Angiotensin II Type 1 Receptor Antagonists Inhibit Basal As Well As Low-Density Lipoprotein and Platelet-Activating Factor-Stimulated Human Monocyte Chemoattractant Protein-1

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ABSTRACT

Monocyte chemoattractant protein-1 (MCP-1) is a potent chemotactic agent for monocytes and other cells and is thought to be involved in atherosclerosis, recruiting monocytes to the subendothelial space or to the site of inflammation. Angiotensin II has been demonstrated, at least in animal models, to stimulate MCP-1 expression. We investigated the effect of the angiotensin II type 1 (AT1) receptor antagonists irbesartan and losartan on MCP-1 production by freshly isolated human monocytes. Irbesartan and losartan inhibited basal MCP-1 production in a dose-dependent manner. Low-density lipoprotein (LDL) stimulated MCP-1 in a concentration-dependent manner, with 200 μg/ml LDL protein giving a 2-fold increase in MCP-1. Irbesartan and losartan dose dependently blocked LDL-stimulated MCP-1. An angiotensin II type 2 receptor antagonist, S-(-)-1-[(4-(dimethylamino)-3-methylphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo(4,5-c)pyridine-6-carboxylic acid (PD123319), had no significant effect on basal MCP-1 levels or LDL-stimulated MCP-1. After noting homology between the AT1 receptor and the platelet-activating factor (PAF) receptor, we showed that irbesartan inhibited both [3H]PAF binding to human monocytes and carbamyl-PAF stimulation of MCP-1. However, irbesartan affinity for the PAF receptor was 700 times less than PAF, suggesting that there may be another mechanism for irbesartan inhibition of PAF-stimulated MCP-1. This is the first report showing that AT1 receptor antagonists inhibit basal as well as LDL- and PAF-stimulated MCP-1 production in freshly isolated human monocytes.

Chemotactic cytokines or chemokines are structurally related small proteins involved in trafficking and activation of leukocytes (Bajjigoli, 1998). The chemokine monocyte chemoattractant protein-1 (MCP-1) is highly expressed in human atherosclerotic lesions and is thought to be important in monocyte recruitment into the arterial wall and developing lesions (Nelken et al., 1991; Yla-Herttuala et al., 1991). The development of atherosclerosis has been associated with numerous risk factors, including elevated levels of plasma cholesterol (particularly low-density lipoprotein; LDL), hypertension, diabetes, and smoking (Ross, 1993). High concentrations of plasma LDL lead to higher concentrations in the subendothelial space where LDL may become oxidatively modified. Oxidized LDL may injure the endothelium and play a role in the migration of leukocytes into the vascular wall (Ross, 1993). Oxidized LDL exposure increased MCP-1 mRNA expression and MCP-1 protein levels in rabbit macrophages (Wang et al., 1997). LDL, minimally modified by oxidation, increased MCP-1 mRNA expression in human endothelial cells and smooth muscle cells (Cushing et al., 1990).

Monocyte/macrophages make up the bulk of infiltrated leukocytes in atherosclerotic plaque and are considered to be the main inflammatory mediators in atherosclerosis. Numerous studies suggest that MCP-1 has an important role in the infiltration of monocytes into lesions (Nelken et al., 1991; Yla-Herttuala et al., 1991; Yu et al., 1992). Mice lacking MCP-1 exhibited attenuated atherosclerosis and monocyte accumulation in the artery (Gu et al., 1998). Deletion of the receptor for MCP-1 resulted in decreased lesion formation (Boring et al., 1998).

