The Endothelium in Hypertension: A Short Review

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Introduction

Due to its high prevalence and the severity of its complications, hypertension is the leading independent and modifiable risk factor in cardiovascular disease. However, despite the significant advances in understanding the disease process and the availability of effective diagnosis and treatment methods, identifying the actual cause is still a considerable challenge.

Over the last three decades, the accumulation of evidence from experiments and clinical trials suggests that primary hypertension (95% of cases) results from a complex interaction between genetic and environmental factors. Association studies have identified the involvement of polymorphisms in several candidate genes, genetic variants and genes that are over- or under-expressed as intermediary phenotypes which regulate the renin-angiotensin-aldosterone system, sympathetic nervous system, beta-adrenergic receptors, endothelial nitric oxide synthase (eNOS), cytochrome P450 2C19 system, nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), the kallikrein-kinin system, tubular sodium channels and various genomic sites that may include other genes that contribute to primary hypertension. The environmental factors include social factors (globalization, urbanization, longevity, education, housing, family income), behavioural factors (unhealthy diet, excessive salt intake, smoking, alcohol consumption, physical inactivity) and metabolic factors (obesity, diabetes and dyslipidemia) (Figure 1). [1]

**Key Words:** Endothelium, hypertension, nitric oxide, oxidative stress, blood pressure, smoking, diabetes, arterial stiffness, myocardial ischemia, resistant hypertension

**Figure 1:** Factors contributing to the development of hypertension and its complications. RAAS: renin-angiotensin-aldosterone system; SNS: sympathetic nervous system; eNOS: endothelial nitric oxide synthase; NADPH oxidase: nicotinamide adenine dinucleotide phosphate oxidase

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The endothelium also inhibits vascular smooth muscle cell proliferation, preventing cell migration through the synthesis of NO, which inhibits cell proliferation and the secretion of growth factors and other cytokines that exert autocrine and paracrine control of cell proliferation. [12]

In addition to these actions, the endothelium participates in the metabolic degradation and transformation of numerous endogenous agents, such as norepinephrine, 5-hydroxytryptamine, prostaglandins E and F, leukotrienes, adrenine nucleotides, adenosine and others. [13,14]

The endothelial vasodilator mechanism is mediated by vasoactive substances, primarily NO, but also by prostacyclin (PGI2), bradykinin, endothelium-derived hyperpolarizing factor (EDHF), C-natriuretic peptide, metabolites of monooxygenases and other substances. [15-20] On the other hand, the vasoconstrictor mechanism is mediated by several substances, including angiotensin II (Ang-II) - formed by the action of angiotensin-converting enzyme present in the vascular endothelium, which besides converting angiotensin I to Ang-II, acts on the degradation of the vasodilatory peptide bradykinin by kininase II, an enzyme identical to the angiotensin-converting enzyme. In turn, bradykinin induces the release of endothelial NO, prostacyclin and EDHF.

Lastly, the endothelin-converting enzyme (ECE), present on the endothelial surface, produces a potent vasoconstrictor, endothelin-1 (ET-1), by a catalytic action on big endothelin-1. [21] Other vasoconstrictor molecules, such as thromboxane A2 (TxA2) and endoperoxide (PGH2 or cyclooxygenase-2), are involved in the pathophysiology of vasomotor control (Figure 2).

The main molecules synthesized by the endothelium and their vasoactive and hemostatic effects, cell growth modulation and inflammatory actions are presented in

The concept of vascular tone regulation came from the works of Furchgott et al., who demonstrated the importance of vascular endothelial integrity in vascular response to acetylcholine and other agonists. Furthermore, the discovery of the central vascular tone modulator, NO, raised the possibility of the participation of ED as an essential mechanism in the pathogenesis of hypertension.

Initial studies conducted in the 1980s investigated the behaviour of endothelium-dependent vasodilation in aorta rings from spontaneously hypertensive and normal rats [22]. At this time, researchers also studied other types of experimental hypertension models, such as deoxycorticosterone acetate (DOCA)-salt-sensitive rats with renovascular hypertension (one clip on the renal artery) and rats with coarctation caused by surgical bandages around the aorta in which reduced vasodilator response to acetylcholine was observed [23]. This change remained even after removing the clip from the kidney artery or the bandages from the aorta, suggesting that the change in

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endothelial function is secondary to high blood pressure [24]. Similar results were observed in other experimental models [25,26].

