Blockade of the Angiotensin II Type 1 Receptor Stabilizes Atherosclerotic Plaques in Humans by Inhibiting Prostaglandin E₂–Dependent Matrix Metalloproteinase Activity

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Background—Clinical trials have demonstrated that agents that inhibit the angiotensin II pathway confer benefit beyond the reduction of blood pressure alone. However, the molecular mechanism underlying this effect has yet to be investigated. Recently, we have demonstrated enhanced expression of inducible cyclooxygenase (COX) and prostaglandin (PG)E₂–dependent synthase (COX-2/mPGES-1) in human symptomatic plaques and provided evidence that it is associated with metalloproteinase (MMP)-induced plaque rupture. Thus, the aim of this study was to characterize the effect of the angiotensin II type 1 (AT₁) receptor antagonist irbesartan on the inflammatory infiltration and expression of COX-2/mPGES-1 and MMPs in human carotid plaques.

Methods and Results—Seventy patients with symptomatic carotid artery stenosis were randomized to irbesartan (300 mg/d) or chlorthalidone (50 mg/d) for 4 months before endarterectomy. Plaques were subjected to analysis of COX-1, COX-2, mPGES-1, MMP-2, and MMP-9, angiotensin II, AT₁, AT₂, and collagen content by immunocytochemistry, Western blot, and reverse-transcriptase polymerase chain reaction, whereas zymography was used to detect MMP activity. Immunohistochemistry was also used to identify CD68⁺/H₁₁₀₀₁ macrophages, CD3⁺/H₁₁₀₀₁ T lymphocytes, smooth muscle cells (SMCs), and HLA-DR⁺ inflammatory cells. Plaques from the irbesartan group had fewer (\(P<0.0001\)) macrophages, T lymphocytes, and HLA-DR⁺ cells; less (\(P<0.0001\)) immunoreactivity for COX-2/mPGES-1 and MMPs; reduced (\(P<0.0001\)) gelatinolytic activity; and increased (\(P<0.0001\)) collagen content. It is worth noting that COX-2/mPGES-1 inhibition was observed after incubation in vitro with irbesartan but not with the selective AT₂ blockade PD123,319.

Conclusions—This study demonstrates that irbesartan decreases inflammation and inhibits COX-2/mPGES-1 expression in plaque macrophages, and this effect may in turn contribute to plaque stabilization by inhibition of MMP-induced plaque rupture. (Circulation. 2004;109:1482-1488.)

Key Words: inflammation ■ metalloproteinases ■ plaque ■ prostaglandins

Several experimental¹ and clinical trials² have definitely established the key role of the renin-angiotensin system in the pathogenesis of atherosclerosis by demonstrating that agents that inhibit the renin-angiotensin system confer cardiovascular benefit beyond the reduction of blood pressure alone.²,³ In particular, in agreement with the hypothesis generated by the results of the placebo-controlled Heart Outcomes Prevention Evaluation (HOPE) trial² that drugs blocking the angiotensin II (Ang II) pathway could protect against stroke beyond reducing blood pressure, the 25% further reduction in stroke with losartan with respect to atenolol observed in the recent Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) study⁴ was largely independent of its effect on blood pressure. These data may be explained by the observations that Ang II promotes several critical processes in atherosclerosis such as oxidative stress, cell growth and migration, release of growth factors, adhesion and chemoattractant molecules, and cytokines.⁵ More interestingly, Ang II may induce in vascular cells the expression of the inflammatory cyclooxygenase (COX)-2 gene⁶ and influence the extracellular matrix turnover by regulating the activity of prostaglandin (PG)E₂–dependent metalloproteinases (MMPs),⁷ enzymes that degrade extracellular matrix and thus weaken the fibrous cap of the athero-
matous lesion, finally promoting its rupture.\(^8\)\(^9\) Notably, all these effects appear mediated by Ang II type 1 (AT\(_1\)) receptors, as reflected by in vitro studies using selective AT receptor antagonists.\(^6\)

In light of an AT\(_1\)-driven evolution of atherosclerotic plaque toward instability, increased angiotensin-converting enzyme (ACE) in culprit lesions in acute coronary syndromes has been recently demonstrated, indicating that enhanced ACE activity is related to a causative mechanism of atherosclerotic lesion rupture.\(^10\) Furthermore, Schieffer et al\(^11\) have recently demonstrated that Ang II, AT\(_1\) receptor, and ACE are coexpressed in human coronary arteries and colocalize with CD68 macrophages at the shoulder region of atherosclerotic plaques in patients with unstable angina. However, the intimate molecular mechanism(s) by which Ang II–AT\(_1\) interaction may influence plaque stability and blockade of the AT\(_1\) receptor may reduce cardiovascular risk are still unclear.