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ABBREVIATIONS: MCP-1, monocyte chemoattractant protein-1; LDL, low-density lipoprotein; Ang II, angiotensin II; AT1, angiotensin II type 1; PAF, platelet-activating factor; AT2, angiotensin II type 2; c-PAF, carbamyl-PAF; HBSS, Hanks’ balanced salt solution; HIFCS, heat-inactivated fetal calf serum; BSA, bovine serum albumin; HSA, human serum albumin; DMSO, dimethyl sulfoxide; fMLP, N-formylmethionyl-leucyl-phenylalanine; TXA2, thromboxane A2; HUVEC, human umbilical vein endothelial cell; PD123319, S-(-)-1-[(4-(dimethylamino)-3-methylphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo(4,5-c)pyridine-6-carboxylic acid; WEB 2086, 3-[4-(2-chlorophenyl)-9-methyl-6-thienol-[3,2-f][1,2,4]triazolo-[4,3-a][1,4]diazepine-2-yl]-1-(4-morpholynil)-1-propionate; CV11974, 2-ethoxy-1-[(4-(tetrazol-5-yl)bibiphenyl-4-yl)methyl]-1H-benzimidazole-7-carboxylic acid.
Ang II has been implicated in the pathogenesis of atherosclerosis. Ang II can stimulate the production of reactive oxygen species and increase expression of proinflammatory gene products, and both oxidative stress and inflammation are thought to have a role in atherogenesis (Griendling and Alexander, 1997; Ross, 1999). Studies in experimental models of atherosclerosis have shown that inhibition of angiotensin-converting enzyme or blockade of AT1 receptors decreases atherosclerosis (Schuh et al., 1993; Keidar et al., 1997; Makaritsis et al., 1998).

Ang II has been shown to increase MCP-1 mRNA expression in cultured monocyte U937 cells and in rat thoracic aortic vascular smooth muscle cells (Hernandez-Presa et al., 1997). Ang II can also activate MCP-1 gene transcription and stimulates MCP-1 mRNA in rat aortic smooth muscle cells (Chen et al., 1998). The increase in MCP-1 mRNA can be prevented by the AT1 receptor antagonist losartan (Chen et al., 1998).

We investigated the effect of Ang II and the AT1 receptor antagonists irbesartan and losartan on the production of MCP-1 by human monocytes. Ang II had no effect on MCP-1 levels in our study, perhaps due to its metabolism during incubation with monocytes. Irbesartan and losartan inhibited both basal and stimulated release of MCP-1 possibly through a non-AT1 receptor-related mechanism.

Platelet-activating factor (PAF) is a phospholipid with proinflammatory and thrombogenic properties, and PAF has been shown to stimulate MCP-1 production (Sugano et al., 1998; Croft et al., 1991). Briefly, plasma density was increased to 1.07 by addition of NaCl and then a four-step gradient was constructed over the plasma using the following densities: 1.8 ml of 1.063 kg/l NaCl, 1.8 ml of 1.04 kg/l NaCl, 1.8 ml of 1.02 kg/l NaCl, and 2.1 ml of water. Samples were ultracentrifuged at 205,000g (average) for 20 h using a Centrifon T-1190 ultracentrifuge (Kontron Instruments, Milan, Italy). The LDL band was collected by aspiration and passed through a PD10 Sephadex column (Pharmacia AB, Uppsala, Sweden) to remove the excess salt and the majority of the EDTA. The LDL was stored in the dark at 4°C. LDL protein was determined by a modification of the Lowry method (Markwell et al., 1978) using BSA protein standard (Sigma-Aldrich). Just before use, isolated LDL was passed through a second PD10 column to remove the remaining EDTA.

**Assay for MCP-1.** Human MCP-1 OptEIA (BD PharMingen, San Diego, CA) and Costar enzyme immunoassay/radioimmunoassay one-half-area flat bottom 96-well plates (Corning Glassworks, Corning, NY) were used to measure MCP-1 levels in cell supernatants. The sensitivity of the assay was 50 pg/ml, the intra-assay coefficient of variation was 10%, and the interassay coefficient of variation was 12%.

