

Pathophysiology, Clinical Manifestations, and Prevention of Ischemia-Reperfusion Injury

Charles D. Collard, M.D.,* Simon Gelman, M.D., Ph.D.†

ISCHEMIA contributes to the pathophysiology of many conditions faced by anesthesiologists, including myocardial infarction, peripheral vascular insufficiency, stroke, and hypovolemic shock. Although restoration of blood flow to an ischemic organ is essential to prevent irreversible cellular injury, reperfusion *per se* may augment tissue injury in excess of that produced by ischemia alone. For example, the histologic changes of injury after 3 h of feline intestinal ischemia followed by 1 h of reperfusion are far worse than the changes observed after 4 h of ischemia alone.¹ Cellular damage after reperfusion of previously viable ischemic tissues is defined as ischemia-reperfusion (I-R) injury.

Ischemia-reperfusion associated with thrombolytic therapy, organ transplantation, coronary angioplasty, aortic cross-clamping, or cardiopulmonary bypass results in local and systemic inflammation. If severe enough, the inflammatory response after I-R may result in the systemic inflammatory response syndrome or multiple organ dysfunction syndrome (MODS), which account for 30–40% of the mortality in tertiary referral intensive care units.² Thus, I-R injury may extend beyond the ischemic area at risk to include injury of remote, nonischemic organs.

Basic Pathophysiology of Ischemia-Reperfusion Injury

Cellular Effects of Ischemia

Prolonged ischemia results in a variety of cellular metabolic and ultrastructural changes (table 1). Ischemia-induced decreases in cellular oxidative phosphorylation results in a failure to resynthesize energy-rich phos-

phates, including adenosine 5'-triphosphate (ATP) and phosphocreatine. Membrane ATP-dependent ionic pump function is thus altered, favoring the entry of calcium, sodium, and water into the cell. Furthermore, adenine nucleotide catabolism during ischemia results in the intracellular accumulation of hypoxanthine, which is subsequently converted into toxic reactive oxygen species (ROS) upon the reintroduction of molecular oxygen (see below). Within the endothelium, ischemia promotes expression of certain proinflammatory gene products (*e.g.*, leukocyte adhesion molecules, cytokines) and bioactive agents (*e.g.*, endothelin, thromboxane A₂), while repressing other “protective” gene products (*e.g.*, constitutive nitric oxide synthase, thrombomodulin) and bioactive agents (*e.g.*, prostacyclin, nitric oxide).^{3,4} Thus, ischemia induces a proinflammatory state that increases tissue vulnerability to further injury on reperfusion.

Role of Reactive Oxygen Species

Reperfusion of ischemic tissues results in the formation of toxic ROS, including superoxide anions (O₂⁻), hydroxyl radicals (OH⁻), hypochlorous acid (HOCl), hydrogen peroxide (H₂O₂), and nitric oxide-derived peroxynitrite. During ischemia, cellular ATP is degraded to form hypoxanthine. Normally, hypoxanthine is oxidized by xanthine dehydrogenase to xanthine. However, during ischemia, xanthine dehydrogenase is converted to xanthine oxidase. Unlike xanthine dehydrogenase, which uses nicotinamide adenine dinucleotide as its substrate, xanthine oxidase uses oxygen and therefore, during ischemia, is unable to catalyze the conversion of hypoxanthine to xanthine, resulting in a buildup of excess tissue levels of hypoxanthine. When oxygen is reintroduced during reperfusion, conversion of the excess hypoxanthine by xanthine oxidase results in the formation of toxic ROS.

Reactive oxygen species are potent oxidizing and reducing agents that directly damage cellular membranes by lipid peroxidation.⁵ In addition, ROS stimulate leukocyte activation and chemotaxis by activating plasma membrane phospholipase A₂ to form arachidonic acid, an important precursor for eicosanoid synthesis (*e.g.*, thromboxane A₂ and leukotriene B₄).⁵ ROS also stimu-

*Assistant Professor, †Leroy D. Vandam/Benjamin G. Covino Professor.

