Published in final edited form as:

Arch Physiol Biochem. 2007 February ; 113(1): 1–12. doi:10.1080/13813450601118976.

Lung ischemia–reperfusion injury: implications of oxidative stress and platelet–arteriolar wall interactions

ALEXANDER V. OVECHKIN¹, DAVID LOMINADZE¹, KARA C. SEDORIS³, TONYA W. ROBINSON², SURESH C. TYAGI¹, and ANDREW M. ROBERTS¹

¹Department of Physiology & Biophysics, University of Louisville; Louisville, Kentucky, USA

²Brown Cancer Center, School of Medicine, University of Louisville; Louisville, Kentucky, USA

³Department of Pediatrics, University of Louisville; Louisville, Kentucky, USA

Abstract

Pulmonary ischemia–reperfusion (IR) injury may result from trauma, atherosclerosis, pulmonary embolism, pulmonary thrombosis and surgical procedures such as cardiopulmonary bypass and lung transplantation. IR injury induces oxidative stress characterized by formation of reactive oxygen (ROS) and reactive nitrogen species (RNS). Nitric oxide (NO) overproduction via inducible nitric oxide synthase (iNOS) is an important component in the pathogenesis of IR. Reaction of NO with ROS forms RNS as secondary reactive products, which cause platelet activation and upregulation of adhesion molecules. This mechanism of injury is particularly important during pulmonary IR with increased iNOS activity in the presence of oxidative stress. Platelet–endothelial interactions may play an important role in causing pulmonary arteriolar vasoconstriction and post-ischemic alveolar hypoperfusion. This review discusses the relationship between ROS, RNS, P-selectin, and platelet–arteriolar wall interactions and proposes a hypothesis for their role in microvascular responses during pulmonary IR.

Keywords

Ischemia–reperfusion; oxidative stress; nitric oxide; platelets; P-selectin; pulmonary arterioles

Introduction

Pulmonary ischemia–reperfusion (IR) injury may result from trauma, atherosclerosis, pulmonary embolism, pulmonary thrombosis and surgical procedures such as cardiopulmonary bypass and lung transplantation (Ambrosio & Tritto, 1999). These conditions are associated with respiratory distress syndrome which is a form of acute lung injury where there is a significant inflammatory response, as well as extravascular fibrin deposition and thrombosis. During IR, ischemia-elicited injury is amplified by incomplete restoration of blood flow due to postischemic vasoconstriction with prolongation of ischemia (Gilroy *et al.*, 1993; Miller & Roberts, 1999). Vascular abnormalities may include pulmonary hypertension, altered vascular reactivity, vascular obstruction, intrapulmonary shunting, increased vascular permeability (pulmonary edema), and ventilation/perfusion mismatch (Carden & Granger, 2000; Fullerton *et al.*, 1993; Gilroy *et al.*, 1993).

High morbidity and mortality associated with IR, and complications induced by IR, require further research to understand the pathophysiological mechanisms of pulmonary IR injury. The mechanisms causing increased platelet interaction with the endothelium which lead to pulmonary arteriolar constriction and decreased alveolar blood flow are unclear. The emphasis of this review is to hypothesize the relationship between inducible nitric oxide synthase (iNOS), reactive oxygen species (ROS), reactive nitrogen species (RNS), P-selectin, platelet–arteriolar wall rolling/adhesion, and microvascular constriction in the intact ventilated lung during pulmonary IR injury. In view of the importance of maintaining alveolar perfusion, elucidation of mechanisms of microvascular responses at the alveolar level will improve the effectiveness of therapy for IR injury (De Greef *et al.*, 1998; Idell, 2002).

Ischemia–reperfusion injury

Generally, during IR in systemic vascular beds, the vascular response appears to occur in at least two phases: (1) ischemia, which is associated with lack of oxygen, cell damage, and activation of cytotoxic enzymes, and (2) reperfusion, which is associated with formation of reactive oxygen intermediates, platelet and neutrophil activation, endothelial cell injury, increased vascular permeability, cytokine activity and complement activation (Grace, 1994; Zimmerman & Granger, 1994). During the ischemic phase of IR injury, when oxygen, ATP and other high-energy phosphates are depleted, conversion from aerobic to anaerobic cellular metabolism causes formation of cytotoxic metabolites (Chintala & Bernardino, 1994; Hamvas, 1992; Sakuma *et al.*, 1999). With re-establishment of perfusion, the injury elicited by reperfusion can be more severe than that caused by ischemia *per se* (Zimmerman & Granger, 1994). The dysfunction of ATP-dependent enzymes affects ion transport systems, causing accumulation of intracellular calcium. This cytosolic calcium leads to activation of several calcium-sensitive enzymes, such as phospholipases, with degradation of the membrane phospholipids (Steinle *et al.*, 1992).

IR causes oxidative stress characterized by the formation of superoxide anion, hydrogen peroxide and hydroxyl radicals, collectively known as ROS (Al-Mehdi *et al.*, 1997; Perrot *et al.*, 2003). It has been suggested that tissue injury mainly occurs during reperfusion (Parks & Granger, 1986). When oxygenated blood returns, it leads to the formation of ROS such as superoxide anion, hydrogen peroxide, and hypochlorous acid (Matata & Galinanes, 2002). The importance of oxygen radicals in the pathophysiological process of IR injury was demonstrated when injection of free radical scavengers or enzymes, such as superoxide dismutase, catalase or glutathione peroxidase, prevented damage occurring during reperfusion (Greef *et al.*, 1998; Mullane *et al.*, 1987; Oredsson *et al.*, 1994).

Pulmonary IR injury also results from the combined effects of ischemia and reperfusion (Zimmerman & Granger, 1994) and involves changes in cellular energy metabolism (Fisher *et al.*, 2004) as well as production of ROS. Amplification of the inflammatory response and adherence of activated platelets and neutrophils to damaged endothelial cells may cause pulmonary vascular dysfunction and obstruction (Colombat *et al.*, 2004). Although some investigators thought that ATP levels remain high during IR of the ventilated lung due to a high alveolar PO₂ (Fisher *et al.*, 1991), disturbances in energy metabolism during ischemia can promote the breakdown of ATP (Hoidal *et al.*, 2001). Hypoxic regions result in a breakdown of high energy phosphates such as ATP which triggers xanthine dehydrogenase to be converted to its O₂-dependent oxidase form in the vascular endothelium (Granger, 1988). Therefore, the pulmonary microcirculation is especially vulnerable to the damaging effects of ROS. A decrease in ATP leads to accumulation of xanthine and hypoxanthine, which are substrates for xanthine oxidase. In the ventilated lung, interaction with oxygen during ischemia and reperfusion can lead to massive generation of superoxide and peroxide,

which may react with iron and form hydroxyl radicals (Bartlett *et al.*, 1995). Cytochrome p450, found in mitochondria, can also generate superoxide and may be an important source of endothelial-derived superoxide in the lungs (Fleming *et al.*, 2001). Mitochondria are a significant source of oxidants in vascular tissue when apoptotic mechanisms are activated (Cadenas, 2004) and when electrons from the electron transport chain lead onto O₂ (Brookes, 2005). Furthermore, NO, which has protective effects during normal physiologic circumstances (Kubes, 1993), is greatly increased and is likely to have a predominant role during IR due to its high reactivity. A high flux of NO can act to inhibit cytochrome oxidase resulting in increased ROS production (Kadenbach, 2003), which can react with NO to form peroxynitrite (Wink & Mitchell, 1998). Nitric oxide synthase is a source of superoxide in endothelial cells (Bouloumie *et al.*, 1997) and can result in generation of superoxide if the reduction of molecular oxygen to L-arginine oxidation is uncoupled (Landmesser *et al.*, 2003). High levels of NO are generated via inducible nitric oxide synthase (iNOS) and have been associated with acute respiratory distress syndrome and IR (Grace, 1994) and related to lung injury (Numata *et al.*, 1998).

Pulmonary ischemic and reperfusion damage can be identified separately, and are not simply caused by endothelial cell dysfunction due to lack of oxygen (Messent *et al.*, 1993). This finding suggests that pulmonary IR injury, requires both ischemia and reperfusion (Pearse & Sylvester, 1996; Seibert *et al.*, 1993), and like IR in other vascular beds is a specific type of injury associated with oxidative stress (Williams *et al.*, 1999; Lases *et al.*, 2000). Pulmonary IR is different from IR in other systemic vascular beds, where oxygen is re-introduced during reperfusion. It was initially thought that the lung was resistant to ischemic injury because of its independent source of oxygen, available from the alveolar space (Deffebach *et al.*, 1987; Esme *et al.*, 2006). However, multiple studies have described that because of unique structure and function, the lung becomes a vulnerable target during IR injury (Adkins *et al.*, 1990; Esme *et al.*, 2006). Interestingly, accumulation of toxic metabolites from aerobic metabolism and free radical injury may occur when the lungs are inflated with oxygen (Haniuda *et al.*, 1995; Kuratani *et al.*, 1992; Watanabe *et al.*, 1997). Alveolar oxygen helps maintain aerobic metabolism and prevents hypoxic ROS formation (Eckenhoff *et al.*, 1992; Fisher & Dodia, 1981). However, the absence of blood flow with a low oxygen gradient in the lung tissue and a high concentration of oxygen in the gas mixture can cause ROS formation (Fisher *et al.*, 1991; Yang & Block, 1995). High inspired oxygen concentrations administered to the lungs during ventilation, amplify the formation of ROS in the form of oxygen free radicals and RNS (Gothard, 2006) at toxic levels causing the molecular and ultimately cellular damage.

As in systemic vascular beds, ROS such as superoxide have a key role in causing pulmonary IR injury (Demiryurek *et al.*, 1999; Wolin, 2000). ROS are produced by enzymes such as NADPH oxidase, xanthine oxidase, and nitric oxide synthase which are found in high concentrations in endothelial cells (Demiryurek *et al.*, 1999). During ischemia in the ventilated lung, NADPH oxidase in endothelial cells, vascular smooth muscle, and macrophages is a major source of ROS generation (Fisher, 2004). In addition, as part of an inflammatory response, NADPH oxidase in macrophages and neutrophils is a significant source of superoxide (Granger & Korthuis, 1995). The toxicity from oxygen metabolites released by stimulated platelets, neutrophils, macrophages, and other cells has been proposed as one of the significant mechanisms of lung injury (Lang *et al.*, 2002). It has been found that the levels of H₂O₂ in expired breath condensates is significantly greater in patients with acute hypoxemic respiratory failure and focal pulmonary infiltrates than in those without pulmonary infiltrates indirectly implicating increased oxidation during IR injury (Quinlan *et al.*, 1997; Lang *et al.*, 2002). Pulmonary IR induces systemic effects associated with liver and heart injury characterized by activated neutrophil sequestration and release of significant amounts of ROS (Esme *et al.*, 2006; McMillen *et al.*, 1993). Whereas

macrophages are activated during ischemia and early reperfusion, neutrophils are involved during a later phase of reperfusion injury (Naidu *et al.*, 2003b).

