Ischemia-Reperfusion Injury

Pathophysiology and Clinical Implications

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Abstract

The term ischemia-reperfusion injury describes the experimentally and clinically prevalent finding that tissue ischemia with inadequate oxygen supply followed by successful reperfusion initiates a wide and complex array of inflammatory responses that may both aggravate local injury as well as induce impairment of remote organ function. Conditions under which ischemia-reperfusion injury is encountered include the different forms of acute vascular occlusions (stroke, myocardial infarction, limb ischemia) with the respective reperfusion strategies (thrombolytic therapy, angioplasty, operative revascularization) but also routine surgical procedures (organ transplantation, free-tissue-transfer, cardiopulmonary bypass, vascular surgery) and major trauma/shock. Since the first recognition of ischemia-reperfusion injury during the 1970s, significant knowledge has accumulated and the purpose of this review is to present an overview over the current literature on the molecular and cellular basis of ischemia-reperfusion injury, to outline the clinical manifestations and to compile contemporary treatment and prevention strategies. Although the concept of reperfusion injury is still a matter of debate, it is corroborated by recent and ongoing clinical trials that demonstrated ischemic preconditioning, inhibition of sodium-hydrogen-exchange and administration of adenosine to be effective in attenuating ischemia-reperfusion injury.

Key Words
Ischemia · Reperfusion · Injury · Pathophysiology · Review

Eur J Trauma Emerg Surg 2007;33:600–12
DOI 10.1007/s00068-007-7152-z

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Received: October 14, 2007; accepted: October 30, 2007;
Published Online: November 20, 2007

Abbreviations: (A)MI: (Acute) Myocardial infarction; ARDS: Acute respiratory distress syndrome; ATP, ADP, AMP: Adenosine-tri/-di/-mono-phosphate; Bax, Bcl2: Regulators of apoptosis (Bax: Pro-apoptotic, Bcl2: Pro-survival); BH_4: Tetrahydrobiopterin; C1-INH: Complement component 1-Inhibitor; C3a, C5a: Anaphylatoxins, Soluble fragments of C3 and C5; C5b-9: Terminal complement complex, Membrane attack complex; CABG: Coronary artery bypass graft; CXC: Subfamily of chemokines (including Interleukin-8); CXC-R: Receptor for CXC-chemokines; DNA: Deoxyribonucleic acid; e/iNOS: endothelial/inducible Nitric Oxide Synthase; EC: Endothelial cell; FAS (CD95): Cell death receptor of the TNF-Receptor superfamily; H_2O_2: Hydrogen peroxide; ICAM-1,-2: Intercellular adhesion molecule-1,-2; IL-1β, *-6, -8*: Interleukin-1β, *-6, -8*; IRI: Ischemia-reperfusion injury; LFA-1: Leukocyte function antigen; LTB_4: Leukotriene B_4; MCP-1: Monocyte chemotactic protein-1; MMP-9: Matrixmetalloproteinase-9; MODS: Multi-organ dysfunction syndrome; MOF: Multi-organ failure; *N-ACC*: *N-acetylcystein*; NAD: Nicotinamid dinucleotid; NFκB: Nuclear factor κB; NHE: Sodium–Hydrogen exchanger; NO: Nitric oxide; NO_2: Nitrogen dioxide; O_2^-: Superoxide anion; $OH^{\cdot}$: Hydroxyl radical; $ONOO^-$: Peroxynitrite; PARP: Poly-(ADP-Ribose)-Polymerase; PLA_2: Phospholipase A_2; PMN: Polymorphonuclear granulocytes; ROS: Reactive oxygen species; sCR1: Soluble complement receptor 1; SIRS: Systemic inflammatory response syndrome; SOD: Superoxide dismutase; TLR: Toll-like receptor; TNF-α: Tumor necrosis factor-α; TXA_2: Thromboxane A_2

Introduction

Tissue ischemia represents the underlying pathophysiological event in a broad variety of clinical conditions

faced by physicians including the explosively growing field of cardiovascular diseases that has emerged as the leading cause of death worldwide [1]. Here, vascular occlusions treated by thrombolytic therapy, angioplasty or operative revascularization is the underlying pathologies of stroke, myocardial infarction and acute limb ischemia. In addition, tissue ischemia is induced during a wide array of routine surgical procedures including organ transplantation, free-tissue-transfer, cardiac surgery using cardiopulmonary bypass, vascular surgery (cross clamping) and orthopedic surgery (tourniquet). Furthermore, conditions leading to hypoperfusion as evidenced in shock or major trauma also represent considerable inductors of tissue ischemia. In cells deprived of oxygen-carrying blood, cellular respiration slows down with irreversible damage occurring rapidly within minutes in sensitive tissues such as myocardium [2]. Timely reestablishment of blood flow is essential to salvage the ischemic tissue, however, reperfusion itself can paradoxically cause further damage to the ischemic tissue, which has been characterized by the term ''ischemia-reperfusion injury'' (IRI). Furthermore, the systemic remote effects of reperfusion that manifest as systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) can be devastating and fatal. Since the first encounter of ischemia-reperfusion injury in the coronary [3] and the peripheral vascular circulation [4] during the 1970s, significant knowledge has accumulated in the literature, which is reflected by the exponentially growing number of publications. The purpose of this review is to present an overview of the current knowledge on the cellular and molecular basis of ischemia-reperfusion injury, to outline the clinical manifestations of ischemia-reperfusion injury with special focus on the skeletal muscle and remote injury as this is of priority interest for the physician involved in trauma and emergency medicine and, finally, to compile contemporary treatment strategies.

Cellular and Molecular Basis of Ischemia-Reperfusion Injury

General Considerations

In general, tissue ischemia can be described as a situation of inadequate oxygen delivery to cover metabolic demands and the concept of a reperfusion injury fosters on the conclusive results of experiments comparing ischemia/reperfusion to sustained ischemia where cell death was only 17% in the group of sustained ischemia versus 73% in the reperfused group [5]. Whilst ischemic injury is mainly due to oxygen-deprived cell death, reperfusion produces a wide array of inflammatory responses that both heightens local damage and leads to systemic insult as well [6].

The theoretical basis for IRI is built upon two assumptions: firstly, that there is a number of cells that are irreversible damaged during tissue ischemia and will ultimately suffer cell death whereas another group of cells will maintain a definitely viable state. Secondly, that there is a putative group of cells that can be considered as potentially viable although at risk for cell death. The latter subgroup represents cells that are potentially salvageable and as the fraction of the dead cells will definitively be lost, the extent of the resulting reperfusion injury is merely determined by the fate of the subgroup of cells at risk (Figure 1): In complete recovery (A), all cells at risk resume normal function and no additional cell death is encountered. In contrast, maximum reperfusion injury results, if the subgroup of cells at risk does not recover but collectively undergoes cell death (C). In cases of partial recovery (B), a varying fraction of cells at risk will recover while the remainder will suffer cell death (adapted from 7).

The underlying pathophysiological changes during ischemia and reperfusion and the complex effects of IRI will be explained in detail in the following sections (A summarized presentation is given in Table 1).

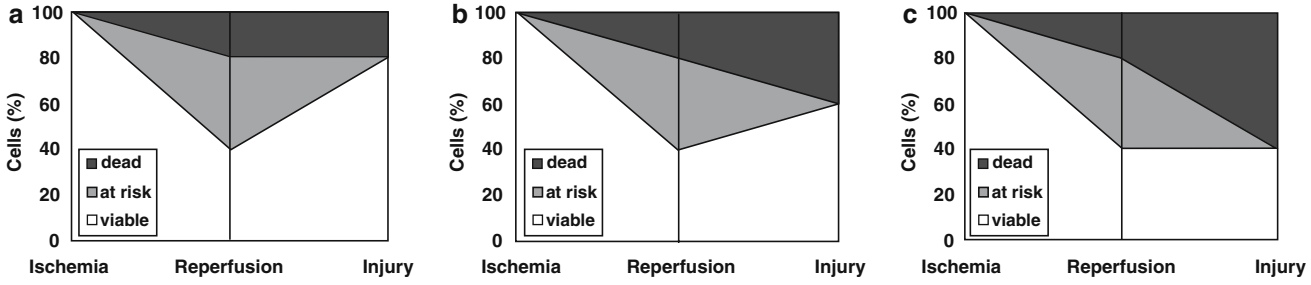

Figure 1. Concept of ischemia-reperfusion injury. Schematic presentation of reperfusion injury where the fate of the cells at risk (grey) determinates the extent of injury with either complete recovery (a), partial recovery (b) or full reperfusion injury (c).

Table 1. Effects of ischemia-reperfusion injury (References in the text).