Received from the Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts. Submitted for publication August 17, 2000. Accepted for publication December 21, 2000. Supported by grant No. HL-03854 from the National Heart, Lung and Blood Institute (to C.D.C.), Bethesda, Maryland.

Address reprint requests to Dr. Collard: Department of Anesthesiology, Pain and Perioperative Medicine, Brigham & Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115. Address electronic mail to: collard@zeus.bwh.harvard.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

The illustrations for this section are prepared by Dmitri Karetnikov, 7 Tennyson Drive, Plainsboro, New Jersey 08536.

Table 1.

Cellular effects of ischemia.
Altered Membrane Potential
Altered Ion Distribution (\uparrow Intracellular $\text{Ca}^{2+}/\text{Na}^{2+}$)
Cellular Swelling
Cytoskeletal Disorganization
Increased Hypoxanthine
Decreased Adenosine 5'-Triphosphate (ATP)
Decreased Phosphocreatine
Decreased Glutathione
Cellular Acidosis

late leukocyte adhesion molecule and cytokine gene expression *via* activation of transcription factors such as nuclear factor- κ B.⁵ In addition to causing direct cell injury, ROS thus increase leukocyte activation, chemotaxis, and leukocyte-endothelial adherence after I-R.

Role of Complement

Ischemia-reperfusion results in complement activation and the formation of several proinflammatory mediators that alter vascular homeostasis.⁶ Particularly important are the anaphylatoxins, C3a and C5a, and complement components, iC3b and C5b-9. The most potent of these proinflammatory mediators is C5a, which is approximately 20 times more potent than C3a. In addition to stimulating leukocyte activation and chemotaxis, C5a may further amplify the inflammatory re-

sponse by inducing production of the cytokines monocyte chemoattractant protein 1, tumor necrosis factor α , interleukin-1, and interleukin-6.⁶

C5b-9 and iC3b may also alter vascular homeostasis. iC3b is formed after C3b cleavage and is a specific ligand for leukocyte adhesion to the vascular endothelium *via* the β_2 integrin, CD11b-CD18 (Mac-1). In addition, C5b-9 may activate endothelial nuclear factor- κ B to increase leukocyte adhesion molecule transcription and expression.⁶ Endothelial leukocyte adhesion molecules influenced by complement include vascular cell adhesion molecule 1, intercellular adhesion molecule 1, E-selectin, and P-selectin.⁶ C5b-9 also promotes leukocyte activation and chemotaxis by inducing endothelial interleukin-8 and monocyte chemoattractant protein 1 secretion.⁶ Finally, C5b-9 may alter vascular tone by inhibiting endothelium-dependent relaxation and decreasing endothelial cyclic guanosine monophosphate.⁶ Thus, complement may compromise blood flow to an ischemic organ by altering vascular homeostasis and increasing leukocyte-endothelial adherence.

Role of Leukocytes

Ischemia-reperfusion results in leukocyte activation, chemotaxis, leukocyte-endothelial cell adhesion, and transmigration.^{3,7} Leukocytes interact with the vascular endothelium *via* a series of distinct steps characterized by leukocyte "rolling" on the endothelium, firm adherence of leukocytes to the endothelium, and endothelial transmigration (fig. 1). The first step is initiated by I-R-induced increases in endothelial P-selectin surface expression, which interacts with its leukocyte counterreceptor, P-selectin glycoprotein 1. This initial low affinity interaction results in intermittent leukocyte-endothelial binding characterized as leukocyte "rolling." Subsequent interaction of leukocyte β_2 integrins, such as CD11a/CD18 (leukocyte function-associated antigen-1) or Mac-1,

