

# Inflammation in early atherogenesis: impact of ACE inhibition

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Cytokines and other inflammatory mediators induce functional changes in the endothelium ("endothelium activation"), which have been shown to be markers of atherosclerotic vascular disease. Endothelial activation accompanies and promotes vascular disease, and is associated with overexpression of chemoattractants and adhesion molecules, which in turn lead to leukocyte binding to the endothelium. The nuclear factor- $\kappa$ B (NF- $\kappa$ B) system appears to regulate the expression of many of

the genes involved in this process. Angiotensin II contributes to atherogenesis by increasing expression of many pro-inflammatory genes, in part by inducing oxidative stress, which activates NF- $\kappa$ B.

(Eur Heart J Supplements 2003; 5 (Suppl A): A15–A24)

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**Key Words:** ACE inhibitors, oxidative stress, inflammation.

## Introduction

This paper reviews the impact of pharmacological interferences with the renin-angiotensin system (RAS) on vascular inflammation, which has a role in all phases of the development of atherosclerosis, from inception to progression and clinical emergence. In all of these conditions the function of the endothelial lining appears to be crucial. Inflammatory aspects are particularly prominent in early atherogenesis. Therefore this paper's focus is on the effects of angiotensin II (Ang II) and its inhibition on pathogenetic aspects collectively referred to as "endothelial activation," which are considered pivotal in early atherogenesis. The impact of therapeutic approaches inhibiting the RAS on atherogenesis in general has been suggested by results from recent clinical trials<sup>[1]</sup> and supported by results in animal models of atherosclerosis<sup>[2–5]</sup>. In particular, these experimental studies support the idea that the attenuation of atherosclerosis is independent of the blood pressure-lowering effect of these drugs<sup>[2,4]</sup> and of any effect on plasma lipids<sup>[2]</sup>. Therefore, this paper begins with a review of the basic inflammatory mechanisms leading to early atherosclerosis, and sets the framework of where to place novel data on the effects of angiotensin-converting enzyme (ACE) inhibitors and Ang II type I (AT<sub>1</sub>)-receptor antagonists in these processes. An article elsewhere in this supplement reviews inflammatory phenomena occurring later in the course of atherosclerosis

progression, which are also affected by treatments affecting the RAS<sup>[6]</sup>.

### *Definitions: endothelial activation as a special set of endothelial dysfunctions*

In normal physiologic conditions the vascular endothelium contributes to vascular homeostasis, adaptively altering its functional state. This happens through a continuous "monitoring" by endothelial cells of blood-borne and locally generated stimuli, and through immediate or long-term subsequent responses to changes in the environment<sup>[7,8]</sup>. Functional properties of the endothelium include the active control of haemostasis (exerting control on platelets, the coagulation, and the fibrinolytic system), control of vascular tone, control of endothelial permeability, and control of medial smooth muscle cell growth<sup>[8]</sup>. Maladaptive changes in any such endothelial functions induced by qualitatively or quantitatively abnormal stimuli can result in localized alterations in the interactions of cellular and macromolecular components acting at the blood-vessel wall interface and in the control of the functional state of more albuminal layers of the vessel wall. Such changes include, therefore, altered anti-haemostatic properties of the endothelium, altered control of vascular tone and permeability to plasma lipoproteins, development of a hyperadhesiveness to blood leucocytes, and increased cytokine and growth factor production (Table 1). These alterations can be adequately and collectively termed "endothelial dysfunctions"<sup>[7]</sup>. The term "endothelial activation" specifically designates one subset of endothelial

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**Table 1** Functional properties of vascular endothelium

Property	Mediators
A) Modulation of thromboresistance	
Antiplatelet	Prostacyclin Nitric oxide
Anticoagulant	Ecto-ADPase (CD39) Heparin-like proteoglycans Thrombomodulin
Profibrinolytic	Tissue plasminogen activator, urokinase
Antifibrinolytic	Plasminogen activator inhibitor-type 1
B) Modulation of vascular tone	Prostacyclin (-) Nitric oxide (-) Endothelium-derived hyperpolarizing factor (-) Angiotensin-converting enzyme*
C) Modulation of smooth muscle cell growth	Endothelium Heparin-like molecules (-) Nitric oxide (-) TGF $\beta$ (-)
D) Selective permeability barrier	Platelet-derived growth factor A/B (+) Junctional proteins Endocytic receptors Cell surface glycocalix
E) Leukocyte recruitment	Inducible ELAMs (Selectins, ICAM-1, VCAM-1) Chemoattractant/Activators (IL-8, MCP-1, PAF)

\*Catalyzes conversion of angiotensin (Ang) I into Ang II, which causes vasoconstriction (+)=increases; (-)=decreases; TGF $\beta$ =transforming growth factor- $\beta$ ; ELAM=endothelium leukocyte adhesion molecule; ICAM-1=intercellular adhesion molecule; VCAM-1=vascular cell adhesion molecule-1; IL=interleukin; MCP-1=monocyte chemoattractant protein-1; PAF=platelet activating factor.

dysfunctions characterized by functional changes in the endothelium under the influence of various stimuli (the best studied of which are inflammatory cytokines), with the acquisition of new functional and antigenic properties mostly influencing interactions with blood leucocytes<sup>[9]</sup>. Endothelial dysfunctions are currently thought to be not only a marker of vascular disease, but also to play an important role in its initiation, progression, and clinical emergence. Most recent progress in the understanding of intimate mechanisms of vascular disease has occurred in the areas of endothelial activation and endothelium-dependent vasodilatory dysfunctions.

### New advances in the understanding of endothelial activation and the initiation of atherosclerosis

#### *Endothelial activation: an inflammatory state accompanying and promoting vascular disease*

Leukocyte binding to endothelium features prominently in a number of inflammatory and immunological disorders. In acute inflammation, polymorphonuclear leucocytes (PMNs) bind to the endothelium in post-capillary venules. However, adhesion of monocytes, but not of PMNs, to a morphologically normal arterial endothelium typifies

dietary-induced atherosclerosis in animals<sup>[10]</sup> and, most likely, many forms of human atherosclerosis, at least those accompanying increased levels of plasma lipids. Leukocyte binding to cultured endothelial cells has been studied extensively in vitro in an attempt to identify and study the mechanisms mediating this cell-cell interaction. It is now clear that activation of the endothelial cell features prominently in this process and largely drives relatively passive or secondary phenomena involving the leucocyte. Multiple protein families on the endothelial surface, each with a distinct function, provide "traffic signals" for leucocytes. These include, among others, the selectin family of adhesion molecules, chemoattractants, among which the recently described chemokines such as monocyte chemoattractant protein-1 (MCP-1) and interleukin (IL)-8 have selectivity for leucocyte subsets, and the immunoglobulin superfamily (intercellular adhesion molecules [ICAM]-1,-2,-3, and vascular cell adhesion molecule-1 [VCAM-1]), that recognise "integrin" ligands on the leucocyte surface (Table 2). The possible sequential interactions of selectin-carbohydrates, chemoattractant-receptors and immunoglobulin-integrins (the so-called "three-step paradigm"), and the multiple molecular choices available for each of these ligand-ligand interactions, provide great diversity in signals, allowing the selective responses of different leucocyte classes to inflammatory agents, the preferential re-circulation patterns of lymphocyte subpopulations, or the selective binding of monocytes to the arterial endothelium during early phases of atherogenesis.

