Endothelial dysfunction and inflammation: What is the link?

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Endothelial dysfunction and inflammation: What is the link? Cardiovascular disease, resulting from arteriosclerotic remodeling of the vasculature, is the main cause of death in end-stage renal disease (ESRD) patients. Early during the course of arteriosclerosis, endothelial dysfunction can be detected in various vascular beds, including peripheral forearm arteries, as well as the coronary circulation. Furthermore, endothelial dysfunction seems to predict the prognosis of cardiovascular disease. Therefore, the question deserves attention whether endothelial dysfunction is simply a marker of cardiovascular disease, or an active player in the progress of the disease. A possible link between arteriosclerosis, endothelial dysfunction, and cardiovascular disease is increased oxidative stress. Inflammatory processes involved in the pathogenesis of arteriosclerosis enhance vascular \( \text{O}_2^- \) formation, leading to endothelial dysfunction. An activated renin angiotensin system, together with oxidized low-density lipoprotein, may play a prominent role for enhanced vascular oxidative stress. In this context, the endothelium is not only a target of oxygen radicals, but may also contribute to \( \text{O}_2^- \) formation. It is the aim of this article to highlight the interplay of inflammation, endothelial dysfunction, and oxidative stress.

Doubtlessly, one of the major achievements in medicine during the last two decades has been the discovery that the vascular endothelium is not simply a semipermeable membrane, but instead forms a most active organ with endocrine and paracrine functions [1]. Concerning cardiovascular medicine, most relevant functions of the endothelium are the release of relaxing factors, contractile factors, and of antiaggregatory substances. Prominent representatives of relaxing factors are nitric oxide (NO) and prostacycline (PGI\(_2\)), both of which also have antiaggregatory properties on platelets. Endothelium-derived hyperpolarizing factor (EDHF) is another vasodilator released from the endothelium. Endothelin-1 (ET-1) and thromboxane (TXA\(_2\)) are the best-characterized endothelial contractile factors so far. Endothelial function, with its relevance to cardiovascular medicine, has frequently been reviewed [2].

Arteriosclerotic cardiovascular disease is the main cause of death in patients with ESRD [3]. Arteriosclerosis is considered to be an inflammatory disease associated with enhanced oxygen radical formation [4, 5]; enhanced oxidative stress is likely to be a major cause for endothelial dysfunction. It is the aim of this article to highlight the interplay of inflammation, endothelial dysfunction, and oxidative stress. Particular emphasis is placed on the role of angiotensin II (AngII) and atherogenic lipoproteins.

**ENDOTHELIAL DYSFUNCTION**

Endothelial dysfunction is a sensitive indicator for cardiovascular disease [6], predicts its prognosis [7], and is closely associated with the development of arteriosclerosis [8]. The term endothelial dysfunction could, of course, implicate a “loss of function” of any of the numerous activities of the endothelium. In the context of vascular diseases, however, endothelial dysfunction usually describes reduced dilatory capacities, particularly reduced NO activity. Probably the most important mechanism leading to reduced NO activity is enhanced oxygen radical formation. Superoxide anion (\( \text{O}_2^- \)) scavenges NO, yielding peroxynitrite (ONOO\(^-\)), which is rather stable but can rearrange to form nitrate and the highly reactive \( \text{OH}^- \) [9]. Many studies clearly indicate that endothelial dysfunction is closely correlated with arteriosclerosis, and that \( \text{O}_2^- \) formation is enhanced in hypercholesterolemia and arteriosclerosis [10]. Indeed, endothelial dysfunction can be detected (e.g., by means of reduced forearm blood flow, early in the development of arteriosclerosis, and in patients with hypercholesterolemia) [11]. Importantly, endothelial dysfunction occurs in arteries without visible characteristics of arteriosclerotic lesions such as fatty streaks or plaque formation.

In view of the numerous endocrine and paracrine functions of the endothelium, the question deserves attention whether endothelial dysfunction is simply a sensitive indicator for cardiovascular disease, or whether it is an active player in its pathophysiology. To shed some light on these questions, the development of arteriosclerosis shall briefly be summarized.

**Key words:** atherosclerosis, inflammation, oxidative stress, antioxidants, oxidized LDL.

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PATHOPHYSIOLOGY OF ARTERIOSCLEROSIS