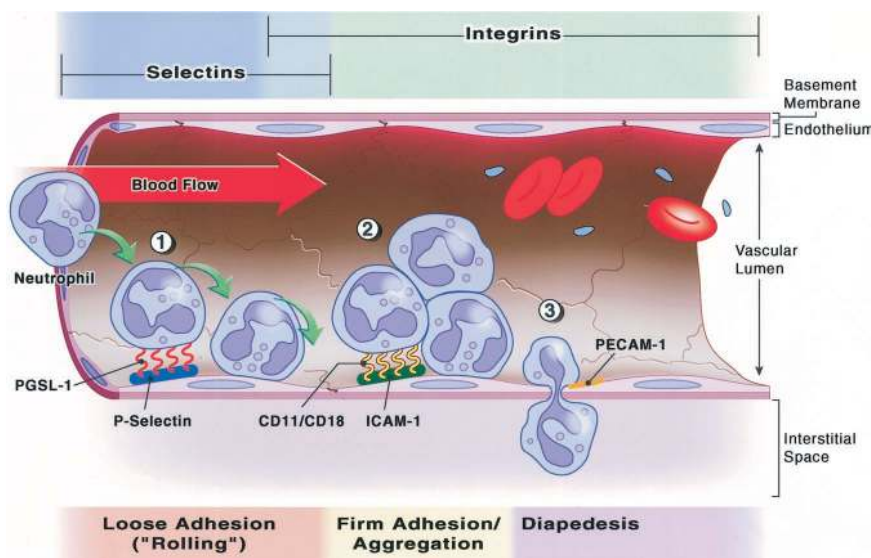


Fig. 1. Leukocyte-endothelial cell adherence and transmigration after ischemia-reperfusion. Activated leukocytes interact with the vascular endothelium *via* a series of distinct steps. The initial "rolling" step is initiated by ischemia-reperfusion-induced increases in endothelial P-selectin expression, which interacts with its leukocyte counterreceptor, P-selectin glycoprotein 1 (PGSL-1) (1). Interaction of leukocyte β_2 integrins, CD11a/CD18 and CD11b/CD18, with endothelial intercellular adhesion molecule 1 (ICAM-1) results in firm leukocyte adherence and aggregation (2). Leukocyte transmigration into the interstitial compartment is facilitated by platelet-endothelial cell adhesion molecule 1 (PECAM-1) within the endothelial cell junctions (3).

with constitutively expressed endothelial intercellular adhesion molecule 1 results in firm leukocyte adherence and cessation of lateral movement. Leukocyte transmigration into the interstitial compartment is facilitated by platelet-endothelial cell adhesion molecule 1, which is constitutively expressed along endothelial cell junctions. On reaching the extravascular compartment, activated leukocytes release toxic ROS, proteases, and elastases, resulting in increased microvascular permeability, edema, thrombosis, and parenchymal cell death.^{3,7}

Clinical Manifestations of Ischemia-Reperfusion Injury

The clinical manifestations of I-R injury are diverse and range from transient reperfusion arrhythmias to the development of fatal MODS. Although the response to I-R varies greatly among individuals, the presence of risk factors such as hypercholesterolemia, hypertension, or diabetes further enhances the vulnerability of the microvasculature to the deleterious effects of I-R.³

Vascular Injury and the “No Reflow” Phenomenon

A common clinical observation is that blood flow to an ischemic organ is often not fully restored after release of a vascular occlusion. Mechanisms of this I-R-associated “no reflow” phenomenon include increased leukocyte-endothelial cell adhesion, platelet-leukocyte aggregation, interstitial fluid accumulation, and decreased endothelium-dependent vasorelaxation, which, together, result in mechanical blood flow obstruction.⁴ Clinically, this may manifest as continued organ dysfunction in the postreperfusion period (*e.g.*, myocardial stunning), failure of a transplanted graft, or increased infarct size. The role of leukocyte adhesion-trapping in the “no reflow” phenomenon is highlighted by canine studies demonstrating that leukocyte depletion improves coronary blood flow, decreases myocardial infarct size, and attenuates the incidence of ventricular arrhythmias.⁸

Myocardial Stunning

Myocardial stunning is defined as myocardial dysfunction that persists after reperfusion despite the absence of irreversible damage. By definition, this transient contractile dysfunction is fully reversible with time, although inotropic or mechanical circulatory support may be required. Postulated mechanisms of myocardial stunning include decreased postreperfusion ATP resynthesis, coronary microvascular spasm or plugging, ROS-mediated cytotoxic injury, and abnormal calcium metabolism.⁴ In contrast, the term “hibernating” myocardium refers to the presence of persistent myocardial dysfunction at rest associated with cardiac ischemia (*i.e.*, reperfusion has not yet occurred).

