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Coronary Artery Disease

Irbesartan, an Angiotensin Type 1 Receptor Inhibitor, Regulates the Vascular Oxidative State in Patients With Coronary Artery Disease

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OBJECTIVES The aim of this study was to determine the effect of angiotensin II type 1 (AT_1) receptor antagonists on pro-oxidant species observed in the pathogenesis of atherosclerosis. Parameters such as low-density lipoprotein (LDL) susceptibility, monocyte binding capacity, superoxide generation and lipid peroxidation were examined in the presence of the AT_1 receptor antagonist irbesartan.

BACKGROUND Low-density lipoprotein oxidation is a key component in the process of atherogenesis. This modification may involve various mechanisms, including changes in nitric oxide levels and superoxide levels. Additionally, compounds that suppress these mechanisms may retard or inhibit the pathogenesis of atherosclerosis.

METHODS Forty-seven patients with documented coronary artery disease were treated with irbesartan for a 12-week period. Patients were randomized to receive irbesartan or placebo. Lipid peroxidation, superoxide levels, monocyte binding and LDL oxidation were measured at 0, 4 and 12 weeks. Findings were statistically evaluated by two-way repeated measures analysis of variance with $p < 0.05$ being significant.

RESULTS Treatment with irbesartan significantly decreased the pro-oxidative environment seen in our study population. Lag time for LDL oxidation increased 32% at 12 weeks, suggesting an increased resistance of LDL modification in the serum. Thiobarbituric acid reactive substances activity indicated that lipid peroxidation decreased by 36% in comparison to placebo. In addition, superoxide levels and monocyte-binding capacity were also significantly reduced in coronary artery disease patients receiving irbesartan.

CONCLUSIONS Our results indicate that irbesartan may suppress the atherosclerotic process by inhibiting the intravascular oxidative state and the production of reactive oxygen species, compounds that may cause damage to the vasculature. (J Am Coll Cardiol 2001;38:1662–7) © 2001 by the American College of Cardiology

Ongoing research indicates that the abundance of reactive oxygen species in the vasculature results in an increased oxidation of proteins including oxidized low-density lipoprotein (ox-LDL), which initiates an inflammatory process and causes intimal damage to the arterial wall (1). The mechanisms of this damage are not yet established and may involve the inactivation of nitric oxide (NO) by oxygen-derived free radicals such as superoxide (2). This inflammatory response affects the gene expression of regulatory molecules, such as vascular cell adhesion molecule and tumor necrosis factor-alpha (3–5), which in turn promote foam cell formation. The reduction in NO levels along with an increase in ox-LDL may function as immunomodulators of the atherosclerotic process (6). Recent studies imply that ox-LDL stimulates an immunological response through the formation of autoantibodies, resulting in further damage to the endothelium and acceleration of the atherosclerotic process (7,8). This antibody response may represent a marker for the extent of atherosclerosis seen in affected individuals.

Agents that inhibit the renin-angiotensin system, such as angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type 1 (AT_1) receptor blockers, have considerable benefit in hypertension and heart failure (9–11). Studies have shown that AT_1 receptor blockers and ACE inhibitors improve endothelium-dependent vasomotor responses (12–15). Yet it appears that the AT_1 receptor blockers can produce greater inhibition of effects mediated by angiotensin II (12). Recently, we have demonstrated that treatment with AT_1 receptor blockers in patients with early atherosclerosis decreases markers of inflammation that may be sensitive to the oxidative state in the vasculature (16). The use of AT_1 receptor blockers may be beneficial in retarding the progression of atherosclerosis because of their ability to prevent native low-density lipoprotein (LDL) from being oxidatively modified to ox-LDL (17). It is possible to speculate that inhibitors of the renin-angiotensin system such as AT_1 receptor blockers may improve endo-

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Abbreviations and Acronyms

ACE	= angiotensin-converting enzyme
ANOVA	= analysis of variance
AT_1	= angiotensin II type 1 receptor
BP	= blood pressure
CAD	= coronary artery disease
13-HPODE	= 13 hydroperoxy linoleic acid
LDL	= low-density lipoprotein
MI	= myocardial infarction
NO	= nitric oxide
ox-LDL	= oxidized low-density lipoprotein
PTCA	= percutaneous transluminal coronary angioplasty
TBARS	= thiobarbituric acid reactive substances

thelial function by regulation of antioxidant-sensitive mechanisms in the vasculature.