Expression of 72-kDa (MMP-2) and 92-kDa (MMP-9) gelatinase has been shown within human atherosclerotic lesions and critically implicated in plaque rupture and thus in acute ischemic events in humans.\(^8\)\(^9\) Biosynthesis of these MMPs by macrophages has been demonstrated to occur through a PGE\(_2\)-dependent pathway.\(^8\)\(^9\) Signaling through this pathway involves the modulation of COX and PGE synthase (PGES).\(^8\)\(^9\) Two isoforms of COX and 3 isoforms of PGES have been identified, called COX-1, COX-2, cytosolic PGES (cPGES), and type 1/type 2 microsomal PGES (mPGES), respectively. Although COX-1 and cPGES are constitutively expressed, COX-2 and mPGES-1 are induced in response to inflammatory stimuli, suggesting that these enzymes are involved in PG generation in inflammatory diseases.\(^12\) Consistent with the hypothesis that COX-2/mPGES-1 contributes to the clinical instability of atherosclerosis, we have recently reported the overexpression of COX-2/mPGES-1 as a pathway underlying the enhanced release of active MMPs in symptomatic atherosclerotic plaques.\(^8\)\(^9\)

Thus, it is time to hypothesize that blockade of the AT\(_1\) receptor could contribute to plaque stabilization by inhibiting COX-2/mPGES-1-dependent MMP biosynthesis, leading in turn to matrix degradation and plaque rupture.

The aim of this study was to test the hypotheses that plaque inflammation is an Ang II–dependent event and that AT\(_1\) blockade would modulate MMP production by macrophages in human atherosclerotic plaques. Thus, we treated hypertensive patients before their endarterectomy with irbesartan, a potent, long-acting, orally active Ang II receptor blocker with high selectivity for the AT\(_1\) receptor subtype and no interaction with the AT\(_2\) receptor,\(^13\) or chlorthalidone, a thiazide diuretic that acts directly on the kidney to increase the excretion of sodium chloride and water and does not have any described intrinsic anti-inflammatory characteristic.

Our data report reduced MMP production by macrophages in carotid plaques of patients randomized to irbesartan, most likely because of a reduction in PGE\(_2\) synthesis as a result of the suppression of COX-2/mPGES-1.

### Methods

**Patients**

We studied 70 of 131 consecutive hypertensive surgical inpatients (43 men, 27 women; mean age, 74±3 years) enlisted to undergo carotid endarterectomy for extracranial high-grade (≥70%) internal carotid artery stenosis. The remaining 61 patients were excluded from the study because they did not meet the blood pressure criteria for the study. All patients were “symptomatic” according to North American Symptomatic Carotid Endarterectomy Trial (NASCET) classification.\(^14\) We randomly assigned participants to irbesartan-based (300 mg/d, Aprovel, Sanofi-Synthelabo) or chlorthalidone-based (50 mg/d, Igroton, Novartis Pharma) regimens for 4 months after 1 to 2 weeks of placebo if sitting blood pressures were 160 to 180 mm Hg systolic, 95 to 110 mm Hg diastolic, or both. After the treatment period, all patients had undergone endarterectomy. Procedural methods, risk factors, blood pressure values at baseline, and concomitant therapy did not differ between the 2 groups (Table 1). The local ethics review committees approved the study. Written informed consent was obtained from all patients before each examination.

### Immunohistochemistry

After the surgical procedure, samples were immediately frozen in isopentane and cooled in liquid nitrogen. Serial sections of the study plaques were prepared and analyzed as previously described.\(^8\)

### Reverse-Transcriptase Polymerase Chain Reaction

COX-2 mRNA was evaluated by reverse-transcriptase polymerase chain reaction (RT-PCR) as previously described.\(^8\) RNA was reverse transcribed, and first-strand cDNA was used as a template in PCR. cDNA aliquots were amplified with primers specific for COX-2 and glyceraldehyde-3-phosphate dehydrogenase.

