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Effect of Angiotensin II Type I Receptor Blockade with Valsartan on Carotid Artery Atherosclerosis: A Double Blind Randomized Clinical Trial Comparing Valsartan and Placebo (EFFERVESCENT)

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Abstract

Background—Progression of atherosclerosis is associated with a greater risk for adverse outcomes. Angiotensin II plays a key role in the pathogenesis and progression of atherosclerosis. We aimed to investigate the effects of Angiotensin II type-1 receptor (AT₁R) blockade with Valsartan on carotid wall atherosclerosis, with the hypothesis that Valsartan will reduce progression of atherosclerosis.

Methods—Subjects (n= 120) with carotid intima-media thickness >0.65mm by ultrasound were randomized (2:1) in a double-blind manner to receive either Valsartan or placebo for 2 years. Bilateral T2-weighted black-blood carotid magnetic resonance imaging was performed at baseline, 12 and 24 months. Changes in the carotid bulb vessel wall area (VWA) and wall thickness (WT) were primary endpoints. Secondary endpoints included changes in carotid plaque thickness, plasma levels of aminothiols, C-reactive protein, fibrinogen, and endothelium-dependent and -independent vascular function.

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Clinical Trial Registration

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Results—Over 2 years, the carotid bulb VWA decreased with Valsartan (-6.7 , 95% CI: $(-11.6, -1.9)$ mm²) but not with placebo (3.4 , 95% CI: $(-2.8, 9.6)$ mm²), $p=0.01$ between groups. Similarly, mean WT decreased with Valsartan (-0.18 , 95% CI: $(-0.30, -0.06)$ mm), but not with placebo (0.08 , 95% CI: $(-0.07, 0.23)$ mm), $p=0.009$ between groups. Furthermore, plaque thickness decreased with Valsartan (-0.35 , 95% CI: $(-0.63, -0.08)$ mm) but was unchanged with placebo ($+0.28$, 95% CI: $(-0.11, 0.69)$ mm), $p=0.01$ between groups. These findings were unaffected by statin therapy or changes in blood pressure. Notably, there were significant improvements in the aminothiols cysteine-glutathione disulfide, and trends to improvements in fibrinogen levels and endothelium-independent vascular function.

Conclusions—In subjects with carotid wall thickening, AT₁R blockade was associated with regression in carotid atherosclerosis. Whether these effects translate into improved outcomes in subjects with subclinical atherosclerosis warrants investigation.

Keywords

Angiotensin II Receptor Blocker; Atherosclerosis; Carotid Magnetic Resonance Imaging; Clinical Trial

BACKGROUND

Complications of atherosclerosis constitute the leading cause of morbidity and mortality worldwide (1). Compared to stable atherosclerosis, progressive atherosclerotic plaques impart a greater risk for clinical complications (2,3), sparking interest in interventions capable of slowing progress of disease. Accumulating evidence supports the role of angiotensin II, via angiotensin type-1 receptor (AT₁R) activation and/or overexpression in both the pathogenesis and progression of atherosclerosis. In experimental studies, AT₁R stimulation increases oxidative stress that leads to endothelial dysfunction, inflammation, thrombosis, fibrosis, proliferation of vascular smooth muscle cells, production of extracellular matrix, and ultimately to plaque formation, progression and rupture (4–6). In humans, AT₁R antagonism improves oxidative stress, endothelial dysfunction, inflammation and fibrosis, which may result in plaque stabilization and reduction of progression of atherosclerosis (7–12). These effects have also translated into improvements in cardiovascular outcomes with AT₁R antagonist therapy in patients with, or at increased risk for, atherosclerotic disease (13–16).