Pulmonary endothelial cells, smooth muscle cells, and lung macrophages generate superoxide under both basal and stimulated conditions (Al Mehdi *et al.*, 1998a; Barnard *et al.*, 1997; Jian *et al.*, 1995; Lansman, 1988; Yang & Block, 1995). The endothelial cell content of superoxide is modulated by mechanical forces such as physiological shear stress which was found to reduce superoxide (De Keulenaer *et al.*, 1998; Stenberg *et al.*, 1985). During ischemia in the lung, the absence of shear stress triggers endothelial cell membrane depolarization which leads to activation of NADPH oxidase, nuclear factor- κ B, and iNOS (Al Mehdi *et al.*, 1998a, 1998b). Mechanisms of ROS formation include accumulation of hypoxanthine and conversion of the enzyme xanthine dehydrogenase into xanthine oxidase during hypoxia, with the degradation of hypoxanthine into superoxide after reoxygenation (Fisher *et al.*, 1991). An NADPH oxidase-dependent mechanism forms ROS by reduction of oxygen to hydrogen peroxide and superoxide anion (Eckenhoff *et al.*, 1992). The predominant source of NADPH oxidase during the early phase of pulmonary IR is the endothelium (Al Mehdi *et al.*, 1998a). In addition, others presented evidence for the NADPH oxidase-dependent O_2^- release from platelets in response to stimulation (Krotz *et al.*, 2004), which could cause injury during early reperfusion (Figure 1). ROS have diverse actions on pulmonary tissue, including smooth muscle contraction, interaction with redox enzymes, cell proliferation, and gene transcription (Demiryörek & Wadsworth, 1999; Schreck & Baeuerle, 1994; Tate *et al.*, 1984). They produce endothelial cell injury by lipid peroxidation of their membranes (Al-Mehdi *et al.*, 1997; Wiklund *et al.*, 1996; White *et al.*, 1991), and downregulate NO released from the endothelium, thus compromising endothelium-dependent relaxation (Cherry *et al.*, 1990; Liu *et al.*, 1977). In addition, overproduction of superoxide antagonizes the effects of a wide range of NO donors by reacting with NO (Marczin *et al.*, 1992). Reactive oxygen metabolites can promote the formation of inflammatory agents by lipid peroxidation and subsequently activate and recruit leukocytes to post-ischemic tissue (Zimmerman & Granger, 1994).

Microvascular effects of pulmonary IR

Post-ischemic lung perfusion is characterized by progressive microvascular obstruction associated with formation of thrombi and vasoconstriction (Grace, 1994; Zimmerman & Granger, 1994). Hypoxia can induce endothelial cells and macrophages to develop procoagulant properties, which may contribute to the formation of microvascular thrombosis and compromise blood flow during reperfusion (Ogawa *et al.*, 1990). Clinical and experimental studies have shown that IR induces a rapid release of thrombin and proinflammatory cytokines such as tumor necrosis factor alpha (TNF α), interleukin 1beta (IL-1 β), IL-6, IL-9 and IL-10 which are potent cell activators and promoters of vasoactive mediators (Chauvet *et al.*, 1999; Oz *et al.*, 1995; Perrot *et al.*, 2002; Sekido *et al.*, 1993; Yadav *et al.*, 1999). Proinflammatory cytokines participate in IR injury of the heart, kidney, small bowel, skin, and liver, but little is known about their roles in pulmonary IR injury (Krishnadasan *et al.*, 2003). Cytokines modulate vascular smooth muscle tone and modify the inflammatory response by regulating the expression of adhesion molecules Barillari *et al.*, 2001; Grace, 1994; Koksoy *et al.*, 2000). This process leads to platelet–and leukocyte–endothelial adhesion and formation of obstructive microembolic debris in the capillary network (Ambrosio & Tritto, 1999; Nishijima *et al.*, 2001; Okada *et al.*, 1997; Roberts *et al.*, 2004). In addition, activated and adhered platelets and leukocytes can release mediators such as serotonin, thromboxane A₂, and platelet activating factor, which contribute to vasoconstriction and edema formation (Blann *et al.*, 2003; Fetkovska, 1992; Golino *et al.*, 1993) (Figure 1).

Role of leukocytes and platelets in pulmonary IR

Leukocyte–endothelial cell adhesion and activation is implicated in microvascular dysfunction associated with IR in different tissues (Butcher, 1991; Kubes *et al.*, 1995; Wickersham *et al.*, 1999), but their role in reperfusion injury is not yet clear (Greef *et al.*, 1998). Some studies demonstrated a role of leukocytes in the process of IR lung injury (Eppinger *et al.*, 1995a). Others, however (Bottiger *et al.*, 1996; Deeb *et al.*, 1990; Doerschuk, 2001; Lu *et al.*, 1997; Shimoaka *et al.*, 1996), observed tissue damage in the absence of leukocytes, and concluded that neutrophils did not participate in the damage during ischemia and reperfusion. For example, neutrophil depletion in rats failed to prevent or decrease lung injury that was caused by clamping the bronchus, pulmonary artery and vein for 90 or 180 min before reperfusion (Steimle *et al.*, 1992). On further analysis, it appears that there is a bimodal pattern of IR injury, consisting of an early neutrophil-independent and a later neutrophil-mediated increase in microvascular permeability (Eppinger *et al.*, 1995a, 1995b; Kuhnle *et al.*, 1998; Mulligan *et al.*, 1992).

During reperfusion, platelets have a very important role in post-ischemic systemic hypoperfusion (Massberg *et al.*, 1998; Okada *et al.*, 1997; Hampton *et al.*, 1967; Itoh *et al.*, 1998; Kroll *et al.*, 2003). Although platelet activation and accumulation are implicated in causing reperfusion injury in tissues such as intestine, liver, pancreas, brain, and kidney (Cooper *et al.*, 2003; Massberg *et al.*, 1998; Zimmerman & Granger, 1994), information is lacking about their response and involvement over the time-course of reperfusion in the ischemic lung (Eichorn *et al.*, 2002). Okada *et al.* (1997) found that accumulation of platelets in preserved and subsequently reperfused rat lungs, correlated with the degree of reperfusion injury in a lung transplant model. Platelet accumulation significantly correlated with capillary congestion, suggesting that platelets contributed to the lung injury, at least in part, by promoting hemostasis and forming microthrombi (Cooper *et al.*, 2003; Eichorn *et al.*, 2002; Khandoga *et al.*, 2003; Massberg *et al.*, 1999; Nishijima *et al.*, 2001). Platelets that adhere to post-ischemic vascular endothelium, cause tissue damage by releasing free radicals and inflammatory mediators, such as serotonin, leukotrienes and thromboxane A₂ (Piccardoni *et al.*, 1996) which are involved in post-ischemic leukocyte recruitment (Carvalho-Tavares *et al.*, 2000; Faint, 1992; Frenette *et al.*, 1998; Ruf & Patscheke, 1995). Platelets have been implicated early during the IR injury process, when formation of reactive oxygen radicals, endothelial disruption, and release of cytokines promote their activation and adhesion to the vascular wall (Massberg *et al.*, 1998, 1999; Itoh *et al.*, 1998). ROS can promote platelet activation and cause up-regulation of the adhesion molecule, P-selectin, which is rapidly expressed on the surface of platelets and endothelial cells (Carden & Granger, 2000; Cooper *et al.*, 2003; Minamino *et al.*, 1998) and can lead to platelet–endothelial adhesion during pulmonary IR (Roberts *et al.*, 2004). Platelet activation could mediate IR injury through their endothelial adhesion and formation of thrombi, as well as by interactions with leukocytes (Celi *et al.*, 1994; Kirton & Nash, 2000) (Figure 1).

Role of adhesion molecules in pulmonary IR

Selectins are responsible for initial adhesive interactions of platelets and leukocytes and integrins are involved in their subsequent firm adhesion to the endothelium and to each other (Blann *et al.*, 2003; Dardik *et al.*, 2002; Petruzzelli *et al.*, 1999; Schurmann, 1997). Platelet and leukocyte adhesion both involve the sequential events of activation, rolling, and firm adhesion. Rolling is mainly dependent on selectin-mediated interaction between endothelial cells (P-selectin and E-selectin), platelets (P-selectin) and leukocytes (L-selectin). Firm adherence and activation of leukocytes occurs when leukocyte β_1 -integrin or β_2 -integrin binds to endothelial cells expressing intercellular adhesion molecule-1 (ICAM-1), or vascular endothelial adhesion molecule-1, respectively (Jung & Ley, 1997; Khandoga *et al.*,

2002; Love & Barber, 2001; Perrot *et al.*, 2003). Blockade of adhesion molecules such as E-selectin, P-selectin, intercellular adhesion molecule-1, and CD18 can reduce lung reperfusion injury (Bombeli *et al.*, 1998; DeMeester *et al.*, 1996; Kapelanski *et al.*, 1993; Minamiya *et al.*, 1998; Moore *et al.*, 1995; Naka *et al.*, 1997). L-selectin blockade may also be beneficial for prevention of leukocyte adhesion after several hours of reperfusion when neutrophils have a predominant role (Demertzis *et al.*, 1999; Moore *et al.*, 1995; Steinberg *et al.*, 1994). When given before reperfusion, analogs of the oligosaccharides, potent ligands for selectin adhesion molecules, have also been shown to reduce IR injury (Perrot *et al.*, 2003; Reignier *et al.*, 1997; Schmid *et al.*, 1997; Stammberger *et al.*, 2000).

