Irbesartan, an Angiotensin Type 1 Receptor Inhibitor, Regulates Markers of Inflammation in Patients With Premature Atherosclerosis

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OBJECTIVES This study assessed the role of angiotensin II type 1 (AT_1) receptor antagonists on inflammatory mechanisms involved in atherogenesis. Specific inflammatory markers included solubilized tumor necrosis factor-alpha receptor II (sTNF-αRII), vascular cell adhesion molecule-1 (VCAM-1) and superoxide. In addition, the AT_1 receptor blocker irbesartan was evaluated for its ability to suppress these markers in individuals with atherosclerosis.

BACKGROUND Mechanisms involved in the complex process of atherogenesis include alterations in the inflammatory responses. The use of compounds that suppress these responses may reduce the degree of damage seen in atherosclerosis.

METHODS With a cross-sectional study design, 33 normotensive patients with stable coronary artery disease (CAD) were treated with irbesartan for a 24-week period. These patients were compared against a control population with no known coronary atherosclerosis. Marker levels were measured by enzyme-linked immunosorbent assay technique and lucigenin chemiluminescence assay and statistically evaluated by two-way repeated measures analysis of variance.

RESULTS All patients with coronary artery disease had increased levels of inflammatory molecules over those of control patients. Treatment with irbesartan in these patients significantly reduced levels of inflammatory molecules measured. Soluble VCAM-1 levels were reduced by 36%; soluble TNF-alpha levels were reduced by 54% and superoxide level decreased by 52%. Maximal suppression of inflammatory markers by irbesartan therapy in patients with CAD was seen at 12 weeks.

CONCLUSIONS The effect of irbesartan on each inflammatory marker is significant. Our results show that use of irbesartan may retard the inflammatory process seen in premature forms of atherosclerosis. (J Am Coll Cardiol 2001;37:440–4) © 2001 by the American College of Cardiology

Atherosclerosis is a pervasive condition responsible for more than half of all mortality in the U.S. (1). Its impact has reached epidemic proportions and has generated significant research into the components that initiate and form atherosclerotic lesions. One such component involves an inflammatory process related to the oxidative modification of low-density lipoproteins (ox-LDL) (2). In the presence of ox-LDL, inflammatory markers such as vascular cell adhesion molecule-1 (VCAM-1) and tumor necrosis factor-alpha receptor II (sTNF-αRII) (3) are elevated. Studies by Keidar et al. (4) imply that the use of compounds with antioxidant properties, such as losartan, may be therapeutic in the treatment of atherosclerosis by decreasing the oxidation of LDL. These compounds appear to target the oxidation of LDL and may reduce the impact of the inflammatory process seen in atherogenesis.

Current treatment modalities are used to lower cholesterol and LDL levels as well as control hypertension. The association between hypertension and atherosclerosis has not fully been established, but preliminary studies indicate a causal relationship (5). Each class of antihypertensive medications has a different effect on atherosclerotic lesions (5). For example, alpha-blockers benefit atherosclerotic lesions by changing the lipid profile (increasing high density lipoproteins [HDL] and decreasing triglycerides) (6), whereas angiotensin-converting enzyme inhibitors and calcium channel blockers have potential antiatherogenic effects at different points of the lesion development (7,8). Specific beta-blockers, such as carvedilol, have been shown to possess possible antioxidant properties and may prevent LDL oxidation, thereby slowing the atherosclerotic process (9).

Type 1-specific angiotensin receptor blockers are promising antihypertensive drugs, but there is little research on their effect on atherosclerosis. The majority of research concerning inhibitors of the angiotensin type 1 (AT_1) receptor has focused on their mechanism of action in treating hypertension, which involves blocking the renin-angiotensin system. This system regulates blood pressure through salt and water homeostasis via vasoconstriction and aldosterone secretion (10). Any imbalance in this system results in elevated blood pressure. The major component involved in the renin-angiotensin system is angiotensin II. Angiotensin II acts by binding to plasma membrane receptors, creating a cascade of cellular responses leading to an increase in blood pressure (10). The discovery of nonpeptide angiotensin II receptor antagonists, losartan being the

prototype, led to the elucidation of angiotensin II receptor subtypes 1 and 2 (AT$_1$ and AT$_2$)(11,12). The AT1 receptor appears to play the major role in blood pressure mechanics and is the target in the treatment of hypertension (13). Initial clinical evidence has indicated that the use of angiotensin II receptor blockers is effective in treating hypertension (14). It also appears that the use of AT$_1$ receptor blockade increases AT$_2$ receptor activity. This agonist effect is postulated to work by increasing nitric oxide levels, which may result in diminished cell proliferation and activation of antioxidant properties (15). This mechanism may also be influential in retarding the atherosclerotic process. Our hypothesis is that inhibitors of the AT$_1$ receptor suppress the activity of the renin-angiotensin system in patients who are susceptible to the inflammatory reactions that are critical in the pathogenesis of atherosclerosis.