In addition, studies with hypertensive patients showed changes in endothelium-dependent vasodilation as a response to acetylcholine infusion and maintenance of endothelium-independent response after the injection of nitrodiolators in different vascular beds including the brachial and coronary arteries, and the cutaneous microcirculation [27-30].

Subsequently, the significance of ED as a primary or secondary phenomenon in hypertension was documented.
by Taddei et al. [31], who demonstrated alterations in the
vasodilating response to acetylcholine in descendants of hypertensive individuals. However, this effect was
not observed in children of normotensive subjects. Successively, lower NO bioavailability was demonstrated in
the progeny of hypertensive patients compared to those
from normotensive individuals. [32] These observations
underlined the participation of ED and reduced NO
bioavailability in the pathogenesis of hypertension. These
findings were supported by the publication of studies
demonstrating antihypertensive drugs’ ability to increase
NO’s bioavailability and restore endothelial function. [33,34]

Endothelial dysfunction

The term ED has been widely used to characterize
any alteration of regular endothelial activity, such as
changes in vasomotor response, cell proliferation, adhesion
and aggregation of platelets, vascular permeability and
leukocyte interaction with the vascular wall. It is also the
first detectable functional change in the evolution of
atherosclerotic disease and has an excellent correlation
with cardiovascular events.

ED has been documented in almost all atherosclerosis
and cardiovascular disease conditions. In humans, ED
has been observed in patients with hypertension, [2,
27] in normotensive individuals with a family history of
hypertension, [31] in active smokers, [35-37] in passive
smokers, [38] dyslipidemic patients, [39,40] diabetes,
[41,42] obesity, [43] and hyperhomocysteinemia, [44]
as well as in ageing [45] and patients with infectious and
inflammatory diseases. [46,47]

Commonly, ED is described as an imbalance
between vasodilator and vasoconstrictor substances that
directly affects vascular function. However, the primary
manifestation of ED is characterized by decreased NO
bioavailability secondary to decreased synthesis and release
by eNOS or increased degradation by reactive oxygen
species (ROS). In the early stages, the endothelial function
can be partially maintained by compensatory upregulation
of prostacyclin and EDHF.

The reduction in NO bioavailability can be the result
of decreased eNOS activity due to genetic factors, such
as polymorphisms in genes that encode eNOS [48] or by
environmental factors, such as hypoxia, decreased blood flow,
shear stress, reduced bioavailability of L-arginine substrate,
 deficiency of cofactors such as tetrahydrobiopterin (BH4),
 altered signal transduction due to enzyme phosphorylation
and its interaction with proteins such as heat shock protein
90 (HSP90) and calmodulin (which modulates the activity
of eNOS), or inhibition of eNOS function induced by
endogenous inhibitors such as asymmetric dimethylarginine
(ADMA). High concentrations of ADMA have been found in
patients with hypercholesterolemia, [49] individuals with
hypertension [50-52] and patients with renal failure. [53] In
addition, ADMA seems to be an excellent predictor of acute
coronary events [54] and cardiovascular mortality. [55]

ADMA is selectively degraded by the dimethylarginine
dimethylaminohydrolase enzyme (DDAH) [56] and eliminated
by renal excretion. This enzyme appears extremely sensitive
to oxidative stress, an effect that is probably responsible
for elevated ADMA levels in the plasma of patients with
hypercholesterolemia, hypertension, diabetes mellitus and
other conditions associated with cardiovascular disease.

Inactivation of NO also occurs because of binding to
various molecules such as haemoglobin, albumin, and
mainly due to its interaction with ROS (superoxide anions).

