Published online October 19, 2004

Vascular ischaemia and reperfusion injury

Holger K. Eltzschig* and **Charles D. Collard†**

**Department of Anesthesiology and Intensive Care Medicine, University Hospital, Tübingen, Germany and †Department of Cardiovascular Anesthesia, Texas Heart Institute, Saint Luke's Episcopal Hospital, Houston, TX 77030, USA*

Although restoration of blood flow to an ischaemic organ is essential to prevent irreversible tissue injury, reperfusion *per se* may result in a local and systemic inflammatory response that may augment tissue injury in excess of that produced by ischaemia alone. Cellular damage after reperfusion of previously viable ischaemic tissues is defined as ischaemia–reperfusion (I–R) injury. I–R injury is characterized by oxidant production, complement activation, leucocyte–endothelial cell adhesion, platelet–leucocyte aggregation, increased microvascular permeability and decreased endothelium-dependent relaxation. In its severest form, I–R injury can lead to multiorgan dysfunction or death. Although our understanding of the pathophysiology of I–R injury has advanced significantly in the last decade, such experimentally derived concepts have yet to be fully integrated into clinical practice. Treatment of I–R injury is also confounded by the fact that inhibition of I–R-associated inflammation might disrupt protective physiological responses or result in immunosuppression. Thus, while timely reperfusion of the ischaemic area at risk remains the cornerstone of clinical practice, therapeutic strategies such as ischaemic preconditioning, controlled reperfusion, and anti-oxidant, complement or neutrophil therapy may significantly prevent or limit I–R-induced injury in humans.

Introduction

Correspondence to: Charles D. Collard, Clinical Associate Professor, Department of Cardiovascular Anesthesia, Texas Heart Institute, St. Luke's Episcopal Hospital, 6720 Bertner Avenue, Room 0520, MC1-226, Houston, TX 77030, USA. E-mail: ccollard@heart.thi.tmc.edu

The consequences of depriving an organ of its blood supply have long been recognized as a critical factor in the clinical outcome of stroke, haemorrhagic shock, myocardial infarction and organ transplantation. Although restoration of blood flow to an ischaemic organ is essential to prevent irreversible tissue injury, reperfusion *per se* may augment tissue injury in excess of that produced by ischaemia alone. For example, the histological changes of injury after 3 h of liver or intestinal ischaemia followed by 1 h of reperfusion are far worse than the changes observed after 4 h of ischaemia alone.[1,2] Cellular damage after reperfusion of previously viable ischaemic tissues is defined as ischaemia–reperfusion (I–R) injury.[3]

DOI: 10.1093/bmb/ldh025

I–R injury may occur in a variety of clinical settings, including reperfusion after thrombolytic therapy, coronary angioplasty, organ transplantation, aortic cross-clamping or cardiopulmonary bypass. Reperfusion of ischaemic tissues results in both a local and a systemic inflammatory response that, in turn, may result in widespread microvascular dysfunction and altered tissue barrier function. If severe enough, the inflammatory response after I–R may even result in the systemic inflammatory response syndrome (SIRS) or the multiple organ dysfunction syndrome (MODS), which account for up to 30–40% of intensive care unit mortality.[4] Thus I–R injury may extend beyond the ischaemic area at risk to include injury of remote non-ischaemic organs. The pathophysiology, clinical manifestations and therapeutic strategies for the prevention or treatment of vascular I–R injury are reviewed.

Pathophysiology of ischaemia–reperfusion injury

Cellular effects of ischaemia

Oxygen homeostasis is fundamental to human physiology. The essential requirement for adenosine 5′-triphosphate (ATP) generation via oxidative phosphorylation is balanced by the risk of oxidative damage to cellular lipids, nucleic acids, and proteins. Thus cellular and systemic oxygen concentrations are tightly regulated via short- and long-acting response pathways affecting cellular protein expression and activity.[5]

Prolonged ischaemia results in multiple cellular metabolic and ultrastructural changes (Table 1). Although the hypoxic tolerance amongst cell types differs depending on the metabolic rate and intrinsic adaptive mechanisms, cellular necrosis inevitably follows extended periods of anoxia (i.e. oxygen absent) or severe hypoxia (i.e. oxygen supply decreased relative to metabolic demand). Ischaemia-induced decreases in cellular oxidative phosphorylation

Table 1 Cellular effects of ischaemia or hypoxia

Cellular acidosis
Altered membrane potential
Altered ion distribution (increased intracellular Ca^{2+}/Na^{+} ratio)
Cellular swelling
Cytoskeletal disorganization
Increased hypoxanthine
Decreased ATP
Decreased phosphocreatine
Decreased glutathione
Stabilization and nuclear translocation of hypoxia-inducible factor 1 (HIF-1)
Increased leucocyte adhesion molecule expression
Increased nucleotide phosphohydrolysis (via CD39 and CD73) and adenosine signalling

result in a failure to resynthesize energy-rich phosphates including ATP and phosphocreatine. Thus, membrane ATP-dependent ionic pump function is altered, favouring the entry of calcium, sodium and water into the cell. Furthermore, adenine nucleotide catabolism during ischaemia results in the intracellular accumulation of hypoxanthine, which is subsequently converted into toxic reactive oxygen species (ROS) when molecular oxygen is reintroduced.[6] Within the endothelium, ischaemia promotes expression of certain proinflammatory gene products (e.g. leucocyte adhesion molecules, cytokines) and bioactive agents (e.g. endothelin, thromboxane A_2), while repressing other 'protective' gene products [e.g. constitutive nitric oxide (NO) synthase, thrombomodulin] and bioactive agents (e.g. prostacyclin, NO).[3,7] Thus ischaemia induces a proinflammatory state that increases tissue vulnerability to further injury on reperfusion.