METHODS

Subjects. Thirty-three patients were enrolled in the study. All study group subjects had documented coronary artery disease (CAD) and had undergone coronary artery bypass graft (CABG), percutaneous transluminal coronary angioplasty or both at least 12 months prior to the start of this study. Those individuals who demonstrated a significant stenosis of at least 50% of at least one main branch of the coronary arteries were considered as having CAD (16). Systolic blood pressure for all patients was <150 mm Hg. All patients were informed of the purpose of the study and voluntarily consented to participate in accordance with procedures established by the Human Studies Committee at Emory University. Medical history of each patient indicated no present or past history of diabetes mellitus or collagen vascular disease. Patients were given irbesartan at 75 to 150 mg/day depending upon the level of blood pressure measured. Patients with a systolic blood pressure of ≤135 mm Hg were started on 75 mg of irbesartan per day, whereas patients with a systolic blood pressure of >135 mm Hg were started on 150 mg irbesartan per day. No patients experienced hypotension during the study period.

Table 1. Demographic Characteristics of Study Population and Control Population

	Control (n = 24)	Study Group (CAD) (n = 33)
Male/female	12/12	17/16
Age range (yrs)	41–59	42–55
Body mass index (kg/m^2)	26.6 (20–38)	27.0 (21–35)
Systolic blood pressure range (mm Hg)	124–139	120–135
Diastolic blood pressure range (mm Hg)	75–87	72–85
Leukocytes (nl^{-1})	7.9 (4.5–11.1)	7.1 (4.2–10.4)
Total cholesterol (ng/dl)	209 (144–248)	206 (171–233)
Triglycerides (ng/dl)	125 (79–189)	142 (100–216)
Previous history of smoking	25%	32%*
History of myocardial infarction	0	48%
Ejection fraction		
>60%	100%	78%
55–40%	0	15%
39–20%	0	6%
<20%	0	0

*25% men, 39% women.
CAD = coronary artery disease.

Blood sampling and laboratory determination. The study spanned 24 weeks with serum samples collected at 0, 4, 12 and 24 weeks. Blood samples were drawn in seated position from the antecubital vein. All samples were cooled with ice. Serum was extracted by centrifugation at 3,000 *g* for 10 min. Serum samples remained stored at −70°C until assays were performed.

Serum vascular cell adhesion molecule-1 (sVCAM-1) (ng/dl) was used as a representative circulating adhesion molecule. Recent research has indicated that various adhesion molecules may be specific to atherogenesis (17,18) with serum VCAM-1 being a potential marker for atherosclerosis (19). Serum tumor necrosis factor-alpha receptor II (TNF-αRII) (ng/dl) was measured as an indirect marker of monocyte/macrophage stimulation (16). Serum levels of VCAM and TNF-αRII were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) technique (BioSound Technology, Chicago, Illinois). Superoxide production has been associated with vascular diseases such as atherosclerosis (20). Superoxide levels were measured using the lucigenin chemiluminescence assay (21).

Statistical analysis. Statistical analysis was performed using analysis of variance. All analyses were tested as two-sided and p values <0.05 were considered statistically significant.

RESULTS

Subject profile. Clinical parameters of all subjects are presented in Table 1. Of the 33 patients, 17 were male and 16 female. Their ages ranged from 42 to 55 years in the CAD group. Cholesterol levels ranged from 171 to 233 mg/dl (mean = 206). Triglyceride levels ranged from
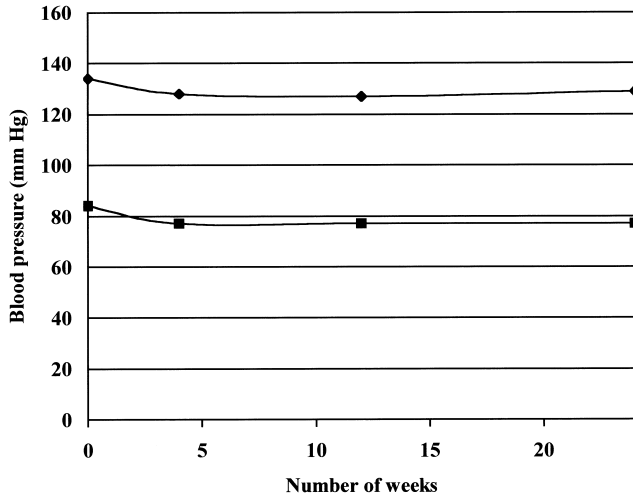
Figure 1. Patients with premature CAD were placed on a daily dose of irbesartan (75 mg daily if systolic blood pressure ≤135 mm Hg and 150 mg daily if systolic blood pressure ≥135 mm Hg) for a 24-week period. Blood pressure was monitored at 0, 4, 12 and 24-week intervals. The average systolic and diastolic blood pressure for the group at each interval is noted. ◆ = systolic; ■ = diastolic.