Atherosclerosis is a systemic process with a predilection for certain vascular beds including the carotid arteries. The relative ease of imaging carotid atherosclerotic plaque, and its association with coronary and cerebrovascular disease and adverse outcomes makes imaging of the carotid arteries an especially attractive diagnostic and prognostic tool. Importantly, atherosclerotic plaque formation tends to preferentially localize at arterial bifurcations and curves where blood flow is oscillatory and shear stress is low, a mechanism that is at least partly explained by up-regulation of AT₁R expression by the low shear stress in these locations (17,18). Whether progression of carotid arterial wall disease is modulated with AT₁R antagonists has been studied previously using carotid intima-media thickness (CIMT) measurements with ultrasound, with conflicting results (19–24). Notably, ultrasound measures CIMT in the common carotid arteries where atherosclerosis and plaques develop

less frequently as compared to the carotid bulb (25). In contrast, magnetic resonance imaging (MRI) provides accurate and reproducible assessment of carotid arterial wall atherosclerosis in the carotid bulb and permits reliable quantification of disease progression, making it a superior technology for assessing therapeutic changes over time in a relatively smaller sample size (26,27).

Whether MRI-assessed atherosclerosis in the carotid arterial bulb is responsive to AT₁R blockade in humans has not been previously studied. In subjects with abnormal CIMT, we used MRI of the carotid bulb to measure the effects of long-term AT₁R blockade with Valsartan, with the hypothesis that Valsartan will reduce progression of carotid wall thickness (WT) and inhibit atherosclerotic plaque progression. As secondary aims, we postulated that the salutary effects of Valsartan on carotid disease would be mediated by improvements in oxidative stress, inflammation, and vascular function.

METHODS

The trial was designed as a prospective, single center, double blind, placebo-controlled, randomized study to investigate the effects of Valsartan on the progression of carotid bulb disease in patients with abnormal CIMT over a 2-year treatment period. The work was supported by an investigator initiated research grant from Novartis, and by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR000454. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper and its final contents.

Patients

Men and women aged between 21 and 80 years were recruited by advertisement between March 2005 and October 2008. Those with a CIMT >0.65 mm measured by a screening ultrasound (28) were enrolled. Exclusion criteria included (1) premenopausal females with potential for pregnancy, (2) angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker therapy in the previous 3 months, (3) initiation or change in dose of statin therapy within 2 months, (4) anticipated change in lipid lowering therapy, (5) LDL cholesterol level >160 mg/dl or >130 mg/dl in the presence of atherosclerotic plaque during screening carotid ultrasound and not receiving statin therapy, (6) acute coronary or cerebrovascular event within 2 months, (7) blood pressure >140 mmHg systolic or >90 mm Hg diastolic, (8) HbA1c >8.5, (9) serum creatinine > 2.5 mg/dL, (10) current neoplasm, (11) inability to give informed consent, and (12) inability to undergo MRI. The Emory University Institutional Review Board approved the study and written informed consents were obtained from all subjects.

Protocol

Subjects were randomized (2:1 drug vs. placebo) in a double-blind manner to receive either Valsartan (n=80) titrated up from 160 mg to 320 mg daily over 2 weeks, or matching placebo (n=40) for 24 months, Figure 1. An independent pharmacist dispensed either Valsartan or placebo in an identical form and appearance and according to a computer

generated randomization list. The allocation sequence was concealed from all researchers enrolling and assessing participants and all outcome assessors were kept blinded to the treatment allocation. Half the enrolled subjects (n=60) were already on long-term statin therapy and were stratified for statin use before randomization. Subjects on statin therapy were switched to simvastatin 40 mg daily unless they were already on high dose statin therapy and thus were placed on 80 mg daily of simvastatin for the duration of the study. Concomitant therapy with aspirin or anti-hypertensive medications except for angiotensin antagonists was permitted. All patients were on stable medical therapy for at least 2 months before recruitment.

Assessments made at 2 weeks, 3, 12, and 24 months included vital signs, physical exam, adverse effects, lipid profile, and safety parameters (creatinine, potassium, creatinine kinase, aspartate aminotransferase, and alanine aminotransferase). MRI was performed at randomization, 12 and 24 months. Measurements of endothelial function and blood sampling for biomarkers were performed at baseline, 12, and 24 months, Figure 2.

