THE ROLE OF NITRIC OXIDE (NO) AND STATINS IN ENDOTHELIAL DYSFUNCTION AND ATHEROSCLEROSIS

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Abstract

Vascular endothelium plays an essential role in the regulation of the vascular tone, contributing to maintaining the balance between vasodilatation – vasoconstriction, owing to the production of endogenous nitric oxide (NO) under the action of different endogenous and exogenous factors. The loss of the functional integrity of the vascular endothelium, called endothelial dysfunction, in different heart diseases, is associated with the damage of NO production. Atherosclerosis, an inflammatory chronic disease, is characterised from the beginning through leukocytes adhesion to the vascular endothelium, the first step of endothelial dysfunction in atherosclerosis. Statins, recently introduced in the therapy for lowering the cholesterol level, through this effect and also the pleiotropic effects, improve the endothelial dysfunction through the increase of endogenous production of NO in different pathways.

Keywords: nitric oxide, statins, endothelial dysfunction, atherosclerosis

The role of nitric oxide in endothelial dysfunction and atherosclerosis

Until 1980 vascular endothelium was considered a passive, semi permeable barrier with role in bidirectional changes between blood and tissue gases, ions, endogenous and exogenous substances with a small molecular weight. In 1980, this perception was modified with the studies of Furchgott and Zawadzki, who established the necessity of an intact vascular endothelium in order to obtain a vasodilatation induced by acetylcholine. From this moment, vascular endothelium demonstrated to have an active
role in the vascular homeostasis regulation. Later, these scientists pointed out an endothelial relaxation factor EDHF (endothelium derived hyperpolarizing factor) liberated by acetylcholine in the presence of an intact endothelium. When the endothelium is impaired, acetylcholine produces constriction as a result of the vasoconstrictor effects on the smooth muscle, which aren’t counteracted [1]. This was identified as the nitric oxide, because both have similar chemical and biological properties [2, 3].

In the vascular endothelial cells (figure 1), NO is formed from the terminal guanidino nitrogen of the amino acid L-arginine during the conversion to L-citrulline. This reaction is catalysed by the endothelial isoform of NO syntase (eNOS), which is constitutive expressed, activated and regulated by calcium and calmoduline [5].

Figure 1

Synthesis of NO-derived endothelium (Richard E. Klabunde, Cardiovascular Physiology Concepts) (GC – guanylyl cyclase; cGMP- 3,5,-cyclic guanosine monophosphate, iNOS- inducible isoform of nitric oxide synthase)

Figure 2

Once it was synthesized, NO diffuses from the endothelial cell to the vascular smooth muscle cell, where it activates the enzyme guanylate cyclase (GC) which catalyses the transformation of GTP into cGMP (figures 1 and 2). The increase of cGMP activates a protein kinase GMP-dependent which induces the kinase phosphorylation of the light chain of myosin and has as result the stabilization of the inactive form of myosin [5]. The result of this action is the consecutive vasodilatation.

Recent studies have shown that beside the role in the control of the vascular tone, NO endothelial derived (EDNO) has other roles in the prophylaxis of atherosclerosis: regulation of the platelet function (platelet aggregation and adhesion), vascular increasing and remodelling (inhibits the proliferation and migration of the vascular and myocardial smooth muscle cells), role in the adhesion of the inflammatory cells on the endothelial surface, role in the modulation of the oxidative stress and oxidation of LDL and also regulation of the apoptosis in atherosclerosis [6].

The sum-up of two factors: the loss of the vascular relaxation directly dependent of the endothelium (flow – dependent) and the NO dependent are the main reason for endothelial dysfunction and play a very important role in the pathogenesis of heart failure. Endothelial dysfunction on vascular peripheral levels contributes to the increased peripheral resistance in patients with heart failure [7]. Endothelial dysfunction, as a pathophysiology disorder, is early present in the initiation of the atherosclerotic process. It represents an important risk factor for atherosclerosis, as well as for other vascular diseases.

The bound between atherosclerosis and the impairment of EDNO was first shown by Ludmer and his colleagues, who demonstrated that intracoronary perfusion with acetylcholine produces vasoconstriction in the epicardic coronary arteries in patients with angiographic evidence of atherosclerosis [8]. Otherwise the response of these patients to infusion with nitro-glycerine is vasodilatation, which demonstrates that the vascular endothelium has kept its capacity of response to exogenous NO; it only lost the capacity to generate EDNO, endogenous NO. The injury of EDNO activity seems to appear early in the atherosclerotic process, which is proved by the impaired coronary vasodilatation response to perfusion with acetylcholine at a lot of patients with hypertension [9], hypercholesterolemia [10], diabetes [11] or smokers [12], before the appearance of the angiographic signs of atherosclerosis. In addition to the early role in the initiation of the atherosclerotic process, the generation of the atheromatous plaque in stable coronary diseases, the injury of EDNO activity plays an important role itself or with the consequences it produces, and in the splitting of the plaque, contributing then to the pathogenesis of acute
coronary syndromes, which is demonstrated by the fact that the loss of NO-dependent vasodilatation leads to increasing shear stress [13] from the vessels, which can increase the risk for plaque rupture.

Mechanisms (figure 3) which are essential for the impairment of EDNO activity, respectively for the appearance of endothelial dysfunction [14, 15] are:

- dysfunctional signal transduction receptor – endothelial cell;
- decreased bioavailability of the substrate L-arginine;
- altered expression of gene NOS3 and stability of mARN; polymorphism NOS3;
- altered eNOS activity;
- increased destruction of NO;
- changes in the balance between NO derived endothelium and the hyperpolarizing factor (EDHF);
- decreased sensitivity of atherosclerotic smooth muscle to NO.