### Western Blot

Proteins were extracted, detected, and quantified by Western blot as previously described.\(^8\) Bands were expressed as densitometric units (DU).

### Zymography

Zymography was performed as previously described.\(^8\) This method detects both activated and zymogen forms of MMPs because, in the

### Table 1. Characteristics of Study Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chlorthalidone (n=35)</th>
<th>Irbesartan (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>73±3</td>
<td>75±4</td>
</tr>
<tr>
<td>Sex, n, male/female</td>
<td>21/14</td>
<td>22/13</td>
</tr>
<tr>
<td>No. patients with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recent TIA and stroke</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Family history of IHD</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Hypertension</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>Diabetes</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>NSAID or glucocorticoid treatment, n</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PRA at end of treatment, ng·mL(^{-1})·h(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>1.7±0.6</td>
<td>1.6±0.8</td>
</tr>
<tr>
<td>Standing (4–6 h)</td>
<td>4.2±1.2</td>
<td>4.4±1.1</td>
</tr>
<tr>
<td>Stenosis severity, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>75±5</td>
<td>75±6</td>
</tr>
<tr>
<td>Range</td>
<td>70–92</td>
<td>70–93</td>
</tr>
<tr>
<td>Reduction after treatment</td>
<td>−1.3±1.1</td>
<td>−1.2±0.9</td>
</tr>
<tr>
<td>Blood pressure reduction at end of treatment, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>−8±3</td>
<td>−9±4</td>
</tr>
<tr>
<td>Diastolic</td>
<td>−4±0.5</td>
<td>−4±0.7</td>
</tr>
</tbody>
</table>

TIA indicates transient ischemic attack; IHD, ischemic heart disease; NSAID, nonsteroidal antiinflammatory drug; and PRA, plasma renin activity.
presence of SDS, otherwise inactive forms can lyse the substrate contained in the gel because of detergent-induced conformational change. Conditioned medium of human fibrosarcoma cell line HT1080 was used as positive control.

Sirius Red Staining for Collagen Content
Sections were performed as previously described. After dehydration, the sections were observed under polarized light after coverslipping. The sections were photographed with identical exposure settings for each section.

Oil Red O Staining for Lipid Content
Two parallel sections from each plaque specimen were incubated in 60% isopropanol for 2 minutes and then in Oil Red O (Carlo Erba) solution for 20 minutes and rinsed in water. One section was counterstained with hematoxylin.

Blood Monocyte Isolation and Culture
Peripheral monocytes were purified and cultured as previously described. Control or stimulated (Ang II, 10^{-7} mol/L, Sigma Chemical Co) monocytes (2×10^5/4 mL, DME) were incubated for 4 hours at 37°C in the presence or absence of 10^{-8} mol/L irbesartan (Sanofi-Synthelabo) and 10^{-6} mol/L of the AT2 receptor antagonist PD123,319 (Sigma), each added 30 minutes before Ang II. PGE2 (10^{-7} mol/L, Cayman) was also added to some cultures. At the end of the incubation, media were collected; the activity of released MMP-2 and MMP-9 was analyzed by zymography; adherent monocytes were scraped, collected, and lysed; and COX-2, mPGES-1, and MMP expression was evaluated by Western blot.

Macrophage Extraction From Atherosclerotic Plaques
Macrophages were extracted from plaques as described by de Vries et al. Then, immunocytochemistry, Western blot, and zymography were carried out as described.

Statistical Analysis
Clinical and histological variables were compared by χ² test. Differences in enzymes expression and inflammatory infiltrate were analyzed by Student’s t test. Statistical significance was indicated by a value of P<0.05. All calculations were performed with the SPSS 11.0.1 computer program.

Results
Clinical Outcomes
Percentages of carotid diameter reduction (−1.3±1.1% versus −1.2±0.9%) did not differ between the 2 groups (Table 1). Blood pressures were equally reduced in both groups after treatment. Systolic/diastolic blood pressure at the end of follow-up fell by 8/4 and 9/4 mm Hg in the irbesartan and chlorthalidone groups, respectively (Table 1).