Carotid MRI

The MRI protocol has been previously described (29). Briefly, examinations were performed on either a 1.5 or 3T MRI system using a multi-channel carotid phased-array coil. Image analysis was done using a dedicated vessel analysis package (VesselMASS, LUMC, Leiden, Netherlands) that simultaneously displayed the entire stack of images. Images were magnified and contrast was adjusted to ensure optimal viewing and to enable accurate tracing of the vessel wall boundaries. A single trained investigator (AK), who was blinded to treatment allocation and timing of the scans, manually traced the outer vessel boundary (defined as the vessel wall-soft tissue interface) and the inner vessel boundary (defined as the vessel lumen-wall interface) in the left and right carotid of each patient. Since the largest amount of disease is expected in the carotid bulb, slices from the bulb were chosen for analysis. Images from all time points were reviewed together to ensure alignment of carotid bulb slices between baseline and each follow-up time point. After the contours were manually traced, the following measurements were generated for each bulb slice using the automated software, Figure 3: (1) vessel wall area (VWA) defined as the difference between the outer vessel wall area and the lumen area; (2) mean circumferential WT, defined as the mean of all individual chords; and (3) maximum WT as the length of the longest chord. For inter-observer variability of carotid MRI assessment in our lab, Pearson correlations ranged from 0.94 to 0.99 and intraclass correlations from 0.92 to 0.99. For intra-observer variability, Pearson correlations ranged from 0.93 to 0.95 and intraclass correlations from 0.83 to 0.95 (29).

For analysis of plaque progression, each cross-sectional image of the vessel was divided into six contiguous sectors by the software and the mean WT of each sector was automatically calculated, Figure 3. Subjects were considered to have an atherosclerotic plaque (n=86) if they had a maximum WT >2 mm (30). Subsequently, the mean WT of the maximum carotid bulb sector in these individuals was tracked over time to estimate changes in plaque progression.

Plasma Biomarkers

Markers of Oxidative Stress—Oxidative stress was estimated by measuring plasma amino thiols (cysteine, glutathione, and their oxidized disulfides cystine, glutathione disulfide (GSSG), and cysteine-glutathione disulfide) using high performance liquid chromatography as previously described (31). Higher levels of the oxidized derivatives of these plasma thiols, such as cystine and cysteine-glutathione disulfide, and lower levels of the reduced forms, such as cysteine and glutathione, represent increased oxidative stress.

Markers of Inflammation—C-reactive protein (CRP) levels were measured using the Dade-Behring Nephelometry system (Deerfield, IL). The Clauss assay was used for quantification of plasma fibrinogen levels.

Measurement of Vascular Function

Endothelium-dependent and -independent vascular function was estimated using brachial artery flow-mediated vasodilation (FMD) and nitroglycerin-mediated vasodilation, respectively, as described previously (32). In our laboratory, the mean difference in FMD between assessments performed in 11 subjects on consecutive days was $1.26 \pm 0.76\%$, with a correlation coefficient of 0.75. The mean difference in the FMD between 2 readings was $0.82 \pm 0.48\%$ ($r = +0.97$).

Statistical Analysis

Results for normally distributed continuous variables are summarized as means $\pm$ standard error, or as proportions for categorical variables. For assessing between group differences at baseline, the two-sample t test was used for comparison of normally distributed continuous variables, and the Mann-Whitney U test for non-normally distributed variables. The chi-square test was used for the comparison of categorical variables. The average value of cross-sectional slices from each carotid bulb (ranging from 1 to 3 slices per side, per subject) was used for analysis. Statistical modeling by linear mixed effects models took into account that each subject was represented by two carotid arteries and that repeated measurements over time on the same subjects were correlated. The model-based means are unbiased with unbalanced and incomplete data, provided that the incomplete data are missing at random. Conditional on the observed data, dropouts were assumed to be independent of the unobserved measurements. After examining a number of within-subject error covariance structures, compound symmetry was assumed. Notably, analysis of non-missing data using paired-sample t test for within group differences and independent-samples t test for changes between groups over time yielded similar results (not shown) to our model-based analysis. We also performed a last value carried forward analysis, and the results did not qualitatively differ from those obtained by the mixed effects models (Appendix Table 1).