In the present study, we wished to determine whether the regulation of angiotensin II activity via blockade of the angiotensin II type 1 receptor might affect pro-oxidant mechanisms critical in the pathogenesis of vascular diseases such as atherosclerosis.

METHODS

Subjects. Forty-seven subjects (26 men and 21 women), all with documented coronary atherosclerosis, were enrolled into the study. All patients had undergone coronary artery bypass graft and/or percutaneous transluminal coronary angioplasty at least one year before the start of the study. No patient had any subjective evidence of acute coronary syndrome in the year preceding the study. Evidence of myocardial infarction (MI) was determined by electrocardiographic findings, previous history of elevated creatine kinase-MB isoenzymes, and/or regional wall motion abnormality noted on either ventriculography or echocardiography. Left ventricular ejection fraction was determined by echocardiography. All patients were considered stable on their medical regimen and free of any acute coronary syndrome (i.e., MI or unstable angina) for at least 12 months. All subjects were on aspirin, and an equal number of subjects in each group were on nitrates, beta-blockers and HMG-CoA reductase inhibitors. No subject had taken either ACE inhibitors or AT_1 receptor inhibitors for at least six months before the beginning of the study.

A survey was performed on each potential subject, and the following were excluded: current smokers, patients with an ejection fraction of <40% by echocardiography or ventriculography, diabetes with a glycosylated hemoglobin >7.5%, systolic blood pressure (BP) >150 mm Hg, renal impairment, hepatic impairment or malignancy. The protocol was approved by the research committee, and all subjects gave written informed consent.

Study design. Under a randomized, placebo-controlled protocol, subjects received either irbesartan 150 mg daily (provided by Bristol-Myers Squibb, Princeton, New Jersey) or placebo for a 12-week period. Serum samples were collected at 0, 4 and 12 weeks. Clinical characteristics and medications are denoted in Table 1. All patients tolerated irbesartan or placebo and completed the 12-week study without difficulty.

Measurement of LDL oxidation in patients with atherosclerosis. The plasma was isolated from patients at 0-, 4- and 12-week intervals, and LDL was isolated via ultracentrifugation at 39,000 rpm at 4°C. The LDL was then oxidized to ox-LDL by an in vitro assay utilizing $CuSO_4$, as previously described (18). The lag time indicating the susceptibility of the LDL to oxidize was measured using a spectrophotometer at 280 nm (18). Values were performed at least in triplicate.

Monocyte binding to the human CD11b receptor. The serum of the patients in either the irbesartan or placebo group was collected at 0, 4 and 12 weeks. Whole-blood immunotyping was performed in blood samples using fluorescence-activated cell sorting analysis with a monoclonal antibody to the human CD11b receptor, a protein with

Table 1. Patient Demographics and Background Medications

	Irbesartan (n = 24)	Placebo (n = 23)
Age, years	62.3 ± 8.9	63.0 ± 7.8
Male/female	13/11	13/10
Hypertension	16	16
Former smoking history	20	19
Diabetes mellitus	10	8
Hyperlipidemia	18	20
Left ventricular ejection fraction (%)	53.6 ± 8.4	52.3 ± 5.9
Previous myocardial infarction	8	6
Starting blood pressure (mm Hg)	136 ± 13 (108–150)	133 ± 12 (111–147)
Starting LDL cholesterol (mg/dl)	126 ± 22 (93–149)	129 ± 19 (100–145)
Background medications		
Aspirin	24 (100%)	23 (100%)
Nitrates	8 (33%)	6 (26%)
Beta-blockers	16 (67%)	13 (56%)
Calcium channel blockers	5 (22%)	5 (23%)
HMG-CoA reductase inhibitors	20 (83%)	21 (91%)
Other hypolipidemics	3 (13%)	4 (18%)

LDL = low-density lipoprotein.

human binding sites and a very high binding affinity to human monocytes. The monoclonal antibody to the CD11b receptor was obtained through Pharmingen. The changes in monocyte CD11b expression were determined with flow cytometry analysis, as previously described (19).