Over-expression of selectins is a major cause of platelet–endothelial cell adherence (Body, 1996; Celi *et al.*, 1997). P-selectin (CD62P, granule membrane protein 140 or platelet-dependent granule or external membrane protein) is the largest of the selectins with a mass of 140 kDa. It is constitutively expressed in platelet alpha granules (Stenberg *et al.*, 1985) and Weibel–Palade bodies of endothelial cells (Bonfanti *et al.*, 1989). P-selectin is a membrane protein with an N-terminal lectin domain followed by an epidermal growth factor motif, nine regulatory protein repeats, a transmembrane section and a short intracytoplasmic C-tail (Andre, 2004; Blann *et al.*, 2003; Johnston *et al.*, 1989). P-selectin is trans-located to the surface of platelets and endothelial cells within minutes when these cells are activated by stimuli such as thrombin, histamine, hydrogen peroxide or hypoxia (Blann *et al.*, 2003; Jang *et al.*, 1994; Reed *et al.*, 1999). As rapidly as two minutes after stimulation, P-selectin is expressed at the surface, but this expression can be short lived, reaching its peak after only 10 minutes and declining to baseline after 3 hours (Gotsch *et al.*, 1994; Kameda *et al.*, 1997; Khew-Goodall *et al.*, 1996). Additional synthesis of P-selectin is brought about within 2 hours by different stimuli such as cytokines, tumor necrosis factor- α , thrombin or oxygen radicals (Gotsch *et al.*, 1994; Khew-Goodall *et al.*, 1996; Weller *et al.*, 1992). Following platelet stimulation, P-selectin expressed on the platelet surface, becomes rapidly shed and forms the soluble fraction of plasma P-selectin (Michelson, 1998), which may have its own physiological activity (Andre, 2004; Michelson *et al.*, 1991). In contrast, P-selectin expressed on the surface of the endothelial cells does not contribute to the soluble P-selectin fraction (Hartwell *et al.*, 1998). Therefore, plasma P-selectin content is a sufficient indicator for platelet P-selectin expression and the level of tissue P-selectin is an indicator for the endothelial P-selectin fraction.

The primary ligand for P-selectin is P-selectin glycoprotein ligand (PSGL-1), a homodimeric mucin rich in O- and N-glycans consisting of two 120 kDa polypeptide chains (Vandendries *et al.*, 2004). It is constitutively expressed mainly on endothelial cell and leukocyte membranes. However, PSGL-1 is a non-specific ligand and also acts as a ligand for the other selectins (Yang *et al.*, 1999). From P-selectin and PSGL-1 interactions, multiple downstream signaling events are generated involving their cytoplasmic tails. These events include intra-cellular signaling with tyrosine phosphorylation of cytoplasmic proteins (Hidari *et al.*, 1997) that triggers a functional upregulation of the integrins (CD11b/CD18, Mac-1) which mediates firm adhesion of platelets and leukocytes (Andre, 2004).

Physiological intravascular shear stress that results in stimulation of endothelial cells and release of normal concentration of NO is an inhibitory regulator of P-selectin expression (Armstead *et al.*, 1997; Minamino *et al.*, 1998). In addition to platelet–endothelial interactions, P-selectin expression on activated platelets may also be an important factor in platelet aggregation (Merten & Thiagarajan, 2000; Merten *et al.*, 2000). In addition, it has been suggested that P-selectin plays a role in inflammation and atherogenesis by further activating the endothelium and regulating production of platelet activating factor by monocytes (Elstad *et al.*, 1995), and inducing the formation of tissue factor (Celi *et al.*, 1994; Furie & Furie, 1996).

Expression of P-selectin appears to be involved in myocardial IR injury, where it may cause early reperfusion-induced tissue damage attributed to neutrophil infiltration (Omata *et al.*, 1997). Cooper *et al.* (2003) found that IR induces time-dependent platelet–endothelial adhesion in postcapillary venules via a mechanism that involves both platelet and endothelial P-selectin, with platelet P-selectin playing a greater role. Recent studies suggest that platelets are important in IR injury, not only due to their adhesion and thrombi formation, but also through their role in inflammatory interactions with leukocytes (Kirtan & Nash, 2000). IR leads to an inflammation reaction by leukocyte infiltration and subsequent organ dysfunction (Hernandez *et al.*, 1987; Jaeschke *et al.*, 1990). Activated platelets contribute to leukocyte adhesion to the vascular wall enabling them to transfer to the surface of the endothelium (Hampton *et al.*, 1967; Theilmeier *et al.*, 1999). Moreover, Nishijima *et al.* (2004) demonstrated *in vivo* that platelet depletion suppresses leukocyte rolling and accumulation in post-ischemic tissues. They showed that platelets play a major role in the leukocyte recruitment after IR through expression of platelet P-selectin.

Role of nitric oxide in pulmonary IR

The superoxide anion (O_2^-) is central to ROS chemistry, because it may be converted into other physiologically relevant ROS by enzymatic or non-enzymatic reactions including formation of peroxynitrite (Krotz *et al.*, 2004). This may be especially important in pulmonary IR because of the exposure of alveolar microvessels to a high oxygen tension during ischemia. Nitric oxide synthase is a source of superoxide in endothelial cells and can result in generation of superoxide if there is uncoupling of the reduction of molecular oxygen to L-arginine oxidation (Landmesser *et al.*, 2003). It has been shown that ROS exert critical regulatory functions within the vascular wall and platelets, supporting a net prothrombotic effect of vascular-derived and platelet-derived ROS (Krotz *et al.*, 2004).

When NO reacts with ROS, it forms secondary reactive products such as nitrosonium cation (NO^+), nitroxil anion (NO^-) and peroxynitrite ($ONOO^-$) (Naidu *et al.*, 2003a; Stamper *et al.*, 1992) known as RNS (Demiryürek & Wadsworth, 1999; Moro *et al.*, 1994). Peroxynitrite, the most common form of RNS, has been suggested to be responsible for significant cytotoxic effects and platelet activation (Moro *et al.*, 1994). Reperfusion injury in skeletal muscle tissue (Oredsson *et al.*, 1991), and platelet activation *in vitro* (Porta *et al.*, 2000), can be prevented by superoxide free-radical scavengers (Seki *et al.*, 1999). Following intestinal ischemia, increased iNOS expression and NO overproduction caused pulmonary damage by nitrosylation of tyrosine residues (Virlos *et al.*, 2003). Peroxynitrite reacts with most biological molecules, making peroxynitrite, a selective oxidant. Peroxynitrite modifies tyrosine in proteins to create nitrotyrosine, which is a RNS footprint that has been detected *in vivo* in major pathological conditions including IR (Beckman & Koppenol, 1996; Nakazawa *et al.*, 2000). NO has become a well known signaling molecule critical to maintaining many physiological functions, including vascular tone. However, it has been shown that NO can be both protective (Bhabra *et al.*, 1997; Ishibe *et al.*, 1999; Weinberger *et al.*, 1999) and deleterious to vascular homeostasis (Kurose *et al.*, 1994; Radomski & Salas, 1995) through its direct effects on cell signaling, as well as indirect actions. The direct effects of NO are related to low and brief NO production. These effects are usually involved in protective mechanisms such as arterial vasodilatation, which leads to improved perfusion and oxygen delivery, and antithrombotic activity, principally by inhibiting platelet function under normal physiologic conditions. In contrast, indirect effects occur under high and sustained flux of NO under pathophysiological circumstances (Naidu *et al.*, 2003a). Overproduction of NO via the iNOS pathway is an important component in the pathogenesis of IR injury (Al-Majed *et al.*, 2003; Liaudet *et al.*, 2000; McDonald *et al.*, 2003; Zhou *et al.*, 2003). It has been shown that high flux of NO can act to inhibit cytochrome oxidase, and resulting in increased ROS production (Kadenbach, 2003).

During IR, excess NO production has been attributed to iNOS that is not stimulated under normal conditions, but can be induced within 2 h of lung reperfusion (Naidu *et al.*, 2003a) and results in upregulation of adhesion molecules (Cuzzocrea *et al.*, 2002). Administration of NO during ischemia in a systemic vascular bed was reported to be protective in IR (Okabayashi *et al.*, 1996), but administration of NO during reperfusion is associated with endothelial dysfunction and increased vascular permeability (Eppinger *et al.*, 1995b). These pathological effects could relate to the interaction of NO with ROS, since they are prevented by superoxide free radical scavengers (Oredsson *et al.*, 1991; Porta *et al.*, 2000). Physiological concentrations of NO inhibit platelet activation by downregulating platelet P-selectin (Loscalzo, 2001; Murohara *et al.*, 1995) through activation of platelet soluble guanylate cyclase and increasing levels of cGMP (Radomski & Moncada, 1993). A regulatory effect of NO on endothelial P-selectin expression that modulates leukocyte–endothelial cell interactions to preserve vascular homeostasis also has been shown (Armstead *et al.*, 1997).

NO is synthesized by NOS, a complex enzyme which acts on a pair of substrates (molecular oxygen and L-arginine) to produce NO and L-citrulline (Moncada *et al.*, 1991; Vural & Oz, 2000). This process requires five essential cofactors (FMN, FADH, NADPH, calmodulin and tetrahydrobiopterin) and two divalent cations: Ca^{2+} and heme Fe^{2+} . Three distinct isoforms of NOS have been identified: neuronal NOS (nNOS or NOS 1), endothelial NOS (eNOS or NOS 3), and inducible NOS (iNOS or NOS 2). All three known NOS isoforms are expressed in the lung, including nNOS and eNOS, which are usually expressed constitutively and are calcium-dependent. However, iNOS is calcium-independent and is expressed only when cells are stimulated (Forstermann *et al.*, 1994; Moncada & Higgs, 1993; Nathan, 2002). The iNOS has been implicated in the pathogenesis of IR injury, shock and inflammation (Al-Majed *et al.*, 2003; Carden & Granger, 2000; McDonald *et al.*, 2003; Zhou *et al.*, 2003). Following intestinal ischemia, Virlos *et al.* (2003) found that pulmonary damage is associated with increased iNOS expression, NO overproduction and consequent nitrosylation of protein tyrosine residues in the lung which leads to, and aggravates pulmonary injury. Under stress, when cellular L-arginine is depleted, NO synthase is capable of generating superoxide (Xia *et al.*, 1996). In addition, inhibition of physiological NO production leads to increased leukocyte (Kanwar & Kubes, 1995) and platelet (Horowitz *et al.*, 2000; Loscalzo, 2001) rolling and adhesion in various vascular beds with P-selectin playing a significant role in these processes (Kanwar & Kubes, 1995).

