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Reactive Species-Induced Microvascular Dysfunction in Ischemia/Reperfusion

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Abstract

Vascular endothelial cells line the inner surface of the entire cardiovascular system as a single layer and are involved in an impressive array of functions, ranging from the regulation of vascular tone in resistance arteries and arterioles, modulation of microvascular barrier function in capillaries and postcapillary venules, and control of proinflammatory and prothrombotic processes, which occur in all segments of the vascular tree but can be especially prominent in postcapillary venules. When tissues are subjected to ischemia/reperfusion (I/R), the endothelium of resistance arteries and arterioles, capillaries, and postcapillary venules become dysfunctional, resulting in impaired endothelium-dependent vasodilator and enhanced endothelium-dependent vasoconstrictor responses along with increased vulnerability to thrombus formation, enhanced fluid filtration and protein extravasation, and increased blood-to-interstitium trafficking of leukocytes in these functionally distinct segments of the microcirculation. The number of capillaries open to flow upon reperfusion also declines as a result of I/R, which impairs nutritive perfusion. All of these pathologic microvascular events involve the formation of reactive species (RS) derived from molecular oxygen and/or nitric oxide. In addition to these effects, I/R-induced RS activate NLRP3 inflammasomes, alter connexin/pannexin signaling, provoke mitochondrial fission, and cause release of microvesicles in endothelial cells, resulting in deranged function in arterioles, capillaries, and venules. It is now apparent that this microvascular dysfunction is an important determinant of the severity of injury sustained by parenchymal cells in ischemic tissues, as well as being predictive of clinical outcome after reperfusion therapy. On the other hand, RS production at signaling levels promotes ischemic angiogenesis, mediates flow-induced dilation in patients with coronary artery disease, and instigates the activation of cell survival programs by conditioning stimuli that render tissues resistant to the deleterious effects of prolonged I/R. These topics will be reviewed in this article.

Graphical Abstract

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Keywords

ischemia; reperfusion; reactive species; arterioles; capillaries; venules; endothelium; endothelium-dependent vasodilators; capillary no-reflow; leukocyte adhesion; endothelial permeability; inflammasome; connexins; pannexins; mitochondrial fission; microvesicles; angiogenesis; cell survival programs

INTRODUCTION

When blood flow to a tissue is decreased secondary to blockade of its arterial blood supply, cellular hypoxia ensues which induces metabolic disturbances, cell membrane permeability changes, alterations in ion channel function, and depletion of ATP (reviewed in 1-5). These changes are largely responsible for the cellular injury and organ dysfunction induced by ischemia, which can progress to frank necrosis unless the blood supply is rapidly re-established. However, the reintroduction of molecular oxygen to ischemic tissues by the inflow of reperfusion blood is not without peril because it fuels over-exuberant production of reactive species (RS) derived from molecular oxygen and/or nitric oxide that, if sufficiently large, overwhelms the ability of tissue defense systems to detoxify these toxic metabolites or to readily repair resulting damage. The effects of RS depend on the magnitude of their production. At low levels, RS have been shown to serve as signaling molecules that are involved in regulating normal tissue function. When produced at higher, but still moderate levels, cells may be able to overcome these relatively modest perturbations through repair mechanisms and restore function. However, when produced at high levels that overwhelm a cell's ability to detoxify or repair the ensuing damage in conditions such as I/R, RS directly affect the structure and function of important cellular molecules (eg, DNA, proteins, and lipids), thereby producing modifications in subcellular organelles such as the nucleus, mitochondria, endoplasmic reticulum, and plasma membrane. As a consequence, I/R-induced RS disrupt cellular calcium and hydrogen ion homeostasis and promote mitochondrial permeability transition, which can ultimately lead to cell death by a variety of mechanisms (1-5). In addition, pathologic pro-inflammatory changes are initiated by events occurring during ischemia that set the stage for activation of innate and adaptive immune systems during reperfusion, effects that are amplified by the oxidative stress induced by re-establishing the blood supply. While the postischemic injury response varies in accord with the diverse functional responses displayed by different organs, it is now clear that all segments of the microcirculation in any given tissue become dysfunctional in response to I/R

by mechanisms related in large part to RS generation. Moreover, these microvascular changes contribute to pathogenesis of tissue injury in I/R (1-5).

The microvasculature consists of arterioles, capillaries and postcapillary venules. Like all structures in the cardiovascular system, each microcirculatory segment is lined by a single layer of endothelial cells. In addition to endothelial cells, the walls of arterioles also contain a variable number of vascular smooth muscle layers, which vary by branching order. On the other hand, capillaries and postcapillary venules are not surrounded by vascular smooth muscle cells, although both are invested by pericytes at varying densities, as are arterioles.

Endothelial cells (EC) lining the microvasculature are particularly vulnerable to the deleterious effects of I/R, a susceptibility that is mediated by increased RS generation and a diminution in nitric oxide (NO) production by these cells (1-8). Characteristic endothelial changes that occur in response to I/R include disrupted cytoskeletal architecture, swelling, diminished production of certain bioactive agents (e.g., prostacyclin, NO) and accelerated formation of others (e.g., endothelin, Ang II, thromboxane A2, chemotactic mediators), and enhanced expression of some endothelial genes (e.g., adhesion molecules, cytokines) while suppressing others (e.g., eNOS, thrombomodulin) (1,2,4,5). Even though all endothelial cells in the microcirculation are exposed to the detrimental effects of I/R, the characteristics of their dysfunction are manifested in a site-specific manner within the functionally distinct segments of the microvasculature, as shown in Figure 1 and outlined below. The purpose of this review is to summarize the evidence implicating RS in the pathogenesis of I/R-induced dysfunction in microvessels comprising the microcirculation, ie, arterioles, capillaries, and postcapillary venules.

