Nitric oxide and endothelial cell aging

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1 author:

Judith Haendeler
Universitätsklinikum Düsseldorf
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REVIEW ARTICLE

Judith Haendeler

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Abstract Nitric oxide (NO) is a crucial factor for the integrity and function of the endothelium. Besides its role in blood pressure regulation, NO acts antithrombotically and antiapoptotically. The laminar flow in the blood vessel, called shear stress, is the most potent endogenous protective force against endothelial cell apoptosis, mostly by increasing the expression of the endothelial NO synthase (eNOS) and thereby increasing NO bioavailability in endothelial cells. However, during the process of endothelial cell aging, shear stress is unable to induce eNOS expression and protect against apoptosis induction. Moreover, apoptosis induction is correlated with aging in vivo, suggesting a link between NO bioavailability, aging, and apoptosis. Moreover, cellular aging is accompanied with an increase in reactive oxygen species (ROS), which results in an imbalance of the redox status of the cell. Thioredoxin is an important redox regulator in endothelial cells and can "bind" NO. *S*-nitrosylation of thioredoxin increases its enzymatic activity, which in turn leads to reduced intracellular ROS and apoptosis, suggesting that thioredoxin may play an important role in NO bioavailability. One crucial step in the process of cellular aging is the telomerase activity that is reduced in aged endothelial cells. NO can inhibit the decrease in telomerase activity and thereby delay the onset of replicative senescence. Thus, the reduction in NO bioavailability is a crucial factor for endothelial cell aging and apoptosis.

Keywords Nitric oxide bioavailability · Endothelial cells · Thioredoxin

J. Haendeler (✉)
Molecular Cardiology,
Department of Internal Medicine III,
University of Frankfurt,
Theodor Stern-Kai 7
60590 Frankfurt, Germany
e-mail: j.haendeler@em.uni-frankfurt.de
Tel.: +49-69-63017340
Fax: +49-69-630183462

Introduction

The endothelium is located in a strategically anatomical position within the blood vessel wall. It acts as a barrier between the blood and the vascular smooth muscle cells and thereby plays an important role in maintaining blood vessel homeostasis. Therefore, the functional integrity of the endothelium monolayer is essential for preventing vascular leakage and the formation of atherosclerotic lesions [1]. Injury of the endothelial monolayer results in inflammatory remodeling of the vessel wall. This process is characterized by invasion of inflammatory cells and proliferation of smooth muscle cells and leads to the development of atherosclerotic lesions. In the case of atherosclerosis, the relevant pathology occurs in the coronary arteries. The endothelial cells that line these arteries play a definite role in the cascade of pathology, and these cells show profound changes with age [2, 3]. Aging is one of the major risk factors for the development of cardiovascular disease.

Nitric oxide (NO) plays an important role in regulating the functional integrity of the endothelium, which acts as a barrier between the circulating blood and the underlying tissue [4, 5]. Thus, NO regulates the vascular tone, provides antithrombotic and antiinflammatory activity, and inhibits endothelial cell apoptosis [5–7]. Recent studies support the hypothesis that advanced age leads to impaired endothelial NO synthesis. The following review will summarize the evidence for a potential role of NO bioavailability in endothelial cell aging and apoptosis.

NO bioavailability in endothelial cell apoptosis and aging

NO is a short-lived free radical gas with multiple biological effects. It is synthesized from L-arginine by three different isoforms of NO synthases (NOS) [8], and it is a key molecule in regulating diverse biological processes. In the endothelium, the most relevant isoform is the endothelial nitric oxide synthase (eNOS), which produces NO at moderate rates. The important antiatherosclerotic function of

NO is evidenced by experimental and clinical studies. eNOS-deficient mice develop hypertension, exhibit a reduced growth-factor-induced angiogenesis, and show accelerated atherosclerotic lesion formation [9–11]. Moreover, impaired endothelial NO synthesis predicts a worse outcome in patients with coronary artery disease [12].