During IR, excess NO production has been attributed to iNOS that is not present under normal conditions, but can be induced within 2 h, reaching significant levels by 4 to 6 h of lung reperfusion (Moncada & Higgs, 1993; Nathan, 2002). The iNOS inhibitor aminoguanidine (Bryk & Wolff, 1998), as we showed previously (Roberts *et al.*, 2002), attenuates increased pulmonary vascular resistance during lung IR. The local release of large amounts of NO by iNOS is linked to the production of harmful oxidative products such as peroxynitrite, when NO reacts with other molecules such as superoxide (Buttery *et al.*, 1996; Luoma *et al.*, 1988). In experiments with “iNOS-knock out” mice Cuzzocrea *et al.* (2002) showed that the induction of iNOS and NO production are essential for the upregulation of the inflammatory response in IR. Both iNOS and NO participate in end-organ damage by upregulation of adhesion molecules, neutrophil infiltration, as well as oxidative and nitrosative stress. ROS, produced during IR, have also been implicated in a number of signal transduction pathways (Lander, 1997). Normal concentrations of NO act as an inhibitory regulator of P-selectin expression (Armstead *et al.*, 1997), and inhibition of physiological NO production leads to increased platelet and leukocyte rolling and adhesion in various vascular beds (Kanwar & Kubes, 1995; Loscalzo, 2001). In contrast, high and sustained flux of NO under pathophysiological circumstances leads to increased P-selectin expression (Armstead *et al.*, 1997; Naidu *et al.*, 2003a) which may promote platelet–vascular wall

interactions. The work from our laboratory showed that number of platelet–arterial wall interactions were highly correlated with the duration of lung ischemia and were associated with the levels of RNS and P-selectin strongly suggesting that the time of the ischemia is critical (Ovechkin *et al.*, 2005). Recently we observed iNOS dependent platelet–endothelial adhesion after 2 h of pulmonary ischemia during early reperfusion (2 h of reperfusion). However, leukocyte adhesion was not observed during the same time course of reperfusion. The present results show that postischemic platelet–endothelial and leukocyte–endothelial interactions in pulmonary arterioles of the intact ventilated lung correlate with the bimodal pattern of neutrophil involvement (Ovechkin *et al.*, 2005). Because of the great number of regulatory enzymatic sources that are potentially involved in platelet activation, many questions about the particular role of ROS, RNS and proinflammatory cytokines for platelet activation remain unclear. In particular, the specific significance of the different types of ROS with respect to platelet activity has not definitely been determined (Krotz *et al.*, 2004). The most common pharmacological approach to RNS has been attributed to the scavenging of the peroxynitrite anion and related ROS by molecules endowed with antioxidant activity. Many research lines are currently in progress to establish the true potential of RNS deactivators in the therapy including IR damage and lung injury (Olmos, 2006). It has been reported also that prevention of platelet and leukocyte activation appears to attenuate IR-induced lung injury (Roberts *et al.*, 2004; Wu *et al.*, 2006).

Summary

Pulmonary IR injury significantly contributes to the high mobility and mortality associated with numerous disease processes. Research in this area continues to provide a better understanding of the complex interactions involved with IR injury and how those interactions relate to pathophysiologic consequences. During the oxidative stress of ischemia and reperfusion, over-expression of iNOS and subsequent formation of RNS may promote platelet adhesion to the vascular wall with subsequent formation of microthrombi and release of vasoactive substances. Platelets play an important role in causing reperfusion injury in the systemic vasculature and consequences of their interactions with the arteriolar wall in the pulmonary microcirculation over the time course of lung reperfusion are becoming recognized. A mechanism causing increased platelet interaction with the endothelium could lead to pulmonary arteriolar constriction and decreased alveolar blood flow. Pulmonary IR induces platelet–arteriolar wall interactions via a P-selectin dependent mechanism and contributes to pulmonary microvascular constriction during reperfusion. Increased P-selectin expression may result from elevated iNOS activity and subsequent RNS generation (Figure 1).

Acknowledgments

American Lung Association of Kentucky, Community Trust Fund of Norton Health Care, American Heart Association, National Center, 0235317N, HL-71010, and HL-74185.

References

- Adkins WK, Taylor AE. Role of xanthine oxidase and neutrophils in ischemia–reperfusion injury in rabbit lung. *J Appl Physiol.* 1990; 69:2012–8. [PubMed: 2076995]
- Al Mehdi A, Zhao G, Dodia C, Tozawa K, Costa K, Muzykantov V, Ross C, Blecha F, Dinanuer M, Fisher A. Endothelial NADPH oxidase as the source of oxidants in lungs exposed to ischemia or high K⁺ *Circ Res.* 1998a; 83:730–7. [PubMed: 9758643]
- Al Mehdi A, Zhao G, Fisher A. ATP-independent membrane depolarization with ischemia in the oxygen-ventilated isolated rat lung. *Am J Respir Cell Mol Biol.* 1998b; 18:653–61. [PubMed: 9569235]

- Al-Majed A, Khattab M, Raza M, Al-Shabanah O, Mostafa A. Potentiation of diclofenac-induced anti-inflammatory response by aminoguanidine in carrageenan-induced acute inflammation in rats: The role of nitric oxide. *Inflamm Res*. 2003; 52(9):378–82. [PubMed: 14504665]
- Al-Mehdi A, Shuman H, Fisher A. Intracellular generation of reactive oxygen species during nonhypoxic lung ischemia. *Am J Physiol*. 1997; 272:L294–300. [PubMed: 9124381]
- Ambrosio G, Tritto I. Reperfusion injury: experimental evidence and clinical implications. *Am Heart J*. 1999; 138(2 pt 2):69–75. [PubMed: 10385767]
- Andre P. P-selectin in haemostasis. *Brit J Haematol*. 2004; 126:298–306. [PubMed: 15257701]
- Armstead VA, Minchenko AG, Schuhl RA, Hayward R, Nossuli TO, Lefer AM. Regulation of P-selectin expression in human endothelial cells by nitric oxide. *Am J Physiol*. 1997; 273(2 Pt 2):H740–6. [PubMed: 9277491]
- Barillari G, Albonici L, Incerpi S, Bogetto L, Pistrutto G, Volpi A, Ensoli B, Manzari V. Inflammatory cytokines stimulate vascular smooth muscle cells locomotion and growth by enhancing $\alpha_5\beta_1$ integrin expression and function. *Atherosclerosis*. 2001; 154(2):377–85. [PubMed: 11166770]
- Bartlett D, Church DF, Bounds PL, Koppenol WH. The kinetics of the oxidation of L-ascorbic acid by peroxynitrite. *Free Radic Biol Med*. 1995; 18(1):85–92. [PubMed: 7896175]
- Barnard M, Robertson B, Watts B, Turrens J. Role of nitric oxide and superoxide anion in spontaneous lung chemiluminescence. *Am J Physiol*. 1997; 272:L262–7. [PubMed: 9124377]
- Beckman J, Koppenol W. Nitric oxide, superoxide, and peroxynitrite: The good, the bad, and ugly. *Am J Physiol*. 1996; 271(5 Pt 1):C1424–37. [PubMed: 8944624]
- Bhabra M, Hopkinson D, Shaw T, Hooper T. Attenuation of lung graft reperfusion injury by a nitric oxide donor. *J Thorac Cardiovasc Surg*. 1997; 113(2):327–34. [PubMed: 9040627]
- Blann A, Nadar S, Lip G. The adhesion molecule P-selectin and cardiovascular disease. *European Heart Journal*. 2003; 24(24):2166–79. [PubMed: 14659768]
- Body S. Platelet activation and interactions with the microvasculature. *Journal of Cardiovascular Pharmacology*. 1996; 27(1):13–25.
- Bombeli T, Schwartz BR, Harlan JM. Adhesion of activated platelets to endothelial cells: evidence for a GPIIb/IIIa-dependent bridging mechanism and novel roles for endothelial intercellular adhesion molecule 1 (ICAM-1), $\alpha_3\beta_1$ integrin, and GPIIb/IIIa. *J Exp Med*. 1998; 187:329–39. [PubMed: 9449713]
- Bonfanti R, Furie BC, Furie B, Wagner DD. PADGEM (GMP140) is a component of Weibel–Palade bodies of human endothelial cells. *Blood*. 1989; 73:1109–12. [PubMed: 2467701]
- Bottiger B, Bohrer H, Boker T, Motsch J, Aulmann M, Martin E. Platelet factor 4 release in patients undergoing cardiopulmonary resuscitation—can reperfusion be impaired by platelet activation? *Acta Anaesthesiol Scandinavica*. 1996; 40:631–5.
- Bouloumie A, Bauersachs J, Linz W, Scholkens BA, Wiemer G, Fleming I, Busse R. Endothelial dysfunction coincides with an enhanced nitric oxide synthase expression and superoxide anion production. *Hypertension*. 1997; 30(4):934–41. [PubMed: 9336396]
- Brookes PS. Mitochondrial H(+) leak and ROS generation: An odd couple. *Free Radic Biol Med*. 2005 Jan 1; 38(1):12–23. [PubMed: 15589367]
- Bryk R, Wolff D. Mechanism of inducible nitric oxide synthase inactivation by aminoguanidine and L-N⁶-(1-iminoethyl) lysine. *Biochemistry*. 1998; 37(14):4844–52. [PubMed: 9538001]
- Butcher E. Leukocyte–endothelial cell recognition: Three (or more) steps to specificity and diversity. *Cell*. 1991; 77:1033–6. [PubMed: 1760836]
- Buttery L, Springall D, Chester A, Evans Y, Standfield E, Parums D, Yacoub M, Polak J. Inducible nitric oxide synthase is present within human atherosclerotic lesions and promotes the formation and activity of peroxynitrite. *Lab Invest*. 1996; 75:77–85. [PubMed: 8683942]
- Cadenas E. Mitochondrial free radical production and cell signaling. *Mol Aspects Med*. 2004; 25(1–2):17–26. [PubMed: 15051313]
- Carden DL, Granger DN. Pathophysiology of ischaemia–reperfusion injury. *J Pathol*. 2000; 190:255–66. [PubMed: 10685060]