Arteriosclerosis, a generic term for several diseases in which the arterial wall becomes thickened and loses elasticity, is not a static condition; rather, it reflects a continuous development over decades from macroscopically intact arteries to grossly damaged and ruptured sclerotic plaques, with different stages being present simultaneously within one individual. Important steps during atherogenesis include enhanced endothelial permeability, expression of adhesion molecules, monocyte adhesion and immigration, foam cell formation, fatty streaks, smooth muscle cell migration, plaque formation, and finally plaque rupture and thrombus formation. Earlier concepts on the pathophysiology of arteriosclerosis were based on the assumption of an initial injury leading to intimal lesions, and subsequent repair mechanisms (“response to injury” hypothesis) [12]. Today we know that even in macroscopically intact arteries, processes take place that can be best defined as chronically inflammatory [4]. Arguments that support the hypothesis of a chronic inflammation in arteriosclerosis include the following: Cells found in early atherosclerotic lesions are typically inflammatory cells (monocytes/macrophages and T-lymphocytes); early in arteriosclerosis, enhanced vascular oxygen radical formation can be detected, as well as enhanced activity of lipoxygenases; arteriosclerosis is associated with enhanced serum levels of inflammation parameters; the atherosclerotic artery produces different hydrolytic enzymes, adhesion molecules, cytokines, and growth factors, as seen in chronic inflammation. In addition, large clinical studies clearly show a strong correlation between markers of inflammation and clinical outcome in patients with coronary heart disease. In particular, high sensitive C-reactive protein (hs-CRP) has been identified as a predictor for cardiovascular events in patients with and without known coronary heart disease [13, 14].

As mentioned above, at very early stages of arteriosclerosis (characterized by enhanced endothelial permeability, expression of adhesion molecules, monocyte adhesion, and immigration), endothelial dysfunction can be detected. For example, in one study, the intima-media thickness and plaque formation of the common carotid artery of 34 men with arteriosclerosis was correlated with flow mediated dilation (FMD) of the brachial artery. A significant negative correlation of FMD with the intima-media thickness of the common carotid artery was found, supporting the concept that endothelial dysfunction is significantly related to atherogenesis [8]. In another study, a close relation between coronary artery endothelium-dependent vasomotor responses to acetylcholine and FMD in the brachial artery was found [6]. The relevance of endothelial dysfunction for the prognosis of patients was shown in a study of the Mayo Clinic: coronary endothelial dysfunction is a strong predictor for future cardiovascular events in patients with coronary heart disease [7].

Thus, there is strong evidence that arteriosclerosis is an inflammatory disease, and that endothelial dysfunction is an early event in this process. How could inflammation contribute to endothelial dysfunction, and how could the endothelium contribute to inflammation?

PATHOPHYSIOLOGY OF INFLAMMATION AND CONSEQUENCES OF ENHANCED OXIDATIVE STRESS FOR VASCULAR AND RENAL FUNCTION

Certainly, many different factors may contribute to the state of inflammation in the vasculature, as summarized in Figure 1. For patients with ESRD, it makes sense to distinguish between endogenous and exogenous factors, because the dialysis treatment, in addition to uremia, may be a source for continuous exposure to proinflammatory stimuli. What these different factors have in common is that they all—albeit by different mechanisms—enhance oxidative stress. Endothelial dysfunction through scavenging of NO by $\text{O}_2^-$ is one important consequence of enhanced oxidative stress, as outlined above. In various vascular or renal diseases, enhanced formation of reactive oxygen species is considered to be pathogenic (e.g., in atherosclerosis, glomerular diseases, renal failure, pyelonephritis, or aminoglycoside nephropathy) [15, 16]. Furthermore, oxidative stress importantly influences vascular cell cycle decisions; induction of apoptosis [17, 18], as well as induction of cell proliferation [19], has been described.
The scope of this article would be disrupted if all the influences listed in Figure 1 which contribute to inflammation and oxidative stress should be discussed in detail. Instead, we will focus on two factors, AngII and oxidized low density lipoprotein (OxLDL), for two reasons: First, AngII and OxLDL are well characterized as potentially endothelium-damaging agents, and, second, we possess pharmacologic tools to interfere with their activity.

**ANGII AND OXIDIZED LDL**

Accumulation of OxLDL in atherosclerotic plaques is a well-known event in the development of atherosclerosis [20]. Only recently did it become apparent that atherosclerotic arteries (human atherectomy preparations and arteries of hypercholesterolemic monkeys) show enrichment with AngII, co-localizing with resident macrophages [21]. Thus, AngII accumulates in the same vascular region as OxLDL. Not only do AngII and OxLDL co-localize in atherosclerotic plaques, there is strong experimental evidence that these agents interact with each other, with relevance for vascular biology and atherosclerosis.

**EVIDENCE FOR A POTENTIAL INTERACTION BETWEEN ANGII AND OXIDIZED LDL**

Clinical studies suggest that ACE-inhibitors are of particular benefit for endothelial function in hypercholesterolemic patients with high LDL levels [22, 23]. Furthermore, several studies have shown that the expression of the OxLDL receptor LOX-1, and of the AT1 receptor, is stimulated by the respective other agonist [24, 25]. In cultured smooth muscle cells, LDL induced expression of the AT1 receptor [24]. Thus, LDL may sensitize the vascular tissue to AngII. On the other hand, expression of the OxLDL receptor LOX-1 and uptake of OxLDL in human umbilical vein endothelial cells (HUVEC) is increased by AngII [25]. Together, these studies imply that AngII and OxLDL amplify the effect of the respective other agonist. But how could AngII and OxLDL contribute to atherosclerosis, inflammation, and endothelial dysfunction?

