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Microcirculation and oxidative stress

E. CRIMI[1], L. J. IGNARRO[2], & C. NAPOLI[3,4,5]

[1]*Department of Anesthesia and Critical Care, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA,* [2]*Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles, CA, USA,* [3]*Department of General Pathology, Clinical Pathology and Excellence Research Center on Cardiovascular Diseases, 1st School of Medicine, II University of Naples, Italy,* [4]*IRCCS Multimedica, Milan, Italy, and* [5]*Evans Department of Medicine and Whitaker Cardiovascular Institute, School of Medicine, Boston University, MA, USA*

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Abstract
The microcirculation is a complex and integrated system, transporting oxygen and nutrients to the cells. The key component of this system is the endothelium, contributing to the local balance between pro and anti-inflammatory mediators, hemostatic balance, as well as vascular permeability and cell proliferation. A constant shear stress maintains vascular endothelium homeostasis while perturbed shear stress leads to changes in secretion of vasodilator and vasoconstrictor agents. Increased oxidative stress is a major pathogenetic mechanism of endothelial dysfunction by decreasing NO bioavailability, promoting inflammation and participating in activation of intracellular signals cascade, so influencing ion channels activation, signal transduction pathways, cytoskeleton remodelling, intercellular communication and ultimately gene expression. Targeting the microvascular inflammation and oxidative stress is a fascinating approach for novel therapies in order to decrease morbidity and mortality of chronic and acute diseases.

Keywords: *Microcirculation, oxidative stress, endothelial dysfunction, antioxidants*

Abbreviations: *ACE-I, angiotensin-converting enzyme inhibitors; ADMA, asymmetric dimethylarginine; AGEs, advanced glycation products; AP-1, Activator protein-1; BH4, Tetrahydrobiopterin; BMC, bone-marrow cells; cNOS, Constitutive NOS; COX, Cyclooxygenase; CRP, C-reactive protein; CVD, Cardiovascular damage; eNOS, endothelial NOS; ERK, Extracellular signal-regulated kinase; ET-1, Endothelin-1; ET-A, Endothelin-A receptor; HPV, Hypoxic pulmonary vasoconstriction; JNK, Jun N-terminal kinase; KATP, Potassium-Adenosine triphosphate sensitive K+ channels; Kv, Voltage-dependent potassium channels; ICAM-1, Intercellular adhesion molecule-1; IL-1, Interleukin-1; IFN-γ, interferon-gamma; iNOS, Inducible NOS; I/R, Ischemia reperfusion injury; LDL, Low density lipoprotein; LTB4, Leukotriene B4; MAPK, Mitogen-activated protein kinase; MCP-1, Monocyte chemotactic protein-1; MMPs, Matrix metalloproteinases; MPO, Myeloperoxidase; NADPH, Reduced nicotinamide adenine dinucleotide phosphate; NO, Nitric oxide; NOS, Nitric oxide synthase; NF-κB, Nuclear factor-κB; nNOS, neuronal NOS; PAF, Platelet-activating factor; PAI-1, Plasminogen activator inhibitor-1; PGI2, Prostacyclin; PI3 K, phosphatidyl-inositol-3-kinase; ROS, Reactive oxygen species; SOD, superoxide dismutase; TNF-α, Tumour necrosis factor alpha; TRP, Transient receptor potential channels; TXA2, Thromboxane; VCAM-1, Vascular cell adhesion molecule-1.*

Correspondence: Ettore Crimi, MD, Department of Anesthesia and Critical Care, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA. Tel: (617) 726-5322. Fax: (617) 724-8511. E-mail: ecrimi@partners.org

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Introduction

The microcirculation is a complex system delivering oxygen in order to meet the cellular oxygen demand. The key component of this system is the endothelium, mediating, under physiologic conditions, several functions to ensure a normal homeostasis.

