Effects of AT$_1$-receptor blockade on progression of left ventricular dysfunction in dogs with heart failure

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Tanimura, Mitsuhiro, Victor G. Sharov, Hisashi Shimoyama, Takayuki Mishima, T. Barry Levine, Sidney Goldstein, and Hani N. Sabbah. Effects of AT$_1$-receptor blockade on progression of left ventricular dysfunction in dogs with heart failure. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1385–H1392, 1999.—The objective of the present study was to determine the effects of early long-term monotherapy with the angiotensin II AT$_1$-receptor antagonist valsartan on the progression of left ventricular (LV) dysfunction and remodeling in dogs with moderate heart failure (HF). Studies were performed in 30 dogs with moderate HF produced by multiple sequential intracoronary microembolizations. Embolizations were discontinued when LV ejection fraction was 30–40%. Two weeks after the last embolization, dogs were randomized to 3 mo of oral therapy with low-dose valsartan (400 mg twice daily, n = 10), to high-dose valsartan (800 mg twice daily, n = 10), or to no treatment at all (control, n = 10). Treatment with valsartan significantly reduced mean aortic pressure and LV end-diastolic pressure compared with control. In untreated dogs, LV ejection fraction decreased (37±1 vs. 29±1%, P = 0.001) and end-systolic volume (ESV) and end-diastolic volume (EDV) increased (81±5 vs. 92±5 ml, P < 0.001; 51±3 vs. 65±3 ml, P = 0.001, respectively) after 3 mo of follow-up compared with those levels before follow-up. In dogs treated for 3 mo with low-dose valsartan, ejection fraction was preserved (37±1 vs. 38±2%, pretreatment vs. posttreatment) as was ESV but not EDV. In dogs treated for 3 mo with high-dose valsartan, ejection fraction decreased (35±1 vs. 31±2%, P = 0.02) and ESV and EDV increased in a manner comparable to those levels in controls.Valsartan had no significant effects on cardiomyocyte hypertrophy or on the extent of interstitial fibrosis. We conclude that, for dogs with moderate HF, early long-term therapy with the AT$_1$-receptor blocker valsartan decreases preload and afterload but has only limited benefits in attenuating the progression of LV dysfunction and chamber remodeling.

Congestive heart failure; angiotensin II-receptor antagonists; ventricular function; ventricular remodeling; animal models

Heart failure (HF) is a progressive disorder whereby left ventricular (LV) dysfunction, once established, can worsen over time despite the absence of clinically apparent intercurrent adverse events. Although the mechanisms responsible for this progressive hemodynamic deterioration are not fully understood, studies both in patients with HF and in animals with experimentally induced HF have shown that this process can be attenuated by long-term treatment with angiotensin-converting enzyme (ACE) inhibitors (18, 32). The cardioprotective effects of this class of drugs have been attributed to the blockade of angiotensin II formation and/or to the prevention of degradation of bradykinins (4, 11–13, 20). Several studies (3, 8, 17) have suggested that alternative enzymatic pathways, independent of ACE, exist for the production of angiotensin II within the myocardium, specifically chymase. If this is indeed the case, and if prevention of formation of angiotensin II at the receptor site may potentially be even more effective in preventing or attenuating the progressive deterioration of LV function characteristic of the HF state.

Angiotensin receptors comprise two major subtypes, AT$_1$ and AT$_2$ (42). Although both subtypes have been cloned and sequenced, it is generally believed that only the AT$_1$ receptor has an important physiological or pathophysiological role (40). ATI and AT$_2$ receptors are found both in normal and in failing cardiac tissue (40). The receptors are found on myocytes, endothelial cells, fibroblasts, coronary arterial smooth muscle cells, and peripheral sympathetic nerves (40). Orally active, nonpeptide angiotensin II, AT$_1$-receptor antagonists such as losartan, valsartan, and irbesartan have been developed and shown to be effective in the treatment of hypertension (2, 6, 7, 25, 41). In patients with symptomatic HF [New York Heart Association (NYHA) class II–IV], administration of the AT$_1$-receptor antagonist losartan showed beneficial short-term (24 h) hemodynamic effects with additional beneficial effects also seen after 12 wk of therapy (7). Eight weeks of oral therapy with losartan in patients with moderate or severe HF (NYHA class III–IV) showed efficacy in exercise capacity and clinical status that was comparable to that seen with enalapril (29). In the ELITE (Evaluation of Losartan in the Elderly Study) trial, 48 wk of oral therapy with losartan in elderly (>65 yr) patients with HF was associated with lower mortality than mortality rates found with captopril therapy (9). Although not without important limitations, the above clinical studies support the notion that AT$_1$-receptor antagonists may be effective in the treatment of HF. The present study was designed to determine the effects, if any, of early long-term monotherapy with a selective AT$_1$-receptor antagonist, valsartan, on the progression of LV dysfunction and chamber remodeling in dogs with moderate HF defined as a reduced LV ejection fraction between 30 and 40%. 