100 to 216 mg/dl (mean = 142). Sixteen patients (48%) had documented history of myocardial infarctions in the past. Twenty-six patients (78%) had an ejection fraction of >60%, with the rest of the subject population ranging between 20% and 60% ejection fractions. None of the patients fell below 20% ejection fraction. Body mass index ranged from 21 to 35 (mean = 27). All patients had normal to borderline blood pressure, with no one above 135/85. Eleven patients (32%) were smokers. All patients were on antianginal therapy.

Analysis of blood pressure in patients with CAD treated with irbesartan. Figure 1 displays the blood pressure change in patients with CAD treated with irbesartan over the study period. There was a decrease in systolic and diastolic blood pressure measured at the four-week interval of 6 mm Hg systolic and 4 mm Hg diastolic. Subsequent measurements at 4, 12, and 24 weeks showed no change in blood pressure after the decrease at the 4-week interval.

Analysis of serum levels of adhesion molecules and markers. The data in Figure 2 show that serum levels of soluble VCAM-1 were significantly increased at interval 0 weeks compared to corresponding levels in control patients (466.6 ± 69.7 ng/dl vs. 331.6 ± 39.5 ng/dl at 0 weeks). The graph also indicates serum VCAM-1 levels decreased significantly in patients with CAD treated with irbesartan as compared against control patients over the 24-week study interval (325.3 ± 42.3 ng/dl vs. 466.6 ± 69.7 ng/dl at 24 weeks). Serum VCAM levels remained constant at 331.6 ng/dl in the control population throughout the study interval. We studied the levels of sVCAM-1 in a separate group of patients with CAD that would have met the inclusion criteria for the study but were not treated with irbesartan (data not shown). No significant changes in
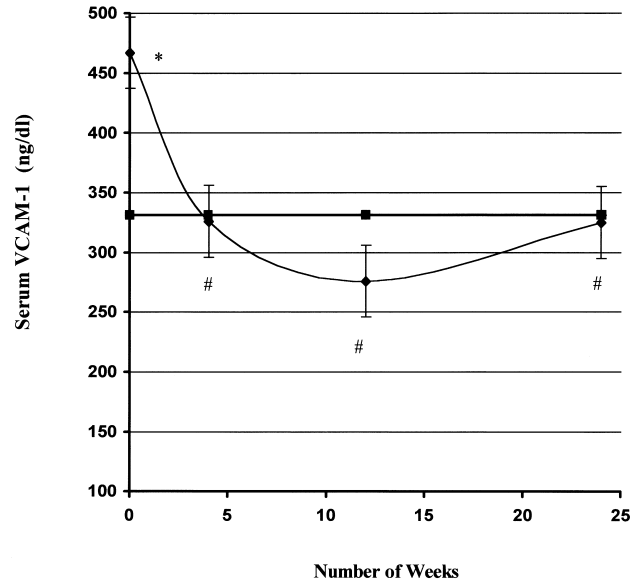

Figure 2. Serum levels of soluble VCAM-1 (ng/dl) were measured in subjects with known coronary artery disease (CAD) by enzyme-linked immunosorbent assay technique. **Diamonds** represent the change in sVCAM-1 levels over the 24-week study period at intervals of 0, 4, 12 and 24 weeks in patients with CAD treated with irbesartan. **Squares** represent sVCAM-1 levels measured in control patients over the study period at the same interval schedule. Values for CAD patient group represent mean ± SD. * = value differs ($p < 0.001$) from the control group. # = value differs ($p < 0.001$) from the CAD group before treatment with irbesartan. ◆ = CAD; ■ = control.