Superoxide anion generation by neutrophils. A modification of the lucigenin-based chemiluminescence assay of NADPH oxidase activity to measure basal and postactivation levels of superoxide generation by neutrophils was used (20,21). Neutrophils were separated from venous blood using a Ficoll-Hypaque density gradient, and activation of the neutrophil respiratory burst was achieved using 1 micromole/l of N-formyl-methionyl-leucyl-phenylalanine (Sigma Chemical Corp., St. Louis, Missouri). The peak level of superoxide generated after activation was recorded in mV/5 $\times$ 10^5 neutrophils (16,20).

Measurement of lipid peroxidation in plasma. The polyunsaturated fatty acid linoleic acid (18:2) is converted into 13-hydroperoxy linoleic acid (13-HPODE) by treatment with the enzyme lipoxygenase. Previous investigations suggest that 13-HPODE may be involved in the initiating and propagating steps toward atherosclerosis (22,23). The thiobarbituric acid reactive substances (TBARS) assay was performed as an indication of lipid peroxidation in the plasma by using a colorimetric assay as previously described (24). One molecule of either malonedialdehyde or 4-hydroxyalkenal reacts with thiobarbituric acid to yield a stable chromophore (20). Spectrophotometric analysis was performed at 568 nm.

Statistical analysis. All data are presented as the mean value ± SEM. Comparisons were determined within the placebo and irbesartan groups (0, 4 and 12 weeks) and between the placebo and irbesartan groups with two-way analysis of variance (ANOVA). We determined a p value of <0.05 to be statistically significant. (Bonferroni correction applied; p values are related to ANOVA).

RESULTS

The clinical characteristics of the study group are noted in Table 1. The average age of the study population was 62.7 years; 68% of the study population had a previous history of elevated BP. Patients in the irbesartan group had a starting BP of 136 ± 13 mm Hg (range 108 to 150 mm Hg) and those in the placebo group had a starting BP of 133 ± 12 mm Hg (range 111 to 147 mm Hg). Eighty-three percent of the study population were former smokers; 38% of the study population had diabetes mellitus. Eighty percent of the study population had hyperlipidemia. Starting LDL-cholesterol levels measured at 126 ± 22 mg/dl (range 93 to 149 mg/dl) and 129 ± 19 mg/dl (range 100 to 145 mg/dl) for the irbesartan and placebo groups, respectively. Thirty percent of the study population suffered from a previous MI (eight patients in the irbesartan group; six patients in the placebo group). The average left ventricular ejection fraction for the irbesartan group was 53.6% versus 52.3% for the placebo group.

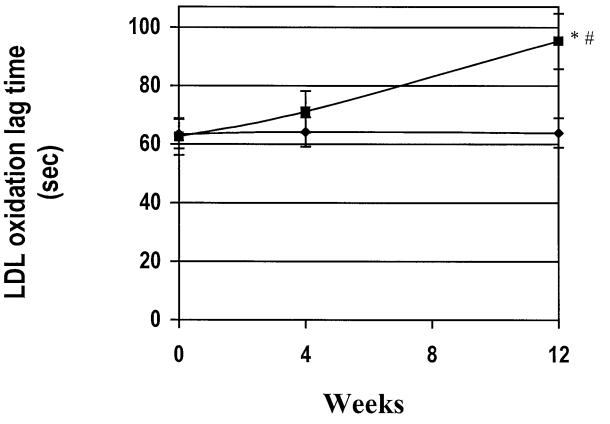

Figure 1. Irbesartan decreases susceptibility to low-density lipoprotein (LDL) oxidation in patients with coronary artery disease (CAD). Patients with CAD were placed on placebo or irbesartan (150 mg/day) for a 12-week period. Serum samples were collected at the start of the study (0 weeks) and at 4 and 12 weeks of treatment. The LDL was isolated by centrifugation, and rate of oxidation was measured by spectrophotometric analysis as described in the Methods section. The p value, as determined by analysis of variance, was 0.027 for time, <0.0001 for group and 0.014 for the group-time interaction. **Black diamond** = placebo; **black square** = irbesartan; *$p < 0.05$ = difference between 0 weeks and 4 or 12 weeks within each group; #$p < 0.05$ = difference between placebo and irbesartan groups.

