ISSN 0735-1097/03/$30.00
doi:10.1016/S0735-1097(03)00846-5

Pleiotropic Effects of Angiotensin II Receptor Blocker in Hypertensive Patients

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OBJECTIVES We investigated the vascular effects of candesartan in hypertensive patients.

BACKGROUND The renin-angiotensin system may contribute to atherogenesis through the promotion of endothelial dysfunction. The plausible mechanisms are that angiotensin II promotes superoxide anion generation, endothelial dysfunction, inflammation, and impaired fibrinolysis. The effects of candesartan on these conditions have not been clearly observed.

METHODS We administered placebo or candesartan 16 mg daily during two months to 45 patients with mild-to-moderate hypertension. This was a randomized, double-blind, placebo-controlled, crossover study in design.

RESULTS Candesartan did not significantly change lipoprotein levels. However, compared with placebo, candesartan significantly reduced plasma levels of malondialdehyde from 1.50 ± 0.07 to 1.29 ± 0.09 μM ($p = 0.009$); improved the percent flow-mediated dilator response to hyperemia from 5.17 ± 0.24 to 6.22 ± 0.26% ($p < 0.001$); and, furthermore, reduced plasma levels of monocyte chemoattractant protein (MCP-1) from 213 ± 8 to 190 ± 7 pg/ml ($p = 0.003$), tumor necrosis factor-alpha from 2.93 to 2.22 pg/ml ($p = 0.026$), and plasminogen activator inhibitor type 1 from 74 ± 4 to 53 ± 4 ng/ml ($p < 0.001$) but not C-reactive protein (CRP), matrix metalloproteinase protein, and fibrinogen. There were no significant correlations between these changes and reduction of systolic blood pressure (BP) ($-0.247 \leq r \leq 0.195$) and between these changes and reduction of diastolic BP ($-0.262 \leq r \leq 0.197$). There were no significant correlations between markers of inflammation and flow-mediated dilation percent or reduction of oxidant stress ($-0.119 \leq r \leq 0.127$). Furthermore, we observed no significant correlations between CRP and MCP-1 levels ($r = -0.162$).

CONCLUSIONS Inhibition of the angiotensin II type 1 (AT1) receptor in hypertensive patients reverses endothelial dysfunction, measured as an improvement in flow-mediated dilation and fibrinolysis and reduction of oxidant stress and inflammatory cytokines, suggesting that AT1 receptor blocker therapy has antiatherogenic effects. (J Am Coll Cardiol 2003;42:905–10)

The Losartan Intervention For Endpoint Reduction in Hypertension study (LIFE) demonstrated that losartan, angiotensin II type 1 (AT1) receptor blocker, therapy prevented or retarded the progression of coronary heart disease. This study suggests benefits beyond reduction in

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blood pressure (BP) (1). Endothelial dysfunction of epicardial coronary arteries precedes development of atherosclerotic disease that is either angiographically apparent or of sufficient obstructive severity to cause myocardial ischemia and angina pectoris (2). Hypertensive patients have impaired functions of the endothelium, which is a marker of future cardiovascular events (3). The vessel wall in endothelial dysfunction may promote inflammation, plaque rupture, and thrombus formation, which contribute to development and clinical expression of atherosclerosis (4).

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Manuscript received November 7, 2002; accepted December 6, 2002.

Recent experimental studies have confirmed that angiotensin II (AII) accelerates the development of atherosclerosis (5,6). The plausible mechanisms are that AII promotes superoxide anion generation and endothelial dysfunction (7,8). Angiotensin II activates nuclear transcription factor (NFκB) induced by oxidative stress (9). This effect is mediated by AT1 receptor (10). Nuclear transcription factor activates proinflammatory transcription factors and, thus, stimulates the synthesis of protein products such as cell adhesion molecules and chemokines (9,11,12). In hypertensive patients AII infusion leads to a rapid increase in plasma intercellular adhesion molecule-1 via an AT1 receptor-dependent mechanism (13). Angiotensin II type 1 receptor blocker could diminish intracellular production of superoxide anions via reduced activity of AII-dependent oxidases in the endothelium and vascular smooth muscle (14,15), thus protecting nitric oxide (NO) from oxidant degradation to biologically inert or toxic molecules (4,16). Inhibition of the production of superoxide anions could also limit the oxidation of low-density lipoprotein (LDL), thus contributing to increased NO bioactivity by enhancing NO syn-