A dysfunction of normally protective endothelium may contribute to initiation and progression of several diseases, including cardiovascular damage associated to hypercholesterolemia, hypertension, diabetes, ischemia/reperfusion injury and sepsis [1–6]. Increased oxidative stress, resulting from an exceeding production of reactive oxygen species (ROS) and other oxidants, plays an important role in determining microvascular injury [7].

The goal of this review is to highlight the complex interactions between microcirculation and oxidative stress in some acute and chronic diseases.

Microcirculation

The microcirculation is a hidden organ consisting of the smallest blood vessels, including resistance arterioles, capillaries and venules. It is a complex and integrated system, transporting oxygen and nutrients to the cells. The endothelial cells, forming the inner lining of all blood vessels, represent the main cell types of the microcirculation.

Endothelial physiology

The endothelium, an active biologic organ, contributes to the local balance between pro- and anti-inflammatory mediators, hemostatic balance, as well as vascular permeability and cell proliferation [8]. Normal endothelial cells show vasodilator, anti-coagulant and anti-adhesive properties.

The endothelium mediates the vasomotor tone of the microcirculation by release of vasodilators (nitric oxide, prostacyclin, bradykinin and endothelium-derived hyperpolarizing factor) and vasoconstrictors (endothelin-1, angiotensin II and thromboxane) factors. Nitric oxide (NO) plays a predominant role in vasodilation [9]. NO is synthesized from the aminoacid L-arginine in a reaction catalysed by a family of nitric oxide synthases (NOSs), requiring tetrahydrobiopterin (BH4) as cofactor, and leads to relaxation of smooth muscle cells by increasing intracellular cyclic guanosine-monophosphate levels.

The small physiologic amounts of NO generated by the constitutive NOSs (neuronal NOS, nNOS, NOS I and endothelial NOS, eNOS, NOS III) are responsible for most of its beneficial effects (vasodilation, inhibition of platelet aggregation and leukocyte adhesion to the endothelium). A constant production of NO contributes to regulation of arterial systemic pressure by maintaining a continuous vasodilator tone as shown by hypertensive phenotype in eNOS knockout mouse [10].

The inducible NOS (iNOS, NOS II), activated by inflammatory stimuli, produces larger and more persistent concentration of NO, leading to most of its detrimental actions: hypotension, negative inotropic effect, pro-oxidant properties, apoptosis, mediation of the effects of cytokines, cytotoxic innate immunity [11].

The most important physiologic factor for NO synthesis is shear stress, that is a tangential distortion of the endothelial cells produced by blood flow [12]. NO is also released in response to pharmacological agonists such as acetylcholine.

The counterpart of NO is endothelin-1 (ET-1), which causes vasoconstriction, smooth muscle cell proliferation by activation of endothelin-A receptor (ET-A) and release of inflammatory mediators such as interleukin-1 (IL-1), IL-6 and IL-8.

A constant shear stress maintains vascular endothelium homeostasis, preventing cell apoptosis/proliferation, coagulation, leukocyte adhesion and atherogenesis [13,14], while perturbed shear stress leads to changes in secretion of vasoactive factors as well as in gene expression by activating different signal transduction pathways [15]. For example, acute loss of shear stress, as observed in ischemia, results in membrane depolarization of lung microvascular endothelial cells, secondary to adenosine triphosphate sensitive K^+ (K_{ATP}) channels closure; this response is followed by activation of endothelial nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, ROS production, activation of transcriptor factors such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) and increase of intracellular Ca^{2+}, with enhanced eNOS activity and NO release (Figure 1). Ultimately, the endothelial cells try to restore the blood flow by promoting angiogenesis and vasodilation [15,16]. In pulmonary endothelium, calcium influx, via voltage-gated T-Type calcium channels, promotes secretion of von Willebrand factor and expression of P-selectin, so influencing hemostasis and inflammation [17].