Molecular effects	Cellular effects
ATP depletion	Endothelial cell dysfunction/ swelling
Defective ATP-resynthesis	Leukocyte (PMN) recruitment
Increase in hypoxanthine	Oxidative burst (PMN)
Activation of xanthine oxidase	Impaired vasodilatation (NO-mediated)
Generation of ROS (O_2^-, H_2O_2, $OH\cdot$, $ONOO^-$)	Enhanced vasoconstriction (endothelin-mediated)
Antioxidant (glutathione) depletion	Endothelial barrier disruption
Intracellular Na^+ and Ca^{++}-overload	Expression of adhesion molecules (P-/L-selectin, ICAM-1/2)
Activation of NHE	Liberation of matrix-degrading proteases (Elastase, MMP-9)
Activation of PLA_2	
Activation of PARP	
Activation of NFκb	
Activation of Toll-like-receptor-signaling	
Subcellular effects	**Mediators**
Mitochondrial dysfunction/swelling	Arachidonic acid metabolites (LTB_4, TXA_2)
Translocation of *bax*	Cytokines (IL-1β, IL-6, TNF-α)
Efflux of cytochrome c	Chemokines (IL-8, MCP-1)
Lipid peroxidation	Activated complement (C3a, C5a, C5b-9)
DNA strand breaks	
Cell membrane damage	
Increased cell membrane permeability	
Cytoskeletal derangements	

Energy Depletion, Generation of Reactive Oxygen Species (ROS), Ion Dysregulation and Cell Death

The initial metabolic change during tissue ischemia is energy depletion as a result of defective resynthesis of adenosintriphosphat (ATP) and degradation of energy-rich phosphates (ATP) via ADP and AMP to adenosine and finally hypoxanthine. Under physiologic conditions, hypoxanthine is converted to xanthine by the enzyme xanthine dehydrogenase with consumption of nicotinamid adenine dinucleotide (NAD). However, under ischemic conditions, xanthine dehydrogenase (D-form) undergoes a conformational change to xanthine oxidase (O-form) that is capable of generating reactive oxygen species (ROS) [8, 9]. Of note, this conformational change is also promoted by intracellular Ca^{++} increase [10]. In addition, cellular ATP-depletion results in mitochondrial dysfunction and initiates the translocation of bax, a proapoptotic bcl2 family member protein, from the cytosol to the outer mitochondrial membrane. This causes mitochondrial swelling and induces the efflux of cytochrome c via opening of the permeability transition pore into the cytosol where cytochrome c activates effector caspases and initiates apoptosis [11].

In the setting of ischemia and subsequent reperfusion, the carefully orchestrated homeostasis of oxygen metabolism is altered and highly reactive oxygene species accumulate: under normal conditions, 95% of oxygen is reduced in the mitochondrium to H_2O via tetravalent reduction without any free radical intermediates, whereas 5% is reduced by univalent pathway in which free radicals like superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) are produced and are safely metabolized to H_2O by dismutase, catalase and the glutathion peroxidase system [5]. With ischemia, antioxidant defenses become eroded and elevating H_2O_2 increasingly generates the highly destructive hydroxyl radical ($\cdot OH$) that causes direct damage to cellular membranes as well as proteins and induces lipid peroxidation already at this early stage [5, 12]. Following restoration of oxygen supply the production of ROS by dysfunctional mitochondria rises dramatically [5] and additionally, xanthineoxidase further accentuates the problem ROS production by converting hypoxanthine and O_2 into highly reactive superoxide (O_2^-) [7]. An additional source of ROS is the production of superoxide (O_2^-) by eNOS/iNOS as a result of depletion of their actual substrate L-arginine and the cofactor tetrahydrobiopterin (BH_4). Furthermore, NO itself can also be a mediator of tissue damage during I/R injury, as it reacts with the abundantly prevalent superoxide anion to form peroxynitrite ($ONOO^-$) and subsequently dissociates into the highly cytotoxic species NO_2 and OH^-. However, as NO also exerts cytoprotective effects the exact role of NOS enzymes in I/R injury is still under investigation [13]. Under the above circumstances of increasing ROS levels and the fact that cellular antioxidant defenses like glutathione, protecting cellular proteins and lipids against oxidation, are depleted [7], ROS react directly with cellular lipids, proteins and DNA leading to cell injury/death and activation of NFκB [14]. Of note, NFκB activation in I/R occurs not only through ROS [15] but also via cytokines (TNF-α and IL-1β) and Toll-like receptor (TLR)-signaling. In IRI TLR-signaling can be induced by hyaluronan-fragments as a consequence of tissue breakdown and this mechanism was shown to play an important role in chemokine upregulation in endothelial cells in vitro and in an TLR4 knockout model [16]. Furthermore, TLR4-deficient mice exhibit

reduced infarct size following myocardial ischemia [17]. Additionally, production of reactive oxygen species (ROS) during ischemia/reperfusion provokes DNA-strand breaks that in turn activate the nuclear enzyme Poly(ADP-ribose) polymerase (PARP). PARP cleaves NAD(+) into nicotinamide and ADP-ribose that is polymerized and used for subsequent DNA repair and maintenance of genomic stability. The ROS-induced overactivation of PARP consumes ATP and may culminate in cell dysfunction and cell death [18]. PARP activation in ischemia/reperfusion injury was first described in 1997 in a rabbit model of myocardial and skeletal ischemia [19] and subsequently also identified operative in ischemia-reperfusion injury of the lung [20], the kidneys [21] and the brain [22]. Lastly, a major pathological effect of oxygen radicals lies in the attraction of neutrophils (PMN) and the triggering of their oxidative burst reaction [23].

A crucial feature of IRI is dysregulation of cellular ion homeostasis resulting in ischemia-induced acidosis and calcium overload. The key mechanism for restoration of cellular pH is activation of the sodium (Na^+)-hydrogen (H^+)-exchanger (NHE) that is the major pH-regulatory protein and promotes the efflux of excess protons coupled to the influx of sodium-ions in an electroneutral process. Unfortunately, the sodium-(Na^+)-potassium (K^+)-ATPase, that under physiologic conditions effectively removes sodium from the cytosol, is inhibited during ischemia-induced ATP-depletion and therefore, the sodium (Na^+)-calcium (Ca^{++})-exchanger takes over their role to circumvent intracellular sodium accumulation. By moving sodium out of the cell, extracellular calcium is consequently transported into the cytosol and results in intracellular calcium overload [24]. Unregulated calcium-influx exerts detrimental effects as it activates a multitude of intracellular enzymes including proteases and endonucleases important in proapoptotic signaling [25] and it activates plasmamembrane phospholipase A_2 which leads to formation of arachidonic acid metabolites (leukotriene B_4, thromboxane A_2) [26] that increase neutrophil adhesiveness [27, 28] and perpetuate the generation of reactive oxygene radicals via stimulation of neutrophil oxidative burst [29].

A special point of interest is the mechanism of cell death during IRI and here, it has been established that cell death occurs both via necrosis and apoptosis: during the degenerative process of necrosis, cellular integrity is lost and the concomitant release of cytosolic contents elicits an inflammatory response whereby the extent of necrotic cell loss is a function of the duration of ischemia and is localized in the central zone of the infarct. In contrast, apoptosis is a highly regulated, energy (ATP) dependent mechanism that leads to cellular degradation into membrane-covered apoptotic bodies that are removed by macrophages without provoking an inflammatory response [30]. Of note, the contribution of apoptosis and necrosis to ischemia-reperfusion injury is differential: although myocardial injury results from a significant increase in both necrosis and apoptosis, the contribution of necrosis to infarct size is significantly greater than that of apoptosis [11] which is plausible in the light that apoptosis is an energy-consuming process. However, there is evidence, that ischemia itself triggers apoptosis whereas reperfusion accelerates the process and may lead to an enhancement of apoptosis as reperfusion restores the energy required for the completion of apoptosis [31].

Endothelial Cell-Dysfunction and Leukocyte Adhesion

Endothelial cell dysfunction occurs as a consequence of cell injury during I/R and is likely caused by the concert of oxidative damage to membranes, dysregulation of ion homeostasis and osmotic stress. In addition to endothelial cell swelling, IRI is known to cause many additional changes in endothelial cells including increased membrane permeability, cytoskeletal derangements and recruitment of inflammatory cells. One of the most sensitive indicators of EC-dysfunction is impaired endothelium-dependent vasodilatation that is mediated by NO and during IRI synthesis of NO by eNOS or iNOS may be decreased by reduced availability of the precursor L-arginine or by depletion of the cofactor tetrahydrobiopterin (BH_4) [25]. The release of endothelin-1, the most powerful vasoconstrictor, is dramatically increased following reperfusion and further capacity to cause vasospasm is conferred by leukotriene B_4, activated complement components and thromboxan A_2 that are liberated during the inflammatory reaction induced by reperfusion [32]. In addition, the acute endothelial dysfunction may result in endothelial swelling a result of influx of water and sodium during ischemia and enhanced vasoconstriction, all of which can work in concert narrowing the capillary lumen and elevating hydraulic resistance to impair perfusion at the microvascular level despite adequate restoration of blood flow [33, 34]. Additionally, disruption of the endothelial barrier due to disorganization of junctional adhesion proteins, increased fluid filtration at the capillary level and macromolecular leakage results in reduced capillary perfusion [25]. The pathological sequence of endothelial integrity disrup-

tion and fluid loss with the consequence of intravascular hemoconcentration seems to be the most likely cause for the capillary ''no-reflow'' phenomenon whereas blood coagulation, platelet aggregation and leukocyte plugging appear not to be a significant mechanism [34], although early reports have attributed the so called ''no-reflow'' phenomenon to blockage of capillaries by neutrophils preventing reperfusion and resulting in extensive capillary damage and myocardial cell swelling [35].

