Ischemia–reperfusion injury pathophysiology, part I

Maureen McMichael, DVM, DACVECC and Rustin M. Moore, DVM, PhD, DACVS

Abstract

Objective: To review the current scientific literature on ischemia–reperfusion (IR) injury in both human and veterinary medicine. To describe the normal antioxidant defense mechanisms, the pathophysiology of IR injury, and the role of neutrophils in IR injury.
Data sources: Data sources include scientific reviews and original research publications in both human and veterinary medicine.
Summary: IR injury is a complex pathophysiological process involving numerous pathways and body systems. Normal antioxidant defense mechanisms function to limit oxidative injury during times of health. Ischemia is the period that occurs before oxygenated blood is re-introduced and the severity of injury has been shown to correlate with the magnitude and length of ischemia in dogs. During ischemia, there is a buildup of substances (i.e., xanthine oxidase, hypoxanthine, etc.) that, upon re-introduction of oxygen, form reactive oxygen species (ROS). ROS, produced in large part upon reperfusion, can cause extensive damage to DNA, proteins, carbohydrates, and lipids. Although mammalian systems are endowed with abundant antioxidant defenses, the generation of large amounts of ROS can overwhelm these mechanisms leading to cell dysfunction and death. Neutrophils play a critical role in IR injury and may mediate the majority of mucosal and microvascular injury that occurs by releasing ROS and proteolytic enzymes. Although experimental studies have been carried out on cats, dogs, and horses there are few clinical studies on companion animals.
Conclusions: The pathophysiology of IR injury is complex and involves damage by ROS to all biological membranes. Neutrophils play a major role in IR injury and initiate and propogate much of the damage. This article is intended as a review of the pathophysiology of IR injury.

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Introduction

Ischemia–reperfusion (IR) injury is a complex cascade of events involving a multitude of pathophysiological processes. These include activation of neutrophils, platelets, cytokines, reactive nitrogen species, reactive oxygen species (ROS), the coagulation system, the endothelium, and the xanthine-oxido-reductase enzyme system. This can eventually lead to cell damage, cell death, increased vascular permeability, tissue necrosis, and multi-organ dysfunction.[1] Cell death due to both necrosis and apoptosis is triggered by the substances released during IR injury.[2] ROS are believed to play a central role in IR injury in addition to several other diseases including neoplasia, atherosclerosis, and neurodegenerative diseases.

In humans, the most common syndromes involving IR injury include myocardial infarction, cardiac bypass

From the Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, Texas A&M University, College Station, TX (McMichael), and Department of Veterinary Clinical Sciences, Director of Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA (Moore).

Address correspondence and reprint requests to:
Dr. Maureen McMichael, Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843. E-mail: mmcmichael@cvm.tamu.edu

surgery, and organ transplantation. In veterinary medicine, the most common syndromes include gastric dilatation volvulus (GDV), arterial thromboembolism (ATE), resuscitation from hemorrhagic shock, organ transplantation, diaphragmatic hernia, head trauma, mesenteric torsion, intestinal incarceration, and spinal cord trauma.

This article is intended as a review of endogenous antioxidant defense mechanisms, of the pathophysiology of IR injury, and of the role of neutrophils in IR injury.

Normal anti-oxidant defense mechanisms

A free radical is a molecule with one or more unpaired electrons in the outer shell. Since not all of the species that cause oxidative injury are technically free radicals (i.e., hydrogen peroxide is not a free radical, but is a key player in oxidative damage), a more appropriate term is ROS. ROS are capable of reacting with all biological molecules, including nucleic acids, proteins, carbohydrates, and lipids.

In health, the major source of ROS formation in cells is electron leakage from electron transport chains. It is estimated that ~ 90–95% of the oxygen passing through the mitochondria is converted to water and that the remaining 5–10% is reduced, creating ROS.[3] The generation of ROS is kept to a minimum by the high efficiency of electron transfer and sequestration of metal ions. Separate microenvironments exist for the mitochondria, the lysosome, and the peroxisome; each contains a ROS-generating system coupled to immediately adjacent antioxidant defense mechanisms. Some suggest that this compartmentalization may be the most important endogenous defense mechanism against ROS.[4] Other sources of ROS are the cytochrome P450 in the endoplasmic reticulum, lipoxygenases, cyclooxygenases, xanthine oxidase (XO), and NADPH oxidase.[5]

Although all molecules are susceptible to ROS injury, lipids are targeted most frequently. This is believed to be due to lipids' propensity to contain double bonds and their ubiquitous presence in cell membranes.[6] Mammalian cells are rich in polyunsaturated fatty acids (PUFA), which are highly susceptible to oxidative stress. These PUFAs include linoleic, linolenic, and arachidonic acids and along with other fatty acids form part of the structure of triglycerides and phospholipids.[7] Once formed, ROS can either react with another radical to form a covalent bond or, more commonly, react with a non-radical.[8] When a free radical reacts with a non-radical, the non-radical loses an electron, transforming into a free radical. This is the essence of the chain reaction that propagates extensive damage to cell membranes. When the radical combines with another radical, the product can be more damaging than the original radical. An example is when nitric oxide (NO) combines with superoxide ($O_2^{\bullet -}$) creating peroxynitrite ($OONO^-$), which is 2000 times more damaging than hydrogen peroxide (H_2O_2).[9] Alternatively, the reaction of two radicals can result in a termination of the cascade. The interaction of ROS with lipids in the presence of free iron results in lipid peroxidation.[10,11]

The 2 major free radicals that can initiate lipid peroxidation are the hydroxyl radical ($OH^{\bullet}$) and peroxynitrite. The hydroxyl radical is formed when hydrogen peroxide combines with metals (i.e., ferrous iron) or by fission of water molecules by radiation (i.e., UV light or radiation). Peroxynitrite is formed when NO combines with superoxide. The hydroxyl radical and peroxynitrite initiate lipid peroxidation by abstraction of a proton from the PUFA, causing the formation of a peroxy radical ($RO_2^{\bullet}$). The $RO_2^{\bullet}$ can now attack other PUFAs in the membrane and propagate a chain reaction of lipid peroxidation, ending when the substrate (i.e., the lipids of cell membranes) is eliminated or when the $RO_2^{\bullet}$ encounters a chain-breaking antioxidant such as vitamin E.[12] Lipid peroxidation severely damages cell membranes, causing alterations in enzyme systems and receptors, alterations in ionic channels and increased permeability to calcium and other ions.[7] In addition, the products of lipid peroxidation are also thought to initiate inflammation, apoptosis, and inactivation of thiol-containing enzymes.[13–15]