![Figure 3](image)

**Figure 3**
Mechanisms of NO-related vascular endothelial dysfunction in atherosclerosis and hypercholesterolemia [14]

(THB- tetrahydrobiopterin; EDHF- endothelium-derived hyperpolarizing factor; ADMA- asymmetric dimethylarginine; DDAH- dimethylarginine dimethylaminohydrolase; AT1- angiotensin II subtype 1 receptor)

**The role of the statins in endothelial dysfunction and atherosclerosis**

Atherosclerosis, considered a long time as a passive process of cholesterol accumulation in the arteries walls, is now identified to be an inflammatory chronic process, where the inflammation is present in all its steps, from the initiation, progression and splitting of the plaque to the thrombosis complications of the atherosclerosis [16]. During the atherosclerosis process the normal functional homeostasis of the vascular
endothelium is affected as a response to any kind of aggression to the endothelium, leukocyte adhesion to the impaired endothelial cells representing the first step of this inflammatory process [17].

The discovery of the statins represents an important progress in the treatment of atherosclerosis through its direct action on the cholesterol through competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), which has as result the decrease of endogenous cholesterol synthesis in the liver and the increase of the LDL cholesterol catabolism (it results the decrease of the cholesterol level in the organism) and also through the pleiotropic effects (figure 4) which interfere with a lot of atherosclerotic components, like cell migration, endothelial function, splitting of the plaque, tendency to thrombosis, etc [18].

**The role of statins related to lowering of cholesterol**

Mechanisms, which contribute to the lowering of cholesterol, increased in atherosclerosis, are [19]:

- inhibition of HMG CoA reductase, the enzyme that converts HMG-CoA into mevalonic acid, a cholesterol endogenous precursor;
- direct effects of HMG CoA reductase inhibition, inhibiting liverwort the synthesis of apolipoprotein B-100, determining a reduction of the synthesis and secretion of triglyceride rich lipoproteins;
- reduction of LDL susceptibility towards oxidation;
- inhibition of the expression of type A scavenger receptor in THP -1 cells and human monocytes, which decreases the receptor-mediated degradation of oxidised LDL.

![Figure 4](image)

*Immunomodulatory mechanisms of statins [20]*

(SMC- smooth muscle cells; IFNγ- interferon γ; LFA-1- leukocyte function antigen-1; ICAM-1- intercellular adhesion molecule 1)
Pleiotropic effects of statins

Beside the effects on the cholesterol, the statins present a lot of other actions, known as the pleiotropic effects of the statins. These are defined to be the effects other than those due to lowering LDL levels and independent of the LDL level, like:

- improved endothelial function
- diminish vascular inflammation
- improve ventricular function of heart failure
- antithrombotic effect
- reduce the rate of vascular events
- antioxidant effect

but also other effects: reduction in blood pressure, reduced incidence of type II diabetes, reduction of allograft organ rejection, regression of tumour cells, reduced incidence of dementia, reduction in osteoporosis and risks of fracture, all of them contributing to a better quality life of the patient.

The role of the statins on the endothelial function

Vascular Endothelium

The essential role in the regulation of the vascular homeostasis belongs to the vascular endothelium, which in conditions of a good functionality is responsible to maintain a balance between vasodilatation – vasoconstriction, between inhibition – stimulation of proliferation and migration of smooth muscle cells, between thrombosis – fibrinolysis \([22, 23]\). Thus, a dysfunction of the vascular endothelium is considered a first sign in the atherosclerotic process, before angiographic or ultrasonic changes, which show the atheromatous plaque \([22]\). Therefore is relieved the interest for the role of statins in lowering the cholesterol (directly and indirectly) improving the endothelial function. Statins improve endothelial function through the following mechanisms (figure 5):

- enhanced endothelial NO production by decrease of cholesterol, by up regulating posttranscriptional mRNA of eNOS \([24]\) and by antioxidative effects (reduction of reactive oxygen species, increase of superoxide elimination and decrease of oxidized LDL) \([25]\);
- reduced production of endothelin-1, endothelial vasoconstrictor factor \([26]\);
- diminish the affinity for AT1 receptors \([26]\);
- stimulation of angiogenesis through proliferation, migration and surviving of the circulating endothelial progenitor cells in part by enhancing vascular endothelial growth factor production (VEGF) \([27, 28]\).
The direct and indirect role of statins by improving the endothelial function [29] (ET-1- endothelin-1; Ang II- angiotensin II; oxLDL- oxidized LDL; VLDL- very low-density lipoprotein; TNFα- tumor necrosis factor α; IGF-1- insulin-like growth factor-1; VCAM-1- vascular cell adhesion molecule; ICAM-1- intracellular adhesion molecule)

**Vascular swell**

Statins decrease the swell of the vascular wall [26, 30] by:
- decreasing the level of C-reactive protein,
- decreasing the synthesis of proinflammatory cytokines (IL-1, IL-6, IL-8, TNFα),
- diminishing the leukocyte adhesion to endothelial cells,
- inhibiting macrophage growth and smooth muscle cell migration and proliferation.

Because of the aspects presented above, it can be supposed that improve of the activity EDNO can reduce the endothelial dysfunction, the risk of appearance of the atheromatous plaque or the splitting of one already existent, thus reducing the risk of complications of vascular diseases. Also the association of a treatment by reducing the cholesterol levels could improve endothelial dependent vasodilatation at patients with high levels of cholesterol, contributing to the primary/secondary prevention of cardiovascular diseases (figure 6).
Figure 6
Relationship NO-statins in endothelial dysfunction and atherosclerosis as treatment basis in cardiovascular diseases [30]
(RhoA- Ras homolog gene family; MMPs- matrix metalloproteinases; TF- tissue factor; t-PA- tissue-type plasminogen activator; PAI-1 – plasminogen activator inhibitor-1; TXA2- thromboxane A2)

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