Overexuberant RS Production and Impaired Antioxidant Defenses Create Oxidative Stress in I/R

There are a large number of RS sources in I/R including xanthine oxidase, several NADPH oxidase (NOX) isoforms, uncoupled endothelial nitric oxide synthase, the mitochondria (via dysfunctional electron transport chain and activation of p66^{Shc}, NADPH oxidase 4 (NOX-4), monoamine oxidase), and infiltrating leukocytes (NOX-2, myeloperoxidase) (Figure 1, ref 1-5). Importantly, components of the electron transport chain generate reactive species in response to RS exposure, a phenomenon termed RS-induced RS release (RIRR) (9-14) (Figure 1). This self-amplifying process exacerbates oxidative stress in I/R and plays an important role in flow-induced vasodilation in arterioles in patients with coronary artery disease (15,16). Oxidative injury secondary to postischemic RS production by these sources occurs when the buffering capacity of RS scavengers or antioxidant defense systems (superoxide dismutases (SOD), catalase, glutathione peroxidase, peroxiredoxins, and thioredoxins) become defective in I/R (Figure 1). These RS sources and antioxidant defense mechanisms have been extensively reviewed elsewhere (1-5,17-26) and will not be covered here. However, several recent reports have identified a previously unrecognized RS source in I/R, vascular adhesion protein-1 (VAP-1, also known as semicarbazide-sensitive amine oxidase (SSAO), primary amine oxidase, or plasma amine oxidase, (EC 1.4.3.21))) (Figure 1). VAP-1 is an endothelial adhesion molecule that is also expressed on pericytes and acts

uniquely as an ectoenzyme to catalyze the oxidative deamination of primary amines. Tanaka et al (27) have shown that VAP-1 enhances neutrophil infiltration into postischemic kidneys by generating a local hydrogen peroxide gradient. Other studies conducted in VAP-1-deficient mice or in WT-mice treated with VAP-1 inhibitors have demonstrated an important role for this enzyme in postischemic neutrophil infiltration and injury after stroke, myocardial infarction, intestinal I/R and subsequent acute lung injury, by a mechanism dependent on VAP-1-generated hydrogen peroxide to induce selectin expression (28-33).

I/R-induced, RS-mediated Vasomotor Dysfunction in Resistance Vessels of the Microcirculation

Endothelial nitric oxide synthase (eNOS) is the major source of nitric oxide (NO) that mediates vasodilator responses to acetylcholine and increased shear stress in many vascular beds. This enzyme catalyzes the conversion of L-arginine to L-citrulline. It performs this function at the expense of NADPH, using tetrahydrobiopterin (BH₄), flavin adenine dinucleotide, flavin mononucleotide, and Ca²⁺/calmodulin as cofactors (34,35). In vessels exposed to I/R, electron transfer from eNOS flavins becomes uncoupled from L-arginine oxidation and superoxide is released from the oxygenase domain, a condition referred to as eNOS uncoupling (35). This occurs in endothelial cells exposed to H/R via ROS-mediated tetrahydrobiopterin depletion and eNOS S-glutathionylation (36) (Figure 2). Postischemic superoxide production can interact with NO to produce peroxynitrite, thereby eliminating NO-mediated vasodilation. The downstream target of NO, soluble guanylate cyclase, also becomes oxidized secondary to I/R, reducing the ability of NO to produce vasodilation (37-41) (Figure 2). Endothelial cells also produce endothelin, a very powerful direct vasoconstrictor that also provokes RS production, which reduces NO bioavailability, thereby decreasing the bioavailability of this gaseous signaling molecule (39). On the other hand, RS also activate the endothelin-1 promoter to increase synthesis of big endothelin-1 (42). ROS-induced changes in prostaglandin synthesis by peroxide tone of cyclooxygenases and nitration/inhibition of prostacyclin may also play a role in altered arterial responses (42).

A well-established consequence of I/R is altered responses of resistance arteries and arterioles to vasoconstrictor and endothelium-dependent vasodilator agonists (44-74) (Figure 2). Similar changes have been observed in larger conduit arteries, where superoxide and other RS play a major role the development of impaired endothelium-dependent vasodilation induced by I/R. Interestingly, neutrophils adhere to the endothelium of conduit arteries after I/R, an effect that is rarely observed or is much less pronounced in arterioles, even at very low shear rates (44,50,63-65,75-80) (Figure 2). Adherent neutrophils appear to contribute to postischemic impaired vasorelaxation to acetylcholine in conduit arteries because such responses are preserved in wild-type mice treated with reagents that prevent neutrophil adhesion and in mice genetically deficient in the expression of P-selectin, ICAM-1 or CD11/CD18 (50,51,57,59-65,70).

Impaired endothelium-dependent vasodilator responses also occur in resistance arteries and arterioles after I/R, an effect which is not explained by vascular smooth muscle dysfunction because these vessels respond appropriately to endothelium-independent vasodilators

(1,2,44,48,49,51,55,58,59,63-71,81). However, endothelium-dependent vasodilator responses are maintained in arterioles treated with superoxide scavengers or inhibitors of xanthine oxidase or NOX prior to I/R. These observations indicate that RS production contributes to postischemic deficits in endothelium-dependent vasorelaxation in arterioles by a mechanism that reduces NO bioavailability (Figure 2). Increased arginase activity, which reduces the availability of L-arginine required for NO synthesis by nitric oxide synthase, may also contribute to impaired endothelium-dependent vasodilation exhibited by coronary arterioles after I/R (58,82) (Figure 2). More recently, it was shown that I/R depletes endothelial NADPH secondary to CD38 (cyclic ADP ribose hydrolase) activation, the net effects of which are to impair eNOS function and limit BH₄ recycling, thereby uncoupling eNOS to favor superoxide generation and impairing endothelium-dependent vasorelaxation. (83-85) (Figure 2). Although the role of RS in postischemic CD38 activation was not directly tested in these studies, NADPH oxidase-derived hydrogen peroxide has been shown to activate this hydrolase in immune cells (86), suggesting a potential role for RS in I/R-induced, CD38-mediated eNOS uncoupling. In addition, NADPH oxidase derived oxidants activate xanthine oxidase in postischemic myocardium, an observation previously reported in aortic endothelial cells (87-89)

Oxidative stress invoked by I/R also contributes to impaired responses to the vasoconstrictors norepinephrine, angiotensin II and vasopressin (53-55,73) (Figure 2). On the other hand, hypoxia/reoxygenation (H/R), an ex vivo (for isolated microvessels) or in vitro (for cultured cells) surrogate for I/R, enhances the vasoconstrictor responses to angiotensin II by an RS-dependent mechanism in renal afferent arterioles (90). Paradoxical coronary vasoconstriction during ischemia has been shown to involve the release of the endothelial-derived peptide vasoconstrictor, endothelin-1 (91,92). Interestingly, the potent vasoconstrictor effects of this peptide are enhanced by its ability to induce RS generation, which decreases NO bioavailability (91,92) (Figure 2).

A number of proteases have been implicated in the pathogenesis of I/R-induced microvascular dysfunction and tissue injury. Of these, matrix metalloproteinases appear to be of particular importance. Expressed by a variety of cell types, including leukocytes and endothelial cells, MMPs are secreted as inactive zymogens that can be activated by proteolytic cleavage and by hypochlorous acid (HOCl), a potent RS formed by neutrophil-derived myeloperoxidase (MPO) (93,94). Through its action as an NO oxidase, neutrophil-derived myeloperoxidase (MPO) can reduce NO bioavailability, thereby contributing to impaired responsiveness to endothelium-dependent vasodilators (24,95). In addition, MPO-derived hypochlorous acid reduces endothelial NO production via superoxide-dependent reduction in nitric oxide synthase dimer stability (25). Importantly, treatment with the MMP inhibitor doxycycline improves postischemic endothelium-dependent vasodilator function by inhibiting oxidative stress and improving NO bioavailability (96).