The term ‘oxidative stress’ describes conditions
involving increased levels of ROS, also called free radicals
or ‘oxidants’. These molecules are intermediary products of
the oxidation-reduction (redox) reaction of oxygen (O2) and
water (H2O) and comprise two main groups, free radicals
such as superoxide anions (·SO2−), hydroxyl radicals (OH−)
and NO, and non-radical derivatives such as hydrogen
peroxide (H2O2) and peroxynitrite (ONOO−). [57]

Under physiological conditions, the extent to which
oxidants are generated is counteracted by the action of
endogenous antioxidant enzymes superoxide dismutase
(SOD), catalase and glutathione peroxidase (GSH), thereby
limiting the interaction between NO and free radicals. The
main sources and pathways that generate ROS are shown
in Figure 3.

Endothelial dysfunction and hypertension

Change in the vasomotor function is an important
feature of the vascular bed in hypertensive patients.
In the broadest sense, this modification refers to an
imbalance in the endothelial production and release of
substances that regulate vascular tone (vasodilators and
vasoconstrictors) and cell proliferation and to structural
changes accompanying vascular remodeling.

However, in conditions of rest, the arterial bed exhibits
a baseline vasoconstriction called vascular tone that
is modulated by central (sympathetic nervous system)
and peripheral (participation of the renin-angiotensin-
aldosterone system) control mechanisms and a local
mechanism (endothelial) whose effect is greater than the
central and peripheral mechanisms.

Thus, the endothelium participated in the
pathophysiology of hypertension and decreased NO
bioavailability is a significant marker of this functional
change. [2, 58] However, the mechanisms involved in
ED differ according to the experimental model, the type
of human hypertension and the vascular bed evaluated (microcirculation or macrocirculation).

The primary studies that evaluated ED in hypertensive individuals were performed on the peripheral microcirculation with the venous plethysmography and brachial artery perfusion techniques using local infusions of vasoactive substances and observing the vasodilator or vasoconstrictor response to the different substances administered. [27] This technique undeniably demonstrated the critical association between ED and NO-mediated hypertension.

Thus, ED in hypertensive individuals may be related to decreased NO bioavailability due to its reduced synthesis and release by eNOS or increased degradation by ROS. It can also be associated with the release of vasoconstrictor substances derived from the endothelium, such as endoperoxides, thromboxane A2, prostaglandin H2 (PGH2), ET-1 and Ang-II, which stimulates NADPH oxidase and the production of ROS. Furthermore, conditions involving increased ROS levels - involved in the inactivation of NO - are present in patients with primary hypertension, renovascular hypertension, malignant hypertension, salt-sensitive hypertension and preeclampsia. Under these conditions, there is an increase in oxidative stress due to the increased generation of ROS from sources that produce ROS such as xanthine oxidase enzymes, free eNOS, NADPH oxidase (NOX), cyclooxygenases, lipoxygenases, and mitochondrial electron transport chain enzymes and decreased activity of the major endogenous antioxidant enzymes, SOD, catalase and glutathione peroxidase.

However, in this condition, a complex interaction between NO and ET-1 seems to contribute to the development of ED and effectively, ET-1 plays a vital role in blood pressure increases and vascular remodelling in moderate to severe hypertensive patients, especially those sensitive to salt and Afro-descendants. [59]

Furthermore, the role of oxidative stress in ED-induced hypertension in humans is also supported by data showing that vitamin C restores NO production and improves endothelial function in essential hypertension. [60] Thus, oxidative stress is one of the primary mechanisms, if not the main contributor, to the development of ED.

This reduced NO bioavailability resulting from an increase in ‘oxidative stress’ can be offset, in part, by the activation of alternative routes, including by the production and release of prostacyclin derived from the cyclooxygenase (COX) pathway and by EDHFs, which contribute to maintaining endothelium-dependent dilation. However, this same COX pathway appears to be involved in the synthesis and release of vasoconstrictor prostanoids, which operate independently, and are not produced in basal conditions. Thus, the presence of risk factors such as hypertension, diabetes and ageing stimulates the release of vasoconstrictive substances derived from the COX cycle, such as thromboxane A2 and PGH2 (endoperoxide). These substances act on smooth muscle cells' thromboxane prostanoid receptor (FP), promoting vasoconstriction and contributing to ED. [61]

Experimental studies in humans have demonstrated that inhibition of COX-1 and not COX-2 is associated with reversing ED induced by prostanoid vasoconstrictors.

