

Endothelial effects of antihypertensive treatment: focus on irbesartan

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Abstract: The endothelium is characterized by a wide range of important homeostatic functions. It participates in the control of hemostasis, blood coagulation and fibrinolysis, platelet and leukocyte interactions with the vessel wall, regulation of vascular tone, and of blood pressure. Many crucial vasoactive endogenous compounds are produced by the endothelial cells to control the functions of vascular smooth muscle cells and of circulating blood cells. These complex systems determine a fine equilibrium which regulates the vascular tone. Impairments in endothelium-dependent vasodilation lead to the so called endothelial dysfunction. Endothelial dysfunction is then characterized by unbalanced concentrations of vasodilating and vasoconstricting factors, the most important being represented by nitric oxide (NO) and angiotensin II (AT II). High angiotensin-converting enzyme (ACE) activity leads to increased AT II generation, reduced NO levels with subsequent vasoconstriction. The net acute effect results in contraction of vascular smooth muscle cells and reduced lumen diameter. Furthermore, when increased ACE activity is chronically sustained, increase in growth, proliferation and differentiation of the vascular smooth muscle cells takes place; at the same time, a decrease in the anti-proliferative action by NO, a decrease in fibrinolysis and an increase in platelets aggregation may be observed. AT II is then involved not only in the regulation of blood pressure, but also in vascular inflammation, permeability, smooth muscle cells remodelling, and oxidative stress which in turn lead to atherosclerosis and increased cardiovascular risk. Given the pivotal role exerted by AT II in contributing to alteration of endothelial function, treatment with ACE inhibitors or angiotensin receptor blockers (ARBs) may be of particular interest to restore a physiological activity of endothelial cells. In this view, the blockade of the renin-angiotensin system (RAS), has been shown to positively affect the endothelial function, beyond the antihypertensive action displayed by these compounds. In this review, attention has been specifically focused on an ARB, irbesartan, to examine its effects on endothelial function.

Keywords: angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, endothelial dysfunction, irbesartan

Introduction

The endothelium is a monolayer that covers the inner surface of the entire vascular system; its total weight is more than a liver, and if extended, it covers various tennis courts area. Other than being a barrier between blood and tissues, endothelial cells have multiple functional activities, that are impaired in common diseases like hypertension, diabetes, and the metabolic syndrome. The main feature of the endothelial dysfunction is an impaired endothelium-dependent vasodilation, that is mainly due to reduced nitric oxide (NO) availability and increased angiotensin II (AT II) levels. This altered balance induces an increase of oxidative stress, free radicals, inflammation, and coagulation. In this picture, the use of angiotensin-receptor blockers (ARBs) or angiotensin-converting enzyme inhibitors (ACEis), has demonstrated that these drugs display multiple beneficial effects on endothelial function; then, while in the past, the blood pressure control was considered the most important therapeutic target,

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nowadays these drugs have shown such favorable effects on the endothelial cells that these properties can not be considered just ancillary.

Endothelial function

Endothelial cells line the internal lumen of all the vasculature and serve as an interface between circulating blood and vascular smooth muscle cells. Other than being a physical barrier between blood and tissues, the endothelium displays multiple interactions with vascular smooth muscle cells and blood components. Then the endothelium cannot be considered just as a barrier, as it plays a pivotal role in vasculature function: it is involved in vasodilation and vasoconstriction, inflammation, regulation of the thrombotic state, proliferation, and apoptosis of vascular smooth muscle cells (Haller 1997; De Meyer and Herman 1997).

Among the functions of endothelial cells, the NO production is certainly one of the most important. NO is a free radical produced from an essential amino acid,

L-arginine, which in turn is converted in L-citrulline and subsequent production of NO (Palmer et al 1987) (Figure 1). This reaction is catalyzed by the endothelial NO synthase (eNOS). The physiologic event that leads to an increased activity of eNOS is represented by the shear stress, ie, the force produced by the blood flow per surface unit of the vascular wall (Vallance et al 1989). Once produced, NO diffusing in vascular smooth muscle cells, activates the guanylate cyclase (cGMP), which induces relaxation and then vasodilation. Other relevant effects of NO include inhibition of platelets activation, limitation of vascular smooth muscle cells proliferation, monocytes adhesion, platelets aggregation, and apoptosis of endothelial cells (Radomski et al 1987a; Garg and Hassid 1989). Other factors with vasodilating action are represented by prostacyclins and hyperpolarizing factor (EDHF). The prostacyclin PGI_2 is the main prostaglandin produced by the endothelium; its functions are represented by vasodilation, inhibition of platelets aggregation, and inhibition of vascular smooth

