Angiotensin II and Atherosclerosis: Relevance for Renal Disease

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Localization of Ang II in Atherosclerotic Arteries

Angiotensin II (Ang II) has been identified within macrophages in atherosclerotic lesions of primates [1] and humans [2], and the angiotensin-converting enzyme (ACE) is expressed in human atherosclerotic plaques in areas of clustered macrophages [3]. Colocalization of ACE with Ang II has recently been shown in atherosclerotic lesions of human coronary arteries obtained at autopsy [4] or from patients undergoing heart transplantation [5]. Interestingly, Ang II was identified in close proximity to the presumed rupture sites of human coronary arteries [5]. Ang II cannot only be formed by ACE, but also by other enzymes such as kallikrein [6], cathepsin G [7], and chymase [8]. Since only a few chymase-containing mast cells were identified in the latter studies, these authors suggested that ACE is the major Ang II-forming enzyme in atherosclerotic human coronary arteries. However, a recent study suggested that chymase is primarily responsible for Ang II-forming activity in atherosclerotic arteries [2].

Thus, the question whether ACE or chymase is primarily responsible for the enhanced Ang II-forming activity in atherosclerotic arteries remains to be clarified, but localization of Ang II within atherosclerotic lesions has frequently been described.

Certainly, the detection of Ang II in atherosclerotic lesions does not prove or define a pathophysiological role in atherosclerosis, and could simply be an epiphenomenon of this process. What is the evidence that Ang II is indeed causatively involved in the development of atherosclerosis, and what are the potential underlying mechanisms?
Experimental Evidence for Pro-Atherogenic Effects of Ang II

It is well established that Ang II has numerous biological actions, all of which could contribute to atherosclerosis: in vascular tissue, Ang II induces cell proliferation [9], stimulates smooth muscle cell migration [10], induction of adhesion molecules [11, 12], activation of monocytes [13], and matrix production [14]. More recently, it has become apparent that Ang II is a potent stimulator of vascular oxygen radical production [15–17]. Cell culture studies with rat smooth muscle cell preparations provided first experimental evidence for stimulation of \( \text{O}_2^- \) formation by Ang II [15]. In experiments with rat smooth muscle cell membranes, Ang II-induced stimulation of \( \text{O}_2^- \) formation could be inhibited by diphenylene iodonium, suggesting that \( \text{O}_2^- \) was produced by membrane bound NAD(P)H oxidases [15]. In the meantime, it has been shown that Ang II also induces \( \text{O}_2^- \) formation in endothelial cells and nonvascular tissue [18–21]. In a recent investigation, a constitutive \( \text{O}_2^- \)-generating activity was localized in the rabbit aortic adventitia [22]. This constitutive \( \text{O}_2^- \) formation could be enhanced by Ang II, confirming the initial study [15], and involvement of NAD(P)H oxidase was also confirmed. In numerous studies it has been demonstrated that Ang II stimulates vascular NAD(P)H oxidase-dependent \( \text{O}_2^- \) formation via the AT1 receptor. The NAD(P)H oxidase consists of 4 major subunits: a plasma membrane spanning cytochrome b558 (composed of the large subunit gp91phox and the small subunit p22phox), and 2 cytosolic components, p47phox and p67phox [23]. In smooth muscle cells, the NADPH subunits p22phox, p47phox, and eventually p67phox, are involved in \( \text{O}_2^- \) formation [23]. In endothelial cells, mRNAs for gp91phox, p22phox, p47phox, and p67phox have been detected, and the gp91phox [24] and p22phox [25, 26] subunits seem to be of particular importance for \( \text{O}_2^- \) formation in endothelial cells.

AngII-induced vascular \( \text{O}_2^- \) formation is considered to be importantly involved in some of its pro-atherogenic effects. Therefore, the pathophysiological consequences of enhanced oxidative stress will be considered in more detail.

General Consequences of Enhanced Oxidative Stress for Vascular and Renal Function

Detection of oxygen radicals and reactive oxygen species does not always indicate a pathological situation; in contrast, reactive oxygen species are produced continuously in many – not only vascular or renal – tissues, and are part of the unspecific defence system. However, in various vascular or renal diseases enhanced formation of reactive oxygen species is considered to be pathogenic, e.g. in atherosclerosis, glomerular diseases, renal failure, pyelonephritis, or
aminoglycoside nephropathy [27–30]. In the vascular system, the formation of superoxide anion (O$_2^-$) is a major source of reactive oxygen species (ROS). O$_2^-$ may react with NO to yield peroxynitrite (ONOO$^-$) which is rather stable but can rearrange to form nitrate and the highly reactive OH$^\cdot$. OH$^\cdot$ can also result from the Haber-Weiss and the Fenton reaction [107], and may cause cellular damage and contribute to inflammation [107].