Abbreviations and Acronyms

AII = angiotensin II
AT1 = angiotensin II type 1
BP = blood pressure
CRP = C-reactive protein
LDL = low-density lipoprotein
MCP = monocyte chemoattractant protein
MDA = malondialdehyde
MMP = matrix metalloproteinase protein
NFκB = nuclear transcription factor
NO = nitric oxide
PAI-1 = plasminogen activator inhibitor type-1
TNF = tumor necrosis factor

thesis and limiting oxidative degradation of NO (4,16). In this regard, AT1 receptor blocker inhibited LDL oxidation and attenuated atherosclerosis independent of lowering BP (17). In addition to improving vasomotor tone, increased NO bioactivity within the vasculature may prevent activation of proinflammatory transcription factors and, thus, reduce synthesis of protein products such as chemokines (18). On the other hand, plasminogen activator inhibitor type-1 (PAI-1) expression is affected by AII and mediated by the AT1 receptor, and interestingly, PAI-1 expression was suppressed by candesartan (19,20), in contrast with losartan (21), showing no effects.

However, the effects of AT1 receptor blocker on endothelium-dependent vasomotor responsiveness are conflicting in patients (22–25). Furthermore, the effects of AT1 receptor blockers on inflammation (26–31) and fibrinolysis (31–34) are conflicting in patients. In this study, we investigated the potential benefit of candesartan, an AT1 receptor blocker, on endothelium-dependent vasomotor responsiveness, oxidant stress, and markers of inflammation, hemostasis, and plaque stability in hypertensive patients.

METHODS

Study population and design. Forty-seven patients with mild-to-moderate hypertension participated in this study. We used World Health Organization/International Society of Hypertension definitions (35) for hypertension defined as systolic and diastolic BP ≥140 or ≥90 mm Hg, respectively. In order to minimize acute side effects to candesartan, study medication was titrated from 8 to 16 mg upwards over a two-week period if no hypotension (systolic BP <100 mm Hg) was noted. At the end of this time, participants were receiving either placebo or candesartan per day. The patients were seen, at a minimum, at 14-day intervals during the study. None was diabetic or a current smoker. Forty-five of 47 patients tolerated candesartan at 16 mg to maintain a systolic BP >100 mm Hg for 3 h after drug administration, experiencing no adverse effects. One patient was hypotensive, and the other suffered from a dry cough. Thus, data from a total of 45 patients were analyzed. Baseline total cholesterol, triglyceride, high-density lipoprotein cholesterol, and LDL cholesterol levels were 209 ± 8, 195 ± 14, 49 ± 2, and 116 ± 7 mg/dl, respectively. Baseline systolic and diastolic pressures were 165 ± 2 and 101 ± 1 mm Hg. The mean age was 50 ± 2 years, and 33 (73%) were male. This was a randomized, double-blind, placebo-controlled, crossover study in design. Study participants received placebo or candesartan 16 mg daily during two months, with the second treatment period initiated upon completion of the first treatment period (without washout phase). In order to avoid effects from other drugs, no other medications were allowed during the study period. The study was approved by the Gil Hospital Institute Review Board, and all participants gave written, informed consent.

Laboratory assays. Blood samples for laboratory assays were obtained at approximately 8:00 AM after overnight fasting, at baseline, and at the end of each treatment period and immediately coded so that investigators performing laboratory assays were blinded to subject identity or study sequence. Assays for lipids, plasma malondialdehyde (MDA), monocyte chemoattractant protein (MCP)-1, tumor necrosis factor (TNF)-alpha, matrix metalloproteinase protein (MMP)-9, PAI-1, and serum C-reactive protein (CRP) were performed in duplicate by ELISA (R & D Systems, Inc., Minneapolis, Minnesota; BIOXYTECH LPO-586, OxisResearch, Portland, Oregon; BioPool, Ventura, California; Rate nephelometry; IMMAGEO, Beckman Coulter, Brea, California), as previously described (36–40). C-reactive protein levels were determined with an immunonephelometry system according to methods described by the manufacturer. The measurement range is 0.1 to 98 mg/dl. All samples from the same patient (batch samples) were measured in blinded pairs on the same ELISA kit to minimize run-to-run variability. The inter-assay and intra-assay coefficients of variation were <6%.