In general, there are 3 lines of antioxidant defense against damage caused by ROS. Antioxidants are defined as substances that can delay or prevent oxidation of lipids, DNA, or proteins.[16] Antioxidant proteins, such as albumin, haptoglobin, ferritin, and ceruloplasmin are abundant in plasma.[17] Intracellular enzymatic antioxidants include superoxide dismutase (SOD), catalase, and glutathione peroxidase.[17] These are expressed in most mammalian cells and prevent the generation of ROS. Small molecule antioxidants are divided into water-soluble and lipid-soluble categories. Water-soluble antioxidants include ascorbic acid (vitamin C), uric acid, bilirubin, glutathione (GSH), zinc, and selenium. Lipid-soluble antioxidants include tocopherols (vitamin E), β-carotene, ubiquinol-10 (co-enzyme Q10), and lycopene.[18] Cell membranes contain tocopherols and β-carotene within their lipid layer and these can act to quench chain reactions of lipid peroxidation.[8] The extracellular fluids within the body contain numerous molecules that have antioxidant properties, including ascorbic acid, bilirubin, transferrin, haptoglobin, albumin, urate, and ceruloplasmin.[17]

Glutathione peroxidase, synthesized in mammalian cells, is generally considered the first line of defense against ROS formation. It is a sulfur-containing tripeptide (glycine, cysteine, glutamine) that reduces hydrogen peroxide to water, using GSH as a substrate.[19] There are two forms, one catalyzes the conversion of hydrogen peroxide and lipid peroxides and requires selenium as a cofactor and the other form does not require selenium, but only catalyzes the reduction of hydrogen peroxide. Oxidative stress has been shown to be associated with a depletion of GSH[20] and this has been shown to induce apoptosis of hepatocytes.[21] Alpha tocopherol is believed to be the most abundant of the tocopherols and tocotrienols that comprise vitamin E, and is considered the second line of defense against ROS. Vitamin E inhabits the lipophilic interior of the cell membrane, where the PUFAs are located, and is a chain-breaking scavenger, halting lipid peroxidation.[22] When a wave of lipid peroxidation reaches vitamin E, it is oxidized to a free radical, sparing any adjacent PUFAs from oxidation. Vitamin C (ascorbic acid) then combines with the E radical forming a poorly reactive, water-soluble, vitamin C radical, and regenerating vitamin E. Vitamin C is the most abundant water-soluble antioxidant and it can directly scavenge ROS or regenerate vitamin E.[23]

SOD is an oxido-reductase that contains copper, zinc, or manganese at the active site. It catalyzes the dismutation of superoxide to oxygen and hydrogen peroxide. It is present in the cytosol (requires copper and zinc), the mitochondria (requires manganese), and on the extracellular surface (requires copper and zinc). Superoxide is often written as an anion (O_2^-), but the appropriate representation is as a radical anion $(O_2^{\bullet-})$.[24] Mitochondrial SOD is believed to play a major role in antioxidant defense mechanisms.[25,26]

Catalase is a heme protein located in peroxisomes, which converts hydrogen peroxide to water and oxygen. Catalase functions in conjunction with SOD; SOD converts superoxide to hydrogen peroxide, and catalase then converts the hydrogen peroxide to water and oxygen.

Ischemia

Reperfusion injury starts with a variable period of ischemia before oxygenated blood is re-introduced. Cold ischemia refers to the absence of blood flow in organs outside the body (i.e., organ transplantation). Warm ischemia occurs in organs or tissues inside the body (i.e., during GDV).[27] Interestingly, the predominant cell type affected during IR injury differs in warm compared with cold ischemia. Hepatocyte death predominates in warm IR injury, while endothelial cells and Kupffer cells are damaged earlier in cold IR injury.[2] The severity of ischemic injury is determined by the magnitude and length of decreased blood flow to an organ or tissue.[28]

Several events combine during ischemia to set the stage for massive ROS formation. When cells cannot maintain adequate adenosine triphosphate (ATP) synthesis (i.e., during hypoxia) they compensate by degrading existing ATP into its components adenosine, then inosine, and finally hypoxanthine. With continued ischemia, hypoxanthine accumulates. Intracellular pH decreases during ischemia due to anaerobic metabolism as lactate and hydrogen ion accumulate. Intracellular enzymes and regulatory proteins are damaged leading to even greater cellular dysfunction.[29] Decreased ATP inactivates the ATP-dependent cell membrane pumps, allowing a net efflux of potassium and an influx of sodium, calcium, and chloride, which causes acute cellular swelling. The increase in calcium appears to be due to several causes including decreased extrusion owing to inactivation of ATP-dependent cell membrane pumps, calcium release from organelles, and calcium influx.[30,31] Since the rise in calcium can be inhibited by calcium channel blockers, increased influx appears to play a prominent role in the increase in intracellular calcium.[2] This increase in intracellular calcium is one of the earliest events in IR injury and the damage is dependent upon both the duration and the extent of the increase.[2] (Figure 1)

Increased cytosolic calcium has been shown to cause both apoptosis and necrosis of the cell.[32,33] The increased intracellular calcium activates a protease, calpain, which converts xanthine dehydrogenase (XD) to XO. Xanthine oxido-reductase is unique in that it exists in two active forms. In health, the XD form predominates and converts hypoxanthine to xanthine and xanthine to uric acid. XD does not require oxygen for its activation, and uses nicotinamide adenine dinucleotide, a carrier that can accept two electrons and transfer them. During ischemia, due to increased calcium, the XO form predominates and requires oxygen for its activation.[4] There is, in essence, a roadblock during ischemia, with continued accumulation of hypoxanthine and XO, neither of which can be utilized because the enzyme XO requires oxygen.

Several other important events occur during ischemia. Activation of nuclear factor-κB (NFκB) leads to increases in inflammatory mediators. Increased expression of adhesion molecules, specifically synthesis of intracellular adhesion molecule-1 (ICAM-1) and E-selectin, via activation of NFκB, occurs, leading to increased leukocyte adhesion at the site of IR injury during reperfusion. Inactivation of endothelial NO
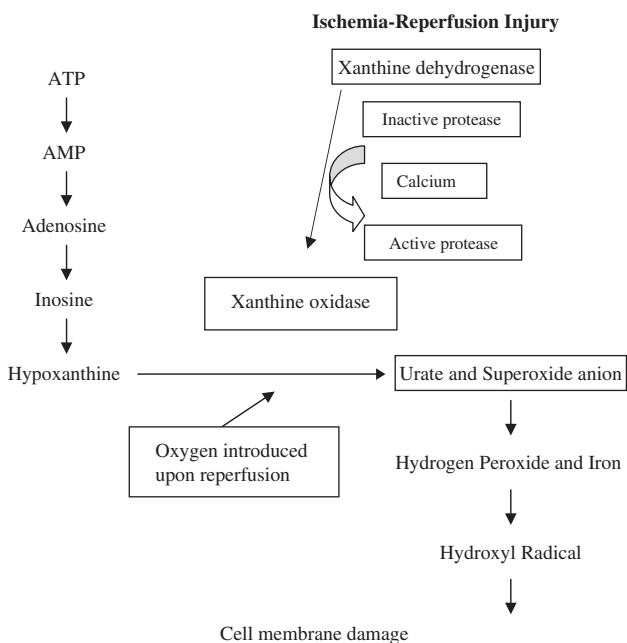
Ischemia-Reperfusion Injury