Postischemic Capillary No-reflow: Role of I/R-induced RS Production

Over 50 years ago, it was first shown that some capillaries fail to reperfuse despite successful recanalization of feed arteries supplying blood flow to previously ischemic myocardium, brain and skeletal muscle (97-101). This postischemic impairment in nutritive

perfusion was subsequently shown to occur in most other organs and is referred to as capillary no-reflow (102-107). Capillary no-reflow appears to result from endothelial cell swelling and bleb formation, capillary luminal obstruction by leukocytes, fibrin, and platelet microaggregates, extravascular compression secondary to interstitial edema, parenchymal cell and astrocyte swelling, formation of neutrophil extracellular traps, and constriction of capillaries as a result of pericyte contraction (97-117) (Figure 3). I/R-induced, RS-dependent disruption of the capillary endothelial glycocalyx likely contributes to plugging of capillaries by neutrophils (118-119) (Figure 3). Indeed, most of the aforementioned factors contributing to capillary no-reflow have been linked directly or indirectly to postischemic RS formation (120-127). It is of interest to note that postschismic capillary no-reflow is reduced in transgenic mice overexpressing Cu/Zn-SOD, but is not decreased in wild-type mice treated with Cu/Zn-SOD or Mn-SOD by intravascular injection (128). This suggests that RS contribute to the development of this perfusion impairment after I/R and that the cellular localization of SOD activity is critical to its potential for protection.

RS-induced deficits in endothelium-dependent arteriolar vasodilator function and enhanced vasoconstrictor responsiveness (discussed above) induced by I/R may also contribute to the reduced nutritive capillary perfusion, as suggested by Granger and Kvietys (129). Pericytes surrounding cerebral capillaries are actively contracted during ischemia and remain contracted for several hrs after reperfusion is initiated, resulting in segmental constrictions along the length of these microvessels that restrict nutritive perfusion (126,130-133) (Figure 3). Similar results have been reported in postschismic heart (134,135). In the brain, postschismic pericyte contraction is mediated by RS and peroxynitrite, and perhaps ATP and thromboxane (125,136,137), while other studies implicate calpain-dependent cleavage of talin and RhoGTPase in modulating pericyte contractility (138,139). Pericyte Nox4 is upregulated by cerebral ischemia and may enhance blood brain barrier disruption via activation of NFkB and MMP-9 (140-142). Pro-nerve growth factor (precursor to NGF) is a cytokine that is upregulated after myocardial infarction that appears to target p75^{NTR} receptors on pericytes to induce endothelial cell activation, leukocyte transmigration, fibrin and platelet deposition, and microvascular thrombosis (143). Interestingly, pericytes, which play a key role in vascular and muscle regeneration, become dysfunctional secondary to activation of pro-RS-PKC β II-p66^{Shc} signaling in skeletal muscles of patients with diabetes and critical limb ischemia (144,145). Use of squalenoyl adenosine nanoparticles effectively reduces cerebral no-reflow, perhaps by a mechanism involving pericyte relaxation and prevention of glial endfeet swelling (146,147). Similar beneficial effects of adenosine on postschismic pericyte contraction and coronary no-reflow were reported by O'Farrell et al (135). While the notion that pericytes actively regulate capillary perfusion in normal and ischemic brain should be interpreted with caution (148,149), the concept is appealing since precise identification of structures corresponding to functional precapillary sphincters have been difficult.

RS-induced Capillary and Postcapillary Venular Endothelial Barrier Dysfunction in I/R

The formation of edema occurs rapidly following the onset of reperfusion as a result of I/R-induced disruption of the endothelial barrier and increased microvascular hydrostatic pressure secondary to arteriolar vasodilation. In many organs, the enhanced fluid (and protein) efflux is magnified by increased numbers of perfused capillaries, an effect which increases microvascular surface area available for exchange. Over time, the number of capillaries that are open to flow declines with the development of the no-reflow phenomenon, discussed above. These effects are relatively mild when the duration of ischemia is less than an hr and little tissue necrosis, inflammation, or damage to the microvascular barrier. However, when ischemic times exceed 2-6 hrs, the necrotic area expands in size, neutrophil infiltration becomes conspicuous, and microvascular damage can progress to permit frank hemorrhage (129). Interestingly, venular endothelial cells demonstrated an exaggerated inflammatory profile in response to oxidative stress, relative to that seen in arterial endothelium (150).

Edema formation and/or frank hemorrhage increases the diffusion distance for oxygen and nutrient delivery to the tissues, thereby reducing the transport capacity of the cardiovascular system as it struggles to supply the materials needed to re-establish ATP synthesis during reperfusion. As edema accumulates in tissues that cannot readily expand such as the kidneys (with their capsule investment), many skeletal muscles (with tight fascial sheaths), and the brain (encased in the cranial vault), interstitial fluid pressure rises precipitously and chokes off the blood supply by compressing those vessels with the lowest intravascular pressure, ie, capillaries and postcapillary venules, producing no-reflow (Figure 3). RS-dependent edema formation in the lung can arise as a direct consequence of pulmonary I/R, but can also result when distant organs are reperfused after prolonged ischemia (151,152). Pulmonary edema is a particularly devastating complication of increased microvascular permeability, especially if fluid accumulation progresses to alveolar flooding, owing to marked impairments in gas exchange.

Although capillary hydraulic conductance and permeability to macromolecules are increased by hypoxia or I/R, effects that are exacerbated by the presence of coexisting risk factors (153-158), postcapillary venules are thought to represent the major site of reperfusion-induced fluid and protein leakage (159-163). The structural components of the microvascular barrier that limit convective fluid flux and protein exchange are the glycocalyx coat on the endothelial cell surface, the tight junctions between adjacent endothelial cells and vesiculo-vesicular organelles that shuttle across the cytoplasm or form patent channels, and the basement membrane with its pericyte investment. Of these serial barriers to macromolecule flux, the endothelial glycocalyx may be the earliest structure to be compromised by I/R (164-169).