Cellular Composition
Plaque area occupied by macrophages and T cells was significantly smaller (P<0.0001) in plaques from the irbesartan group compared with plaques from the chlorthalidone group (Figure 1 and Table 2). On the contrary, staining for α-actin was higher in plaques from irbesartan-treated patients (Table 2).

Macrophage Activation Status
Plaques from patients randomized to chlorthalidone were always characterized by strong expression of HLA-DR antigens, which contrasted with the low expression of HLA-DR in the irbesartan-treated plaques (Figure 1 and Table 2).

HLA-DR expression was observed mainly on macrophages and lymphocytes.

COX-2 Expression in Plaques Is Reduced by Irbesartan
After treatment, COX-2 was significantly (P<0.0001) more abundant in lesions from patients randomized to chlorthalidone than in plaques from subjects treated with irbesartan (Figure 2 and Table 2). COX-2 accumulated mainly in the

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chlorthalidone (n=35)</th>
<th>Irbesartan (n=35)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages</td>
<td>28±9</td>
<td>7±3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T lymphocytes, cells/mm²</td>
<td>76±15</td>
<td>21±7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SMCs</td>
<td>13±4</td>
<td>21±7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>22±5</td>
<td>14±4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>COX-2</td>
<td>25.1±4</td>
<td>7.2±2.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>mPGES-1</td>
<td>22.4±5.1</td>
<td>5.6±1.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MMP-2</td>
<td>25.8±4.9</td>
<td>5.8±1.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MMP-9</td>
<td>28.2±4</td>
<td>6.2±2.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Collagen content</td>
<td>9.3±2.6</td>
<td>18.4±2.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lipid content</td>
<td>22.3±5.3</td>
<td>15±4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Oxidized LDL content</td>
<td>23.4±4.5</td>
<td>13.8±3.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ang II</td>
<td>17.8±3.1</td>
<td>15.6±3.2</td>
<td>NS</td>
</tr>
<tr>
<td>AT1 receptor</td>
<td>21.1±2.2</td>
<td>19.8±2.6</td>
<td>NS</td>
</tr>
<tr>
<td>AT2 receptor</td>
<td>14.9±1.5</td>
<td>19.5±1.2</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
macrophages at the shoulder region and the periphery of the lipid core in subjects treated with chlorthalidone, whereas it localized prevalently in smooth muscle cells (SMCs) in irbesartan-treated patients. Finally, Western blot and semi-quantitative RT-PCR analyses confirmed lower COX-2 expression in the irbesartan-treated patients in both plaque homogenates (6876±143 versus 1156±121 DU; mean±SD) for protein expression; n=35; P<0.0001; Figure 3) and plaque-derived macrophages. In contrast, no significant effects were observed with respect to COX-1 expression.

mPGES-1 Expression in Plaques Is Reduced by Irbesartan
Immunohistochemistry (Figure 2) revealed strong mPGES-1 immunoreactivity in all plaques from patients randomized to chlorthalidone but only weak staining in plaques from patients randomized to irbesartan (Table 2). In the chlorthalidone group, mPGES-1 localized in the plaque shoulder, an area characterized as macrophage-rich. Only weak mPGES-1 expression was observed by Western blot in irbesartan-treated patients. In contrast, a 7-fold-higher signal was demonstrated in chlorthalidone-treated patients in both plaque homogenates (978±143 versus 6124±217 DU; n=35; P<0.0001; Figure 3) and plaque-derived macrophages.

Effect of Irbesartan on MMP Expression in Plaques
MMP staining was significantly (P<0.0001) less abundant in the lesions from irbesartan-treated patients compared with that of patients randomized to chlorthalidone (Figure 2). Levels of MMP-2 and MMP-9 in chlorthalidone-treated plaques significantly exceeded those in irbesartan-treated plaques (Table 2). Immunoreactivity localized in the plaque shoulder, corresponding to areas of intense macrophage infiltration.

Effect of Irbesartan on MMPs Activity in Plaques
The higher (P<0.0001) MMP-2 and MMP-9 immunoreactivity documented by Western blot in chlorthalidone-treated plaques (6576±257 versus 1234±152 DU and 7121±241 versus 1167±158 DU, respectively; n=35; Figure 3) does not necessarily correspond to enhanced enzymatic activity because all MMPs require activation before they can digest their substrate.16 Thus, we used zymography to demonstrate that both plaque homogenates and macrophages extracted from plaques of chlorthalidone-treated patients contained activated MMPs (Figure 4). In contrast, only weak positivity for activated MMPs was observed in irbesartan-treated plaques (Figure 4).