ATP

AMP

Adenosine

Inosine

Hypoxanthine

Xanthine dehydrogenase

Inactive protease

Calcium

Active protease

Xanthine oxidase

Urate and Superoxide anion

Oxygen introduced upon reperfusion

Hydrogen Peroxide and Iron

Hydroxyl Radical

Cell membrane damage

Figure 1: Ischemia–reperfusion (IR) injury, a schematic of what occurs in the cell during IR injury. There is breakdown of ATP into its components. Xanthine dehydrogenase is converted to xanthine oxidase due to the presence of calcium, which converts a protease, calpain, to its active form. Hypoxanthine is metabolized by xanthine oxidase to urate and superoxide anion upon re-introduction of oxygen. ATP – adenosine triphosphate, AMP – adenosine monophosphate.

causes vasoconstriction, and inhibition of the prostacyclin arm of the arachidonic acid cascade favors vasoconstriction and platelet aggregation. Oxygen is also required for NO synthesis and during hypoxia, oxygen can become a limiting substrate. In addition, complement activation and synthesis of platelet activating factor (PAF) occur.[34]

Reperfusion

Although necessary for cellular salvage, reperfusion paradoxically creates more injury than ischemia. Several studies have shown an increase in toxic by-products of IR injury after reperfusion but not after ischemia alone.[35,36] Reperfusion is associated with severe endothelial dysfunction. This is most likely due to a combination of factors including ROS damage to the endothelium, decreased NO release from the endothelium, and increased endothelin causing marked vasoconstriction further impairing blood flow.[37] Great variability exists in the amount of XO present in different species and in different organs. Dogs and rats have significantly greater amounts of XO than do humans or rabbits.[38–40] The endothelium and the

mucosal villi of the GI tract have the greatest amount of XO in the body and are highly susceptible to IR injury.[41] The first area believed to generate ROS during reperfusion is at the interface between the endothelium and the blood and XO-mediated endothelial injury is believed to be a major factor in IR injury.[42]

When oxygen is re-introduced, it combines with XO and H_2O to convert hypoxanthine to uric acid and superoxide. A burst of ROS formation is seen within 10–30 seconds after the onset of reperfusion.[43] Superoxide is released when XO combines with oxygen and hypoxanthine. Superoxide, by itself, is not a very damaging molecule. It is, however, a source of hydrogen peroxide. SOD converts superoxide to hydrogen peroxide. Catalase then converts hydrogen peroxide to water and oxygen. Hydrogen peroxide can combine with transition metals, usually free iron, to form the hydroxyl radical. Similar to superoxide, hydrogen peroxide is not very reactive and its main action of toxicity is in forming the hydroxyl radical. Neither superoxide nor hydrogen peroxide can initiate lipid peroxidation, but can stimulate it when added to lipids in the presence of iron by forming the hydroxyl radical. Because the hydroxyl radical is so potent, the body has several defenses against its formation, including SOD and catalase.[44] In addition, iron is very carefully sequestered in biological systems and free or loosely bound iron is never present in health.[45] Iron is bound in hemoglobin and myoglobin in the blood and muscle, to transferrin while circulating in plasma, and to ferritin in cells.

During ischemia, intracellular iron that is bound to ferritin is released, most likely as a result of a combination of acidosis and increased concentrations of reducing equivalents.[46] In addition, superoxide mobilizes iron from ferritin[47] and hydrogen peroxide mobilizes iron from heme.[48] Proteolytic degradation of ferritin occurs during times of oxidative stress and may contribute to the release of free iron. Red blood cells contain large amounts of iron and hemolysis may contribute to free iron release during oxidative stress.

In 1934, it was discovered that the hydroxyl radical $(OH^\bullet)$ could be generated from the interaction of superoxide $(O_2^{\bullet-})$ and H_2O_2.[49] The Haber–Weiss reaction $(O_2^{\bullet-} + H_2O_2 \rightarrow OH^\bullet + O_2 + OH^-)$ has a second-order rate constant of zero and cannot occur in biological systems without a metal catalyst.[49] The *iron-catalyzed* Haber–Weiss reaction was postulated to account for the presence of transition metals in biological systems $(Fe^{2+} + H_2O_2 \rightarrow OH^\bullet + OH^- + Fe^{3+})$.[49] The ferrous (Fe^{2+}) form of iron is required for hydroxyl radical formation, but the ferric (Fe^{3+}) form is more common in biological systems. The conversion of the ferric to the ferrous form of iron occurs via XO, $O_2^{\bullet-}$, and NO. The ability of $O_2^{\bullet-}$

to liberate bound iron and to convert ferric to ferrous iron may contribute to its toxicity.[50]

The hydroxyl radical is the one of the most destructive and potent oxidizing agents known reacting with almost every molecule in the living cell.[24] It causes a chain reaction of lipid peroxidation that leads to loss of membrane selective permeability, damage to DNA, and degradation of structural proteins and membrane-bound enzyme activity.[51] The chain reaction will continue until the hydroxyl radical is scavenged, two radicals combine to form a non-reactive species, or the substrate is consumed.

Damage due to ROS includes lipid peroxidation with increased cell membrane permeability, damage to the sarcoplasmic reticulum causing negative inotropy, impaired cell function, and cell death. Since the heart and lungs receive ROS and products of IR injury first after portal blood, they appear to be affected quite commonly.[52,53] After reperfusion of ischemic intestine, acute lung injury has been documented with increased damage to alveolar cells, infiltration of neutrophils, and increased microvascular permeability, in an experimental model.[53] Decreased contractility was observed in the myocardium after 2 hours of IR injury in rats.[52] In experimental IR injury of the cat small intestine, there was increased mucosal structural damage and increased microvascular permeability.[54]

Interestingly, the brain appears to be one of the least protected organs from ROS damage. The brain has a high concentration of unsaturated fatty acids (i.e., perfect media for lipid peroxidation), a large iron store with low metal binding capacity (i.e., perfect for generation of hydroxyl radical), low antioxidant capacity, and is incapable of neuronal regeneration.[5]