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H1385
METHODS

Animal model. Thirty healthy mongrel dogs weighing between 19 and 32 kg were used in the study. Chronic LV dysfunction was produced by multiple sequential intracoronary embolizations with polystyrene latex microspheres (77–102 μm diameter), as previously described (33). Coronary microembolizations were performed during sequential cardiac catheterizations under general anesthesia and sterile conditions. Anesthesia consisted of a combination of intravenous injections of oxymorphone (0.22 mg/kg), diazepam (0.17 mg/kg), and pentobarbital sodium (150–250 mg/kg to achieve a surgical plane of anesthesia). This anesthesia regimen has been shown to be effective in preventing the tachycardia, systemic hypertension, and myocardial depression associated with the use of pentobarbital alone (32). In all dogs, coronary microembolizations were discontinued when LV ejection fraction, determined angiographically, was between 30 and 40%. To achieve this target ejection fraction, dogs underwent an average of 5.9 microembolization procedures performed over an average period of 6.6 wk. A minimum of 1 wk was allowed between embolizations. In all instances, selective microembolization of the left coronary artery (subselective injections of microspheres into the left anterior descending coronary artery and circumflex coronary artery) was performed. The right coronary artery was not embolized because it only perfuses <70% of the right ventricular free wall and none of the LV myocardium (interventricular septum and free wall). The study was approved by the institution’s Care of Experimental Animals Committee and conformed to the “Position of the American Heart Association on Research Animals Use” and the Guiding Principles of the American Physiological Society.

Determination of doses of valsartan. The high and low doses of valsartan used in the study were selected based on studies performed on three normal conscious dogs instrumented for intra-arterial blood pressure measurement. In each dog, systolic pressure was measured at baseline and after a 3-min intravenous administration of exogenous angiotensin II (50 ng·kg⁻¹·min⁻¹) sufficient to increase blood pressure by 30 mmHg or more. After this initial pressor test, each dog received 30 mg/kg of oral valsartan. The pressor response with exogenous angiotensin II was then repeated at 1, 4, 8, 12, and 24 h after administration of valsartan. The results showed that, for an average single dose of valsartan (680 ± 81 mg), the pressor response decreased by 80% and the reduction was maintained for up to 8 h before rising gradually (Fig. 1). This degree of suppression of the pressor response was considered a surrogate to acceptable blockade of the AT₁ receptor. On the basis of these data, two fixed doses of valsartan were chosen for the study, namely, a low dose of 400 mg twice daily and a high dose of 800 mg twice daily.

Study protocol. Two weeks after the last coronary microembolization, all dogs underwent a prerandomization left and right heart catheterization. The 2-wk period was allowed to ensure that all infarctions produced by the last microembolization session were completely healed. One day after the cardiac catheterization, dogs were randomized to 3 mo of oral monotherapy with low-dose valsartan (400 mg twice daily, n = 10), high-dose valsartan (800 mg twice daily, n = 10), or to no treatment at all (control group, n = 10). No other drugs were used in any of the study groups during the 3 mo of follow-up. After a final hemodynamic and angiographic study was performed at the end of 3 mo of therapy, dogs were killed and the heart of each dog was removed for histological examination.

Study end points. The primary study end point was progression of LV systolic dysfunction based on ejection fraction determined angiographically. A secondary end point was the extent of LV chamber remodeling. The latter was based on 1) progression of ventricular chamber enlargement assessed on the basis of end-systolic volume (ESV) and end-diastolic volume (EDV), 2) extent of cardiac myocyte hypertrophy assessed histologically on the basis of myocyte cross-sectional area, and 3) the extent of interstitial fibrosis assessed histologically as the volume fraction of interstitial collagen.

