Expression of Angiotensin II and Interleukin 6 in Human Coronary Atherosclerotic Plaques
Potential Implications for Inflammation and Plaque Instability

Bernhard Schieffer, MD; Elisabeth Schieffer, MD; Denise Hilfiker-Kleiner, PhD; Andres Hilfiker, PhD; Petri T. Kovanen, MD; Maija Kaartinen, MD; Jörg Nussberger, MD; Wolfgang Harringer, MD; Helmut Drexler, MD

Background—Patients with an activated renin-angiotensin system (RAS) or genetic alterations of the RAS are at increased risk of myocardial infarction (MI). Administration of ACE inhibitors reduces the risk of MI, and acute coronary syndromes are associated with increased interleukin 6 (IL-6) serum levels. Accordingly, the present study evaluated the expression of angiotensin II (Ang II) in human coronary atherosclerotic plaques and its influence on IL-6 expression in patients with coronary artery disease.

Methods and Results—Immunohistochemical colocalization of Ang II, ACE, Ang II type 1 (AT1) receptor, and IL-6 was examined in coronary arteries from patients with ischemic or dilated cardiomyopathy undergoing heart transplantation (n=12), in atherectomy samples from patients with unstable angina (culprit lesion; n=8), and in ruptured coronary arteries from patients who died of MI (n=13). Synthesis and release of IL-6 was investigated in smooth muscle cells and macrophages after Ang II stimulation. Colocalization of ACE, Ang II, AT1 receptor, and IL-6 with CD68-positive macrophages was observed at the shoulder region of coronary atherosclerotic plaques and in atherectomy tissue of patients with unstable angina. Ang II was identified in close proximity to the presumed rupture site of human coronary arteries in acute MI. Ang II induced synthesis and release of IL-6 shortly after stimulation in vitro in macrophages and rat smooth muscle cells.

Conclusions—Ang II, AT1 receptor, and ACE are expressed at strategic sites of human atherosclerotic coronary arteries, suggesting that Ang II is produced primarily by ACE within coronary plaques. The observation that Ang II induces IL-6 and their colocalization with the AT1 receptor and ACE is consistent with the notion that the RAS may contribute to inflammatory processes within the vascular wall and to the development of acute coronary syndromes. (Circulation. 2000;101:1372-1378.)

Key Words: interleukins ■ angiotensin ■ angina ■ myocardial infarction ■ arteries ■ receptors

Rupture of atherosclerotic plaques occurs predominantly at the edges of the plaque’s fibrous cap, the shoulder region, that is, areas of accumulated inflammatory cells, for example, macrophages, T-lymphocytes, and mast cells in close proximity to vascular smooth muscle cells (SMC).1-6 The activated inflammatory cells stimulate their neighboring cells to erode the extracellular matrix through the release of inflammatory cytokines. The decay of the framework that forms the plaque cap leads to plaque rupture1,7,8 and resembles the morphological correlate of an acute coronary syndrome. Serum levels of interleukin 6 (IL-6) are increased in patients with unstable angina9 and may trigger the onset of an acute coronary syndrome.10 IL-6 is known to be involved in the stimulation of matrix-degrading enzymes, for example, metalloproteinases.11

In parallel, the renin-angiotensin system (RAS) has been suggested to be involved in the development of acute coronary syndromes, based on the observations that (1) increased circulating levels of renin were associated with a higher incidence of myocardial infarction (MI),12 (2) genetic polymorphisms of the ACE gene revealed a higher risk for coronary events for the ACE/ID phenotype, as compared with the DD-phenotype,13,14 and (3) clinical trials in patients with left ventricular dysfunction demonstrated that long-term ACE inhibition reduces the incidence of MI.15,16

The present study investigated the localization of angiotensin II (Ang II), the Ang II type 1 (AT1) receptor, and ACE within human coronary atherosclerotic plaques. Since IL-6 is increased in patients with acute coronary syndromes, we also...
investigated whether and how Ang II interacts with IL-6 in vitro and in atherosclerotic plaques of patients with coronary artery disease.

Results

In SMC culture, Ang II induced a transient increase of IL-6 transcription (upper transcript) by ~7-fold, peaking at 30 minutes and lasting up to 60 minutes after receptor ligand binding (Figure 1A). Losartan (10^-5 mol/L), a selective nonpeptide AT1 antagonist, abolished IL-6 mRNA increase. Losartan alone or serum-free conditions showed no effect on IL-6 transcription. Increasing dosages of Ang II caused an increase in IL-6 transcription up to 3-fold (Figure 1C). Similarly, Ang II induced a rapid increase in IL-6 transcription in human macrophages (Figure 1B).