The tone of microcirculation is tightly coupled to parenchymal oxygen consumption, resulting in vasodilation or vasoconstriction according to the actual interstitial Po_2 concentration [18]. Endothelial cells might act as oxygen sensor and, when exposed to hypoxia, mediate vasodilation by increased production of NO, prostacyclin (PGI_2) or by activation of K_{ATP} channels in smooth muscle cells with subsequent hyperpolarization and reduced calcium inflow [19,20]. Conversely, hypoxic pulmonary vasoconstriction (HPV) is a feature of pulmonary circulation in response to alveolar hypoxia in order to preserve gas exchange. ROS may participate in HPV by regulating potassium and/or calcium channels [21,22].

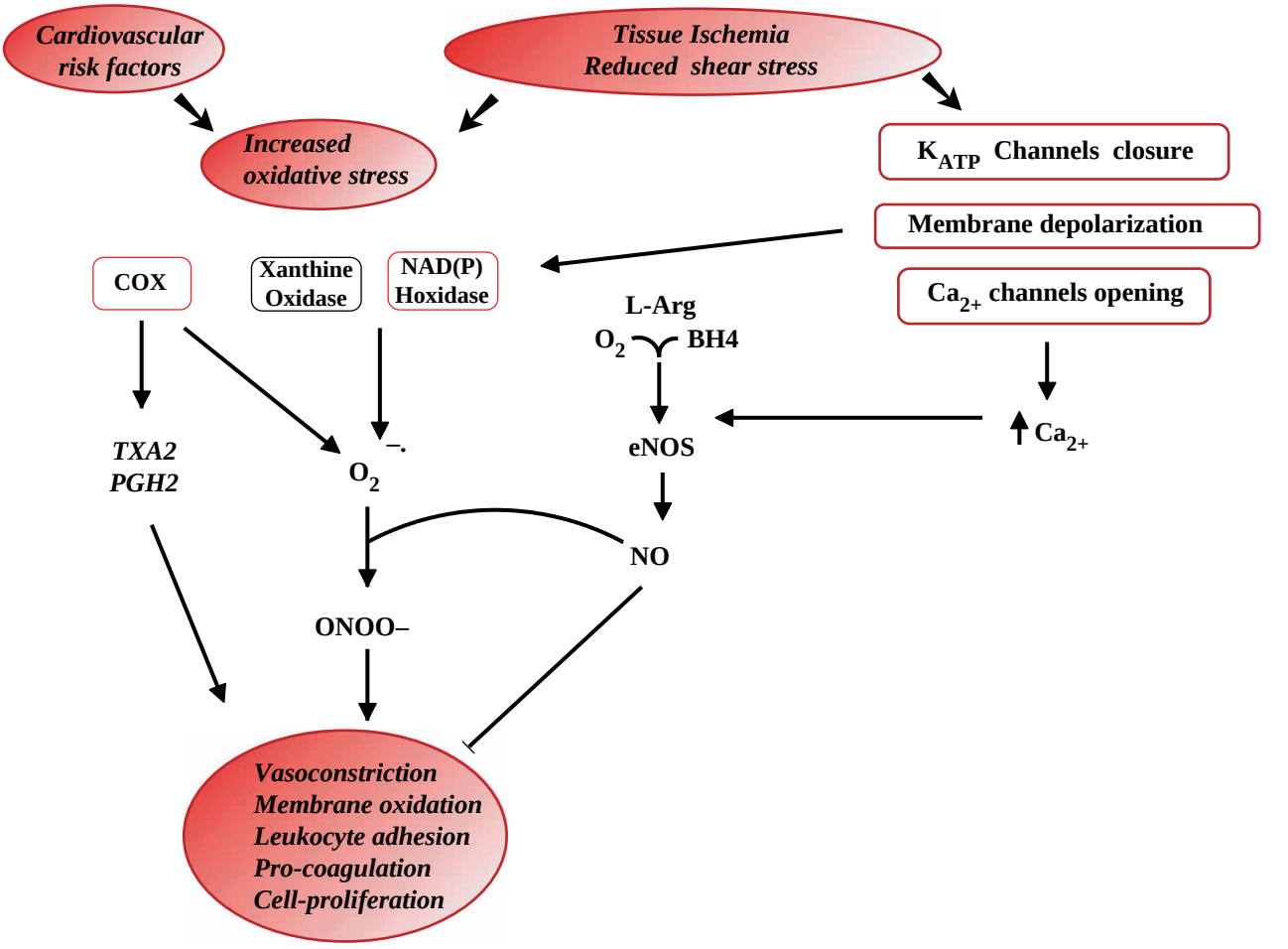

Figure 1. Role of increased oxidative stress and perturbed shear stress on endothelial dysfunction. Main source of ROS are NADPH oxidase, xanthine oxidase and cyclooxygenase (COX). The superoxide anion ($O2^{-}\cdot$) scavenges NO to form peroxynitrite (ONOO). Acute loss of shear stress can result in potassium-adenosine triphosphate sensitive K^{+} (KATP) channels closure with membrane depolarization, leading on one side to activation of endothelial NADPH oxidase, ROS generation and on the other side to increase of intracellular Ca^{2+} by activation of voltage dependent Ca^{2+} channels with activation of endothelial NO synthase and NO production in the attempt to restore blood flow.

Endothelial dysfunction

An impairment of endothelial-mediated vasodilation characterizes endothelial dysfunction; it is the result of reduced NO production, mainly due to its inactivation by ROS and increased release of vasoconstrictor factors [23–25]. L-arginine and tetrahydrobiopterin deficiencies cause NOS dysfunction, affecting NO production and increasing the concentration of oxidants such as superoxide and hydrogen peroxide, a phenomenon described as 'NOS uncoupling' [26,27].

Endothelial dysfunction includes a pro-inflammatory, pro-coagulant and proliferative condition, called endothelial activation. Different signals can activate the endothelial cells: cell-to-cell interactions, soluble mediators and hemodynamic forces [28]. Haematic hyperviscosity and reduced erythrocyte deformability with worse hemorheological parameters can increase the risk of ischemia [29].