Furthermore, recent demonstrations of the reversibility of ED using inhibitors of TP and COX-1 receptors raise the possibility of interfering with the progression of the disease and restoring normal endothelial function, thus reducing the risk of cardiovascular events. [62,63]

### Microparticles

Extracellular vesicles are a heterogeneous population of small membrane fragments released into the circulation from several cell types under normal conditions but more so in pathological conditions. [64] According to their size, content and mechanism of formation, these particles are divided into three categories: exosomes (40-100 μm in diameter), apoptotic bodies (1-5 μm in diameter) and microparticles [1] (0.1-1.0 μm in diameter). In the past, microparticles were considered inert waste. However, nowadays they represent a major pathway of exchanges and biological signalling, capable of transferring information from the parent cell to several target cells by direct contact (cell-cell) or, alternatively, by the secretion of soluble mediators and effector molecules. In humans, the microparticles primarily originate from platelets and, to a lesser extent, from leukocytes and endothelial cells. Increased concentrations of microparticles are associated with vascular injury and inflammation and are biomarkers of cardiovascular disease, including myocardial infarction, diabetes, hypertension, preeclampsia and metabolic syndrome. The endothelium is one of the main targets of circulating microparticles. Endothelial response to increased levels of circulating microparticles may be acute, resulting in the release of several factors or prolonging the altered expression of genes involved in the structural and functional regulation of the vascular wall. Although this response assists in regulating functions under normal conditions, under pathological conditions, microparticles undergo phenotypic changes in their operations, taking on procoagulant, pro-inflammatory and apoptotic actions, leading to ED and the development of cardiovascular diseases.

Microparticles originating from T lymphocytes decrease NO production and increase the production of ROS, with consequent changes in the vascular tone. These effects
are related to reduced eNOS activity, which depends on phosphatidylinositol-3-kinase (PI3K), extracellular signal-regulated kinase 1 and 2 (ERK 1/2), and nuclear factor kappa beta (NF-kB), which are directly affected by microparticles. In addition, elevations in the microparticle concentration also promote ED in conductance arteries and small resistance arteries in response to agonists and shear stress, thus aggravating the existing pathological condition.

Strategies to remove microparticles may have a therapeutic indication to prevent cardiovascular disease. Accordingly, due to their anti-inflammatory effect, inhibitors of 3-hydroxy-3-methyl glutaryl-coenzyme A reductase (3-HMG-CoA reductase) reduce the release of microparticles from the endothelium. Similarly, agonists of the proliferator peroxisome activated receptors (PPAR), such as rosiglitazone, inhibit the release of microparticles originating from leukocytes and thus reduce inflammatory activity and vascular dysfunction mediated by leukocytes and by the increased expression of NF-kB.

Dihydropyridine calcium channel blockers improve endothelial function due to the reduction of circulating microparticles. Nifedipine, in patients with type 2 diabetes, showed a significant decrease in levels of endothelial microparticles. [72]

Circulating microparticles from platelets and endothelial cells are elevated in hypertensive patients with type 2 diabetes. The administration of the angiotensin receptor blocker (losartan) reduces the concentration of these circulating microparticles.

These observations point to a series of pharmacological interventions that aim to reduce or eliminate pathological microparticle production and thus prevent or slow the progression of ED and the development of cardiovascular diseases. Therefore, a reduction in microparticles is a new potential therapeutic target in the treatment of diseases affecting the vascular endothelium.

**Methods to evaluate endothelial function**

**Flow-mediated vasodilation**

The principle of assessment by flow-mediated vasodilation is quite simple. The large conductance arteries, such as the brachial and radial arteries, are initially subjected to a temporary exclusion of blood flow due to artery occlusion using a blood pressure cuff inflated to a level above the systolic blood pressure and subsequently deflated. Then, normal arteries respond with reactive hyperemia due to the change in flow pattern (change from laminar flow to swirling flow) called flow-mediated vasodilation (VMF). This stimulus is sufficient to promote the release of NO and other vasodilators from the vascular endothelium of the artery segment being evaluated in the presence of functionally intact endothelium. Celermajer et al. [66] introduced this technique in the arsenal of endothelial function evaluation tools.