Vascular studies. Imaging studies of the right brachial artery were performed using a ATL HDI 3000 ultrasound machine equipped with a 10-MHz linear-array transducer, based on a previously published technique (36,37,39,40). All images were transmitted to a personal computer via Ethernet with DICOM format (Digital Imaging and Communication in Medicine) and then saved on the hard disk of a personal computer as a bitmap format. Arterial diameters were measured with Image Tool for Windows version 2.0 (University of Texas Health Science Center, San Antonio, Texas). Measurements were performed by two independent investigators (D.K.J. and H.S.K.) blinded to the subject's identity and medication status. Measurements of maximum diameter and percent flow-mediated dilation were made in 10 studies selected at random. The interobserver and intraobserver variability for repeated measurement of maximum diameter were 0.004 ± 0.039 mm and 0.005 ± 0.089 mm, respectively. The interobserver and intraobserver variability for repeated measurement of percent flow-mediated dilation were 0.07 ± 1.27% and 0.15 ± 1.24%, respectively.

Statistical analysis. Data are expressed as mean ± SEM or median (range: 25% to 75%). After testing data for normal-

Table 1. Effects of Placebo or Oral Candesartan on Lipids and Endothelial Function in Hypertensive Patients

	Placebo	Candesartan
Lipids (mg/dl)		
Total cholesterol	208 ± 7	202 ± 6
HDL cholesterol	50 ± 2	51 ± 3
LDL cholesterol	116 ± 5	113 ± 5
Triglycerides	203 ± 22	193 ± 19
Vasomotor function		
Flow-mediated dilation (%)	5.17 ± 0.24	6.22 ± 0.26‡
Nitroglycerin dilation (%)	13.73 ± 0.58	14.18 ± 0.57
MDA (μM)	1.50 ± 0.07	1.29 ± 0.09†
Inflammation		
MCP-1 (pg/ml)	213 ± 8	190 ± 7†
TNF-α (pg/ml)	2.93 (1.87–4.31)	2.22 (1.85–3.75)*
CRP (mg/dl)	0.16 (0.11–0.27)	0.17 (0.11–0.26)
Hemostasis		
Antithrombin III (mg/dl)	28.8 ± 0.5	29.0 ± 0.5
PAI-1 (ng/ml)	74 ± 4	53 ± 4‡
Fibrinogen (mg/dl)	285 ± 10	285 ± 11
MMP-9 (ng/ml)	24.4 ± 1.7	27.2 ± 2.5

Data are expressed as means ± SEM or median (25% to 75%). *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$ vs. placebo.

CRP = C-reactive protein; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MCP = monocyte chemoattractant protein; MDA = malondialdehyde; MMP = matrix metalloproteinase protein; PAI = plasminogen activator inhibitor; TNF = tumor necrosis factor.

ity, we used the Student paired *t* or Wilcoxon signed rank test to compare values after placebo and candesartan therapies, as reported in Table 1. We decided to use the end of a two-month treatment period without washout and the second baseline. Indeed, we found no carryover effect in this study (see Results section). Pearson correlation coefficient analysis was used to assess associations between measured parameters. We calculated that 30 subjects would provide 80% power for detecting an absolute increase of ≥2.1% (α = 0.05, based on our previous studies) (39) flow-mediated dilation of the brachial artery between baseline and candesartan. A value of $p < 0.05$ was considered statistically significant.

RESULTS

To assess the possibility of a carryover effect from the initial treatment periods to the next treatment period, we compared the percent changes of: 1) the first treatment placebo, and the second treatment placebo 2) the first treatment candesartan and the second treatment candesartan, relative to baseline values. There were no significant differences in age and baseline values—vascular function (diameter and flow) and markers of inflammation—between each group. No significant differences were found in the above two comparisons (data not shown).

Effects of therapies on BP and lipids. Compared with placebo, candesartan therapy reduced systolic (160 ± 2 vs. 140 ± 2 mm Hg, $p < 0.001$) and diastolic (99 ± 1 vs. 87 ± 1 mm Hg, $p < 0.001$) BP after two months' administration of oral candesartan. Resting heart rate (83 ± 1 vs. 84 ± 1, $p = 0.575$) was similar after candesartan therapy. Candesartan therapy did not change lipoprotein levels (Table 1).