The most important physiological stimulus for the activation of eNOS and the continuous release of NO is the laminar flow in the blood vessel, called shear stress. Shear stress is the most potent endogenous protective force against endothelial cell apoptosis. Classical risk factors, which are known to promote endothelial dysfunction in vivo, can induce endothelial cell apoptosis in vitro. Pro-inflammatory cytokines and the peptide hormone angiotensin II induce apoptosis in endothelial cells [13, 14]. Likewise, oxidized low-density lipoprotein (LDL) and high concentrations of reactive oxygen species (ROS) trigger apoptosis of endothelial cells [15–19]. All of the apoptosis-inducing stimuli mentioned above have been shown to be completely inhibited in vitro by the application of shear stress, demonstrating the powerful protective role of shear stress. However, it has to be noted that in aged endothelial cells, apoptosis induced by TNFα or oxidized LDL could not be prevented by shear stress [2]. Interestingly, shear-stress-induced increase in NO bioavailability is impaired in aged endothelial cells [2], suggesting a potential link between NO bioavailability, aging, and apoptosis (Fig. 1). Studies in animals and humans further suggest a link between aging and NO bioavailability. In rats, NO bioavailability in the aorta as well as endothelium-dependent relaxation decline with age [20]. Aging in humans could also be correlated with reduced NO bioavailability [21]. A recent study demonstrated that endothelial cell apoptosis is enhanced in aged monkeys, suggesting a link between aging and apoptotic cell death [22]. Moreover, further studies demonstrated that senescent endothelial cells are detectable in reendothelialized areas of the vascular wall after repeated balloon injury [23, 24]. These regenerated endothelial cells are also characterized by reduced NO synthesis [23]. Therefore, one may hypothesize that the reendothelialized regions are predisposed to apoptosis induction, which may accelerate endothelial cell aging and further reduce NO synthesis.

Signaling pathways involved in reduced NO bioavailability and aging

Aging is an independent risk factor for the development of endothelial dysfunction. Furthermore, endothelium-dependent vasodilatation and the basal release of NO are reduced during aging. The most important physiological stimulus for the basal release of NO is shear stress. Shear stress increases protein expression of eNOS and further post-translationally increases enzymatic activity [25, 26] (Fig. 1). The reduction of bioavailable NO and the content of *S*-nitrosylated proteins in aged endothelial cells may be explained by a downregulation of the phosphorylation and protein expression of eNOS [2]. This is in accordance with in vivo studies demonstrating downregulation of eNOS expression in aged rats [27] and in atherosclerotic human vessels [28]. Moreover, loss of eNOS protein expression is paralleled by reduced Akt protein expression in aged endothelial cells, which may further reduce NO synthesis because Akt is required for eNOS activation [2, 25, 26].

In addition to downregulation of eNOS expression, an increase in superoxide production may decrease NO bioavailability [29, 30]. Indeed, there is growing evidence that an accumulation of age-related damage to mitochondria occurs that leads to enhanced ROS formation [31, 32]. Moreover, vascular peroxynitrite formation has been shown to increase with age [33]. Therefore, elevated formation of peroxynitrite from superoxide and NO could be another possible mechanism for the reduced NO bioavailability during the process of endothelial aging.

In line with the reduced NO bioavailability in endothelial cells is the correlation between aging and endothelial cell apoptosis. Endothelial cell apoptosis is executed via