Reperfusion Arrhythmias

Reperfusion arrhythmias are commonly observed in patients undergoing thrombolytic therapy or cardiac surgery and have been postulated to be a cause of sudden death after relief of coronary ischemia. Support for this concept comes from studies demonstrating that reperfusion of the ischemic myocardium in animals with normal coronaries often leads to the occurrence of ventricular tachycardia, ventricular fibrillation, or an accelerated idioventricular rhythm, particularly if performed abruptly after 15–20 min of ischemia.⁹ The occurrence of reperfusion arrhythmias may partly be a result of rapid and sudden alterations in ion concentrations within the ischemic region on reperfusion. Staged, gradual reflow or transient acid reperfusion substantially decreases the frequency of malignant arrhythmias.^{9,10} Nonetheless, most reperfusion arrhythmias are clinically nonsignificant, and studies of thrombolytic therapy in patients with acute myocardial infarction have clearly shown an overall lower incidence of ventricular fibrillation or tachycardia in treated than in nontreated patients, suggesting that reperfusion lowers the overall risk of myocardial arrhythmias.⁴

Central Nervous System Ischemia-Reperfusion Injury

Ischemia-reperfusion injury of the central nervous system (CNS) may occur after stroke, traumatic head injury, carotid endarterectomy, aneurysm repair, or deep hypothermic circulatory arrest. CNS I-R injury is characterized by disruption of the blood-brain barrier, resulting in leukocyte transmigration into the surrounding brain tissues.¹¹ Release of various proteases, lipid-derived mediators, and ROS by leukocytes into the brain tissue irreversibly damages potentially salvageable cells, particularly within the ischemic penumbra. Disruption of the blood-brain barrier after I-R also results in the development of cerebral edema and increased intracranial pressure. Compounding the cerebral edema is a loss of cerebral vasoreactivity resulting in a reactive hyperemia. Thus, CNS I-R injury may clinically manifest as significantly worsened sensory, motor, or cognitive functioning, or death.

Gastrointestinal Ischemia-Reperfusion Injury

Ischemia-reperfusion of the gastrointestinal tract is associated with a variety of pathologic conditions and surgical procedures, including strangulated bowel, vascular surgery, and hemorrhagic shock. Similar to the CNS, a key consequence of gastrointestinal I-R is the breakdown of intestinal barrier function, which normally protects the body from the hostile environment within the bowel lumen. Thus, in addition to impaired gut motility and absorption, I-R injury of the bowel is associated with increased intestinal permeability and bacterial translocation into the portal and systemic cir-

Table 2.

Therapeutic strategies to attenuate I/R injury.		
Controlled, Graded Reperfusion		
Ischemic Preconditioning		
Aspirin-Triggered Lipoxin (ATL) Analogues		
Antioxidant Therapy		
Superoxide Dismutase (SOD)	Iron Chelators	Mannitol
N-acetylcysteine (NAC)	Vitamin E	Thiols
Allopurinol	Catalase	
Calcium Antagonists		
Angiotensin-Converting Enzyme (ACE) Inhibitors		
Anti-Complement Therapy		
Recombinant, Humanized, Single-Chain Anti-C5 Antibody (h5G1.1-scFv)		
Soluble Complement Receptor-1 (sCR1)		
Leukocyte Depletion / Filtration		
Anti-Cytokine or Leukocyte Adhesion Molecule mAb		
Antisense-Oligodeoxynucleotides (ODNs) and Transcription Factor Decoys		
Endothelin Receptor Antagonists		
Platelet Activation Factor (PAF) Antagonists		
Leukotriene-B ₄ (LTB ₄) Antagonists		