Effect of Irbesartan on Plaque Extracellular Components
Sirius Red polarization showed an increased content of interstitial collagen in the tissue sections of patients random-
ized to irbesartan (Table 2). In contrast, the plaque content of total lipids (Table 2) and oxidized LDLs (Table 2 and Figure 5) was significantly reduced after irbesartan therapy.

Effect of Treatment on Ang II Pathway in Plaques

Immunohistochemistry and Western blot did not show differences in Ang II and AT1 expression in both tissue preparations and plaque-derived macrophages between patients randomized to irbesartan or chlorthalidone (Table 2). In contrast, AT2 expression was significantly enhanced after irbesartan therapy (Table 2).

AT1-Dependent Modulation of MMPs by Ang II

Ang II caused an enhancement in COX-2, mPGES-1, MMP-2, and MMP-9 levels over that detected in control monocytes (Figure 6). Enzyme induction by Ang II was significantly \((P<0.0001)\) inhibited by irbesartan but was not affected by the selective AT2 antagonist PD 123,319. However, the inhibitory effect of irbesartan on MMPs was reversed by the addition of PGE2.

Discussion

We have previously reported that COX-2 and mPGES-1 contribute to the clinical instability of atherosclerotic plaques by promoting plaque rupture induced by MMPs, key enzymes in the final step of this process.\(^8\),\(^9\) Now, in the present report, we provide evidence for the critical involvement of COX-2/mPGES-1 downregulation in the process of plaque stabilization induced by AT1 antagonists.

In particular, the present findings are, to the best of our knowledge, the first to (1) demonstrate by randomized study the anti-inflammatory effect of AT1 antagonists in human carotid atherosclerotic plaques, (2) show a direct inhibitory effect of irbesartan on COX-2/mPGES-1 in human atherosclerotic lesion, and (3) relate the inhibition of COX-2/mPGES-1 to the reduction of MMP activity observed after irbesartan therapy.

Concomitantly higher expression of COX-2/mPGES-1, MMP-2, and MMP-9 was found in specimens obtained from the “culprit” carotid lesions of hypertensive patients randomized to chlorthalidone compared with specimens obtained from patients randomized to irbesartan. Notably, the real impact of MMP suppression in the process of irbesartan-dependent plaque stabilization was confirmed in this study by the parallel increment in plaque collagen content after irbesartan therapy.

In this study, macrophages were more abundant in plaques randomized to chlorthalidone and represented the major source of COX-2/mPGES-1 and MMPs. Furthermore, the site of inflammatory infiltration in plaques randomized to chlorthalidone was always characterized by strong expression of HLA-DR antigens on inflammatory cells, which contrasted with the low expression of HLA-DR elsewhere in the irbesartan-treated plaques. Thus, these data suggest the ability...
of irbesartan to reduce the inflammatory reaction in symptomatic atherosclerotic plaques.

In fact, in agreement with the difference in COX-2/mPGES-1 and MMP staining patterns, the histological milieu of the lesions appears different with regard to cellularity, presence of foam cells, cholesterol clefts, and collagen content but not in the degree of vessel stenosis, suggesting that lesions treated with irbesartan or chlorthalidone are different only in terms of inflammatory burden and that differences in plaque behavior stem from differences between irbesartan and chlorthalidone in the ability to influence the expression of ≥1 enzymes capable of disrupting plaque stability.

The recent LIFE study has reported the ability of AT1 antagonists to reduce in carotid arteries the evolution of atherosclerotic lesion toward rupture. However, this study did not provide any evidence about the involvement of COX-2/mPGES-1 in the pathophysiology of AT1 antagonist–dependent plaque stabilization. COX-2 is an intermediate enzyme in the metabolic pathway of arachidonic acid, and the COX bioproduct PGH2 is further metabolized by other isomerases to various prostanoids. Thus, the concomitant expression of COX-2 and mPGES-1 is necessary for the biosynthesis of PGE2-dependent MMPs in the setting of atherosclerotic plaque.