The primary endpoints were changes in the mean circumferential WT and the mean VWA of the carotid bulb between groups over 24 months. Secondary endpoints included change over 24 months in the mean lumen area, maximum WT, maximum sector mean WT, plaque wall thickness, biomarkers, and vascular function. Based on a previous study with simvastatin, we estimated that 39 subjects in each group would provide 80% power with an alpha of 0.05 to observe a similar difference (25). A larger group was recruited to allow for 20% dropout

rate. Data was analyzed using SAS version 9.3 (SAS Institute Inc.) and SPSS version 20.0 (SPSS, Inc., Chicago, Illinois). Statistical tests were 2-tailed, and a 0.05 level significance was used.

RESULTS

Sample characteristics

Of 216 screened subjects, 120 were randomized for study participation (Figure 1). Notably, our study population had a relatively low cardiovascular risk profile with 7% diabetics and 12% with a history of coronary artery disease. Moreover, they had normal blood pressures and a favorable lipid profile at baseline (Table 1). Importantly, there were no differences in baseline characteristics between those randomized to Valsartan or placebo with respect to demographics, risk factor profile, hemodynamic parameters, biomarkers, or vascular function, except for a lower baseline HDL level in the placebo group. There were significant changes in blood pressure during the 24-month period, but the magnitude of changes in both systolic and diastolic blood pressures between the Valsartan and placebo groups was not significant ($p=0.56$ and $p=0.78$, respectively), Table 2. Serum potassium level increased significantly with Valsartan, with a trend toward a greater change compared to placebo, Table 2. Although HDL levels increased in both groups, LDL levels significantly decreased in the Valsartan group. However, the magnitude of change in LDL and HDL levels at 24 months between the groups was not significantly different, Table 2.

A total of 34 subjects (28%) did not complete the study for various reasons as outlined in Figure 1. Specifically, twenty subjects (25%) in the Valsartan group and 7 subjects (17.5%) in the placebo group dropped out of the study before 12-months and an additional 7 subjects from the Valsartan group dropped out before the 24-month MRI examination, Figure 1.

Carotid Arterial Wall Dimensions

Over the 24 month period, the mean VWA of the carotid bulb decreased significantly with Valsartan (-6.7 , 95% CI: $(-11.6, -1.9)$ mm²) compared to an insignificant change with placebo ($+3.4$, 95% CI: $(-2.8, 9.6)$ mm²), $p=0.01$ between groups, Figure 4A. Similarly, after 24 months, the mean circumferential WT of the carotid bulb decreased with Valsartan (-0.18 , 95% CI: $(-0.30, -0.06)$ mm) compared to an insignificant change with placebo (0.08 , 95% CI: $(-0.07, 0.23)$ mm), $p=0.01$ between groups, Figure 4B. Importantly, the effects of Valsartan were unaffected by statin use (p for interaction= 0.59 and 0.20 for VWA and mean WT, respectively). Notably, there was no significant difference in the change of mean vessel lumen area between both groups during follow up.

We further investigated the impact of treatment on the thickest carotid arterial wall segments (where plaque deposition is most likely to be present) by evaluating its effect on (1) maximum WT, and (2) the *sector* with the greatest mean WT at baseline. After 24 months, maximum WT of the carotid bulb increased with placebo ($+0.87$, 95% CI: $(0.45, 1.29)$ mm) compared to an insignificant change with Valsartan (-0.08 , 95% CI: $(-0.41, 0.25)$ mm), $p=0.0008$ between groups, Figure 4C. The sector with the maximum mean WT at baseline increased significantly with placebo after 24 month ($+0.36$, 95% CI: $(0.03, 0.69)$ mm), as

compared to a significant decrease with Valsartan (-0.26 , 95% CI: $(-0.51, -0.01)$), $p=0.004$ between groups, Figure 4D, that was unaffected by statin use (p for interaction= 0.15). Finally, plaque thickness (defined as mean WT of the sector containing maximum WT >2 mm) decreased significantly with Valsartan (-0.35 , 95% CI: $(-0.63, -0.08)$ mm) but was unchanged with placebo ($+0.28$, 95% CI: $(-0.11, 0.69)$ mm) after 24 months of treatment, a difference that was significant between the groups, $p=0.01$, Figure 4E.