I/R = ischemia-reperfusion.

culations.¹² Intestinal bacterial translocation, along with the cascading activation of cytokines, is thought to contribute to the development of the systemic inflammatory response syndrome.¹²

Multiorgan Dysfunction Syndrome

A devastating consequence of I-R is the development of remote organ injury, including MODS. MODS is the leading cause of death in critically ill patients² and may be a consequence of gut, liver, and skeletal muscle I-R, as well as aortic occlusion-reperfusion and the resuscitation of circulatory shock.³ Additional risk factors for MODS include sepsis, major trauma, burns, pancreatitis, and immunologic disorders. The pulmonary system is the most frequently injured organ in MODS, and onset of the syndrome is usually heralded by the development of acute respiratory insufficiency within 24–72 h of the initiating ischemic event. The pulmonary injury may rapidly progress to respiratory failure and the acute respiratory distress syndrome. Respiratory failure is followed by hepatic, renal, gastrointestinal, myocardial, and CNS dysfunction. In addition to increased microvascular permeability, MODS is characterized by dysfunction

of the coagulation and immune systems, resulting in thrombosis, disseminated intravascular coagulation, and immunocompromise. Intensive care unit mortality directly correlates with the number of failed organ systems, with associated mortality rates of 30–40%, 50–60% or 80–100% when one, two, or more than three organ systems fail, respectively.²

Therapeutic Strategies To Prevent Ischemia-Reperfusion Injury

Many therapeutic strategies that have successfully limited or prevented I-R injury in controlled, experimental models (table 2) have yielded equivocal results in clinical practice or have not reached human clinical trials. Furthermore, few studies have examined the efficacy of combined strategies in attenuating I-R injury. Thus, at present, timely reperfusion of the ischemic area at risk remains the cornerstone of clinical practice.

Ischemic Preconditioning

Ischemic preconditioning refers to the phenomenon by which exposure of tissues to brief periods of ischemia

protects them from the harmful effects of prolonged I-R. Specifically, preconditioning has been shown experimentally to improve ventricular function and to decrease myocardial neutrophil accumulation and apoptosis after I-R.^{13,14} Although the beneficial effects of ischemic preconditioning have been demonstrated in many species, human clinical data are limited. Recently, ischemic preconditioning was demonstrated to have a protective effect on recovery of right ventricular contractility in patients who had coronary artery bypass grafting¹⁵ and to reduce liver injury in humans undergoing hepatic resection.¹⁶ Different mechanisms underlie the protective effects of acute and delayed ischemic preconditioning. Adenosine or α_1 -adrenergic receptor activation of pertussis-sensitive G proteins appears to be a critical initiator of acute preconditioning *via* stimulation of phospholipase C or D, which in turn activates protein kinase C. The beneficial effects of acute preconditioning may be partly a result of protein kinase C-dependent phosphorylation of ATP-sensitive potassium channels.¹³ Acute preconditioning also induces protein kinase C-dependent translocation of 5'-nucleotidase to the cell surface, an effect that increases cellular adenosine production and may confer protection by augmenting cellular energy stores and/or inhibiting leukocyte adherence.¹³ Interestingly, both of these mechanisms may also account for the beneficial myocardial effects of isoflurane, which mimics the cardioprotective effects of ischemic preconditioning.^{17,18} Although the acute beneficial effect of preconditioning is lost as the interval between the brief and prolonged ischemic insults is extended beyond 2 h, a delayed protective effect of preconditioning is observed if the prolonged ischemic insult occurs 24 h after the initial brief periods of ischemia.³ Unlike the acute response, delayed preconditioning is dependent on altered gene expression as well as new protein synthesis, including antioxidant enzymes, nitric oxide synthase, and heat shock proteins.³