The process of leukocyte adhesion is initiated by P-selectin that is expressed on endothelial cells and is primarily responsible for the initial tethering of granulocytes (PMN) in the microvessels [36] whereas L-selectin becomes the principal mediator of leukocyte rolling after aprox. 20 min [37] via binding of their respective ligand Sialyl-Lewis-X [38]. Secondly, rolling neutrophils become activated and more firmly attached to the endothelium via interaction of binding proteins of the integrin-family, namely LFA-1 (CD11/18) and their counterparts ICAM-1 and 2 on endothelial cells [39]. The selectin-family consists of three members, L-, P- and E-selectin of which L-selectin is constitutively expressed on the surface of leukocytes [40] whereas P- and E-selectins are expressed by endothelial cells in response to stimuli such as Interleukin-1β and TNF-α [41]. P-selectin antibody has been successfully used to reduce local skeletal muscle reperfusion injury in the rat [42] and pulmonary/hepatic injury in a mouse model of lower torso ischemia [43]. In a sheep model of ischemia-reperfusion, antibodies directed against both P- and L-selectin significantly reduced pulmonary leakage and neutrophil accumulation [44]. Additionally, application of soluble Sialyl-Lewis-X oligosaccharide, the principal carbohydrate ligand for P-, L- and E-selectin in an ischemia-reperfusion model of the rabbit ear resulted in attenuated reperfusion injury [45]. ICAM-1 is up regulated in a canine model of myocardial ischemia within one hour of reperfusion [46] and targeting of ICAM-1 in animal models either by blocking antibody [47] or ICAM-1 knockout [48] was shown to reduce neutrophil infiltration during myocardial reperfusion. An anti-integrin directed treatment by application of an CD18-antibody resulted in reduced remote liver and lung injury after ischemia-reperfusion in a rat model [49]. Neutrophil granulocytes (PMN) are the pivotal leukocyte population involved in experimental I/R injury [23] and their contribution mainly relies on release of oxygen-derived cytotoxic products including superoxide anion and hypochlorus acid [50] as well as potent cytotoxic and matrix-degrading proteases (Elastase, MMP-9) [51]. Consequently, animal studies revealed reduced reperfusion injury after blocking of PMN adherance or PMN depletion in myocardial [52, 53], intestinal [54] and pulmonary [43, 55] tissue. Additionally, MMP-9, a protease capable of degrading collagen IV, is secreted by neutrophils in response to proinflammatory mediators and upregulation/expression of MMP-9 has been documented in cerebral [56], cardiac [57], pulmonary [58] and skeletal [59] reperfusion injury.

Release of Inflammatory Mediators (Cytokines, Chemokines, Activated Complement)

Conclusive evidence demonstrates the involvement of proinflammatory cytokines such as Tumor necrosis factor (TNF)-α, Interleukin-1β and Interleukin-6 in the postischemic response. This is corroborated by the finding, that both defective IL-1β signaling [60] as well as TNF-α signaling [61] resulted in decreased chemokine upregulation and attenuated neutrophil infiltration and that in a selection of patients with episodes of ischemia/reperfusion (major blunt trauma, ruptured aortic aneurysm) increased levels of TNF-α, IL-1β and IL-6 are associated with increased mortality and increased risk for ARDS and MOF [62].

Concerning the role of chemokines, upregulation of IL-8 as well as successful prevention of neutrophil infiltration by neutralizing antibodies directed against IL-8 have been documented in animal models of ischemia-reperfusion injury of myocardial, pulmonary and cerebral tissue [63–65]. IL-8 is the prototypic member of the CXC-chemokine family that exhibits strong chemotactic activity for neutrophil granulocytes [66]. Research focussing on the role of the IL-8-receptor (CXC-R1 and -R2) yielded equivocal results as myocardial protection was noted in cases of active [67] as well as defective CXC-R2 [67, 68]. In contrast to IL-8, the chemotactic activity of MCP-1, a member of the CC-chemokine family, is exclusively limited to monocytes and T-cells [69]. Upregulation of MCP-1 could be demonstrated in a canine model of myocardial reperfusion [70] and blocking of MCP-1 signaling either by use of a neutralizing antibody [71] or a MCP-1/CXC-R2 knockout mouse model [72] attenuated reperfusion injury. However, cautious remarks have to be made as chemokine signaling also mediates actions beyond leukocyte chemotaxis including angiogenic and profibrotic effects: for example in the ischemic heart, the chemokine response is an important regulator of cardiac remodeling and in this context, an early transient peak as well as a timely

repression of chemokine synthesis is important for optimal tissue repair [73].

Contribution of the complement system in I/R injury was proposed over three decades ago, when activated complement products were detected in ischemic tissue [32]. Numerous experimental studies have shown subsequently, that I/R injury results in activation of the complement system in several organ systems and that both pathways of activation (classical/alternative) are involved [74] whereby two products of complement activation, C5a and C5b-9, are believed to be mainly responsible for IRI. C5a exerts numerous proinflammatory effects such as chemotaxis of neutrophils [75], release of proteases [76], production of oxygen radicals [77] and it may further amplify the inflammatory response by initiating production of TNF-α, Interleukin-1 and -6 and MCP-1 [78]. C5b-9 was demonstrated to have major contribution to complement-mediated tissue injury [79], to activate NFκB and to induce chemotactic mediators (IL-8, MCP-1) [78]. Complement activation with increased systemic levels of the anaphylatoxins C3a and C5a occurs in patients with severely ischemic limbs and respiratory failure [80]. In C6-deficient animal model, an attenuated IL-8 response accompanied by decreased neutrophil infiltration was noted [81]. C5-deficient mice exhibited decreased lung and liver injury after lower torso ischemia, that was restored after reconstitution with wild-type serum [43] and administration of a specific C5a receptor antagonist abolished upregulation of the CXC-chemokine family and led to reduced neutrophil infiltration [82]. The involvement of both complement activation pathways (classical/alternative) has been ascertained by inhibition studies that targeted the classical pathway [83] and experiments that were carried out in factor-D-deficient mice [84].

Clinical Manifestations of Reperfusion Injury

The clinical manifestations of I/R are diverse and may include myocardial hibernation/stunning, reperfusion arrhythmias, impaired cerebral function, breakdown of the gastrointestinal barrier, systemic inflammatory response syndrome (SIRS) and most devastating, multiorgan dysfunction syndrome (MODS) [78]. However, for the purpose of this review, we have turned our focus towards the local injury of skeletal muscle. The ability of skeletal muscle to anaerobically synthesize ATP confers a relative tolerance to ischemic injury but once energy stores are depleted, reperfusion following ischemia may be complicated by muscle edema, compartment syndrome, muscle necrosis and impaired function. The underlying pathologies include vascular thrombosis and embolism as well as vascular surgical procedures and most importantly, limb trauma and are endowed with a 10–20% rate of amputation [10]. The cellular and biochemical events during ischemia-reperfusion of the skeletal muscle are grossly the same already described: Generation of ROS through xanthine oxidase, lipid peroxidation and Ca^{++}-dyshomeostasis trigger secondary release of leukotriene (LTB_4) and thromboxan (TXA_2) and promote PMN sequestration within skeletal muscle that initiates an amplification loop via further liberation of ROS (oxidative burst) and proteolytic enzymes (elastase) [85, 86]. Those cytotoxic mediators in concert induce endothelial dysfunction and more important, disruption of endothelial integrity, which is associated with increased microvascular permeability and fluid loss into the interstitial space with the result of edema formation. This in particular is devastating as the skeletal muscles are limited in expansion and the rise in interstitial fluid pressure can produce extravascular compression and compartment syndrome [34]. While the local injury reflects microcirculatory failure, release of mediators from the limb may promote remote organ injury contributing to the high mortality seen in these patients [10]. Early work has shown that revascularization of ischemic limbs released K^+, H^+ and myoglobin into the circulation and resulted in impaired renal and pulmonary function and subsequently, a wide array of inflammatory mediators including LTB_4, TXA_2, TNF-α, IL-1β, IL-6 and activated complement components have been identified [10]. The most devastating effects of IRI incur through MODS, the leading cause of death in critically ill patients and acute respiratory insufficiency (ARDS) due to increased permeability in lung vasculature that often is the first clinical sign [25]. The pulmonary injury (ARDS) is mediated by neutrophil sequestration [87] where neutrophils are abundantly present in the pulmonary bed when compared to the normal circulating pool [88] and are activated directly by metabolites produced by the ischemic tissue (e.g. C5a, LTB_4, Thromboxan A_2) [89]. Besides the lung, MODS can involve renal, hepatic, myocardial and CNS dysfunction and neutrophil granulocytes and complement activation in concert with cytokine release (TNF-α, IL-6) have been implicated as the primary mediators of remote organ damage [25, 90]. In this context, PMN immunodepletion was shown to moderate both local and remote organ injury [23] and blockage of complement activation (sCR1) was demonstrated to prevent

Table 2. Contemporary treatment strategies for I/R injury (References in the text).