**Specific Effects of Ang II-Induced Oxygen Radical Formation**

The particular importance of Ang II-induced oxidative stress for vascular biology has been investigated in the context of vasomotor tone and cell cycle regulation. Early studies suggested the importance of O$_2^-$ in the vasculature of spontaneously hypertensive rats by showing that exogenous superoxide dismutase could lower blood pressure [37]. In a rat model of Ang II-induced hypertension, it has been shown that vascular O$_2^-$ formation was enhanced after Ang II treatment, but not after treatment with the control vasoconstrictor...
norepinephrine [38]. Enhanced $O_2^-$ formation resulted in impairment of endothelium-dependent dilations [39] which could be prevented by liposome-encapsulated superoxide dismutase. The AT1-receptor antagonist losartan also prevented impairment of endothelium-dependent dilations, demonstrating a role for the AT1 receptor in these processes. In the rat two-kidney one-clip model of hypertension, similar results were obtained [40]. In that study, endothelial dysfunction was improved by preincubation of vascular tissue with superoxide dismutase and calphostin C, indicating that increased vascular $O_2^-$ formation was secondary to a protein kinase C-mediated activation of a membrane-associated NAD(P)H-dependent oxidase. Indirect evidence that Ang II-induced $O_2^-$ formation takes place in vivo in humans was provided by a study using the forearm plethysmography method which allows direct measurement of Ang II-induced vasomotor actions. Constrictor actions of Ang II in the human forearm were enhanced during NO inhibition and were attenuated during vitamin C infusion, suggesting Ang II-associated stimulation of endothelial NO and of oxygen radicals, respectively [41].

The impact of Ang II on cell cycle decisions via activation of NAD(P)H-dependent oxidases has been demonstrated in vascular and nonvascular tissue. For instance, Ang II causes vascular smooth muscle cell hypertrophy, and inhibition of p22phox mRNA expression in vascular smooth muscle cells results in a significant inhibition of Ang II-stimulated NADH(P)H-dependent superoxide production, subsequent hydrogen peroxide production, and $H^3$-leucine incorporation [42]. In nonvascular tissue, Ang II-induced $O_2^-$ formation in cultured LLC-PK1 and mouse proximal tubule cells induced p27(Kip1) expression, and stimulated hypertrophy, suggesting a novel mechanism of how Ang II can modulate cell cycle regulation [43]. Recent data from this group indicate that the impact of Ang II on hypertrophy and $O_2^-$ formation of tubule cells involves stimulation of mitogen activated kinases [21]. In cultured rat mesangial cells, Ang II-induced $O_2^-$ formation increased $H^3$-leucine incorporation and mesangial cell protein content, two markers of cellular hypertrophy, as well as $H^3$-thymidine incorporation, a marker of hyperplasia [44].

Thus, Ang II-induced $O_2^-$ formation has important consequences for the regulation of vascular tone and for cell cycle decisions.

**Clinical Evidence for the Role of Ang II in Atherosclerosis**

The hypothesis that Ang II is involved in the pathogenesis of atherosclerosis finds support in a number of experimental studies that revealed that ACE inhibitors exert antiatherogenic and antiproliferative effects in the vascular wall.
[45–48], and that ACE inhibition is beneficial for endothelial function in hypercholesterolemia [49].

Consequently, clinical trials have been designed in order to evaluate this hypothesis in humans. With regard to endothelial function, there is good evidence that ACE inhibition is indeed beneficial: e.g. the Trial on Reversal of Endothelial Dysfunction (TREND) study, the Quinapril Ischemic Event Trial (QUIET) [50, 51], as well as the Heart Outcome Prevention Evaluation (HOPE) study [52] clearly showed improvement in endothelial dysfunction by ACE inhibition. However, a beneficial effect of ACE inhibition on the development of atherosclerosis in humans has not yet been proven: in a randomized, placebo-controlled trial with the ACE inhibitor ramipril, MacMahon et al. [53] found no reduction in carotid atherosclerosis in patients with coronary, cerebrovascular or peripheral vascular disease. This result is in accordance with the atherosclerosis substudy of QUIET, which also failed to prove any effect of 3-year quinapril treatment on coronary atherosclerosis [51]. In addition, the Simvastatin/Enalapril Coronary Atherosclerosis Trial (SCAT), which evaluated the effects of cholesterol-lowering therapy with ACE inhibition on coronary atherosclerosis in normocholesterolemic patients, could confirm that lipid-lowering therapy improves atherosclerosis, but found no beneficial effects of ACE inhibition [54]. Thus, the beneficial effects of ACE inhibitors on the mortality of patients with vascular disease [52] seem to result from effects other than a reduction in atherosclerosis, such as lowering of blood pressure and reversal of endothelial dysfunction.