The endothelium produces both NO and endothelin. In arteries, NO can reverse the vasoconstrictive effects of endothelin, but it appears to have the opposite effect in veins.[55,56] During ischemia, endothelial transcription of endothelin, the most potent vasoconstrictor known, is upregulated.[57] NO is a free radical gas that appears to have an effect on almost every tissue in the body. NO is the end product of nitric oxide synthase (NOS), which occurs in several isoforms; inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS). Arginine and molecular oxygen are required for NO synthesis. It is paradoxical in that NO has been shown to be both beneficial and toxic depending on the conditions. In health, low doses of NO cause vasodilation via cGMP-mediated vasodilation, decrease platelet aggregation and leukocyte adhesion, neutralize ROS, and have anti-microbial and anti-apoptotic effects.[58] In the large concentrations generated by iNOS, however, NO is believed to have cytotoxic effects, causing severe, non-responsive vasodilation.[58] NO and superoxide

rapidly combine to form a toxic reaction product, peroxynitrite ($ONOO^-$), which is highly reactive, readily reacting with proteins, lipids, and DNA during times of inflammation.[59] Hypoxia induces the transcription of iNOS, but because oxygen is required as a substrate for NO production, NO may not increase despite significant elevations in iNOS.[60] Upon reperfusion and reintroduction of oxygen, there may be sustained increases in NO.[58] During reperfusion, large bursts of ROS can cause depletion of NO when it binds superoxide. The inhibition of NO by ROS is believed to contribute to increased intestinal mucosal permeability.[61] Endothelial dysfunction was improved after administration of a NO donor in an IR study in cats.[62] In animal models of IR injury, increasing endothelial NO availability significantly improved hemodynamics and liver function post-reperfusion in pigs and rats.[63,64] The beneficial effects may be due to the ability of NO to block the effects of endothelin, which is released from endothelial cells and macrophages during IR injury.[65] Increasing NO may also decrease anti-inflammatory cell activity, decrease the expression of adhesion molecules, or decrease cytokine levels.[66–68]

Blocking iNOS activity markedly reduced liver injury in several studies.[69–71] NO is increased in graft tissue of rejected organs, compared with organs that are not rejected, and NO has been shown to be a mediator of acute graft rejection in liver transplant patients. It is still controversial whether the increased NO synthesized during IR injury in transplant recipients is beneficial or harmful to tissues.[72]

Conflicting results showing NO to be both beneficial and cytotoxic are most likely due to multiple factors that influence the actions of NO, making it difficult to design effective IR treatment protocols using NO.

Role of neutrophils in IR injury

Neutrophils play an important role in IR injury. XO, PAF, and ROS can initiate chemotaxis and subsequent neutrophil infiltration.[73,74] Leukocyte infiltration is a crucial component in the IR cascade. Cell adhesion molecules on the leukocyte surface bind to ligands on endothelial cells, initiating a sequence of events culminating in extravasation of leukocytes from the microvasculature. Much of the tissue injury that occurs upon reperfusion results from the oxidants generated and proteolytic enzymes released by immense numbers of either resident or invading neutrophils. Activated neutrophils can cause tissue injury via ROS synthesis during the respiratory burst, release of intrinsic proteolytic enzymes, and by physical obstruction of capillaries.[75,76] Neutrophil infiltration in feline intestinal mucosa following reperfusion has been prevented by

prior administration of allopurinol (a XO inhibitor), SOD, catalase, and deferoxamine, providing additional evidence of the link between ROS, neutrophil infiltration, and subsequent tissue injury.[77,78] Additionally, the increase in microvascular permeability that occurs with IR is attenuated with administration of XO inhibitors, neutropenia, dimethyl sulfoxide (hydroxyl radical scavenger), or monoclonal antibodies directed against the CD18 neutrophil adhesion molecule.[79–81] It has been suggested that neutrophils mediate the majority of mucosal and microvascular injury subsequent to IR.[42,82,83]

The microvasculature is exquisitely sensitive to IR and the increased adhesiveness of neutrophils to endothelial cells contributes to the microvascular injury observed with IR.[84] Neutrophil emigration from the microvasculature involves a multi-step series of events involving selectins and integrins, and in general includes tethering/rolling, firm adhesion, transmigration, and chemotaxis.[85] During flow, neutrophils normally undergo tethering and rolling via interaction of E and P selectins on activated endothelial cells and leukocyte L-selectin with various membrane glycoproteins. Rolling is initiated principally by activation of the endothelium. Rolling is mediated early on by constitutively expressed endothelial P-selectin and neutrophil L-selectin. E-selectin only becomes involved at a later time because it requires *de novo* synthesis. Once neutrophils are tethered to the endothelium by selectin interactions, neutrophil integrin receptors are activated by endothelial cell-expressed PAF, chemokines, or locally secreted chemoattractants. Integrin receptor activation increases their affinity for their endothelial cell ligands, ICAM-1 and -2.[85] Once neutrophils become adherent to the endothelium, they move over the endothelial cell surface and then diapedese through intercellular endothelial cell junctions and migrate to the site of inflammation. This migration may be facilitated by disruption of the intercellular junction integrity by elastase or other mediators. The recruitment of neutrophils is terminated by several mechanisms, including removal of E and P selectins from endothelial cells via endocytosis and cleavage of L-selectin from neutrophils by a membrane protease.

Studies have shown that there is an early phase (phase 1) and a later phase (phase 2) of neutrophil adhesion to endothelial cells subsequent to anoxia and reoxygenation.[86–89] The early phase appears to be mediated by PAF- and XO-derived hydrogen peroxide, whereas phase 2 neutrophil adhesion appears to be mediated by PAF as well as oxidants generated intracellularly within the mitochondria. The pattern of neutrophil adhesion during phase 1 suggests that it is due to constitutively expressed ICAM-1 and the rapidly

mobilized preformed pool of P-selectin as well as upregulation of neutrophilic surface expression of β2-integrins.[86–89] Nuclear transcription factor (NFκB) seems to play an important role in the upregulation of endothelial cell adhesion molecules during phase 2 neutrophil adhesion. Unlike many transcription activators, NFκB is normally present in the cytoplasm and must be translocated into the nucleus to elicit a response. The phase 2 E-selectin-dependent pattern of neutrophil adhesion is consistent with a transcriptional-dependent upregulation of the adhesion protein mediated by NFκB. Inhibition of NFκB activation prevented increased surface expression of E-selectin that was directly correlated with a decrease in neutrophil adhesion to endothelial cell monolayers.[90]