Finally, there were no correlations between the magnitude of change in carotid wall dimensions and the changes in systolic or diastolic blood pressure, LDL, or HDL levels over the treatment period.

Vascular Function

FMD did not change significantly in either group. Conversely, nitroglycerin-mediated vasodilation improved by $2.8\pm0.8\%$, $p=0.002$ at 12 months and by $3.1\pm1.0\%$, $p=0.004$ at 24 months with Valsartan compared to baseline, but remained unchanged with placebo. However, the magnitude of change was not significantly different between the groups, Table 2.

Biomarkers

Plasma aminothiols levels changed over the 24-month period, and the increase in cysteine-glutathione disulfide was greater with placebo than with Valsartan ($p=0.007$), indicating improved oxidative stress with Valsartan, Table 2. Serum CRP levels did not change significantly in either group. Finally, plasma fibrinogen level increased by 14% ($p=0.007$) with placebo but remained unchanged with Valsartan ($p=0.32$) at 24 months, however, the magnitude of difference was not statistically significant between the groups, Table 2.

DISCUSSION

In a randomized double-blind, placebo controlled study, we found that long term blockade of AT₁R with Valsartan resulted in significant reverse remodeling of the carotid arteries manifested as regression in carotid WT and carotid plaque, without significant changes in lumen size (33). These effects of Valsartan were independent of changes in blood pressure or lipid levels, or statin use, indicating that the anti-atherosclerotic effects of AT₁R blockade extend beyond its effects on traditional risk factors (16). Finally, Valsartan therapy was associated with lower oxidative stress and trends to improvement in markers of inflammation and endothelium-independent vascular function, providing potential mechanistic explanations for the observed beneficial effects.

Since greater carotid WT is associated with angiographically obstructive coronary artery disease and major adverse cardiovascular events (34,35), our findings imply that Valsartan therapy may be associated with long-term reduction in cardiovascular events in subjects with early atherosclerosis. Although controversial in meta-analyses, reduction in cardiovascular events with Valsartan and other AT₁R antagonists have been observed in subjects with hypertension, stable angina, diabetes, heart failure, and after myocardial infarction (13,15,36,37). Angiotensin II promotes endothelial dysfunction through AT₁R-mediated generation of superoxide anions from reduced nicotinamide adenine dinucleotide-

dependent oxidase (38). Potential mechanisms underlying the beneficial effects of AT₁R antagonists in atherosclerosis include modification of risk factors such as blood pressure, as well as improvement in oxidative stress, inflammation, and endothelial dysfunction. Improvements observed in our study are unlikely to be due to changes in blood pressure, which were similar in placebo and Valsartan groups. Indeed, previous studies have also shown that improvement in endothelial dysfunction with AT₁R antagonists is independent of blood pressure lowering (16,39).

AT₁R activation stimulates production of reactive oxygen species (40), and systemic oxidative stress can be quantified in vivo by assessing plasma protein and non-protein amino thiols that represent the two major pools modulating redox potential and oxidant balance (38,41). Of these pools, glutathione constitutes the major non-protein intracellular antioxidant that eliminates peroxides and maintains cellular redox state, and cysteine represents the major protein antioxidant thiols extracellularly (41). Modification of cysteine by S-glutathionylation forms cysteine-glutathione disulfide, which is uniquely positioned to assess overall body oxidative stress by connecting non-protein and protein thiols (41–43). Although we found evidence of increased oxidative stress over time in both treatment groups, probably due to aging (44), cysteine-glutathione disulfide levels increased less with Valsartan compared to placebo, suggesting evidence of improved oxidative stress. Indeed, S-glutathionylation is emerging as an important signaling mechanism and effect of oxidative insult in the cardiovascular system and has been linked to processes responsible for plaque formation and progression (41–43).