Regulation of vascular endothelial growth factor (VEGF) expression illustrates how reduced oxygen availability (hypoxia) can elicit physiological responses via a variety of molecular mechanisms. VEGF plays an essential role in angiogenesis. Hypoxia induces VEGF expression, thus providing a mechanism by which tissue perfusion can be optimized to demand. VEGF mRNA levels increase in hypoxic cells as a result of both increased production (transcriptional activation) and decreased destruction (mRNA stabilization). Whereas overall cell protein synthesis is inhibited in response to hypoxia, VEGF mRNA is efficiently translated into protein.[8] The essential first step in this process, transcriptional activation, is mediated by the binding of hypoxia-inducible factor 1 (HIF-1) to a hypoxia-response element localized upstream from the 5′ transcriptional start site of the human VEGF gene.[9] HIF-1 is a basic helix–loop–helix Per-ARNT-Sim (PAS) protein consisting of HIF-1α and HIF-1β subunits.[10] HIF-1α protein expression is negatively regulated in non-hypoxic cells by ubiquitination and proteasomal degradation. Under hypoxic conditions, HIF-1α protein levels increase dramatically and the fraction that is ubiquitinated decreases.[11] Thus hypoxia results in rapid nuclear accumulation of HIF-1α, where it dimerizes with HIF-1β and binds to the core DNA sequence 5′-RCGTG-3′, leading to the transcriptional activation of VEGF and many other known target genes.[12]

Vascular effects of hypoxia

The pathogenesis of I–R injury begins with a hypoxic insult to the vascular endothelium which not only serves as a vascular barrier, but also orchestrates polymorphonuclear leucocyte (PMN) trafficking.[13,14] As noted previously, hypoxia activates both transcriptional and non-transcriptional pathways, and several parallels exist between tissue responses to hypoxia and to acute inflammation.[13,15] For example, PMNs are mobilized from

the intravascular space to the interstitium during hypoxia, and such responses may contribute significantly to tissue damage during subsequent reperfusion.[16–18] Moreover, myeloid cell migration to sites of inflammation is highly dependent on hypoxia-adaptive pathways.[13,19] PMN migration through the endothelial barrier may disrupt such tissue barriers and create the potential for extravascular fluid leakage and oedema formation.[20]

Many endogenous protective mechanisms exist to fortify the vascular barrier during ischaemic insults. Activated PMNs release a variety of soluble mediators which regulate vascular permeability, including glutamate[16] and adenine nucleotides [in the form of ATP or adenosine monophosphate (AMP) which, through metabolic conversion, liberate adenosine at the vascular surface].[17,18,21,22] In particular, adenosine protects the microvascular endothelial barrier function by helping to re-establish endothelial cell–cell contact following PMN transmigration.[23] Recent studies demonstrate that hypoxia differentially influences vascular permeability in response to activated PMNs. Hypoxic endothelia demonstrate increased protective responses to activated PMNs that are mediated in part through PMN-derived ATP. Further, extracellular ATP metabolism and signalling are enhanced by hypoxia-induced transcriptional increases in functional endothelial surface apyrase (CD39), 5′-ecto-nucleotidase (CD73) and adenosine A_{2B}-receptors ($AdoRA_{2B}$). Thus, hypoxia initiates a coordinated endothelial-barrier-protective response associated with enhanced extracellular adenosine concentrations and signalling (Fig. 1).[17,18]

In contrast, PMNs also liberate factors that may disrupt the endothelial barrier. Activation of PMNs through β_2 integrins stimulates the release of soluble factor(s) which induce endothelial cytoskeletal rearrangement and gap formation, and increase permeability.[24] One PMN-derived permeabilizing factor is heparin-binding protein (HBP), also known as azurocidin or CAP37,[24] which is a member of the serprocidin family of cationic peptides. HBP, unlike other neutrophil granule proteins (e.g. elastase, cathepsin G), induces Ca^{2+}-dependent cytoskeletal changes in cultured endothelia and triggers macromolecular leakage *in vivo*. Interestingly, endothelial cells themselves were recently shown to release HBP,[25] suggesting self-regulation of permeability under some conditions.

Role of leucocytes

I–R injury is characterized by leucocyte activation, chemotaxis, leucocyte–endothelial cell adhesion, and transmigration.[3] Leucocytes interact with the vascular endothelium via a series of distinct steps characterized by leucocyte 'rolling' on the endothelium, firm adherence of leucocytes to the endothelium and endothelial transmigration (Fig. 2).[14] According