Monocyte recruitment into the intima of large arteries is specific for atherosclerosis as compared to other forms of

**Table 2 Selected endothelial leukocyte adhesion molecules, endothelial chemoattractants, and their cognate ligands implicated in atherosclerosis**

Property	Molecules/CD nomenclature	Cellular/tissue expression	Cognate ligand
<i>Surface-associated</i>			
Integrins	$\beta 2$ CD11a/CD18 (LFA-1)	Leukocytes (monocyte, lymphocyte)	ICAM-1,2,3
	CD11b/CD18 (Mac-1) CD11c/CD18	Leukocytes (monocyte) Leukocytes (monocyte)	ICAM-1,2,3
Selectins	$\beta 1$ $\alpha 4\beta 1$ (VLA-4)	Leukocytes (monocyte, lymphocyte)	VCAM-1
	L-selectin (CD62L)	Leukocytes	sialyl Lewis <sup>x</sup> and <sup>a</sup>
	E-selectin (ELAM-1, CD62E) P-selectin (CD62P, PADGEM)	Endothelium Endothelium, platelets	sialyl Lewis <sup>x</sup> and <sup>a</sup> sialyl Lewis <sup>x</sup> and <sup>a</sup>
Immunoglobulins	ICAM-1	Endothelium and certain leukocyte cell lines	LFA-1, Mac-1, CD11c/CD18
	ICAM-2	Endothelium, platelets	LFA-1, Mac-1, CD11c/CD18
	ICAM-3	Leukocytes	LFA-1, Mac-1, CD11c/CD18
	VCAM-1 PECAM-1 (CD31)	Endothelium, smooth muscle Endothelium, platelets, leukocytes	$\alpha 4\beta 1$ (VLA4) —
Mucin-like	PSGL-1	Leukocytes	sialyl Lewis <sup>x</sup> and <sup>a</sup>
<i>Secreted</i>			
Cytokine	IL-1	Monocytes, smooth muscle	IL-1 receptor
	TNF	Monocytes, smooth muscle	TNF receptor
	MCP-1	Endothelium, smooth muscle	MCP-1 receptor
	M-CSF	Endothelium, smooth muscle	M-CSF receptor
Lipid	PAF	Endothelium, leukocytes	PAF receptor

ELAM=endothelium leukocyte adhesion molecule; ICAM=intracellular adhesion molecule; VCAM-1=vascular cell adhesion molecule-1; PECAM-1=platelet endothelial cell adhesion molecule-1; LFA-1=leukocyte function-associated antigen-1; VLA-4=very late activation antigen-4; IL=interleukin; TNF=tumor necrosis factor; MCP-1=monocyte chemoattractant protein-1; M-CSF=macrophage colony stimulating factor; PAF=platelet activating factor.

leucocyte-endothelial interactions, a finding suggesting that specific molecular changes in the adhesive properties of the endothelial surface lead to endothelial surface expression of athero-endothelium-leucocyte adhesion molecules (athero-ELAM), i.e. adhesion molecules expressed in the early phases of atherosclerosis. The first such protein to be identified originally in hypercholesterolemic rabbits was VCAM-1<sup>[11]</sup>. Endothelial cells express VCAM-1 early during cholesterol feeding in rabbits; before the appearance of macrophages/foam cells in the intima of developing fatty streaks and in a temporal pattern consistent with its pathogenetic role in lesion development<sup>[12]</sup>. Genetically engineered mice with disruption of the fourth immunoglobulin domain of the VCAM-1 gene (*Vcam1*<sup>D4D</sup>) showed reduced VCAM-1 mRNA and protein to 2%–8% of wild-type allele (*Vcam1*<sup>+</sup>) levels, but levels were sufficient to partially rescue the otherwise lethal phenotype of VCAM-1-null embryos. Findings in these animals conclusively prove the causal role of VCAM-1 in early atherogenesis. After crossing into the LDL receptor-null background, both *Vcam1*<sup>+/+</sup> and *Vcam1*<sup>D4D/D4D</sup> paired littermates were generated from heterozygous inter-crosses and fed a cholesterol-enriched diet for 8 weeks. The area of early atherosclerotic lesions in the aorta, quantified by *en face* oil-red O staining, was reduced significantly in *Vcam1*<sup>D4D/D4D</sup> mice, although cholesterol levels, lipoprotein profiles, and numbers of circulating leucocytes were comparable to wild-type. In contrast, deficiency of

ICAM-1, either alone or in combination with VCAM-1 deficiency, did not alter nascent lesion formation<sup>[13]</sup>. Therefore, although expression of both VCAM-1 and ICAM-1 is upregulated in atherosclerotic lesions, these data indicate that VCAM-1 plays a dominant role in the initiation of atherosclerosis.

Similar experiments in mice with selective disruptions of other candidate genes have also indicated causal roles for other proteins. These include monocyte chemoattractant protein-1 (MCP-1), which controls monocyte adhesion<sup>[14]</sup>, and macrophage-colony stimulating factor (M-CSF), which controls macrophage differentiation and replication once monocytes transform into macrophages within the arterial intima<sup>[15]</sup>.

### *Endothelial activation as a transducer of atherogenic risk factors*

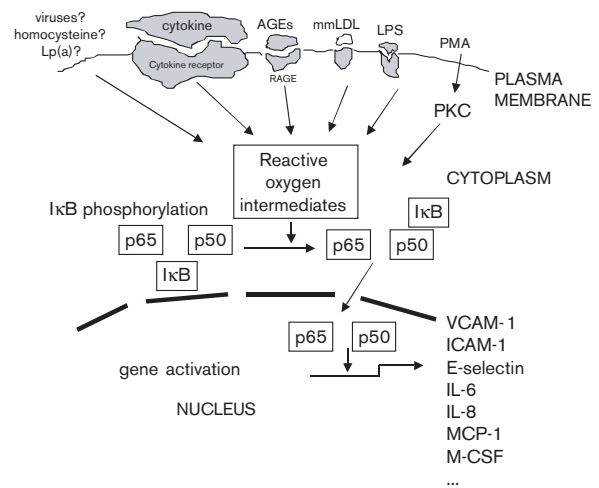
Evidence for the participation of leukocyte adhesion molecules, chemoattractants and cytokines in early atherogenesis has led to extensive study of the signals that regulate this expression. Resting, inactivated endothelial cells express negligible or low levels of these molecules (with the notable exception of ICAM-1). The gene expression of VCAM-1, as well as of other adhesion molecules (such as ICAM-1, E-selectin, and of inducible

soluble endothelial products such as MCP-1, M-CSF, IL-6, and IL-8) is augmented several-fold in response to bacterial endotoxin and cytokines such as IL-1 or tumor necrosis factor (TNF).

Following the interaction of endotoxin or cytokines with their specific cell-surface receptors, a cascade of intracellular events follows, ultimately leading to the surface appearance or secretion of these products of endothelial activation. Cytokine activation requires initiation of transcription<sup>[16]</sup>. Also, the expression of different adhesion molecules, which are products of separate genes, mostly proceeds simultaneously and together with increased gene expression for other endothelial products such as MCP-1, M-CSF, IL-6, IL-8, or tissue factor. Thus, a concerted activation of genes occurs by the activation of one or few transcription factors, including the early-response genes (*c-jun*, *c-fos*) and the nuclear factor- $\kappa$ B (NF- $\kappa$ B) system. NF- $\kappa$ B, particularly, has received increasing attention over the past years as a common denominator of endothelial activation, possibly causally linked to adhesion molecule expression<sup>[17]</sup>.