The endothelial surface layer facing the lumen of blood vessels is composed of the glycocalyx and soluble components derived from endothelial cells (such as xanthine oxidase and extracellular superoxide dismutase (ecSOD or SOD3)) and plasma proteins (eg, albumin, tissue factor pathway inhibitor, and anti-thrombin III) that adhere to this structure. The

glycocalyx is a delicate meshwork composed of membrane-bound proteoglycans, glycolipids, and sialic acid-containing glycoproteins that together with its bound components affects numerous functions of endothelial cells. For example, the dense molecular meshwork of heavily sulfated glycosaminoglycan chains in the glycocalyx offers steric hindrance to macromolecule movement, which present a negative charge barrier that limits transfer of anionic plasma proteins and thus fluid from the blood to the interstitial space (166,167,17-176). This surface layer also participates in sensing changes in the shear stress exerted by flowing blood on the vessel wall, thereby regulating vascular tone via modulation of endothelial nitric oxide synthase (eNOS) activity and NO formation (172). The glycocalyx can also bind proteins in the coagulation cascade as well as mask endothelial cell adhesion molecules, preventing their interaction with counterligands on circulating leukocytes and platelets, thereby regulating thrombogenicity and inflammation (118,166-168,177,178). Damage to this endothelial surface layer during I/R can thus contribute to arteriolar, capillary and postcapillary venular dysfunction.

Destruction of the glycocalyx in I/R is mediated by postischemic RS production secondary to activation of endothelial NADPH oxidase and xanthine oxidase bound to glycosaminoglycans in the endothelial surface layer (118,165-167,179-181). This leads to release of its component molecules in the circulation, which can be used as biomarkers for glycocalyx damage and ischemic injury (172). In addition to direct effects of RS to fragment the glycocalyx, reactive species can activate matrix metalloproteinases (MMPs) while incapacitating tissue inhibitors MMPs (TIMPs). Activation of MMPs (and other membrane bound proteases, glycosidases or lyases) secondary to I/R leads to cleavage of glycosaminoglycans in the glycocalyx and may involve G protein signaling (177,179,182-184). Crosslinking endothelial cell adhesion molecules induces Rac-1-dependent endothelial NADPH oxidase activation to promote MMP activation and disruption of interendothelial junctional complexes (180,185,186). This may involve activation of protein kinases and phosphatases to alter the balance of junctional protein phosphorylation status and integrity (187). Moreover, dissociation of glycocalyx-bound eSOD from the endothelium in postischemic tissues renders these cells more vulnerable to reactive species produced by adherent leukocytes, glycocalyx-bound and circulating xanthine oxidase, and other sources (22,188-191). Postischemic disruption of the glycocalyx can be prevented by ischemic preconditioning or adenosine A2 receptor agonist treatment, interventions that also abrogate I/R-induced RS production (192,193). Targeting antioxidants to determinants on the glycocalyx, endothelial cell adhesion molecules or endothelial caveolae decreases RS-induced endothelial dysfunction in I/R and other forms of oxidative stress (189,190,193-197). Similarly, binding therapeutic agents such as thrombomodulin to determinants on red blood cells reduces postischemic endothelial injury more effectively than non-bound agents by confining them to the intravascular space, prolonging their residence time in the circulation, and protecting them from degradation (198). These factors increase the bioavailability of therapeutic agents to the endothelium, enhancing their protective actions.

In an ischemic organ, release and activation of xanthine oxidase from the endothelial surface layer into the blood stream allows this circulating enzyme to produce RS-dependent injury in distant tissues remote from its site of origin (166,199,200). The ability of circulating

xanthine oxidase to extend RS-mediated injury to remote tissues appears to depend on the binding of the enzyme to the intact sulfated cell surface proteoglycans in the endothelial glycocalyx in distant organs. As a consequence, bound xanthine oxidase may then be concentrated several thousand fold on the endothelial cell surface at distant sites, followed by migration into the endothelium via endocytosis (201). Interestingly, the highly anionic nature of the glycocalyx also allows it to bind the highly basic MPO enzyme, which detaches the core glycocalyx protein syndecan-1, causing a reduction in thickness of this meshwork surface coat via shedding and physical collapse, through a mechanism independent of MPO's enzymatic activity (202). This not only contributes to microvascular barrier dysfunction, but may also facilitate the action of MPO to promote leukocyte adhesion by exposing adhesion molecules that were masked by the non-collapsed glycocalyx, as well as by attenuating the electrostatic repulsion between the negatively charged glycocalyxes of the leukocyte and endothelium (203). It is likely that RS produced secondary to the enzymatic activity of endothelial cell-bound MPO may also contribute to endothelial dysfunction.

Exposing cultured endothelial cells to H/R, an in vitro surrogate for I/R in vivo, results in NADPH oxidase-dependent disruption of tight and adherens junctions, cytoskeletal rearrangements and increased cellular tension (stiffening), the net effects of which are to induce endothelial gap formation and increase monolayer albumin permeation (204-212). Because white cells were absent in these in vitro models, these observations suggest that H/R induces RS-dependent disruption of endothelial barrier integrity secondary to activation of NADPH oxidase expressed by the endothelium. In contrast to these in vitro results, RS generation by xanthine oxidase or mitochondria in microvessels appear to play a dominant role in postischemic endothelial barrier disruption in vivo (153,213-216).

Neutrophils also appear to contribute to I/R-induced microvascular permeability changes by MMP and Nox-2/RS-dependent mechanisms (1,2,217-221). Pericytes surrounding cerebral microvessels contribute to blood-brain-barrier disruption after I/R by a mechanism involving RS produced Nox4 (141,222). Mast cells are activated by I/R and induce neutrophil infiltration and microvascular barrier disruption by a mechanism that may involve chymase-induced, angiotensin II-dependent NOX activation as well as by release a host of mediators that influence endothelial barrier function (223).

It is of interest to note that while platelet activation is invoked by I/R and contributes to a prothrombotic phenotype, platelets may also limit postischemic increases in microvascular permeability. This may be due in part to adherent platelets covering endothelial gaps, but also to release of soluble factors (eg, adenosine nucleotides, angiopoietin 1, sphingosine-1-phosphate) that enhance microvascular barrier function (221). However, I/R also induces the formation of platelet-neutrophil aggregates that increase pulmonary vascular permeability in mice with sickle cell disease, a disorder in which erythrocytes adopt a crescent shaped (sickled) and become stiff and sticky, causing them to lodge in microvessels and produce ischemia (224). Neutrophils in these heterotypic aggregates produce superoxide at enhanced rates compared to with platelet-free neutrophils, while aggregated platelets release substantially more PAF, which may explain their effect to increase pulmonary permeability (221). Indeed, the recurring appearance of sickle cell crises

is related to repeated polymerization/depolymerization of sickle hemoglobin, which can lead to a cyclic cascade of RS generation, blood cell adhesion, hemolysis, and vaso-occlusion (225,226).