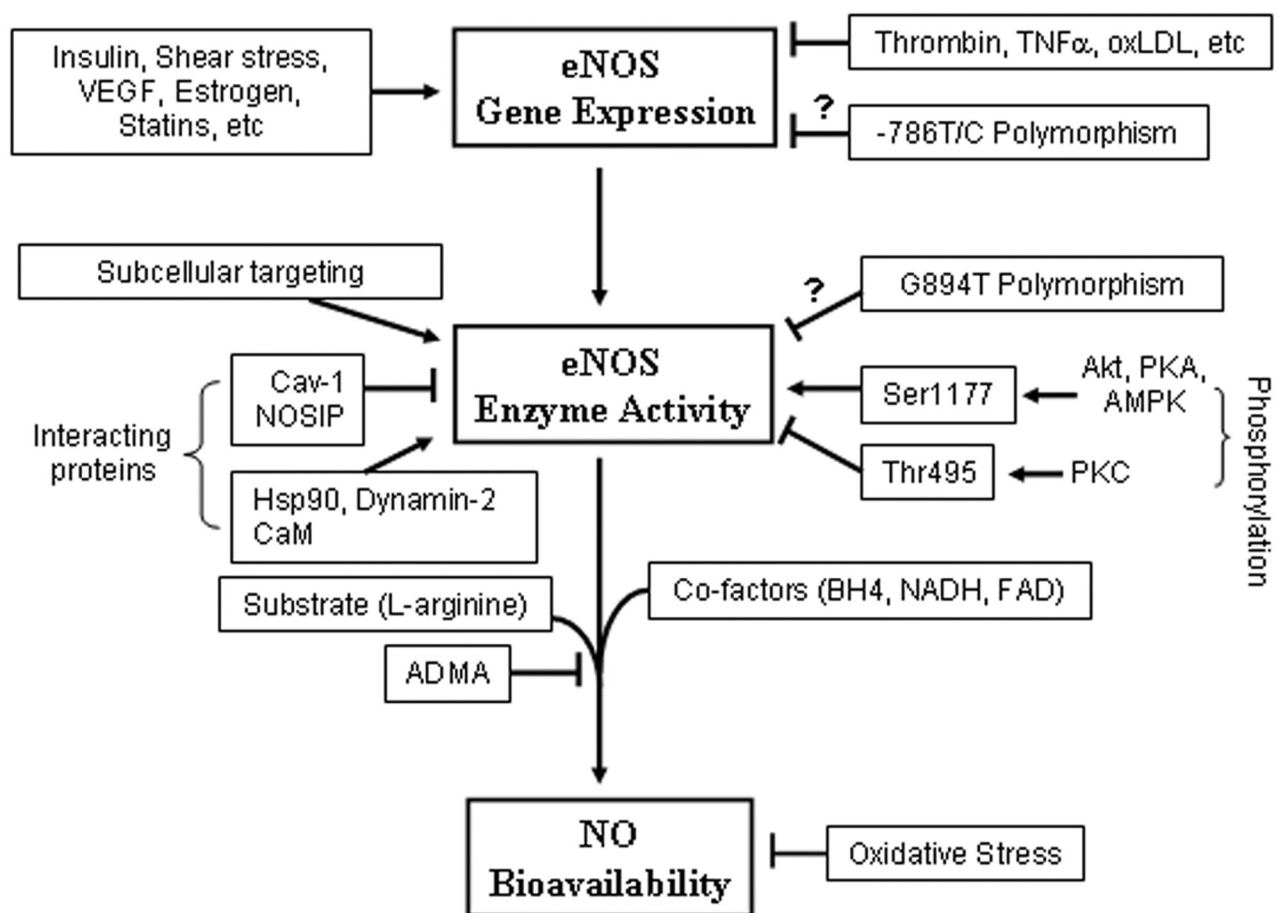


Figure 1 Regulatory mechanisms of endothelial NO production. Reproduced with permission from Yang Z, Ming XF. 2006. Recent advances in understanding endothelial dysfunction in atherosclerosis. *Clin Med Res*, 4:53–65. Copyright © 2006 Marshfield Clinic.

muscle cells proliferation (Moncada and Higgs 1987). EDHF is an endothelium-derived factor which exerts a vasodilating action mainly on small vessels, and whose action is reduced in presence of diabetes (Chen et al 1988; Matsumoto et al 2003). As endothelial cells contribute to regulate the vascular tone, they are able to produce not only vasodilating but also vasoconstricting factors. AT II exerts opposite actions in respect to NO in the regulation of the vascular tone (Dzau 1989). AT II induces at vascular level proliferation and migration of smooth muscle cells; furthermore, it is involved in the production of reactive species of oxygen (ROS), such altering the NO mediated vasodilation (Luscher and Tanner 1993). AT II, other than determining the proliferation and migration of vascular smooth muscle cells, induces the expression of adhesion molecules and chemokines which mediate the adhesion and the migration of the monocytes in the vascular wall (Tummala et al 1999; Kintscher et al 2001). The key enzyme that regulates the local generation of AT II is the angiotensin-converting enzyme (ACE). This proteolytic enzyme is synthesized by the endothelial cells and exerts its activity upon the blood-borne angiotensin I. AT II binds to and regulates vascular smooth muscle cells tone via specific angiotensin receptors (Studdy et al 1983). Elevated ACE concentrations antagonize NO activity not only by increasing AT II generation but also by decreasing concentrations of bradykinin (Mombouli 1997). High ACE activity leads to vasoconstriction due to reduced NO levels and increased AT II generation. The net acute effect results in contraction of vascular smooth muscle cells and reduced lumen diameter. Furthermore, when increased ACE activity is chronically sustained, a stimulation of growth, proliferation, and differentiation of the vascular smooth muscle cells takes place; at the same time, a decrease in the antiproliferative action by NO, a decrease in fibrinolysis and an increase in platelets aggregation may be observed. Another factor involved in vascular tone regulation is represented by endothelin-1 (ET-1) (Yanagisawa et al 1988). ET-1 induces vasodilation at low concentrations while vasoconstriction at high concentrations (Seo et al 1994). The interactions of ET-1 with its receptors, ETA and ETB, are responsible either for vasoconstriction, or induction of vascular smooth muscle cells proliferation (Arai et al 1990; Sakurai et al 1990). Other factors which display vasoconstricting properties are thromboxane A_2 and prostaglandin H_2 , which represent products of the cyclooxygenase pathway. Both these factors exert actions which antagonize NO and prostacyclin activities not just at endothelial level but also at vascular smooth muscle cells and platelets level. Furthermore, the cyclooxygenase