Antioxidant Therapy

Numerous experimental animal studies have demonstrated the efficacy of antioxidant therapy in preventing or attenuating I-R injury, including the use superoxide dismutase, catalase, mannitol, allopurinol, vitamin E, *N*-acetylcysteine, iron chelating compounds, angiotensin-converting enzyme inhibitors, or calcium channel antagonists.⁴ In a small prospective trial of human recombinant superoxide dismutase in patients with hemorrhagic shock, Marzi *et al.*¹⁹ demonstrated that patients receiving a continuous infusion of superoxide dismutase for 5 days had significantly less severe organ failure, fewer days in the intensive care unit, and lower serum phospholipase and polymorphonuclear neutrophil elastase concentrations. In addition, superoxide dismutase has been shown to increase graft survival and reduce the

incidence of acute rejection after cadaveric renal transplantation.²⁰ Despite promising results such as these, many studies have yielded equivocal outcomes regarding the efficacy of antioxidant therapy in attenuating human I-R injury.²¹ Nonetheless, considerable clinical and experimental data support the role of oxidative stress in I-R injury and emphasize the importance of antioxidant defense mechanisms in tissue protection.

Anticomplement Therapy

Tissue injury after I-R is significantly reduced by complement inhibition, complement depletion, or in complement-deficient animals.⁶ Administration of the C3 convertase inhibitor, soluble complement receptor 1, was shown to decrease infarct size by 44% in a rat model of myocardial I-R.²² More recently, a "humanized," recombinant, single-chain antibody specific for human C5 (h5G1.1-scFv) was demonstrated to significantly attenuate complement activation, leukocyte activation, myocardial injury, blood loss, and cognitive dysfunction in humans undergoing coronary artery bypass graft surgery with cardiopulmonary bypass.²³ C5 inhibition was also recently shown to significantly decrease myocardial infarct size, apoptosis, and leukocyte infiltration in a rat model of I-R.²⁴ Although soluble complement receptor 1 and h5G1.1-scFv are still being studied in clinical trials, these data suggest that anticomplement therapy may prove effective for attenuating human myocardial I-R injury.

Antileukocyte Therapy

In general, experimental therapeutic strategies to limit leukocyte-mediated I-R injury have focused on inhibition of inflammatory mediator release or receptor engagement, leukocyte adhesion molecule synthesis, or leukocyte-endothelial adhesion.⁷ Leukocyte activation after I-R is facilitated by release of such inflammatory mediators as histamine, platelet activation factor, leukotriene B₄, and tumor necrosis factor α . Inhibition of inflammatory mediator release or receptor engagement using therapeutic agents such as soluble interleukin-1 receptor antagonists, anti-tumor necrosis factor α antibodies, or platelet activation factor-leukotriene B₄ antagonists attenuates I-R-induced leukocyte activation.⁷ Recently, aspirin was found to trigger the biosynthesis of a novel group of bioactive eicosanoids termed 15-epi-lipoxins, or aspirin-triggered lipoxins.²⁵ Lipoxins are lipoxygenase products generated from arachidonic acid. In many assay systems, lipoxins prevent chemotaxis, adhesion, and transmigration of neutrophils induced by leukotrienes and other mediators, suggesting that lipoxins may act as endogenous braking signals in host inflammatory reactions.²⁵ Administration of novel, biostable aspirin-triggered lipoxin analogs has been shown to attenuate neutrophil-mediated changes in vascular permeability and second organ injury in a murine model of hind-limb I-R.²⁵

Aspirin-triggered lipoxin analog therapy may thus represent a novel therapeutic strategy for preventing neutrophil-mediated tissue injury and the development of the systemic inflammatory response syndrome after I-R.