Dysfunction of the normally protective endothelium is a key component in several diseases, including atherosclerosis, hypertension, diabetes, ischemia reperfusion injury and sepsis [30–33]. Specialized microvasculature is present in the central nervous system where endothelial cells and astrocytes participate in the blood–brain barrier. There is increasing interest in elucidating the relation between the integrins and matrix adhesion receptors and extracellular matrix in the regulation of cerebral vascular permeability as novel therapeutic target in conditions such as cerebral ischemia and multiple sclerosis [34].

The identification of biological markers of endothelial dysfunction could help for an early diagnosis and new effective treatment. Some of the most studied indexes are citrulline/arginine ratio, an index of NOS activity, asymmetric dimethylarginine (ADMA), a potent endogenous inhibitor of NO, synthesis endothelial progenitor cells, myeloperoxidase (MPO) and C-reactive protein (CRP) [35–41].

Oxidative stress

Oxidative stress describes an imbalance in production of free radical species and their effective removal by antioxidants and scavenger enzymes [42]. ROS are normally produced during cellular respiration and inflammatory defense mechanisms [43]. There are several scavenger systems including enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase, a selenium dependent enzyme (GSH-Px), and non-enzymatic antioxidants such as vitamin E, vitamin C, beta-carotene and hemebinding proteins [44].

ROS deplete the cellular levels of NO, increase the expression of adhesion molecules (P-selectin), lipid inflammatory mediators such as platelet-activating factor (PAF), leukotriene B4 (LTB4) and cytokines such as IL-8 [7,25], so contributing to endothelial dysfunction. The superoxide anion ($O_2^{-}\cdot$) scavenges NO to form peroxynitrite, triggering different proinflammatory signals and inhibiting endothelial repair and preventing angiogenesis [45,46].

