Additive effects of combined blockade of AT$_1$ receptor and HMG-CoA reductase on left ventricular remodeling in infarcted rats

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Lee, Tsung-Ming, Mei-Shu Lin, Tsai-Fwu Chou, and Nan-Chung Chang. Additive effects of combined blockade of AT$_1$ receptor and HMG-CoA reductase on left ventricular remodeling in infarcted rats. Am J Physiol Heart Circ Physiol 291: H1281–H1289, 2006. First published March 24, 2006; doi:10.1152/ajpheart.00792.2005.—Both angiotensin receptor antagonists and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors have been shown to attenuate cardiomyocyte hypertrophy after myocardial infarction. Whether combination treatment may be superior to either drug alone on cardiomyocyte hypertrophy remains unclear. After ligation of the left anterior descending artery, rats were randomized to both, one, or neither of the angiotensin receptor antagonists olmesartan (0.01, 0.1, 1, and 2 mg/kg·day$^{-1}$) and HMG-CoA reductase inhibitor pravastatin (5 mg·kg$^{-1}$·day$^{-1}$) for 4 wk. Each drug, when given alone, decreased cardiomyocyte sizes isolated by enzymatic dissociation at the border zone when compared with vehicles. However, compared with either drug alone, combined olmesartan and pravastatin prevent cardiomyocyte hypertrophy to a larger extent, which was further confirmed by downregulation of the left ventricular atrial natriuretic peptide mRNA. The myocardial endothelin-1 levels at the border zone were 6.5-fold higher ($P <0.0001$) in the vehicle group compared with the sham group, which can be inhibited after pravastatin administration. Combination treatment significantly attenuated cardiac hypertrophy in a dose-dependent manner, although tissue endothelin-1 levels remained stable in combination groups of different olmesartan doses. Measurements of the arrhythmic score mirrored those of cardiomyocyte hypertrophy. Dual therapy with pravastatin and olmesartan, which produced an additive reduction in cardiomyocyte hypertrophy and cardiac fibrosis after myocardial infarction through different mechanisms, decreases the propensity of the heart to arrhythmogenesis. Pravastatin administration provided favorable ventricular remodeling, probably through decreased tissue endothelin-1 level. In contrast, olmesartan-related attenuated cardiomyocyte hypertrophy is independent of endothelin-1 pathway.

arrhythmias; hypertrophy; myocardial infarction; myocytes; 3-hydroxy-3-methylglutaryl coenzyme A

CARDIAC REMODELING was associated with myocardial hypertrophy and left ventricular (LV) dilation after myocardial infarction (37). Myocardial hypertrophy is characterized by altered phenotype; typically, this is manifest within the LV as a reexpression of fetal isoforms such as atrial natriuretic peptide (ANP) (4). Accumulating evidence indicates that angiotensin II plays a key role in the pathophysiology of myocardial hypertrophy after myocardial infarction (34). Angiotensin receptor blockers (ARBs) favorably modulate extracellular signal-regulated protein kinase to elicit attenuated cardiac hypertrophy (32). There is considerable evidence that electromechanical changes were associated with the hypertrophied myocardium (30). Agents with the regression of ventricular hypertrophy have been shown to decrease the susceptibility of ventricular arrhythmias (3). Recent trials attributed the survival benefit of ARB to reduction of arrhythmic death in animals (14) and in patients (29).

We have previously demonstrated that pravastatin can attenuate ventricular hypertrophy separate from its cholesterol-lowering actions in hyperlipidemic patients (20) and in normolipidemic rats (21). 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (statin) therapy has many effects independent of changes in plasma cholesterol concentrations, such as attenuated endothelin-1 (ET-1) expression (21). An alteration in the expression of ET-1 has been proposed as a potential regulation of cell hypertrophy after infarction (21). ET-1 can activate ERK1/ERK2 cascade, which plays an important role in the hypertrophic response (5). Attenuated increase of ET-1 concentrations effectively prevents cellular hypertrophy (11) It is of interest to identify drugs other than ARBs to reduce cardiac hypertrophy. The usefulness of ARB and statins after infarction has been demonstrated individually. However, pharmacological profiles are substantially different between these two classes. Whether their coadministration has a synergistic or additive effect on ventricular remodeling and the pharmacological mechanisms underlying the benefit of the combination therapy remain poorly understood. Thus we assessed whether pravastatin provides an additive effect on ventricular hypertrophy by inhibiting ET-1 expression after myocardial infarction in animals cotreated with olmesartan, a nonpeptide ARB.