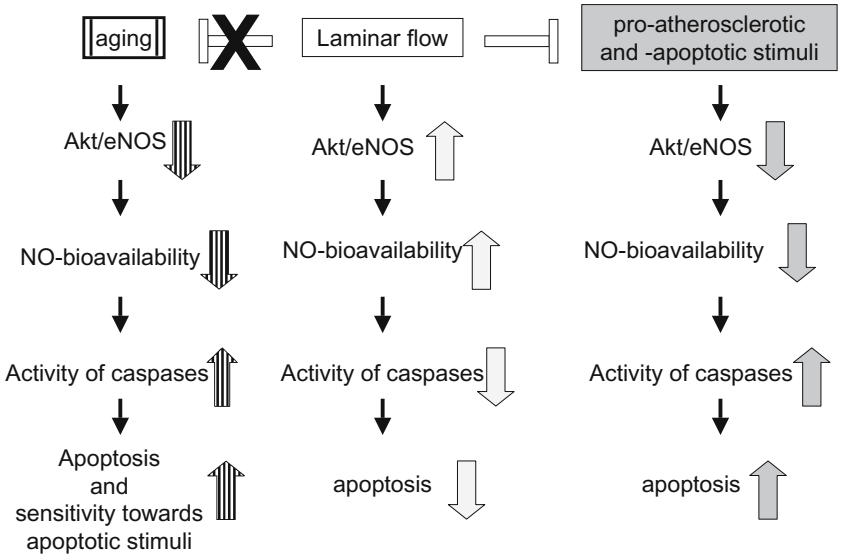

Fig. 1 Laminar flow is the most potent inhibitor of apoptosis-inducing stimuli in endothelial cells. One mechanism for apoptosis inhibition by laminar flow is the activation of Akt and endothelial nitric oxide synthase (eNOS), which subsequently leads to an increase in NO bioavailability and a decrease in activation of caspases. In contrast, laminar flow does not protect from apoptosis induction during the process of endothelial cell aging

the activation of the cysteine protease family, the caspases [34, 35]. NO can interfere with the execution of apoptosis by inhibiting caspases via *S*-nitrosylation of essential cysteine moieties [35, 36, 37]. During the aging process of endothelial cells, NO bioavailability is reduced, resulting in a dramatic caspase activation and suggesting an important link between aging and apoptosis [2]. Importantly, laminar flow, the most potent inhibitor of apoptosis inhibition in endothelial cells, is incapable of increasing eNOS activation and NO bioavailability in aged endothelial cells, which may explain the higher sensitivity of aged endothelial cells toward apoptotic stimuli (Fig. 1).

In addition, an important redox regulator of endothelial cells is the protein thioredoxin [38]. The thioredoxin system consists of two oxidoreductases, thioredoxin-reductase and thioredoxin. Thioredoxin is ubiquitously expressed in mammalian cells, and thioredoxin deficiency results in a lethal phenotype, underscoring the fundamental importance of the enzyme [39]. Thioredoxin exerts its enzymatic activity as an oxidoreductase via cysteine-32 and cysteine-35. Recently, thioredoxin has been identified as a novel target for *S*-nitrosylation [19]. *S*-nitrosylation of thioredoxin at cysteine-69 increases its antioxidative and antiapoptotic capacity. Thereby, NO exerts an effect on the redox state of endothelial cells, besides the chemical interaction with H_2O_2. However, during the process of endothelial cell aging, ROS are increased [32] and expression of eNOS is decreased [2], which in turn could result in a reduction in thioredoxin activity, one of the most important antioxidants in endothelial cells. In fact, prolonged treatment with the antioxidant *N*-acetylcysteine can delay the onset of replicative senescence in endothelial cells [32], giving rise to the hypothesis that the redox status of the cell during aging processes is altered.

Another important mechanism that is involved in the process of aging and can also be regulated by NO is the activation of telomerase. It is well known that telomeres are the ends of the chromosomes, which are shortened during each cell division [40]. Thus, somatic cells can undergo only a limited number of cell divisions and are then arrested in a state of replicative senescence. The enzyme responsible for telomere maintenance is the ribonucleoprotein telomerase reverse transcriptase (TERT) [41]. During the process of endothelial cell aging, TERT activity is reduced and precedes the onset of replicative senescence. Preincubation with NO inhibits the decrease in TERT activity and delays the onset of replicative senescence [42]. The underlying mechanisms by means of which NO can interfere with the reduction in TERT activity during the process of aging are not yet clear. Further studies are needed to determine the mechanisms involved.

Conclusion

Taken together, the maintenance of an intact endothelial monolayer and an intact endothelial function are necessary to protect against the initiation of atherogenesis. However, the process of aging, which is an independent risk factor for atherosclerosis, leads to reduced NO bioavailability and enhanced sensitivity toward apoptosis. Thus, aging may importantly contribute to the destruction of the endothelial cell monolayer. The disturbance of the redox status seems to play an important role in the process of aging. However, further studies are needed to delineate the underlying mechanisms.

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