Postischemic Oxidative Stress Promotes Interactions of Blood Cells with Postcapillary Venules

I/R, as well as inflammatory conditions that predispose individuals to cardiovascular disease and enhance the risk for adverse events such as myocardial infarction and stroke (eg; hypercholesterolemia, hypertension, smoking, diabetes/obesity/metabolic syndrome), are associated with increased interactions between blood cells (leukocytes and platelets) and endothelial cells. As noted above, each of these conditions are associated with impaired vessel (especially the endothelium) function in all segments of the microcirculation. However, blood cell-endothelial cell interactions occur primarily in postcapillary venules in these proinflammatory states. Importantly, I/R induces homotypic and heterotypic interactions amongst leukocytes, platelets and postcapillary venules that are enhanced by co-existing risk factors (reviewed in 1,2,220,221,227,228).

The adhesion of circulating leukocytes to postcapillary venular endothelium and subsequent migration into the tissue occurs by a complex, highly dynamic, and tightly regulated process that involve specific ligand/receptor interactions between immunocytes and the endothelium. In addition to leukocyte-endothelial cell interactions, I/R is also associated with platelet adhesion to the endothelium, as well as the formation of platelet/platelet and platelet/leukocyte aggregates (1,2,220,221,227,228). These adhesive interactions induce a prothrombotic phenotype in postischemic tissues that can lead to a perfusion impairment during reperfusion secondary to vessel occlusion, as discussed above.

The fact that leukocyte adhesion and emigration induced by I/R are attenuated by treatment with RS scavengers or antioxidant enzymes (eg, superoxide dismutase (SOD) or cell permeant SOD mimetics) and are limited in transgenic mice overexpressing antioxidant enzymes (eg, CuZn SOD overexpressors) provides strong support for the concept that postischemic RS generation is critical to the recruitment of leukocytes to ischemic sites (1,2,220,221,227,228). It is now clear that postischemic neutrophil infiltration occurs in two phases, both of which require RS generation (229,230). The first phase is immediate and depends on pre-existing adhesion molecules that become activated and cluster to support adhesive interactions or are mobilized from preformed intracellular pools to the cell surface. The second phase, which also provokes T-lymphocyte adhesion, arises 1-4 hrs later and is dependent on transcription-dependent gene expression of new adhesion molecules (229,230). Hydrogen peroxide generated by the enzymatic action of xanthine oxidase, as well as RS-dependent formation of PAF and LTB₄, drives adhesion in the early phase while RS generation in the second phase is derived from the adherent and emigrating leukocytes, which invokes NF- κ B-dependent adhesion molecule expression (229-232). Moreover, leukocytes require a functional gp91phox to effectively infiltrate tissues in the presence of risk factors for cardiovascular disease and in I/R (233-236). I/R also reduces eNOS activity and the bioavailability of its anti-adhesive product NO, which also contributes to enhanced

postischemic leukocyte adhesion and emigration (1,2,220,221,227). NOS uncoupling also occurs in I/R and has been implicated in postischemic RS generation (18) and could play a role in I/R-induced adhesion molecule expression and leukocyte-endothelial cell adhesive interactions. Since NO can serve as a brake on xanthine oxidase activity (237), the postischemic decrease in NO bioavailability may enhance xanthine oxidase-driven leukocyte adhesion.

RS-activated NLRP3 Inflammasome Activation in I/R

An emerging body of evidence supports a role for RS-mediated activation of inflammasomes, particularly the nucleotide-binding domain oligomerization domain-like receptor containing pyrin domain 3 (NLRP3) inflammasome, in sterile inflammatory conditions such as I/R (refers to inflammation that occurs in the absence of bacterial contamination, and can result from products released from dead cells, non-penetrating trauma, immunogenic antigens, and autoimmune conditions, to name just a few). In I/R, release of ATP and/or mitochondrial DNA following mitochondrial permeability transition pore opening and/or rupture of mitochondrial membranes serve as strong danger signals that initiate sterile inflammation. RS-induced NLRP3 activation appears to play a critical role in the maturation of inflammatory cytokines as well as the activation of pyroptosis, an inflammatory form of programmed cell death (238,239). This may occur by several mechanisms. For example, dysfunctional mitochondria participate secondary to their excessive RS production in I/R and exposure of mitochondrial cardiolipin, both of which serve as potent triggers for NLRP activation (239-243). Postischemic RS production by mitochondria also induces detachment of thioredoxin from the potent NLRP3 activator thioredoxin interacting protein (TXNIP) in microvascular endothelial cells (244,245). Agents that target postischemic RS production, NLRP3 activation, TXNIP/NLRP3 signaling reduce postischemic cytokine production, neutrophil infiltration, endothelial barrier dysfunction and cell death (242,249-249). It is of interest to note that once activated by danger signals released in postischemic tissues, NLRP3 forms an inflammasome composed of apoptosis-associated Speck-like protein containing a caspase activation and recruitment domain (ASC), which recruits and activates caspase-1 (250). Although NLRP3-deficient mice exhibited less inflammation and injury after hepatic I/R, animals lacking ASC or caspase-1 were not protected, suggesting that NLRP3 plays an inflammasome-independent role in the development of postischemic liver injury (251).

Microvascular Connexins And Pannexins, RS, and I/R

Connexins (Cx) and pannexins (Panx) form channels between adjacent vascular cells that allow intercellular communication. In the microcirculation, connexin channels play a key role in conducted vasomotor responses in arterioles, modulate inflammatory responses in postcapillary venules, and mediate crosstalk between inflammation and thrombogenesis (252-258). Opening connexin hemichannels occurs in cerebral I/R where they play a role in excitotoxicity, inflammation, blood-brain-barrier disruption, and neuronal death (258,259). Connexin hemichannels also provide a route for release of intracellular ATP and UTP to the extracellular compartment from postischemic cells, where these nucleotides serve as important danger signals to activate the innate immune system and disrupt the blood-brain-

barrier (259). Endothelial Panx1 appears to play a key role to modulate the severity of ischemic stroke by promoting leukocyte infiltration and inhibiting myogenic arterial tone (260). This pannexin also mediates increased vascular permeability and edema, release of endothelial cell-derived ATP and leukocyte infiltration, and lung dysfunction induced by pulmonary I/R (261). I/R also disrupts conducted vasomotor responses in arterioles (262). Although a role for postischemic RS in the aforementioned effects of I/R has not been tested, hypoxia/reoxygenation reduced electrical coupling between wild-type, but not Cx40-deficient microvascular endothelial cells. The H/R-induced reductions in cell coupling noted in wild-type endothelial cells was associated with enhanced RS production and reduced PKA activity, effects that were prevented by antioxidant treatment (263). On the other hand, treatment with a cell-permeant analog of cAMP prevented the reduction in coupling. PKA inhibition in normoxic cells mimicked the effects of H/R to reduce endothelial electrical coupling (263). When tested in vivo, I/R reduced conducted vasoconstrictor responses to upstream arterioles elicited by local application of KCl at focal downstream sites and this effect was abolished by 8-Br-cAMP. In a subsequent study, it was shown that enhanced RS production by endothelial cells exposed to H/R requires Cx40, independent of its role in gap junctional communication (264). Interestingly, these investigators also showed that H/R reduced electrical coupling in wild type cells and cells deficient in gp91phox, but not in p47phox knockout cells, suggesting a role for gp91phox rather than RS derived from NADPH oxidase in modulating cell coupling, ie, direct crosstalk between Cx40 and NADPH oxidase.