Treatment	Positive clinical trials
Ischemic preconditioning	Yes
Na-H-Exchange-inhibition	Yes
Adenosine	Yes
Anti-complement (sCR-1/C1-INH)	No/yes
Aprotinin	Yes (side effects!)
Controlled reperfusion	Ongoing (CRAIL)
Antioxidants (SOD, catalase, *N*-ACC, vitamin E)	No
Anti-apoptotic	No
Neutrophil depletion	No
PARP-inhibition	No

pulmonary albumin leak after lower torso ischemia-reperfusion [91].

Contemporary Treatment Strategies

Due to the complexity of IRI, multiple treatment strategies have been investigated and Table 2 summarizes the currently available treatment options for IRI that are compiled in the following sections.

Numerous experimental and preclinical studies have underlined the efficacy of antioxidants in preventing or attenuating ROS-mediated I/R injury including the use of superoxide dismutase (SOD), catalase, allopurinol, vitamin E and *N*-acetyl-Cysteine (*N*-ACC) [78]. However, large clinical trials testing SOD and vitamin E failed to detect any benefit from antioxidant administration. Therefore, despite a wide array of antioxidant agents available, there is to date no clinical indication established recommending the routine use of antioxidants in the setting of ischemia/reperfusion [5].

The same is true for strategies that have been used to target proapoptotic pathways. Here, the literature is equivocal as there were reports in animal models where blockage of apoptotic pathways was successful [31], but also reports demonstrating that caspase inhibition during early reperfusion did not improve immediate postischemic recovery of cardiac function in an experimental model of isolated rabbit heart [11]. In addition, many approaches have little relevance for the clinical setting and the results obtained from clinical trials so far have been disappointing [31].

One promising approach of preventing I/R injury currently under investigation is Na^+/H^+-exchange (NHE) inhibitors. NHE is generally inactive in the normal cell and there is little evidence that NHE-inhibitors affect other cellular regulatory processes aside from NHE, which results in a relatively low risk of this group of drugs to exhibit undesirable side effects. The search for specifically targeted inhibitors of the sodium/hydrogen exchanger led to the development of Cariporide (HOE 642), an selective inhibitor of NHE-1 that has been shown to protect the ischemic myocardium in cardiac surgery by reducing death and MI after CABG in the post-hoc analysis of the GUARDIAN-trial [92] and the prospective EXPEDITION-trial [93]. However, the salubrious effects of cariporide were abrogated in the latter study by an unexpectedly high incidence of neurologic complications. Furthermore, the timing of Na/H-exchange: inhibition seems to be critical as in both GUARDIAN and EXPEDITION trials, cariporide was administered prior to onset of ischemia and a trial that tested eniporide, another inhibitor of NA/H-exchange inhibition at the time of reperfusion in patients with AMI (ESCAMI) did not show any improvement in infarct size or clinical outcome [94]. However, ongoing clinical trials are awaited to assure the definitive proof of this therapy in humans [24].

One of the most powerful and reproducible protective interventions in I/R identified to date that consistently limits infarct size in every animal model and in every species examined and is effective in protecting human myocardium as well is ischemic preconditioning [95]. Ischemic preconditioning was first described in 1986 and uses a brief (sub-lethal) exposure to hypoxia prior to an ischemic period thereby leading to a cellular protection during the following reperfusion phase [5]. Interestingly, ROS and free radicals were shown to play a crucial role during preconditioning, as the protective effects of preconditioning were abolished by antioxidants [96]. Although the mechanisms are not completely clear, brief periods of ischemia seem to induce a phenotype of resistance to subsequent ischemic injury in a variety of tissues including the endothelium by increasing NOS-activity, levels of heat-shock-proteins and antioxidants and activation of ATP-dependent K^+-channels that prevent major shifts in Na^+ which can cause cell swelling and no-reflow [25]. Preconditioned tissues exhibit a improved ''ischemic tolerance'' by reduced energy requirements, altered energy metabolism, better electrolyte homeostasis as well as improved ''reperfusion tolerance'' with less ROS and neutrophils released, reduced apoptosis, better microcirculatory perfusion and diminished systemic I/R injury [6]. Interestingly, in parallel to remote injury in I/R, remote effects of ischemic preconditioning have been postulated as brief periods of skeletal muscle ischemia were shown to

provide protection against subsequent ischemia in myocardium, lung and gut [25]. This is corroborated by a recent randomized controlled trial that documented a benefit in terms of reduced cardiac enzyme relase during elective coronary artery bypass following remote ischemic preconditioning using transient upper limb ischemia in a group of 57 patients [97].

Pioneered by Beyersdorf, the principle of controlled reperfusion of ischemic limbs was introduced into clinical practice [98] after experimental observations in an isolated hindlimb model [99]. Subsequently, several groups have used this method successfully to reduce postischemic reperfusion injury experimentally [100] and clinically [101, 102]. Perfusate solutions mostly were crystalloid, partially combined with adjuncts like antioxidants or initial venous drainage of the perfusate. Recently, controlled reperfusion was successfully used to improve outcomes after surgical treatment of acute limb ischemia in a preliminary clinical study utilizing a mixture of oxygenated blood and crystalloid solution [103] and a randomized controlled multicenter study is currently under way to prove the preliminary findings (Controlled Reperfusion of the Acutely Ischemic Limb (CRAIL)-Trial).

Despite the above-mentioned strong preclinical data demonstrating neutrophil depletion to result in reduced damage by the no-reflow-phenomenon [104], there are reports with contradictory results where neutrophil depletion failed to reduce infarct volume in a rat model of ischemia-reperfusion injury of the brain [105]. Furthermore, all forms of reperfusion injury can be observed under neutrophil-free conditions and a wide range of ''anti-neutrophil'' interventions (antisera, filters, inhibitors of adhesion) did surprisingly not limit infarct size in different models and species [106]. In addition, results of phase 2 and 3 trials of neutrophil adhesion blockage in ischemia-reperfusion disorders (stroke, myocardial infarction, hemorrhagic shock) have been disappointing [106] and to date, two controlled trials exist (FESTIVAL, LIMIT-AMI), that failed to detect a benefit attributable to neutrophil depletion [107].

The following drugs and inhibitors already introduced in the clinical routine have been investigated in I/R injury: Simvastatin inhibits neutrophil infiltration but does not effect tissue damage in rat hindlimb model of skeletal muscle ischemia-reperfusion injury [108].

Aprotinin, a single chain polypeptide that reversibly inhibits a broad spectrum of serine proteases (trypsin, chymotrypsin, plasmin, elastase, kallikrein) with a short half-life kinetic [109], improves myocardial recovery and viability after ischemia and reperfusion in different animal models [110–113]. Aprotinin additionally limits myocardial reperfusion injury by reducing neutrophil infiltration and myocyte apoptosis as well as expression of proinflammatory genes like P-/E-selectin, ICAM-1, TNF-α, IL-6, MCP-1 and Fas (CD95) in a rat model of cardiac ischemia [114, 115], it significantly attenuates expression of P-selectin and reduces leukocyte rolling, adherence and transendothelial migration in a rat model of mesenteric ischemia-reperfusion [116] and it enhances hepatic microcirculation and reduces hepatic reperfusion injury in a rat model of warm liver ischemia [117]. Aprotinin was also successfully employed to improve skin flap survival in a rat model of warm skin-flap ischemia [118]. In summary, preclinical data strongly suggest that aprotinin may reduce aspects of ischemia-reperfusion injury, but prospective clinical trials are needed to evaluate the impact of aprotinin on patient outcomes [112]. In this light, it is noteworthy, that aprotinin had strong beneficial effects on patient outcomes and decreased the incidence of transplant ischemia-reperfusion injury in lung transplantation in a prospective series of 59 patients [119]. However, cautious remarks have to be made about the use of aprotinin, as there were reports of serious side affects including renal impairment, myocardial infarction and stroke [120] as well as anaphylactic reactions [121].