Hemodynamic, angiographic, and neurohumoral measurements. In all study dogs, hemodynamic and angiographic measurements were obtained during left and right heart catheterizations performed at baseline before any microembolizations, repeated before randomization, and at the end of 3 mo of therapy. Aortic and LV pressures were measured with catheter-tipped micromanometers (Millar Instruments). Peak pressure development over time (+dP/dt) and LV end-diastolic pressure were measured from the phasic LV pressure waveform. Mean pulmonary artery wedge pressure and mean right atrial pressure were measured using a Swan-Ganz catheter in conjunction with a P23 XL pressure transducer (Spectramed). Cardiac output was measured in duplicate using the thermodilution method. Cardiac index was calculated as the ratio of cardiac output to body surface area. Systemic vascular resistance was calculated as the difference between mean aortic pressure and mean right atrial pressure times 80 divided by cardiac output (33). Single-plane left ventriculograms were obtained during each cardiac catheterization after the hemodynamic measurements were completed with the dog placed on its right side. Ventriculograms were recorded on 35-mm cine film at 30 frames/s during the injection of 20 ml of contrast material (Reno-M-60, Squibb). Correction for image magnification was made using a radiopaque calibrated scale placed at the level of the left ventricle. LV ESV and EDV were calculated from angiographic silhouettes using the area-length method (10). Ejection fraction was calculated as [(EDV – ESV)/EDV] × 100. Extrasystolic and postextrasystolic beats were excluded from all angiographic analyses. Venous blood samples were obtained from conscious dogs 1 day before cardiac catheterization for evaluation of plasma concentration of norepinephrine and angiotensin II. To minimize possible variations, blood samples were always obtained between 8:00 and 10:00 AM. Plasma norepinephrine concentration was measured using aluminum oxide absorption by high-performance liquid chromatography. Plasma immunoreactive angiotensin II was measured by radioimmu-
nos assay after extraction by reversible absorption by phenyl-
silyl-silica.

Histological and morphometric assessments. At the end of
3 mo of therapy and immediately after the final hemodynamic
and angiographic evaluation, the dog chest was opened and
the heart was rapidly removed and placed in ice-cold cardio-
plegia solution. We obtained an transverse slice from each heart
(∼3 mm thick) from the LV at the midventricular level. Transmu-
sal tissue blocks were obtained from the free wall segment of the slice, mounted on cork using Tissue-Tek
embedding medium (Miles), rapidly frozen in isopentane
precooled in liquid nitrogen, and stored at −70°C until use.
Cryostat sections (∼8 µm thick) were prepared from each
block and stained with fluorescein-labeled peanut agglutinin
(Vector Laboratories) after pretreatment with 3.3 U/ml neu-
roaminidase type V (Sigma Chemical) to delineate the myo-
cyte border and the interstitial space including capillaries as
previously described (21). Sections were double stained with
rhodamine-labeled Griffonia simplicifolia lectin I (GSL I) to
identify capillaries. Ten radially oriented microscopic fields
(magnification ×100, objective ×40, and ocular 2.5) were
selected at random from each section and photographed using
35-mm color film. Fields containing scar tissue (infarcts) were
excluded. Images were projected with a photo magnifier, and
a cross-sectional area of each myocyte was measured using
computer-based planimetry. An average myocyte cross-
sectional area was calculated for each dog using data ob-
tained from all fields. The total surface area occupied by
interstitial space and the total surface area occupied by
capillaries were measured from each randomly selected field
using computer-based video densitometry (J AVA, J andel
Scientific). The volume fraction of interstitial collagen (intersti-
tial fibrosis) was calculated as the percent total surface area
occupied by interstitial space minus the percent total area
occupied by capillaries (21). An average volume fraction of
interstitial fibrosis was calculated for each dog using data
obtained from all fields. For comparison purposes, measure-
ments of myocyte cross-sectional area and volume fraction
of interstitial fibrosis were made by employing identical tech-
niques in LV tissue sections obtained from seven normal dogs.

Data analysis. Intragroup comparisons of hemodynamic,
angiographic, and neurohumoral variables within each of the
three study groups were made by employing identical tech-
niques in LV tissue sections obtained from seven normal dogs.