pathway represents a source of anion superoxide which in turn is a potent NO inactivator (Juliet et al 2003). Under physiologic conditions PGI_2 and NO prevent platelets aggregation and adhesion to the endothelium, such underlining a key role exerted by the endothelial cells in the regulation of the coagulative state. Of note, NO inhibits monocytes adhesion to vascular wall, an event that triggers the development of atherosclerotic plaque (Böger et al 2000). Then, one of the actions exerted by endothelial cells concerns coagulation. Physiologically, the most important activator of the conversion of plasminogen to plasmin is the tissue plasminogen activator (t-PA). This peptide has a critical role in the dissolution of clots and maintenance of vessel lumen. The most important regulator of t-PA is the plasminogen activator inhibitor-1 (PAI-1) (Dawson and Henney 1992). AT II is able to stimulate platelets aggregation and to induce a procoagulative state by the activation of PAI-1 expression (Vaughan et al 1995).

The endothelium is also involved in the production of specific molecules which may have a role in inflammation (Biegelsen and Loscalzo 1999). The most important are the intracellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM). These molecules act attracting and anchoring those cells involved in the inflammatory reaction. Not by chance, the atherosclerotic process is associated with increased levels of acute phase proteins (Tracy et al 1997).

Endothelial dysfunction

Endothelial dysfunction is characterized by a defect in endothelium-dependent vasodilation which precedes a series of structural changes of the vascular wall. It may occur at any level in the arterial system and contribute to the development and progression of atherosclerosis by favoring coagulation, cells adhesion, and inflammation, by promoting inappropriate vasoconstriction, and/or vasodilation, and by enhancing trans-endothelial transport of atherogenic lipoproteins. The main alteration ascribable to the endothelial dysfunction is a reduced (or absent) availability of NO, essentially as a consequence of increased oxidative stress. The endothelial dysfunction may contribute to the onset and progression of atherosclerosis and several studies have reported that endothelial dysfunction represents an independent predictor of cardiovascular events not only at coronary district but also in peripheral vasculature (Perticone et al 2001; Gokce et al 2002).

Hypertension is a pathological condition which activates endothelial cells leading to the production of contracting

factors, including thromboxane A₂, prostaglandin H₂, cyclooxygenase-derived endothelium-dependent contracting factors (EDCF), and oxygen free radicals, which antagonize the relaxing activity of NO (Miller and Vanhoutte 1985; Aldere et al 1986; Katusic and Vanhoutte 1989). Furthermore, oxygen free radicals can impair endothelial function by causing NO breakdown (Gryglewski et al 1986).

Endothelial dysfunction has been extensively explored by evaluating the response to pharmacological or mechanical endothelium-dependent stimuli (Lüscher and Noll 1996). Impaired response to acetylcholine, methacholine, bradykinin, and substance P has been documented in the forearm vasculature of essential hypertensive patients compared with normotensive controls (Linder et al 1990; Panza et al 1990; Panza et al 1993a, b, c; Taddei et al 1994; Creager and Roddy 1994; Panza et al 1995; Taddei et al 1995; Taddei et al 1997). Since in the same experimental conditions the vasodilating effect of an endothelium-independent vasodilator such as sodium nitroprussiate was found to be preserved, this line of evidence clearly indicates the presence of endothelial dysfunction in essential hypertension. Moreover, the evidence that reduced response to acetylcholine is detected in young normotensive offspring of essential hypertensive patients, and that this abnormality does not correlate with blood pressure levels, suggests that impaired endothelium-dependent vasodilation, may be at least in part, genetically determined (Taddei et al 1996). Both the animal and human data strongly suggest that the production of COX-dependent EDCF is one of the principal mechanisms leading to an impaired availability of NO, at least in aging or essential (spontaneous) hypertension. Therefore, endothelial dysfunction accompanied by the production of EDCF must play a key role in the progression of cardiovascular disease (Radomski et al 1987a, b; Dubey and Lucher 1993; Kubes et al 1991; De Caterina et al 1995). NO is an important autoregulatory inhibitor of inflammation (Napoli and Ignarro 2001). It limits oxidative stress in the microvasculature through its ability to scavenge ROS (Miles et al 1996). Noteworthy, even if the relationship between angiotensin receptors and NO is not fully clear, it has to be outlined that ACEis and ARBs exerted protective effects on AT II-mediated inflammatory response (Tamarat et al 2002; Chen et al 2003). Interestingly, there is accumulating evidence to suggest a central role for inflammatory response in the pathogenesis of endothelial dysfunction, hypertension, and coronary artery disease. Elevated C-reactive protein (CRP) and interleukin-6 (IL-6) levels have been shown to be related with a poor outcome in patients with unstable angina, probably reflecting an important inflammatory component in