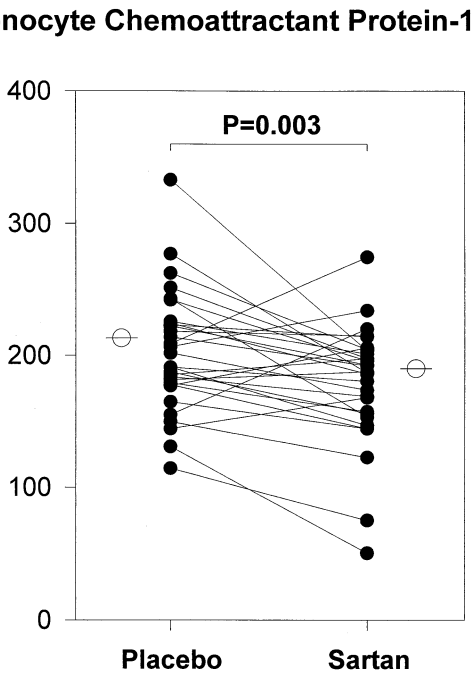

Figure 1. Compared with placebo, candesartan therapy significantly lowered plasma levels of monocyte chemoattractant protein-1 from the respective baseline levels ($p = 0.003$). **Open circles** = mean values.

Effects of therapies on malondialdehyde and vasomotor function. Basal brachial artery diameter and forearm blood flows were similar to those during placebo or candesartan treatment periods, as were the peak brachial artery diameters and forearm blood flows during reactive hyperemia and the percent increase in flow during hyperemia (data not shown). Compared with placebo, candesartan therapy significantly reduced plasma levels of MDA and improved the percent flow-mediated dilator response to hyperemia by 14 ± 5% and 29 ± 7%, respectively ($p = 0.009$ and $p < 0.001$, respectively). The brachial artery dilator response to nitroglycerin between each therapy was not significantly different ($p = 0.296$).

Effects of therapies on markers of inflammation. Compared with placebo, candesartan therapy significantly lowered plasma levels of MCP-1 by 9 ± 3% and TNF-alpha by 10 ± 4% from the respective baseline levels ($p = 0.003$ and $p = 0.026$, respectively, Fig. 1). We observed that patients with the highest baseline MCP-1 levels showed the greatest extent of reductions with candesartan ($r = -0.363$, $p = 0.013$). However, candesartan therapy did not significantly lower serum levels of CRP ($p = 0.799$).

Effects of therapies on markers of hemostasis and plaque stability. Compared with placebo, candesartan therapy significantly lowered plasma levels of PAI-1 by 21 ± 5% from the respective baseline levels ($p < 0.001$; Fig. 2). However, candesartan therapy did not significantly lower plasma levels of antithrombin III, fibrinogen, and MMP-9 ($p = 0.284$, $p = 0.768$, and $p = 0.282$, respectively).

We investigated whether candesartan-induced changes in the percent flow-mediated dilator response to hyperemia

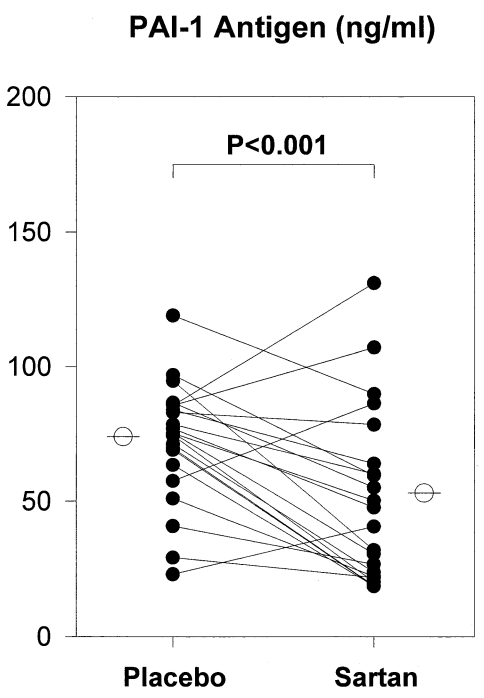

Figure 2. Compared with placebo, candesartan therapy significantly lowered plasma levels of plasminogen activator inhibitor type-1 (PAI-1) from the respective baseline levels ($p < 0.001$). **Open circles** = mean values.

and serological markers of oxidant stress, inflammation, and fibrinolysis were mediated by reduction of systolic or diastolic BP after candesartan. There were no significant correlations between these changes and reduction of systolic BP ($-0.247 \leq r \leq 0.195$) and between these changes and reduction of diastolic BP ($-0.262 \leq r \leq 0.197$). We also investigated whether the candesartan-induced reduction in serological markers of inflammation was mediated by improvement in NO bioactivity or reduction of oxidant stress. There were no significant correlations ($-0.119 \leq r \leq 0.127$). Furthermore, despite an experimental study showing a mechanism for the regulation of CRP and MCP-1 levels (41), we observed no significant correlations between CRP and MCP-1 levels ($r = -0.162$, $p = 0.307$).