In addition to the endogenous inhibitor nicotinamid, more specific and potent inhibitors of PARP (Poly (ADP-ribose) polymerase) have been synthesized (e.g. PJ34, INO1001) and have been successfully used to attenuate ischemia-reperfusion injury in different animal models in an experimental setting [18]. In a dog model of hypothermic cardiac arrest, inhibition of PARP resulted in decreased intestinal reperfusion injury by reducing neutrophil adhesion and restoring nitric oxide production [122] and in improved recovery of myocardial and endothelial function [123]. Using rodent models of ischemia-reperfusion, PARP inhibition was shown to exhibit beneficial effects on renal damage [21], neuron survival [22] and overall mortality [124]. However, as the physiological role of PARP is to repair injured DNA, targeting PARP may act as a ''double-edged sword'', thus increasing cytotoxicity and therefore, clinical proof of the therapeutic effect of PARP inhibitors in human disease has to be awaited [125].

Due to his established role in I/R, the complement system became a major target for therapeutical intervention and to date, numerous strategies including C3 depletion (via infusion of cobra venom factor), C3-blockage by soluble Complement receptor 1 (sCR1),

administration of C1-esterase inhibitor (C1-INH), antibody blockage of C5 and complement-deficient (C3, C4, C5, C6) mice are available that consistently showed beneficial effects in experimental ischemia reperfusion injury [83]. For example, blockage of complement activation in animal models by inhibiting C3 activation via use of the recombinant soluble form of complement receptor 1 (sCR1) prevents local and pulmonary albumin leak after lower torso ischemia-reperfusion [43, 91], shows reduced local and remote organ injury after ischemia-reperfusion of skeletal muscle and gut [126] and limits myocardial infarction size [127]. However, despite these profound experimental data, the only available clinical trial addressing the role of sCR1 in ischemia-reperfusion injury yielded disappointing therapeutic results [128]. In contrast, the efficacy of C1-Esterase inhibitor (C1-INH) in preventing reperfusion injury has been shown experimentally in rats [129] and pigs [83, 130] as well as clinically [131], where application of C1-INH in patients with coronary bypass surgery in acute ST-elevation myocardial infarction resulted in reduced myocardial reperfusion injury [132, 133] and improved cardiac function [134].

Adenosine has been shown to prevent leukocyte adhesion and emigration following I/R [25] and was found to trigger the early protective response against I/R injury [6]. Clinically, 2 large trials in patients with AMI have demonstrated a marked reduction in the infarct size but no significant difference in clinical outcomes after administration of adenosine (AMISTAD I, [135], AMISTAD II, [136]), although a trend towards improved clinical outcome was noted in AMISTAD II. However, in the setting of high-risk CABG-procedures, administration of adenosine before, during and after clamping has been reported to reduce perioperative infarction and to improve outcome [137].

Conclusion

The concept of reperfusion injury has been a subject of debate for the past three decades where some investigators believed that all injury develops during the ischemic period whereas others argued that restored blood flow is capable of extending tissue injury [138]. While the data on the cellular level reveal mechanisms for true reperfusion injury under conditions of simulated ischemia, the presence of true reperfusion injury in whole animal models remains uncertain [5]. However, the beneficial effects of adenosine and Na/H-exchange inhibitors in clinical trials strengthen the concept of a distinct reperfusion injury [95, 138].

The vast majority of I/R research has been performed focussed on cardiac ischemia with an enormous number of publications (>13.000 according to PubMed since 1971), the majority of them being ''positive'', hundreds of different experimental interventions proposed and significant resources invested by pharmaceutical companies and federal funding agencies [95]. However, despite significant progress in understanding the mechanisms and the complexity of I/R-injury, there currently is no established targeted therapy to safely and significantly reduce its effects [139]. This is largely due to lack of reproducibility and failure to improve outcome in clinical trials, and to date, only the concept of timely reperfusion has been translated into clinical practice [95]. However, adenosine and Na/H-exchange inhibitors are probably the most promising candidates to enter the clinical setting in the future [95, 138].

References

1. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJL. Global and regional burden of disease and risk factors 2001: systematic analysis of population health data. Lancet 2006;367:1747–57.
2. Farber NE, Pieper GM, Gross GJ. Regional differences in postischemic recovery in the stunned canine myocardium. Am Heart J 1987;114:1086–95.
3. Maroko PR, Libby P, Ginks WR, Bloor CM, Shell WE, Sobel BE, Ross J. Coronary artery reperfusion. J Clin Invest 1972;51:2710–6.
4. Haimovici H. Muscular, renal and metabolic complications of acute arterial occlusions: myonephrotic-metabolic syndrome. Surgery 1979;85:461–8.
5. Becker LB. New concepts in reactive oxygen species and cardiovascular reperfusion physiology. Cardiovasc Res 2004; 61:461–70.
6. Pasupathy S, Homer-Vanniasinkam S. Ischaemic preconditioning protects against ischaemia-reperfusion injury: emerging concepts. Eur J Vasc Endovasc Surg 2005;29:106–15.
7. Maxwell SR, Lip GY. Reperfusion injury: a review of the pathophysiology, clinical manifestations and therapeutic options. Int J Cardiol 1997;58:95–117.
8. Chambers DE, Parks DA, Patterson G, Roy R, McCord JM, Yoshida S, Parmley LF, Downey JM. Xanthine oxidase as a source of free radical damage in myocardial ischemia. J Mol Cell Cardiol 1985;17:145–52.
9. Schaffer SW, Roy RS, McCord JM. Possible role for calmodulin in calcium paradox-induced heart failure. Eur Heart J 1983;4:H81–7.
10. Homer-Vanniasinkam S, Crinnion JN, Gough MJ. Post-ischaemic organ dysfunction: a review. Eur J Vasc Endovasc Surg 1997; 14:195–203.
11. McCully JD, Wakiyama H, Hsieh YJ, Jones M, Levitsky S. Differential contribution of necrosis and apoptosis in myocardial ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol 2004;286:1923–35.
12. Granger DN. The role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. Am J Physiol 1988;255:H1269–75.
13. Khanna A, Cowled PA, Fitridge RA. Nitric oxide and skeletal muscle reperfusion injury: current controversies. J Surg Res 2005;128:98–107.

14. Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA. Reactive oxygen species, cell signaling and cell injury. Free Radic Biol Med 2000;28:1456–62.
15. Stancovski I, Baltimore D. NFκB activation: the I kappaB-kinase revealed? Cell 1997;91:299–302.
16. Taylor KR, Trowbridge JM, Rudisill JA, Termeer CC, Simon JC, Gallo RL. Hyaluronan fragments stimulate endothelial recognition of injury through TLR4. J Biol Chem 2004;279: 17079–84.
17. Oyama J, Blais C Jr, Liu X, Pu M, Kobzik L, Kelly RA, Bourcier T. Reduced myocardial ischemia-reperfusion injury in toll-like receptor 4-deficient mice. Circulation 2004;109:784–9.
18. Szabo G, Liaudet L, Hagl S, Szabo C. Poly(ADP-ribose) polymerase activation in the reperfused myocardium. Cardiovasc Res 2004;61:471–80.
19. Thiemermann C, Bowes J, Myint FP, Vane JR. Inhibition of the activity of poly(ADP ribose) synthetase reduces ischemia-reperfusion injury in the heart and skeletal muscle. Proc Natl Acad Sci 1997;94:679–83.
20. Woolley SM, Farivar AS, Naidu BV, Salzman A, Szabo C, Thomas R, Fraga C, Mulligan MS. Role of poly(ADP) ribose synthase in lung ischemia-reperfusion injury. J Heart Lung Transplant 2004;23:1290–6.
21. Chatterjee PK, Zacharowski K, Cuzzocrea S, Otto M, Thiemermann C. Inhibitors of poly (ADP ribose) synthetase reduce renal ischemia-reperfusion injury in the anesthetized rat *in vivo*. FASEB J 2000;14:641–51.
22. Hamby AM, Suh SW, Kauppinen TM, Swanson RA. Use of a poly(ADP ribose) polymerase inhibitor to suppress inflammation and neuronal death after cerebral ischemia-reperfusion. Stroke 2007;38:632–6.
23. Welbourn CR, Goldman G, Paterson IS, Valeri CR, Shepro D, Hechtman HB. Pathophysiology of ischemia reperfusion injury: central role of the neutrophil. Br J Surg 1991;78:651–5.
24. Mentzer RM, Lasley RD, Jessel A, Karmazyn M. Intracellular sodium hydrogen exchange inhibition and clinical myocardial protection. Ann Thorac Surg 2003;75:S700–8.
25. Seal JB, Gewertz BL. Vascular dysfunction in ischemia-reperfusion injury. Ann Vasc Surg 2005;19:572–84.
26. Ernster L. Biochemistry of reoxygenation injury. Crit Care Med 1988;16:947–53.
27. Gimbrone MA, Brock AF, Schafer AI. Leukotriene B4 stimulates polymorpho nuclear leukocyte adhesion to cultured vascular endothelial cells. J Clin Invest 1984;74:1552–5.
28. Spagnuolo PJ, Ellner JJ, Hassid A, Dunn MJ. Thromboxane A2 mediates augmented polymorpho-nuclear leukocyte adhesiveness. J Clin Invest 1980;66:406–14.
29. Paterson IS, Klausner JM, Goldman G, Kobzik L, Welbourn R, Valeri CR, Shepro D, Hechtman HB. Thromboxane mediates the ischemia-induced neutrophil oxidative burst. Surgery 1989;106:224–9.
30. Hamacher-Brady A, Brady NR, Gottlieb RA. The interplay between por-death and pro-survival signaling pathways in myocardial ischemia/reperfusion injury: apoptosis meets autophagy. Cardiovasc Drugs Ther 2006;20:445–62.
31. Eefting F, Rensing B, Wigman J, Pannekoek WJ, Liu WM, Cramer MJ, Lips DJ, Doevendans PA. Role of apoptosis in reperfusion injury. Cardiovasc Res 2004;61:414–26.
32. Hill JH, Ward PA. The phlogistic role of C3 leukotactic fragment in myocardial infarcts in rats. J Exp Med 1971;133:855–900.
33. Boyle EM, Pohlman TH, Cornejo CJ, Verrier ED. Ischemia-reperfusion injury. Ann Thorac Surg 1997;64:S24–30.
34. Menger MD, Rucker M, Vollmar B. Capillary dysfunction in striated muscle ischemia/reperfusion: on the mechanisms of capillary "no-reflow". Shock 1997;8:2–7.
35. Kloner RA, Ganote CE, Jennings RB. The "no-reflow" phenomenon after temporary coronary occlusion in the dog. J Clin Invest 1974;54:1496–508.
36. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 1994; 76:301–4.
37. Lasky LA. Selectin carbohydrate interactions and the initiation of the inflammatory response. Ann Rev Biochem 1995;64:113–39.
38. Moore KL, Stults NL, Diaz S, Smith DF, Cummings RD, Varki A, McEver RP. Identification of a specific glycoprotein ligand for P-selectin (CD62) on myeloid cells. J Cell Biol 1992;118:445–56.
39. Diamond MS, Springer TA. A subpopulation of Mac-1 (CD11b/CD18) molecules mediates neutrophil adhesion to ICAM-1 and fibrinogen. J Cell Biol 1993;120:545–56.
40. Bevilacqua MP, Nelson RM. Selectins. J Clin Invest 1993;91:379–87.
41. Bevilacqua MP, Stengelin S, Gimbrone MA, Seed B. Endothelial leukocyte adhesion molecules: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. Science 1989;243:1160–5.
42. Weiser MR, Gibbs SA, Valeri CR, Shepro D, Hechtman HB. Anti-selectin therapy modifies skeletal muscle ischemia and reperfusion injury. Shock 1996;5:402–7.
43. Kyriakides C, Austen WG Jr, Wang Y, Favuzza J, Moore FD Jr, Hechtman HB. Neutrophil mediated remote organ injury after lower torso ischemia and reperfusion is selectin and complement dependent. J Trauma 2000;48:32–8.
44. Seekamp A, Regel G, Rother K, Jutila M. The effect of anti-L-Selectin (EL-246) on remote lung injury after infrarenal ischemia/reperfusion. Shock 1997;7:447–54.
45. Han KT, Sharar SR, Phillips ML, Harlan JM, Winn RK. Sialyl Lewis (x) oligosaccharide reduces ischemia-reperfusion injury in the rabbit ear. J Immunol 1995;155:4011–15.
46. Kukielka GL, Hawkins HK, Michael L, Manning AM, Youker K, Lane C, Entman ML, Smith CW, Anderson DC. Regulation of intercellular adhesion molecule-1 (ICAM-1) in ischemic and reperfused canine myocardium. J Clin Invest 1993;92:1504–16.
47. Ma XL, Lefer DJ, Lefer AM, Rothlein R. Coronary endothelial and cardiac protective effects of a monoclonal antibody to intercellular adhesion molecule-1 in myocardial ischemia and reperfusion. Circulation 1992;86:937–46.
48. Palazzo AJ, Jones SP, Girod WG, Anderson DC, Granger DN, Lefer DJ. Myocardial ischemia-reperfusion injury in CD18 and ICAM-1 deficient mice. Am J Physiol 1998;275:H2300–7.
49. Welbourn R, Goldman G, Kobzik L, Paterson IS, Valeri CR, Shepro D, Hechtman HB. Role of neutrophil adherence receptors (CD18) in lung permeability following lower torso ischemia. Circ Res 1992;71:82–6.
50. Park JL, Lucchesi BR. Mechanisms of myocardial reperfusion injury. Ann Thorac Surg 1999;68:1905–12.
51. Weiss SJ. Tissue destruction by neutrophils. N Engl J Med 1989;320:365–76.
52. Simpson PJ, Todd RF, Fantone JC, Mickelson JK, Griffin JD, Lucchesi BR. Reduction of experimental canine myocardial reperfusion injury by a monoclonal antibody (Anti-MO1) that inhibits leukocyte adhesion. J Clin Invest 1988;81:624–9.
53. Romson JL, Hook BG, Kunkel SL, Abrams GD, Schork MA, Lucchesi BR. Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. Circulation 1983;67:1016–23.