I/R-induced, RS-dependent Mitochondrial Fission in Endothelial Cells

Simulated I/R in vitro, induced by exposing HUVEC to hypoxia followed by oxygenated flow at shear stress of 10 dyn/cm², results in large increases in mitochondrial RS production, dynamin-related guanosine triphosphatase protein 1 (Drp1) activation, and significant mitochondrial fission compared to hypoxia followed by reoxygenation with static flow (265). Because these effects were attenuated by treatment with an antioxidant or inhibitors of NO synthase, succinate dehydrogenase, or mitochondrial division, it was suggested that shear-induced NO, RS, and Drp1 activation are essential for mitochondrial fission in endothelial cells exposed to simulated I/R. Since Drp1 activation and excessive mitochondrial fission are required for apoptotic mitochondrial fission and intrinsic apoptosis, it is possible that these processes contribute to endothelial dysfunction, cell fragility, and/or death in postischemic tissues (Figure 4). Two recent studies support this concept (266,267). In the first, I/R was shown to cause upregulation of mitochondrial fission factor (Mff), a receptor for Drp1 (266). This was associated with increased mitochondrial fission (and reduced fusion), which in turn promoted oligomerization of voltage-dependent anion channel 1 (VDAC1), which lowers the affinity of VDAC1 for hexokinase 2 (HK2). As a consequence, HK2 separates from the outer mitochondrial membrane, resulting in the opening of mitochondrial permeability transition pores (mPTP). Enhanced mitochondrial fission was also associated with mitochondrial RS production, resulting in cardiolipin peroxidation and cytochrome c release. Opening of mPTP and cytochrome c release activate mitochondrial-dependent apoptosis pathways in coronary microvascular endothelial cells to precipitate microvascular perfusion defects, reduced eNOS activation, and endothelial

barrier disruption (Figure 4). The second study examined events upstream of Mff activation in coronary microvascular endothelium by I/R (267). It was demonstrated that I/R increased the expression of nuclear receptor subfamily 4 group A member 1 (NR4A1), which in turn activated serine/threonine kinase casein kinase2 α (CK2 α) to phosphorylate and activate Mff. Activated Mff enhanced translocation of cytosolic Drp1 to mitochondria where it activates RS production and cytochrome c release, mPTP opening, and endothelial cell apoptosis, the net effect of which was to increase endothelial permeability, reduce microvascular perfusion, and limit eNOS activation. Later work implicated I/R-induced reductions in Bax inhibitor 1 (BI1) expression in postischemic mitochondrial fission (268) (Figure 4). Downregulation of BI1 activates xanthine oxidase-dependent RS production to promote F-actin depolymerization and mitochondrial fission. On the other hand, overexpression of BI1 inactivates this xanthine oxidase/RS/F-actin pathway and suppresses mitochondrial fission, thereby preserving microvascular endothelial structure and function (268). A very recent study has shown that the actin binding protein filamin A (FLNa) functions as a scaffold to couple Drp1 with actin at fission sites where it acts as a guanine nucleotide exchange factor to activate Drp1 and enhance mitochondrial fission under hypoxic conditions (269) (Figure 4).

Endothelial Microvesicle/Microparticle-Derived RS and I/R

Microvesicles (sometimes referred to as microparticles or exosomes) released by human umbilical vein endothelial cells (HUVEC) exposed to H/R promote apoptosis and oxidative stress in cardiac myocytes (270). Other studies from this same group showed that endothelial microvesicles derived from H/R-treated HUVECs also impaired endothelium-dependent vasodilator responses to acetylcholine, an effect that was associated with reduced eNOS phosphorylation and NO formation (271). Vesicle crosstalk between the myocardium and immune cells following myocardial infarction has also been demonstrated (273,273). In these studies, coronary artery ligation produced an increase in myocardial extracellular vesicles, which upon exposure to monocytes in vitro, induced the release of proinflammatory cytokines (IL-6, CCL2, CCL7). Recent work reported by Hervera et al (274) suggests that exosomes derived from inflammatory cells deliver RS to promote axon regeneration in injured neurons. Interestingly, treating human brain microvascular endothelial cells with extracellular vesicles derived from serum-starved endothelial progenitor cells (EPCs) abrogated H/R-induced RS production and apoptosis whereas extracellular vesicles prepared from EPCs exposed to TNF α exacerbated these responses (275).

RS Signaling is Required for Ischemic Angiogenesis, Flow-mediated Dilation in Patients with Coronary Artery Disease, and for Conditioning-activated Cell Survival Programs in I/R

Although data presented in the aforementioned references support a role for oxidative stress in the pathogenesis of acute I/R injury to the microcirculation, RS are also required for angiogenesis after I/R (reviewed in 276-278). This indicates that when RS are produced at lower levels, stimulates growth factor-dependent cell signaling required for new vessel

growth by modifying proteins (276-278) (Figure 5). These RS-dependent proangiogenic effects appear to require interactions between RS and reactive nitrogen species and glutathione (GSH) adducts on proteins, such as p65 NFkB and sarcoplasmic reticulum calcium ATPase-2, that are essential to endothelial cell growth factor responses. Generation of superoxide/hydrogen peroxide and NO derived from NOX2/NOX4/p66Shc and NOS, respectively, mediate these angiogenic responses (278) (Figure 5).

A growing body of evidence indicates that hydrogen peroxide is an endothelium-derived hyperpolarizing factor that contributes to the vasodilatory action of acetylcholine and bradykinin as well as linking myocardial metabolism to blood flow (reviewed in 279). Hydrogen peroxide has also been shown to act as a compensatory mediator of flow-mediated dilation in coronary arterioles obtained from patients with coronary artery disease (279). Recent work indicates that ceramide and plasminogen activator inhibitor-1 provoke the release of endothelium-derived extracellular vesicles by a mechanism-dependent on mitochondrial RS generation (280). This mitochondria RS-regulated formation of endothelial vesicles shifts the mediator of flow-mediated dilation from nitric oxide to hydrogen peroxide in human adipose resistance arteries. The transition from NO- to hydrogen peroxide-mediated vasodilation in coronary artery disease may also involve a reduction in telomerase activity and upregulation of the transcription coactivator PGC-1 α (peroxisome proliferator-activated receptor γ coactivator 1 α) (281,282).