All of the patients completed the 12-week course of either irbesartan or placebo without significant adverse effect. No significant changes in serum creatinine, blood urea nitrogen or potassium were noted in either group before or after treatment. There was an average reduction of systolic BP by 7 ± 3 mm Hg and diastolic BP by 4 ± 2 mm Hg in the group of patients treated with irbesartan. These changes were statistically significant, but no patient developed hypotension (i.e., systolic BP of <90 mm Hg) at any time during the study.

Irbesartan affects the susceptibility of LDL to oxidation in patients with coronary artery disease (CAD). Using time-course analysis, irbesartan increased the lag time of LDL oxidation in patients, with a slight increase of 11% from placebo (Fig. 1) at four weeks of treatment (71.1 ± 8.3 s vs. 63.1 ± 5.6 s for placebo, NS). Furthermore, there was an increase of 32% in LDL lag time in the irbesartan group from placebo at 12 weeks of treatment (93.2 ± 8.4 vs. 62.7 ± 7.6 s, $p < 0.05$). There was no change in serum cholesterol or LDL cholesterol in any of the patients who were treated with irbesartan.

Treatment with irbesartan regulates monocyte binding in the vasculature of patients with stable CAD. Using flow cytometry analysis, treatment with irbesartan suppressed monocyte binding to CD11b at four weeks and 12 weeks of treatment as compared to placebo. Figure 2 indicates that there was a significant decrease in monocyte binding capacity in the group of patients treated with irbesartan. This decreased capacity remained constant through the course of the study. On the other hand, monocyte binding capacity

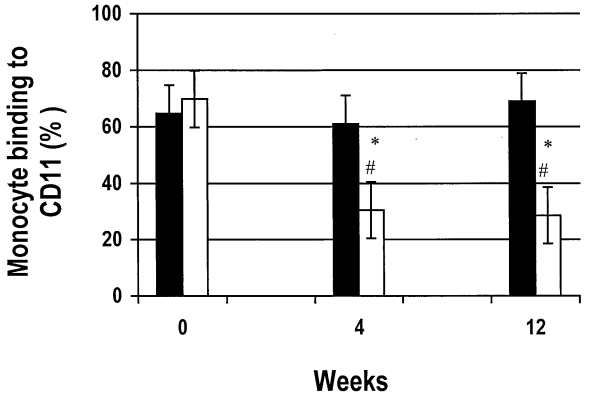

Figure 2. Irbesartan decreases monocyte binding the CD11b monoclonal antibody in patients with coronary artery disease (CAD). Patients with CAD were placed on placebo or irbesartan (150 mg/day) for a 12-week period. Serum samples were collected and red blood cells were lysed. Using flow cytometry analysis, whole-blood monocyte CD11b expression was performed. The values expressed are a percentage of expression of CD11b by circulating monocytes. The p value, as determined by analysis of variance, was <0.0001 for time, for group and for the group-time interaction. **Black square** = placebo; **white square** = irbesartan; *p < 0.05 = difference between 0 weeks and 4 or 12 weeks within each group; #p < 0.05 = difference between placebo and irbesartan groups.

continued to increase in the placebo group throughout the study (from 61% binding at four weeks to 68.9% binding at 12 weeks).

Treatment with irbesartan modifies neutrophil superoxide-generating capacity in patients with stable CAD. Figure 3 indicates that neutrophil superoxide generating capacity was markedly reduced in patients treated with irbesartan at both four and 12 weeks. The figure shows that treatment with irbesartan reduced neutrophil superoxide-generating capacity by 42% at 12 weeks. In contrast, the placebo group showed no significant change in neutrophil superoxide-generating capacity during the 12-week study period.

