Different Effects of Antihypertensive Therapies Based on Losartan or Atenolol on Ultrasound and Biochemical Markers of Myocardial Fibrosis

Results of a Randomized Trial

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Background—In hypertensive left ventricular hypertrophy (LVH), myocardial texture is altered by a disproportionate increase in fibrosis, but there is insufficient clinical evidence whether antihypertensive therapy or individual agents can induce regression of myocardial fibrosis.

Methods and Results—We compared the effects of an angiotensin II receptor antagonist with a β-blocker on myocardial collagen volume (assessed by echoreflectivity and serum collagen markers) in 219 hypertensive patients with echocardiographically documented LVH. Patients were allocated randomly to receive losartan 50 to 100 mg/d (n=111) or atenolol 50 to 100 mg/d (n=99) with or without hydrochlorothiazide 12.5 to 25 mg/d for 36 weeks. Echoreflectivity analysis was conducted on ultrasound tracings of the midapex septum with specifically designed and validated software. A color histogram of reflecting echoes was obtained, and its spread (broadband [BB], previously shown to correlate directly with collagen volume fraction on endomyocardial biopsies) was used as the primary outcome measure. Mean color scale and serum markers of collagen synthesis (PIP, PIIIP) or degradation (CITP) were secondary outcome variables. Echoreflectivity analysis proved feasible in 106 patients (losartan 52, atenolol 54). Losartan reduced BB over 36 weeks (from 114.5 to 104.3 color levels, \( P=0.02 \)), whereas atenolol treatment was associated with an increase in BB (from 109.0 to 113.6 color levels, \( P=NS \)), the difference between treatments being -12.8 color levels (95% CI -23.6 to -2.0, \( P=0.02 \)). Secondary end points (mean color scale and collagen markers) also changed in the direction of decreased collagen in patients receiving losartan, but differences between groups were not statistically significant.

Conclusions—In hypertensive patients with LVH, losartan decreases myocardial collagen content, whereas atenolol does not. The difference between the 2 treatments is statistically significant. (Circulation. 2004;110:552-557.)

Key Words: hypertension myocardium collagen angiotensin

In hypertensive left ventricular hypertrophy (LVH), myocardial texture is altered, at least in part, by increased fibrosis. Both postmortem and endomyocardial biopsy studies have shown that along with a variable increase in left ventricular mass (LVM), myocardial collagen volume fraction (CVF) is increased in hypertensive patients compared with normotensive controls.

Endomyocardial biopsies for antemortem measurements of myocardial collagen content have been limited to small numbers of patients, for obvious reasons. Recently, noninvasive ultrasound and biochemical markers of myocardial collagen content have been validated against CVF as measured in endomyocardial biopsies. In particular, backscatter analysis of returning echoes and quantitative echoreflectivity analysis have been shown to correlate with CVF in myocardial tissue from animals and humans. Serum markers of collagen synthesis or degradation have also been investigated and found to be altered in hypertension. The carboxy-terminal propeptide of procollagen type I, PIP, correlated with CVF on endomyocardial biopsies and with its regression during antihypertensive treatment. The LVH Regression with the Angiotensin Antagonist Losartan (REGAAL) study compared the effects of losartan and atenolol on LVM index (LVMI) in 225 hypertensive patients. 15
patients with LVH, with a noninferiority design. Despite similar antihypertensive effects, the reduction in LVMi was statistically significant in losartan-treated patients (−6.56 g/m², \( P<0.001 \)) but not so in those treated with atenolol (−3.71 g/m², \( P=NS \)). Losartan was found to be significantly noninferior (\( P<0.01 \)) to atenolol in reducing LVMi, because the 95% CI for the treatment difference (−2.5 g/m² in favor of losartan, 95% CI −7.36 to 2.37 g/m²) was entirely within preset equivalence limits (±5 g/m²). Furthermore, plasma levels of the cardiac natriuretic peptides were reduced by losartan and increased by atenolol (at least \( P=0.01 \) for all between-treatment differences).³¹

When REGAAL was designed, further analyses were planned regarding changes in myocardial collagen content as assessed by echoreflectivity analysis and serum markers of collagen synthesis (PIP and the amino-terminal propeptide of procollagen type III, PIIIP) and degradation (decarboxy-terminal telopeptide of collagen type I, CTTP). Here, we report results of these measurements.