- Carvalho-Tavares J, Hickey MJ, Hutchison J, Michaud J, Sutcliffe IT, Kubes P. A role for platelets and endothelial selectins in tumor necrosis factor- α -induced leukocyte recruitment in the brain microvasculature. *Circ Res*. 2000; 87:1141–8. [PubMed: 11110771]
- Celi A, Lorenzet R, Furie B, Furi B. Platelet–leukocyte–endothelial cell interaction on the blood vessel wall. *Seminars Hematol*. 1997; 34(4):327–35.
- Celi A, Pellegrini G, Lorenzet R, Blasi A, Ready N, Furie BC, Furie B. P-selectin induces the expression of tissue factor on monocytes. *Proc Natl Acad Sci USA*. 1994; 91(19):8767–71. [PubMed: 7522321]
- Chauvet P, Bienvenu J, Theoret J, Latour J, Merhi Y. Inhibition of platelet–neutrophil interactions by fucoidan reduces adhesion and vasoconstriction after acute arterial injury by angioplasty in pigs. *J Cardiovasc Pharm*. 1999; 34(4):597–603.
- Cherry P, Omar H, Farrell K, Stuart J, Wolin M. Superoxide anion inhibits cGMP-associated bovine pulmonary arterial relaxation. *Am J Physiol*. 1990; 259:H1056–62. [PubMed: 2171363]
- Chintala M, Bernardino V. Cyclic GMP but not cyclic AMP prevents renal platelet accumulation after ischemia–reperfusion in anesthetized rats. *J Pharm Experim Therap*. 1994; 271(3):1203–8.
- Colombat M, Castier Y, Leseche G, Rufat P, Mal H, Thabut G, Fournier M, Groussard O, Degott C, Couvelard A. Early expression of adhesion molecules after lung transplantation: evidence for a role of aggregated P-selectin-positive platelets in human primary graft failure. *J Heart Lung Transplant*. 2004; 23(9):1087–92. [PubMed: 15454176]
- Cooper D, Chitman KD, Williams MC, Granger DN. Time-dependent platelet–vessel wall interactions induced by intestinal ischemia–reperfusion. *Amer J Physiol Gastrointest Liver Physiol*. 2003; 284(6):G1027–33. [PubMed: 12736150]
- Cuzzocrea S, Chatterjee P, Mazzon E, Dugo L, Sarro A, Van de Loo F, Caputi A, Thiemermann C. Role of induced nitric oxide in the initiation of the inflammatory response after posts ischemic injury. *Shock*. 2002; 18(2):169–76. [PubMed: 12166782]
- Dardik R, Shenkman B, Tamarin I. Factor XIII mediates adhesion of platelets to endothelial cells through $\alpha(v)\beta(3)$ and glycoprotein IIb/IIIa integrins. *Thromb Res*. 2002; 105:317–23. [PubMed: 12031826]
- De Greef K, Ysebaert D, Ghielli M, Vercauteren S, Nouwen E, Eyskens E, De Broe M. Neutrophils and acute ischemia–reperfusion injury. *J Nephrol*. 1998; 11(3):110–22. [PubMed: 9650119]
- Deffebach ME, Charan NB, Lakshminarayan S, Butler J. The bronchial circulation. Small, but a vital attribute of the lung. *Am Rev Respir Dis*. 1987; 135:463–81. [PubMed: 3544986]
- De Keulenaer G, Chappell D, Ishizaka N, Nerem R, Alexander R, Griendling K. Oscillatory and steady laminar shear stress differentially affect human endothelial redox state: role of a superoxide-producing NADH oxidase. *Circ Res*. 1998; 82:1094–101. [PubMed: 9622162]
- Deeb GM, Grum CM, Lynch MJ, Guynn TP, Gallagher KP. Neutrophils are not necessary for induction of ischemia reperfusion lung injury. *J Appl Physiol*. 1990; 68:374–81. [PubMed: 2312480]
- DeMeester SR, Molinari MA, Shiraishi T, Okabayashi K, Manchester JK, Wick MR, Cooper JD, Patterson GA. Attenuation of rat lung isograft reperfusion injury with a combination of anti-ICAM-1 and anti- $\beta(2)$ integrin monoclonal antibodies. *Transplantation*. 1996; 62:1477–85. [PubMed: 8958275]
- Demertzis S, Langer F, Graeter T, Dwenger A, Georg T, Schafers H. Amelioration of lung reperfusion injury. *Eur J Cardiothorac Surg*. 1999; 16:174–80. [PubMed: 10485417]
- Demiryürek A, Wadsworth R. Superoxide in the pulmonary circulation. *Pharmacology & Therapeutics*. 1999; 84(3):355–65. [PubMed: 10665834]
- Doerschuk C. Mechanisms of leukocyte sequestration in inflamed lungs. *Microcirculation*. 2001; 8:71–88. [PubMed: 11379793]
- Eckenhoff R, Dodia C, Tan Z, Fisher A. Oxygen-dependent reperfusion injury in the isolated rat lung. *J Appl Physiol*. 1992; 72:1454–60. [PubMed: 1592738]
- Eichorn M, Ney L, Massberg S, Goeltz A. Platelet kinetics in the pulmonary microcirculation *in vivo* assessed by intravital microscopy. *J Vasc Res*. 2002; 39:330–9. [PubMed: 12187123]

- Elstad MR, La Pine TR, Cowley FS, McEver RP, McIntyre TM, Prescott SM, Zimmerman GA. P-selectin regulates platelet-activating factor synthesis and phagocytosis by monocytes. *J Immunol.* 1995; 155(4):2109–22. [PubMed: 7543539]
- Eppinger M, Jones M, Deeb G, Bolling S, Ward P. Pattern of injury and the role of neutrophils in reperfusion injury of rat lung. *J Surg Res.* 1995; 58:713–8. [PubMed: 7791351]
- Eppinger M, Ward P, Jones M, Bolling S, Deeb G. Disparate effects of nitric oxide on lung ischemia–reperfusion injury. *Ann Thorac Surg.* 1995; 60:1169–75. [PubMed: 8526595]
- Esme H, Fidan H, Koken T, Solak O. Effect of lung ischemia–reperfusion on oxidative stress parameters of remote tissues. *Eur J Cardiothorac Surg.* 2006; 29(3):294–8. [PubMed: 16439150]
- Faint RW. Platelet–neutrophil interactions: Their significance. *Blood Rev.* 1992; 6:83–91. [PubMed: 1633510]
- Fetkovska N. Platelet activation by low-density lipoprotein and serotonin: effects of calcium antagonists. *J Cardiovasc Pharmacol.* 1992; 19(3):25–8.
- Fisher AB. Reactive oxygen species and cell signaling with lung ischemia. *Undersea Hyperb Med.* 2004 Spring;31(1):97–103. [PubMed: 15233164]
- Fisher A, Dodia C, Tan Z, Ayene I, Eckenhoof R. Oxygen-dependent lipid peroxidation during lung ischemia. *J Clin Invest.* 1991; 88:674–9. [PubMed: 1864976]
- Fisher A, Dodia C. Lung as a model for evaluation of critical intracellular PO₂ and PCO. *Am J Physiol.* 1981; 241:E47–50. [PubMed: 7246767]
- Fleming I, Michaelis UR, Bredenkotter D, Fisslthaler B, Dehghani F, Brandes RP, Busse R. Endothelium-derived hyperpolarizing factor synthase (Cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries. *Circ Res.* 2001 Jan 19; 88(1):44–51. [PubMed: 11139472]
- Forstermann U, Closs E, Pollock J, Nakane M, Schwarz P, Gath I, Kleinert H. Nitric oxide synthase isozymes: Characterization, purification, molecular cloning, and functions. *Hypertension.* 1994; 23:1121–31. [PubMed: 7515853]
- Frenette P, Moyna C, Hartwell D, Lowe J, Hynes R, Wagner D. Platelet–endothelial interactions in inflamed mesenteric venules. *Blood.* 1998; 9(4):1318–24. [PubMed: 9454762]
- Fullerton D, Hahn A, Koike K, Banerjee A, Harken A. Intracellular mechanisms of pulmonary vasomotor dysfunction in acute lung injury caused by mesenteric ischemia–reperfusion. *Surgery.* 1993; 114(2):360–7. [PubMed: 8393598]
- Furie B, Furie B. P-selectin induction of tissue factor biosynthesis and expression. *Haemostasis.* 1996; 26(1):60–5. [PubMed: 8904175]
- Gilroy R, Bhatte M, Wickersham N, Pou N, Loyd J, Overholser K. Postischemic hypoperfusion during unilateral lung reperfusion in vivo. *Am Rev Respir Dis.* 1993; 147:276–87. [PubMed: 8430948]
- Golino P, Ambrosio G, Ragni M, Pascucci I, Triggiani M, Oriente A, McNatt J, Buja L, Condorelli M, Chiariello M, Willerson J. Short-term and long-term role of platelet activation factor as a mediator of *in vivo* platelet aggregation. *Circulation.* 1993; 88(3):1205–14. [PubMed: 8353882]
- Gothard J. Lung injury after thoracic surgery and one-lung ventilation. *Curr Opin Anaesthesiol.* 2006; 19(1):5–10. [PubMed: 16547427]
- Gotsch U, Jager U, Dominis M, Vestweber D. Expression of P-selectin on endothelial cells is upregulated by LPS and TNF-alpha in vivo. *Cell Adhes Commun.* 1994; 1:7–14. [PubMed: 7526954]
- Grace P. Ischaemia–reperfusion injury. *British J Surg.* 1994; 81:637–47.
- Granger DN. Role of xanthine oxidase and granulocytes in ischemia–reperfusion injury. *Am J Physiol.* 1988; 255:H1269–75. [PubMed: 3059826]
- Granger DN, Korthuis RJ. Physiologic mechanisms of postischemic tissue injury. *Annu Rev Physiol.* 1995; 57:311–32. [PubMed: 7778871]
- Greef K, Ysebaert D, Ghielli M, Vercauteren S, Nouwen E, Eyskens E, Broe M. Neutrophils and acute ischemia–reperfusion injury. *J Nephrol.* 1998; 3(11):110–22. [PubMed: 9650119]
- Hampton JR, Harrison AJ, Honour AJ, Mitchell JR. Platelet behavior and drugs used in cardiovascular disease. *Cardiovasc Res.* 1967; 1:101–6. [PubMed: 4964626]