The percentage change in the diameter of the brachial or radial artery in response to shear stress induced during reactive hyperemia is the flow-mediated vasodilation (response dependent on the endothelium-mediated release of NO), which shows excellent correlation with coronary endothelial function evaluated using an invasive technique. [67] However, although the method is relatively simple, its standardization and examiner training are necessary, as is the proper implementation of the method according to the guidelines and protocols of medical societies in order to validate the results. [68] Another test used to assess peripheral endothelial function is peripheral arterial tonometry, a non-invasive test that does not depend on the examiner and has an excellent correlation with endothelial function.

**Venous occlusion plethysmography**

This technique is limited due to the semi-invasive nature of the procedure; it requires catheterization of the brachial artery and venous plethysmography of the forearm. However, it has the advantage of allowing the infusion of vasoactive drugs, such as acetylcholine (ACh) or nitroglycerin (NTG), and thereby it can quantify the endothelium-dependent and -independent vasodilation, respectively. It also allows low doses of both agonists and antagonists to be infused simultaneously, and by using bilateral plethysmography, a comparison of the vasomotor response of both limbs can be made, with the contralateral extremity serving as a control. The results are expressed as a change in flow rate relative to the applied stimulus. [45] Although the microcirculation of the forearm is assessed, the response to ACh has an independent predictive value for future cardiovascular events. [4]

**Evaluation of arterial stiffness**

Changes in the vascular wall's structural and functional properties modify the arterial bed's viscoelastic properties, which can lead to vascular stiffness. The hardening of the arterial wall is due to a complex interaction of genetic, metabolic and hormonal aspects and exposure to cardiovascular risk factors. The pathophysiological modulation is mediated by a balance between the production and degradation of elastin and collagen, which are influenced by pro-inflammatory factors, the action of metalloproteinases and rheological factors. In this context, although it is not the only contributor, endothelial function plays an important role in the pathogenesis of arterial wall stiffness.
The evaluation of arterial stiffness can be achieved by applying non-invasive methods such as measuring the pulse wave velocity (PWV), profile analysis of blood pulse wave and assessments of the central systolic blood pressure and the augmentation index (AI).

Arterial PWV, by definition, is the speed of the pulse wave along the arterial bed; expressed in meters per second (m/s), it is calculated from a distance travelled by the blood flow wave divided by the time taken to travel that distance. As the vascular wall becomes rigid, the elasticity is reduced, and the PWV is faster. Currently, this method is considered the ‘gold standard’ for measuring arterial stiffness. In addition, several epidemiological studies stress the importance of this method. For example, data from the Framingham study suggested a strong relationship between arterial stiffness and risk factors for cardiovascular disease. Likewise, the Copenhagen Heart Study findings found that high PWV is associated with a 50% higher risk of cardiovascular events. Another method, tonometry, allows an analysis of the pressure wave by summing the forward pulse wave (ejected by the left ventricle) and the backward or reflected wave (caused by reflection when large calibre arteries bifurcate into smaller resistance vessels - arteries and arterioles). With the application of an integral transfer function, the waveform analysis allows inference of the central pressure and the augmentation index, an indicator of arterial stiffness and endothelial function, can be calculated.

Conclusion

This brief review shows some of the main mechanisms involved in the pathogenesis of hypertension, especially of ED. Any change in the normal activity of endothelium characterizes this dysfunction. Hypertension is associated with reduced NO bioavailability and the release of endothelium-derived vasoconstrictive substances.

This review also discusses ways to measure ED and its markers, such as arterial stiffness. In addition, it discusses new issues, such as the presence of microparticles that can act either as regulators of physiological processes or as cardiovascular disease biomarkers. Finally, it examined aspects of the treatment of ED and, consequently, showed that antihypertensive medications can reverse or minimize the imbalance in the vasoconstriction/vasodilatation mechanisms present in ED.

References