Methods
Patient characteristics, procedures, and principal results of the REGAAL study have been reported elsewhere.³² Briefly, the study included 225 men and women (67% men) aged 21 to 80 years (mean 57 years) with seated diastolic blood pressures of 95 to 115 mm Hg (mean 98 mm Hg) or seated systolic pressures of 160 to 200 mm Hg (mean 167 mm Hg). The screening LVMi, read at the central laboratory, was >120 g/m² for men and >105 g/m² women (mean 149 g/m²). Seventy-six percent had received previous antihypertensive treatment, which was withdrawn 4 weeks before randomization. Patients were randomized in a double-blind manner to receive losartan or atenolol (50 mg once daily) for 36 weeks. Those not achieving goal blood pressure (<140/90 mm Hg) were prescribed hydrochlorothiazide 12.5 mg/d after 6 weeks of treatment, a double dose of study drug after 12 weeks, and 25 mg/d hydrochlorothiazide after 24 weeks.

Echocardiography
Two-dimensionally guided M-mode echocardiographic recordings were performed for measurement of LVMi, first before entry into the study (screening echocardiogram), then on completion of the placebo run-in period (baseline), and third, at the end of the study after 36 weeks of treatment. Recordings were made by commercially available ultrasound systems with 3.0- to 5.5-MHz or 2.0- to 2.5-MHz transducers and super-VHS or standard VHS recorders. Quality control was ensured by centralized quantitative reading of all recordings on completion of the study by readers blinded to both the sequence of tape acquisition and to drug treatment.

Echoreflectivity Analysis
Baseline and 36-week echocardiographic scans used for LVMi measurements were analyzed for echoreflectivity in the imaging laboratory of the Centro Interuniversitario di Fisiologia Clinica e Ipertensione, University of Milan, Italy, by a single experienced reader blinded to study treatment and scan sequence. From parasternal long-axis echocardiographic scans, 3 subsequent sinus rhythm cardiac cycles were selected and digitized offline onto a personal computer (Power Macintosh G4, 867 MHz, 640 MB of RAM, 60-GB hard disk; Apple Computer, Inc) with an external video digitizer card, operating at 30 frames/s, 8 bits/pixel, on a standard PAL pixel matrix. The region for analysis was positioned by means of a square selection tool (10×10 mm) in the midapex septum, the region where echoreflectivity analysis had been validated previously against endomyocardial biopsy histology.³³ By the use of anatomic landmarks on the image, the same myocardial area could be analyzed in each patient at baseline and treatment end. To obtain optimal visualization of structures, the original 256 echogray scale (0=white, 256=black) was recoded as a color scale (0=yellow, 128=magenta, 256=black).

Analysis algorithms were developed as a set of macros written in Pascal.³⁴ The macros were executed with NIH Image, an integrated image-processing software,³⁵ distributed on a freeware basis by the National Institutes of Health (Bethesda, Md). All values were normalized for blood echoreflectivity (black=256) assessed along the same axis within the left ventricle. In addition to mean color scale (MCS), a color histogram representing the pixel color-level frequency distribution was derived for estimation of broadband (BB), indicating the spread of echoes about the distribution.

Intraobserver and interobserver variabilities were tested³⁶ on 10 scans that were selected randomly. Mean bias between 2 independent measurements made by the reader responsible for the study was −0.33 (95%CI −1.76 to 1.10) for BB and 0.53 (95% CI −2.71 to 3.77) for MCS. Mean bias between measurements of this reader and another experienced investigator was 0.20 (95% CI −2.13 to 2.17) for BB and 0.68 (95% CI −2.24 to 3.60) for MCS.