The NF- $\kappa$ B system provides a potential common link to coordinate the expression of the variety of endothelial genes involved in endothelial activation. A common feature of stimuli able to activate the NF- $\kappa$ B system appears to be the induction of oxidant stress<sup>[18,19]</sup> in the form of reactive oxygen species (ROS), namely superoxide anion and hydrogen peroxide. Antioxidants can inhibit such activation<sup>[18,19]</sup>, thus giving an important molecular rationale for the use of such substances in vascular disease initiation and progression (Fig. 1).

Cytokines such as IL-1, tumor-necrosis factor (TNF), and IL-4 can induce the expression of adhesion molecules and secondary products of endothelial activation in vitro. These cytokines can be produced by monocyte-macrophages (and, to some extent, by T-lymphocytes) infiltrating developing lesions<sup>[20]</sup>. Therefore, such stimuli might provide a paracrine mechanism to amplify the local reaction at the site of a fatty streak, enhancing local monocyte recruitment. But what might initiate the entire process? Some clues may come from the notion that, on the one hand, cholesterol-induced atherosclerosis in animals is invariably accompanied by both endothelial activation and the focal expression of VCAM-1<sup>[12]</sup> and, on the other hand, the focal accumulation of low-density lipoproteins (LDL) in the arterial intima<sup>[21]</sup> or some of their biotransformation products may stimulate monocyte recruitment. Indeed, several lines of evidence implicate oxidative modifications of LDL occurring in the arterial intima as the critical process. This pro-oxidant microenvironment, protected from circulating antioxidants, may heighten the atherogenicity of these lipoproteins. Indeed, minimally oxidized LDL or  $\beta$ -very-low-density lipoproteins ( $\beta$ -VLDL) can heighten monocyte adhesiveness to endothelial cells and also increase endothelial production of MCP-1 and M-CSF in vitro<sup>[22]</sup>. The exact component of oxidized LDL able to confer such property is still elusive. Conversely, the protective effect of high-density lipoproteins (HDL) on atherosclerosis may result in part from inhibition of LDL oxidation, possibly mediated by the



**Figure 1** The nuclear factor- $\kappa$ B (NF- $\kappa$ B) system of transcription factors comprises a series of heterodimeric molecules (monomers, including Rel-A/p65, p50, p52, Rel-B, c-Rel) normally sequestered in the cytoplasm and bound to an inhibitor (I- $\kappa$ B). Upon the influence of inflammatory cytokines, and likely other stimuli able to activate endothelial cells, the generation of intracellular reactive oxygen species, mostly hydrogen peroxide ( $H_2O_2$ ), leads to the binding of I- $\kappa$ B to ubiquitin, and the proteolytic degradation of I- $\kappa$ B. The heterodimers (represented herein by p65-p50) free from the inhibitor migrate into the nucleus, where they bind specific recognition (consensus) sequences in the promoter region of a variety of genes of endothelial activation, resulting in increased transcription of the respective genes<sup>[19]</sup>. The fact that the system may be activated by various stimuli (e.g. different cytokines) explains the partial overlap of cytokine properties, e.g. the similarity of endothelial activation induced by interleukin (IL)-1 and TNF, a property named “redundancy.” The fact that multiple genes (e.g. E-selectin, VCAM-1, and ICAM-1) are turned on when the system is activated even by one single cytokine explains another property of inflammatory cytokines, named “pleiotropy.” AGEs=advanced glycosylation end products; LPS=lipopolysaccharide; MCP-1=monocyte chemo-attractant protein-1; mmLDL=minimally oxidized low-density lipoprotein; M-CSF=macrophage-colony stimulating factor; PKC=protein kinase C.

presence of paraoxonase, a serum esterase carried on HDL that has been shown to protect against LDL oxidation in vitro<sup>[23]</sup>.

Other circulating products or metabolites might act by similar mechanisms in conditions associated with enhanced atherosclerotic risk independent of the lipid status. Such factors may include the advanced glycosylation end products (AGEs) associated with diabetes<sup>[24,25]</sup>, and lipoprotein (Lp)(a) or homocysteine, which appear to be independent risk factors for atherosclerosis. Ang II is one of the few stimuli for which a proven role in endothelial activation has been established<sup>[26]</sup>.

## Ang II: a trigger for endothelial activation and target for anti-atherosclerotic therapy

Tummala *et al.*<sup>[27]</sup> tested the hypothesis that Ang II may contribute to atherosclerosis by increasing the expression of vascular inflammatory genes such as VCAM-1. Rats infused with noradrenalin or Ang II for 6 days developed similar hypertensive responses, but only Ang II-treated rats at a physiological concentration (1 nmol/L) exhibited significant increases in aortic VCAM-1 protein and mRNA expression. By inducing a specific block of AT<sub>1</sub> receptor, treatment with oral losartan inhibited Ang II-induced hypertension and aortic VCAM-1 mRNA expression. Ang II treatment also significantly increased VCAM-1 mRNA expression in cultured rat aortic smooth muscle cells. In addition, Ang II induced NF-κB-like binding activity and transactivated an NF-κB driven VCAM-1 promoter. Losartan and proteasome inhibitors blocked Ang II-induced NF-κB activation and VCAM-1 mRNA accumulation. Overexpression of IκB-α, the main active IκB component, in rat aortic smooth muscle cells inhibited Ang II-induced VCAM-1 promoter transactivation. Therefore, Ang II may contribute to atherogenesis by activation of VCAM-1 through proteasome-dependent, NF-κB-like transcriptional mechanisms<sup>[27]</sup>.

It has to be pointed out that these results were obtained in rat aortic<sup>[27]</sup> or human<sup>[28]</sup> smooth muscle cells and, so far, have not been reported for human endothelial cells. One explanation is that cultured endothelial cells apparently lose the ability to express AT<sub>1</sub> receptors in early passages<sup>[27]</sup>. This may explain differences in published data on Ang II actions in endothelial cells. Indeed, Grafe *et al.*<sup>[29]</sup> reported that Ang II induces E-selectin gene expression in, and leucocyte adhesion to, human coronary endothelial cells. However, Ang II did not induce VCAM-1 in that study. Kim *et al.*<sup>[30]</sup> reported that Ang II induces monocyte adherence to endothelial cells without inducing VCAM-1, ICAM-1, or E-selectin gene expression. These last findings suggest the possibility of alternative pathways for leukocyte recruitment independent of VCAM-1 expression, but have to be put in the context of the observed early loss of the AT<sub>1</sub> receptor in cultured endothelial cells<sup>[27]</sup>.

The effect of ACE inhibitors in attenuating the expression of VCAM-1 *in vivo* has also been reported by Candido *et al.*<sup>[5]</sup> in a model of atherosclerosis in diabetes. Here, diabetic animals had a four-fold increase in plaque area compared with non-diabetic animals. This accelerated atherosclerosis was associated with a significant increase in aortic ACE expression and activity, and connective tissue growth factor and VCAM-1 expression. Treatment with the ACE inhibitor perindopril significantly attenuated atherosclerosis and the expression of connective tissue growth factor and VCAM-1. In a more clinical setting, ACE inhibitors<sup>[31]</sup> and AT<sub>1</sub>-receptor antagonists<sup>[32]</sup> have been found effective in reducing circulating (plasma) levels of soluble adhesion molecules, including soluble (s)VCAM-1. Other molecules variably affected at the same time have been soluble (s)ICAM-1, soluble (s)MCP-1, and soluble TNF-α

receptor II. These findings, which might be the systemic reflection of what occurs on the vessel surface, have not, however, been unequivocal<sup>[33]</sup>.