A vicious cycle occurs during reperfusion, with continued neutrophil chemotaxis and activation leading to additional ROS formation, endothelial damage, and capillary plugging.[34] Maximal adherence to venular endothelium has been demonstrated using intravital microscopy to occur 10 minutes after reperfusion in feline mesentery.[79] Similar temporal findings have been reported in other species and organs using other techniques, including in the large colon of horses 10 minutes after restoration of blood flow following 3 hours of low-flow ischemia.[91] In several studies, increased myeloperoxidase (MPO) activity has been documented at the site of IR injury, adding to the evidence that neutrophil accumulation occurs and likely contributes to this injury. A correlation between MPO activity and MDA concentrations (i.e., a marker of lipid peroxidation) suggests that lipid peroxidative damage occurred subsequent to neutrophil-derived ROS.[92] Alternatively, the increased MPO could occur subsequent to the increase in phospholipid-derived mediators (e.g., PAF) released during lipoperoxidation of cell membrane phospholipids. Inflammatory mediators generated during IR include tumor necrosis factor alpha (TNFα), interleukin-1β (IL-1β), PAF, complement, and chemokines, all of which are potent chemoattractants and contribute to increase neutrophil sequestration.[93–96] Although activated neutrophils themselves can release TNFα and IL-1β, these cytokines are predominantly released from resident macrophages and mast cells.[97,98] TNFα induces neutrophil adhesion and degranulation, stimulates NADPH oxidase (resulting in ROS synthesis), and enhances the expression of IL-2 receptors and the expression of ICAM-1 on the endothelium.[99] Neutrophil degranulation increases oxidative stress, and also increases tissue activity of elastase and collagenase and augments eicosanoid synthesis. Elastase is the predominant proteolytic enzyme released from neutrophils that cause tissue damage.[100] Xanthine oxidase-derived ROS initiate

recruitment of neutrophils, which then become activated and release ROS, leading to exacerbation of organ damage. It appears that circulating leukocytes contribute to increased microvascular permeability and resident interstitial granulocytes contribute to increased gastrointestinal mucosal permeability associated with IR.[83]

The 'no-reflow phenomenon' is a term used to describe diminished or absent blood flow to an area of tissue after relief of vascular occlusion. The concept was first described by Majno et al.[101] Rabbits were subjected to variable periods of brain ischemia and the authors noted that although blood flow was restored with brief (2.5 minutes) periods of ischemia, it was not restored when the ischemia was of longer duration. This model was repeated in other animal models of ischemia and in other organs including the skin, skeletal muscle, and kidney.[102–104] Kloner et al.[105] studied electron microscopic samples of cardiac vessels in a dog model of proximal coronary artery occlusion. Swollen endothelium, endothelial protrusions, and platelet and fibrin thrombi were observed and were believed to be responsible for the no-reflow in these animals. Experimental studies have shown that longer periods of ischemia are more likely to lead to the no-reflow phenomenon.[106–108]

It is likely that neutrophils play a key role in no-reflow. Neutrophils adhering to the endothelium can cause damage resulting in endothelial swelling and additional neutrophil accumulation, which can lead to further ischemia and a downward spiral in which even more neutrophils are attracted to the area. In several studies, no-reflow was attenuated by decreasing either neutrophils or platelets.[109–112] Platelets rapidly adhere to subendothelium exposed after vascular injury causes endothelial denudation or retraction. These platelets may then recruit additional neutrophils by initiating selectin- and integrin-dependent leukocyte adhesion to surface-bound platelets.[113–115] The accumulated neutrophils adhering to the platelets may promote fibrin deposition and subsequent thrombus formation.[116] Since there is often a period of normal flow after elimination of the occlusion and a subsequent decrease in flow, the term diminishing reflow has been suggested.[117]

Conclusion

IR injury is a complex process involving numerous mediators and intricate pathways. Key players in the IR injury cascade include neutrophils, platelets, cytokines, and ROS. Damage due to ROS is widespread and extensive, since they can react with all biological membranes. In dogs, the severity of ischemic injury is determined by the length of the ischemia,[28] and the

reperfusion phase is associated with severe tissue and endothelial injury. Endogenous antioxidant defense mechanisms become overwhelmed during reperfusion, contributing to the damage. In an experimental cat model of IR injury, significant mucosal injury was seen in the small intestine upon reperfusion and this contributes to the vicious cycle of tissue damage.[54] In experimental studies with cats, administration of a NO donor was shown to be beneficial in IR injury.[62] On the other hand, blocking iNOS activity was shown to reduce liver injury in several studies in rats. Because NO requires molecular oxygen for its synthesis, elevations of iNOS do not necessarily translate into increased NO during hypoxia.[60] Conflicting studies regarding NO make designing treatment strategies very difficult and the timing of treatment appears to be a very critical element.

Neutrophils contribute significantly to tissue injury in IR injury. ROS and proteolytic enzymes are released by both invading and resident neutrophils during the reperfusion phase. The prevention of neutrophil infiltration into feline intestine was accomplished with prior administration of allopurinol, SOD, catalase, and deferoxamine.[77,78] XO inhibitors have been shown to decrease the vascular permeability that occurs upon reperfusion in experimental studies using cats and rats.[79,80] Clinical trials of IR injury in companion animals are lacking. Due to the enormous complexity of the disease process and the lack of clinical research, it is difficult to design effective assessment and treatment strategies.

References

1. Korthuis RJ, Granger DN, Townsley MI, et al. The role of oxygen-derived free radicals in ischemia-induced increases in canine skeletal muscle vascular permeability. Circ Res 1985; 57:599–609.
2. Sakon M, Ariyoshi H, Umeshita K, et al. Ischemia–reperfusion injury of the liver with special reference to calcium-dependent mechanisms. Surg Today 2002; 32:1–12.
3. Esterbauer H. Estimation of peroxidative damage. A critical review. Pathol Biol (Paris) 1996; 44:25–28.
4. Rangan U, Bulkley GB. Prospects for treatment of free radical-mediated tissue injury. Br Med Bull 1993; 49:700–718.
5. Curtin JF, Donovan M, Cotter TG. Regulation and measurement of oxidative stress in apoptosis. J Immunol Methods 2002; 265:49–72.
6. Kelly FJ. Urinary F2-isoprostane metabolite analysis: a step closer to obtaining a reliable measure of oxidative stress? Clin Exp Allergy 2001; 31:355–356.
7. Acworth I, McCabe D, Maher T. The analysis of free radicals, their reaction products, and antioxidants, In: Baskin S, Salem H. eds. Oxidants, Antioxidants, and Free

Radicals. Washington, DC: Taylor & Francis; 1997, pp. 23–77.

8. Pratico D. *In vivo* measurement of the redox state. Lipids 2001; 36(Suppl.):S45–S47.

9. Freeman B. Free radical chemistry of nitric oxide. Looking at the dark side. Chest 1994; 105:79S–84S.

10. Halliwell B. Free radicals and antioxidants: a personal view. Nutr Rev 1994; 52:253–265.

11. Brown MS, Goldstein JL. Receptor-mediated endocytosis: insights from the lipoprotein receptor system. Proc Natl Acad Sci USA 1979; 76:3330–3337.

12. Radi R, Beckman JS, Bush KM, et al. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch Biochem Biophys 1991; 288:481–487.