Table 1. Baseline hemodynamic and angiographic measurements

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>400 mg bid</th>
<th>800 mg bid</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>80 ± 5</td>
<td>74 ± 2</td>
<td>72 ± 3</td>
</tr>
<tr>
<td>Mean AoP, mmHg</td>
<td>112 ± 5</td>
<td>108 ± 4</td>
<td>108 ± 3</td>
</tr>
<tr>
<td>LV EDP, mmHg</td>
<td>8 ± 1</td>
<td>9 ± 1</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Peak LV +dP/dt,</td>
<td>2,233 ± 168</td>
<td>2,266 ± 142</td>
<td>2,354 ± 119</td>
</tr>
<tr>
<td>mmHg/s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean PAWP, mmHg</td>
<td>8 ± 1</td>
<td>7 ± 1</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>CI, L/min</td>
<td>3.3 ± 0.3</td>
<td>3.1 ± 0.2</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>SVR, dyn·s·cm⁻⁵</td>
<td>3,471 ± 346</td>
<td>3,305 ± 195</td>
<td>3,323 ± 276</td>
</tr>
<tr>
<td>LV EDV, ml</td>
<td>68 ± 5</td>
<td>60 ± 5</td>
<td>74 ± 5</td>
</tr>
<tr>
<td>LV ESV, ml</td>
<td>30 ± 1</td>
<td>28 ± 2</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>LV EF, %</td>
<td>57 ± 2</td>
<td>53 ± 1</td>
<td>55 ± 1</td>
</tr>
<tr>
<td>PNE, pg/ml</td>
<td>294 ± 28</td>
<td>300 ± 25</td>
<td>332 ± 37</td>
</tr>
<tr>
<td>Plasma ANG II, pg/ml</td>
<td>50 ± 19</td>
<td>44 ± 9</td>
<td>45 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE. bid, Administered twice daily; HR, heart
rate; AoP, aortic pressure; LV, left ventricular; ED, end-diastolic
pressure; dP/dt, rate of change of LV pressure during isovolumic
contraction; PAWP, pulmonary artery wedge pressure; CI, cardiac
index; SVR, systemic vascular resistance; EDV, end-diastolic volume;
ESV, end-systolic volume; EF, ejection fraction; PNE, plasma norepi-
nephrine concentration.
change did not reach statistical difference. LV ejection fraction remained essentially unchanged after completion of 3 mo of therapy (Table 2, Fig. 2). There was no significant progressive increase of ESV in dogs treated with low-dose valsartan, but EDV increased. Three months of treatment with low-dose valsartan maintained peak +dP/dt, cardiac index, and plasma norepinephrine concentration at levels similar to those present before initiation of treatment, whereas it significantly decreased LV end-diastolic pressure and tended to decrease pulmonary artery wedge pressure. In this low-dose valsartan group, plasma angiotensin II levels did not increase significantly after 3 mo of treatment compared with pretreatment levels (Table 2).

In dogs treated with high-dose valsartan (800 mg twice daily), mean aortic pressure decreased and was accompanied by a significant decrease of systemic vascular resistance. In this treatment group, contrary to the low-dose valsartan group, ejection fraction decreased significantly (Table 2, Fig. 2). The decrease of LV ejection fraction was accompanied by an increase of EDV and ESV. Three months of treatment with high-dose valsartan also tended to decrease peak +dP/dt, but cardiac index and plasma norepinephrine concentration remained unchanged. As with low-dose valsartan, treatment with high-dose valsartan also significantly decreased end-diastolic pressure and tended to decrease pulmonary artery wedge pressure. Oral treatment with high-dose valsartan was associated with a significant increase of plasma angiotensin II concentration compared with pretreatment levels (Table 2).

Comparisons of treatment effect. In the posttreatment analysis, the three treatment groups were compared with one another. The probability values based on ANCOVA are shown in Table 3. LV ejection fraction was significantly higher in dogs treated with low-dose valsartan but not with high-dose valsartan compared with the control group. Similarly, LV ESV was significantly lower in dogs treated with low-dose valsartan but not high-dose valsartan compared with control. There was no difference in EDV with either low- or high-dose valsartan compared with the control group. Low-dose valsartan resulted in marginal improvement of +dP/dt and tended to also decrease plasma norepinephrine concentration, whereas neither parameter was significantly affected by high-dose valsartan compared with the control group.

### Table 2. Hemodynamic, angiographic, and neurohumoral measurements obtained before initiation of therapy and 3 mo after initiation of therapy

