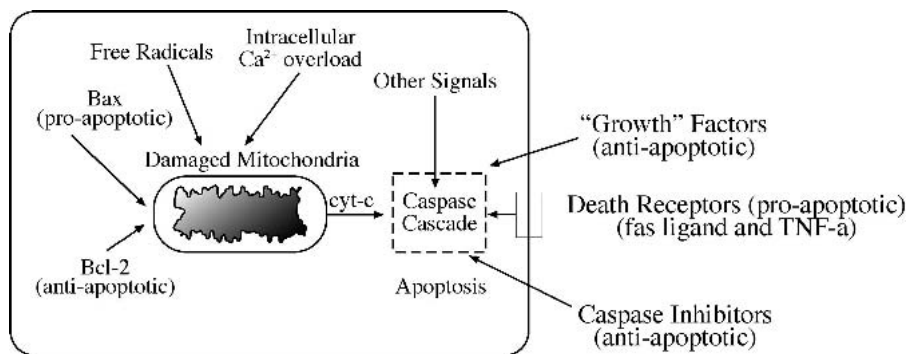


Figure 8. Apoptosis and potential apoptotic inhibition. Although difficult to achieve because the inhibiting molecule has to be very selective, stable, small, and possess the ability to permeate membranes efficiently apoptosis has been inhibited by caspase inhibitors and counter regulatory growth factors. In experimental models, these agents have been found to decrease infarct size when given early during reperfusion. (Adapted from Heart 2000;83:383.)

peptide molecule has to be very selective, stable, small, and possess the ability to permeate membranes efficiently (62). Mocanu and colleagues first achieved caspase inhibition, in which early reperfusion in isolated rat hearts with caspase inhibitors limited infarct size (152). Heat shock proteins (Hsp) increase molecular tolerance to heat but also to other pathophysiological conditions such as ischemia-reperfusion. Hsp are often referred to as “molecular chaperones”, which maintain a protein’s shape when exposed to unusual levels of heat and environmental stress. Potentially, these could be used as an apoptosis reduction treatment (72). Hsp70 decreased the binding of Apaf-1 to the apoptosome, thereby decreasing apoptosome-induced apoptosis (72). Hsp90 binds to Apaf-1, thus reducing Apaf-1 levels that are able to participate in apoptotic activation (70).

Another potential target for apoptosis inhibition is apoptosis inhibiting factor (AIF). Anti-AIF antibody prevents chromatin condensation and inhibition of AIF-induced Apaf-1 release from the mitochondria (74). Another potential apoptotic inhibiting therapy may be found in macromolecule synthesis inhibitors. Macromolecule synthesis inhibitors block RNA and/or protein synthesis, and have been documented to inhibit the activation and/or execution of the apoptotic cascade in various tissues (153). Identifying apoptosis using a noninvasive nuclear medicine scan (Apomate™) may provide a prospective assessment of the risk of injury after reperfusion. Discoveries in other research disciplines such as cancer, will open up avenues for a greater understanding of the apoptotic pathway. Future research will also look at possible ways to maximize and further induce the mitotic proliferative cycle of cardiac myocytes.

INHIBITION OF MYOCYTE HYPERCONTRACTURE

Recent excitement has resulted from the recognition that inhibition of the NHE mechanism is a powerful tool to delay cell death during ischemia (27). NHE inhibitors (NHEI) are hypothesized to decrease I/R injury by causing delayed alkalinization and decreased intracellular Na⁺

load and hence decreased intracellular Ca²⁺ levels (154–156). NHEI also are believed to reduce: toxicity of ischemic metabolites, ischemia-reperfusion -induced hypercontracture, arrhythmias, apoptosis, necrosis, and post-infarction mortality (155,157). NHEI are beneficial in decreasing ventricular fibrillation, and extending the window of myocardial salvage for reperfusion (88). Other beneficial attributes of NHEI include no associated hypotension and potential prevention of cardiac hypotrophy and fibrosis (155). NHEI have low toxicity and the only negative attribute is increased platelet activity (83). In animal models, these inhibitor-compounds have consistently limited infarct size when administered pre-ischemia (4,98). EMD 85131, a NHEI, was administered before ischemia-reperfusion in canine models and resulted in significant cardioprotection and reduction in infarction (80). The fact that the cardiac cell possesses several pH-regulating systems is of great importance regarding the use of NHEI, for inhibition of the antiporter does not totally inhibit acid-base homeostasis, especially in situations of elevated levels of protons found during ischemia (158). The NHE is inactive during basal physiological conditions, so compounds that inhibit the NHE would only affect ionic movement in ischemic situations (88,159). Mechanisms of pH regulating/H⁺ removing in cardiomyocytes include the Na⁺/H⁺ exchanger, lactate/H⁺ symporter, and Na⁺/HCO₃⁻ symporter (81,83,159). Difficulties in the past have occurred with NHE inhibition because the agents used were not specific enough for the NHE. Amiloride, the classic NHEI, allows nonspecific inhibition of the NHE. The non-specificity of amiloride is evidenced by inhibitory activity on the Na⁺/Ca²⁺ exchanger (81). Newer NHEI like dimethylamiloride and cariporide (HOE642) provide specific NHE inhibition (81). Dimethylamiloride demonstrated protection against ischemia-reperfusion injury and preserved functional and ultrastructural integrity of the NHE without affecting cellular energy levels (100). The GUARD During Ischemia Against Necrosis (GUARDIAN) study looked at the effect cariporide had on all-cause mortality and reoccurring MI (66,155,159–161). The overall analysis did not reveal a significant reduction in three groups, although one cohort, patients un-

dergoing coronary artery bypass grafting did show a reduction in incidence of Q-wave MI (66,160). Patients in this cohort received the 120-mg dose of cariporide and exhibited clinical benefit early after treatment was initiated and continued for 6 months (160). Another major clinical study, ESCAMI (Evaluation of the Safety and the Cardioprotective effects of Eniporide in Acute Myocardial Infarction), is still underway in Europe evaluating the effect of eniporide in a setting of acute MI (66).