the pathogenesis of this condition (Liuzzo et al 1994; Liuzzo et al 2001). A large number of studies has demonstrated that AT II is involved in key events of the inflammatory process. AT II increases vascular permeability (by the release of prostaglandins and vascular endothelial cell growth factor or rearrangement of cytoskeletal proteins) that initiates the inflammatory process (Baylis and Brenner 1978; Ichikawa and Harris 1991; Schramek et al 1995). AT II contributes to the recruitment of inflammatory cells into the tissue through the regulation of adhesion molecules and chemokines by resident cells (Pastore et al 1999; Piqueras et al 2000; Pueyo et al 2000). Moreover, AT II could directly activate infiltrating immunocompetent cells, including chemotaxis, differentiation and proliferation (Diet et al 1996; Hansson 2001; Costantinescu et al 1998). Additional data suggest that RAS activation could play a certain role even in immunologically-induced inflammation (Rodriguez-Iturbe et al 2001). Finally, AT II participates in tissue repair and remodeling, through the regulation of cell growth and matrix synthesis (Egido 1996; Nakamura et al 2000; Tunon et al 2000; Border and Noble 2001; Wolf et al 2002). In summary, there are evidences enough to support the hypothesis that RAS is key mediator of inflammation. AT II is then involved not only in the regulation of blood pressure, but also in vascular inflammation, permeability, smooth muscle cells remodelling, and oxidative stress which lead to atherosclerosis and increase cardiovascular risk. While in the past, AT II was considered as a circulating factor, playing a central role in the regulation of blood pressure and electrolyte homeostasis, it has been later ascertained that non-ACE pathways exist and function to generate about 40% of the total AT II (Campbell 1987; Johnston 1992; Gibbons and Dzau 1994; Hollenberg et al 1998; Padmanabhan et al 1999). In fact, vascular inflammatory response has been shown to be more closely related to local AT II than circulating AT II, and recent studies tried to elucidate the consequences of increased AT II production within specific organs (heart, vasculature, pancreas, adipose tissue) (Bader et al 2001; Fleming et al 2006). These data indicate that there is a remarkable production of AT II by tissues and that a complete suppression of the RAS cannot be achieved by ACE inhibition alone (Petrie et al 2001). Inhibition of the RAS by blockade at the AT-1 receptor should, theoretically, result in more complete inhibition of the adverse cardiovascular effects of AT II. On the other hand, ACEis also inhibit the enzyme kininase II, which is responsible for the degradation of bradykinin (Erdos 1975) (Figure 2). Several studies have emphasized the possible role of elevated bradykinin levels resulting from ACE inhibitor therapy.

Favorable hemodynamic effects mediated by bradykinin may include venodilation, vasodilation (coronary and systemic), improved left ventricular relaxation, and contractile function (Trippodo et al 1995; Hornig et al 1998; Cheng 1998). Other potential benefits of bradykinin include a reduction in ventricular dilatation and cardiac hypertrophy, an increase in levels of endogenous tissue plasminogen activator, and an improvement in abnormal endothelial function (Mancini et al 1996; Schlaifer et al 1997). An additional evidence of the importance of the bradykinin system in ACE inhibition comes from a study by Guazzi et al (1997). The authors showed that although losartan and enalapril had similar hemodynamic and clinical effects, the action of enalapril was antagonized by aspirin, whereas that of losartan was not. This may be related to the role of the bradykinin in mediating

prostaglandin release. But bradykinin has been blamed for some of the undesirable complications of ACEis such as cough and angioedema. Cough may occur in up to 10% of patients treated with ACEis, while angioedema represents a potentially lethal complication (Israili and Hall 1992; Fox et al 1996). By directly blocking AT-1 receptors, ARBs can inhibit the action of AT II while having little or no effect on the bradykinin system (Brooks and Ruffolo 1999).

Hypertension is a common condition which is often associated with obesity, diabetes, and dyslipidemia. There is much evidence in support of an activation of the RAS in obesity. This has led to the notion that blockade of the RAS might be a beneficial strategy for management of hypertension associated with obesity (Sharma 2004). Adipose RAS has recently received much attention because experimental