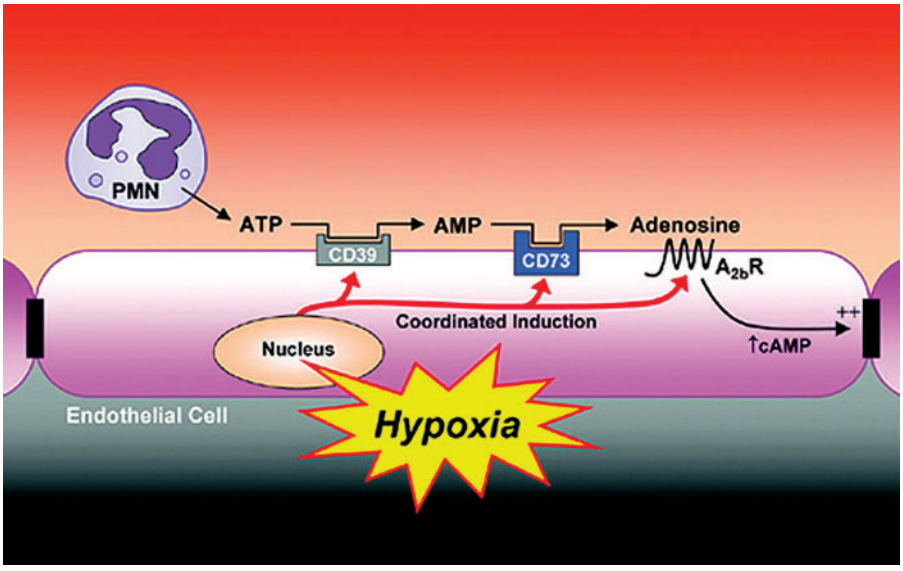

Fig. 1 Proposed model of coordinated nucleotide metabolism and nucleoside signalling in hypoxic endothelial cells. In areas of ongoing inflammation, diminished oxygen supply coordinates the induction of endothelial surface apyrase (CD39), 5′-ecto-nucleotidase (CD73) and adenosine A_{2B} receptors ($AdoRA_{2B}$). At such sites, activated PMNs provide a readily available extracellular source of ATP that, through two enzymatic steps, results in the liberation of extracellular adenosine. Adenosine generated in this fashion is available for surface endothelial adenosine receptor activation, particularly $AdoRA_{2B}$. Post-receptor increases in intracellular cyclic AMP result in enhanced barrier function. Thus this protective mechanism may provide an innate mechanism for preserving vascular integrity and preventing fulminant intravascular fluid loss.[18] Reproduced with permission from Eltzschig *et al.* (2003) *J Exp Med*, **198**, 783–96.

to this multistep paradigm, the first step is initiated by I–R-induced increases in endothelial P-selectin (CD62P) surface expression, which interacts with its leucocyte counter-receptor P-selectin glycoprotein 1 (PSGL-1). This initial low-affinity interaction results in intermittent leucocyte–endothelial binding or 'leucocyte rolling'. Firm leucocyte adherence results from the subsequent interaction of the leucocyte β_2 integrins CD11a/CD18 and CD11b/CD18 with endothelial intercellular adhesion molecule 1 (ICAM-1). Leucocyte transmigration into the interstitial compartment is facilitated by platelet–endothelial cell adhesion molecule 1 (PECAM-1) constitutively expressed along endothelial cell junctions. Upon reaching the extravascular compartment, activated leucocytes release toxic ROS, proteases and elastases, resulting in increased microvascular permeability, oedema, thrombosis and parenchymal cell death.[3] PMN accumulation in the extravascular compartment is also facilitated by interleukin 8 (IL-8) released from hypoxic tissues, resulting in a chemotactic gradient that directs neutrophils from the intravascular space towards the hypoxic interstitium.[26]

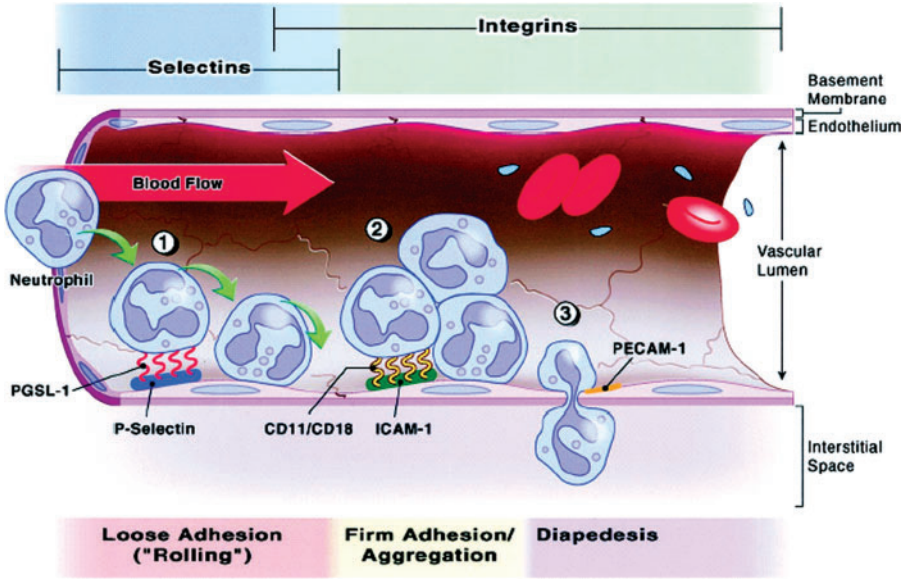

Fig. 2 I–R-induced leucocyte–endothelial cell adherence and transmigration. Leucocyte–endothelial interactions are mediated via a series of distinct steps. (1) Leucocyte 'rolling' is initiated by I–R-induced increases in endothelial P-selectin expression, which interacts with its leucocyte counter-receptor PGSL-1. (2) Interaction of the leucocyte β_2 integrins CD11a/CD18 and CD11b/CD18, with endothelial ICAM-1 results in firm leucocyte adherence and aggregation. (3) Leucocyte transmigration into the interstitial compartment is facilitated by PECAM-1 within the endothelial cell junctions. Reproduced with permission from Collard and Gelman (2001) *Anesthesiology*, **94**, 1133–8.

The no-reflow phenomenon

The no-reflow phenomenon refers to the clinical observation that blood flow to an ischaemic organ is often not fully restored following the release of a vascular occlusion. Possible mechanisms include increased platelet–leucocyte aggregation, leucocyte–endothelial cell adhesion, interstitial fluid accumulation and decreased endothelium-dependent vasorelaxation.[7,27] The no-reflow phenomenon may manifest clinically as continued organ dysfunction in the post-reperfusion period (e.g. myocardial stunning), failure of a transplanted graft or increased infarct size. Experimental canine studies demonstrate a central role of leucocyte adhesion/trapping in the no-reflow phenomenon, as leucocyte depletion in these models improves coronary blood flow, decreases myocardial infarction size and attenuates ventricular arrhythmias.[28]