Interestingly, because macrophages of the shoulder region contain most of the COX-2/mPGES-1 protein within the lesion, they emerge as the principal cellular target of irbesartan in the context of plaque stabilization. This finding may have functional importance and may contribute to the explanation of the controversial findings associated with selective COX-2 inhibition, because different cell types can regulate the production of different eicosanoids. Endothelium predominantly releases PGI2, a vasodilating agent and an inhibitor of platelet activation, and Belton et al reported that COX is responsible for the increase in PGI2 seen in patients with atherosclerosis. In contrast, macrophages, not present in normal arterial tissue, produce an array of prostanoids, including PGE2, considered one of the most atherogenic eicosanoids.

Prostanoids have potent actions on SMCs, regulating contractility, cholesterol metabolism, and proliferation. Reduced expression of COX might thus contribute to the decrease in lipid accumulation in lesional macrophages observed in this study, reducing formation of macrophage-derived foam cells within atheroma. On the other hand, anti-proliferative and antimigratory actions of COX products on SMCs suggest potential contributions of the inhibition of this enzyme to the evolution of a lesion toward an SMC-enriched and macrophage-depleted, and thus more stable, plaque. More importantly, PGE2 induces in human atherosclerotic plaques the expression of MMP-2 and MMP-9, enzymes considered crucial in the degradation of plaque stability. Our description of a strong reduction in these MMPs in plaque treated with irbesartan and found to be macrophage depleted and COX-2/mPGES-1 negative suggests that such arachidonate-dependent inhibition of MMPs by irbesartan may operate in vivo.

Interestingly, these results obtained with irbesartan therapy, despite only modest reductions in blood pressure, are in accord with the results of the recent HOPE and Second Australian National Blood Pressure (ANBP2) studies and provide further support for the hypothesis that AT1 antagonists can have a significant effect on stroke beyond blood pressure. Furthermore, our results are also in agreement with recent studies demonstrating the ability of AT1 antagonists to reduce the development of early atherosclerosis and the inflammatory status in patients with proven atherosclerosis. Finally, the hypothesis that plaque COX-2/mPGES-1 suppression by irbesartan is dependent on the selective blockade of the AT1 receptor is supported in this study by in vitro experiments with the selective AT2 antagonist PD123,319.

Notably, because it has been reported that hypercholesterolemia may induce overexpression of AT1 receptors, an effect reversed by statin therapy, we can speculate that this mechanism of plaque instability may also operate in hypercholesterolemic patients, and association of AT1 antagonists with statins could offer additional benefits in this setting.

One primary reason for our choice of chlorthalidone as the comparative agent was that it has been shown to be better than other first-line molecules in trials of antihypertensive drugs. However, because diuretics could theoretically increase generation of Ang II via the renin pathway, we also analyzed in this study the plaque expression of Ang II and the plasma renin activity. Results clearly demonstrate that both plaque contents of Ang II and plasma renin activity were not different between the 2 groups of patients, thus ruling out any hypothesis of drug-dependent induction of COX-2 in plaques of patients randomized to chlorthalidone. Finally, because in this study we did not include patients treated with either chlorthalidone or irbesartan, we cannot exclude a potentially untoward proinflammatory effect of diuretics. However, this hypothesis also seems unlikely because the amounts of inflammatory infiltration and enzyme expression observed in this study in the plaques from patients treated with chlorthalidone were extremely similar to those recently observed in another study on untreated plaques.

In conclusion, this study addresses the missing link between blockade of the AT1 receptors and MMP inhibition, leading in turn to plaque stabilization, by demonstrating the inhibition of COX-2/mPGES-1 in human atherosclerotic lesions after irbesartan therapy and providing evidence that downregulation of COX-2/mPGES-1 in activated macrophages by irbesartan is associated with plaque stabilization, possibly by suppression of the MMP-induced matrix degradation promoting plaque rupture.

These findings are potentially important from a fundamental standpoint because they indicate a crucial role for COX-2/mPGES-1 in the stabilization of atherosclerotic lesions observed with AT1 antagonists. From a practical standpoint, these findings provide further support for the possibility that AT1 antagonists might provide a novel form of therapy for plaque stabilization in patients with atherosclerotic disease.

Acknowledgments
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References


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