<table>
<thead>
<tr>
<th></th>
<th>Control (No Therapy)</th>
<th>Pre</th>
<th>Post</th>
<th>P value</th>
<th>Valsartan 400 mg bid</th>
<th>Pre</th>
<th>Post</th>
<th>P value</th>
<th>Valsartan 800 mg bid</th>
<th>Pre</th>
<th>Post</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>89 ± 8</td>
<td>79 ± 4</td>
<td>0.17</td>
<td></td>
<td>84 ± 5</td>
<td>76 ± 7</td>
<td>0.33</td>
<td></td>
<td>75 ± 7</td>
<td>80 ± 4</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>Mean AoP, mmHg</td>
<td>107 ± 3</td>
<td>112 ± 6</td>
<td>0.37</td>
<td></td>
<td>108 ± 4</td>
<td>96 ± 4</td>
<td>0.07</td>
<td></td>
<td>99 ± 4</td>
<td>91 ± 4</td>
<td>0.051</td>
<td></td>
</tr>
<tr>
<td>LV EDP, mmHg</td>
<td>20 ± 2</td>
<td>19 ± 2</td>
<td>0.29</td>
<td></td>
<td>22 ± 2</td>
<td>12 ± 2</td>
<td>0.001</td>
<td></td>
<td>18 ± 2</td>
<td>11 ± 2</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Peak LV +dP/dt, mmHg/s</td>
<td>1,894 ± 94</td>
<td>1,635 ± 74</td>
<td>0.003</td>
<td></td>
<td>2,028 ± 63</td>
<td>1,935 ± 94</td>
<td>0.37</td>
<td></td>
<td>1,966 ± 54</td>
<td>1,822 ± 63</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Mean PAWP, mmHg</td>
<td>10 ± 1</td>
<td>11 ± 1</td>
<td>0.85</td>
<td></td>
<td>11 ± 1</td>
<td>8 ± 1</td>
<td>0.07</td>
<td></td>
<td>11 ± 2</td>
<td>8 ± 2</td>
<td>0.15</td>
<td></td>
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<tr>
<td>CI, l/min</td>
<td>3.2 ± 0.4</td>
<td>2.7 ± 0.2</td>
<td>0.11</td>
<td></td>
<td>2.9 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>0.41</td>
<td></td>
<td>2.2 ± 0.2</td>
<td>2.7 ± 0.1</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>SVR, dyn·s·cm⁻⁵</td>
<td>3,280 ± 373</td>
<td>3,675 ± 265</td>
<td>0.11</td>
<td></td>
<td>3,396 ± 259</td>
<td>3,496 ± 208</td>
<td>0.75</td>
<td></td>
<td>3,967 ± 337</td>
<td>3,069 ± 192</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>LV EDV, ml</td>
<td>81 ± 5</td>
<td>92 ± 5</td>
<td>0.001</td>
<td></td>
<td>73 ± 5</td>
<td>83 ± 6</td>
<td>0.03</td>
<td></td>
<td>86 ± 5</td>
<td>95 ± 6</td>
<td>0.08</td>
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<tr>
<td>LV ESV, ml</td>
<td>51 ± 3</td>
<td>65 ± 3</td>
<td>0.001</td>
<td></td>
<td>46 ± 3</td>
<td>51 ± 4</td>
<td>0.10</td>
<td></td>
<td>55 ± 3</td>
<td>65 ± 3</td>
<td>0.007</td>
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<tr>
<td>LV EF, %</td>
<td>37 ± 1</td>
<td>29 ± 1</td>
<td>0.001</td>
<td></td>
<td>37 ± 1</td>
<td>38 ± 2</td>
<td>0.51</td>
<td></td>
<td>35 ± 1</td>
<td>31 ± 2</td>
<td>0.02</td>
<td></td>
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<tr>
<td>PNE, pg/ml</td>
<td>329 ± 21</td>
<td>446 ± 80</td>
<td>0.004</td>
<td></td>
<td>391 ± 35</td>
<td>355 ± 31</td>
<td>0.52</td>
<td></td>
<td>420 ± 50</td>
<td>389 ± 41</td>
<td>0.65</td>
<td></td>
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<tr>
<td>Plasma ANG II pg/ml</td>
<td>22 ± 4</td>
<td>27 ± 6</td>
<td>0.36</td>
<td></td>
<td>44 ± 9</td>
<td>51 ± 6</td>
<td>0.48</td>
<td></td>
<td>34 ± 9</td>
<td>84 ± 20</td>
<td>0.033</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE.