Role of reactive oxygen species

Reperfusion of ischaemic tissues results in formation of toxic ROS, including superoxide anions (O^{2-}), hydroxyl radicals (OH^{-}), hypochlorous

acid (HOCl), hydrogen peroxide (H_2O_2) and peroxynitrite derived from NO.[6] Cellular ischaemia results in ATP degradation to form hypoxanthine. Under normal physiological conditions, hypoxanthine is oxidized by xanthine dehydrogenase to xanthine. However, xanthine dehydrogenase is converted to xanthine oxidase during ischaemia. Unlike xanthine dehydrogenase, which uses nicotinamide adenine dinucleotide (NAD) as its substrate, xanthine oxidase uses oxygen; therefore during ischaemia it is unable to catalyse the conversion of hypoxanthine to xanthine, resulting in a build-up of excess tissue levels of hypoxanthine. When oxygen is re-introduced during reperfusion, conversion of the excess hypoxanthine by xanthine oxidase results in the formation of toxic ROS.[29,30]

ROS may cause tissue injury via several mechanisms. As they are potent oxidizing and reducing agents, ROS directly damage cellular membranes through lipid peroxidation.[29,31] ROS also stimulate leucocyte activation and chemotaxis by inducing plasma membrane phospholipase A_2 mediated formation of arachidonic acid, an important precursor of eicosanoid synthesis (e.g. thromboxane A_2 and leukotriene B_4).[31] Finally, ROS increase leucocyte adhesion molecule and cytokine gene expression by activating transcription factors such as nuclear factor κB (NF-κB) and activator protein 1 (AP-1).[31]

Role of complement

I–R results in complement activation and the formation of several key inflammatory mediators that may alter vascular homeostasis, including the anaphylatoxins C3a and C5a, and the complement components iC3b and C5b-9.[32] C5a is the most potent of these inflammatory mediators, being approximately 20 times more potent than C3a. In addition to directly stimulating leucocyte activation and chemotaxis, C5a may further amplify the inflammatory response to I–R by inducing the production and release of several pro-inflammatory cytokines, including IL-1, IL-6, monocyte chemoattractant protein 1 (MCP-1) and tumour necrosis factor α (TNF-α).[32]

Vascular endothelial function may also be altered by C5b-9 and iC3b. iC3b is formed after C3b cleavage and is a specific ligand for leucocyte adhesion to the vascular endothelium via the β_2 integrin CD11b/CD18 (Mac-1). C5b-9 activates endothelial NF-κB to increase leucocyte adhesion molecule transcription and expression.[32] Leucocyte adhesion molecules known to be regulated by complement include vascular cell adhesion molecule 1 (VCAM-1), ICAM-1, E-selectin and P-selectin.[32] C5b-9 has also been shown to increase leucocyte activation and chemotaxis by inducing endothelial IL-8 and MCP-1 secretion.[32] Finally, C5b-9 may directly alter vascular tone by inhibiting endothelium-dependent relaxation and

decreasing endothelial cyclic guanosine monophosphate (cGMP).[32] Thus complement may further compromise blood flow to ischaemic tissues by altering vascular homeostasis and promoting leucocyte–endothelial adherence.

Clinical manifestations of ischaemia–reperfusion injury

A wide spectrum of clinical manifestations of I–R injury has been described, ranging from transient reperfusion arrhythmias to the development of fatal MODS. While the response to I–R may vary amongst individuals, risk factors such as diabetes, hypertension or hypercholesterolaemia increase the vulnerability of the microvasculature to I–R injury.[3]

Myocardial stunning

Myocardial stunning can be defined as myocardial dysfunction persisting after reperfusion despite the absence of irreversible damage. The contractile dysfunction is transient and fully reversible with time, although temporary inotropic or mechanical support may be required. Myocardial stunning should be differentiated from 'hibernating' myocardium, which is defined as persistent ischaemia-associated myocardial dysfunction at rest (i.e. reperfusion has not yet occurred). Mechanisms of myocardial stunning may include decreased ATP resynthesis, coronary microvasculature spasm or plugging, ROS-mediated cytotoxic injury and altered intracellular calcium release and uptake.[7,29]

Reperfusion arrhythmias

Reperfusion arrhythmias are frequent in patients undergoing thrombolytic therapy or myocardial surgical revascularization, and may be a cause of sudden death after relief of coronary ischaemia. Further, ventricular tachycardia, ventricular fibrillation or accelerated idioventricular rhythms are often observed following myocardial I–R in animals with normal coronary arteries, particularly if reperfusion occurs abruptly after 15–20 min of ischaemia.[33] Reperfusion arrhythmias may be in part due to rapid and sudden ion concentration changes within ischaemic tissues upon reperfusion. Staged gradual reflow or transient acid reperfusion may reduce the incidence of malignant arrhythmias.[33] In contrast, studies of thrombolytic therapy in acute myocardial infarction patients clearly demonstrate a lower incidence of malignant arrhythmias in treated compared with non-treated patients, suggesting that reperfusion lowers the overall risk of sudden death.[7]

Central nervous system ischaemia–reperfusion injury

Reperfusion injury of the central nervous system (CNS) may contribute to the morbidity and mortality of stroke, head trauma, carotid endarterectomy, aortic aneurysm repair and deep hypothermic circulatory arrest. I–R injury of the CNS is characterized by disruption of the blood–brain barrier, resulting in cerebral oedema, increased intracranial pressure and leucocyte transmigration into the surrounding brain tissues.[34] Leucocytes may then release various proteases, lipid-derived mediators and ROS that may result in irreversible tissue damage, particularly within the ischaemic penumbra. Moreover, because of a loss of cerebral vasoreactivity, a reactive hyperaemia may occur which may worsen the cerebral oedema. Thus I–R injury of the CNS may manifest clinically as worsened sensory, motor or cognitive functioning and death.