DISCUSSION

We observed that two months of candesartan therapy significantly improved the percent flow-mediated dilator response to hyperemia and reduced levels of oxidant stress and inflammatory and impaired fibrinolysis markers in hypertensive patients, independent of lipoprotein and BP changes.

Our finding of improved endothelium-dependent vasomotor responsiveness is consistent with other groups' results (23–25). Thus, Schiffrin et al. (24) demonstrated that, in contrast with atenolol, losartan corrected endothelial dysfunction in patients with essential hypertension, and Ghiadoni et al. (25) also demonstrated similar improvement in endothelial function with candesartan. We reasoned that candesartan might improve endothelium-dependent vasomotor responsiveness by reducing oxidant stress and augmenting NO bioactivity, considering that AII is a potent vasoconstrictor that also promotes oxidant stress. We observed that two months of candesartan therapy significantly reduced plasma levels of MDA, compared with placebo. Although we did not measure prostaglandin $F_{2\alpha}$, studies have reported an excellent correlation between prostaglandin $F_{2\alpha}$ and MDA levels (42,43).

In order to gain insight into mechanisms of potential vasculoprotective effects of candesartan, we measured surrogate markers for vascular inflammation. Inflammatory cytokines secreted by macrophages and T lymphocytes can modify endothelial function, smooth muscle cell proliferation, collagen degradation, and thrombosis (4,44). We observed that candesartan therapy significantly reduced plasma levels of MCP-1 and TNF-alpha but not CRP and fibrinogen levels.

Tomita et al. (45) observed that inhibition of NO synthesis with N^{w}-nitro-L-arginine methyl ester induced inflammatory changes and MCP-1 expression in rat hearts and vessels. Activated platelets stimulated MCP-1 production in endothelial cells via an NFκB-dependent mechanism (46). On the other hand, experimental studies demonstrated that AII activates NFκB through AT1 and AT2 receptor stimulation (10) and induces MCP-1 expression in cell culture (47); furthermore, candesartan appears to inhibit the enhancement of MCP-1 expression in rats (48). However, the effects of AT1 receptor blockade on MCP-1 are controversial in patients (29–31). For example, losartan at a mean dose of 77 mg daily did not significantly alter the levels of soluble cell adhesion molecules (consistent with our previous findings [28]) or MCP-1 (29). However, candesartan administered at 16 mg daily for six weeks significantly reduced levels of soluble intercellular adhesion molecule-1 and MCP-1 in hypercholestrolemic patients (30,31), a finding that is consistent with our current study. The difference between losartan and candesartan may partly be attributed to a tenfold stronger AT1 blocking effect of the latter agent. We hypothesize that candesartan may reduce plasma MCP-1 levels via inactivation of NF-κB, due to an improved bioactivity of NO, as shown by improvement in flow-mediated dilation. However, we did not observe a significant correlation between the changes in flow-mediated dilation and plasma levels of MCP-1, a finding that may be explained by the complex pathogenesis of atherosclerosis involving multiple interactions and the relatively small sample size (β error).

We also observed that candesartan reduced plasma levels of TNF-alpha. Other studies have observed this effect in patients with heart failure (26) and coronary artery disease (27). Experimental studies demonstrated that CRP stimulates the synthesis of MCP-1 (41). In the current study, candesartan did not alter serum CRP levels, consistent with other studies (27,28). We observed no correlations between CRP levels and MCP-1 levels in humans.

Previous studies have shown that the AT1 receptor

blocker losartan partially blocks AII-induced PAI-1 expression in cell culture studies (21). In contrast, other reports have suggested that the AT1 receptor does not mediate AII-stimulated PAI-1 expression (49). However, recent studies demonstrated the role of the AT1 receptor in mediating the effects of AII on PAI-1 expression (19,20,50). Furthermore, unlike losartan, candesartan abolished the stimulatory action of AII on PAI-1 expression (19,50). In human studies the effect of losartan on PAI-1 antigen remains controversial. For example, Erdem et al. (33) reported that losartan and perindopril significantly reduced PAI-1 antigen levels in patients with hypertension. Goodfield et al. (34) reported that, in patients with heart failure, losartan 50 mg, but not enalapril, reduced PAI-1 levels. In contrast, other studies demonstrated that losartan did not change in patients (32,51–53). The effect of candesartan on PAI-1 antigen is controversial. In postmenopausal women, candesartan 8 mg daily for 12 weeks significantly increased plasma PAI-1 antigen (32). However, in hypercholesterolemic patients, candesartan 16 mg daily for six weeks significantly decreased plasma PAI-1 antigen (30,31). This discrepancy may result from the duration of treatment, the patient population, and the drug dosage.