sVCAM-1 were observed after 24 to 26 weeks past the collection of the initial samples, suggesting that irbesartan alone was responsible for the reduction of sVCAM-1 in patients with CAD.
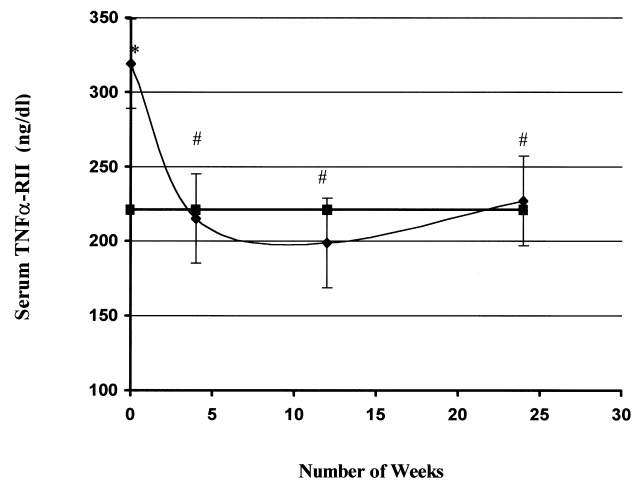
Figure 3 shows a similar pattern for TNF-αRII levels.

Figure 3. Serum levels of TNFα-RII (ng/dl) were measured in subjects with known coronary artery disease (CAD) by enzyme-linked immunosorbent assay technique. **Diamonds** represent the change in TNFα-RII levels over the 24-week study period at intervals of 0, 4, 12 and 24 weeks in patients with CAD treated with irbesartan. **Squares** represent TNFα-RII levels measured in control patients over the study period at the same interval schedule. Values for CAD patient group represent mean ± SD. * = value differs ($p < 0.001$) from the control group. # = value differs ($p < 0.001$) from the CAD group before treatment with irbesartan. ◆ = CAD; ■ = control.
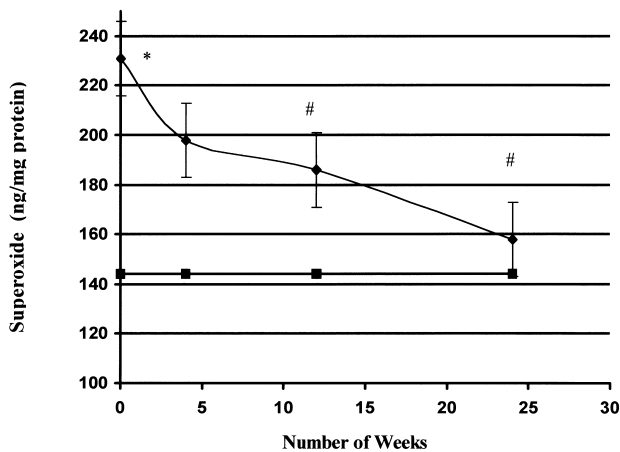
Figure 4. Serum levels of superoxide (ng/mg) were measured in subjects with known coronary artery disease (CAD) by the lucigenin chemiluminescence assay. **Diamonds** represent the change in TNFα-RII levels over the 24-week study period at intervals of 0, 4, 12 and 24 weeks in patients with CAD treated with irbesartan. **Squares** represent superoxide levels measured in control patients over the study period at the same interval schedule. Values for CAD patient group represent mean ± SD. * = value differs (p < 0.01) from the control group. # = value differs (p < 0.01) from the CAD group before treatment with irbesartan. ◆ = CAD; ■ = control.

The graph indicates that patients with CAD had an elevated level of TNF-α RII at the zero time interval as compared with their control counterparts (319.15 ± 30.2 ng/dl vs. 221 ± 63.5 ng/dl). The graph also shows that treatment with irbesartan in the population of patients with CAD significantly reduced TNF-αRII levels to those of the control population (227 ± 27.9 ng/dl vs. 221 ± 63.5 ng/dl at 24 weeks). Marker levels of TNF-αRII in control patients remained constant at 221 ng/dl for the length of the study.

Figure 4 displays the change in superoxide levels in patients with CAD as compared to control patients. Initial values indicate that superoxide levels were significantly greater in patients with CAD as compared to control (231.2 ± 43.7 ng/mg vs. 143.6 ± 40.1 ng/mg at 0 weeks). Treatment with irbesartan decreased superoxide levels to that of control patients in patients with CAD (158 ± 25.9 ng/mg vs. 143.6 ± 40.1 ng/mg at 24 weeks).