**PAF Binding Assay.** Cells were washed twice with cold HEPES-Tyrode’s buffer (Izumi et al., 1997) containing 0.25% (w/v) fatty acid-free human serum albumin (HSA) and kept on ice. Cells (1–2 × 10^6/200-μl/tube) were preincubated for 5 min with (non-specific binding) or without (total binding) unlabeled PAF (10 μM) or test reagents before addition of [3H]PAF (4 nM final concentration) and incubation on ice for 10 min. Cells were then washed three times with the cold buffer and solubilized with 1% Triton X-100. Radioactivity associated with the cells was measured with liquid scintillation counting. Separate experiments were carried out using varying concentrations of [3H]PAF, and saturation binding data were analyzed with nonlinear regression using GraphPad Prism 3 (GraphPad Software Inc., San Diego, CA). Competitive binding experiments were carried out measuring the binding of 4 nM [3H]PAF in the presence of varying concentrations of irbesartan and unlabeled PAF, and IC_{50} (inhibitory concentration 50%) concentrations were determined for each ligand using GraphPad Prism 3.
Results

Time Course of MCP-1 Production. Isolated human monocytes were incubated at 37°C, and cell supernatants were collected at varying time points. MCP-1 production increased over time (Fig. 1), so future incubations were carried out overnight.

Effect of Ang II and Irbesartan on MCP-1 Production by Human Monocytes. Human monocytes in HBSS were preincubated for 20 min with or without irbesartan (50 μM) and then incubated overnight with or without Ang II (10⁻⁷ M) (Fig. 2). Levels of MCP-1 produced by cells incubated with DMSO (vehicle for irbesartan) were not different from control cells incubated with medium alone. There was no significant effect of Ang II on basal MCP-1 production. Irbesartan reduced basal MCP-1 by more than 90% under these conditions.

AT1 Receptor Antagonists Dose Dependently Inhibit Basal and LDL-Stimulated MCP-1 Production. Fig. 3A shows that increasing concentrations of irbesartan resulted in increasing inhibition of basal MCP-1 production by human monocytes. At 50 μM irbesartan, MCP-1 was reduced by more than 60% (p < 0.02), whereas at 5 μM irbesartan, MCP-1 inhibition was greater than 95% (p = 0.001). A similar effect was seen with losartan, another AT1 antagonist, at concentrations 2 times higher than irbesartan (Fig. 3B).

Addition of increasing concentrations of LDL before the overnight incubation resulted in dose-dependent increases in MCP-1, with 200 μg/ml LDL protein resulting in a 2-fold increase in MCP-1 levels compared with control cells (p < 0.04) (Fig. 3A). LDL-stimulated MCP-1 levels were reduced by irbesartan in a dose-dependent manner (200 μg/ml LDL).
plus 50 μM irbesartan, p < 0.02; 100 μg/ml LDL plus 50 μM irbesartan, p < 0.02; 50 μg/ml LDL plus 50 μM irbesartan, p < 0.03) (Fig. 3A). Losartan dose dependently reduced MCP-1 levels stimulated with 200 μg/ml LDL (Fig. 3B).

Specificity of AT1 Antagonist. To determine whether this result was specific for AT1 receptor antagonists, the effect of an AT2 receptor antagonist, PD123319, was examined. Figure 4 shows that the AT2 antagonist had no significant effect on basal MCP-1 levels, whereas the same concentration of AT1 antagonist almost completely blocks MCP-1 production. The LDL-stimulated increase in MCP-1 was blocked by the AT1 antagonist, but the same concentration of AT2 antagonist had no significant effect. These data suggest that the effect on both basal and LDL-stimulated MCP-1 is due mainly to the AT1 type receptor antagonist.

Possible Binding of Irbesartan to Other Receptors. The inhibition of basal MCP-1 production by irbesartan and losartan suggested the possibility that these reagents are able to bind to cell receptors other than AT1. A previous study (Raiden et al., 1997) reported that losartan blocked the binding of [3H]fMLP to the fMLP receptor, which was found to have 25 to 30% structural homology with the AT1 receptor. The PAF receptor is in the same family of chemotactant receptors as the fMLP receptor. A search conducted using GenBank found 22% sequence similarities between the angiotensin receptor and the PAF receptor. This raised the possibility that irbesartan and losartan may bind to the PAF receptor.