Serum Collagen Markers
In 207 patients, venous serum samples were obtained at baseline and at 36 weeks and stored at −40°C for measurements of PIP, PIIIP, and CTTP by specific radioimmunoassays³⁷–³⁹ at the Center of Applied Medical Research, University of Navarra, Pamplona, Spain, by staff blinded to study treatment and sample sequence. The interassay and intra-assay coefficients of variation were 7% and 3% for PIP, 8% and 6% for PIIIP, and 6% and 4% for CTTP, respectively. Assay sensitivities were 1.2 μg/L for PIP, 1.5 μg/L for PIIIP, and 0.5 μg/L for CTTP.

Statistical Analyses
The primary outcome variable was the change over 36 weeks in BB, the variable that was most strongly correlated with CVF in our previous validation study against myocardial biopsy histology.³³ Secondary outcome variables were changes in MCS and serum concentrations of PIP, PIIIP, CTTP, and the ratio PIP/CTTP.

Differences in changes from baseline at week 36 in BB and MCS between the 2 treatment groups were analyzed in the context of covariance analyses (ANCOVA), which included terms for treatment and baseline values as covariates. Treatment effects were assessed by the least-squares mean change from baseline from the above ANCOVA model. Changes in the prevalence of excessive fibrosis as a consequence of the 2 treatments were investigated by the \( \chi^2 \) test. Relations of the changes in echoreflectivity and in LVMi or septum thickness were investigated by multiple regression analysis. For changes in serum collagen markers, the Wilcoxon signed rank test of median percent change from baseline was used for analyses within treatment groups and the Wilcoxon rank sum test for testing differences between treatment groups. Correlation analyses were made with Spearman correlation coefficients and their 95% CIs.

Results
Changes in Ultrasound Parameters of Myocardial Fibrosis
Of the 219 patients included in the intention-to-treat analysis of change in LVMi, 106 (52 losartan, 54 atenolol) had both baseline and on-treatment echocardiographic scans adequate for texture evaluation. No other exclusion criteria were used, and the reader’s decision was taken without knowledge of randomized treatment. Baseline characteristics of the 106 patients (Table 1) were similar to those of the entire REGAAL cohort or of those excluded from the present study.

At baseline, the mean BB was 114.5 color levels in the losartan group and 109.0 in the atenolol group (Table 2). Mean BB decreased significantly to 104.3 color levels over 36 weeks with losartan-based treatment (\( P=0.02 \)), whereas in the atenolol group, it increased to 113.6 (\( P=NS \)). The
least-squares difference in change from baseline between the
groups (including treatment and baseline value as covariates) 
was −12.8 (P=0.02). Figure 1 illustrates changes in the 2 
groups by showing pixel color frequency distributions from a 
typical patient treated with atenolol and another treated with 
losartan. Fifteen normotensive subjects (systolic/diastolic 
blood pressure 121.3/76.3 mm Hg), matched for age (58 
years) and gender (73% male) who served as controls were 
found to have a mean BB of 80.1±6.8 color levels, which 
was significantly different from baseline values in hyperten-
sives of the REGAAL study. Figure 2 shows the prevalence 
of excessive myocardial fibrosis using a cutoff BB value of 
33.8 to 55.9 color levels. Negative mean between-treatment differences indicate greater reduction in BB with losartan.

Figure 1. Examples of echoreflectivity analysis in 2 patients who 
received either atenolol (left) or losartan (right). Top, baseline 
tracings; bottom, after 36 weeks of therapy. The 256 echogray 
scale converted to a color scale. MCS and spread of echoes 
about distribution (BB) are indicated. Note that MCS is unmodi-
fied and BB is increased by atenolol treatment, whereas MCS is 
increased and BB is decreased by losartan treatment.

Serum Collagen Markers
No significant differences between treatments were observed 
for any of the serum collagen markers (Table 4), although the 
percent decrease in collagen synthesis markers (PIP and 
PIIIP) was numerically greater in the losartan group. The 
percent decrease in the collagen degradation marker (CITP) 
was numerically lower in the losartan group than in the 
atenolol group. Hence, the percent change in the PIP/CITP 
ratio was numerically decreased in the losartan group and 
numerically increased in the atenolol group (P=NS).