- Hamvas A, Park C, Palazzo R, Liptay M, Cooper J, Schuster D. Modifying pulmonary ischemia–reperfusion injury by altering ventilatory strategies during ischemia. *J Appl Physiol.* 1992; 73(5): 2112–9. [PubMed: 1474092]
- Haniuda M, Dresler CM, Mizuta T, Cooper JD, Patterson GA. Free radical-mediated vascular injury in lungs preserved at moderate hypothermia. *Ann Thorac Surg.* 1995; 60:1376–81. [PubMed: 8526630]
- Hartwell DW, Mayadas TN, Berger G, Frenette PS, Rayburn H, Hynes RO, Wagner DD. Role of P-selectin cytoplasmic domain in granular targeting *in vivo* and in early inflammatory responses. *J Cell Biol.* 1998; 143:1129–41. [PubMed: 9817767]
- Hernandez LA, Grisham MB, Twohig B, Arfors KE, Harlan JM, Granger DN. Role of neutrophils in ischemia–reperfusion-induced microvascular injury. *Am J Physiol.* 1987; 253:699–703.
- Hidari KI, Weyrich AS, Zimmerman GA, McEver RP. Engagement of P-selectin glycoprotein ligand-1 enhances tyrosine phosphorylation and activates mitogen-activated protein kinases in human neutrophils. *J Biol Chem.* 1997; 272:28750–6. [PubMed: 9353345]
- Hoidal JR. Reactive oxygen species and cell signaling. *Am J Respir Cell Mol Biol.* 2001; 25(6):661–3. [PubMed: 11726388]
- Horowitz JD. Nitrovasodilators. In: Loscalzo, J.; Vita, JA., editors. *Nitric Oxide and the Cardiovascular System*. Totowa, NJ: Humana Press; 2000. p. 383–409.
- Idell S. Adult respiratory distress syndrome: Do selective anticoagulants help? *Am J Respir Med.* 2002; 1(6):383–91. [PubMed: 14720025]
- Ishibe Y, Liu R, Ueda M, Mori K, Miura N. Role of inhaled nitric oxide in ischaemia–reperfusion injury in the perfused rabbit lung. *Br J Anaesth.* 1999; 83(3):430–5. [PubMed: 10655914]
- Itoh Y, Tomita M, Tanahashi N, Takeda H, Yokoyama M, Fukuchi Y. Platelet adhesion to aortic endothelial cells *in vitro* after thrombin treatment: Observation with video-enhanced contrast microscopy. *Thrombosis Research.* 1998; 91:15–21. [PubMed: 9700848]
- Jaeschke H, Farhood A, Smith CW. Neutrophils contribute to ischemia/reperfusion injury in rat liver *in vivo*. *FASEB J.* 1990; 4:3355–9. [PubMed: 2253850]
- Jang Y, Lincoff AM, Plow EF, Topol E. Cell adhesion molecules in coronary artery disease. *J Am Coll Cardiol.* 1994; 24:1591–601. [PubMed: 7963103]
- Jian Z-J, Yang Z, Mason GL, Slauson DO, Bochsler PN. Regulation of superoxide anion generation in bovine alveolar macrophages by bacterial lipopolysaccharide, serum proteins, and modulators of signal transduction. *Inflammation.* 1995; 19:637–50. [PubMed: 8595931]
- Johnston G, Cook R, McEver R. Cloning of GMP-140, a granule membrane protein of platelets and endothelium: sequence similarity to proteins involved in cell adhesion and inflammation. *Cell.* 1989; 56:1033–44. [PubMed: 2466574]
- Jung U, Ley K. Regulation of E-selectin, P-selectin, and intercellular adhesion molecule 1 expression in mouse cremaster muscle vasculature. *Microcirculation.* 1997; 4(2):311–9. [PubMed: 9219223]
- Kadenbach B. Intrinsic and extrinsic uncoupling of oxidative phosphorylation. *Biochim Biophys Acta.* 2003 Jun 5; 1604(2):77–94. [PubMed: 12765765]
- Kameda H, Morita I, Handa M. Re-expression of functional P-selectin molecules on the endothelial cell surface by repeated stimulation with thrombin. *Br J Haematol.* 1997; 97:348–55. [PubMed: 9163601]
- Kanwar S, Kubes P. Nitric oxide is an antiadhesive molecule for leukocytes. *New Horiz.* 1995; 3(1): 93–104. [PubMed: 7704596]
- Kapelanski DP, Iguchi A, Niles SD, Mao HZ. Lung reperfusion injury is reduced by inhibiting a CD18-dependent mechanism. *J Heart Lung Transplant.* 1993; 12:294–306. [PubMed: 8097405]
- Khandoga A, Biberthaler P, Enders G, Axmann S, Hutter J, Messmer K, Krombach F. Platelet adhesion mediated by fibrinogen–intercellular adhesion molecule-1 binding induces tissue injury in the postischemic liver *in vivo*. *Transplantation.* 2002; 74:681–8. [PubMed: 12352886]
- Khandoga A, Biberthaler P, Messmer K, Krombach F. Platelet–endothelial cell interactions during hepatic ischemia–reperfusion *in vivo*: A systematic analysis. *Microvasc Res.* 2003; 65(2):71–7. [PubMed: 12686164]

- Khew-Goodall Y, Butcher CM, Litwin MS, Newlands S, Korpelainen EI, Noack LM, Berndt MC, Lopez AF, Gamble JR, Vadas MA. Chronic expression of P-selectin on endothelial cells stimulated by the T-cell cytokine, interleukin-3. *Blood*. 1996; 87(4):1432–8. [PubMed: 8608233]
- Kirton M, Nash GB. Activated platelets adherent to an intact endothelial cell monolayer bind flowing neutrophils and enable them to transfer to the endothelial surface. *J Lab Clin Med*. 2000; 136:303–13. [PubMed: 11039851]
- Koksoy C, Kuzu M, Ergun H, Demirpence E, Zulfikaroglu B. Intestinal ischemia and reperfusion impairs vasomotor functions of pulmonary vascular bed. *Ann Surg*. 2000; 231(1):105–11. [PubMed: 10636109]
- Krishnadasan B, Naidu B, Byrne K, Fraga C, Verrier E, Mulligan M. The role of proinflammatory cytokines in lung ischemia–reperfusion injury. *J Thorac Cardiovasc Surg*. 2003; 125(2):261–72. [PubMed: 12579094]
- Kroll, R.; Resendiz, J. Mechanisms of platelet activation. In: Joseph, L.; Andrew, IS., editors. *Thrombosis and Hemorrhage*. Baltimore: Williams and Wilkins Press; 2003. p. 187–205.
- Krotz F, Sohn HY, Pohl U. Reactive oxygen species: players in the platelet game. *Arterioscler Thromb Vasc Biol*. 2004; 24(11):1988–96. [PubMed: 15374851]
- Kubes P. Polymorphonuclear leukocyte–endothelium interactions: A role for proinflammatory and anti-inflammatory molecules. *Can J Physiol Pharmacol*. 1993; 71(1):88–97. [PubMed: 8513437]
- Kubes P, Jutila M, Payne D. Therapeutic potential of inhibiting leukocyte rolling in ischemia/reperfusion. *J Clin Invest*. 1995; 95:2510–9. [PubMed: 7539452]
- Kuhnle G, Reichenspurner H, Lange T, Wagner F, Groh J, Messmer K, Goetz A. Microchemodynamics and leukocyte sequestration after pulmonary ischemia and reperfusion in rabbits. *Thorac Cardiovasc Surg*. 1998; 115:937–44.
- Kuratani T, Matsuda H, Sawa Y, Kaneko M, Nakano S, Kawashima Y. Experimental study in a rabbit model of ischemia–reperfusion lung injury during cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. 1992; 103(3):564–8. [PubMed: 1545556]
- Kurose I, Wolf R, Grisham M, Granger D. Modulation of ischemia/reperfusion-induced microvascular dysfunction by nitric oxide. *Circ Res*. 1994; 74:376–82. [PubMed: 8118946]
- Lander H. Role of induced nitric oxide in the initiation of the inflammatory response after posts ischemic injury. An essential role for free radicals and derived species in signal transduction. *FASEB J*. 1997; 11:118–24. [PubMed: 9039953]
- Lang JD, McArdle PJ, O'Reilly PJ, Matalon S. Oxidant–antioxidant balance in acute lung injury. *Chest*. 2002; 122(6. Suppl):314S–20S. [PubMed: 12475808]
- Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest*. 2003; 111(8):1201–9. [PubMed: 12697739]
- Lases EC, Durkens VA, Gerritsen WB, Haas FJ. Oxidative stress after lung resection therapy: A pilot study. *Chest*. 2000 Apr; 117(4):999–1003. [PubMed: 10767230]
- Lansman J. Endothelial mechanosensors: Going with the flow. *Nature*. 1988; 331:481–2. [PubMed: 2448647]
- Liaudet L, Soriano F, Szabo C. Biology of nitric oxide signaling. *Crit Care Med*. 2000; 28:N37–52. [PubMed: 10807315]
- Liu J, Simon L, Phillips J, Robin E. Superoxide dismutase (SOD) activity in hypoxic mammalian systems. *J Appl Physiol*. 1977; 42:107–10. [PubMed: 833067]
- Loscalzo J. Nitric oxide insufficiency, platelet activation, and arterial thrombosis. *Circ Res*. 2001; 88:756. [PubMed: 11325866]
- Love S, Barber R. Expression of P-selectin and intracellular adhesion molecule-1 in human brain after focal infarction or cardiac arrest. *Neuropathology and Applied Neurobiology*. 2001; 27:465–73. [PubMed: 11903929]
- Lu Y, Hellewell P, Evans T. Ischemia–reperfusion lung injury: Contribution of ischemia, neutrophils, and hydrostatic pressure. *AJP Lung Cell Mol Physiol*. 1997; 273(17):L46–54.
- Luoma J, Stralin P, Marklund S, Hiltunen T, Sarkioja T, Yla-Hertuala S. Expression of extracellular SOD and iNOS in macrophages and smooth muscle cells in human and rabbit atherosclerotic