Irbesartan Inhibits PAF Binding to Human Monocytes. To determine whether irbesartan binds to the PAF receptor, human monocytes were preincubated with irbesartan, losartan, WEB 2086 (PAF receptor antagonist), or unlabeled PAF (nonspecific binding) before addition of [3H]PAF (Fig. 5). Irbesartan and losartan dose dependently inhibited [3H]PAF binding, indicating that they bind to the PAF receptor. Their efficacy was similar to the PAF antagonist WEB 2086 in our study. PAF is very hydrophobic and exhibits high nonspecific binding to the lipid bilayer of plasma membranes (Chao and Olson, 1993), which may account for some of the uninhibitable [3H]PAF binding.

Competitive Binding. Varying concentrations of [3H]PAF were added to human monocytes to determine binding parameters (Fig. 6). Data were analyzed using nonlinear regression in GraphPad Prism 3 to obtain $B_{\text{max}}$ (36,280) and $K_D$ (41 nM) values for [3H]PAF.

Competitive binding experiments were carried out using
irbesartan and unlabeled PAF (Fig. 7), and the IC50 value (inhibitory concentration 50%) determined. Ks (affinity of PAF receptor) was calculated from Kd and IC50 values using Prism. Using five different monocyte preparations, unlabeled PAF gave a mean IC50 value of 6.4 ± 1.6 × 10−8 M and a Ks value of 5.8 ± 1.5 × 10−8 M. Irbesartan gave a mean IC50 value of 49.0 ± 4.3 × 10−6 M and a Ks value of 43.1 ± 3.9 × 10−6 M. These results indicated that irbesartan had 700 to 800 times lower affinity for the PAF receptor than unlabeled PAF.

Irbesartan Inhibits PAF-Stimulated MCP-1 Production. Human monocytes were preincubated with irbesartan, losartan, or the PAF antagonist WEB 2086, and then incubated overnight with the stable PAF agonist carbamyl-PAF, and supernatant MCP-1 levels were measured. The stable metabolite of PAF was required for stimulation of MCP-1, probably because native PAF is degraded during incubation with cells. This experiment was carried out in HBSS (Fig. 8A) and RPMI 1640 medium containing 1% HIFCS, a more physiological medium (Fig. 8B). In serum-free HBSS (Fig. 8A), irbesartan dose dependently inhibited basal (control plus irbesartan, p < 0.002 for all irbesartan concentrations) and carbamyl-PAF (c-PAF)-stimulated (control plus c-PAF, p < 0.03; c-PAF plus irbesartan, p < 0.002 for all irbesartan concentrations) MCP-1 production. WEB 2086 also dose dependently inhibited basal (control plus WEB 2086, p < 0.05 for all WEB 2086 concentrations) and c-PAF-stimulated (c-PAF plus 20 or 50 μM WEB 2086, p < 0.009) MCP-1 release, but was less effective than irbesartan.

Carbamyl-PAF stimulated monocyte MCP-1 production in a dose-dependent manner (Fig. 8B) (control plus c-PAF 1 μM, p < 0.04). Figure 8B shows that in RPMI 1640 medium containing 1% HIFCS, irbesartan, and losartan inhibited c-PAF MCP-1 stimulation, similar to the PAF antagonist WEB 2086 (c-PAF 1 μM plus irbesartan, p < 0.04; plus losartan, p < 0.09; plus WEB 2086, p < 0.04) (c-PAF 0.6 μM plus irbesartan, p < 0.05; plus losartan, p < 0.04; plus WEB 2086, p < 0.07). Basal MCP-1 levels were significantly reduced in the presence of irbesartan, losartan, and WEB 2086 (control plus irbesartan, p < 0.001; plus losartan, p < 0.001; plus WEB 2086, p < 0.002). Thus, the receptor or mechanism involved in the inhibition of human monocyte MCP-1 is sensitive to LDL, PAF, AT1 antagonists, and PAF antagonists.