A second therapeutic strategy to limit leukocyte-mediated I-R injury has been to inhibit leukocyte adhesion molecule synthesis. Transcription factors regulating leukocyte adhesion molecule synthesis, such as nuclear factor- κ B, are targets for many of the commonly used antiinflammatory drugs, including glucocorticoids, aspirin, salicylates, gold salts, and D-penicillamine.⁷ Antisense oligodeoxynucleotides and transcription factor decoys have also been used successfully in several experimental models of I-R to inhibit leukocyte adhesion molecule and cytokine expression.⁷ Antisense oligodeoxynucleotides are single-stranded DNA sequences that bind in a complimentary fashion to a specific messenger RNA, thereby blocking expression of the gene product. In contrast, transcription factor decoys are double-stranded antisense oligodeoxynucleotides containing specific binding elements that compete for gene regulatory protein binding (e.g., nuclear factor- κ B) with the authentic nuclear binding elements, thereby interfering with gene regulation.

The third therapeutic strategy to limit leukocyte-mediated I-R injury is inhibition of leukocyte-endothelial adhesion. This is exemplified by studies in which antileukocyte adhesion molecule monoclonal antibodies or soluble adhesion molecules (e.g., P-selectin glycoprotein 1, sialyl-Lewis^x, intercellular adhesion molecule 1) were administered to prevent binding of the membrane-bound form of the adhesion molecule to its ligand.⁷ Although these antileukocyte strategies have received limited attention in the clinical setting, they have proven extremely effective in animal models of I-R.⁷

Conclusions

Ischemia-reperfusion results in a local and systemic inflammatory response characterized by oxidant production, complement activation, leukocyte-endothelial cell adhesion, transendothelial leukocyte migration, platelet-leukocyte aggregation, increased microvascular permeability, and decreased endothelium-dependent relaxation.³ In its severest form, I-R injury may clinically result in MODS or death. Although our understanding of the basic pathophysiology of I-R injury has significantly advanced in the last decade, these experimentally derived ideas have yet to be fully integrated into clinical practice, particularly with regard to stroke and hemorrhagic shock. Treatment of I-R injury is also confounded by the fact that inhibition of I-R-associated inflammation might disrupt protective physiologic responses or result in immunosuppression. Although timely reperfusion of the ischemic area at risk remains the cornerstone of clinical practice, therapeutic strategies such as ischemic precon-

ditioning, controlled reperfusion, and antioxidant, complement, or neutrophil therapy may significantly prevent or limit I-R injury in humans.