- lesions: Colocalization with epitopes characteristic of oxidized LDL and peroxynitrite-modified proteins. *Arterioscler Thromb Vasc Biol.* 1998; 18:157–67. [PubMed: 9484979]
- Marczin N, Ryan U, Catravas J. Methylene blue inhibits nitrovasodilator- and endothelium-derived relaxing factor-induced cyclic GMP accumulation in cultured pulmonary arterial smooth muscle cells via generation of superoxide anion. *J Pharmacol Exp Ther.* 1992; 263:170–9. [PubMed: 1328604]
- Massberg S, Enders G, Eisenmenger S, Leiderer R, Vestweber D, Krombach F, Messmer K. Platelet–endothelial cell interactions in the postischemic microvasculature. *Prog Appl Microcirc.* 1999; 23:66–81.
- Massberg S, Enders G, Leiderer R, Eisenmenger S, Vestweber D, Krombach F, Messmer K. Platelet–endothelial cell interactions during ischemia/reperfusion: The role of P-Selectin. *Blood.* 1998; 92(2):507–15. [PubMed: 9657750]
- Matata B, Galinanes M. Peroxynitrite is an essential component of cytokines production mechanism in human monocytes through modulation of nuclear factor-kappa B DNA binding activity. *J Biol Chem.* 2002; 277:2330–5. [PubMed: 11706022]
- McDonald M, Abdelrahman M, Cuzzocrea S, Thiernemann C. Tyrphostin reduces the organ injury in haemorrhagic shock: Role of inducible nitric oxide synthase. *Resuscitation.* 2003; 58(3):349–61. [PubMed: 12969613]
- McMillen MA, Huribal M, Sumpio B. Common pathway of endothelial–leukocyte interaction in shock, ischemia, and reperfusion. *Am J Surg.* 1993; 166:557–62. [PubMed: 8238752]
- Merten M, Thiagarajan P. P-selectin expression on platelets determines size and stability of platelet aggregates. *Circulation.* 2000; 102:1931–6. [PubMed: 11034941]
- Merten M, Chow T, Hellums D, Thiagarajan P. A new role for P-selectin in shear-induced platelet aggregation. *Circulation.* 2000; 102:2045–50. [PubMed: 11044418]
- Messent M, Griffiths J, Evans T. Pulmonary vascular reactivity and ischaemia reperfusion injury in the rat. *Clin Sci.* 1993; 85:71–5. [PubMed: 8149698]
- Michelson A, Ellis P, Barnard M, Matic G, Viles A, Kestin A. Downregulation of the platelet surface glycoprotein Ib-IX complex in whole blood stimulated by thrombin, adenosine diphosphate, or an in vivo wound. *Blood.* 1991; 77(4):770–9. [PubMed: 1704263]
- Michelson AD. Increased platelet reactivity and circulating monocyte–platelet aggregates in patients with stable coronary artery disease. *J Am Coll Cardiol.* 1998; 31:352–8. [PubMed: 9462579]
- Miller D, Roberts A. Pulmonary artery occlusion and reperfusion causes microvascular constriction in the rabbit lung. *Ann Thorac Surg.* 1999; 67:323–8. [PubMed: 10197648]
- Minamino T, Kitakaze M, Sanada S, Asanuma H, Kurotobi T, Koretsune Y, Fukunami M, Kuzuya T, Hoki N, Hori M. Increased expression of P-selectin on platelets is a risk factor for silent cerebral infarction in patients with atrial fibrillation: Role of nitric oxide. *Circulation.* 1998; 17:1721–7. [PubMed: 9788825]
- Minamiya Y, Tozawa K, Kitamura M, Saito S, Ogawa J. Platelet-activating factor mediates intercellular adhesion molecule-1-dependent radical production in the nonhypoxic ischemia rat lung. *Am J Respir Cell Mol Biol.* 1998; 19:150–7. [PubMed: 9651191]
- Moncada S, Higgs E. The L-arginine–nitric oxide pathway. *N Engl J Med.* 1993; 329:2002–12. [PubMed: 7504210]
- Moncada S, Palmer R, Higgs E. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev.* 1991; 43:109–42. [PubMed: 1852778]
- Moore TM, Khimenko P, Adkins WK, Miyasaka M, Taylor AE. Adhesion molecules contribute to ischemia and reperfusion-induced injury in the isolated rat lung. *J Appl Physiol.* 1995; 78:2245–52. [PubMed: 7665425]
- Moro M, Darley-Usmar V, Goodwin D, Read N, Zamora-Pino R, Feelisch M, Radomski M, Moncada S. Paradoxical fate and biological action of peroxynitrite on human platelets. *Proc Natl Acad Sci.* 1994; 91(14):6702–6. [PubMed: 7517561]
- Mullane KM, Salmon JA, Kraemer R. Leukocyte derived metabolites of arachidonic acid in ischemia induced myocardial injury. *Fed Proc.* 1987; 46:2422–33. [PubMed: 3106093]
- Mulligan M, Polley M, Bayer R, Nunn M, Paulson J, Ward P. Neutrophil dependent acute lung injury requirement for P-selectin (GMP-140). *J Clin Invest.* 1992; 90:1600–7. [PubMed: 1383277]

- Murohara T, Parkinson S, Waldman S, Lefer A. Inhibition of nitric oxide biosynthesis promotes P-selectin expression in platelets: Role of protein kinase C. *Arterioscl Thromb Vasc Biol.* 1995; 15:2068–75. [PubMed: 7583591]
- Naidu B, Fraga C, Salzman A, Szabo C, Verrier E, Mulligan M. Critical role of reactive nitrogen species in lung ischemia–reperfusion injury. *J Heart Lung Transplant.* 2003a; 22(7):784–93. [PubMed: 12873547]
- Naidu BV, Krishnadasan B, Farivar AS, Woolley SM, Thomas R, Van Rooijen N, Verrier ED, Mulligan MS. Early activation of the alveolar macrophage is critical to the development of lung ischemia–reperfusion injury. *J Thorac Cardiovasc Surg.* 2003b; 126(1):200–7. [PubMed: 12878956]
- Naka Y, Toda K, Kayano K, Oz M, Pinsky J. Failure to express the P-selectin gene or P-selectin blockade confers early pulmonary protection after lung ischemia or transplantation. *Proc Natl Acad Sci.* 1997; 94:757–61. [PubMed: 9012858]
- Nakazawa H, Fukuyama N, Takizawa S, Tsuji C, Yoshitake M, Ishida H. Nitrotyrosine formation and its role in various pathological conditions. *Free Radic Res.* 2000; 33(6):771–84. [PubMed: 11237099]
- Nathan C. Inducible nitric oxide synthase in the tuberculous human lung. *Am J Respir Crit Care Med.* 2002; 166(2):130–1. [PubMed: 12119220]
- Nishijima K, Kiryu J, Tsujikawa A, Honjo M, Nonaka A, Yamashiro K, Tanihara H, Tojo J, Ogura Y, Honda Y. In vivo evaluation of platelet–endothelial interactions after transient retinal ischemia. *Investig Ophthalmol Vis Sci.* 2001; 42:2102–9. [PubMed: 11481278]
- Nishijima K, Kiryu J, Tsujikawa A, Miyamoto K, Honjo M, Tanihara H, Nonaka A, Yamashiro K, Katsuta H, Miyahara S, Honda Y, Ogura Y. Platelets adhering to the vascular wall mediate postischemic leukocyte–cell interactions in retinal microcirculation. *Investig Ophthalmol Vis Sci.* 2004; 45:977–84. [PubMed: 14985320]
- Numata M, Suzuki S, Miyazawa N, Miyashita A, Nagashima Y, Inoue S, Kaneko T, Okubo T. Inhibition of inducible nitric oxide synthase prevents LPS-induced acute lung injury in dogs. *J Immunol.* 1998; 151:60(6):3031–7.
- Ogawa S, Gerlach H, Esposito C, Pasagian-Macaulay A, Brett J, Stern D. Hypoxia modulates the barrier and coagulant function of cultured bovine endothelium: increased monolayer permeability and induction of procoagulant properties. *J Clin Invest.* 1990; 85:1090–8. [PubMed: 2156893]
- Okabayashi K, Triantafyllou A, Yamashita M, Aoe M, DeMeester S, Cooper J, Patterson G. Inhaled nitric oxide improves lung allograft function after prolonged storage. *J Thorac Cardiovasc Surg.* 1996; 112:293–9. [PubMed: 8751493]
- Okada Y, Marchevsky A, Zuo X, Pass J, Kass R, Matloff J, Jordan S. Accumulation of platelets in rat syngeneic lung transplants. *Transplantation.* 1997; 64(6):801–6. [PubMed: 9326401]
- Olmos A, Giner RM, Manez S. Drugs modulating the biological effects of peroxynitrite and related nitrogen species. *Med Res Rev.* 2006 Jun.2..
- Omata M, Matsui N, Inomata N, Ohno T. Protective effects of polysaccharide fucoidin on myocardial ischemia–reperfusion injury in rats. *J Cardiovasc Pharm.* 1997; 30:717–24.
- Oredsson S, Plate G, Qvarfordt P. Experimental evaluation of oxygen free radical scavengers in the prevention of reperfusion injury in skeletal muscle. *Eur J Surg.* 1994; 160(2):97–103. [PubMed: 8193214]
- Oredsson S, Plate G, Qvarfordt P. Allopurinol—a free radical scavenger—reduces reperfusion injury in skeletal muscle. *Eur J Vasc Surg.* 1991; 160(2):97–103.
- Ovechkin A, Lominadze D, Sedoris K, Gozal E, Robinson T, Roberts A. Inhibition of inducible nitric oxide synthase attenuates platelet adhesion in subpleural arterioles caused by lung ischemia–reperfusion in rabbits. *J Appl Physiol.* 2005; 99:2423–32. [PubMed: 16037396]
- Oz MC, Liao H, Naka Y, Seldomridge A, Becker DN, Michler RE, Smith CR, Rose EA, Stern DM, Pinsky DJ. Ischemia-induced interleukin-8 release after human heart transplantation: A potential role for endothelial cells. *Circulation.* 1995; 92:II428–32. [PubMed: 7586450]
- Parks DA, Granger DN. Contributions of ischemia and reperfusion to mucosal lesion formation. *Am J Physiol.* 1986; 250:G749–53. [PubMed: 3717337]