Exogenous sources of ROS are phagocytes as well as circulating enzymes such as xanthine oxidase. Endothelial cells can also generate ROS in response to tumour necrosis factor alpha (TNF-α), IL-1β, platelet-derived growth factor. Endogenous sources of ROS, such as cyclo-oxygenase and NADPH oxidase, might lead to more subtle effects other than cell injury [13]. In fact, ROS might act as second messenger and have a significant effect on the vascular cells signal transduction pathways involving mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK) as well NF-κB, activator protein-1 activation (AP-1), so influencing gene expression, cytoskeleton organization, cross-talk communication and ion channels activation.

ROS can mediate the endothelial response to mechanical stress, converting the cellular mechanical distortion into biological signals, a process called mechanotransduction [13,15]. NADPH oxidase might represent a specific targeting of signalling modulation and inhibition [47] (Figure 1).

In the pulmonary microcirculation, ROS can induce contraction of pericytes, that surround the endothelial cells, resulting in disruption of the microvasculature barrier and increased permeability [48]. In pulmonary endothelial cells, increased cytosolic calcium level, regulated by T-Type calcium channel and recent identified transient receptor potential channels (TRP) activated by inflammatory mediators, triggers cytoskeleton remodelling responsible for endothelial cell barrier disruption. The inhibition of these channels represents a novel anti-inflammatory strategy [17,49–51].

Ageing

Cell senescence is associated to endothelial dysfunction [52]. Senescent vascular cells show an impairment of endothelium-dependent vasodilation secondary to reduced eNOS activity, NO production and increased ROS production. These cells present a pro-coagulant phenotype, due to increased production of thromboxane A_2 (TXA_2), ET-1 and plasminogen activator inhibitor-1 (PAI-1). Ageing is also associated with higher levels of circulatory inflammatory mediators such as TNF-α, advanced glycation products (AGEs), matrix metalloproteinases (MMPs) and inflammatory cells as mast cells that disrupt the integrity of the microvascular endothelium and, ultimately, reduce blood flow [52–56]. All these changes can exacerbate age-related diseases like hypertension, atherosclerosis and diabetes mellitus.

Targeting senescence mechanisms might represent a new therapeutic strategy. Physical activity and caloric restriction can improve age-related modifications to endothelium and reduce the risk of death from cardiovascular diseases in the elderly [57–59].

Cardiovascualr damage and hypercholesterolemia

Hypercholesterolemia is a major risk factor for coronary atherosclerosis and is associated to endothelial dysfunction [60,61]. Increased oxidative stress can contribute to myocardial vascular dysfunction in hypercholesterolemia [62]. The most important effects of ROS include oxidation of low-density lipoprotein (LDL), arteriolar scavenging of the endothelium-derived NO with reduced vasorelaxation and blood cells–venular endothelial cells interaction.

Oxidative modification of LDL may promote endothelial injury with progression of the fatty lesions [63]. Oxidized LDLs antagonize the endothelial production of NO, reducing the expression of eNOS [64,65], decreasing the uptake of L-arginine and enhancing the level of asymmetric dimethylarginine (ADMA) [66]. Oxidative stress, by activation of NF-κB, can influence the expression of IL-1, vascular cell adhesion molecule-1 (VCAM-1), ICAM-1, pro-angiogenic factors such as ET-1, MMP-2 and MMP-9, thus contributing to vasa vasorum and atherosclerotic plaque neovascularization [67–69]. Angiogenesis can contribute to progression as well as to vulnerability of atherosclerotic lesions [70]. The vasodilation in response to substances like acetylcholine (Ach) is diminished, as a result of increased ROS and decreased bioavailability of NO in arterioles [71,72].

In post-capillary venules, oxidative stress promotes adhesion molecules expression with platelet and leukocyte recruitment, a response attenuated by NO

supplementation [73,74]. Platelets play a complex role, linking thrombosis, inflammation and immune response [75]. Platelet activation produces peroxynitrite, which increases thromboxane levels and inhibits the mechanisms of endothelial repair. The interaction of platelets and endothelial cells promotes the release of IL-8, monocyte chemotactic protein-1 (MCP-1) and ultimately monocyte activation [35].