Ang II induced IL-6 protein release in the supernatant media that peaked at 6 hours (Figure 2A). Losartan completely abolished the IL-6 release. Serum-free conditions do not stimulate the release of IL-6 (Figure 2A). The amount of IL-6 release was dose dependent (Figure 2B).

In serial sections of the left anterior descending coronary artery (LAD) obtained from patients with ischemic cardiomyopathy, atherosclerotic plaques showed a fibrous cap covering the atherosclerotic material. Frequently, the superficial cap at the shoulder region contained inflammatory infiltrates composed of CD68-positive macrophages (Figure 3A). When parallel sections were stained for ACE, Ang II, and AT1 receptor, a strong positivity corresponding with the sites of macrophage accumulation (Figure 3, B through D) was found at the shoulder region. When parallel sections were investigated for IL-6 expression, a colocalization of IL-6 with macrophage-rich areas was observed (Figure 3E). Control experiments with the use of an unspecific IgG as primary antibody revealed no specific staining pattern, as shown in Figure 3F. Further control experiments with a rabbit preimmune serum showed also no specific staining pattern (not shown).

Scattered macrophages within the adventitia were positive for Ang II, IL-6, ACE, and AT1 receptor (data not shown). A weak and dispersed positivity for Ang II and IL-6 only was observed in the media. In the adventitia, chymase-containing mast cells identified by chymase staining were found. However, these mast cells were not positive for Ang II or IL-6.

Control experiments with serial sections of the LAD from patients with dilated cardiomyopathy showed no atherosclerotic lesions. In the intima and adventitial layers, only rare and scattered macrophages were found weakly positive for Ang II, AT1 receptor, and IL-6 (not shown). Chymase-containing mast cells were only found scattered within the adventitia. Similar to the atherosclerotic sections, the chymase-containing mast cells did not stain positive for Ang II or IL-6 in any of the coronary sections from patients with dilated cardiomyopathy.

Human coronary plaques from patients with unstable angina were obtained by directional atherectomy and examined for CD68, Ang II, AT1 receptor, and IL-6 (Figure 4). CD68-positive cells were frequently found scattered throughout these tissues and were colocalized with Ang II, the AT1 receptor, and IL-6 (Figure 4, A through D). The expression of

![Figure 1. A](image1.png)
Ang II, the AT1 receptor, and IL-6 appeared to be more pronounced in atherectomy samples as compared with stable coronary segments. However, morphometric quantification was not applicable in the heterogeneous and altered tissue sections because of the atherectomy procedure. Control experiments with the use of an unspecific IgG as primary antibody revealed no specific staining pattern, as shown in Figure 4E.

Expression of Ang II was investigated in coronary arteries from patients who died within 2 days of an acute MI. Coronary segments containing the presumed ruptured plaque site were isolated and have been characterized previously.17 Immunohistochemical results revealed that in close proximity of the presumed plaque rupture site, Ang II is accumulated (Figure 5). Chymase-containing mast cells were not present. Comparison of adjacent sections revealed that the chymase-containing mast cells did not contain Ang II.

**Discussion**

The present study demonstrates that Ang II, the AT1 receptor, and ACE are expressed at strategically relevant sites of human coronary atherosclerotic plaques in the shoulder region. These findings suggest that ACE is the major Ang II–forming enzyme in atherosclerotic human coronary arteries. Moreover, Ang II was detected in close proximity to the presumed plaque rupture site in coronary artery sections from patients who died acutely after MI. Colocalization of components of the RAS with IL-6 was observed in stable coronary plaques and atherectomy tissues, and Ang II induced the expression of IL-6 in vitro, both in macrophages and in SMC. These findings are consistent with the notion that the RAS may contribute to inflammatory processes within the atherosclerotic vascular wall and to the development of acute coronary syndromes.

Recent observations indicated that the RAS plays an important role in the progression of atherosclerosis and in the development of acute coronary syndromes.12–16 Clinical trials reported that administration of ACE inhibitors after MI reduced not only the cumulative incidence of heart failure but also the incidence of reoccurrence of MI.15,16 These observations support the hypothesis that Ang II, generated by ACE, may contribute to the progression of atherosclerosis and potentially to the disruption of coronary plaques. Experimental studies revealed further that ACE inhibitors might exert antithrombotic and antiproliferative effects in the vascular wall.18–21

We demonstrated that Ang II is expressed in stable, unstable, and ruptured human coronary plaques. Similarly, there is evidence that Ang II is expressed in atherosclerotic lesions in primates.22 Recent observations indicate that ACE is expressed in human atherosclerotic plaques in areas of clustered macrophages.23 Importantly, macrophage-rich areas are more abundant in human atherosclerotic coronary arteries of patients with unstable angina and non-Q-wave infarction as compared with stable atherosclerotic plaques.24 Therefore Ang II expression might be enhanced in unstable plaques as compared with stable coronary plaques. Together with the vast abundance of ACE, Ang II, and CD68-positive macrophages and only the few chymase-containing mast cells, it is conceivable that ACE in macrophages is the primary Ang II–forming pathway in human atherosclerotic plaques. In this regard, preliminary findings suggest that ACE but not chymase generates Ang II in isolated human coronary.