Correlation Analyses
No significant correlations were found at baseline between 
BB and systolic or diastolic blood pressure, LVMI, interven-
tricular septum thickness, PIP, PIP/CITP, or PIIIP. Further-
more, there were no statistically significant correlations 
between treatment-associated changes in BB and concomitant 
changes in any of these variables.

Discussion
On the basis of changes over 36 weeks in ultrasound BB, the 
predetermined primary outcome variable, the present study 
shows that losartan influenced myocardial collagen content to 
a significantly greater extent than atenolol in hypertensive 
patients with LVH despite a comparable decrease in arterial 
pressure. The prevalence of excessive myocardial fibrosis

TABLE 2. Change From Baseline in BB at Week 36

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Baseline, mean±SD</th>
<th>Week 36, mean±SD</th>
<th>Change From Baseline</th>
<th>Between-Treatment Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LSM (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Losartan</td>
<td>52</td>
<td>114.5±31.1</td>
<td>104.3±27.6</td>
<td>−9.2 (−16.9 to −1.5)</td>
<td>0.020</td>
</tr>
<tr>
<td>Atenolol</td>
<td>54</td>
<td>109.0±32.5</td>
<td>113.6±40.2</td>
<td>3.6 (−3.9 to 11.2)</td>
<td>NS</td>
</tr>
</tbody>
</table>

LSM indicates least squares mean from ANCOVA; NS, not significant.
All values are given in color levels. Negative mean between-treatment differences indicate greater reduction in BB with losartan.
Patients with different degrees of LVH19 or different serum concentrations in the backscatter signal apparently distinguish between hypertensives with different degrees of LVH.18,19 Cyclic variations between normotensives and hypertensives and between hypertensives and normotensives were found to be insensitive in detecting differences by other authors to be insensitive in detecting differences in the backscatter analysis because the only index of backscatter analysis is the pixel color level frequency distribution (BB ≥100, ie, 3 SD above the mean in the normotensive group, which was matched for age and gender) was also found to be reduced significantly by losartan-based treatment but not by atenolol-based treatment.

Previous reports have raised the possibility that antihypertensive agents that interfere with the renin-angiotensin system through inhibition of ACE6,7 or blockade of angiotensin II AT1 receptors13,14 can reduce myocardial fibrosis. These studies, however, were limited in size because they were based on endomyocardial biopsy, and 2 were not randomized.6,13 The present study is the first to randomize a large sample of hypertensive patients with LVH. Although only 106 of the original cohort of 219 patients were available for echoreflectivity assessment of fibrosis, it can be assumed that this subgroup is a representative sample of the entire randomized population, because (1) the quality of the scan was the only criterion for inclusion, (2) the decision was taken by a reader blinded to treatment, (3) excluded scans were equal in number in both treatment groups, and (4) baseline characteristics and week 36 LVMI results were similar to those of the entire randomized cohort.

We chose BB of the pixel color level frequency distribution on echoreflectivity as the primary outcome variable because it has been validated against simultaneous measurements of CVF on endomyocardial biopsies and shown to have a robust statistical association (r=0.72).5 This was preferred to backscatter analysis because the only index of backscatter analysis to correlate with histologically measured CVF was integrated backscatter signal intensity,9,10 which was found by other authors to be insensitive in detecting differences between normotensives and hypertensives and between hypertensives with different degrees of LVH.18,19 Cyclic variations in the backscatter signal apparently distinguish between patients with different degrees of LVH19 or different serum levels of collagen markers,18 but this index reflects more directly the contractile properties of the myocardium20 rather than measurement of collagen content. We previously found BB to be a highly reproducible measurement,3 and good reproducibility was confirmed in the present study. BB was measured in the same area of the ventricular septum where histological validation of the technique was previously demonstrated. That the extrapolation of these values to the remaining left ventricular myocardium is valid is supported by a postmortem study of hypertensive human hearts that found that fibrosis present in the septum was representative of fibrosis in the left ventricular free wall.21

MCS was chosen as a secondary outcome variable because in our previous validation study,5 it was shown to be a relatively insensitive index of histologically measured CVF. Perhaps because of this low sensitivity, treatment-related changes in MCS were not significantly different between groups, although the statistically significant losartan-associated increase in the MCS, which suggests less fibrosis, is consistent with the outcome from the primary end point.