### Table 3. Treatment-effect probability values for comparison between the control group and each of the two active treatment groups

<table>
<thead>
<tr>
<th></th>
<th>Valsartan 400 mg bid vs. Control</th>
<th>Valsartan 800 mg bid vs. Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>0.76</td>
<td>0.76</td>
</tr>
<tr>
<td>Mean AoP, mmHg</td>
<td>0.014</td>
<td>0.008</td>
</tr>
<tr>
<td>LV EDP, mmHg</td>
<td>0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>Peak LV +dP/dt, mmHg/s</td>
<td>0.031</td>
<td>0.14</td>
</tr>
<tr>
<td>Mean PAWP, mmHg</td>
<td>0.10</td>
<td>0.073</td>
</tr>
<tr>
<td>CI, l/min</td>
<td>0.54</td>
<td>0.97</td>
</tr>
<tr>
<td>SVR, dyn·s·cm⁻⁵</td>
<td>0.43</td>
<td>0.006</td>
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<tr>
<td>LV EDV, ml</td>
<td>0.73</td>
<td>0.85</td>
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<tr>
<td>LV ESV, ml</td>
<td>0.014</td>
<td>0.42</td>
</tr>
<tr>
<td>LV EF, %</td>
<td>0.001</td>
<td>0.24</td>
</tr>
<tr>
<td>PNE, pg/ml</td>
<td>0.082</td>
<td>0.31</td>
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</tbody>
</table>

Fig. 2. Bar graph depicting left ventricular (LV) ejection fraction before initiation of therapy and at end of 3 mo of therapy. Values are means ± SE for untreated dogs (con, control), dogs treated with low-dose valsartan (400 mg) administered twice daily, and dogs treated with high-dose valsartan (800 mg) administered twice daily. Probabilities refer to within-group comparisons between pretherapy and posttherapy.
pared with the control group. Valsartan regardless of the dose used produced significant reduction of mean aortic pressure and LV end-diastolic pressure compared with the control group (Table 3). When low-dose valsartan was compared with high-dose valsartan, no significant differences were noted between the two groups with respect to hemodynamic, angiographic, and neurohumoral measures except for LV ejection fraction (Table 3). LV ejection fraction was significantly higher with low-dose valsartan compared with high-dose valsartan. LV ESV and systemic vascular resistance tended to be lower with low-dose valsartan compared with high-dose valsartan, but the difference did not reach statistical significance.

Histomorphometric findings are shown in Table 4. Cardiac myocyte cross-sectional area and volume fraction of interstitial fibrosis were both significantly higher in untreated HF dogs compared with normal dogs. Both parameters were also significantly higher in valsartan-treated dogs regardless of dose compared with normal dogs. There was no statistically significant difference in myocyte cross-sectional area and volume fraction of interstitial fibrosis between dogs treated with valsartan and untreated control dogs.

**DISCUSSION**

Consistent with earlier studies from our laboratory (31–33), results of the present study also indicate that, in the absence of any therapeutic intervention, progressive deterioration of LV systolic function and chamber remodeling occur in dogs with moderate HF secondary to loss of viable myocardium. In the present study, long-term therapy with the AT₁-receptor antagonist valsartan at a dose of 400 mg administered twice daily was somewhat effective in attenuating the progression of LV systolic dysfunction but had only a marginal effect, if any, on ventricular remodeling parameters. In contrast, valsartan administered at a higher dose, 800 mg twice daily, did not preserve LV systolic function and did not affect progressive ventricular chamber dilation. Both high- and low-dose valsartan, however, reduced preload and afterload as evidenced by a significant decline of LV end-diastolic pressure and mean aortic pressure. Data from the present study also indicate that cardiac myocyte hypertrophy and interstitial fibrosis, features of LV remodeling at the cellular level, were not affected by long-term therapy with either high- or low-dose valsartan.

Several studies have examined the effects of AT₁-receptor antagonists in animal models of HF. In dogs with LV remodeling evoked by localized myocardial injury resulting from transmyocardial direct current shock, McDonald et al. (22) failed to show any benefit on ventricular remodeling after long-term treatment with the AT₁-receptor antagonist DUP-532. In their study, treatment with DUP-532 did not elicit improvements of LV ejection fraction, EDV, or LV mass compared with untreated control dogs (22). Spinale et al. (37) examined the effects of valsartan in pigs with HF produced by rapid atrial pacing. AT₁-receptor blockade with valsartan at a dose of 60 mg/day, administered simultaneously with the initiation of atrial pacing for 3 wk, did not attenuate the decline in LV percent fractional shortening, the increase in LV end-diastolic dimension, or the increase of plasma norepinephrine concentration seen in dogs subjected to rapid pacing only (37). In these studies, however, monotherapy with valsartan had a significant beneficial effect on systemic and pulmonary resistance (37). In an extension of the above studies in pigs, Spinale et al. (38) showed that the shortening velocity of isolated cardiac myocytes was decreased in pacing-induced HF dogs compared with unpaced dogs and that monotherapy with the AT₁-receptor antagonist valsartan did not improve myocyte shortening velocity.