13. Poli G, Parola M. Oxidative damage and fibrogenesis. Free Radic Biol Med 1997; 22:287–305.

14. Uchida K, Stadtman ER. Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase. A possible involvement of intra- and intermolecular cross-linking reaction. J Biol Chem 1993; 268: 6388–6393.

15. Kruman I, Bruce-Keller AJ, Bredesen D, et al. Evidence that 4-hydroxynonenal mediates oxidative stress-induced neuronal apoptosis. J Neurosci 1997; 17:5089–5100.

16. Halliwell B, Aeschbach R, Loliger J, et al. The characterization of antioxidants. Food Chem Toxicol 1995; 33: 601–617.

17. Halliwell B. Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radic Res 1999; 31:261–272.

18. Oranje WA, Wolffenbuttel BH. Lipid peroxidation and atherosclerosis in type II diabetes. J Lab Clin Med 1999; 134:19–32.

19. Flohe L. Glutathione peroxidase brought into focus, In: Pryor W. ed. Free Radicals in Biology. New York: Academic Press; 1980, pp. 223–254.

20. van den Dobbelsteen DJ, Nobel CS, Schlegel J, et al. Rapid and specific efflux of reduced glutathione during apoptosis induced by anti-Fas/APO-1 antibody. J Biol Chem 1996; 271:15420–15427.

21. Kurose I, Higuchi H, Miura S, et al. Oxidative stress-mediated apoptosis of hepatocytes exposed to acute ethanol intoxication. Hepatology 1997; 25:368–378.

22. Meydani M. Vitamin E. Lancet 1995; 345:170–175.

23. Marino P. The threat of oxidant injury, In: Marino P. ed. The ICU Book. Baltimore, MD: Williams & Wilkins; 1998, pp. 32–50.

24. Baskin S, Salem H. Oxidants, Antioxidants, and Free Radicals. Washington, DC: Taylor & Francis; 1997.

25. Hamet P, Moreau P, Dam TV, et al. The time window of apoptosis: a new component in the therapeutic strategy for cardiovascular remodeling. J Hypertens 1996; 14(Suppl.):S65–S70.

26. Chen C, Ma L, Linfert DR, et al. Myocardial cell death and apoptosis in hibernating myocardium. J Am Coll Cardiol 1997; 30:1407–1412.

27. Southard J. Temperature effects and cooling, In: Zelenock G. ed. Clinical Ischemic Syndromes: Mechanism and Consequence of Tissue Injury. St Louis: The CV Mosby Co.; 1990, pp. 303–325.

28. Parks DA, Grogaard B, Granger DN. Comparison of partial and complete arterial occlusion models for studying intestinal ischemia. Surgery 1982; 92:896–901.

29. Grum CM. Cellular energetics, In: Zelenock G. ed. Clinical Ischemic Syndromes: Mechanism and Consequence of Tissue Injury. St. Louis: The CV Mosby Co.; 1990, pp. 47–62.

30. Murata M, Monden M, Umeshita K, et al. Role of intracellular calcium in superoxide-induced hepatocyte injury. Hepatology 1994; 19:1223–1228.

31. Hatanaka N, Kamike W, Shimizu S, et al. Ca^{2+} release from mitochondria induces cytosolic enzyme leakage in anoxic liver. J Surg Res 1995; 58:485–490.

32. MacDonald HR, Schneider R, Lees RK, et al. T-cell receptor V beta use predicts reactivity and tolerance to Mlsa-encoded antigens. Nature 1988; 332:40–45.

33. Shibasaki F, McKeon F. Calcineurin functions in Ca(2+)-activated cell death in mammalian cells. J Cell Biol 1995; 131:735–743.

34. Ondiveeran HK, Fox-Robichaud A. New developments in the treatment of ischemia/reperfusion injury. Curr Opin Invest Drugs 2001; 2:783–791.

35. Zimmerman BJ, Guillory DJ, Grisham MB, et al. Role of leukotriene B4 in granulocyte infiltration into the postischemic feline intestine. Gastroenterology 1990; 99: 1358–1363.

36. Mangino MJ, Anderson CB, Murphy MK, et al. Mucosal arachidonate metabolism and intestinal ischemia–reperfusion injury. Am J Physiol 1989; 257:G299–G307.

37. Granger DN. Ischemia–reperfusion: mechanisms of microvascular dysfunction and the influence of risk factors for cardiovascular disease. Microcirculation 1999; 6:167–178.

38. Grum CM, Ragsdale RA, Ketai LH, et al. Absence of xanthine oxidase or xanthine dehydrogenase in the rabbit myocardium. Biochem Biophys Res Commun 1986; 141: 1104–1108.

39. Parks DA, Granger DN. Xanthine oxidase: biochemistry, distribution and physiology. Acta Physiol Scand 1986; 548(Suppl.):87–99.

40. Eddy LJ, Stewart JR, Jones HP, et al. Free radical-producing enzyme, xanthine oxidase, is undetectable in human hearts. Am J Physiol 1987; 253:H709–H711.

41. Schoenberg MH, Beger HG. Reperfusion injury after intestinal ischemia. Crit Care Med 1993; 21:1376–1386.

42. Granger DN. Role of xanthine oxidase and granulocytes in ischemia–reperfusion injury. Am J Physiol 1988; 255: H1269–H1275.

43. Zweier JL, Flaherty JT, Weisfeldt ML. Direct measurement of free radical generation following reperfusion of ischemic myocardium. Proc Natl Acad Sci USA 1987; 84:1404–1407.

44. Bannister JV, Bannister WH, Rotilio G. Aspects of the structure, function, and applications of superoxide dismutase. CRC Crit Rev Biochem 1987; 22:111–180.
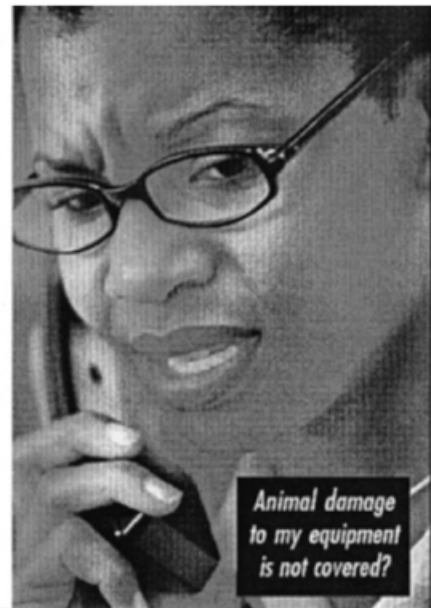
45. Galey JB. Recent advances in the design of iron chelators against oxidative damage. Mini Rev Med Chem 2001; 1:233–242.

46. Voogd A, Sluiter W, van Eijk HG, et al. Low molecular weight iron and the oxygen paradox in isolated rat hearts. J Clin Invest 1992; 90:2050–2055.