The conclusions from our ultrasound analysis receive some support from changes in serum collagen markers. The treatment-associated changes in serum collagen markers, although not significant statistically, were consistent with the hypothesis that losartan and atenolol may exert opposite effects on cardiac fibroblastic collagen turnover. Specifically, the equilibrium between collagen type I synthesis and degradation, as indicated by a 7.4% decrease in the ratio PIP/CITP, moved toward decreased synthesis and increased tissue clearance of collagen type I in patients receiving losartan, whereas in those randomized to atenolol, an increase (1.2%) in the PIP/CITP ratio suggests that degradation of collagen type I may be equally or more strongly inhibited than was synthesis. The significant increase (11.5%) in serum PIIIP levels in atenolol-treated patients and its decrease (−5.0%) with losartan treatment is consistent with collagen type III deposition with atenolol and degradation with losartan.

PIP in serum is not exclusively heart specific, and previous work has shown that the myocardial fibrosis–predictive value of PIP is better at serum concentrations >127 μg/L.10 The patients included in REGAAL were selected on the basis of LVM measurements, not on the degree of myocardial fibrosis. Judging from BB and PIP calibrations against histology,5,8 baseline myocardial fibrosis was only moderate in patients in the present study, although it was significantly greater than in normotensive controls. That the extent of baseline fibrosis influences the response was also evident in our own data, because baseline BB (as well as MCS) values significantly influenced covariance analysis. The calculated change in MCS was significantly associated with baseline BB (as well as MCS) values significantly influenced covariance analysis. The calculated change in MCS was significantly associated with baseline BB (as well as MCS) values significantly influenced covariance analysis. The calculated change in MCS was significantly associated with baseline BB (as well as MCS) values significantly influenced covariance analysis. The calculated change in MCS was significantly associated with baseline BB (as well as MCS) values significantly influenced covariance analysis.
change in CVF was relatively small (a decrease from 3.2% to 2.7% in CVF). Nevertheless, the treatment period of 36 weeks was only slightly longer than the cardiac collagen half-life of 6 months. Furthermore, the results of multiple regression analyses showed that changes in echoreactivity, and hence in fibrosis, were independent of those in LVM. Our previous observations from REGAAL showed that although the E-wave amplitude and the E/A ratios were not more improved by losartan than by atenolol, sensitive indices of myocardial stretch such as plasma levels of cardiac natriuretic peptides decreased significantly with losartan and increased with atenolol.15

In vitro and in vivo experimental studies are supportive of a specific role for angiotensin II in facilitating cardiac fibrosis23 and also show that drugs that block the renin-angiotensin system may induce regression of fibrosis.24-27 The present data are consistent with these experimental observations while also confirming and extending previous preliminary data from studies of ACE inhibition6-7 and angiotensin receptor antagonists13,14 in patients with essential hypertension. The reduction in fibrosis with Losartan in both myocardium, as shown by the present study, and arterioles28 may have contributed to the protective actions that losartan exerted in the Losartan Intervention For Endpoint reduction in hypertension (LIFE) study.29

In conclusion, our results strongly suggest that Losartan-based treatment has significantly greater effects on myocardial fibrosis in hypertensive patients with LVH than the β-blocker atenolol.

Disclosure

The analyses presented in this report were supported by scientific grants of Merck & Co Inc and Merck, Sharp and Dohme (Italy) but were performed independently in the investigators’ laboratories. Drs Smith and Gilles are employees of Merck & Co.

References

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