Gastrointestinal ischaemia–reperfusion injury

Similar to I–R injury to the CNS, gastrointestinal (GI) I–R injury is characterized by decreased intestinal barrier function. Pathological conditions or surgical procedures where GI I–R injury may occur include strangulated bowel, haemorrhagic shock and vascular surgery producing intestinal ischaemia. Under normal physiological conditions, the intestinal barrier protects the body from the hostile environment within the bowel lumen. However, GI I–R disrupts this protective function, resulting in increased intestinal permeability and bacterial translocation into the portal and systemic circulations.[35] GI I–R injury may also be associated with impaired gut motility and absorption. Activation of complement and circulating leucocytes by translocated bacteria may eventually lead to the development of SIRS following GI I–R injury.

Several innate pathways have been identified that protect intestinal barrier function during hypoxia. Under physiological conditions activation of epithelial adenosine receptors regulates chloride secretion and promotes intestinal barrier function.[36] As mentioned previously, generation of extracellular adenosine is in part dependent on CD73-mediated conversion of AMP to adenosine. Recently, CD73 was shown to play a critical role in the maintenance of intestinal barrier function during hypoxia.[22] Such studies may help to identify potential novel therapeutic targets that may prevent or attenuate development of SIRS following GI I–R.

Multiorgan dysfunction syndrome

A devastating consequence of I–R is the development of remote organ injury and MODS. MODS is a leading cause of death in critically ill

patients in intensive care units (ICUs), with mortality directly correlating with the number of failed organ systems.[4] Risk factors for MODS include sepsis, major trauma, circulatory shock, aortic cross-clamping, burns, pancreatitis and immunological disorders.[3] The pulmonary system is the most frequently injured organ in patients suffering from MODS, and the onset of the syndrome is commonly preceded by the development of acute respiratory insufficiency within 24–72 h of the initiating ischaemic event. Respiratory failure and development of acute respiratory distress syndrome (ARDS) may quickly ensue.[37] Respiratory failure is often 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, leading to thrombosis, disseminated intravascular coagulation and immunocompromise.

Therapeutic implications

The biotechnology and pharmaceutical industries have directed considerable effort into developing novel therapeutic strategies to limit or prevent I–R injury. Although many therapeutic strategies have been shown to be effective in controlled experimental models, most have yielded equivocal results in clinical practice or have yet to reach human clinical trials. Therefore timely reperfusion of the ischaemic area at risk remains the cornerstone of clinical practice.

Leucocyte therapy

Therapeutic strategies to prevent leucocyte-mediated I–R injury include inhibition of leucocyte adhesion molecule synthesis, inflammatory mediator release, receptor engagement and endothelial cell adhesion. Inflammatory mediators such as platelet activation factor (PAF), histamine, leukotriene B_4 (LTB_4), and TNF-α facilitate leucocyte activation. One experimental approach has been to inhibit the release or receptor engagement of such mediators using anti-TNF-α antibodies, soluble IL-1 receptor antagonists or PAF–LTB_4 antagonists.[38] Additionally, aspirin has been shown to induce biosynthesis of a group of bioactive eicosanoids known as the 15-epi-lipoxins or 'aspirin-triggered' lipoxins.[39] Lipoxins are potent inhibitors of leucocyte chemotaxis, adhesion and transmigration induced by leukotrienes and other inflammatory mediators, which suggests that they are part of innate protective pathways dampening the host inflammatory response. Administration of novel biostable lipoxin analogues has been shown to attenuate PMN-mediated

changes in vascular barrier function and second-organ injury in several experimental models of I–R.[39] Thus lipoxin analogue therapy may represent a novel therapeutic strategy for preventing PMN-mediated I–R injury.

A second therapeutic approach to limiting or preventing I–R injury is to decrease leucocyte adhesion molecule synthesis. Many commonly prescribed anti-inflammatory drugs, such as aspirin, glucocorticoids, gold salts and D-penicillamine, attenuate activation of transcription factors (e.g. NF-κB, AP-1) which regulate leucocyte adhesion molecule synthesis or cytokine expression.[38] Antisense oligodeoxynucleotides and transcription factor decoys have also been used successfully to attenuate leucocyte adhesion molecule expression and cytokine release.[38] Antisense oligodeoxynucleotides are single-stranded DNA molecules capable of specifically binding to a complementary nucleic acid sequence, thereby blocking expression of a gene product on a translational and/or transcriptional level. In contrast, transcription factor decoys are double-stranded antisense oligodeoxynucleotides containing specific binding elements that compete for gene regulatory protein binding (e.g. NF-κB, AP-1) with the authentic nuclear binding elements, thereby interfering with gene regulation. Recently, small interfering RNA (siRNA) molecules have been successfully used in mammalian cell culture to target translation of specific gene products. Following siRNA 'knockdown', functional assays can be performed to determine the physiological contribution of specific gene products and their regulation.[40] This experimental approach is similar to that of the gene knockout animal (e.g. knockout mice)[41] and, while limited to cell culture, can be performed much more easily.