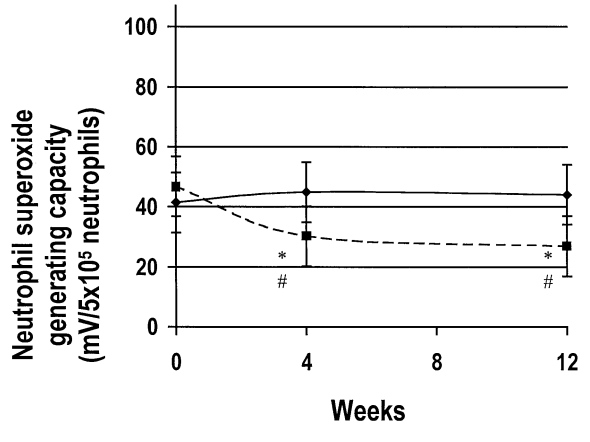

Figure 3. Irbesartan decreases production of superoxide in the neutrophils of patients with coronary artery disease. Serum samples were collected from the patients who were placed on placebo or irbesartan (150 mg/day) for a 12-week period. Samples were collected at the start of the study (0 weeks) and at 4 and 12 weeks of treatment. The neutrophils were isolated and the peak levels of superoxide production after neutrophil respiratory burst were performed. The p value was <0.0001, as determined by analysis of variance, for time, for group and for the group-time interaction. **Black diamond** = placebo; **black square** = irbesartan; *p < 0.05 = difference between 0 and 4 or 12 weeks within each group; #p < 0.05 = difference between placebo and irbesartan groups.

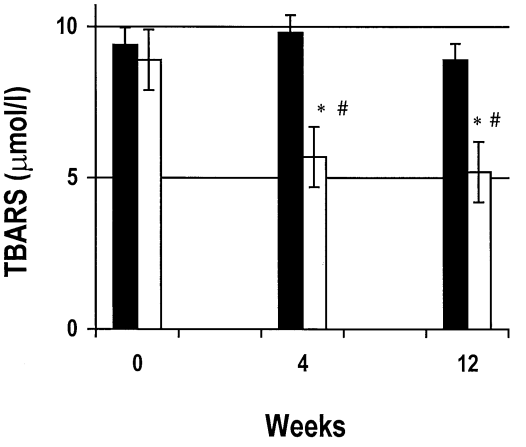

Figure 4. Irbesartan decreases lipid peroxidation in the serum of patients with coronary artery disease. Serum samples were collected during the 12-week period of treatment at the start of the study (0 weeks) and at 4 and 12 weeks of treatment. The thiobarbituric acid reactive substances (TBARS) assay was performed, as described in the Methods section, using spectrophotometric analysis at 568 nm. The p value, as determined by analysis of variance, was 0.027 for time, 0.012 for the group and 0.004 for the group-time interaction. **Black square** = placebo; **white square** = irbesartan; *p < 0.05 = difference between 0 weeks and 4 or 12 weeks within each group; #p < 0.05 = difference between placebo and irbesartan groups.

Irbesartan regulates lipid peroxidation in patients with CAD. To determine whether lipid-derived free-radical production is regulated by inhibition of the AT_1 receptor, the TBARS assay was utilized as a measurement of lipid peroxidation. Patients with stable CAD when treated with irbesartan showed a reduction in TBARS activity that was 33% and 36% lower than placebo (Fig. 4) at four weeks and 12 weeks (both $p \leq 0.05$), respectively.

DISCUSSION

The present study assessed the effect of the angiotensin II type 1 receptor inhibitor irbesartan on oxidative processes seen in patients with CAD. Our findings demonstrate that treatment of these patients with irbesartan decreased levels of lipid peroxidation, monocyte binding and superoxide production and significantly increased the lag time for the oxidation of LDL. These findings suggest that irbesartan possesses certain antioxidant properties in addition to its ability to lower BP.

Potential antioxidant mechanisms seen with the blockade of the angiotensin II type 1 receptor. The binding of angiotensin II to the AT_1 receptor is responsible for most of the peripheral and central effects of angiotensin II on BP, osmoregulation and cell growth, and thus for the effect of the renin-angiotensin system in cardiovascular pathology (25,26). Drugs that block the renin-angiotensin system have been shown to provide benefits beyond BP control because of their inhibition of angiotensin II (27). Recent interest has focused on the role of angiotensin II acting at the AT_1

receptor in the vasculature, specifically in the processes of atherosclerosis (26).