Ang II also up-regulates other inflammatory molecules involved in early atherogenesis such as P-selectin, which is involved in leukocyte rolling<sup>[34]</sup>, IL-6<sup>[35,36]</sup>, which is a further amplifier of the inflammatory reaction<sup>[37]</sup>, and MCP-1<sup>[38-40]</sup>. NF-κB controls the gene expression of most of the above-mentioned proteins. In particular, researchers studied the effect of ACE inhibition<sup>[38,41]</sup> and AT<sub>1</sub> receptor blockade<sup>[40]</sup> on the expression of MCP-1 in hypercholesterolemic rabbits. Since AT<sub>1</sub> receptor blockade is associated with feedback up-regulation of the RAS, Chen *et al.*<sup>[39]</sup> also examined alterations in plasma Ang II levels by losartan therapy. Male New Zealand white rabbits were fed a high-cholesterol diet, one group with and one group without losartan 25 mg per kg of body mass each day. As expected, there was a marked intimal proliferation in association with increase in serum cholesterol. In addition, there was a modest increase in plasma Ang II levels, and a significant increase in the expression of AT<sub>1</sub> receptors, P-selectin, and MCP-1 in aortas of the high-cholesterol diet rabbits. Concurrent administration of losartan with high-cholesterol diet attenuated aortic intimal proliferation, induced a fivefold increase in plasma Ang II levels, and caused a marked decrease in the expression of P-selectin and MCP-1 without change in serum lipid levels and aortic AT<sub>1</sub> receptor expression. These observations in hypercholesterolemic animal models show that AT<sub>1</sub> receptor blockade is associated with modulation of P-selectin and MCP-1 expression concurrent with reduction in intimal proliferation. The rise in plasma Ang II does not appear to limit the potential beneficial effect of losartan<sup>[40]</sup>. Ang II also appears to have other detrimental effects in atherogenesis possibly independent of endothelial activation. For example, the expression of an important lectin-like scavenger ox-LDL receptor, which is expressed throughout early atherogenesis, is enhanced by Ang II, an effect due to AT<sub>1</sub> stimulation, since blocked by losartan<sup>[42]</sup>. Ang II also appears to have a role in the increased matrix metalloproteinase (MMP)-1 expression and in the alteration of the balance between MMP-1 and its inhibitor, tissue inhibitor of metalloproteinases (TIMP)-2, that occurs during the development of experimental atherosclerosis in rabbits, as shown by the partial reversal of these changes with losartan treatment<sup>[43]</sup>. These findings highlight the complexity of interferences with the RAS at the endothelial level in early atherogenesis.

## How do ACE inhibitors and AT<sub>1</sub> antagonists limit the expression of inflammatory mediators of atherogenesis?

Because of the central role of NF-κB in the coordinated expression of adhesion molecules, chemoattractants, and growth factors on endothelial cells, several investigators

have assessed the effects of Ang II and its interferences on NF- $\kappa$ B.

Li *et al.*<sup>[44]</sup> first reported that Ang II can activate NF- $\kappa$ B and further stimulate, in a positive feedback loop, angiotensinogen gene expression through an NF- $\kappa$ B-mediated transcriptional mechanisms in rat hepatocytes. Besides NF- $\kappa$ B, several other transcription factors, including activator protein-1 (AP-1) and signal transducer and activator of transcription (STAT) are induced by Ang II in various cell types<sup>[45,46]</sup>. NF- $\kappa$ B is, however, of particular interest because of the many inflammatory genes (including the angiotensinogen gene) that are transactivated by this factor. That Ang II can activate NF- $\kappa$ B is likely to be a general phenomenon, since it is also reported in monocytes<sup>[47]</sup>. Research by Tummala *et al.*<sup>[27]</sup> in rat aortic smooth muscle cells found that NF- $\kappa$ B activation also occurs in vascular cells. Ang II induced nuclear NF- $\kappa$ B-like binding activity and transactivated an NF- $\kappa$ B driven VCAM-1 promoter. Losartan and proteasome inhibitors blocked Ang II-induced NF- $\kappa$ B activation and VCAM-1 mRNA accumulation. Proteasome inhibitors would act by inhibiting the degradation of the inhibitor I $\kappa$ B- $\alpha$ , which occurs after I $\kappa$ B- $\alpha$  has been phosphorylated by the specific I $\kappa$ B-kinase as a result of activation of the early signal transduction pathway leading to NF- $\kappa$ B activation. Losartan would conversely work by inhibiting the specific Ang II-initiated pathway at its very beginning. I $\kappa$ B- $\alpha$  overexpression in rat aortic smooth muscle cells inhibited Ang II-induced VCAM-1 promoter transactivation, confirming that I $\kappa$ B- $\alpha$  is implicated in Ang II-mediated VCAM-1 expression in vascular smooth muscle cells. Therefore, Ang II may contribute to atherogenesis by activation of VCAM-1 through proteasome dependent, NF- $\kappa$ B-like transcriptional mechanisms<sup>[27]</sup>. Interestingly, the mechanism for Ang II-inducible NF- $\kappa$ B regulation differs between aortic smooth muscle cells and hepatocytes. In smooth muscle cells, Ang II induces nuclear translocation of cytoplasmic transactivation of NF- $\kappa$ B proteins through proteolysis of its inhibitor, I $\kappa$ B. By contrast, in hepatocytes, Ang II induces large nuclear isoforms of NF- $\kappa$ B1 to bind DNA through a mechanism independent of changes in I $\kappa$ B turnover<sup>[45]</sup>.

The next step is a deeper understanding of how Ang II can induce I $\kappa$ B- $\alpha$  phosphorylation. This appears to be deeply related to the ability of Ang II to increase intracellular oxidative stress, of which the NF- $\kappa$ B system is an important target. It has long been known that the activation of AT<sub>1</sub> receptor leads to increased oxidative stress<sup>[48,49]</sup> (see Landmesser and Drexler<sup>[50]</sup> in this supplement). In brief, there is now substantial evidence that AT<sub>1</sub> receptor activation leads to production of ROS in the vessel wall, in part because the AT<sub>1</sub> receptor is linked to activation of an NADH/NADPH (NAD[P]H) oxidase in vascular cells<sup>[51]</sup>. This oxidase system, which has similarities to the neutrophil oxidase, is a major source of ROS in endothelial cells, vascular smooth muscle cells, and adventitial fibroblasts<sup>[52]</sup>. The vascular NAD(P)H oxidase is responsive to hormones, metabolic factors, and mechanical forces<sup>[52,53]</sup>. Besides growth factors and cytokines, Ang II is a principal activator of NAD(P)H oxidase expressed in