Over the 24-week period, serum VCAM-1 levels decreased by 36% in the irbesartan-treated patient group. Serum TNF-αRII levels decreased by 54% and superoxide levels fell 52% in the irbesartan-treated group over the study period. No change in inflammatory markers was noted in any of the control subjects for the span of the study. We studied other serum markers that are associated with inflammation, including total C-reactive protein and erythrocyte sedimentation rate (ESR). We observed that there was no difference in these markers between the control and CAD group (data not shown); moreover, treatment with irbesartan did not affect C-reactive protein or ESR in the CAD group.

DISCUSSION

This study focused on the effect of the AT_1 antagonist irbesartan on subjects who develop early forms of atherosclerosis. We demonstrated that this drug reduces circulating inflammatory markers associated with CAD. Serum VCAM, TNF-αRII and superoxide were elevated to between 40% and 100% higher than controls. Irbesartan decreased the amount of inflammatory molecules to below levels of control.

Adhesion molecules as markers for CAD. The markers measured in our study were chosen for specific reasons. Recent studies by Peter et al. (19) and DeCaterina et al. (22) indicate that soluble VCAM levels may be considered a marker for quantifying atherosclerosis, particularly in the early stages of the disease process. Tumor necrosis factor-alpha and its receptor subtypes (RI and RII) have been considered as potential markers for atherosclerosis (3). Tumor necrosis factor-alpha RII is the stable receptor subtype for TNF-α and appears to be associated with CAD (16). Because of its stability in comparison with TNF-α and TNF-α-RI, TNF-α-RII was considered an ideal investigative marker to use in this study. Our study indicates that TNF-α-RII levels are increased to a significant degree in patients with early atherosclerosis. Superoxide levels have been associated with hypercholesterolemia (23) and endothelial dysfunction (20). These properties result in the oxidation of LDL. Oxidized low density lipoprotein then damages the vascular endothelium and promotes the atherosclerotic lesion (24). In our study, we found that patients with premature CAD had elevated levels of superoxide. This result supports the in vitro studies done by Miller et al. (25) and indicates that superoxide levels are increased secondary to the oxidative environment created by elevated lipid levels.

Irbesartan decreases inflammatory marker levels in premature patients with CAD, which may operate via antioxidant-sensitive mechanisms. Much of the research literature concerning irbesartan involves its efficacy in the treatment of hypertension. The present study addresses the potential effects of AT_1 receptor blockers on the inflammatory markers of atherosclerosis. Our study indicates that irbesartan at therapeutic doses can reduce the inflammatory reaction to atherosclerosis without significant hypotension in normotensive patients with CAD. The mechanism of this reduction is unclear but may involve the inhibition of NAD(P)H-dependent oxidases. Warnholtz et al. (23) proposed that AT_1 receptor activation by angiotensin II increases the amount of superoxide. The increase in superoxide results in LDL oxidation and inhibition of nitric oxide and prostacyclin (26). All of these components ultimately result in the endothelial dysfunction seen in the atherosclerotic process. Blocking the AT_1 receptor decreases the detrimental effect of superoxide on the vasculature. Kurz et al. (27) demonstrated that losartan, the angiotensin receptor blocker prototype, produced this effect through AT_1 block-

ade. Our study indicates that irbesartan also has a significant effect on reducing superoxide production and probably uses a similar mechanism of action.

The issue of using patients with a past history of some intervention, such as CABG or coronary angioplasty, may indicate a potential source for the elevated marker levels. This concern was addressed in the study done by Porsche et al. (16), which found no differences in the marker levels they examined in patients with CABG and those patients who received an interventional procedure. Another concern involves the specificity of inflammatory markers in regards to atherosclerosis. Only a handful of markers have been studied in any detail. The markers used in this study represent inflammatory molecules found in atherosclerosis as determined by other studies (22–24,28).

Our results indicate two conclusions. First, the study demonstrates that patients with a premature form of coronary artery disease have significantly increased levels of VCAM-1, TNF-α-RII and superoxide as compared to their control counterparts. Second, irbesartan significantly reduces the levels of these molecules in patients with CAD. The first conclusion shows that our results mirror work done by other researchers (19,29) establishing an inflammatory process in atherogenesis. The second conclusion indicates that irbesartan, possibly through its mechanism of action, reduces the inflammatory response seen in atherosclerosis. These findings imply that the use of irbesartan in low doses may retard the inflammatory component of the atherosclerotic process without affecting blood pressure.

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