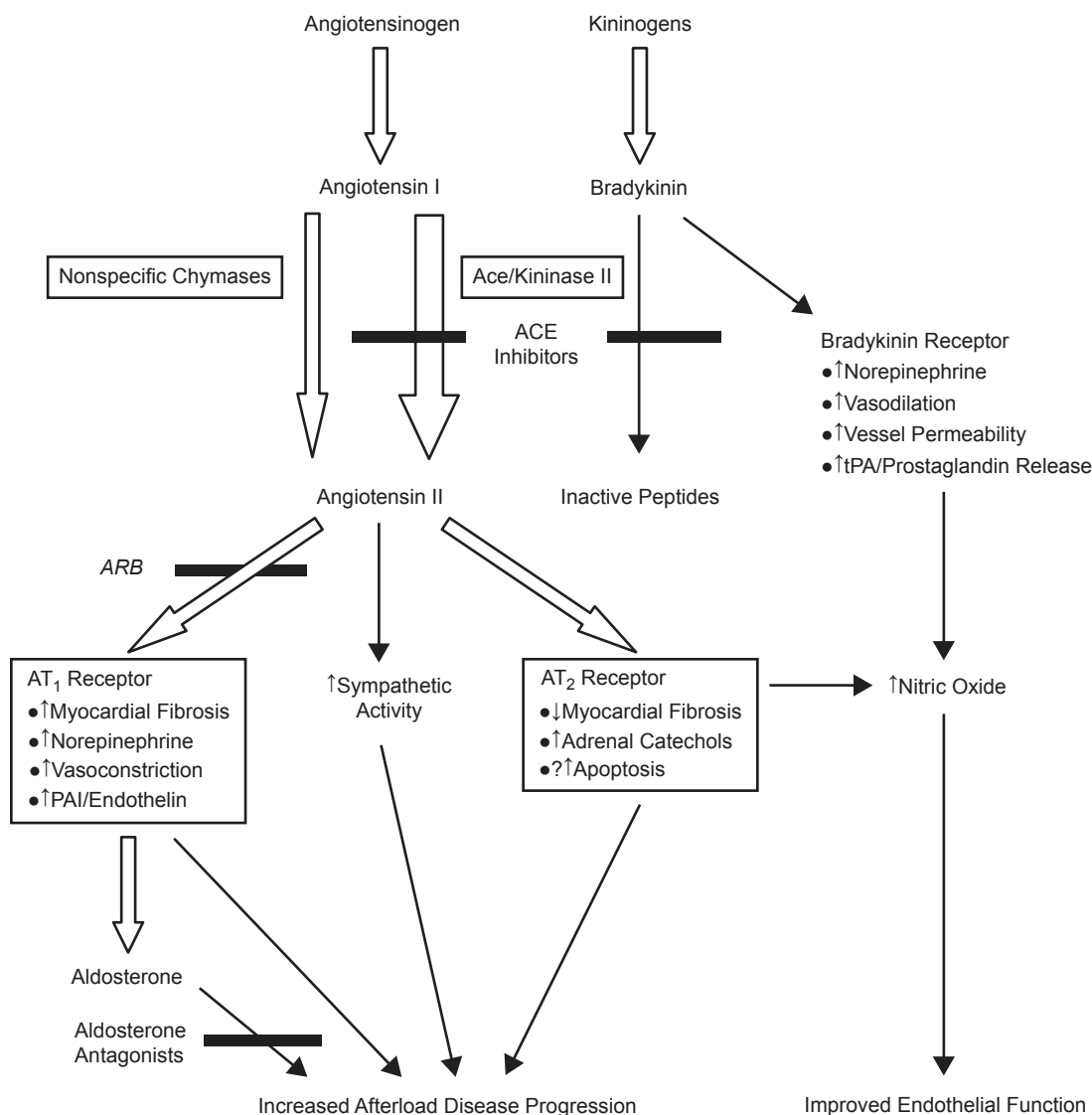


Figure 2 Relationships between angiotensin and kinin cascades.

evidence has shown its involvement in the pathophysiology of obesity-induced hypertension. In mice, adipocyte-derived angiotensinogen can act locally to affect adipocyte growth and differentiation and can also be secreted into the bloodstream, contributing to the circulating pool of angiotensinogen (Massiera et al 2001). The demonstration that angiotensinogen produced by the adipose tissue may be released in the bloodstream suggests that the high circulating levels of angiotensinogen associated with obesity may be attributable in part to increased fat mass. Mice with obesity induced by a high-fat diet exhibit greater angiotensinogen gene expression in intra-abdominal fat but not in other fat depots or non-adipose tissues (Rahmouni et al 2004). Interestingly, increased production of angiotensinogen by intra-abdominal fat appears to explain the high circulating levels of this peptide observed in dietary obesity (Boustany et al 2004). Activation of adipose RAS is also involved in development of high blood pressure in transgenic mice used as a model of visceral obesity. These transgenic mice show an increase in enzyme activity similar to that seen in obese humans and replicate visceral fat accumulation and high blood pressure (Masuzaki et al 2003). The hypertension observed in this model was abolished by selective AT receptor blockade. The above-mentioned data establish that adipose RAS plays an important role in the association between obesity and hypertension. This activation of adipose RAS may also explain the link between excessive visceral fat and cardiovascular diseases. Endothelial dysfunction is a typical feature of the states of insulin resistance and not by chance, and obesity is associated with elevated plasma levels of ET-1 (Steinberg et al 1996; Ferri et al 1996). Caballero et al (1999) demonstrated early abnormalities in vascular reactivity and biochemical markers of endothelial cells activation in individuals at risk of developing diabetes. The investigators measured the increase in blood flow in the microcirculation (laser Doppler flowmetry) and in the macrocirculation (ultrasound) in four groups of subjects: 1) healthy normoglycemic individuals with no history of type 2 diabetes in a first-degree relative (controls); 2) healthy normoglycemic subjects with a history of type 2 diabetes in one or both parents; 3) subjects with impaired fasting glucose; and 4) patients with type 2 diabetes without vascular complications. Moreover the investigators measured plasma concentrations of ET-1, soluble intercellular adhesion molecule (sICAM), and soluble vascular cell adhesion molecule (sVCAM), as indicators of endothelial cells activation. The vasodilatory responses to acetylcholine were reduced in groups 2, 3, and 4 compared with controls and ET-1 was significantly higher in these three