47. Biemond P, van Eijk HG, Swaak AJ, et al. Iron mobilization from ferritin by superoxide derived from stimulated polymorphonuclear leukocytes. Possible mechanism in inflammation diseases. J Clin Invest 1984; 73:1576–1579.

48. Gutteridge JM. Iron promoters of the Fenton reaction and lipid peroxidation can be released from haemoglobin by peroxides. FEBS Lett 1986; 201:291–295.

49. Kehrer JP. The Haber–Weiss reaction and mechanisms of toxicity. Toxicology 2000; 149:43–50.

50. Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine. Oxford: Oxford University Press/Clarendon Press; 1989.

51. Flaherty JT, Weisfeldt ML. Reperfusion injury. Free Radic Biol Med 1988; 5:409–419.

52. Horton JW, White DJ. Cardiac contractile injury after intestinal ischemia–reperfusion. Am J Physiol 1991; 261:H1164–H1170.

53. Schmeling DJ, Caty MG, Oldham KT, et al. Evidence for neutrophil-related acute lung injury after intestinal ischemia–reperfusion. Surgery 1989; 106:195–201.

54. Parks DA, Bulkley GB, Granger DN, et al. Ischemic injury in the cat small intestine: role of superoxide radicals. Gastroenterology 1982; 82:9–15.

55. Lerman A, Burnett JC Jr. Intact and altered endothelium in regulation of vasomotion. Circulation 1992; 86:III12–III19.

56. Luscher TF, Yang Z, Tschudi M, et al. Interaction between endothelin-1 and endothelium-derived relaxing factor in human arteries and veins. Circ Res 1990; 66:1088–1094.

57. Kourembanas S, Marsden PA, McQuillan LP, et al. Hypoxia induces endothelin gene expression and secretion in cultured human endothelium. J Clin Invest 1991; 88:1054–1057.

58. Hon WM, Lee KH, Khoo HE. Nitric oxide in liver diseases: friend, foe, or just passerby? Ann NY Acad Sci 2002; 962:275–295.

59. Beckman JS, Beckman TW, Chen J, et al. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 1990; 87:1620–1624.

60. Otto CM, Baumgardner JE. Effect of culture PO_2 on macrophage (RAW 264.7) nitric oxide production. Am J Physiol Cell Physiol 2001; 280:C280–C287.

61. Kubes P. Nitric oxide modulates epithelial permeability in the feline small intestine. Am J Physiol 1992; 262: G1138–G1142.

62. Carey C, Siegfried MR, Ma XL, et al. Antishock and endothelial protective actions of a NO donor in mesenteric ischemia and reperfusion. Circ Shock 1992; 38: 209–216.

63. Ricciardi R, Foley DP, Quarfordt SH, et al. V-PYRRO/NO: an hepato-selective nitric oxide donor improves porcine liver hemodynamics and function after ischemia reperfusion. Transplantation 2001; 71:193–198.

64. Cottart CH, Do L, Blanc MC, et al. Hepatoprotective effect of endogenous nitric oxide during ischemia–reperfusion in the rat. Hepatology 1999; 29:809–813.

65. Rieder H, Ramadori G, Meyer zum Buschenfelde KH. Sinusoidal endothelial liver cells in vitro release endothelin – augmentation by transforming growth factor beta and Kupffer cell-conditioned media. Klin Wochenschr 1991; 69:387–391.

66. Pannen BH, Al Adili F, Bauer M, et al. Role of endothelins and nitric oxide in hepatic reperfusion injury in the rat. Hepatology 1998; 27:755–764.

67. Liu P, Xu B, Hock CE, et al. NO modulates P-selectin and ICAM-1 mRNA expression and hemodynamic alterations in hepatic I/R. Am J Physiol 1998; 275: H2191–H2198.

68. De Caterina R, Libby P, Peng HB, et al. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. J Clin Invest 1995; 96:60–68.

69. Aoki K, Ohmori M, Takimoto M, et al. Cocaine-induced liver injury in mice is mediated by nitric oxide and reactive oxygen species. Eur J Pharmacol 1997; 336:43–49.

70. Diez-Fernandez C, Sanz N, Alvarez AM, et al. Influence of aminoguanidine on parameters of liver injury and regeneration induced in rats by a necrogenic dose of thioacetamide. Br J Pharmacol 1998; 125:102–108.

71. Gardner CR, Heck DE, Yang CS, et al. Role of nitric oxide in acetaminophen-induced hepatotoxicity in the rat. Hepatology 1998; 27:748–754.

72. Sugioka A, Morita M, Esaki T, et al. Kinetics and significance of graft tissue nitric oxide after rat and mouse liver transplantation. Transplant Proc 1999; 31: 860–862.

73. Hernandez LA, Grisham MB, Twohig B, et al. Role of neutrophils in ischemia–reperfusion injury. Fed Proc 1987; 46:1124.

74. Lewis MS, Whatley RE, Cain P, et al. Hydrogen peroxide stimulates the synthesis of platelet-activating factor by endothelium and induces endothelial cell-dependent neutrophil adhesion. J Clin Invest 1988; 82:2045–2055.

75. Weiss SJ. Tissue destruction by neutrophils. N Engl J Med 1989; 320:365–376.

76. Bagge U, Amundson B, Lauritzen C. White blood cell deformability and plugging of skeletal muscle capillaries in hemorrhagic shock. Acta Physiol Scand 1980; 108: 159–163.

77. Grisham MB, Hernandez LA, Granger DN. Xanthine oxidase and neutrophil infiltration in intestinal ischemia. Am J Physiol 1986; 251:G567–G574.

78. Zimmerman BJ, Grisham MB, Granger DN. Role of oxidants in ischemia/reperfusion-induced granulocyte infiltration. Am J Physiol 1990; 258:G185–G190.

79. Granger DN, Benoit JN, Suzuki M, et al. Leukocyte adherence to venular endothelium during ischemia–reperfusion. Am J Physiol 1989; 257:G683–G688.

80. Hernandez LA, Grisham MB, Twohig B, et al. Role of neutrophils in ischemia–reperfusion-induced microvascular injury. Am J Physiol 1987; 253:H699–H703.

81. Sekizuka E, Benoit JN, Grisham MB, et al. Dimethyl-sulfoxide prevents chemoattractant-induced leukocyte adherence. Am J Physiol 1989; 256:H594–H597.

82. Grisham MB, Granger DN. Neutrophil-mediated mucosal injury. Role of reactive oxygen metabolites. Dig Dis Sci 1988; 33:6S–15S.

83. Kubes P, Hunter J, Granger DN. Ischemia/reperfusion-induced feline intestinal dysfunction: importance of granulocyte recruitment. Gastroenterology 1992; 103:807–812.