PAF Receptor Antagonist Inhibits Basal and LDL-Stimulated MCP-1 Production. Fig. 9 shows that increasing concentrations of WEB 2086 resulted in increasing inhibition of basal MCP-1 production. At 10 μM WEB 2086, there was a 20% reduction (p < 0.05), whereas 50 μM WEB 2086
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Fig. 9. PAF receptor antagonist inhibits basal and LDL-stimulated MCP-1. Monocytes were isolated as described under Materials and Methods, resuspended in HBSS, and preincubated for 20 min with 0, 10, 20, or 50 \( \mu \)M irbesartan or WEB 2086. Medium or LDL (200 \( \mu \)g/ml final concentration) was added, and the cells were incubated overnight. Results are mean ± S.E. from three experiments.

resulted in a 60% decrease \((p < 0.009)\) in basal MCP-1. Irbesartan was more effective, with 10 \( \mu \)M irbesartan reducing basal MCP-1 levels by 40% \((p < 0.05)\), 20 \( \mu \)M giving 75% reduction \((p < 0.02)\), and 50 \( \mu \)M reducing levels by greater than 95% \((p < 0.008)\). LDL (200 \( \mu \)g/ml) significantly increased MCP-1 production 2-fold \((p < 0.01)\). WEB 2086 inhibited LDL-stimulated MCP-1 levels by 30% \((p < 0.02)\) at 20 \( \mu \)M and by 50% \((p < 0.02)\) at 50 \( \mu \)M. Irbesartan was again more effective, reducing LDL-stimulated MCP-1 by 50% \((p < 0.02)\) at 10 \( \mu \)M, 70% \((p < 0.001)\) at 20 \( \mu \)M, and greater than 95% \((p < 0.002)\) at a concentration of 50 \( \mu \)M.

Discussion

Recent studies suggest that AT1 receptor antagonists used in the treatment of hypertension may also be beneficial in the treatment of atherosclerosis. Lesion development in Apo E-deficient mice was significantly reduced by treatment with the AT1 antagonists irbesartan (Dol et al., 2001) and losartan (Keidar et al., 1997). Irbesartan-treated mice had less macrophages in the lesion area, suggesting irbesartan inhibits monocyte/macrophage influx into the vessel wall. Irbesartan treatment also decreased MCP-1 mRNA levels and MCP-1 immunostaining in the lesion area (Dol et al., 2001).

Treatment of patients with coronary artery disease with irbesartan reduced levels of the inflammatory markers vascular cell adhesion molecule-1, tumor necrosis factor-\( \alpha \), and superoxide (Navalkar et al., 2001). Lipid peroxidation, superoxide levels, and monocyte-binding capacity were reduced in subjects with coronary artery disease receiving irbesartan (Khan et al., 2001).

Our in vitro studies with freshly isolated human monocytes suggest that AT1 receptor antagonists may inhibit the inflammatory component of atherosclerosis. We found that irbesartan and losartan inhibited basal production of the inflammatory marker MCP-1 by human monocytes in the absence of any stimulant. Yanagitani et al. (1999) showed that the AT1 antagonist CV11974 decreased basal levels of peroxide production in macrophages. This may be related to our findings, because reactive oxygen species, which include peroxide and superoxide, are involved in MCP-1 production (De Keulenaer et al., 2000).