Although the impact of ACE inhibitors on the development of atherosclerosis in humans has not yet been proven, there seems to be an interesting link between the renin-angiotensin system and hypercholesterolemia, one of the main risk factors for atherosclerosis. Subgroup studies in the TREND and the QUIET studies revealed that the beneficial effect of ACE inhibition on endothelial function was particularly pronounced in patients with high serum low-density lipoprotein (LDL) cholesterol (>125 mg/dl). The authors concluded that the benefits of ACE inhibition were in part likely due to attenuation of the superoxide-generating effects of Ang II. This effect depended on the LDL cholesterol levels. Therefore, the analysis of a potential interaction between the renin-angiotensin system and lipoproteins deserves attention.

Evidence for a Potential Interaction between Ang II and Lipoproteins

Several lines of evidence indicate that there may be an interaction between Ang II and lipoproteins, particular oxidized LDL (OxLDL), and that such an
interaction is relevant for vascular biology and atherosclerosis: (1) as outlined above, clinical studies suggest that ACE inhibitors are of par-ticular benefit for endothelial function in hypercholesterolemic patients [50, 51]; (2) OxLDL and Ang II share strikingly similar effects on vascular function; (3) AngII and OxLDL colocalize in the atherosclerotic plaque [1], and (4) the expression of the OxLDL receptor LOX-1 and of the AT1 receptor is stimulated by the respec-

tive other receptor agonist [55–57].

Oxidative Stress Induced by OxLDL and Functional
Consequences

OxLDL shares many features with Ang II. Animal studies with cholesterol-
fed rabbits provided first indirect evidence for a role of LDL in the induction of oxidative stress. Aortas from hypercholesterolemic rabbits produced signifi-
cantly more superoxide than control aortas [58, 59]. Later, our group was able to show directly that incubation of cultured human umbilical vein endothelial cells (HUVECs) and isolated arteries with oxidized LDL or Lp(a) stimulated O$_2^-$ formation [35, 60–62]. Induction of free radical formation has also been demonstrated directly in macrophages after stimulation with Lp(a) [63], and indirectly in mesangial cells after stimulation with OxLDL [64]. The functional consequences of OxLDL-induced oxidative stress extent to atherosclerosis, vasomotor regulation and endothelial function. OxLDL affects endothelial function and impairs endothelium-dependent dilations [61, 62, 65]. The impact of OxLDL on apoptotic cell death may be a clue to its role in the development of atherosclerosis and glomerulosclerosis. OxLDL induces apoptosis in cultured HUVECs [35, 36, 66] and in smooth muscle cells of isolated aorta [35]. Further-
more, OxLDL induces apoptosis in cultured mouse mesangial cells [64]. In all the cited studies the use of antioxidants (SOD, vitamin C/E, or butylated hydroxy-toluene) prevented the induction of apoptosis. Another effect that OxLDL and Ang II have in common is the stimulation of endothelial cell proliferation [25].

Colocalization of OxLDL and Ang II

Accumulation of OxLDL in atherosclerotic plaques and in the glomerulus is a well-known event in the development of atherosclerosis and glomeruloscle-
rosis [67–70]. Only recently it became apparent that atherosclerotic arteries (human atherectomy preparations and arteries of hypercholesterolemic
monkeys) show enrichment with Ang II, colocalizing with resident macrophages [1, 2]. Thus, Ang II accumulates in the same vascular region as OxLDL.

**Receptor Expression**

Recently, several studies showed that the expression of the OxLDL receptor LOX-1 and of the AT1 receptor is stimulated by the respective other receptor agonist [55–57]. In cultured smooth muscle cells, LDL induced expression of the AT1 receptor [55]. In line with this finding, hypercholesterolemic rabbits expressed the AT1 receptor with higher density on the surface of their aorta, compared to normocholesterolemic animals [56]. Thus, LDL may sensitize the vascular tissue to Ang II. On the other hand, expression of the OxLDL receptor LOX-1 and uptake of OxLDL in HUVEC is increased by Ang II [57]. Taken together, these studies imply that Ang II and OxLDL amplify the effect of the respective other agonist.

**Impact of Ang II in Renal Disease**

Less than a decade ago, Ang II appeared to have no effect other than those on systemic hemodynamics. Recently, large scale clinical studies have provided clear evidence that ACE inhibitors slow the progression of renal disease independent of a reduction in systemic blood pressure [71, 72].

Therefore, effects of Ang II other than its influence on systemic blood pressure may account for their beneficial renal effects. These nonhemodynamic renal Ang II effects (table 1) have been subjected to extensive investigation in the past few years and lead to the disclosure of links between Ang II, renal disease and atherosclerosis.

First evidence for the pivotal role of Ang II in the progression of renal disease was based on treatment studies: ACE inhibitors effectively reduced or even reversed the development of progressive glomerular sclerosis in animal models [73–75]. Furthermore, ACE inhibitors as well as Ang II receptor antagonists were capable of reducing both proteinuria and glomerulosclerosis in remnant kidney [76, 77] and hypertensive glomerulosclerosis models [78–80].