I–R injury may also be attenuated by inhibiting leucocyte–endothelial interaction. Monoclonal antibodies directed against leucocyte adhesion molecules or their soluble forms (e.g. PSGL-1, sialyl-Lewis^x or ICAM-1) have been successfully used in several experimental models to prevent binding of the membrane-bound form of the adhesion molecule to its ligand.[38] Although these strategies have proved extremely effective in animal models of I–R, human clinical data are limited.[38]

Ischaemic preconditioning

Repeated exposure of tissues to brief periods of ischaemia or hypoxia may protect against the harmful effects of prolonged I–R. This phenomenon is referred to as ischaemic preconditioning. In experimental models of I–R, ischaemic preconditioning has been shown to improve ventricular function and decrease myocardial neutrophil accumulation and apoptosis.[42] Nonetheless, human clinical data are limited. Ischaemic preconditioning was recently shown to reduce the risk of postoperative

arrhythmias in patients undergoing coronary artery bypass graft (CABG) surgery[43] and to reduce liver injury in humans undergoing hepatic resection.[44]

Different mechanisms are thought to be responsible for the protective effects of acute and delayed ischaemic preconditioning in humans. Acute ischaemic preconditioning is associated with adenosine or α_1-adrenergic receptor mediated activation of pertussis-sensitive G proteins, which in turn stimulate phospholipase C or D to activate protein kinase C (PKC). Thus, the beneficial effects of acute ischaemic preconditioning may be due in part to PKC-dependent phosphorylation of ATP-sensitive potassium channels[45] and PKC-dependent translocation of 5′-nucleotidase to the endothelial cell surface, leading to increased adenosine production, augmentation of cellular energy stores and decreased leucocyte adherence.[45] Although the beneficial effects of acute ischaemic preconditioning are decreased as the interval between the brief and prolonged ischaemic insults is extended beyond 2 h, a delayed protective effect of preconditioning may be observed when the prolonged ischaemic insult occurs 24 h after the initial brief periods of ischaemia.[3] Unlike the acute response, delayed preconditioning is dependent on altered gene expression and synthesis of new proteins, including antioxidant enzymes, NO synthase and heat shock proteins.[3]

As noted previously, adenosine is a critical factor in tissue protection during ischaemia and/or hypoxia. Ischaemic preconditioning is associated with increased extracellular adenosine formation.[46] While the source of interstitial adenosine in hypoxic tissues has been the basis of much debate, it is generally accepted that CD73-mediated AMP dephosphorylation represents the major pathway of extracellular adenosine formation (Fig. 1). Indeed, CD73 is transcriptionally upregulated by hypoxia *in vitro* and *in vivo*,[22] and myocardial ischaemia has been shown to increase extracellular adenosine production.[47] Similarly, myocardial ischaemic preconditioning is associated with increased CD73 activity and adenosine metabolism, suggesting a protective role for CD73 and adenosine following I–R.[47]

In addition to the critical role of adenosine signalling, a role for inducible NO synthase has also been suggested in ischaemic preconditioning. For example, cyclooxygenase-2 (COX-2) is known to mediate the cardioprotective effects of delayed ischaemic preconditioning. It was recently shown that NO-mediated activation of COX-2 is the underlying signalling pathway by which NO synthase is involved in ischaemic preconditioning of the heart.[48] In addition, inducible NO synthase has been shown to influence ischaemic preconditioning of both kidney[49] and brain.[50] This is supported by data indicating that knockout mice deficient in endothelial or neuronal NO synthases show no protection following rapid ischaemic preconditioning in a model of

transient middle cerebral artery occlusion followed by permanent occlusion of the vessel.[50]

Antioxidant therapy

Experimental data obtained from studies using *N*-acetylcysteine, angiotensin-converting enzyme inhibitors, mannitol, iron-chelating compounds, catalase, superoxide dismutase, allopurinol, calcium-channel antagonists and vitamin E suggest that antioxidant therapy may prevent or attenuate I–R injury.[7] A prospective trial of human recombinant superoxide dismutase in patients with haemorrhagic shock demonstrated that patients receiving 5 days of continuous intravenous superoxide dismutase had significantly less severe organ failure, fewer days in the ICU and lower serum phospholipase and PMN elastase concentrations.[51] Superoxide dismutase has also been shown to promote graft survival and decrease the incidence of acute rejection after renal transplantation.[52] However, many human trials of antioxidant therapy to prevent or attenuate I–R injury have yielded equivocal results. Thus, while experimental data strongly support the role of oxidative stress in I–R injury and indicate a role for antioxidant therapy, further randomized human trials are warranted.[53]

Complement therapy

Inhibition of complement activation has been shown to reduce I–R injury significantly in many experimental models.[32] Administration of soluble complement receptor 1, a C3 convertase inhibitor, significantly decreased myocardial infarction size (by 44%) in an experimental rat model.[54] Similarly, a recombinant single-chain antibody specific for human C5 (pexelizumab) was shown to attenuate complement activation, leucocyte activation, myocardial injury and acute postoperative mortality significantly in humans undergoing CABG surgery.[55,56] Although both these compounds, as well as several other novel anticomplement agents, are still undergoing human clinical trials, anticomplement therapy may represent a novel therapeutic strategy for preventing myocardial I–R injury in humans.

Summary

Vascular I–R injury has been shown to contribute to a variety of adverse outcomes. In its severest form, I–R injury may clinically result in MODS or death. The pathogenesis of I–R is complex and involves both a local

and systemic inflammatory response characterized by oxidant production, complement activation, leucocyte–endothelial cell adhesion, transendothelial leucocyte migration, platelet–leucocyte aggregation, increased microvascular permeability and decreased endothelium-dependent relaxation. At the same time, several protective pathways which may be critical in limiting excessive tissue inflammation and barrier breakdown have recently been described. 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. Further, the treatment of I–R injury is confounded by the fact that inhibition of I–R-associated inflammation might disrupt protective physiological responses or result in immunosuppression. Thus, timely reperfusion of the ischaemic area at risk remains the cornerstone of clinical practice. Nevertheless, therapeutic approaches like ischaemic preconditioning, controlled reperfusion and antioxidant, complement or neutrophil therapy may contribute to preventing or limiting I–R injury. Additionally, novel therapeutic targeting of specific anti-inflammatory and barrier-protective pathways (e.g. specific adenosine receptor activation) may hold future promise.

Conflict of interest statement:

Dr Collard has served as a Principal Investigator in the clinical trials of Alexion Pharmaceuticals and Avant Immunotherapeutics. Dr. Eltzschig has no conflicts.