MPO, released by neutrophils and monocytes, is a powerful generator of oxidizing, nitrating and chlorinating species with proinflammatory and proatherogenic properties. It can oxidize LDL cholesterol, activate metalloproteinases and decrease NO availability. MPO is involved in destabilization and rupture of the atherosclerotic plaque and associated to adverse outcome in patients with acute coronary syndrome [39,40].

Cytokines, such as interferon-γ (IFN-γ), TNF-α, IL-1, released by activated leukocytes, increase oxidative stress in venules [76,77] and arteriolar dysfunction in hypercholesterolemia [78].

Endothelial dysfunction induced by oxidized LDL can be reversed by L-arginine [79,80], pomegranate juice containing polyphenolic antioxidants [81,82], flavanol-rich cocoa [83,84], antioxidants like vitamins C and E [80,85,86], inhibition of the renin angiotensin system [87] and cholesterol lowering statins [88–90]. Metabolic treatment with antioxidant and L-arginine associated to moderate physical exercise in experimental models of hypercholesterolemia showed protective effects, with reduction of atherosclerotic lesion formation, plaque rupture and prolonged survival [91,92]. Interestingly, chronic exercise elicited an increase in production of NOS expression and improved scavenger activities [93].

Notably, the beneficial effects of chronic antioxidant supplementation in hypercholesterolemia are not confirmed under normal conditions, where it can impair myocardial perfusion and coronary endothelial function by an increased level of oxidative stress in the arterial wall [94]. Therefore, high-dose antioxidant vitamins in healthy subjects with presumed low oxidative stress might be detrimental.

A new promising therapeutic approach consists of targeting the processes involved in angiogenesis. Statins decrease vascular wall oxidative stress by eNOS activation with increased NO bioavailability and a direct antioxidant activity [88,89]. Moreover, they reduce angiogenesis and vasa vasorum neovascularization [90].

The blockade of the endothelin system, which is upregulated in hypercholesterolemia, showed protective effects on renal microvasculature [95].

Over the past few years, CRP has been investigated not only as an inflammatory marker but also as a possible mediator of atherosclerosis [96]. In particular, CRP can influence the microcirculation by eNOS downregulation [97], increasing ET-1 [98] and upregulating angiotensin type 1 receptor in smooth muscle cells [99]. CRP also promotes ROS production and upregulation of chemokines and endothelial cell adhesion molecules, so contributing to a proinflammatory and proatherosclerotic environment [100,101]. CRP could represent not only a diagnostic marker during primary prevention of cardiovascular diseases [102] but also a new therapeutic target in cardiovascular protection [103,104].

Cardiovascular damage and hypertension

Hypertension is a significant cardiovascular risk factor, associated to endothelial dysfunction and oxidative stress. An increased production of ROS and a reduced level of SOD are reported in both animal [105–109] and human studies [110]. Oxidative stress participates in increasing systemic arterial pressure, reducing NO availability and vasodilation [111,112]. NADPH oxidase and xanthine oxidase are the main sources of ROS in hypertension [113,114]. Oxidative stress is involved in remodelling of myocardial microvascular architecture and subsequent development of left ventricular hypertrophy [115,116]. Hypertension, combined to hypercholesterolemia, accentuates oxidative stress, showing a synergistic deleterious effect on myocardial microvascular dysfunction [117,118].

Antioxidant supplementation with high dose of vitamins C and E can reduce endothelial dysfunction and improve myocardial perfusion in early hypertension [119]. Simvastatin showed a protective effect, preventing myocardial microvascular remodelling and hypertrophy in experimental renovascular hypertension [120].