**EXPERIMENTAL EVIDENCE FOR PRO-INFLAMMATORY AND PRO-OXIDATIVE EFFECTS OF ANGII**

Recently, it has become apparent that AngII is a potent stimulator of vascular oxygen radical production, thereby contributing to endothelial dysfunction and inflammation [26, 27]. Cell culture studies with rat smooth muscle cell preparations provided first experimental evidence for stimulation of O2 formation by AngII [26]. In experiments with rat smooth muscle cell membranes, AngII-induced stimulation of O2 formation could be inhibited by diphenylene iodonium, suggesting that O2 was produced by membrane-bound NAD(P)H oxidases [26]. In the mean time, it has been shown that AngII also induces O2 formation in endothelial cells and non-vascular tissue [28]. In numerous studies, it has been demonstrated that AngII stimulates vascular NAD(P)H oxidase-dependent O2 formation via the AT1 receptor. The NAD(P)H oxidase consists of four major subunits: a plasma membrane spanning cytochrome b558 (composed of the large subunit gp91phox and the small subunit p22phox), and 2 cytosolic components, p47phox and p67phox [29]. In smooth muscle cells, the NADPH subunits p22phox, p47phox, and eventually p67phox, are involved in O2 formation [29]. In endothelial cells, mRNAs for gp91phox, p22phox, p47phox, and p67phox have been detected, and the gp91phox [30] and p22phox [19] subunits seem to be of particular importance for O2 formation in endothelial cells.

**SPECIFIC EFFECTS OF ANGII-INDUCED OXYGEN RADICAL FORMATION**

The particular importance of AngII-induced oxidative stress for vascular biology has been investigated in the context of vasomotor tone and of cell cycle regulation (e.g., enhanced O2 formation resulted in impairment of endothelium-dependent dilations) [31], which could be prevented by liposome-encapsulated superoxide dismutase. Indirect evidence that AngII-induced O2 formation takes place in vivo in humans was provided by a study using the forearm plethysmography method, which allows direct measurement of AngII-induced vasomotor actions. Constrictor actions of AngII in the human forearm were enhanced during NO inhibition and were attenuated during vitamin C infusion, suggesting AngII-associated stimulation of endothelial NO and of oxygen radicals, respectively [32]. Thus, AngII-induced O2 formation has important consequences for the NO metabolism and for cell cycle decisions, potentially affecting the course of atherosclerosis.

**OXIDATIVE STRESS INDUCED BY OXLDL AND FUNCTIONAL CONSEQUENCES**

Animal studies with cholesterol-fed rabbits provided first the indirect evidence for a role of LDL in the induc-
tion of oxidative stress. Aortas from hypercholesterolemic rabbits produced significantly more superoxide than control aortas [10]. Later, our group was able to show directly that incubation of cultured human umbilical vein endothelial cells (HUVEC), and of isolated arteries with oxidized LDL or Lp(a), stimulated O$_2^-$ formation [34]. Functional consequences of OxLDL-induced oxidative stress extend to atherosclerosis, vasomotor regulation, and endothelial dysfunction. OxLDL affects endothelial function and impairs endothelium-dependent dilations [34]. The impact of OxLDL on apoptotic cell death may be a clue to its role in the development of atherosclerosis. OxLDL induces apoptosis in HUVEC [17, 18, 35] and in smooth muscle cells of isolated aortas [17]. In all the cited studies, the use of antioxidants (SOD, vitamin C/E, or butylated hydroxytoluene) prevented the induction of apoptosis. Another common effect of OxLDL and AngII is the stimulation of endothelial cell proliferation [19].

**CLINICAL RELEVANCE**

The latest argument for the relevance of AngII-induced oxidative stress for endothelial dysfunction is provided by a study in patients with renovascular hypertension [36]. In such patients with activated renin-angiotensin system and endothelial dysfunction, the response of forearm blood flow to acetylcholine, an endothelium-dependent vasodilator, was evaluated before and after renal-artery angioplasty. The forearm blood flow in response to acetylcholine was less in subjects with renovascular hypertension than in control subjects. After angioplasty, the forearm blood flow in response to acetylcholine was increased in the patients with renovascular hypertension. Interestingly, angioplasty decreased serum malondialdehyde-modified LDL, an index of oxidative stress that was positively correlated with endothelial dysfunction. The authors conclude that excessive oxidative stress is involved in endothelial dysfunction in patients with renovascular hypertension.

**SUMMARY**

In early stages of arteriosclerosis, enhanced oxygen radical formation already leads to endothelial dysfunction. The endothelium is a target of oxidative stress by means of attenuated endothelium-dependent dilation, enhanced cellular turnover, and cell death. However, the endothelium also contributes to the vascular state of inflammation because it is one source of O$_2^-$ formation. Beside other factors, AngII and OxLDL are important stimuli for vascular oxygen radical formation, endothelial dysfunction, and cell proliferation.

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