In contrast to observations in large animals, studies of the effects of AT₁-receptor antagonists in rats showed more promise. Studies in spontaneously hypertensive rats receiving monotherapy with losartan showed a reduction in systemic blood pressure accompanied by an attenuation of overall LV weight-to-body weight ratio (26). In rats with HF produced by coronary artery ligation, long-term monotherapy with the AT₁-receptor antagonist L-158809 resulted in an attenuation of the increase of LV EDV and ESV as well as a significant improvement of LV ejection fraction (21). L-158809 in rats also significantly attenuated cardiac myocyte hypertrophy and volume fraction of interstitial fibrosis (21). In rats with experimental pressure-overload LV hypertrophy, the AT₁-receptor antagonist TCV-116 reduced LV end-diastolic wall thickness, cardiac myocyte length, and width compared with vehicle-treated rats (28). In spontaneously hypertensive rats, treatment with TCV-116 was also shown to reduce LV weight, wall thickness, cardiac myocyte diameter, as well as interstitial fibrosis compared with treatment with vehicle and with the vasodilator hydralazine (16). Studies in rats with myocardial infarction produced by coronary artery ligation showed that long-term (1 yr) treatment with the AT₁-receptor antagonist losartan was similar to that of captopril with respect to its effects on mortality (27).

In the dog model of coronary microembolization-induced chronic HF used in this study, there was a benefit of valsartan on the progression of LV systolic dysfunction when the drug was administered in the lower amount of the two doses selected. The prevention of the progressive decline of ejection fraction was similar to that seen in this dog model after long-term (3 mo) oral monotherapy with enalapril (32). Unlike valsartan, however, enalapril also attenuated the increase in EDV as well as the increase in cardiac fibrosis; MCSA, myocyte cross-sectional area. *P < 0.017 vs. normal.

<table>
<thead>
<tr>
<th>Table 4. Histomorphometric findings</th>
<th>Normal</th>
<th>Control (No Therapy)</th>
<th>400 mg bid</th>
<th>800 mg bid</th>
</tr>
</thead>
<tbody>
<tr>
<td>VFIF, %</td>
<td>3.5 ± 0.3</td>
<td>11.1 ± 0.7*</td>
<td>9.4 ± 0.6*</td>
<td>11.0 ± 0.6*</td>
</tr>
<tr>
<td>MCSA, µm²</td>
<td>616 ± 18</td>
<td>814 ± 83*</td>
<td>685 ± 21*</td>
<td>752 ± 52*</td>
</tr>
</tbody>
</table>

Values are means ± SE. VFIF, volume fraction of interstitial fibrosis; MCSA, myocyte cross-sectional area. *P < 0.017 vs. normal.
myocyte cross-sectional area and volume fraction of interstitial fibrosis (14, 32). The failure of valsartan and other AT1-receptor antagonists to inhibit or attenuate remodeling at the cellular level in large animal models of HF suggests that, unlike the situation in rats, angiotensin II acting through the AT1 receptor may not be critical for the remodeling process (24). In studies using the direct current shock canine model of LV, McDonald et al. (23) showed that bradykinin antagonism inhibits the antigrrowth effect of ACE inhibition, suggesting that prevention of bradykinin degradation is an important factor responsible for the antiremodeling effects of ACE inhibitors. Another possible explanation for the essentially negative findings of this study with respect to LV remodeling is that heightened activity of the renin-angiotensin system is prerequisite for AT1 antagonists to elicit a hemodynamic and structural benefit. In the present study, the extent of LV dysfunction was only moderate (LV ejection fraction 30–40%) when therapy was initiated, plasma angiotensin II levels were not elevated, and tissue levels of components of the renin-angiotensin system were not assessed. Nevertheless, treatment with valsartan did elicit significant preload and afterload reduction. In the same animal model and for the same degree of LV dysfunction, long-term therapy with ACE inhibition was effective in attenuating progressive LV dysfunction and chamber remodeling (32). Mechanistic arguments aside, the potential usefulness of AT1-receptor antagonists as adjuncts to the long-term treatment of chronic HF remains a viable option (2, 6, 7, 25, 41). As we alluded to earlier, in the present dog study, valsartan, regardless of dose, lowered mean aortic pressure as well as LV end-diastolic pressure. In studies of patients with symptomatic HF, administration of losartan also lowered mean arterial pressure, systemic vascular resistance, and mean pulmonary artery wedge pressure (7). In patients with moderate and severe HF, losartan was also shown to improve exercise capacity and clinical status to levels comparable to those seen with enalapril (9).