groups. These results suggest that abnormalities in vascular reactivity and biochemical markers of endothelial cells activation are present early in individuals at risk of developing type 2 diabetes, even at stage when normal glucose tolerance exists. Hyperglycemia can cause endothelial dysfunction not only in already diagnosed diabetes but also in mild and transient increases of blood glucose, as demonstrated in studies carried on healthy volunteers undergoing hyperglycemic clamp (Williams et al 1998). The crucial point that determines endothelial dysfunction, without structural alterations of the vascular wall, is the reduced availability of NO. Insulin exerts its vascular effects primarily by increasing the availability of NO. In experimental conditions of insulin resistance, endothelial-mediated vasodilation is impaired, because of insulin's inability to stimulate the activity of eNOS (Vincent et al 2003; Shaul 2002). In the presence of diabetes, several factors may alter NO availability: increased production of oxygen free radicals, increased levels of asymmetric dimethylarginine (ADMA), which is an irreversible inhibitor of NOS, activation of protein-kinase C (PKC), and accelerated production of advanced products of glycosilation (AGEs) (Brownlee 1992; Surdacki et al 2007). Since 1992, ADMA has been recognized as an endogenous inhibitor of eNOS, and in vitro experiments demonstrated that the NO production is inhibited by ADMA in a concentration-dependent manner (Vallance et al 1992). In fact, ADMA represents a cardiovascular risk factor: it is a strong predictor of cardiovascular events and total mortality in hemodialysis patients; high ADMA concentrations are related to increased risk of death in patients on intensive care unit, and it predicts the outcome after percutaneous coronary intervention in patients with stable angina pectoris (Zoccali et al 2001; Nijveldt et al 2003; Lu et al 2003). It has become more and more evident that the production of free oxygen radicals plays a pivotal role in the development of vascular complications of diabetic disease (Ceriello 2006). In vitro studies suggest that endothelial cells exposed to high glucose levels increase the production of superoxide and show alterations of the cell proliferation, which may be completely prevented by the increase in the expression of endogenous anti-oxidants (Nishikawa et al 2000; Zanetti et al 2001). Diabetic patients are particularly exposed to the endothelial damage from free radicals also because they show a reduction of anti-oxidant defences, including a reduction of superoxide dismutase (Crouch et al 1978). Another important mediator of endothelial dysfunction in diabetes is represented by the activation of PKC. In presence of hyperglycemia high levels of diacylglycerol activate PKC which in turn induces alterations of eNOS activity and

NADPH oxidase, and then contributes to an elevated production of free oxygen radicals and to an increase in oxidative stress (Hink et al 2001). NADPH is required for proper NO generation; hyperglycemia may lead to intracellular changes in the redox state with activation of PKC resulting in depletion of the cellular NADPH pool (Williamson et al 1993). If acute hyperglycemia induces endothelial dysfunction mostly by worsening oxidative stress, chronic hyperglycemia exerts further deleterious effects on vascular wall by production of AGEs. It has been demonstrated that in the presence of diabetes, AGEs accumulation is able to inhibit NO production producing defective endothelium-dependent vasodilation (Bucala et al 1991). Furthermore, the binding of AGEs with their cell receptors, receptors for advanced glycation end-products (RAGE) and AGE-R3, may induce endothelial activation with consequent production of growth factors and pro-inflammatory molecules, which lead to development and progression of atherosclerotic process (Kislinger et al 2001). The inflammatory component in diabetic patients is particularly harmful, given the progression towards atherosclerosis. Proinflammatory cytokines, like tumor necrosis factor- α (TNF- α), inhibit insulin-stimulated activation and expression of eNOS resulting in diminished levels of NO (Kim et al 2001; Anderson et al 2004). In vitro studies have demonstrated that CRP, which is particularly elevated in diabetic patients, induces direct damage at the endothelial level leading to a reduction in NO and vasodilating prostaglandins and an increase in ET-1. Furthermore, proinflammatory cytokines like TNF- α and IL-6 have demonstrated a detrimental effect at the endothelial level (Picchi et al 2006). In humans, the relationships between inflammation, endothelial dysfunction, and hyperglycemia were outlined in the Hoorn Study, a population-based cohort study. Results showed that type 2 diabetes was associated with both endothelial dysfunction and low-grade inflammation, whereas impaired glucose tolerance was associated only with low-grade inflammation. These findings were independent of other risk factors that accompany diabetes. Furthermore, endothelial dysfunction and low-grade inflammation were associated with a greater risk of cardiovascular mortality, especially in diabetic patients; and, finally, diabetes-associated endothelial dysfunction and low-grade inflammation explained about 43% of the greater cardiovascular mortality risk conferred by type 2 diabetes (de Jager et al 2006). Not by chance, diabetic patients with myocardial infarction, compared with non-diabetic patients, show impaired endothelium-dependent vasodilation, lower adiponectin levels, and higher concentrations of TNF- α and IL-6 (Nystrom et al 2006). Therefore, not only obesity,

but also diabetes, are associated with proinflammatory states characterized by increased circulating markers of inflammation as well as infiltration of adipose tissue with activated macrophages (Weisberg et al 2003; Xu et al 2003). In particular, CRP has been identified as a risk factor for developing type 2 diabetes, and its levels are correlated with cardiovascular risk (Pradhan et al 2001; Saito et al 2003) (Figure 3).

Effects of irbesartan on endothelial dysfunction

In vitro and in vivo studies have explored the effects of ARBs on endothelial dysfunction. One of the most studied ARB is irbesartan, whose action has been proven to be beneficial in ameliorating endothelial function above all in hypertension and diabetes, two frequent diseases in which alterations of endothelium homeostasis are typically present. In essential hypertensive patients, treatment with irbesartan promoted a significant increase in endothelium-dependent and endothelium-independent vasodilation. In a study performed by Bragulat et al (2003), hypertensive patients were examined at baseline and at the end of a 6-month period of irbesartan treatment. Endothelium-dependent and endothelium-independent responses were determined by measuring changes in forearm blood flow (FBF) in response to intrarterial infusions of acetylcholine (endothelium-dependent vasodilation [EDV]), sodium nitroprusside (endothelium-independent vasodilation [EIV]), with and without the addition of the NO synthase inhibitor L-NMMA. Irbesartan promoted a significant increase in EDV and EIV. L-NMMA-induced vasoconstriction was significantly enhanced after irbesartan treatment. Plasma concentrations of endothelin fell significantly after irbesartan treatment. In addition to a significant increase in endothelium-dependent and endothelium-independent vasodilation, irbesartan restored the vasoconstriction capacity of NO synthase inhibitors, suggesting a direct effect on tonic NO release, and decreased endothelin production. These actions may play an important role in the vascular protecting effects of irbesartan. One of the crucial points in the use of antihypertensive drugs concerns their beneficial effects beyond the reduction of blood pressure. Given the pivotal role that AT II exerts in determining endothelial dysfunction, it is reasonable that ACEis and ARBs may have some additive and favorable effects on endothelium. For example, when irbesartan has been compared with the beta-blocker atenolol, results have shown that both were able to improve endothelium-dependent vasodilation, but only irbesartan was able to reduce fibrinogen, PAI-1,