References

1 Varadarajan R, Golden-Mason L, Young L *et al.* (2004) Nitric oxide in early ischaemia reperfusion injury during human orthotopic liver transplantation. *Transplantation*, **78**, 250–256.
2 Parks DA, Granger DN (1986) Contributions of ischemia and reperfusion to mucosal lesion formation. *Am J Physiol*, **250**, G749–G753.
3 Carden DL, Granger DN (2000) Pathophysiology of ischaemia–reperfusion injury. *J Pathol*, **190**, 255–266.
4 Neary P, Redmond HP (1999) Ischaemia–reperfusion injury and the systemic inflammatory response syndrome. In Grace PA, Mathie RT (eds) *Ischaemia–Reperfusion Injury*. Oxford: Blackwell Science, 123–136.
5 Semenza GL (1999) Perspectives on oxygen sensing. *Cell*, **98**, 281–284.
6 Kaminski KA, Bonda TA, Korecki J, Musial WJ (2002) Oxidative stress and neutrophil activation—the two keystones of ischemia/reperfusion injury. *Int J Cardiol*, **86**, 41–59.
7 Maxwell SR, Lip GY (1997) Reperfusion injury: a review of the pathophysiology, clinical manifestations and therapeutic options. *Int J Cardiol*, **58**, 95–117.
8 Stein I, Itin A, Einat P, Skaliter R, Grossman Z, Keshet E (1998) Translation of vascular endothelial growth factor mRNA by internal ribosome entry: implications for translation under hypoxia. *Mol Cell Biol*, **18**, 3112–3119.
9 Forsythe J, Jiang B, Iyer N *et al.* (1996) Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol*, **16**, 4604–4613.

10 Wang G, Jiang B, Rue E, Semenza G (1995) Hypoxia-inducible factor 1 is a basic-helix–loop–helix-PAS heterodimer regulated by cellular O_2 tension. *Proc Natl Acad Sci USA*, **92**, 5510–5514.
11 Sutter CH, Laughner E, Semenza GL (2000) Hypoxia-inducible factor 1alpha protein expression is controlled by oxygen-regulated ubiquitination that is disrupted by deletions and missense mutations. *Proc Natl Acad Sci USA*, **97**, 4748–4753.
12 Semenza GL (2000) HIF-1: mediator of physiological and pathophysiological responses to hypoxia. *J Appl Physiol*, **88**, 1474–1480.
13 Kong T, Eltzschig HK, Karhausen J, Colgan SP, Shelley CS (2004) Leukocyte adhesion during hypoxia is mediated by HIF-1-dependent induction of beta2 integrin gene expression. *Proc Natl Acad Sci USA*, **101**, 10440–10445.
14 Springer TA (1994) Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell*, **76**, 301–314.
15 Tamura DY, Moore EE, Partrick DA, Johnson JL, Offner PJ, Silliman CC (2002) Acute hypoxemia in humans enhances the neutrophil inflammatory response. *Shock*, **17**, 269–273.
16 Collard CD, Park KA, Montalto MC *et al.* (2002) Neutrophil-derived glutamate regulates vascular endothelial barrier function. *J Biol Chem*, **277**, 14801–14811.
17 Eltzschig HK, Thompson LF, Karhausen J *et al.* (2004) Endogenous adenosine produced during hypoxia attenuates neutrophil accumulation: coordination by extracellular nucleotide metabolism. *Blood*, in press.
18 Eltzschig HK, Ibla JC, Furuta GT *et al.* (2003) Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in posthypoxic endothelium: role of ectonucleotidases and adenosine A2B receptors. *J Exp Med*, **198**, 783–796.
19 Cramer T, Yamanishi Y, Clausen BE *et al.* (2003) HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell*, **112**, 645–657.
20 Luscinskas FW, Ma S, Nusrat A, Parkos CA, Shaw SK (2002) Leukocyte transendothelial migration: a junctional affair. *Semin Immunol*, **14**, 105–113.
21 Thompson LF, Eltzschig HK, Ibla JC *et al.* Critical role for ecto-5′-nucleotidase (CD73) in vascular permeability during hypoxia. Submitted for publication.
22 Synnestvedt K, Furuta GT, Comerford KM *et al.* (2002) Ecto-5′-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. *J Clin Invest*, **110**, 993–1002.
23 Lennon PF, Taylor CT, Stahl GL, Colgan SP (1998) Neutrophil-derived 5′-adenosine monophosphate promotes endothelial barrier function via CD73-mediated conversion to adenosine and endothelial A2B receptor activation. *J Exp Med*, **188**, 1433–1443.
24 Gautam N, Olofsson AM, Herwald H *et al.* (2001) Heparin-binding protein (HBP/CAP37): a missing link in neutrophil-evoked alteration of vascular permeability. *Nat Med*, **7**, 1123–1127.
25 Lee TD, Gonzalez ML, Kumar P, Chary-Reddy S, Grammas P, Pereira HA (2002) CAP37, a novel inflammatory mediator: its expression in endothelial cells and localization to atherosclerotic lesions. *Am J Pathol*, **160**, 841–848.
26 Colgan SP, Dzus AL, Parkos CA (1996) Epithelial exposure to hypoxia modulates neutrophil transepithelial migration. *J Exp Med*, **184**, 1003–1015.
27 Reffelmann T, Hale SL, Dow JS, Kloner RA (2003) No-reflow phenomenon persists long-term after ischemia/reperfusion in the rat and predicts infarct expansion. *Circulation*, **108**, 2911–2917.
28 Romson JL, Hook BG, Kunkel SL, Abrams GD, Schork MA, Lucchesi BR (1983) Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. *Circulation*, **67**, 1016–1023.
29 Li C, Jackson RM (2002) Reactive species mechanisms of cellular hypoxia-reoxygenation injury. *Am J Physiol*, **282**, C227–C241.
30 Berry CE, Hare JM (2004) Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. *J Physiol (Lond)*, **555**, 589–606.
31 Toyokuni S (1999) Reactive oxygen species-induced molecular damage and its application in pathology. *Pathol Int*, **49**, 91–102.
32 Collard CD, Lekowski R, Jordan JE, Agah A, Stahl GL (1999) Complement activation following oxidative stress. *Mol Immunol*, **36**, 941–948.
33 Yamazaki S, Fujibayashi Y, Rajagopalan RE, Meerbaum S, Corday E (1986) Effects of staged versus sudden reperfusion after acute coronary occlusion in the dog. *J Am Coll Cardiol*, **7**, 564–572.