RS generation also initiates the development of protected phenotypes induced by conditioning stimuli, such as short bouts of ischemia or antecedent ethanol ingestion, to limit microvascular and parenchymal cell injury and dysfunction. Under these conditions, RS are generated in much lower amounts than occurs during prolonged I/R and activate cell survival signaling cascades that appear to confer protection by mechanisms that reduce oxidative stress during prolonged I/R (reviewed in 1, 2, 283-287) (Figure 6).

Summary and Future Directions

It is now clear that postischemic microvascular dysfunction is an early and rate-limiting step in the pathogenesis of tissue injury in I/R. Despite effective recanalization of occluded arteries, alterations in endothelium-dependent vasodilator and vasoconstrictor responses in arterioles and resistance arteries, when coupled with the development of postischemic capillary no-reflow, occur during reperfusion following prolonged ischemia, which impair nutritive perfusion to affected sites. Disruption of barrier function in capillaries and postcapillary venules contributes to enhanced fluid and protein leakage from the vascular to extravascular compartment, which increases diffusion distance for delivery of oxygen and nutrients and for removal of potentially toxic metabolic byproducts. Edema formation secondary to postischemic microvascular barrier disruption also increases interstitial fluid pressure, which compresses microvessels and capillary no-reflow. Increased vulnerability to thrombus formation occurring in the microcirculation also contributes to impaired perfusion, while increased blood-to-interstitium trafficking of leukocytes across the walls of postcapillary venules allows for directed attack on parenchymal cells by inflammatory phagocytes. An abundance of evidence supports a pivotal role for RS in the pathogenesis of these devastating microvascular consequences of I/R. In sharp contrast, signaling levels of

RS participate in ischemic angiogenesis, serve as compensatory mediators of flow-mediated vasodilation in patients with coronary artery disease, and can function as endothelium-dependent hyperpolarizing factors that contribute to vasodilation induced by acetylcholine, bradykinin, and changes in shear stress while also acting to couple changes in myocardial metabolism to blood flow. Likewise, short bouts of ischemia and reperfusion or many pharmacologic stimuli (eg, ethanol, BKCa or KATP channel agonists) stimulate RS production at signaling levels to promote the expression of cell survival programs that prevent the overexuberant and deleterious generation of RS during reperfusion after prolonged I/R.

While much has been learned about the role of RS in the development of microvascular dysfunction in I/R and its consequences for parenchymal cell injury, there are still many unanswered questions and areas for fruitful investigation. For example, the lack of specificity of current redox sensors limits identification of particular RS species involved in I/R. However, new advances are rapidly expanding capabilities, and although far from full maturity, have the potential for circumventing many issues involved with in vivo detection of specific RS (288). In addition, it has been suggested that many conditions linked to increased cardiovascular risk for ischemic events may result in circadian clock disruption and vice versa. While others have shown that nocturnal variations in sympathetic activity and RS production may contribute to increase risk for heart and stroke, very little is known about how the circadian clock regulates damage from RS or influences antioxidant defenses in I/R or how these changes might impact on microvascular function (289-292). In addition, Flavahan and coworkers (293-295) have presented compelling evidence that superoxide inhibition can restore endothelium-dependent vasodilator responses by enhancing adherens junctions in aged animals. Whether this applies to dysfunctional endothelium-dependent vasodilation in I/R remains to be determined. Myocardial infarction has been shown to accelerate atherosclerosis by inducing the liberation of hematopoietic stem and progenitor cells from bone marrow niches to increase monocyte production and expression of adhesion molecules in aortic endothelial plaques (296-297). However, the role of reactive species in these responses was not examined, nor is it clear whether such changes contribute to atherosclerosis-induced microvascular dysfunction.

Other important questions that deserve attention in future studies include the role of mast-cell-derived RS in the development of postischemic microvascular dysfunction. While it is clear that these perivascular sentinel cells produce RS to regulate proinflammatory mediator release as well as participate in postischemic leukocyte recruitment (50,298,299), a direct link between these events has not been established. Genetic loss of telomerase reverse transcriptase (TERT), results in excessive RS production and a corresponding reduction in NO bioavailability that is associated with impaired vasodilation and proinflammatory effects (300). While it is unknown whether microvascular endothelial TERT is reduced by I/R, a novel TERT-derived peptide, GV1001, has proven effective in reducing myocardial I/R injury and inflammation (301). Another area deserving attention is the role of microRNAs in regulating RS production in the microcirculation after I/R, especially in view of their demonstrated roles in modulating mitochondrial RS, NOXs, and antioxidant signaling pathways and effectors in postischemic cardiac cells (302). An emerging area of interest relates to the role of the extracellular matrix protein, thrombospondin-1, in the pathogenesis of I/R.

This protein is upregulated by I/R and activates RS-production by a CD47/Nox1/Nox2/signal-regulatory protein- α -dependent mechanism in cultured vascular smooth muscle cells and aorta to modify vasomotor responses (303-305). However, the contribution of this signaling axis in postischemic microvascular dysfunction has not been evaluated.

Despite the large literature supporting a role for MPTP in parenchymal cell death and as a target for the protective actions of conditioning stimuli on myocardial cells (306-312), surprisingly little attention has been focused on the role of mitochondrial permeability transition in postischemic endothelial cell dysfunction, with the exception of its role in postischemic mitochondrial fission/fusion (Figure 4). This may relate to the fact that endothelial cells are resistant to ATP depletion during ischemia because of their reliance on glycolytic pathways. Thus, early dysfunction is likely due mostly to effects of mediators released from dying and necrotic parenchymal cells. However, the role of RS in provoking postischemic mitochondrial permeability transition and endothelial cell dysfunction and death, as well as serving as a target for conditioning stimuli to preserve microvascular dysfunction requires attention. Recent work suggests a role for MPTP opening in endothelial immunogenicity during reperfusion of transplanted lungs (313) and inflammation and impaired microcirculatory flow in other tissues (314,315), observations that support the possibility that inhibition the opening of MPTP may limit I/R-induced leukocyte infiltration, capillary no-reflow, and cell damage in the microcirculation. Despite intense interest regarding the effects of the intestinal microflora in the pathogenesis of tissue injury in I/R (316), little attention has been devoted to examining the influence of the gut microbiome on postischemic microvascular function. However, this seems likely since comparisons of ICAM-1 expression in wild-type versus germ-free mice indicate that indigenous gut microflora are responsible for much of the basal expression of this adhesion molecule in the intestine and other tissues (317). Moreover, it is well known that intestinal I/R leads to bacterial translocation by RS-dependent mechanisms and can induce systemic inflammatory responses (318). However, it is unclear how the composition of the gut microbiome influences the functions of arterioles, capillaries and venules after myocardial infarction or stroke or if such potential effects are influenced or initiated by RS. This intriguing area investigation should be pursued with vigor, given the potential to modify the gut microfloral composition by dietary or other manipulations, as potential avenues for reductions in I/R dysfunction.