In the current study, we determined that irbesartan reduces the susceptibility of LDL to oxidize in patients with CAD. These patients had LDL cholesterol values that were treated with lipid-lowering agents, and treatment with irbesartan did not alter their LDL cholesterol values during the entire course of the study. It has been reported previously that saralasin, an AT_1 receptor blocker, inhibits angiotensin II macrophage-mediated oxidation of LDL (17). Additionally, our findings indicate that treatment with irbesartan reduces the adhesion of monocytes in whole blood. Therefore, it may be that cells such as monocytes are inactivated in their ability to induce mechanisms of inflammation that are significant in atherogenesis.

The study also showed that treatment of CAD patients with irbesartan reduced the capacity of neutrophils to make superoxide anion. The NAD(P)H oxidase system in endothelial cells, smooth muscle cells and phagocytes is an important source of reactive oxygen species in cardiovascular disease (20,28), and this activity may be regulated by angiotensin II operating primarily through the AT_1 receptor. It has been shown that angiotensin II mediates vascular superoxide production via the NAD(P)H oxidase system (29,30) and that cardiovascular diseases exhibit increased levels of superoxide in the vasculature (16,20,31). Our findings suggest that inhibition of the AT_1 receptor regulates the production of reactive oxygen species that may in turn modulate the oxidative state in the vasculature. These findings suggest that the reduction of reactive oxygen species such as superoxide anion by irbesartan may be significant in decreasing irbesartan's ability to modify LDL to an oxidized form. This decrease in turn may reduce the uptake of LDL by inflammatory cells via the scavenger receptor or other receptors that would subsequently form an atherogenic foam cell seen in the pathogenesis of atherosclerosis. Furthermore, these inhibitory mechanisms would then reduce the ability of inflammatory cells such as monocytes to adhere to the vessel wall and thereby reduce the formation of an atherosclerotic plaque. These potential antioxidant mechanisms may be specific to irbesartan and its chemical structure and may not necessarily be an effect of this class of antihypertensive compounds.

Our findings with the TBARS assay indicate that irbesartan reduced the oxidative stress responsible for lipid peroxidation. It has been observed that oxidative stress may contribute to endothelial dysfunction in cardiovascular diseases such as atherosclerosis by reducing NO synthase activity and by reacting with NO to form peroxynitrite (20,32). Recent studies in the aorta suggest that angiotensin II interacts with the bradykinin/NO system by an AT_2 receptor-dependent mechanism (33,34). Recent studies indicate that the AT_2 receptor mediates antiproliferative and potentially antioxidative effects (35–37), and it is conceivable that many if not all of the antioxidant effects by irbesartan may be modulated by upregulation of AT_2 receptor activity. Further investigations must be undertaken to assess the validity of these potential mechanisms of action.

Study limitations. Our findings are primarily confined to patients with known CAD, and it is not clear whether treatment with irbesartan would be beneficial in the primary prevention of CAD. Unlike AT_1 receptor inhibitors such as losartan or valsartan, the pharmacological properties of irbesartan are that the compound blocks 100% of the AT_1 receptor and has insurmountable properties (38). This difference indicates that the properties seen with irbesartan should not be considered a class effect of the AT_1 receptor antagonists and that further investigations with the other AT_1 receptor inhibitors need to be performed. Also, the TBARS assay is not an accurate indicator of the oxidative state in the vasculature, but it is an indirect measurement of lipid peroxidation, and has been utilized with reliable results in previous investigations (20,24). Finally, our results have been observed in only 12 weeks of therapy. A large group study of long-term therapy with an AT_1 receptor inhibitor, similar to previous reported results with ACE inhibitors (39), is required to determine whether these agents would be beneficial in secondary prevention of CAD.

Conclusions. Because inflammatory and pro-oxidant mechanisms are significant in the pathogenesis of atherosclerosis, the findings in the present study suggest that AT_1 receptor inhibitors such as irbesartan may have benefits in treatment of atherosclerosis. We have previously shown that irbesartan reduces markers of inflammation in the serum of patients with early CAD (16). The present findings extend these observations to indicate that antioxidant-sensitive mechanisms may be responsible for reducing these inflammatory markers independent of BP reduction.

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