Ang II stimulated MCP-1 mRNA in the cultured monocytic cell line U937 and in cultured rat thoracic aortic smooth muscle cells (Hernandez-Presa et al., 1997). In rat aortic smooth muscle cells, Ang II stimulated MCP-1 mRNA and the increase was prevented by losartan (Chen et al., 1998). We found that in freshly isolated human monocytes, Ang II had no effect on MCP-1 protein levels, whereas the AT1 receptor antagonists irbesartan and losartan inhibited basal MCP-1. It is possible that the absence of an effect of Ang II was caused by its degradation during the 20-h incubation with cells. It may be necessary to use a stable derivative of Ang II to see an effect under these conditions. It is also possible that there was basal release of Ang II by the cells and that MCP-1 stimulation by basal Ang II was inhibited by irbesartan and losartan. These hypotheses were not examined in our study. Our results suggested the possibility that inhibition of basal MCP-1 was independent of the AT1 receptor. Raiden et al. (1997) reported that the AT1 receptor antagonist losartan inhibited neutrophil recruitment and activation by fMLP, by inhibiting neutrophil binding of fMLP through a mechanism independent of losartan binding to AT1 receptors. The AT1 receptor and the high-affinity receptor for fMLP share 25 to 30% sequence identity.

The receptors for Ang II, fMLP, PAF, complement protein fragment 5a, and the chemokines belong to the family of seven-transmembrane-domain rhodopsin-like G protein-coupled receptors (Murphy, 1994). On searching GenBank, we found that the AT1 receptor has 22% homology with the PAF receptor. We showed that irbesartan and losartan inhibited both binding of \( [3H] \)PAF to monocytes and PAF stimulation of MCP-1 in monocytes. When irbesartan binding to the human monocyte PAF receptor was examined more closely, it was found that the affinity of the PAF receptor for irbesartan \((43 \mu M)\) was about 700 times less than the affinity for PAF \((0.06 \mu M)\). This suggests that the inhibition of PAF-stimulated MCP-1 by irbesartan may be partially independent of irbesartan binding to the PAF receptor.

PFA stimulates thromboxane \( A_2 \) (TXA\(_2\)) production (Ishizuka et al., 1994), a TXA\(_2\) analog stimulates MCP-1 production and TXA\(_2\) receptor antagonists inhibit PAF-induced MCP-1 in human umbilical vein endothelial cells (HUVECs) (Ishizuka et al., 2000). These studies suggest that the TXA\(_2\) receptor may be involved in PAF-stimulated MCP-1 in HUVECs. Irbesartan inhibited TXA\(_2\)-induced vasoconstriction in canine coronary arteries and human platelet aggregation, and high concentrations of irbesartan significantly inhibited TXA\(_2\) receptor antagonist binding (Li et al., 2000). However, the affinity of the TXA\(_2\) receptor for irbesartan \((10 \mu M)\) was 1400 times less than the affinity for the TXA\(_2\) analog \((7 \text{ nM})\) (Li et al., 2000). These findings suggest that inhibition of PAF-stimulated MCP-1 by irbesartan may be partially independent of irbesartan binding to the TXA\(_2\) receptor. Irbesartan was 2-fold more potent than losartan in the inhibition of TXA\(_2\) analog-induced vasoconstriction (Li et al., 2000) as it was in the inhibition of MCP-1 production in monocytes in the current study. Other AT1 antagonists may behave differently to irbesartan and losartan, depending on their binding properties.

A 300-mg dose of irbesartan results in human plasma
concentrations of around 10 μM irbesartan (Pool et al., 1998).
Our results suggest that this concentration of irbesartan inhibits basal and LDL-stimulated MCP-1 production from monocytes (Figs. 3A and 9). Sugano et al. (2001) showed that 10 nM carbamyl-PAF stimulated MCP-1 in cultured human uterine cervical fibroblasts, and this stimulation of MCP-1 was abolished by coincubation with 10 μM (1000-fold excess) WEB 2170, a PAF receptor antagonist, in medium containing 0.1% BSA. We showed that 1 μM carbamyl-PAF stimulated MCP-1 production in human monocytes. This stimulation was inhibited by 50 μM (50-fold excess) WEB 2086, a PAF receptor antagonist, and by 50 μM of the AT1 receptor antagonist irbesartan (Fig. 8B), in medium containing 1% serum, a more physiological environment with more potentially complicating factors than 0.1% BSA.