The diversity of results from the above studies in animal models of hypertrophy and failure and in patients with HF illustrates the differences reported to date with regard to the effectiveness of AT1-receptor antagonists as a viable therapeutic modality for the treatment of chronic HF. The reasons for these differences are not fully understood but may be related, in part, to differences in the distribution and regulation of angiotensin II-receptor subtypes in the heart of different species (5, 24, 30, 34, 40) and to differences in the regulation of angiotensin II receptors in the failing heart (1, 15, 19, 39). Recent studies, for instance, have shown that angiotensin II receptors in the nonfailed human heart are predominantly of the AT1 subtype and that HF is associated with a reduction of the density of the AT1-receptor subtype, whereas the density of the AT2 receptor was unchanged (1). These observations were further supported by studies that showed decreased expression of the AT1 receptor but not the AT2 receptor in the failing human heart (15). Furthermore, the reduced density of AT1 receptor in the failing human heart was greater in myocardial tissue obtained from patients with idiopathic dilated cardiomyopathy compared with myocardial tissue of patients with ischemic cardiomyopathy (1), an observation that also argues in favor of differences in the regulation of angiotensin II receptors that are dependent on the etiology of HF. In contrast, studies by others have suggested that the AT2 receptor is the predominant subtype in the normal human heart and that both AT1 and AT2 receptors are downregulated in HF (30). In addition to the differences described above that may account, in part, for the diversity of findings with respect to the effects of AT1-receptor antagonists in HF, there is also evidence to suggest that available AT1-receptor antagonists do not act in an identical manner even when used in the same species (30), to say nothing of the possible influences of the dose of an antagonist selected for a given study as clearly evident from results of the present investigation.

As alluded to earlier, the present study showed that valsartan at a dose of 800 mg administered twice daily had no effect on the progression of LV dysfunction and remodeling. We do not have a ready answer for this observation. There are some possible explanations, however, that merit consideration. At high doses of valsartan, a substantial increase in the plasma concentration of angiotensin II was observed. Because valsartan, like most other nonpeptide AT1-receptor antagonists, is a competitive blocker, the possibility exists that the increase in plasma angiotensin II, observed with the high dose, may have displaced the block at the receptor site rendering the treatment ineffective. This explanation, although possible, may not be likely because significant afterload and preload reduction was clearly evident after administration of high-dose valsartan. Another possibility is the potential adverse contributory role of the AT2 receptor. Although not known for dogs, AT2 receptors constitute a relatively large proportion of angiotensin II receptors in the failing human heart (1). Blockade of the AT1 receptor that results in increased availability of angiotensin II can further exaggerate the effects mediated by the AT2 receptor (19). Activation of the AT2 receptor may play a role in inducing programmed cell death or apoptosis (39, 43), thus creating a substrate for ongoing loss of viable myocardium. The dog model of HF used in the present study was previously shown to manifest cardiomyocyte apoptosis (35). Studies in rats have also suggested that AT2-receptor antagonists may have favorable effects on postinfarction ventricular remodeling, a finding that points toward additional adverse actions that can result from of stimulation of the AT2 receptor (36). To date, a complete understanding of the physiological function of the AT2 receptor is clearly lacking, and future studies of the function and significance of this angiotensin II-receptor subtype in HF are warranted.

In summary, the results of this study suggest that long-term therapy with the AT1-receptor antagonist valsartan, when administered in doses that do not
significantly increase circulating angiotensin II levels, in dogs with moderate HF attenuates the progression of LV systolic dysfunction but has marginal, if any, effects on overall LV remodeling. At doses of valsartan that significantly increased circulating angiotensin II levels, no benefits were observed on either LV function or remodeling. The inability of valsartan to inhibit or attenuate cellular remodeling in this model suggests that angiotensin II blockade at its AT1 receptor may not be the only component of the remodeling process at the cellular level. Whereas the present study did not compare the effects of monotherapy with valsartan to that of ACE inhibition, low-dose valsartan therapy, like that of treatment with enalapril (2), did prove beneficial in attenuating progressive LV dysfunction in the dog. Results of preliminary studies in animal models of HF in which therapy with AT1-receptor antagonists, including valsartan, was tested in combination with an ACE inhibitor (26, 37, 38) show promising trends and argue in favor of the notion that AT1-receptor antagonists may be useful adjuncts to the treatment of HF. Future studies should explore further the potential positive synergy that may be derived from such combination therapy.

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