We measured CRP and fibrinogen as markers of inflammation (45,46), with the hypothesis that their change with therapy would herald the anti-inflammatory effects of Valsartan. No change in CRP levels was observed, possibly because our study was underpowered to observe small changes (47). Fibrinogen, another acute-phase reactant, may also play a role in promoting atherogenesis (48) with higher levels observed in those with clinically silent atherosclerosis, vulnerable plaque, and increased risk of stroke and cardiovascular disease (46). We found that plasma fibrinogen increased significantly in the placebo- but not the Valsartan-treated group, indicating that these anti-inflammatory and potential anti-thrombotic effects of Valsartan may at least partly be responsible for the observed salutary long-term changes in carotid atherosclerosis.

In a previous study with losartan, we observed both an acute increase in coronary nitric oxide bioavailability and improvement in FMD over a 3-month oral treatment period (7). However, no significant change in FMD was observed at 1 or 2 years after Valsartan in this study. In contrast, endothelium-independent vasodilation, reflecting vascular smooth muscle cell function, improved significantly with Valsartan, possibly by reversing the hypertrophic and fibrotic effects of angiotensin II (9).

Along with the randomized, double blind, placebo-controlled design of this study, other major strengths include MRI based measurements of carotid WT and plaque progression, the long duration of intervention, and simultaneous measurements of potential mechanistic pathways. Previous studies examining the effects of AT₁R antagonists on CIMT have measured changes in the common carotid artery, often with variable results (19–24). Our

investigation is the first to use MRI to study the effects of therapy on atherosclerosis in the carotid bulb where AT₁R are overexpressed and atherosclerosis predominates (17). Weaknesses of our study include the significant dropout rate and number of un-analyzable MRI scans, which may have reduced our ability to observe a significant difference at the 1-year time-point. Additionally, plaques were classified based on wall thickness using T2-weighted imaging of the carotid arteries and not using multi contrast MRI imaging, and thus changes in plaque morphology could not be studied. Lastly, the current ACC/AHA prevention guidelines suggest using the intensity of statin therapy as the goal of treatment rather than lipid profile targets, which is a change from the standard of care during the course of our study. Whether a uniform higher-potency statin therapy could have attenuated the ARB effect that we have demonstrated on stabilizing atherosclerosis warrants consideration.

In summary, in subjects with carotid wall thickening and subclinical atherosclerosis we demonstrate beneficial effects of long-term AT₁R antagonist therapy with Valsartan on arterial wall atherosclerosis, independent of statin therapy and changes in blood pressure or lipid levels. These changes were accompanied by concomitant improvements in markers of oxidative stress, inflammation, and peripheral vascular smooth muscle function. Whether changes in carotid arterial atherosclerosis translate into improved clinical outcomes in subjects with subclinical atherosclerosis is worthy of further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

AT₁R	Angiotensin II Type-1 Receptor
CIMT	Carotid Intima-Media Thickness
CRP	C-Reactive Protein
FMD	Flow-Mediated Vasodilation
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
MRI	Magnetic Resonance Imaging
WT	Wall Thickness
VWA	Vessel Wall Area

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Figure 1.
Study protocol

Study Protocol

Figure 2.
Flow diagram of study design and subject enrollment

Figure 3.

Panel A demonstrates a carotid artery angiogram (Left) through which a cross sectional slice from the bulb is shown as the original source image (zoomed and cropped) (right).

Panel B shows a cross-sectional MRI slice from the inferior aspect of the carotid bulb. (1) manually drawn contours with the red line delineating the inner vessel wall and the green line delineating the outer vessel wall. (2) calculation of Vessel Wall Area (VWA) shaded in yellow, which equals total vascular area (entire area encircled in green) minus lumen area (entire area encircled in red). (3) calculation of mean Wall Thickness (WT) as the mean value of all circumferential cords (yellow lines). (4) division of the vessel wall into sectors (S1 –S6) where the mean wall thickness is calculated automatically for each sector. (5) cross sectional image of a subject with a maximum wall thickness (orange line) exceeding 2mm. (6) mean wall thickness of the maximum sector (orange filled) represents plaque.

A. **B.**

C.

D.

Figure 4. Changes in the mean carotid bulb (A) vessel wall area, (B) circumferential wall thickness, (C) maximum wall thickness, (D) wall thickness of maximal sector, and (E) plaque wall thickness, in the Valsartan- and placebo-treated subjects at 12 and 24 months compared to baseline. Data shows mean change and SEM calculated using a mixed effects model.