54. Hernandez LA, Grisham MB, Twohing B, Arfors KE, Granger DN. Role of neutrophils in ischemia reperfusion-induced microvascular injury. Am J Physiol 1987;253:H699–703.
55. Horgan MJ, Wright SD, Malik AB. Protective effect of monoclonal antibody IB4 directed against leukocyte adhesion glycoprotein CD18 in reperfusion lung injury. Am Rev Respir Dis 1989;139:303.
56. Fujimura M, Gasche Y, Morita-Fujimura Y, Massengale J, Kawase M, Chan PH. Early appearance of activated matrix metalloproteinase-9 and blood–brain barrier disruption in mice after focal cerebral ischemia and reperfusion. Brain Res 1999;842:92–100.
57. Lindsey M, Wedin K, Brown MD, Keller C, Evans AJ, Smolen J, Burns AR, Rossen RD, Michael L, Entman M. Matrix-dependent mechanism of neutrophil mediated release and activation of matrix metalloproteinase-9 in myocardial ischemia/reperfusion. Circulation 2001;103:2181–7.
58. Yano M, Omoto Y, Yamakawa Y, Nakashima Y, Kiriyama M, Saito Y, Fujii Y. Increased matrix metalloproteinase-9 activity and mRNA expression in lung ischemia-reperfusion injury. J Heart Lung Transplant 2001;20:679.
59. Roach DM, Fitridge RA, Laws PE, Millard SH, Varelias A, Cowled PA. Up-regulation of MMP-2 and MMP-9 leads to degradation of type IV collagen during skeletal muscle reperfusion injury; protection by the MMP inhibitor doxycycline. Eur J Vasc Endovasc Surg 2002;23:260.
60. Kato A, Gabay C, Okaya T, Lentsch AB. Specific role of interleukin-1 in hepatic neutrophil recruitment after ischemia/reperfusion. Am J Pathol 2002;161:1797–803.
61. Maekawa N, Wada H, Kanda T, Niwa T, Yamada Y, Saito K, Fujiwara H, Sekikawa K, Seishima M. Improved myocardial ischemia/reperfusion injury in mice lacking tumor necrosis factor-alpha. J Am Coll Cardiol 2002;39:1229–35.
62. Roumen RMH, Hendriks T, Ven-Jongekrijg J, Nieuwenhuijzen G, Sauerwein RW, van der Meer JWM, Goris RJA. Cytokine patterns in patients after major vascular surgery, hemorrhagic shock and severe blunt trauma. Ann Surg 1993;218:769–76.
63. Kukielka GL, Smith CW, LaRosa GJ, Manning AM, Mendoza LH, Daly TJ, Hughes BJ, Youker KA, Hawkins HK, Michael LH. Interleukin-8 gene induction in the myocardium after ischemia and reperfusion in vivo. J Clin Invest 1995;95:89–103.
64. Sekido N, Mukaida N, Harada A, Nakanishi I, Watanabe Y, Matsushima K. Prevention of lung reperfusion injury in rabbits by a monoclonal antibody against Interleukin-8. Nature 1993;365:654–7.
65. Matsumoto T, Ikeda K, Mukaida N, Harada A, Matsumoto Y, Yamashita J, Matsushima K. Prevention of cerebral edema and infarct in cerebral reperfusion injury by an antibody to interleukin-8. Lab Invest 1997;77:119–25.
66. Clark-Lewis I, Kim KS, Rajarathnam K, Gong JH, Dewald B, Moser B, Baggiolini M, Sykes BD. Structure-activity relationships of chemokines. J Leukocyte Biol 1995;57:703–11.
67. Tarzami ST, Miao W, Mani K, Lopez L, Factor SM, Berman JW, Kitsis RN. Opposing effects mediated by the chemokine receptor CXCR2 on myocardial ischemia-reperfusion injury: recruitment of potentially damaging neutrophils and direct myocardial protection. Circulation 2003;108:2387–92.
68. Souza DG, Bertini R, Vieira AT, Cunha FQ, Poole S, Allegretti M, Colotta F, Teixeira MM. Repertaxin, a novel inhibitor of rat CXCR2 function, inhibits inflammatory responses that follow intestinal ischemia and reperfusion injury. Br J Pharmacol 2004;143:132–42.
69. Gu L, Tseng SC, Rollins BJ. Monocyte chemoattractant protein-1. Chem Immunol 1999;72:7–29.
70. Kumar AG, Ballantyne CM, Michael LH, Kukielka GL, Youker KA, Lindsey ML, Hawkins HK, Birdsall HH, MacKay CR, LaRosa GJ, Rossen RD, Smith CW, Entman ML. Induction of monocyte chemoattractant protein-1 in the small veins of the ischemic and reperfused canine myocardium. Circulation 1997;95:693–700.
71. Ono K, Matsumori A, Furukawa Y, Igata H, Shioi T, Matsushima K, Sasayama S. Prevention of myocardial reperfusion injury in rats by an antibody against monocyte chemoattractant protein-1. Lab Invest 1999;79:195–203.
72. Furuichi K, Wada T, Iwata Y, Kitagawa K, Kobayashi K, Hashimoto H, Ishiwata Y, Asano M, Wang H, Matsushima K, Takeya M, Kuziel WA, Mukaida N, Yokoyama H. CCR2 signaling contributes to ischemia-reperfusion injury in kidney. J Am Soc Nephrol 2003;14:2503–15.
73. Frangogiannis NG. Chemokines in ischemia and reperfusion. Thromb Haemostas 2007;97:738–47.
74. Arumugam TV, Shiels IA, Woodruff TM, Granger DN, Taylor SM. The role of the complement system in ischemia-reperfusion injury. Shock 2004;21:401–9.
75. Shin HS, Snyderman R, Friedman E, Mellors A, Mayer MM. Chemotactic and anaphylatoxic fragment cleaved from the fifth component of guinea pig complement. Science 1968;162:361–3.
76. Goldstein IM, Weissmann G. Generation of C5-derived lysosomal enzyme-releasing activity (C5a) by lysates of leukocyte lysosomes. J Immunol 1974;113:1583–8.
77. Sacks T, Moldow CF, Craddock PR, Bowers TK, Jacob HS. Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes. An in vitro model of immune vascular damage. J Clin Invest 1978;61:1161–7.
78. Collard CD, Gelman S. Pathophysiology, clinical manifestations and prevention of ischemia-reperfusion injury. Anesthesiology 2001;94:1133–8.
79. Ito W, Schafer HJ, Bhakdi S, Klask R, Hansen S, Schaarschmidt S, Schofer J, Hugo F, Hamdoch T, Mathey D. Influence of the terminal complement-complex on reperfusion injury, no-reflow and arrhythmias: a comparison between C6-competent and C6-deficient rabbits. Cardiovasc Res 1996;32:294–305.
80. Heideman M, Norder-Hansson B, Bengtson A, Mollnes TE. Terminal complement complexes and anaphylatoxins in septic and ischemic patients. Arch Surg 1988;123:188–92.
81. Kilgore KS, Park JL, Tanhehco EJ, Booth EA, Marks RM, Lucchesi BR. Attenuation of interleukin-8 expression in C6-deficient rabbits after myocardial ischemia/reperfusion. J Mol Cell Cardiol 1998;30:75–85.
82. De Vries B, Köhl J, Leclercq WK, Wolfs TG, van Bijnen AA, Heeringa P, Buurman WA. Complement factor C5a mediates renal ischemia-reperfusion injury independent from neutrophils. J Immunol 2003;170:3883–9.
83. Riedemann NC, Ward PA. Complement in ischemia reperfusion injury. Am J Path 2003;162:363–7.
84. Stahl GL, Xu Y, Hao L, Miller M, Buras JA, Fung M, Zhao H. Role for the alternative complement pathway in ischemia/reperfusion injury. Am J Path 2003;162:449–55.
85. Choudhury NA, Sakaguchi S, Koyano K, Matin AF, Muro H. Free radical injury in skeletal muscle ischemia and reperfusion. J Surg Res 1991;51:392–8.
86. Homer-Vanniasinkam S, Gough MJ. The role of leukotrienes in controlling post-ischaemic skeletal muscle function. Vasc Surg 1993;27:585–90.

87. Donnelly SC, Strieter RM, Kunkel SL, Walz A, Robertson CR, Carter DC, Grant IS, Pollok AJ, Haslett C. Interleukin-8 and development of adult respiratory distress syndrome in at-risk patient groups. Lancet 1993;341:643–7.
88. Staub NC, Albertine KH. Leukocytes and pulmonary microvascular injury. Ann NY Acad Sci 1982;384:332–4.
89. Klausner JM, Paterson IS, Mannick JA, Valeri R, Shepro D, Hechtman HB. Reperfusion pulmonary edema. JAMA 1989; 261:1030–35.
90. Klausner JM, Anner H, Paterson IS, Kobzik L, Valeri CR, Shepro D, Hechtman HB. Lower torso ischemia-induced lung injury is leukocyte dependent. Ann Surg 1988;208:761–7.
91. Lindsay TF, Hill J, Ortiz F, Rudolph A, Valeri CR, Hechtman HB, Moore FD. Blockage of complement activation prevents local and pulmonary albumin leak after lower torso ischemia-reperfusion. Ann Surg 1992;216:677–83.
92. Theroux P, Chaitman BR, Danchin N, Erhardt L, Meinertz T, Schroeder JS, Tognoni G, White HD, Willerson JT, Jessel A. Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations. Main results of the GUARDIAN trial. Circulation 2000;102:3032–8.
93. Mentzer RMthe EXPEDITION study investigators Effects of Na^+/H^+ exchange inhibition by cariporide on death and nonfatal myocardial infarction in patients undergoing coronary artery bypass graft surgery: the EXPEDITION study. Circulation 2003;108:3M.
94. Zeymer U, Suryapranata H, Monassier JP, Opolski G, Davies J, Rasmanis G, Linssen G, Tebbe U, Schroder R, Tiemann R, Machnig T, Neuhaus KLand the ESCAMI Investigators The Na(+)/H(+) exchange inhibitor eniporide as an adjunct to early reperfusion therapy for acute myocardial infarction. Results of the evaluation of the safety and cardioprotective effects of eniporide in acute myocardial infarction (ESCAMI) trial. J Am Coll Cardiol 2001;38:1644–50.
95. Bolli R, Becker L, Gross G, Mentzer R Jr, Balshaw D, Lathrop DANHLBI Working Group on the Translation of Therapies for Protecting the Heart from Ischemia Myocardial protection at a crossroads: the need for translation into clinical therapy. Circ Res 2004;95:125–34.
96. Vanden Hoek TL, Becker LB, Shao Z, Li C, Schumacker PT. Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. J Biol Chem 1998;273:18092–8.
97. Hausenloy DJ, Mwamure PK, Venugopal V, Harris J, Barnard M, Grundy E, Ashley E, Vichare S, Di Salvo C, Kolvekar S, Hayward M, Keogh B, MacAllister RJ, Yellon DM. Effect of remote ischemic preconditioning on myocardial injury in patients undergoing coronary artery bypass graft surgery: a randomized controlled trial. Lancet 2007;370:575–9.
98. Beyersdorf F, Mitrev Z, Eckel L, Sarai K, Satter P. Controlled limb reperfusion as a new surgical technique to reduce postischemic syndrome. J Thorac Cardiovasc Surg 1993;106:378–80.
99. Beyersdorf F, Matheis G, Kruger S, Hanselmann A, Freisleben HG, Zimmer G, Satter P. Avoiding reperfusion injury after limb revascularization: experimental observations and recommendations for clinical application. J Vasc Surg 1989;9:757–66.
100. Luther B, Lehmann C, Grune T, Meyer R, Schwenke G, Beutel H, Burger K. Controlled reperfusion of ischemic extremity musculature to prevent free radical induced lesions. Zentralbl Chir 1999;124:336–43.
101. Allen BS, Hartz RS, Buckberg GD, Schuler JJ. Prevention of ischemic damage using controlled limb reperfusion. J Card Surg 1998;13:224–7.
102. Walker PM, Romaschin AD, Davis S, Piovesan J. Lower limb ischemia: phase 1 results of salvage perfusion. J Surg Res 1999;84:193–8.
103. Wilhelm MP, Schlensak C, Hoh A, Knipping L, Mangold G, Rojas DD, Beyersdorf F. Controlled reperfusion using a simplified perfusion system preserves function after acute and persistent limb ischemia: a preliminary study. J Vasc Surg 2005;42:690–4.
104. Engler RL, Dahlgren MD, Morris DD, Peterson MA, Schmidt-Schoberlein GW. Role of leukocytes in response to acute myocardial ischemia and reflow in dogs. Am J Physiol 1986;251: H314–23.
105. Harris AK, Ergul A, Kozak A, Machado LS, Johnson MH, Fagan SC. Effect of neutrophil depletion on galetinase expression, edema formation and hemorrhagic transformation after focal ischemic stroke. BMC Neurosci 2005;6:49.
106. Harlan JM, Winn RK. Leukocyte-endothelial interactions: clinical trials of anti-adhesion therapy. Crit Care Med 2002;30:S214–9.
107. Baxter GF. The neutrophil as a mediator of myocardial ischemia-reperfusion injury: time to move on. Basic Res Cardiol 2002;97:268–75.
108. Cowled PA, Khanna A, Laws PE, Field JBF, Varelias A, Fitridge RA. Statins inhibit neutrophil infiltration in skeletal muscle reperfusion injury. J Surg Res 2007;141:267–76.
109. Westaby S. Aprotinin in perspective. Ann Thorac Surg 1993; 55:1033–41.
110. Gurevitch J, Barak J, Hochhauser E, Paz Y, Yakirevich V. Aprotinin improves myocardial recovery after ischemia and reperfusion. Effects of the drug on isolated rat hearts. J Thorac Cardiovasc Surg 1994;108:109–18.
111. Sunamori M, Sultan I, Suzuki A. Effect of aprotinin to improve myocardial viability in myocardial preservation followed by reperfusion. Ann Thorac Surg 1991;52:971–8.
112. Bull DA, Maurer J. Aprotinin and preservation of myocardial function after ischemia-reperfusion injury. Ann Thorac Surg 2003;75:S735–9.
113. Khan TA, Bianchi C, Voisine P, Feng J, Hart M, Takahashi M, Stahl G, Sellke FW. Reduction of myocardial reperfusion injury by aprotinin after regional ischemia and cardioplegic arrest. J Thorac Cardiovasc Surg 2004;128:602–8.
114. Pruefer D, Buerke U, Khalil M, Dahm M, Darius H, Oelert H, Buerke M. Cardioprotective effects of the serine protease inhibitor aprotinin after regional ischemia and reperfusion on the beating heart. J Thorac Cardiovasc Surg 2002;124:942–9.
115. Buerke M, Pruefer D, Sankat D, Carter JM, Buerke U, Russ M, Schlitt A, Friedrich I, Boergermann J, Vahl CF, Werdan K. Effects of aprotinin on gene expression and protein synthesis after ischemia and reperfusion in rats. Circulation 2007;116:I121–6.
116. Pruefer D, Makowski J, Dahm M, Guth S, Oelert H, Darius H, Buerke M. Aprotinin inhibits leukocyte-endothelial cell interactions after hemorrhage and reperfusion. Ann Thorac Surg 2003;75:210–5.
117. Maksan SM, Maksan MO, Gebhard MM, Herfarth C, Klar E. Reduction of hepatic reperfusion injury by antithrombin III and aprotinin. Transpl Int 2000;13:S562–4.
118. Stadelman WK, Hess DB, Robson MC, Greenwald DP. Aprotinin in ischemia-reperfusion injury: flap survival and neutrophil response in a rat skin flap model. Microsurgery 1998;18:354–61.