34 Collard CD, Park KA, Montalto MC *et al.* (2002) Neutrophil-derived glutamate regulates vascular endothelial barrier function. *J Biol Chem*, **277**, 14801–14811.
35 Kong SE, Blennerhassett LR, Heel KA, McCauley RD, Hall JC (1998) Ischaemia-reperfusion injury to the intestine. *Aust NZ J Surg*, **68**, 554–561.
36 Madara JL, Patapoff TW, Gillece-Castro B *et al.* (1993) 5′-adenosine monophosphate is the neutrophil-derived paracrine factor that elicits chloride secretion from T84 intestinal epithelial cell monolayers. *J Clin Invest*, **91**, 2320–2325.
37 Ware LB, Matthay MA (2000) The acute respiratory distress syndrome. *N Engl J Med*, **342**, 1334–1339.
38 Panes J, Perry M, Granger DN (1999) Leukocyte–endothelial cell adhesion: avenues for therapeutic intervention. *Br J Pharmacol*, **126**, 537–550.
39 Chiang N, Gronert K, Clish CB, O'Brien JA, Freeman MW, Serhan CN (1999) Leukotriene B4 receptor transgenic mice reveal novel protective roles for lipoxins and aspirin-triggered lipoxins in reperfusion. *J Clin Invest*, **104**, 309–316.
40 Brummelkamp TR, Bernards R, Agami R (2002) A system for stable expression of short interfering RNAs in mammalian cells. *Science*, **296**, 550–553.
41 Teoh N, Field J, Sutton J, Farrell G (2004) Dual role of tumor necrosis factor-alpha in hepatic ischemia–reperfusion injury: studies in tumor necrosis factor-alpha gene knockout mice. *Hepatology*, **39**, 412–421.
42 Kloner RA, Jennings RB (2001) Consequences of brief ischemia: stunning, preconditioning, and their clinical implications: Part 1. *Circulation*, **104**, 2981–2989.
43 Wu Z-K, Iivainen T, Pehkonen E, Laurikka J, Tarkka MR (2002) Ischemic preconditioning suppresses ventricular tachyarrhythmias after myocardial revascularization. *Circulation*, **106**, 3091–3096.
44 Clavien PA, Yadav S, Sindram D, Bentley RC (2000) Protective effects of ischemic preconditioning for liver resection performed under inflow occlusion in humans. *Ann Surg*, **232**, 155–162.
45 Jerome SN, Akimitsu T, Gute DC, Korthuis RJ (1995) Ischemic preconditioning attenuates capillary no-reflow induced by prolonged ischemia and reperfusion. *Am J Physiol*, **268**, H2063–H2067.
46 Linden J (2001) Molecular approach to adenosine receptors: receptor-mediated mechanisms of tissue protection. *Annu Rev Pharmacol Toxicol*, **41**, 775–787.
47 Minamino T, Kitakaze M, Morioka T *et al.* (1996) Cardioprotection due to preconditioning correlates with increased ecto-5′-nucleotidase activity. *Am J Physiol*, **270**, H238–H244.
48 Shinmura K, Xuan Y-T, Tang X-L *et al.* (2002) Inducible nitric oxide synthase modulates cyclooxygenase-2 activity in the heart of conscious rabbits during the late phase of ischemic preconditioning. *Circ Res*, **90**, 602–608.
49 Park KM, Byun J-Y, Kramers C, Kim JI, Huang PL, Bonventre JV (2003) Inducible nitric-oxide synthase is an important contributor to prolonged protective effects of ischemic preconditioning in the mouse kidney. *J Biol Chem*, **278**, 27256–27266.
50 Atochin DN, Clark J, Demchenko IT, Moskowitz MA, Huang PL (2003) Rapid cerebral ischemic preconditioning in mice deficient in endothelial and neuronal nitric oxide synthases. *Stroke*, **34**, 1299–1303.
51 Marzi I, Buhren V, Schuttler A, Trentz O (1993) Value of superoxide dismutase for prevention of multiple organ failure after multiple trauma. *J Trauma*, **35**, 110–120.
52 Land W, Schneeberger H, Schleibner S *et al.* (1994) The beneficial effect of human recombinant superoxide dismutase on acute and chronic rejection events in recipients of cadaveric renal transplants. *Transplantation*, **57**, 211–217.
53 Salvemini D, Cuzzocrea S (2003) Therapeutic potential of superoxide dismutase mimetics as therapeutic agents in critical care medicine. *Crit Care Med*, **31**, S29–S38.
54 Weisman HF, Bartow T, Leppo MK *et al.* (1990) Soluble human complement receptor type 1: *in vivo* inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science*, **249**, 146–151.
55 Fitch JCK, Rollins S, Matis L *et al.* (1999) 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*, **100**, 2499–2506.
56 Shernan SK, Fitch JC, Nussmeier NA *et al.* (2004) Impact of pexelizumab, an anti-C5 complement antibody, on total mortality and adverse cardiovascular outcomes in cardiac surgical patients undergoing cardiopulmonary bypass. *Ann Thorac Surg*, **77**, 942–950.