Ang II may be involved in the development of an acute coronary syndrome, based on the observations that (1) Ang II may increase biomechanical stress at the shoulder of atherosclerotic lesions26 and (2) the site of plaque rupture is characterized by an inflammatory process and an accumulation of macrophages.4,23,24 The present study demonstrated that IL-6 is expressed in areas of clustered macrophages colocalized with Ang II and that Ang II induces IL-6 expression in macrophages in vitro. Although IL-6 is thought to be an anti-inflammatory cytokine, recent obser-
Observations emphasized the proinflammatory potency of IL-6 as a central regulator of inflammation and macrophage differentiation. IL-6 induces the expression of acute-phase proteins in SMC and the migration and differentiation of activated macrophages. IL-6 may contribute to the development of an acute coronary syndrome by stimulating the synthesis of matrix degrading enzyme and LDL receptors in macrophages and the stimulation of LDL-uptake in macrophages. Moreover, IL-6 activates macrophages to secrete monocyte chemotactic protein-1, pivotal for monocyte recruitment into tissues and a central mediator of inflammatory events in atherosclerosis.

Finally, IL-6 regulates the expression of adhesion molecules and other cytokines, for example, IL-1β and tumor necrosis factor-α, which potentially enhance the inflammatory reaction.

The present study demonstrated (1) that Ang II stimulates the synthesis and release of IL-6 in vitro and (2) the colocalization of both factors in vivo at the shoulder region of coronary plaques. These observations may point to Ang II as a potential modulator of inflammatory processes that occur chronically at the shoulder region of atherosclerotic coronary plaques. It is
conceivable, therefore, that these 2 factors interact and thereby amplify the development of an acute coronary syndrome.

Furthermore, Ang II may contribute to the development of an acute coronary syndrome through the migration of macrophages into a neointimal area or by producing reactive oxygen species and thereby increasing oxidative stress. Increased secretion of macrophage-derived interleukins was observed in cells exposed to oxidative stress, such as oxidized LDL or cellular lipid peroxidation induced by iron ions. In contrast, administration of ACE inhibitors abolished macrophage recruitment in this experimental model, and blockade of the AT₁ receptor by losartan was shown to prevent the accumulation of oxidative reactants, which abolished lipid peroxidation and the progression of atherosclerosis in an apolipoprotein E-deficient animal model.

The present study may have potential clinical implications by pointing to mechanisms by which ACE inhibitors reduce the incidence of reinfarctions, that is, the attenuation of proinflammatory processes in atherosclerotic plaques. If so, ACE inhibition should reduce serum markers of inflammation in patients treated with ACE inhibitors. Preliminary observations indicated that long-term ACE inhibition reduces circulating levels of C-reactive protein in patients with coronary artery disease. These findings are consistent with the notion that an interaction between the RAS and proinflammatory cytokines occurs, which may affect the balance between stabilizing and destabilizing factors at the fibrous cap and thereby promote the instability of a former stable coronary plaque.

**Study Limitations**

Our analysis of coronary arteries obtained during transplantation reveals that chymase-containing mast cells are consis-

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**Figure 4.** Immunohistochemical stainings from atherectomy tissues obtained from culprit lesion from patients with unstable angina. Plaques stained with CD-68 positive cells revealed that at presumed luminal edge, macrophages accumulate (A). Serial sections stained for Ang II (B), AT₁ receptor (C), and IL-6 (D) revealed strong positivity at presumed luminal edge of culprit lesion corresponding with site of macrophage accumulation. Control experiments with unspecific IgG demonstrated no specific staining pattern (E). Magnification ×200 (A through E).
tently seen in the adventitia but did not stain for Ang II. We cannot exclude that chymase secreted by activated mast cells provides an alternative pathway for Ang II formation, but cellular colocalization and abundance of Ang II in macrophage-rich areas suggests that mast cell–derived chymase is not the major contributor of Ang II formation in human atherosclerotic coronary arteries.

Second, diffusion of Ang II from its areas of generation cannot be excluded but rather would be explained by the metabolism of ACE. As a transmembrane enzyme with its extracellular catalytic domain, some ACE is cleaved from the plasma membrane and appears as a catalytically active ACE in the extracellular space. This would explain the diffuse staining pattern of ACE and Ang II.

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