The involvement of PAF in atherosclerosis was suggested in a study where the PAF receptor antagonist WEB 2086 inhibited fatty streak development in LDL receptor null mice fed a western diet (Subbanagounder et al., 1999). They found that the in vitro inhibitory effects of WEB 2086 on monocyte binding to endothelial cells did not seem to be due to blocking the PAF receptor. Similarly, in our experiments, the inhibitory effects of irbesartan, losartan, and WEB 2086 on carbamyl-PAF stimulation of MCP-1 may not be due entirely to blocking the PAF receptor.

Ether- and ester-containing PAF-like lipids are generated during oxidation of LDL (Tokumura et al., 1996), and PAF and PAF-like oxidized phospholipids have been shown to activate monocytes via the PAF receptor (Heery et al., 1995; Tokumura et al., 1996; Lehr et al., 1997). Hayek et al. (2000) have shown that the AT1 receptor losartan significantly reduced human monocyte-derived macrophage uptake of oxidized LDL and also decreased CD36 (an ox LDL receptor) mRNA expression. These reports raise the possibility that LDL incubated overnight with monocytes, as in our study, becomes oxidized and PAF-like lipids are generated and stimulate MCP-1 production. LDL may not be working exclusively through the PAF receptor because the PAF receptor antagonist did not completely inhibit LDL-stimulated MCP-1 (Fig. 9), although it blocked PAF-stimulated MCP-1 (Fig. 8A). It is possible that other PAF receptor antagonists with different binding characteristics may show greater inhibition.

Our study shows that the AT1 receptor antagonists irbesartan and losartan decreased basal MCP-1 levels in human monocytes possibly through a mechanism independent of binding to the AT1 receptor. LDL-stimulated and carbamyl-PAF-stimulated MCP-1 levels in human monocytes were reduced by these AT1 receptor antagonists (Figs. 3, 8, and 9) through a mechanism partially independent of binding to the PAF receptor. Another possible mechanism of action is reduction of MCP-1 levels by nitric oxide. It has been shown (Kalinowski et al., 2002) that AT1 receptor antagonists stimulate nitric oxide release in rat platelets and HUVECs. Increased nitric oxide release in the presence of AT1 receptor antagonists may decrease MCP-1 levels because nitric oxide has been shown to inhibit MCP-1 production (Zeher et al., 1995; Tsao et al., 1997). Li et al. (2000) found that a nitric-oxide synthase inhibitor had no effect on inhibition of TXA2-induced vasoconstriction by irbesartan. However, the effect of a nitric-oxide synthase inhibitor on the reduction of monocyte MCP-1 production by irbesartan has not been studied.

Because PAF and Ang II may both be involved in athero-
sclerosis, AT1 receptor antagonists may inhibit atherosclero-
sis through more than one pathway; blocking AT1 receptors and the effects of Ang II, as well as inhibiting the effects of PAF or PAF-like lipids through some as yet unidentified mechanism. We have shown that the AT1 receptor antagonists irbesartan and losartan inhibit basal as well as LDL- and PAF-stimulated MCP-1 production by human monocytes, important inflammatory mediators in atherosclerosis. At atherogenic sites, LDL and/or LDL oxidation products as well as PAF are likely to be present. AT1 receptor antagonists may reduce the levels of MCP-1 at these sites by inhibiting basal release of MCP-1 and by blocking the stimulation of MCP-1 production by these molecules. Lower levels of MCP-1 may result in less circulating monocytes entering the vessel wall. In this way, AT1 receptor antagonists may inhibit the inflammatory component of atherosclerosis. Further work is required to determine the mechanism of the anti-inflammatory effect of AT1 receptor antagonists.

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References

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