Table 1

Baseline Characteristics

Characteristics	All Patients N=120	Treatment N=80	Placebo N=40	P value
Age (years, Mean $\pm$ SD)	60 $\pm$ 9	59 $\pm$ 9	62 $\pm$ 9	0.17
Male sex (% , N)	51 (61)	53 (42)	48 (19)	0.61
Caucasian race (% , N)	80 (96)	79 (63)	83 (33)	0.69
Hypertension (% , N)	39 (47)	35 (28)	48 (19)	0.19
Diabetes Mellitus (% , N)	7 (8)	6 (5)	8 (3)	0.8
Current and previous tobacco smoking (% , N)	35 (42)	33 (26)	40 (16)	0.65
Body mass index (kg/m ² , Mean $\pm$ SD)	29 $\pm$ 9	29 $\pm$ 6	28 $\pm$ 5	0.22
Statin use (% , N)	60 (50)	40 (50)	20 (50)	1
Prior History of:				
Coronary artery disease (% , N)	12 (14)	11 (9)	13 (5)	0.84
Myocardial infarction (% , N)	7 (8)	6 (5)	8 (3)	0.8
Cerebrovascular disease (% , N)	13 (15)	13 (10)	13 (5)	1
Stroke (% , N)	3 (3)	4 (3)	0 (0)	0.22
Screening Hemodynamic Measures (Mean $\pm$ SD)				
Systolic blood pressure (mmHg)	126 $\pm$ 12	126 $\pm$ 10	127 $\pm$ 16	0.75
Diastolic blood pressure (mmHg)	72 $\pm$ 10	72 $\pm$ 10	71 $\pm$ 12	0.47
Heart rate (bpm)	67 $\pm$ 10	68 $\pm$ 10	65 $\pm$ 9	0.11
Fasting Lipid Profile (Mean $\pm$ SD)				
Total cholesterol (mg/dL)	180 $\pm$ 30	179 $\pm$ 30	180 $\pm$ 32	0.96
Triglycerides (mg/dL)	114 $\pm$ 58	116 $\pm$ 57	109 $\pm$ 62	0.53
High density lipoprotein (mg/dL)	53 $\pm$ 17	51 $\pm$ 18	57 $\pm$ 14	0.048
Low density lipoprotein (mg/dL)	102 $\pm$ 26	104 $\pm$ 25	99 $\pm$ 27	0.32
Biomarkers (Mean $\pm$ SD)				
Potassium (mmol/L)	4.4 $\pm$ 0.36	4.3 $\pm$ 0.31	4.5 $\pm$ 0.41	0.07
Creatinine (mg/dL)	0.95 $\pm$ 0.18	0.95 $\pm$ 0.17	0.95 $\pm$ 0.20	0.94
<u>Oxidative Stress</u>				
Cystine (μ M)	85.6 $\pm$ 15.7	86.5 $\pm$ 16.9	83.9 $\pm$ 13.3	0.41
Glutathione (μ M)	1.34 $\pm$ 0.55	1.3 $\pm$ 0.59	1.3 $\pm$ 0.48	0.91
Cysteine-Glutathione Disulfide (μ M)	2.4 $\pm$ 0.85	2.4 $\pm$ 0.84	2.4 $\pm$ 0.89	0.89
<u>Inflammation</u>				
C-Reactive Protein (mg/L)	3.3 $\pm$ 6.3	3.8 $\pm$ 7.5	2.2 $\pm$ 2.4	0.19
Fibrinogen (g/L)	2.4 $\pm$ 0.78	2.5 $\pm$ 0.78	2.3 $\pm$ 0.76	0.26
Vascular Function (Mean $\pm$ SD)				
Endothelium-Dependent (FMD)	5.6 $\pm$ 3.7	5.7 $\pm$ 3.7	5.2 $\pm$ 3.7	0.52
Endothelium-Independent (NMD)	19.7 $\pm$ 6.8	19.5 $\pm$ 6.9	20.1 $\pm$ 6.8	0.67