Cardiovascular damage and diabetes

Diabetes mellitus is a major cardiovascular risk factor, associated with increased morbidity and mortality [121]. Endothelial dysfunction with impaired endothelium-dependent vasodilation has been documented in type 1 [122] and type 2 diabetes [123] as well as in the syndrome of insulin resistance [124]; it also plays an important role in the development of macro and microvascular disease [125]. Suggested cellular mechanisms of impaired endothelium-dependent vasodilation include decreased production of vasodilators (NO, prostacyclin, endothelium-derived hyperpolarizing factor) and increased release of vasoconstrictors (thromboxane, ET-1) [4]. Disturbance in voltage-gated K^+ channel function [126] and impaired vasodilation to hypoxia can contribute to microvascular dysfunction [127]. A defect of insulin action on phosphatidyl-inositol-3-kinase (PI3-K) pathway that normally mediates the vasodilator effect of insulin by increasing eNOS

gene expression and NO bioavailability can explain the vascular dysfunction in insulin resistance [128,129].

Hyperglycemia-induced oxidative stress plays a central role in endothelial dysfunction [130,131]. Hyperglycemia, the hallmark of diabetes, increases superoxide production by NADPH depletion with glutathione regeneration impairment and activation of vascular NADPH oxidase due to increased AGE production and protein kinase C activation [131]. Other possible sources of ROS include uncoupled eNOS, xanthine oxidase and mitochondria [4]. Hyperglycemia and oxidative stress, through increased LDL oxidation, can accelerate the atherosclerotic disease in diabetic patients [132].

Antioxidants (N-acetylcysteine, vitamins E and C) can restore endothelial function [133–135]. In an experimental model of metabolic syndrome, characterized by insulin resistance, pomegranate fruit extract, rich in polyphenolic antioxidants, reduced the expression of oxidation-sensitive genes at the sites of perturbed shear-stress and increased eNOS expression [136]. However, antioxidant therapy does not reduce cardiovascular complications in diabetic patients [137].

ACE inhibitors can decrease NADH oxidase activity and free radicals by inhibition of angiotensin II [125]. Statins improved endothelial dysfunction in diabetes, independently of changes in cholesterol levels [138,139].

Cardiovascular damage and ischemia/repurfusion injury

Ischemia/reperfusion injury (I/R) defines the cellular structural changes occurring after reperfusion of ischemic tissue [5]. Endothelial cells exposed to the deleterious effects of ischemia and reperfusion show depletion of energy stores, altered ion distribution, membrane depolarization, increased hypoxanthine level, increased membrane fluidity, cellular swelling and cytoskeleton derangement [140].

The components of the microcirculation including arterioles, capillaries and venules manifest different responses to I/R injury.

I/R results in severe dysfunction of the endothelium in arterioles characterized by impaired endothelium-dependent vasodilation and increased arterial resistance. A reduction in NO release is responsible for impaired arteriolar vasodilation [141–144]. The mechanisms responsible for this inhibition are still unclear. Increased activity of arginase [145], enzyme competing with NOS for the substrate L-arginine, and depletion of BH4, cofactor of NOS [146], have been described following I/R injury. A burst of oxidant production at the onset of the reperfusion can impair the NO-dependent vasodilation by direct inhibition [147] and stimulating leukocyte adhesion molecule, promoting leukocytes activation, chemotaxis and ultimately leading to further ROS production. Interestingly, inhibition of arginase [145], administration of BH4 [148] as well as gene deficiency of leukocyte adhesion molecules (CD11/18, P selectin, ICAM-1) [149] can restore and/or preserve endothelium-dependent NO-mediated relaxation.

The microvascular dysfunction in capillaries includes increased fluid filtration with interstitial edema and less perfused capillaries. Increased permeability of the endothelium rather than elevated intracapillary pressure is the main mechanism for increased filtration [150]; it can be due to NO inhibition [151] as well as to intercellular adhesion disruption secondary to ROS production and energy depletion. Decreased endothelium-dependent vasorelaxation, interstitial fluid accumulation, obstruction and/or narrowing of the vessels by platelet-leukocyte aggregation and leukocyte-endothelial cell adhesion can lead to mechanical blood flow obstruction and capillary malperfusion following the reperfusion of an ischemic organ, known as 'no reflow phenomenon' [152]; it may be clinically evident as persistence and/or worsening of organ dysfunction after the reperfusion.