On the other hand, the complete blockade of AII-induced PAI-1 expression by candesartan may be attributed to its higher affinity and slower dissociation kinetics than losartan (54,55). Thus, candesartan reduces maximal AII binding to the AT1 receptor, whereas losartan causes a rightward shift in the AII dose response without reducing maximal AII binding (55). It is therefore likely that the partial reduction of AII-induced PAI-1 expression by losartan (21) was due to incomplete antagonism of the AT1 receptor. In this regard, we observed that candesartan 16 mg daily for eight weeks significantly reduced plasma levels of PAI-1 antigen. Further, of the six postmenopausal women in our study, five showed candesartan-induced reduction in PAI-1 antigen levels relative to baseline values. The mean reduction of PAI-1 antigen levels in all six were similar to the effect of candesartan in all 45 patients (27% vs. 21%).

Several studies hypothesized that the effects of AT1 receptor blockers on endothelial function go beyond the reduction of BP (1,24,30,56). We observed that candesartan changed the percent flow-mediated dilator response to hyperemia and serological markers of oxidant stress, inflammation, and fibrinolysis independent of lowering BP.

In summary, we observed that candesartan therapy in hypertensive patients reversed endothelial dysfunction, measured as an improvement in flow-mediated dilation and fibrinolysis and reduction of oxidant stress and inflammatory cytokines independent of BP changes, suggesting that AT1 receptor blocker therapy has antiatherogenic effects.

Acknowledgments

The authors greatly appreciate the critical review and comments of Richard O. Cannon III, MD (Clinical Director, National Heart, Lung, and Blood Institute, Bethesda, Maryland), and Arshed A. Quyyumi, MD (Professor, Division of Cardiology, Emory University Hospital, Atlanta, Georgia). They would also like to express their gratitude to Mi Jung Kim, MT, Han Gyu Kim, MT, Sang Kyoon Kwon, MT, and Soo Jin Kim for their excellent assistance.

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REFERENCES

1. Dahlof B, Devereux RB, Kjeldsen SE, et al., the LIFE Study Group. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Lancet 2002;359:995–1003.
2. Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986;315:1046–51.
3. Perticone F, Ceravolo R, Pujia A, et al. Prognostic significance of endothelial dysfunction in hypertensive patients. Circulation 2001; 104:191–6.
4. Koh KK. Effects of statins on vascular wall: vasomotor function, inflammation, and plaque stability. Cardiovasc Res 2000;47:648–57.
5. Weiss D, Kools JJ, Taylor WR. Angiotensin II-induced hypertension accelerates the development of atherosclerosis in apoE-deficient mice. Circulation 2001;103:448–54.
6. Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. J Clin Invest 2000;105:1605–12.
7. Laursen JB, Rajagopalan S, Galis Z, Tarpey M, Freeman BA, Harrison DG. Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. Circulation 1997;95:588–93.
8. Fukai T, Siegfried MR, Ushio-Fukai M, Griendling KK, Harrison DG. Modulation of extracellular superoxide dismutase expression by angiotensin II and hypertension. Circ Res 1999;85:23–8.
9. 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.
10. Ruiz-Ortega M, Lorenzo O, Ruperez M, Konig S, Wittig B, Egido J. Angiotensin II activates nuclear transcription factor kappaB through AT(1) and AT(2) in vascular smooth muscle cells: molecular mechanisms. Circ Res 2000;86:1266–72.
11. Zeiher AM, Fisslthaler B, Schray-utz B, et al. Nitric oxide modulates the expression of monocyte chemoattractant protein 1 in cultured human endothelial cells. Circ Res 1995;76:980–6.
12. Schieffer B, Schieffer E, 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.
13. Pastore L, Tessitore A, Martinotti S, et al. Angiotensin II stimulates intercellular adhesion molecule-1 (ICAM-1) expression by human vascular endothelial cells and increases soluble ICAM-1 release in vivo. Circulation 1999;100:1646–52.
14. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res 1994;74:1141–8.
15. Mohazzab KM, Kaminski PM, Wolin MS. NADH oxidoreductase is a major source of superoxide anion in bovine coronary artery endothelium. Am J Physiol 1994;266:H2568–72.
16. Nickenig G, Harrison DG. The AT1-type angiotensin receptor in oxidative stress and atherogenesis: part I: oxidative stress and atherogenesis. Circulation 2002;105:393–6.
17. Hayek T, Attias J, Coleman R, et al. The angiotensin-converting enzyme inhibitor, fosinopril, and the angiotensin II receptor antagonist, losartan, inhibit LDL oxidation and attenuate atherosclerosis independent of lowering blood pressure in apolipoprotein E deficient mice. Cardiovasc Res 1999;44:579–87.