vascular smooth muscle cells and fibroblasts<sup>[51,52,54]</sup>. Ang II activates the NAD(P)H oxidase via AT<sub>1</sub> receptor activation, through stimulation of intracellular signalling pathways including arachidonic acid metabolites<sup>[55]</sup>. Furthermore, Ang II induces a rapid translocation of the small GTPase *rac1* to the cellular membrane, a prerequisite of NAD(P)H oxidase activation<sup>[56]</sup>. Besides these rapid effects, Ang II exerts long-term alterations by enhancing the gene expression of some of its components such as *rac1*, *p22phox*, and *NOX-1* (Nickenig *et al.* <sup>[48]</sup>). NAD(P)H oxidase is probably not the only source of RAS stimulated by Ang II. Recently, it has become apparent that endothelial nitric oxide synthase (eNOS) can produce large amounts of superoxide under certain pathophysiological conditions<sup>[57]</sup>. One mechanism for this relates to oxidative destruction of tetrahydrobiopterin, which is a critical cofactor for eNOS function<sup>[57]</sup>. In the absence of tetrahydrobiopterin, eNOS transfers electrons to molecular oxygen rather than to L-arginine, resulting in superoxide production. Recent studies have suggested that any condition that increases superoxide production (so-called "kindling radicals") in the endothelium may ultimately lead to production of a large amount of superoxide ("bonfire radicals") from uncoupled eNOS<sup>[58]</sup>. Other potential sources of ROS on stimulation with Ang II, such as xanthine oxidase and the mitochondrial respiratory chain, are currently under investigation<sup>[59]</sup>. Of the many ROS generated in mammalian cells, hydrogen peroxide seems particularly important in cell signaling<sup>[60]</sup>. Hydrogen peroxide is relatively stable and uncharged, and therefore able to diffuse from one cell to the next. Numerous intracellular targets for hydrogen peroxide and other ROS have been described, including the mitogen-activated protein kinase (MAPK) family, the cell survival kinase *Akt*, *Ras/Rac*, *c-Src*, protein kinase C, and tyrosine phosphatases. ROS also modulates intracellular Ca<sup>2+</sup> levels, altering numerous early signalling events. Many of these events have also been shown to be downstream of AT<sub>1</sub> receptor activation and are known to be redox-sensitive signalling targets (reviewed herein<sup>[48]</sup>). The intracellular formation of hydrogen peroxide is linked to NF- $\kappa$ B activation<sup>[17]</sup>, and its inhibition can quench the gene expression of VCAM-1<sup>[60]</sup>. Therefore, the increased formation of ROS, particularly hydrogen peroxide, upon engagement of the AT<sub>1</sub> receptor by Ang II is a likely mechanism linking Ang II to endothelial activation and the expression of a variety of genes conferring endothelial cells a pro-atherogenic phenotype.

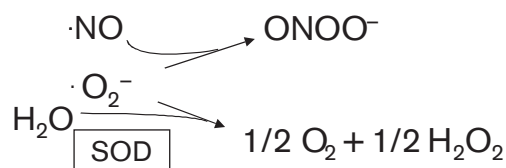
Mitochondrial production of hydrogen peroxide has been recently called into play by findings that Ang II stimulated the degradation of both inhibitors of NF- $\kappa$ B (I $\kappa$ B $\alpha$  and I $\kappa$ B $\beta$ ) with different kinetics. The degradation of I $\kappa$ Bs induced by Ang II was not modified by incubation with exogenous superoxide dismutase and catalase, suggesting that this effect was not mediated by the extracellular production of superoxide anion. In contrast, rotenone and antimycin, two inhibitors of the mitochondrial respiratory chain, inhibited the Ang II-induced I $\kappa$ B degradation, showing that generation of ROS in the mitochondria is involved on Ang II action<sup>[59]</sup>. BXT-51702, a glutathione peroxidase mimic, inhibited the effect of Ang II, and

aminotriazole, an inhibitor of catalase, enhanced it, suggesting a role for hydrogen peroxide in I $\kappa$ B degradation. This was also confirmed by experiments showing that Ang II stimulates the intracellular production of hydrogen peroxide in endothelial cells<sup>[59]</sup>. These results demonstrate that Ang II induces an intracellular oxidative stress in endothelial cells, which stimulates I $\kappa$ B degradation and NF- $\kappa$ B activation. This activation enhances the expression of VCAM-1, and probably other genes, involved in the early stages of atherosclerosis.

### Endothelial activation and the loss of endothelium-dependent vasodilation: two faces of the same basic derangement

Research on the assessment of endothelial activation (mostly in vitro), on one hand, and endothelial dysfunction as loss of normal endothelium-dependent vasodilation, on the other hand, have proceeded as parallel and independent until the relatively recent discovery that nitric oxide (NO), the main mediator of endothelium-dependent vasodilation, is also a potent suppressor of endothelial activation<sup>[60,61]</sup>. Contrary to the vasodilating and platelet-inhibiting properties of NO, the inhibition of adhesion molecule expression and monocyte adhesion occurs through a cyclic GMP-independent pathway, possibly related to the extreme reactivity of NO with superoxide anion and the subsequent prevention of hydrogen peroxide generation<sup>[62]</sup> (Fig. 2).

The notion that regulation of endothelial activation by a substance such as NO, itself a product of the vessel wall, adds complexity to the entire schema of the regulation of endothelial activation, and may be another link with therapy. One may speculate that, in a normal endothelial cell, endothelium-derived NO contributes to maintaining an anti-atherogenic profile. Conversely, endothelial vasodilatory dysfunction, primarily manifested by an alteration of NO bioavailability and endothelium-derived vasodilation, also might have a longer-term counterpart in allowing endothelial expression of leucocyte adhesion molecules and chemoattractants. In more general terms, the notion of negative regulation of endothelial activation by NO identifies a novel, previously unknown mechanism of action for disease-promoting or protective factors. Increased superoxide production, such as that occurring in hypertension, hyperlipidemia, and other conditions favouring atherosclerosis, might act at least partially by inhibiting NO availability, due to the extremely rapid reaction of NO and superoxide, with the formation of the toxic compound peroxynitrite<sup>[63]</sup>. Ang II, by its now well-known stimulating action on NAD(P)H oxidase, would not only directly cause endothelial activation, but also decrease the counter-regulatory production of NO, impairing what has been termed "the biochemical baroreflex" that accompanies coronary risk conditions (as well as, likely, heart failure)<sup>[64]</sup>. RAS inhibitors would add to the list of protective factors (such as dietary enrichment in omega-3 fatty acids<sup>[65,66]</sup> or some monounsaturated fatty acids<sup>[67]</sup>),