In the post-capillary venules, endothelium dysfunction is characterized by an intense inflammatory response. ROS play a pivotal role in this response. On reperfusion of ischemic tissues, ROS production is enhanced by high levels of xanthine oxidase converting the intracellular excess of hypoxanthine as a result of ATP degradation [5]. ROS promote activation of circulating leukocytes, platelets, with generation of inflammatory mediators (PAF, LTB, IL-8), cytokine and adhesion molecule expression, resulting in neutrophil mediated tissue injury [153,154]. Potential strategies to prevent I/R, including ischemic pre-conditioning, antioxidant, antileukocyte and anticomplement therapy, did not provide a definite and unequivocal benefit in the clinical settings [140].

Cardiovascular damage and sepsis

Microvascular dysfunction participates in the pathogenesis of sepsis. Pathogens, lipopolysaccharide as well as inflammatory mediators can be responsible for endothelial cell activation and dysfunction [155].

An increased heterogeneity in microvascular blood flow has been described in various experimental models of sepsis [156–159] and in patients with septic shock, using polarized light microscopy [160]. The early phases of sepsis are characterized by reduced number of perfused capillaries, misdistribution of oxygen delivery and subsequent tissue hypoxia [161].

Oxidative stress is the result of activation of phagocytes, production of NO and ROS, release of iron, copper ions, metalloproteins and contributes to experimental microvascular dysfunction in sepsis. ROS can directly disrupt endothelial cells and impair cellular interaction, so promoting microvascular thrombosis and organ dysfunction [162,163].

NO is an important factor for the integrity of microvascular endothelium and blood flow [164]. Bacterial endotoxin or inflammatory cytokines increase iNOS activity. The enhanced production of NO contributes to vascular collapse and myocardial dysfunction, mediating the depressant effects of proinflammatory cytokines (TNF-α, IL-1β) [165–168]. Endotoxin produced less hypotension in iNOS deficient mice [169] and in mice treated with a selective pharmacological inhibitor of iNOS [170]. The heterogeneous expression of iNOS in different vascular beds can result in flow shunting [171]. NO could have beneficial effects in sepsis related to counteraction of the released vasoconstrictors substances, to inhibition of leukocyte rolling and adhesion and to inhibition of NF-κB [172]. Interestingly, myocardial over-expression of NOSIII showed a protective effect against endotoxin-induced myocardial dysfunction [173].

Conclusions

The endothelium represents a dynamic interface with activation of different and highly integrated cellular pathways in response to mechanical and metabolic stimuli. An imbalance between ROS production and NO availability leads to main changes in endothelial function and ultimately to impaired microcirculatory perfusion. Oxidative stress might play a more sophisticated role in endothelial dysfunction other than cellular damage. It can act as an important mediator in the inflammatory cascade and participate in mechanotransduction, transforming the mechanical forces changes determined by perturbed shear stress in biological signals.

Microcirculatory dysfunction is emerging as an important factor in the pathogenesis of acute and chronic diseases. Interestingly, senescence is also associated to increased oxidative stress and endothelial dysfunction, contributing to the exacerbation of age-related cardiovascular disease.

Targeting the oxidant response by antioxidants and modulating specific enzymes (e.g. NO synthases, NADPH oxidase) represents a potential therapeutic strategy.

Recent evidence indicates that treatment with antioxidants and L-arginine combined to bone-marrow cells (BMC) transplantation can provide beneficial effects beyond those achieved by BMC transplantation alone in the healing process of the injured cardiovascular system [174]. There is growing interest on the protective effects of statins on the endothelial dysfunction, beyond their lipid lowering action. Healthy lifestyle, including regular physical activity and balanced diet, is an easy practice to reduce the endothelium age-related modifications.

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