18. De Caterina R, Libby P, Peng HB, 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.
19. Chen HC, Bouchie JL, Perez AS, et al. Role of the angiotensin AT(1) receptor in rat aortic and cardiac PAI-1 gene expression. Arterioscler Thromb Vasc Biol 2000;20:2297–302.
20. Takeda K, Ichiki T, Tokunou T, et al. Critical role of Rho-kinase and MEK/ERK pathways for angiotensin II-induced plasminogen activator inhibitor type-1 gene expression. Arterioscler Thromb Vasc Biol 2001;21:868–73.
21. Feener EP, Northrup JM, Aiello LP, King GL. Angiotensin II induces plasminogen activator inhibitor-1 and -2 expression in vascular endothelial and smooth muscle cells. J Clin Invest 1995;95:1353–62.
22. Anderson TJ, Elstein E, Haber H, Charbonneau F. Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF study). J Am Coll Cardiol 2000;35:60–6.
23. Prasad A, Tupas-Habib T, Schenke WH, et al. Acute and chronic angiotensin-1 receptor antagonism reverses endothelial dysfunction in atherosclerosis. Circulation 2000;101:2349–54.
24. Schiffrin EL, Park JB, Intengan HD, Touyz RM. Correction of arterial structure and endothelial dysfunction in human essential hypertension by the angiotensin receptor antagonist losartan. Circulation 2000;101:1653–9.
25. Ghiadoni L, Virdis A, Magagna A, Taddei S, Salvetti A. Effect of the angiotensin II type 1 receptor blocker candesartan on endothelial function in patients with essential hypertension. Hypertension 2000; 35:501–6.
26. Tsutamoto T, Wada A, Maeda K, et al. Angiotensin II type 1 receptor antagonist decreases plasma levels of tumor necrosis factor alpha, interleukin-6 and soluble adhesion molecules in patients with chronic heart failure. J Am Coll Cardiol 2000;35:714–21.
27. 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.
28. 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.
29. 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.
30. Wassmann S, Hilgers S, Laufs U, Bohm M, Nickenig G. Angiotensin II type 1 receptor antagonism improves hypercholesterolemia-associated endothelial dysfunction. Arterioscler Thromb Vasc Biol 2002;22:1208–12.
31. Wassmann S, Hilgers S, Laufs U, Bohm M, Nickenig G. Candesartan improves endothelial dysfunction associated with hypercholesterolemia (abstr). Deutsche Medizinische Wochenschrift 2001;126 Suppl 3:S160.
32. Fogari R, Zoppi A, Malamani G, et al. Effects of four angiotensin II-receptor antagonists on fibrinolysis in postmenopausal women with hypertension. Curr Ther Res 2001;62:68–78.
33. Erdem Y, Usalan C, Haznedaroglu IC, et al. Effects of angiotensin converting enzyme and angiotensin II receptor inhibition on impaired fibrinolysis in systemic hypertension. Am J Hypertens 1999;12: 1071–6.
34. Goodfield NE, Newby DE, Ludlam CA, Flapan AD. Effects of acute angiotensin II type 1 receptor antagonism and angiotensin converting enzyme inhibition on plasma fibrinolytic parameters in patients with heart failure. Circulation 1999;99:2983–5.
35. 1999 World Health Organization-International Society of Hypertension. 1999 World Health Organization-International Society of Hypertension guidelines for the management of hypertension. J Hypertens 1999;17:151–83.
36. Koh KK, Cardillo C, Bui MN, et al. The effect of estrogen and cholesterol-lowering therapies on vascular function in hypercholesterolemic postmenopausal women. Circulation 1999;99:354–60.
37. Koh KK, Jin DK, Yang SH, et al. Vascular effects of synthetic or natural progestagen combined with conjugated equine estrogen in healthy postmenopausal women. Circulation 2001;103:1961–6.