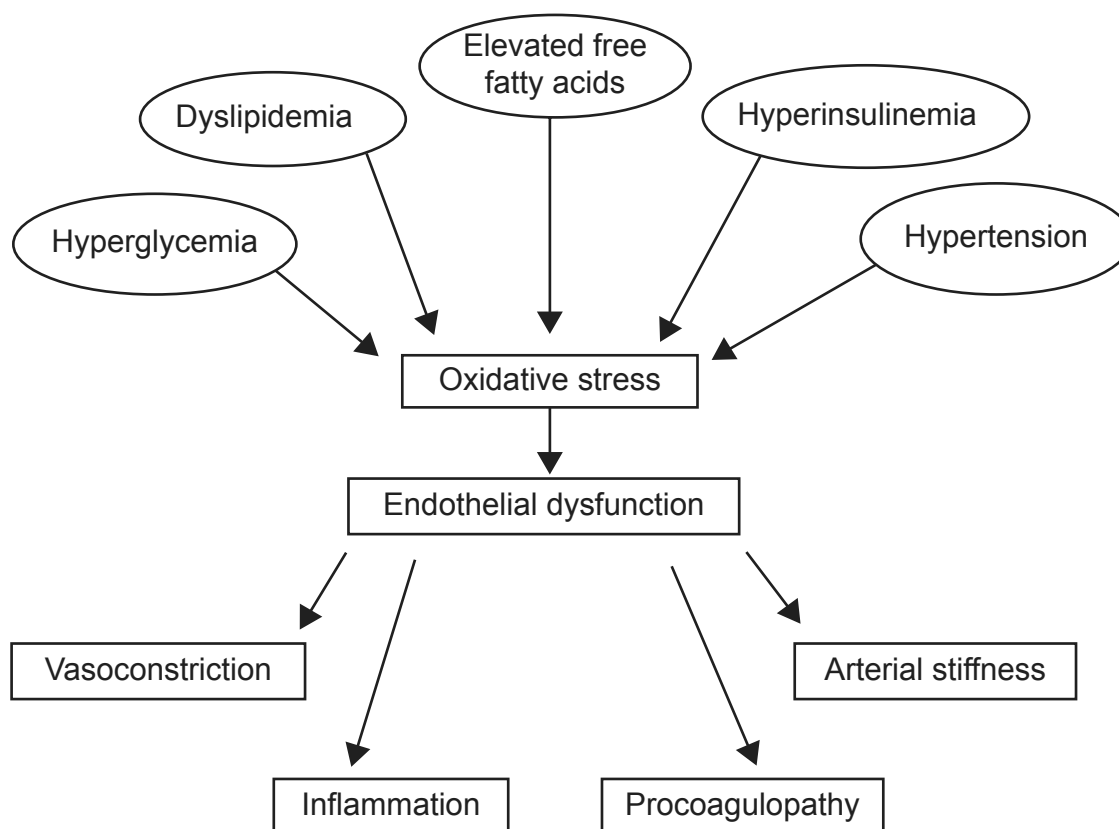


Figure 3 Factors contributing to endothelial dysfunction in type 2 diabetes.

and thrombomodulin. These results showed that, despite an equally controlled blood pressure, the ARB treatment was associated with a more favorable modification of hemostatic/fibrinolytic status (Makris et al 2000; von zur Muhlen et al 2001). The hypothesis that the blockade of AT-1 receptor may exert a protective effect on vasculature was also tested in a study that compared equihypotensive doses of irbesartan and amlodipine in apolipoprotein E-null mice, rendered diabetic by streptozotocin. Diabetes was associated with an increase in plaque area and complexity in the aorta, in association with a significant increase in aortic AT-1 receptor expression, cellular proliferation, collagen content, and macrophage-positive and alpha-smooth muscle actin-positive cell infiltration, as well as an increased expression of platelet-derived growth factor-B (PDGF-B), monocyte chemoattractant protein-1 (MCP-1), and vascular cell adhesion molecule-1 (VCAM-1). Irbesartan but not amlodipine treatment attenuated the development of atherosclerosis, collagen content, cellular proliferation, and macrophage infiltration as well as diabetes-induced AT-1 receptor, PDGF-B, MCP-1, and VCAM-1 overexpression in the aorta, despite similar blood pressure reductions by both treatments (Candido et al

2004). It has been also demonstrated that irbesartan exerts a favorable effect on vascular inflammation, which is a feature of endothelial dysfunction and promotes the development of atherosclerosis. In diabetic patients, irbesartan 300 mg/day alone or in combination with atorvastatin 40 mg/day was able to significantly increase flow-mediated dilatation while decreasing nitrotyrosine, CRP, ICAM-1, and IL-6; these beneficial effects on endothelial function were more marked when irbesartan was associated with atorvastatin (Ceriello et al 2005). The effects on vascular inflammation in type 2 diabetic patients were further assessed in the IRMA 2 substudy, a 2-year, multicenter, randomized, double-blind trial which compared irbesartan (150 or 300 mg/day) versus placebo. A subgroup ($n = 269$) was analyzed for biomarkers of inflammatory activity at baseline and after 1 and 2 years. In this substudy, irbesartan was able to reduce markers of inflammatory activity, ie, CRP and fibrinogen; IL-6 showed a 1.8% increase per year compared with a 6.5% increase for placebo, and changes in IL-6 were associated with changes in albumin excretion. There was no treatment effect on the other biomarkers (ICAM, VCAM, E-selectin, transforming growth factor- β , and AGEs). Then, in this study, irbesartan