References

1. Parks DA, Granger DN: Contributions of ischemia and reperfusion to mucosal lesion formation. *Am J Physiol* 1986; 250:G749-53
2. Neary P, Redmond HP: Ischaemia-reperfusion injury and the systemic inflammatory response syndrome, *Ischemia-Reperfusion Injury*. Edited by Grace PA, Mathie RT. London, Blackwell Science, 1999, pp 123-36
3. Carden DL, Granger DN: Pathophysiology of ischaemia-reperfusion injury. *J Pathol* 2000; 190:255-66
4. Maxwell SR, Lip GY: Reperfusion injury: A review of the pathophysiology, clinical manifestations and therapeutic options. *Int J Cardiol* 1997; 58:95-117
5. Toyokuni S: Reactive oxygen species-induced molecular damage and its application in pathology. *Pathol Int* 1999; 49:91-102
6. Collard CD, Lekowski R, Jordan JE, Agah A, Stahl GL: Complement activation following oxidative stress. *Mol Immunol* 1999; 36:941-8
7. Panés J, Perry M, Granger DN: Leukocyte endothelial cell adhesion: Avenues for therapeutic intervention. *Br J Pharmacol* 1999; 126:537-50
8. Romson JL, Hook BG, Kunkel SL, Abrams GD, Schork MA, Lucchesia BR: Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. *Circulation* 1983; 67:1016-23
9. Yamazaki S, Fujibayashi Y, Rajagopalan RE, Meerbaum S, Corday E: Effects of staged versus sudden reperfusion after acute coronary occlusion in the dog. *J Am Coll Cardiol* 1986; 7:564-72
10. Ibuki C, Hearse DJ, Avkiran M: Mechanisms of antifibrillatory effect of acidic reperfusion: Role of perfusate bicarbonate concentration. *Am J Physiol* 1993; 264:H783-90
11. Winquist RJ, Kerr S: Cerebral ischemia-reperfusion injury and adhesion. *Neurology* 1997; 49:S23-6
12. Kong SE, Blennerhassett LR, Heel KA, McCauley RD, Hall JC: Ischaemia-reperfusion injury to the intestine. *Aust NZ J Surg* 1998; 68:554-61
13. Jerome SN, Akimitsu T, Gute DC, Korthuis RJ: Ischemic preconditioning attenuates capillary no-reflow induced by prolonged ventricular function after coronary artery bypass grafting. *Ann Thorac Surg* 2000; 70:1551-7
14. Wang NP, Bufkin BL, Nakamura M, Zhao ZQ, Wilcox JN, Hewan-Lowe KO, Guyton RA, Vinten-Johansen J: Ischemic preconditioning reduces neutrophil accumulation and myocardial apoptosis. *Ann Thorac Surg* 1999; 67:1689-95
15. Wu ZK, Tarkka MR, Pehkonen E, Kaukinen L, Honkonen EL, Kaukinen S: Beneficial effects of ischemic preconditioning on right ventricular function after coronary artery bypass grafting. *Ann Thorac Surg* 2000; 70:1551-7
16. Clavien PA, Yadav S, Sindram D, Bentley RC: Protective effects of ischemic preconditioning for liver resection performed under inflow occlusion in humans. *Ann Surg* 2000; 232:155-62
17. Roscoe AK, Christensen JD, Lynch C III: Isoflurane, but not halothane, induces protection of human myocardium via adenosine A1 receptors and adenosine triphosphate-sensitive potassium channels. *ANESTHESIOLOGY* 2000; 92:1692-701
18. Belhomme D, Peynet J, Louzy M, Launay JM, Kitakaze M, Menasche P: Evidence for preconditioning by isoflurane in coronary artery bypass graft surgery. *Circulation* 1999; 100:II340-4
19. Marzi I, Buhren V, Schuttler A, Trentz O: Value of superoxide dismutase for prevention of multiple organ failure after multiple trauma. *J Trauma* 1993; 35:110-9
20. Land W, Schneeberger H, Schleibner S, Illner WD, Abendroth D, Rutili G, Arfors KE, Messmer K: The beneficial effect of human recombinant superoxide dismutase on acute and chronic rejection events in recipients of cadaveric renal transplants. *Transplantation* 1994; 57:211-7
21. Dhalla NS, Elmoselhi AB, Hata T, Makino N: Status of myocardial antioxidants in ischemia-reperfusion injury. *Cardiovasc Res* 2000; 47:446-56
22. Weisman HF, Bartow T, Leppo MK, Marsh HC Jr, Carson GR, Concino MF, Boyle MP, Roux KH, Weisfeldt ML, Fearon DT: Soluble human complement receptor type 1: In vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science* 1990; 249:146-51
23. Fitch JC, Rollins S, Matis L, Alford B, Aranki S, Collard CD, Dewar M, Elefteriades J, Hines R, Kopf G, Kraker P, Li L, O'Hara R, Rinder C, Rinder H, Shaw R, Smith B, Stahl G, Shernan SK: Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass. *Circulation* 1999; 100:2499-506
24. Vakeva AP, Agah A, Rollins SA, Matis LA, Li L, Stahl GL: Myocardial infarction and apoptosis after myocardial ischemia and reperfusion: Role of the terminal complement components and inhibition by anti-C5 therapy. *Circulation* 1998; 97:2259-67
25. Chiang N, Gronert K, Clish CB, O'Brien JA, Freeman MW, Serhan CN: Leukotriene B4 receptor transgenic mice reveal novel protective roles for lipoxins and aspirin-triggered lipoxins in reperfusion. *J Clin Invest* 1999; 104:309-16