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Microvascular inflammation in atherosclerosis

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ABSTRACT

Atherogenesis is the pathogenetic process leading to formation of the atheroma lesion. It is associated to a chronic inflammatory state initially stimulated by an aberrant accumulation of lipid molecules beyond the endothelial barrier. This event triggers a cascade of deleterious events mainly through immune cell stimulation with the consequent liberation of potent pro-inflammatory and tissue damaging mediators. The atherogenetic process implies marked modifications of endothelial cell functions and a radical change in the endothelial–leukocyte interaction pattern. Moreover, accumulating evidence shows an important link between microvascular and inflammatory responses and major cardiovascular risk factors. This review illustrates the current knowledge on the effects of obesity, hypercholesterolemia and diabetes on microcirculation; their pathophysiological implications will be discussed.

1. Introduction

In most organs, microcirculation is composed of three anatomically and functionally distinct segments: arterioles, capillaries and venules. Arterioles are well innervated and anatomically characterized by endothelium surrounded by a smooth muscle cell containing wall and a diameter ranging from 10 to 100 μm. Typically, arterioles display a divergent branching pattern so that blood flows from one arteriole into two branches at each bifurcation. Arterioles are highly responsive to sympathetic vasoconstriction and represent a major player in the regulation of systemic vascular resistance. Because of these characteristics, the regulation of blood flow and ultimately oxygen delivery represent the primary function of arterioles. In addition arterioles, by participating to the regulation of capillary hydrostatic pressure, influence capillary fluid exchange [1,2].

Blood flows from arterioles into capillaries, 5–10 μM calibre single endothelial cell layer vessels surrounded by basement membrane and devoid of smooth muscle cell wall and innervation. In some organs, however, a circular band of smooth muscle at the entrance of the capillary (precapillary sphincter) can regulate the number of perfused capillaries [1,2].

Capillaries ensure large surface area and relatively high permeability to favour the exchange of fluids, gases, electrolytes and macromolecules. In different organs, the endothelial wall differs for its structural organization and permeability. In the liver, spleen and bone marrow gaps in both the endothelial layers and the basement membrane result in very high permeability of capillaries. Fenestrated capillaries are present in the intestinal mucosa, renal glomeruli and exocrine glands where endothelial cells display wide intercellular clefts surrounded by a continuous basement membrane. Continuous capillaries are found in the central nervous system, skin, muscle and the lung where intercellular gaps are tight and basement membrane is continuous. These capillaries display the lowest permeability [1–3].

Venules are small exchange vessels but with a larger caliber than the corresponding arterioles (10–50 μM caliber), resulting in a lower flow velocity and wall shear stress that promote leukocyte margination and facilitate adhesion to the vessel wall. Venules are composed of an endothelial cell layer surrounded by a basement membrane. Smooth muscle cells are present in larger venules but not in small postcapillary venules. The presence of smooth muscle and sympathetic innervation in larger

Abbreviations: ADMA, dimethylarginine; ECs, endothelial cells; ESAM, endothelial cell-selective adhesion molecule; ICAM-1, intercellular adhesion molecule 1; JAM, junction adhesion molecule; LDL, low density lipoprotein; MAdCAM-1, mucosal addressin cell adhesion molecule 1; NO, nitric oxide; NOS, NO synthase; PSGL-1, P-selectin glycoprotein ligand 1; ROS, reactive oxygen species; SOD, superoxide dismutase; V-CAM-1, vascular cell adhesion molecule 1; VSMC, vascular smooth muscle cell.

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venules allows the regulation of venule tone and therefore capillary hydrostatic pressure [2,3]. Due to their anatomical architecture resulting in relatively low permeability, fluid and macromolecules exchange occurs predominantly at venular junctions. Leukocyte margination is favoured in venules because of erythrocyte tendency to aggregate at low shear stress forces occurring in the central portion of venules. Erythrocyte aggregation in turn pushes leukocytes toward the vessel wall. In addition, the flow discharged by a capillary into a venule at site of confluent junctions is located to a region close to the venule wall thus promoting leukocytes to take contact with the endothelium. Finally, the most important factor determining the prevalence of leukocyte adhesion to venule walls during inflammation is the selective expression of adhesion molecules by venule but not arteriole or capillary endothelial cells [1,2,4].

2. Inflammatory state of microcirculation associated to atherogenesis

Under normal conditions, vascular endothelial cells subserve several tasks that constitute the "endothelial function" including the regulation of blood flow, blood fluidity, vessel wall permeability and interactions with circulating leukocytes.

Blood flow fluidity is modulated through the regulation of the tone of surrounding vascular smooth muscle cells, that in turn, depends on the balance between vasoconstriction and vasorelaxant signals. Endothelial cell-dependent vasorelaxation of SMC is achieved through the production of nitric oxide. Nitric oxide is highly reactive (having a lifetime of a few seconds), yet diffuses freely across membranes. NO acts through the stimulation of the heterodimeric enzyme soluble guanylate cyclase, with subsequent formation of cyclic GMP. Cyclic GMP activates protein kinase G, which causes phosphorylation of myosin light chain phosphatase, and therefore inactivation of myosin light-chain kinase, causing smooth muscle relaxation through the dephosphorylation of the myosin light chain [5].