119. Bittner HB, Richter M, Kuntze T, Rahmel A, Dahlberg P, Hertz M, Mohr FW. Aprotinin decreases reperfusion injury and allograft dysfunction in clinical lung transplantation. Eur J Cardiothorac Surg 2006;29:210–5.
120. Mangano DT, Tudor IC, Dietzel C. The risk associated with aprotinin in cardiac surgery. N Engl J Med 2006;354:353–65.
121. Beierlein W, Scheule AM, Dietrich W, Ziemer G. Forty years of clinical aprotinin use: a review of 124 hypersensitivity reactions. Ann Thorac Surg 2005;79:741–8.
122. Andrasi TB, Blazovics A, Szabo G, Vahl CF, Hagl S. Poly(ADP-ribose) polymerase inhibitor PJ-34 reduces mesenteric vascular injury induced by experimental cardiopulmonary bypass with cardiac arrest. Am J Physiol Heart Circ Physiol 2005;288:H2972–8.
123. Szabo G, Buhmann V, Andrasi T, Stumpf N, Baehrle S, Kekesi V, Hagl S, Szabo C, Juhasz-Nagy A. Poly-ADP-ribose polymerase inhibition protects against myocardial and endothelial reperfusion injury after hypothermic cardiac arrest. J Thorac Cardiovasc Surg 2003;126:651–8.
124. Black JH, Casey PJ, Albadawi H, Cambria RP, Watkins MT. Poly adenosine diphosphate-ribose polymerase inhibitor PJ34 abolishes systemic proinflammatory responses to thoracic aortic ischemia and reperfusion. J Am Coll Surg 2006;203:44–53.
125. Graziani G, Szabo C. Clinical perspectives of PARP inhibitors. Pharmacol Res 2005;52:109–18.
126. Hill J, Lindsay TF, Ortiz F, Yeh CG, Hechtman HB, Moore FD. Soluble complement receptor type 1 ameliorates the local and remote organ injury after intestinal ischemia-reperfusion in the rat. J Immunol 1992;149:1723–8.
127. 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.
128. Zimmermann JL, Dellinger RP, Straube RC, Levin JL. Phase I trial of the recombinant soluble complement receptor 1 in acute lung injury and acute respiratory distress syndrome. Crit Care Med 2000;28:3149–54.
129. Buerke M, Pruefer D, Dahm M, Oelert H, Meyer J, Darius H. Blocking of classical complement pathway inhibits endothelial adhesion molecule expression and preserves ischemic myocardium from reperfusion injury. J Pharm Exp Ther 1998;286:429–38.
130. Horstick G, Berg O, Heimann A, Götze O, Loos M, Hafner G, Bierbach B, Petersen S, Bhakdi S, Darius H, Horstick M, Meyer J, Kempski O. Application of C1-esterase inhibitor during reperfusion of ischemic myocardium: dose-related beneficial versus detrimental effects. Circulation 2001;104:3125–31.
131. Horstick G. C1-esterase inhibitor in ischemia and reperfusion. Immunobiology 2002;205:552–62.
132. Bauernschmitt R, Böhrer H, Hagl S. Rescue therapy with C1-esterase inhibitor concentrate after emergency coronary surgery for failed PTCA. Int Care Med 1998;24:635–8.
133. Thielmann M, Marggraf G, Neuhaeuser M, Forkel J, Herold U, Kamler M, Massoudy P, Jakob H. Administration of C1-esterase inhibitor during emergency coronary artery bypass surgery in acute ST-elevation myocardial infarction. Eur J Cardiothor Surg 2006;30:285–93.
134. Fattouch K, Bianco G, Speziale G, Sampognaro R, Lavalle C, Guccione F, Dioguardi P, Ruvolo G. Beneficial effects of C1-esterase inhibitor in ST-elevation myocardial infarction in patients who underwent surgical reperfusion: a randomized double-blind study. Eur J Cardiothor Surg 2007;32:326–32.
135. Mahaffey KW, Puma JA, Barbagelata NA, DiCarli MF, Leesar MA, Browne KF, Eisenberg PR, Bolli R, Casas AC, Molina-Viamonte V, Orlandi C, Blevins R, Gibbons RJ, Califf RM, Granger CB. Adenosine as an adjunct to thrombolytic therapy for acute myocardial infarction: results of a multicenter, randomized, placebo-controlled trial: the acute myocardial infarction study of adenosine (AMISTAD) trial. J Am Coll Cardiol 1999;34:1711–20.
136. Ross A, Gibbons R, Stone GW, Kloner RA, Marder VJ, Alexander RW. A randomized, double blinded, placebo-controlled multicenter trial of adenodine as an adjunct to reperfusion in the treatment of acute myocardial infarction (AMISTAD II). J Am Coll Cardiol 2005;45:1775–80.
137. Mentzer RM, Birjiniuk V, Khuri S, Lowe JE, Rahko PS, Weisel RD, Wellons HA, Barker ML, Lasley RD. Adenosine myocardial protection: preliminary results of a phase II clinical trial. Ann Surg 1999;229:643–9.
138. Gross GJ, Auchampach JA. Reperfusion injury: does it exist? J Mol Cell Cardiol 2007;42:12–8.
139. De Silva R, Dunning J, Trull A, Vuylsteke A. A review of ischemia-reperfusion injury in the cardiorespiratory system. Ann Card Anaesthesia 2003;6:126–31.

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