The addition of NHEI to cardioplegia is of particular interest to those within the cardiac surgery arena. Castella and colleagues compared buffered (pH 7.7) and non-buffered (pH 7.2) cardioplegia with and without pretreatment of cariporide. They discovered that NHEI played a more significant role in I/R recovery than pH management (162). The management of pH played a larger role in less jeopardized hearts than in severely jeopardized hearts (i.e., ischemia time of approximately 30 minutes) (162). In a porcine model, pretreatment with cariporide or cardioplegic reperfusion with cariporide resulted in decreased ROS damage, myocyte cellular and endothelial damage, water accumulation, and improved preload recruitable stroke work (163). Cariporide was also added to the cardioplegia solution in a canine model, yielding results such as decreased infarct size, neutrophil accumulation, endothelial dysfunction and tissue edema, however, neutrophils were not directly inhibited (164). Eniporide, another NHEI, was added to cardioplegia in a guinea pig model and resulted in lower peak systolic and diastolic Ca^{2+} loading and decreased infarct size (165). Another study with eniporide in pigs did not demonstrate any beneficial effect provided by NCEI (166). More studies are necessary to elucidate the mechanisms involved with NHE inhibition.

Another possible mechanism that could reduce ischemia-reperfusion injury in cardiac myocytes is the inhibition of NCE in the reverse mode. NCE permits the ionic exchange between Ca^{2+} and Na^+ , thus in the forward mode there is a Ca^{2+} efflux and in the reverse mode there is a Ca^{2+} influx (167). Approximately 15% of intracellular Ca^{2+} is removed from the cell via NCE whereas the rest is transported via the Ca^{2+} ATPase into the SR (168). If the activity of the NCE could be minimized when myocytes are exposed to elevated intracellular levels of Na^+ , myocyte protection would be achieved via the inhibition of Ca^{2+} influx. Intracellular myocyte Ca^{2+} concentrations were reduced through the activity of dichlorobenzamil, a NCE inhibitor (169). Using a rat model, Schafer et al. demonstrated that the NCE inhibitor (NCEI) KB-R7934 protected cardiomyocytes and whole hearts during early reperfusion (170). Other studies have demonstrated that NCEIs such as KB-R7943 and SEA0400 have not been specific enough to inhibit the NCE, which has led to uncertainty about the role NCE has in ischemia-reperfusion

injury (167). In a study by Ohtsuka et al., the researchers wanted to determine how ischemia-reperfusion injury would affect NCE knockout mice. They found that knockout type mice had better cardiac function and decreased infarct size compared to wild type mice, and NCE inhibition may indeed contribute to cardioprotection against I/R injury (167).

Other potential agents are being studied for their calcium controlling attributes. ATP-sensitive potassium channel (K_{ATP}) openers such as nicorandil, bimakalim, and pinacidil are another potential therapy for minimizing reperfusion injury (151). Morphine, an analgesic, also provides ischemic protection by the opening the K_{ATP} (131). Opening of the K_{ATP} channels allows a K^+ efflux, resulting in an increased rate of repolarization and a decreased period of Ca^{2+} influx during the plateau phase of the action potential (171). Opioids can also inhibit the myocardial NHE (172). Blockade of Ca^{2+} cycling across the mitochondrial membrane has been found to reduce Ca^{2+} overloading which causes uncoupling of respiration from ATP synthesis (92,93). Cyclosporin A, an immunosuppressant, blocks the cyclosporin A sensitive pore (allows Ca^{2+} out of the mitochondria) and blocks the Na^+/Ca^{2+} antiport in the mitochondria, and ruthenium red blocks the Ca^{2+} uniport (allows Ca^{2+} into the mitochondria) in the mitochondria (79,93). Dantrolene, a drug that is used for the treatment of malignant hyperthermia via reduced Ca^{2+} release from the SR, has been tested for I/R myocardial protection. *In vitro* studies showed a decrease in lethal cellular injury, but *in vivo* studies in rabbits showed no myocardial improvement in clinically appropriate dosages (173). Magnesium ion (Mg^{2+}) is an antagonist to Ca^{2+} , a cofactor to more than 300 enzymes, and when added to cardioplegia solutions myocardial recovery is enhanced (174). This effect is believed to transpire via the Ca^{2+} channel blocking mechanism derived from Mg^{2+} (174–176). Mg^{2+} effects on Ca^{2+} are believed to also decrease MPT incidence and mitochondria injury (79). Mg^{2+} may also possess antiarrhythmic (176) and ROS attenuation properties (177).

SUMMARY

Myocardial reperfusion injury is a potentially lethal combination of a number of intricate pathophysiologic mechanisms that lead to further myocyte death in an unfortunately already compromised myocardium. It is expectedly a significant partner to ischemia itself, and warrants considerable review and future study. During the next 20 years, the number one global killer is expected to be coronary artery disease, and in many of patients suffering from myocardial ischemia, reperfusion will be an undesired co-morbid condition. No single therapy will address all of the known and potential etiological factors or pathophysiologic mechanism; therefore, this complex pro-

cessed will likely be addressed by a multifaceted pharmacological approach, combined with modern mechanical methods to restore and maintain coronary blood flow. It will be essential to view reperfusion injury with the same fervor that frank myocardial ischemia has received during the past two decades, and ensure that clinicians develop strategies to keep viable myocytes healthy in times of stress and prevent salvageable tissue from undesirable death.

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