Endothelial cells actively inhibit blood coagulation ensuring blood fluidity through several mechanisms such as the expression of coagulation cascade inhibitors including heparane sulphate proteoglycans, inhibitors of tissue factor pathways, and thrombomodulin. In addition ECs inhibit platelet activation by releasing NO and prostacyclin [6,7].

In physiological conditions, plasmatic proteins are contained inside vascular lumen of continuous capillaries, the structure of junctions between adjacent ECs that include tight and adherens junctions. The permeability properties of capillaries depend on the structural organization and the presence of intercellular junction structures that vary considerably in different anatomical locations. Junctional structures are tighter in the capillaries of the central nervous system, in the liver and spleen sinusoidal capillaries and are characterized by discontinuous intercellular junctions that enable blood contact with underlying tissue. However, in most tissues, capillary ECs normally block extravasation of plasmatic proteins as small as albumin. On the other hand, they operate active transport of proteins from capillary lumen to tissue via vesicular transport [8].

In normal conditions, endothelial cells have rare interactions with circulating leukocytes, thus representing a barrier between tissues and inflammatory cells. In non-inflamed vasculature leukocyte–ECs, interaction is limited because of the very low constitutive expression of surface adhesion molecules such as, ICAM-1, VCAM-1 and E-selectin as well as the compartmentalization of P-selectin and chemokines into endothelial intracellular vesicles named Weibel–Palade bodies [2,9]. In addition, resting ECs stimulated by shear stress express NO that inhibits proinflammatory gene transcription, release of Wiebel–Palade body content and leukocyte activation (discussed below).

Atherosclerosis involves the formation of lesions in the arteries characterized by lipid accumulation, inflammation, cell death and fibrosis. These lesions known as atherosclerotic plaques grow and evolve over time with the consequent decrease of the vasal section. A limited and often insufficient blood flow accompanied by stenosis due to modification of blood vessel plasticity, leads to clinical complications. However, the most serious complications arise from lesion rupture and sudden exposure of thrombotic substances to circulating blood leading to abrupt formation of blood clots and occlusion. The atherogenic process involves three major steps: an early and persisting inflammatory component, a proliferative response and, ultimately, a mural thrombosis that is potentially responsible for vascular occlusion.

Although atherosclerotic lesions occur in large arteries, the upregulated expression of adhesion molecules characteristic of EC activation, the reduced endothelium-dependent vasodilatation as well as oxidative stress are not confined to lesion-prone arteries where factors other than EC activation (e.g. elevated shear stress) might act to determine atheroma formation. Atherosclerosis is associated with a systemic inflammatory state characterized by endothelial and blood cell activation as well as increased plasmatic concentration of inflammatory factors and endothelial dysfunction consisting in reduced endothelium-dependent vasodilation [10]. Parameters such as the circulating concentrations of proinflammatory factors or soluble isoforms of adhesion molecules have been proposed as biomarkers for the assessment of cardiovascular risk. Enhanced production of inflammatory mediators such as cytokines, chemokines and reactive oxygen species occurs in the atherosclerotic lesions determining and sustaining local intramural inflammation. However, inflammatory mediators can also be released in the circulation determining systemic inflammation with the involvement of the microcirculation. Alternatively, cardiovascular risk factors as for example elevated blood cholesterol levels, hypertension, diabetes, obesity and cigarette smoke might directly stimulate microvascular endothelial cell activation with consequent release of inflammatory mediators and soluble isoforms of adhesion molecules, thus determining microvascular dysfunction and the atherosclerosis-associated systemic inflammatory state [11]. In support of this hypothesis, many observations have indicated that the presence of cardiovascular risk factors such as hypercholesterolemia, obesity, hypertension and diabetes induces microvascular responses consistent with the induction of an inflammatory phenotype [12]. In both scenarios, due to its preponderant surface area, microcirculation would quantitatively represent the major source of circulating inflammatory mediators.

Accumulating evidence suggests that oxidative stress represents a main pathogenic mechanism underlying the development and the progression of atherosclerosis. Oxidative stress is defined as an imbalance between oxidants and antioxidants in favour of the former. Reactive oxygen species (ROS), including superoxide anions (O_2^-), hydrogen peroxide, and hydroxyl radicals are produced by a variety of cell types including endothelial cells, phagocytes and smooth muscle cells. Free radicals such as O_2^- and hydroxyl radicals are highly reactive and hold exalted oxidizing activity. The activity of enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase, as well as the mitochondrial redox cycle can generate ROS.

Endothelial cells, vascular smooth muscle cells, and monocyte/macrophage cells contain potent defence systems against ROS, including the enzymes superoxide dismutase, catalase, and glutathione peroxidase, and non-enzymatic antioxidants. Nonetheless, excessive ROS production may occur and cause endothelial dysfunction, inflammation of the arterial wall, and atherosclerosis. Endothelial dysfunction is a very early marker of endothelial suffering. Being located between the blood and the vessel wall, the vascular endothelium has a strategic position in the cardiovascular system. NO produced by the endothelium has potent vasorelaxant activity, but it is also highly reactive with O_2. NO oxidation generates peroxynitrite, which is devoid of vasodilatory properties. Reduced NO bioavailability is therefore associated with impaired response to all vasodilators that act primarily by stimulating the endothelial release of NO. Notably, increased oxidative stress and endothelial dysfunction have been demonstrated in subjects with cardiovascular risk factors such as diabetes, hypercholesterolemia, and hypertension,