84. Granger DN, Kvietys PR, Perry MA. Leukocyte – endothelial cell adhesion induced by ischemia and reperfusion. Can J Physiol Pharmacol 1993; 71:67–75.

85. Harlan JM, Winn RK. Leukocyte-endothelial interactions: clinical trials of anti-adhesion therapy. Crit Care Med 2002; 30:S214–S219.

86. de Fougerolles AR, Stacker SA, Schwarting R, et al. Characterization of ICAM-2 and evidence for a third counter-receptor for LFA-1. J Exp Med 1991; 174:253–267.

87. Patel KD, Zimmerman GA, Prescott SM, et al. Oxygen radicals induce human endothelial cells to express GMP-140 and bind neutrophils. J Cell Biol 1991; 112:749–759.

88. Bonfanti R, Furie BC, Furie B, et al. PADGEM (GMP140) is a component of Weibel–Palade bodies of human endothelial cells. Blood 1989; 73:1109–1112.

89. Smith CW, Marlin SD, Rothlein R, et al. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and trans-endothelial migration of human neutrophils in vitro. J Clin Invest 1989; 83:2008–2017.

90. Kokura S, Yoshida N, Yoshikawa T. Anoxia/reoxygenation-induced leukocyte–endothelial cell interactions. Free Radic Biol Med 2002; 33:427–432.

91. Moore RM, Bertone AL, Bailey MQ, et al. Neutrophil accumulation in the large colon of horses during low-flow ischemia and reperfusion. Am J Vet Res 1994; 55:1454–1463.

92. Otamiri T, Lindahl M, Tagesson C. Phospholipase A2 inhibition prevents mucosal damage associated with small intestinal ischaemia in rats. Gut 1988; 29:489–494.

93. Essani NA, Fisher MA, Farhood A, et al. Cytokine-induced upregulation of hepatic intercellular adhesion molecule-1 messenger RNA expression and its role in the pathophysiology of murine endotoxin shock and acute liver failure. Hepatology 1995; 21:1632–1639.

94. Serizawa A, Nakamura S, Suzuki, et al. Involvement of platelet-activating factor in cytokine production and neutrophil activation after hepatic ischemia–reperfusion. Hepatology 1996; 23:1656–1663.

95. Jaeschke H, Farhood A, Bautista AP, et al. Complement activates Kupffer cells and neutrophils during reperfusion after hepatic ischemia. Am J Physiol 1993; 264:G801–G809.

96. Colletti LM, Kunkel SL, Walz A, et al. The role of cytokine networks in the local liver injury following hepatic ischemia/reperfusion in the rat. Hepatology 1996; 23:506–514.

97. Chensue SW, Remick DG, Shmyr-Forsch C, et al. Immunohistochemical demonstration of cytoplasmic and membrane-associated tumor necrosis factor in murine macrophages. Am J Pathol 1988; 133:564–572.

98. Frangogiannis NG, Lindsey ML, Michael LH, et al. Resident cardiac mast cells degranulate and release preformed TNF-alpha, initiating the cytokine cascade in experimental canine myocardial ischemia/reperfusion. Circulation 1998; 98:699–710.

99. Ikeda U, Ikeda M, Kano S, et al. Neutrophil adherence to rat cardiac myocyte by proinflammatory cytokines. J Cardiovasc Pharmacol 1994; 23:647–652.

100. Jordan JE, Zhao ZQ, Vinten-Johansen J. The role of neutrophils in myocardial ischemia–reperfusion injury. Cardiovasc Res 1999; 43:860–878.

101. Majno G, Ames A III, Chiang J, et al. No reflow after cerebral ischemia. Lancet 1967; 2:569–570.

102. Ames AIII, Wright RL, Kowada M, et al. Cerebral ischemia. II. The no-reflow phenomenon. Am J Pathol 1968; 52:437–453.

103. Allen DM, Chen LE, Seaber AV, et al. Pathophysiology and related studies of the no reflow phenomenon in skeletal muscle. Clin Orthop 1995; 122–133.

104. Summers WK, Jamison RL. The no reflow phenomenon in renal ischemia. Lab Invest 1971; 25:635–643.

105. Kloner RA, Ganote CE, Jennings RB. The 'no-reflow' phenomenon after temporary coronary occlusion in the dog. J Clin Invest 1974; 54:1496–1508.

106. Ambrosio G, Weisman HF, Mannisi JA, et al. Progressive impairment of regional myocardial perfusion after initial restoration of postischemic blood flow. Circulation 1989; 80:1846–1861.

107. Hickey MJ, Hurley JV, Morrison WA. Temporal and spatial relationship between no-reflow phenomenon and postischemic necrosis in skeletal muscle. Am J Physiol 1996; 271:H1277–H1286.

108. Kloner RA. Does reperfusion injury exist in humans? J Am Coll Cardiol 1993; 21:537–545.

109. Barroso-Aranda J, Schmid-Schonbein GW, Zweifach BW, et al. Granulocytes and no-reflow phenomenon in irreversible hemorrhagic shock. Circ Res 1988; 63:437–447.

110. Humphrey SM, Gavin JB, Herdson PB. Catecholamine-depletion and the no-reflow phenomenon in anoxic and ischaemic rat hearts. J Mol Cell Cardiol 1982; 14:151–161.

111. Humphrey SM, Seelye RN, Gavin JB. The influence of adenosine on the no-reflow phenomenon in anoxic and ischaemic hearts. Pathology 1982; 14:129–133.

112. Westin M, Heden P. Calcitonin gene-related peptide delays the no-reflow phenomenon in the rat island flap. Ann Plast Surg 1988; 21:329–334.

113. Kuijper PH, Gallardo Torres HI, van der Linden JA, et al. Platelet-dependent primary hemostasis promotes selectin- and integrin-mediated neutrophil adhesion to damaged endothelium under flow conditions. Blood 1996; 87:3271–3281.

114. Weber C, Springer TA. Neutrophil accumulation on activated, surface-adherent platelets in flow is mediated

by interaction of Mac-1 with fibrinogen bound to alphaIIbbeta3 and stimulated by platelet-activating factor. J Clin Invest 1997; 100:2085–2093.

115. Kirchhofer D, Riederer MA, Baumgartner HR. Specific accumulation of circulating monocytes and polymorphonuclear leukocytes on platelet thrombi in a vascular injury model. Blood 1997; 89:1270–1278.

116. Palabrica T, Lobb R, Furie BC, et al. Leukocyte accumulation promoting fibrin deposition is mediated *in vivo* by P-selectin on adherent platelets. Nature 1992; 359:848–851.

117. Vedder NB, Winn RK, Rice CL, et al. Inhibition of leukocyte adherence by anti-CD18 monoclonal antibody attenuates reperfusion injury in the rabbit ear. Proc Natl Acad Sci USA 1990; 87:2643–2646.