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Highlights

- I/R-induced RS impair arteriolar vasomotor responsiveness.
- RS-induced capillary no-reflow limits postischemic nutritive perfusion.
- RS mediate postischemic leukocyte adhesion and endothelial barrier dysfunction in venules.
- RS induce EC microvesicle release, connexin dysfunction, and mitochondrial fission in I/R.
- Signaling levels of RS activate EC survival programs, flow-mediated vasodilation in CAD patients, and ischemic angiogenesis.

Figure 1.

Ischemia/reperfusion (I/R) induces the generation of damaging levels of reactive species (RS) from a number of cellular sources in the vasculature (and parenchymal cells) and mitochondria. In addition to producing cell damage, reactive species derived from these sources can also induce RS formation by mitochondria and vice versa, a phenomenon designated as RS-induced RS release (RIRR). Neutrophils, other immunocytes, and platelets represent other major sources of reactive species in I/R. RS produced from these sources cause damage to all biomolecules in cells. The enhanced RS flux induced by I/R overwhelms cellular antioxidant systems, which results in oxidative stress. In the microcirculation, postischemic RS produces vasomotor dysfunction and thrombus formation in arterioles, elicits impaired nutritive perfusion (no-reflow) and increased fluid filtration in capillaries, and enhances fluid and protein efflux and provokes adhesive interactions between blood cells (e.g., platelets, neutrophils, lymphocytes) and the endothelium of postcapillary venules.

Figure 2. Ischemia/reperfusion (I/R) causes the formation of reactive species (RS) that causes impaired vasomotor responses in resistance arteries and arterioles by several mechanisms. Endothelium-dependent vasodilator dysfunction results from impaired eNOS protein expression, reduced bioavailability of eNOS-derived nitric oxide (NO), eNOS uncoupling, oxidation of soluble guanylyl cyclase (sGC), mitochondrial permeability transition (MPT) pore opening, increased arginase expression and activity, and reductions in cofactors required for eNOS function. Postischemic RS production also impairs arteriolar vasoconstrictor responses to norepinephrine (NEPI), angiotensin II (AII), and vasopressin (AVP). On the other hand, I/R results in enhanced arteriolar production and responsiveness to endothelin, which produces paradoxical coronary vasoconstriction by direct receptor activation and indirectly by induction of RS formation, which reduces NO bioavailability.

Figure 3.

Ischemia/reperfusion (I/R)-induced formation of reactive species (RS) elicits capillary no-reflow, which impairs nutritive perfusion, even though feed arteries were successfully recanalized. Mechanisms induced by dysfunction arising in endothelial cells relate primarily to promotion of adhesive interactions with leukocytes and platelets, disruption of the endothelial glycocalyx, as well swelling and formation of blebs. Contributions attributable to leukocytes include the formation of neutrophil extracellular traps (NETs) and disruption of endothelial barrier function secondary to leukocyte transmigration. Contraction of pericytes localized at capillary orifices and along the length of capillaries compress luminal diameter, thereby impairing nutritive perfusion. Disruption of endothelial barrier function leads to increased microvascular fluid filtration from the blood to the tissue space. The excessive accumulation of fluid in the tissue spaces (interstitial edema) raises tissues pressure and physically compresses capillaries and postcapillary venules, an effect exacerbated by endothelial cell swelling and cell edema in the parenchyma). Postischemic fluid efflux across microvessels also increases microvascular hematocrit, which in turn reduces blood fluidity, thereby increasing vascular resistance to blood flow.

Figure 4.

Mechanisms underlying I/R-induced, RS-mediated, Drp1-dependent mitochondrial fission causes mitochondrial permeability transition and apoptosis to evoke microvascular dysfunction (decreased eNOS activity, capillary no-reflow, and endothelial barrier disruption). See text for further explanation. Abbreviations: I/R = ischemia/reperfusion, BI1 = Bax inhibitor, XO = xanthine oxidase, RS = reactive species, NRF4A1 = nuclear receptor subfamily 4 group A member 1, CK2α = serine/threonine kinase casein kinase2α (CK2α), p-Mff = phosphorylated mitochondrial fission factor, Drp1 = dynamin-related guanosine triphosphatase protein 1, FLNa = actin binding protein filamin A, MitoRS = mitochondrial RS, VDAC1 = voltage-dependent anion channel 1, HK2 = hexokinase 2, MPTP = mitochondrial permeability transition pore, eNOS = endothelial nitric oxide synthase.

Figure 5.

Ischemia/reperfusion (I/R) causes the expression of growth factors (eg, vascular endothelial growth factor (VEGF)) to provoke angiogenesis days after reperfusion is initiated. VEGF interacts with VEGF receptor 2 (VEGFR2) to directly provoke the formation of reactive species (RS) by NADPH oxidases isoform 4 (NOX4). This induces NOX2-dependent RS formation, which can be enhanced by VEGF-initiated, Rac1-dependent signaling. VEGF-induced NOX4/NOX2 signaling leads to phosphorylation of p66^{Shc}, a RS source in mitochondria. This RS-induced RS release signaling mechanism results in phosphorylation of VEGF (p-VEGF), which activates VEGFR2-dependent downstream effectors to promote angiogenesis.

Figure 6.

Antecedent ethanol ingestion evokes the appearance of an anti-inflammatory phenotype in postcapillary venules by a mechanism initiated by reactive species (RS) derived from NADPH oxidase (NOX) and xanthine oxidase. These RS interact with nitric oxide formed by endothelial nitric oxide synthase (eNOS) to form reactive nitrogen oxide species which activate transient receptor potential dependent vanilloid 1 (TRPV1) channels on sensory neurons. Subsequent neuronal release of calcitonin gene-related peptide (CGRP) activates CD4⁺ T cells to provoke the release of tumor necrosis factor- α (TNF α). Stimulation of resident and/or emigrated neutrophils by TNF α results in matrix metalloproteinase (MMP) release and activation. MMP-dependent cleavage of extracellular matrix proteins exposes matricryptins such as $\alpha_v\beta_3$ ligands that ligate and activate large conductance, calcium-activated potassium channels (BKCa), which form RS and activate Nrf2/ARE-dependent heme oxygenase-1 (HO-1) expression and activity. The enzymatic products of HO-1 are anti-adhesive and exert anti-oxidant effects to prevent postischemic RS production, leukocyte adhesion, and microvascular barrier function, thereby producing an anti-inflammatory phenotype and limit I/R injury. Conditioning with short bouts of I/R (ischemic conditioning) or a variety of pharmacologic agents, such as BKCa agonists TNF receptor agonists, also activate cell survival programs by RS-dependent signaling.