(300 mg/day) reduced low-grade inflammation in diabetic patients, possibly reducing the risk of micro- and macrovascular disease (Persson 2006). The ISLAND study (Irbesartan and Lipoic Acid in Endothelial Dysfunction) evaluated the ability of irbesartan and lipoic acid, an antioxidant, to affect endothelial function and inflammation in patients with the metabolic syndrome. Treatment with irbesartan significantly ameliorated the endothelium-dependent vasodilation by 67%, treatment with lipoic acid by 44%, and combined treatment by 75%. In this study the two drugs reduced markers of inflammation such as IL-6 and PAI-1, while just irbesartan significantly reduced plasma levels of isoprostane-8, a marker of oxidative stress. Of note, these results were independent of blood pressure values, underlining the direct effects of AT-1 receptor blockade (Sola et al 2005). The increased cardiovascular risk encountered by women during the menopause is associated with endothelial dysfunction. It has been demonstrated that in ovariectomized rats, estrogen replacement therapy prevents endothelial dysfunction, and that irbesartan exerts a similar protective action, reducing oxidative stress and increasing NO availability (Wassmann et al 2001; Riveiro et al 2002). Of particular interest are the studies concerning the effects of ARBs on myocardial vasculature. One study evaluated the effect of irbesartan on the coronary and peripheral endothelial function in patients with coronary artery disease (CAD). Blockade of AT-1 receptor significantly improved flow-mediated dilation (FMD) of the brachial artery, while no changes in the coronary district were observed (Warnholtz et al 2006). It has to be noted that even if no significant improvement in coronary endothelial function was detected, since reduced FMD of the brachial artery is associated with high cardiovascular event rate, improvement of FMD may lead to better the prognosis in patients with CAD (Brevetti et al 2003). Two studies investigated endothelial function in congestive heart failure (CHF). In the first, treatment either with trandolapril or irbesartan significantly improved endothelium-dependent relaxation in rat aortic rings, but just the ARB was able to reduce superoxide aortic formation (Schäfer et al 2004). In the second, left and right ventricular (LV and RV) coronary vasodilatation reserve (CVR) were investigated in rats with hypertension or CHF. Results showed that treatment with irbesartan had no early effects on LV and RV CVR, and improved RV CVR over the long term, mainly by limiting RV hypertrophy and by preventing the development of pericoronary fibrosis and coronary endothelial function (Richer et al 2001). Taken together these results lead to the conclusion that irbesartan treatment can ameliorate endothelial function not only in

peripheral vasculature, but also in the coronary district, ie, that these properties have a cardiovascular protective effect beyond blood pressure reduction.

Perspectives and conclusions

Recent studies have addressed the fundamental role exerted by the endothelial progenitor cells (EPCs) in endothelial function and cardiovascular risk. EPCs are bone-marrow derived cells which are responsible for vascular repair (Kocher et al 2001). It was observed a strong correlation between the number of circulating EPCs and the subjects' combined Framingham risk factor score. Measurement of flow-mediated brachial-artery reactivity also revealed a significant relation between endothelial function and the number of progenitor cells, and the levels of circulating EPCs were a better predictor of vascular reactivity than was the presence or absence of conventional risk factors. In addition, endothelial progenitor cells from subjects at high risk of cardiovascular events had higher rates of in vitro senescence than cells from subjects at low risk (Hill et al 2003). Therefore, reduced EPCs concentrations are associated with endothelial dysfunction, and, most important, their levels represent a strong predictor for the occurrence of cardiovascular events and death from cardiovascular causes (Werner et al 2005). In patients with high cardiovascular risk such as diabetic patients, the number and/or function of EPCs is significantly altered, and importantly, EPCs' proliferation is inversely related to HbA1c levels and duration of diabetes (Tepper et al 2002). Therefore, the chance of improving the number and/or function of EPCs represents a new frontier in the treatment of endothelial dysfunction. Just one study evaluated the effects of ARBs on EPCs. Bahlmann et al (2005) studied the effect of two ARBs, olmesartan and irbesartan, on the number of EPCs in diabetic patients. The main findings of this study were that EPCs were reduced in diabetic patients compared with healthy controls, and just 4 weeks' treatment with ARBs significantly increased the number of EPCs. This evidence suggest that the stimulatory action on EPCs number may be of therapeutic relevance and may help explain the beneficial effects of ARBs in preventing cardiovascular disease.

In conclusion, the pathophysiological basis of endothelial dysfunction outlines the crucial role exerted in particular by AT II and NO and the related increased risk of cardiovascular diseases. Studies globally agree on the protective effects induced by the blockade of RAS, and many of them involved irbesartan, demonstrating its beneficial actions beyond the blood pressure control. In vitro and in vivo studies, in fact, have confirmed that other than the classical and well known

cardiovascular risk factors such as hypertension, cellular and molecular mechanism involving endothelial cells are pivotal for the development of atherosclerosis and coronary artery disease. The most recent discoveries help to explain and confirm the cardiovascular protective effect derived from irbesartan treatment, and at the same time open new and fascinating perspectives of care.

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