Table 2

Comparison of Blood Pressure, Biomarkers, and Vascular Function at baseline and after 24 Months of Treatment with Valsartan and Placebo

Characteristics		Valsartan		Placebo		Change between Groups			
	Baseline	24 Months	n	P value	Baseline	24 Months	n	P value	P Value
Blood Pressure (mean ± SD)									
Systolic blood pressure (mmHg)	122±13	114±20	51	0.011	129±16	124±15	33	0.14	0.56
Diastolic blood pressure (mmHg)	74±10	69±8	51	0.003	76±12	72±12	33	0.043	0.78
Fasting Lipid Profile (mean ± SD)									
All Patients									
Total cholesterol (mg/dL)	180±31	173±34	49	0.11	172±28	175±33	32	0.42	0.1
Triglycerides (mg/dL)	119±61	120±72	49	0.87	108±67	106±55	32	0.83	0.79
High density lipoprotein (mg/dL)	50±16	55±18	49	<0.001	56±14	62±15	32	0.005	0.39
Low density lipoprotein (mg/dL)	104±26	94±28	49	0.01	93±25	91±27	32	0.68	0.11
Statin Group									
Total cholesterol (mg/dL)	170±33	161±30	29	0.13	167±33	166±35	19	0.92	0.31
Triglycerides (mg/dL)	111±64	120±71	29	0.34	118±77	114±51	19	0.77	0.4
High density lipoprotein (mg/dL)	48±14	52±16	29	0.008	58±14	61±15	19	0.25	0.76
Low density lipoprotein (mg/dL)	97±29	85±26	29	0.037	85±25	83±27	19	0.59	0.23
No Statin Group									
Total cholesterol (mg/dL)	193±24	191±32	20	0.61	179±20	188±26	13	0.12	0.13
Triglycerides (mg/dL)	130±57	120±74	20	0.37	94±49	94±61	13	0.95	0.52
High density lipoprotein (mg/dL)	53±19	58±21	20	0.006	54±14	65±15	13	0.004	0.1
Low density lipoprotein (mg/dL)	114±16	108±25	20	0.13	103±21	104±22	13	0.93	0.27
Biomarkers (mean ± SD)									
Potassium (mmol/L)	4.3±0.3	4.4±0.3	50	0.045	4.5±0.4	4.4±0.3	32	0.45	0.07
Creatinine (mg/dL)	0.98±0.17	0.92±0.27	50	0.054	0.96±0.21	0.94±0.23	32	0.31	0.46
Oxidative Stress (mean ± SD)									
Cysteine (µM)	9.3±2.5	9.2±4.0	41	0.87	9.2±2.3	8.9±2.5	23	0.71	0.91
Cystine (µM)	85.9±19.6	93.6±21.5	41	0.016	82.1±11.3	92.1±20.1	23	0.023	0.64

Characteristics	Valsartan			Placebo			Change between Groups		
	Baseline	24 Months	n	P value	Baseline	24 Months	n	P value	P Value
Glutathione (µM)	1.3±0.6	1.5±0.6	41	0.069	1.31±0.52	1.65±0.61	23	0.018	0.39
Glutathione Disulfide (µM)	0.03±0.02	0.06±0.05	41	<0.001	0.03±0.02	0.06±0.04	23	0.023	0.42
Cysteine-glutathione disulfide (µM)	2.4±0.8	3.1±1.2	41	0.001	2.28±0.91	4.04±2.06	23	<0.001	0.007
Inflammation (mean ± SD)									
C-reactive protein (mg/L)	3.5±6.1	2.9±3.4	49	0.47	2.26±2.59	2.32±2.65	32	0.91	0.55
Fibrinogen (g/L)	2.5±0.8	2.7±0.8	49	0.32	2.23±0.48	2.54±0.53	32	0.007	0.35
Vascular Function (mean ± SD)									
Flow-mediated dilation (% change)	5.5±3.9	6.0±3.6	45	0.43	5.0±3.8	5.0±3.5	30	0.99	0.64
Nitroglycerin-mediated dilation (% change)	19.2±5.0	22.4±7.3	35	0.004	19.9±7.3	21.4±7.7	24	0.48	0.49