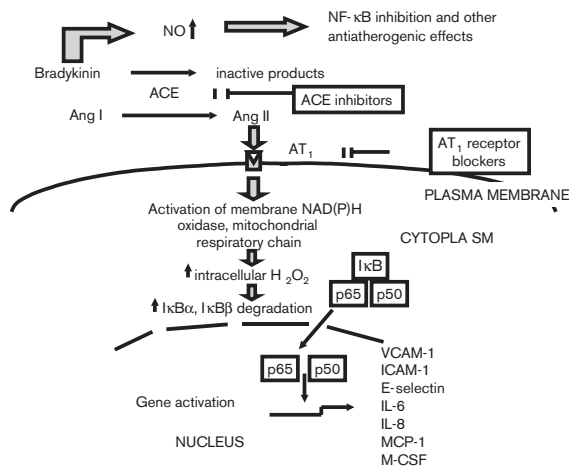


**Figure 2** The reaction between superoxide anion ( $\text{O}_2^-$ ) and nitric oxide (NO) is probably the fastest reaction in the body, about three times faster than the enzymatic dismutation of superoxide to hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) catalyzed by superoxide dismutase (SOD)<sup>[62]</sup>. Endothelial dysfunction is characterized by both an increased production of superoxide<sup>[79,80]</sup> and a reduced bioavailability of NO. This last may partially derive from the rapid formation of peroxynitrite ( $\text{ONOO}^-$ ) in the presence of increased generation of  $\text{O}_2^-$ . In the presence of NO, lesser  $\text{H}_2\text{O}_2$  is formed due to a diversion of superoxide from its spontaneous or enzymatic dismutation to  $\text{H}_2\text{O}_2$ . Since  $\text{H}_2\text{O}_2$  is ultimately responsible for the activation of NF- $\kappa$ B, increased generation of NO is likely to be anti-atherogenic due to inhibition of NF- $\kappa$ B. Conversely, decreased production or bioavailability of NO, besides impairing endothelium-dependent vasodilation, would favor endothelial activation and atherogenesis.

L-arginine<sup>[68]</sup>, estrogens<sup>[69,70]</sup>, and long-acting NO donors<sup>[71]</sup> acting on this balance.

### Interferences with the RAS: are all strategies equivalent?

The various data revised above suggest that inhibition of Ang II signaling by ACE inhibitors or Ang II-receptor blockers might work as a vascular anti-inflammatory therapy. But are these two strategies equivalent? The question arises because of pharmacologic differences in the actions of these two classes of compounds<sup>[72]</sup>. ACE inhibitors, in addition to curtailing production of Ang II, also increase levels of the peptide mediator, bradykinin. Bradykinin, an endothelial-dependent vasodilator, augments local production of NO. As noted earlier, in addition to its vasodilatory properties, NO may mitigate atherogenesis because of anti-inflammatory properties mediated by interference with the NF- $\kappa$ B transcriptional control pathway<sup>[60,73]</sup>. Bradykinin, which is also produced upon stimulation of the  $\text{AT}_2$  receptor, elevates intracellular levels of the second messenger cGMP<sup>[74]</sup>. The increased cGMP may contribute to some of the beneficial actions of ACE inhibitors, e.g. by promoting vasodilation resulting from smooth muscle relaxation<sup>[75]</sup>. In this regard, bifunctional inhibitors of ACE and the neutral endopeptidase that catabolizes certain other peptide hormones, including atrial and brain natriuretic peptides, should also augment intracellular cGMP levels. These differential pharmacological properties of ACE inhibitors



**Figure 3** A scheme of the main postulated effects of ACE inhibitors and angiotensin II (Ang II) type-1 (AT<sub>1</sub>)-receptor blockers in early atherosclerosis. H<sub>2</sub>O<sub>2</sub>=hydrogen peroxide; I-κB=inhibitor κB; IL=interleukin; MCP-1=monocyte chemoattractant protein-1; M-CSF=macrophage-colony stimulating factor; NO=nitric oxide.

and Ang II-receptor blockers are particularly prominent with regard to interferences with the fibrinolytic system, where the effect of ACE inhibitors prevails due to increased bradykinin-dependent stimulation of endothelial release of tissue plasminogen activator<sup>[72]</sup>.

Two animal studies that compare ACE inhibitors and Ang II-receptor blockers support the idea that ACE inhibitors may be superior to Ang II blockers in inhibiting atherosclerosis<sup>[76,77]</sup>. While these results may well be pertinent to the long-term favorable results of ACE inhibitors independent of, or beyond the quite small blood pressure-lowering effect of ramipril in the HOPE study<sup>[1]</sup>, they are in need of further confirmation. (The relevance of bradykinin in explaining the clinical effects of ACE inhibitors is the subject of another review in this supplement<sup>[78]</sup>.)

Figure 3 shows a comprehensive scheme of the mechanism of action of agents interfering with the RAS in endothelial activation and early atherosclerosis.

## Conclusions

A host of animal and in vitro studies strongly indicates the antiatherogenic potential of agents that interfere with the RAS to reduce vascular inflammation. These results provide a strong mechanistic basis for understanding the particularly favourable outcome of clinical studies with ACE inhibitors independent of the blood pressure-lowering effects of these drugs. Future research will lead to a better understanding of the relative role of the available classes of agents with action on the RAS.

## References

- [1] Heart Outcomes Prevention Evaluation Study Investigators. Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. *Lancet* 2000; 355: 253–9.
- [2] Kowala MC, Grove RI, Aberg G. Inhibitors of angiotensin converting enzyme decrease early atherosclerosis in hyperlipidemic hamsters: fosinopril reduces plasma cholesterol and captopril inhibits macrophage-foam cell accumulation independently of blood pressure and plasma lipids. *Atherosclerosis* 1994;108: 61–72.
- [3] Hayek T, Attias J, Smith J, *et al.* Antiatherosclerotic and antioxidative effects of captopril in apolipoprotein E-deficient mice. *J Cardiovasc Pharmacol* 1998; 31: 540–4.
- [4] Keidar S, Attias J, Coleman R, Wirth K, Scholkens B, Hayek T. Attenuation of atherosclerosis in apolipoprotein E-deficient mice by ramipril is dissociated from its antihypertensive effect and from potentiation of bradykinin. *J Cardiovasc Pharmacol* 2000; 35: 64–72.
- [5] Candido R, Jandeleit-Dahm KA, Cao Z, *et al.* Prevention of accelerated atherosclerosis by angiotensin-converting enzyme inhibition in diabetic apolipoprotein E-deficient mice. *Circulation* 2002; 106: 246–53.
- [6] Schieffer B. Interaction of interleukin-6 and angiotensin II and atherosclerosis: culprit for inflammation. *Eur Heart J* 2003; 5(Suppl. A): A25–A30.
- [7] Gimbrone MA, Jr., Kume N, Cybulsky MI. Vascular endothelial dysfunction and the pathogenesis of atherosclerosis. In: Weber PC, Leaf A, eds. *Atherosclerosis Reviews*. New York: Raven Press; 1993.
- [8] Gimbrone MA, Jr. Vascular endothelium in health and disease. (Chapter 4). In: Haber E, ed. *Molecular Cardiovascular Medicine*. New York, NY: Scientific American Medicine; 1995.
- [9] De Caterina R, Gimbrone MA Jr. Leukocyte-endothelial interactions and the pathogenesis of atherosclerosis. In: Kristensen SD, Schmidt EB, De Caterina R, Endres S, eds. *n-3 Fatty Acids—Prevention and Treatment in Vascular Disease*. London: Springer Verlag; 1995:9–24.
- [10] Cybulsky MI, Gimbrone MA, Jr. Endothelial-leukocyte adhesion molecules in acute inflammation and atherosclerosis. In: Simionescu N, Simionescu M, eds. *Endothelial cell dysfunctions*. New York: Plenum Press; 1992: 129–40.
- [11] Cybulsky MI, Gimbrone MA Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherosclerosis. *Science* 1991; 251:788–91.
- [12] Li H, Cybulsky MI, Gimbrone MA Jr, Libby P. An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. *Arterioscl Thromb* 1993; 13: 197–204.
- [13] Cybulsky MI, Iiyama K, Li H, *et al.* A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. *J Clin Invest* 2001; 107: 1255–62.
- [14] Gu L, Okada Y, Clinton SK, *et al.* Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. *Mol Cell* 1998; 2: 275–81.
- [15] Rajavashisth T, Qiao JH, Tripathi S, *et al.* Heterozygous osteopetrotic (op) mutation reduces atherosclerosis in LDL receptor-deficient mice. *J Clin Invest* 1998; 101: 2702–10.
- [16] Kishimoto T, Taga T, Akira S. Cytokine signal transduction. *Cell* 1994; 76: 253–62.
- [17] Collins T. Endothelial nuclear factor-κB and the initiation of the atherosclerotic lesion. *Lab Invest* 1993; 68: 499–508.
- [18] Marui N, Offermann MK, Swerlick R, *et al.* Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *J Clin Invest* 1993; 92: 1866–74.
- [19] Collins T, Read MA, Neish AS, Whitley MZ, Thanos D, Maniatis T. Transcriptional regulation of endothelial cell adhesion molecules: NF-κB and cytokine-inducible enhancers. *FASEB J* 1995; 9: 899–909.
- [20] Clinton SK, Libby P. Cytokines and growth factors in atherosclerosis. *Arch Pathol Lab Med* 1992; 116: 1292–1300.