38. Koh KK, Mincemoyer R, Bui MN, et al. Effects of hormone-replacement therapy on fibrinolysis in postmenopausal women. N Engl J Med 1997;336:683–90.
39. Koh KK, Bui MN, Hathaway L, et al. Mechanism by which quinapril improves vascular function in coronary artery disease. Am J Cardiol 1999;83:327–31.
40. Koh KK, Son JW, Ahn JY, et al. Comparative effects of diet and statin on nitric oxide bioactivity and matrix metalloproteinases in hypercholesterolemic patients with coronary artery disease. Arterioscler Thromb Vasc Biol 2002;22:e19–23.
41. Pasceri V, Chang J, Willerson JT, et al. Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by anti-atherosclerosis drugs. Circulation 2001;103:2531–4.
42. Jang Y, Lee JH, Kim OY, Park HY, Lee SY. Consumption of whole grain and legume powder reduces insulin demand, lipid peroxidation, and plasma homocysteine concentrations in patients with coronary artery disease: randomized controlled clinical trial. Arterioscler Thromb Vasc Biol 2001;21:2065–71.
43. Ide T, Tsutsui H, Ohashi N, et al. Greater oxidative stress in healthy young men compared with premenopausal women. Arterioscler Thromb Vasc Biol 2002;22:438–42.
44. Koh KK. Effects of HMG-CoA reductase inhibitor on hemostasis. Int J Cardiol 2000;76:21–30.
45. Tomita H, Egashira K, Kubo-Inoue M, et al. Inhibition of NO synthesis induces inflammatory changes and monocyte chemoattractant protein-1 expression in rat hearts and vessels. Arterioscler Thromb Vasc Biol 1998;18:1456–64.
46. Gawaz M, Neumann F-J, Dickfeld T, et al. Activated platelets induce monocyte chemotactic protein-1 secretion and surface expression of intercellular adhesion molecule-1 on endothelial cells. Circulation 1998;98:1164–71.
47. Funakoshi Y, Ichiki T, Shimokawa H, et al. Rho-kinase mediates angiotensin II-induced monocyte chemoattractant protein-1 expression in rat vascular smooth muscle cells. Hypertension 2001;38:100–4.
48. Kato S, Luyckx VA, Ots M, et al. Renin-angiotensin blockade lowers MCP-1 expression in diabetic rats. Kidney Int 1999;56:1037–48.
49. Kerins DM, Hao Q, Vaughan DE. Angiotensin induction of PAI-1 expression in endothelial cells is mediated by the hexapeptide angiotensin IV. J Clin Invest 1995;96:2515–20.
50. Skurk T, Lee YM, Hauner H. Angiotensin II and its metabolites stimulate PAI-1 protein release from human adipocytes in primary culture. Hypertension 2001;37:1336–40.
51. Levy PJ, Yunis C, Owen J, Brosnihan KB, Smith R, Ferrario CM. Inhibition of platelet aggregability by losartan in essential hypertension. Am J Cardiol 2000;86:1188–92.
52. Li-Saw-Hee FL, Beevers DG, Lip GY. Effect of antihypertensive therapy using enalapril or losartan on haemostatic markers in essential hypertension: a pilot prospective randomised double-blind parallel group trial. Int J Cardiol 2001;78:241–6.
53. Fogari R, Mugellini A, Zoppi A, et al. Losartan and perindopril effects on plasma plasminogen activator inhibitor-1 and fibrinogen in hypertensive type 2 diabetic patients. Am J Hypertens 2002;15:316–20.
54. Morsing P. Candesartan: a new-generation angiotensin II AT1 receptor blocker: pharmacology, antihypertensive efficacy, renal function, and renoprotection. J Am Soc Nephrol 1999;10 Suppl 11:S248–54.
55. Vanderheyden PM, Fierens FL, De Backer JP, Fraeyman N, Vauquelin G. Distinction between surmountable and insurmountable selective AT1 receptor antagonists by use of CHO-K1 cells expressing human angiotensin II AT1 receptors. Br J Pharmacol 1999;126:1057–65.
56. Kramer C, Sunkomat J, Witte J, et al. Angiotensin II receptor-independent antiinflammatory and antiaggregatory properties of losartan: role of the active metabolite EXP3179. Circ Res 2002;90:770–6.