These results are based on the nonhemodynamic, pleiotropic effects of Ang II which include increased expression of various growth factors, protooncogenes, and vasopeptides, aberrant growth responses and elaboration of inflammatory and fibrogenic cytokines [81–84]. In consequence, these alterations contribute to changes in renal structure and function (table 1) [85] some of which are similar to vascular alterations in endothelial dysfunction and atherosclerosis.
One nonhemodynamic effect of Ang II, recently described in smooth muscle cells and relevant to renal injury, is the induction of oxidative stress by stimulation of the membrane-bound NADH/NADPH oxidase [15, 38]. More recently, similar effects of Ang II have been described in mesangial cells indicating the mediation of oxidative stress by Ang II in the kidney itself [21, 43]. Since membrane-bound NAD(P)H oxidase is located in glomerular mesangial cells [86], podocytes [87], and tubular epithelial cells [88], Ang II may increase superoxide production in virtually each renal tissue and therefore may widely spread intrarenal oxidative stress. This elevated oxidative stress may not only support the progression of renal disease, but may also contribute to the development of atherosclerosis in the renal vascular bed, thus reflecting parallels in the pathophysiology of glomerulosclerosis and atherosclerosis [89].

Ang II stimulates glomerular and tubular growth as well as the synthesis of glomerular matrix (table 1), thus leading to glomerular sclerosis and tubulointerstitial fibrosis. In cell culture, Ang II stimulates the proliferation of glomerular endothelial and mesangial cells [90], and induces hypertrophy of tubular cells.

**Table 1.** Renal effects of angiotensin II (Ang II)

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<tr>
<th>Hemodynamic effects</th>
<th>Pleiotropic effects</th>
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<td>Renal vasoconstriction [82]</td>
<td>Increase in glomerular capillary permeability [97]</td>
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<tr>
<td>Aldosterone release [108]</td>
<td>Induction of renal hypertrophy [91]</td>
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<tr>
<td>Increase of glomerular capillary pressure [80]</td>
<td>Induction of renal proliferation [90]</td>
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- **Modulation of renal function**: Mesangial cell contraction [109], Modification of tubular transport [88], Stimulation of mesangial uptake and processing of macromolecules [85], Modulation of nitric oxide release [44, 110], Stimulation of endothelin production [98], Stimulation of cytokine production (VEGF, TGF-β) [92, 97], Stimulation of superoxide production [15, 21, 111].

- **Modulation of renal structure**: Induction of cell proliferation [90], Stimulation of extracellular matrix synthesis [93], Inhibition of extracellular matrix degradation [105].

- **Immunomodulatory effects**: [86, 96].

- **Hemodynamic effects**
  - Renal vasoconstriction [82]
  - Aldosterone release [108]
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The observation that tubular hypertrophy partly depends on transcription and synthesis of transforming growth factor-β (TGF-β), was the first evidence demonstrating that Ang II is capable of inducing TGF-β in the kidney [92].

In cultured mesangial cells, Ang II has clearly been proven to increase the production of TGF-β [93], which in turn is known to play a pivotal role in the progression of renal disease [94] and therefore has been identified as a potential target in the treatment of glomerular fibrosis [95].

Ang II exerts immunomodulatory effects on the kidney through the induction of chemokines such as monocyte chemoattractant protein-1 and RANTES [96]. Furthermore, it stimulates the release of vascular endothelial growth factor from human mesangial cells [97] and promotes endothelin synthesis by endothelial and mesangial cells [98]. In stimulating superoxide anion and endothelin production, Ang II antagonizes the beneficial effects of NO in the renal microcirculation and thus contributes to augmented vasoconstriction, proliferation and cell adhesion.

A large part of our knowledge on the pleiotropic effects of Ang II has been elucidated in models of diabetic nephropathy, which is among the most frequent indications for renal replacement therapy. Diabetic nephropathy is characterized by marked expansion of glomerular matrix. Studies with ACE inhibitors suggest that inhibition of Ang II formation decreases matrix synthesis and therefore contributes to reduction in the progression of renal disease [99–102]. Ang II directly increases intrarenal cytokine formation and matrix accumulation and stimulates growth and protooncogenes [103] as well as matrix protein production [93, 104] and inhibits mesangial cell collagenase activity [105]. It therefore may contribute to persistent changes not only in function but also in structure of renal tissue.

Advanced knowledge on the pleiotropic effects of Ang II in the kidney and its contribution to oxidative stress disclosed alterations in renal function and structure which are at least in part similar to the pathophysiology of atherosclerosis. Hopefully the development of novel treatment strategies specifically targeting Ang II-mediated effects may be beneficial for both the progression of renal disease and atherosclerosis.

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