- Pearse D, Sylvester J. Vascular injury in isolated sheep lungs. *Am J Respir Crit Care Med*. 1996; 153:196–202. [PubMed: 8542116]
- Perrot M, Liu M, Waddell T, Keshavjee S. Ischemia–reperfusion–induced lung injury. *Am J Respir Crit Care Med*. 2003; 167:490–511. [PubMed: 12588712]
- Perrot M, Sekine Y, Fischer S, Waddell TK, McRae K, Liu M, Wagle D, Keshavjee S. Interleukin-8 release during early reperfusion predicts graft function in human lung transplantation. *Am J Respir Crit Care Med*. 2002; 165:211–5. [PubMed: 11790657]
- Petruzzelli L, Takami M, Humes H. Structure and function of cell adhesion molecules. *Am J Med*. 1999; 106(4):467–76. [PubMed: 10225251]
- Piccardoni P, Evangelista V, Piccoli A, de Gaetano G, Walz A, Cerletti C. Thrombin activated human platelets release two NAP 2 variants that stimulate polymorphonuclear leukocytes. *Thromb Haemost*. 1996; 76:780–5. [PubMed: 8950790]
- Porta C, Maiolo A, Tua A, Grignani G. Amifostine, a reactive oxygen species scavenger with radiation- and chemo-protective properties, inhibits *in vitro* platelet activation induced by ADP, collagen or PAF. *Haematologica*. 2000; 85(8):820–5. [PubMed: 10942928]
- Quinlan GJ, Lamb NJ, Tilley R, Evans TW, Gutteridge JM. Plasma hypoxanthine levels in ARDS: Implications for oxidative stress, morbidity, and mortality. *Am J Respir Crit Care Med*. 1997; 155:479–84. [PubMed: 9032182]
- Radomski M, Moncada S. Regulation of vascular homeostasis by nitric oxide. *Thromb Haemost*. 1993; 70(1):36–41. [PubMed: 7694388]
- Radomski M, Salas E. Nitric oxide–biological mediator, modulator and factor of injury: Its role in the pathogenesis of atherosclerosis. *Atherosclerosis*. 1995; 118:S69–80. [PubMed: 8821467]
- Reed G, Hough A, Bianchi C. Comparative biochemical and ultrastructural studies of P-selectin in rabbit platelets. *Comp Biochem Physiol*. 1999; 119:729–38.
- Reignier J, Sellak H, Lemoine R, Lubineau A, Mazmanian G, Detruit H, Chapelier A, Herve P. Prevention of ischemia–reperfusion lung injury by sulfated Lewis(a) pentasaccharide: The Paris-Sud University Lung Transplantation Group. *J Appl Physiol*. 1997; 82:1058–63. [PubMed: 9104839]
- Roberts A, Ovechkin A, Collins K, Li R, Gozal E, Gozal D. Inhibition of inducible nitric oxide synthase attenuates increased pulmonary vascular resistance during lung ischemia–reperfusion in rabbits. *FASEB J*. 2002:A77.
- Roberts AM, Ovechkin AV, Mowbray JG, Robinson TW, Lominadze D. Effects of pulmonary ischemia–reperfusion on platelet adhesion in subpleural arterioles in rabbits. *Microvasc Res*. 2004; 67(1):29–37. [PubMed: 14709400]
- Ruf A, Patscheke H. Platelet-induced neutrophil activation: Platelet-expressed fibrinogen induces the oxidative burst in neutrophils by an interaction with CD11C/CD18. *Br J Haematol*. 1995; 90:791–6. [PubMed: 7669656]
- Sakuma T, Takahashi K, Ohya N, Kajikawa O, Martin T, Albertine K, Matthay M. Ischemia–reperfusion lung injury in rabbits: Mechanisms of injury and protection. *Am J Physiol Lung Cell Mol Physiol*. 1999; 276:L137–45.
- Schmid R, Yamashita M, Boasquevisque C, Ando K, Fujino S, Phillips L, Cooper J, Patterson G. Carbohydrate selectin inhibitor CY-1503 reduces neutrophil migration and reperfusion injury in canine pulmonary allografts. *J Heart Lung Transplant*. 1997; 16:1054–61. [PubMed: 9361248]
- Schreck R, Baeuerle PA. Assessing oxygen radicals as mediators in activation of inducible eukaryotic transcription factor NF-kappa B. *Methods Enzymol*. 1994; 234:151–63. [PubMed: 7808288]
- Schurmann G. Cell adhesion: Molecular principles and initial implications for surgery. *Chirurg*. 1997; 68(5):477–87. [PubMed: 9303836]
- Seibert A, Haynes J, Taylor A. Ischemia–reperfusion injury in the isolated rat lung. *Am Rev Respir Dis*. 1993; 147:270–5. [PubMed: 8381629]
- Seki S, Flavahan NA, Smedira NG, Murray PA. Superoxide anion scavengers restore NO-mediated pulmonary vasodilation after lung transplantation. *Am J Physiol*. 1999; 45:H42–6. [PubMed: 9887015]

- 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. [PubMed: 8413628]
- Shimoaka M, Ikeda M, Iida T, Taenaka N, Yoshiya I, Honda T. Fucoidin, a potent inhibitor of leukocyte rolling, prevents neutrophil influx into phorbol ester-induced inflammatory sites in rabbit lungs. *Am J Respir Crit Care Med*. 1996; 153:307–11. [PubMed: 8542135]
- Stammberger U, Hamacher J, Hillinger S, Schmid RA. sCR1sLe ameliorates ischemia/reperfusion injury in experimental lung transplantation. *J Thorac Cardiovasc Surg*. 2000; 120:1078–84. [PubMed: 11088029]
- Stamper J, Single D, Loscalzo J. Biochemistry of nitric oxide and its redox-activated forms. *Science*. 1992; 258:1898–902. [PubMed: 1281928]
- Steinle C, Guynn TP, Morganroth ML, Bolling SF, Carr K, Deeb GM. Neutrophils are not necessary for ischemia reperfusion lung injury. *Ann Thorac Surg*. 1992; 53:64–73. [PubMed: 1728243]
- Steinberg J, Mao H, Niles S, Jutila M, Kapelanski D. Survival in lung reperfusion injury is improved by an antibody that binds and inhibits L- and E-selectin. *J Heart Lung Transplant*. 1994; 13:306–18. [PubMed: 7518251]
- Stenberg PE, McEver RP, Shuman MA, Jacques YV, Bainton DF. A platelet alpha-granule membrane protein (GMP-140) is expressed on the plasma membrane after activation. *J Cell Biol*. 1985; 101:880–6. [PubMed: 2411738]
- Tate RM, Morris HG, Schroeder WR, Repine JE. Oxygen metabolites stimulate thromboxane production and vasoconstriction in isolated saline-perfused rabbit lungs. *J Clin Invest*. 1984; 74:608–13. [PubMed: 6547730]
- Theilmeier G, Lenaerts T, Remacle C, Collen D, Vermeylen J, Hoylaerts MF. Circulating activated platelets assist THP-1 monocytoid/endothelial cell interaction under shear stress. *Blood*. 1999; 94:2725–34. [PubMed: 10515876]
- Vandendries ER, Furie BC, Furie B. Role of P-selectin and PSGL-1 in coagulation and thrombosis. *Thromb Haemost*. 2004; 92(3):459–66. [PubMed: 15351841]
- Virlos IT, Inglott FS, Williamson RC, Mathie RT. Differential expression of pulmonary nitric oxide synthase isoforms after intestinal ischemia–reperfusion. *Hepatogastroenterol*. 2003; 50(49):31–6.
- Vural K, Oz M. Endothelial adhesivity, pulmonary hemodynamics and nitric oxide synthesis in ischemia–reperfusion. *Eur J Cardiothorac Surg*. 2000; 18:348–52. [PubMed: 10973546]
- Watanabe A, Kawaharada N, Kusajima K, Komatsu S, Abe T, Takahashi H. Influence of oxygen in inflation gas during lung ischemia on ischemia–reperfusion injury. *J Thorac Cardiovasc Surg*. 1997; 114(3):332–8. [PubMed: 9305184]
- Weinberger B, Heck D, Laskin D, Laskin J. Nitric oxide in the lung: Therapeutic and cellular mechanisms of action. *Pharmacol Ther*. 1999; 84(3):401–11. [PubMed: 10665837]
- Weller A, Isenmann S, Vestweber D. Cloning of the mouse endothelial selectins. Expression of both E-selectin and P-selectin is inducible by tumor necrosis factor alpha. *J Biol Chem*. 1992; 267:15176–83. [PubMed: 1378846]
- White CW, Avraham KB, Shanley PF, Groner Y. Transgenic mice with expression of elevated levels of copper–zinc superoxide dismutase in the lungs are resistant to pulmonary oxygen toxicity. *J Clin Invest*. 1991; 87:2162–8. [PubMed: 2040698]
- Wickersham N, Loyd J, Johnson J, McCain R, Christman J. Acute inflammation in a sheep model of unilateral lung ischemia: The role of interleukin-8 recruitment of polymorphonuclear leukocytes. *Am J Respir Cell Mol Biol*. 1999; 9:99–204.
- Wiklund L, McGregor CGA, Miller VM. Effects of prolonged exposure to oxygen-derived free radicals in canine pulmonary arteries. *Am J Physiol*. 1996; 270:H2184–90. [PubMed: 8764272]
- Williams EA, Quinlan GJ, Anning PB, Goldstraw P, Evans TW. Lung injury following pulmonary resection in the isolated, blood-perfused rat lung. *Eur Respir J*. 1999; 14:745–50. [PubMed: 10573215]
- Wink DA, Mitchell JB. Chemical biology of nitric oxide: Insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. *Free Radic Biol Med*. 1998 Sep; 25(4–5):434–56. [PubMed: 9741580]

- Wolin MS. Interactions of oxidants with vascular signaling systems. *Arterioscler Thromb Vasc Biol.* 2000 Jun; 20(6):1430–42. [PubMed: 10845855]
- Wu H, Ren B, Zhu J, Dong G, Xu B, Wang C, Zheng X, Jing H. Pretreatment with recombinant human erythropoietin attenuates ischemia–reperfusion-induced lung injury in rats. *Eur J Cardiothorac Surg.* 2006; 29(6):902–7. [PubMed: 16675226]
- Xia Y, Dawson V, Dawson T, Snyder S, Zweier J. Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. *Proc Natl Acad Sci.* 1996; 93:6770–4. [PubMed: 8692893]
- Yadav S, Howell D, Steeber D, Harland R, Tedder T, Clavien P. P-selectin mediates reperfusion through neutrophil and platelet sequestration in the warm ischemic mouse liver. *Hepatology.* 1999; 29(5):1494–501. [PubMed: 10216134]
- Yang J, Furie BC, Furie B. The biology of P-selectin glycoprotein ligand-1: Its role as a selectin counter-receptor in leukocyte-endothelial and leukocyte platelet interaction. *Thromb Haemost.* 1999; 81:1–7. [PubMed: 10348699]
- Yang W, Block E. Effect of hypoxia and regeneration on the formation and release of reactive oxygen species by porcine pulmonary artery cells. *J Cell Physiol.* 1995; 164:414–23. [PubMed: 7622587]
- Zhou J, Jin G, Yi Y, Zhang J, Huang X. Role of nitric oxide and peroxynitrite anion in lung injury induced by intestinal ischemia–reperfusion in rats. *World J Gastroenterol.* 2003; 9(6):1318–22. [PubMed: 12800248]
- Zimmerman B, Granger D. Mechanisms of reperfusion injury. *Am J Med Sci.* 1994; 307(4):284–92. [PubMed: 8160724]

Figure 1.

General concept of the early phase of pulmonary ischemia–reperfusion. In the oxidative stress induced by pulmonary ischemia–reperfusion (IR), formation of reactive nitrogen species (RNS) may promote platelet and endothelial cell activation with subsequent platelet adhesion to the arteriolar wall and release of vasoactive substances. We propose that pulmonary IR induces platelet–arteriolar wall interactions via a P-selectin-dependent mechanism and contributes to pulmonary microvascular constriction during reperfusion. Increased P-selectin expression results from elevated inducible nitric oxide synthase (iNOS) activity and subsequent over-production of nitric oxide (NO) and RNS generation.