- [21] Schwenke DC, Carew TE. Initiation of atherosclerotic lesions in cholesterol-fed rabbits. II. Selective retention of LDL vs. selective increases in LDL permeability in susceptible sites of arteries. *Arteriosclerosis* 1989; 9: 908–18.
- [22] Berliner JA, Navab M, Fogelman AM, *et al.* Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. *Circulation*. 1995; 91: 2488–96.
- [23] Shih DM, Gu L, Hama S, *et al.* Genetic-dietary regulation of serum paraoxonase expression and its role in atherogenesis in a mouse model. *J Clin Invest* 1996; 97: 1630–9.
- [24] Schmidt AM, Hori O, Chen JX, *et al.* Advanced glycation end-products interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. A potential mechanism for the accelerated vasculopathy of diabetes. *J Clin Invest* 1995; 96: 1395–403.
- [25] Basta G, Lazzarini G, Massaro M, *et al.* Advanced glycation end products activate endothelium through signal-transduction receptor RAGE: a mechanism for amplification of inflammatory responses. *Circulation* 2002; 105: 816–22.
- [26] Weiss D, Sorescu D, Taylor WR. Angiotensin II and atherosclerosis. *Am J Cardiol* 2001; 87: 25C–32C.
- [27] Tummala PE, Chen XL, Sundell CL, *et al.* Angiotensin II induces vascular cell adhesion molecule-1 expression in rat vasculature: a potential link between the renin-angiotensin system and atherosclerosis. *Circulation* 1999; 100: 1223–9.
- [28] Kranzhofer R, Schmidt J, Pfeiffer CA, Hagl S, Libby P, Kubler W. Angiotensin induces inflammatory activation of human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 1999; 19: 1623–9.
- [29] Grafe M, Auch-Schwelk W, Zakrzewicz A, *et al.* Angiotensin II-induced leukocyte adhesion in human coronary endothelial cells is mediated by E-selectin. *Circ Res* 1997; 81: 804–11.
- [30] Kim JA, Berliner JA, Nadler JL. Angiotensin II increases monocyte binding to endothelial cells. *Biochem Biophys Res Commun* 1996; 226: 862–8.
- [31] Jilma B, Li-Saw-Hee FL, Wagner OF, Beevers DG, Lip GY. Effects of enalapril and losartan on circulating adhesion molecules and monocyte chemotactic protein-1. *Clin Sci (Lond)* 2002; 103: 131–6.
- [32] Navalkar S, Parthasarathy S, Santanam N, Khan BV. Irbesartan, an angiotensin type 1 receptor inhibitor, regulates markers of inflammation in patients with premature atherosclerosis. *J Am Coll Cardiol* 2001; 37: 440–4.
- [33] Prasad A, Koh KK, Schenke WH, *et al.* Role of angiotensin II type 1 receptor in the regulation of cellular adhesion molecules in atherosclerosis. *Am Heart J* 2001; 142: 248–53.
- [34] Furie B, Furie BC, Flaumenhaft R. A journey with platelet P-selectin: the molecular basis of granule secretion, signalling and cell adhesion. *Thromb Haemost* 2001; 86: 214–21.
- [35] Han Y, Runge MS, Brasier AR. Angiotensin II induces interleukin-6 transcription in vascular smooth muscle cells through pleiotropic activation of nuclear factor-kappa B transcription factors. *Circ Res* 1999; 84: 695–703.
- [36] Schieffer B, Schieffer A, Hilfiker-Kleiner D, *et al.* Expression of angiotensin II and interleukin 6 in human coronary atherosclerotic plaques: potential implications for inflammation and plaque instability. *Circulation* 2000; 101: 1372–8.
- [37] Keidar S, Heinrich R, Kaplan M, Hayek T, Aviram M. Angiotensin II administration to atherosclerotic mice increases macrophage uptake of oxidized LDL: a possible role for interleukin-6. *Arterioscler Thromb Vasc Biol* 2001; 21: 1464–9.
- [38] Hernandez-Presa MA, Bustos C, Ortego M, *et al.* Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-kappa B activation, monocyte chemoattractant protein-1 expression, and macrophage infiltration in a rabbit model of early accelerated atherosclerosis. *Circulation* 1997; 95: 1532–41.
- [39] Chen X-L, Tummala PE, Olbrych MT, Alexander RW, Medford RM. Angiotensin II induces monocyte chemoattractant protein-1 gene expression in rat vascular smooth muscle cells. *Circ Res* 1998; 83: 952–9.
- [40] Chen H, Li D, Saldeen T, Phillips MI, Mehta JL. Attenuation of tissue P-selectin and MCP-1 expression and intimal proliferation by AT(1) receptor blockade in hyperlipidemic rabbits. *Biochem Biophys Res Commun* 2001; 282: 474–9.
- [41] Hernandez-Presa MA, Bustos C, Ortego M, Tunon J, Ortega L, Egido L. ACE inhibitor quinapril reduces the arterial expression of NF-kappaB-dependent proinflammatory factors but not of collagen I in a rabbit model of atherosclerosis. *Am J Pathol* 1998; 153: 1825–37.
- [42] Chen H, Li D, Sawamura T, Inoue K, Mehta JL. Upregulation of LOX-1 expression in aorta of hypercholesterolemic rabbits: modulation by losartan. *Biochem Biophys Res Commun* 2000; 276: 1100–4.
- [43] Chen H, Li D, Mehta JL. Modulation of matrix metalloproteinase-1, its tissue inhibitor and nuclear factor-kappa B by losartan in hypercholesterolemic rabbits. *J Cardiovasc Pharmacol* 2002; 39: 332–9.
- [44] Li J, Brasier AR. Angiotensinogen gene activation by angiotensin II is mediated by the rel A (nuclear factor-kappaB p65) transcription factor: one mechanism for the renin angiotensin system positive feedback loop in hepatocytes. *Mol Endocrinol* 1996; 10: 252–64.
- [45] Brasier AR, Jamaluddin M, Han Y, Patterson C, Runge MS. Angiotensin II induces gene transcription through cell-type-dependent effects on the nuclear factor-kappaB (NF-kappaB) transcription factor. *Mol Cell Biochem* 2000; 212: 155–69.
- [46] Brasier AR, Recinos A 3rd, Eleidrisi MS. Vascular inflammation and the renin-angiotensin system. *Arterioscler Thromb Vasc Biol* 2002; 22: 1257–66.
- [47] Kranzhofer R, Browatzki M, Schmidt J, Kubler W. Angiotensin II activates the proinflammatory transcription factor nuclear factor-kappaB in human monocytes. *Biochem Biophys Res Commun* 1999; 257: 826–8.
- [48] Nickenig G, Harrison DG. The AT(1)-type angiotensin receptor in oxidative stress and atherogenesis: part I: oxidative stress and atherogenesis. *Circulation* 2002; 105: 393–6.
- [49] Nickenig G, Harrison DG. The AT(1)-type angiotensin receptor in oxidative stress and atherogenesis: part II: AT(1) receptor regulation. *Circulation* 2002; 105: 530–6.
- [50] Landmesser U, Drexler H. Oxidative stress, the renin-angiotensin system, and atherosclerosis. *Eur Heart J* 2003; 24(Suppl. A): A3–A7.
- [51] Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH activity in cultured vascular smooth muscle cells. *Circ Res* 1994; 74: 1141–8.
- [52] Griendling K, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res* 2000; 86: 494–501.
- [53] Meyer JW, Schmitt ME. A central role for the endothelial NADPH oxidase in atherosclerosis. *FEBS Lett* 2000; 472: 1–4.
- [54] Mollnau H, Wendt M, Szocs K, *et al.* Effects of angiotensin II infusion on the expression and function of NAD(P)H oxidase and components of nitric oxide/cGMP signaling. *Circ Res* 2002; 90: E58–65.
- [55] Griendling K, Sorescu D, Lassegue B, Ushio-Fukai M. Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology and pathophysiology. *Arterioscler Thromb Vasc Biol* 2000; 20: 2175–83.
- [56] Wassmann S, Laufs U, Baumer AT, *et al.* Inhibition of geranylgeranylation reduces angiotensin II-mediated free radical production in vascular smooth muscle cells: involvement of angiotensin AT1 receptor expression and Rac1 GTPase. *Mol Pharmacol* 2001; 59: 646–54.
- [57] Wang W, Wang S, Yan L, *et al.* Superoxide production and reactive oxygen species signaling by endothelial nitric-oxide synthase. *J Biol Chem* 2000; 275: 16899–903.
- [58] Hink U, Li H, Mollnau H, *et al.* Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circ Res* 2001; 88: e14–22.
- [59] Pueyo ME, Gonzalez W, Nicoletti A, Savoie F, Arnal JF, Michel JB. Angiotensin II stimulates endothelial vascular cell adhesion molecule-1 via nuclear factor-kappaB activation induced by intracellular oxidative stress. *Arterioscler Thromb Vasc Biol* 2000; 20: 645–51.
- [60] De Caterina R, Libby P, Peng H-B, *et al.* Nitric oxide decreases

- cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest* 1995; 96: 60–8.
- [61] Armstead VE, Minchenko AG, Schuhl RA, Hayward R, Nossuli TO, Lefu AM. Regulation of P-selectin expression in human endothelial cells by nitric oxide. *Am J Physiol* 1997; 273: H740–6.
- [62] Huie RE, Padmaja S. Reaction of NO with superoxide. *Free Radic Res Commun* 1993; 18: 195–9.
- [63] Landmesser U, Harrison DG. Oxidant stress as a marker for cardiovascular events: Ox marks the spot. *Circulation* 2001; 104: 2638–40.
- [64] Münzel T, Harrison DG. Increased superoxide in heart failure: a biochemical baroreflex gone awry. *Circulation* 1999; 100: 216–8.
- [65] De Caterina R, Cybulsky MI, Clinton SK, Gimbrone MA Jr, Libby P. The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. *Arterioscl Thromb* 1994; 14: 1829–36.
- [66] De Caterina R, Libby P. Control of endothelial leukocyte adhesion molecules by fatty acids. *Lipids* 1996; 31 (Suppl. 1): S557–63.
- [67] Carluccio MA, Massaro M, Bonfrate C, *et al.* Oleic acid inhibits endothelial activation: a direct vascular antiatherogenic mechanism of a nutritional component in the mediterranean diet. *Arterioscler Thromb Vasc Biol* 1999; 19: 220–8.
- [68] Cooke JP, Singer AH, Tsao P, Zera P, Rowan RA, Billingham ME. Antiatherogenic effects of L-arginine in the hypercholesterolemic rabbit. *J Clin Invest* 1992; 90: 1168–72.
- [69] Simoncini T, De Caterina R, Genazzani AR. Selective estrogen receptor modulators: different actions on vascular cell adhesion molecule-1 (VCAM-1) expression in human endothelial cells. *J Clin Endocrinol Metab* 1999; 84: 815–8.
- [70] Simoncini T, Maffei S, Basta G, *et al.* Estrogens and glucocorticoids inhibit endothelial vascular cell adhesion molecule-1 expression by different transcriptional mechanisms. *Circ Res* 2000; 87: 19–25.
- [71] Zampolli A, Basta G, Lazzarini G, Feelisch M, De Caterina R. Inhibition of endothelial cell activation by nitric oxide donors. *J Pharmacol Exp Ther* 2000; 295: 818–23.
- [72] Vaughan D. Pharmacology of ACE inhibitors versus AT1 blockers. *Can J Cardiol* 2000; 16 (Suppl. E): 36E–40E.
- [73] Peng H-B, Libby P, Liao J. Induction and stabilization of I kappa B alpha by nitric oxide mediates inhibition of NF-kappa B. *J Biol Chem* 1995; 270: 14214–9.
- [74] Gohlke P, Pees C, Unger T. AT2 receptor stimulation increases aortic cyclic GMP in SHRSP by a kinin-dependent mechanism. *Hypertension* 1998; 31: 349–55.
- [75] Searles CD, Harrison DG. The interaction of nitric oxide, bradykinin, and the angiotensin II type 2 receptor: lessons learned from transgenic mice. *J Clin Invest* 1999; 104: 1013–4.
- [76] Schuh JR, Blehm DJ, Friedrich GE, McMahon EG, Blaine EH. Differential effects of renin-angiotensin system blockade on atherogenesis in cholesterol-fed rabbits. *J Clin Invest* 1993; 91: 1453–8.
- [77] Fennessy PA, Campbell JH, Mendelsohn FAO, Campbell GR. Angiotensin-converting enzyme inhibitors and atherosclerosis: relevance of animal models to human disease. *Clin Exp Pharmacol Physiol* 1996; 23(8): S30–2.
- [78] Murphey L, Vaughan D, Brown N. Contribution of bradykinin to the cardioprotective efforts of ACE inhibition. *Eur Heart J* 2003; 24(Suppl. A): A37–A41.
- [79] Harrison DG, Ohara Y. Physiologic consequences of increased vascular oxidant stresses in hypercholesterolemia and atherosclerosis: implications for impaired vasomotion. *Am J Cardiol* 1995; 75: 75B–81B.
- [80] Huraux C, Makita T, Kurz S, *et al.* Superoxide production, risk factors, and endothelium-dependent relaxations in human internal mammary arteries. *Circulation* 1999; 99: 53–9.