METHODS

Animals. Male normocholesterolemic Wistar rats that weighed 300–350 g were fed a normal sodium diet and offered tap water ad libitum. On the study day, 24 h after myocardial infarction induced by ligating the left anterior descending artery, rats were randomly assigned into 10 groups: 1) vehicle group; 2) pravastatin (5 mg·kg$^{-1}$·day$^{-1}$; Sankyo, Tokyo, Japan) in the drinking water; 3) olmesartan (0.01, 0.1, 1, and 2 mg·kg$^{-1}$·day$^{-1}$; Sankyo) given orally by gastric gavage once a day; and 4) pravastatin (5 mg·kg$^{-1}$·day$^{-1}$) + olmesartan (0.01, 0.1, 1, and 2 mg·kg$^{-1}$·day$^{-1}$). The doses of pravastatin used in this study were based on our previous reports (21). The dose of olmesartan $>0.1$ mg·kg$^{-1}$·day$^{-1}$ has been shown to be effective in lowering blood pressure (36). To differentiate...
the hemodynamic and angiotensin effect of olmesartan on ventricular remodeling, a nonpressor dose (0.01 mg·kg\(^{-1} \cdot \text{day}^{-1}\)) was administered. The drugs were started 24 h after myocardial infarction, during which drugs can exert maximum benefits at this timing window (38). In each treated group, drugs were withdrawn ~24 h before the end of the experiment to eliminate their pharmacological actions. The study duration was designed to be 4 wk because the majority of the myocardial remodeling process in the rat (70–80%) is complete within 3 wk (27). Sham operation served as controls. The animal experiments were approved and conducted in accordance with local institutional guidelines for the care and use of laboratory animals in the Chi-Mei Medical Center, Tainan, Taiwan.

To create the model, rats were anesthetized with ketamine (90 mg/kg) intraperitoneally. After adequate anesthesia, the anterior descending artery ligation or sham operation was performed as described previously (21). Twenty-eight days after the first operation, the second operation was performed. With the use of a 2-Fr micro-manometer-tipped catheter (model SPR-407, Miller Instruments, Houston, TX) inserted through the right carotid artery, we measured LV systolic and diastolic pressure as the mean of measurements of five consecutive pressure cycles. The maximal rate of LV pressure rise and decrease was measured. Next, the heart was rapidly excised and suspended for retrograde perfusion with a Langendorff apparatus. At the completion of electrophysiological tests, the heart was then rapidly divided into right and left atria, right ventricle, LV, and the scarred area. Each tissue was then weighed individually. To evaluate the degree of pulmonary edema, lungs were also weighed. They have shown that hypertrophy of the residual myocardium progresses after infarction, only if infarct size is larger than 30% of the LV (28). Thus, with respect to clinical importance, only rats with infarction larger than 30% of the LV were selected for analysis.

Additionally, heart sections were stained with Masson’s Accustain trichrome stain (Sigma, St. Louis, MO) to distinguish areas of connective tissue. The details of the methodology have been described previously (3). The percentage of blue staining, indicative of fibrosis, measured (10 randomly selected fields) from the infarct zone (0–2 mm outside the infarct) and nonischemic areas (>2 mm outside the infarct) on two sections from each heart and averaged. Collagen was quantified by computer-assisted image analysis (Image Pro Plus) as previously described (22). The value was expressed as the ratio of trichrome-stained fibrosis area to total area.

Spontaneous and induced arrhythmias. To prevent the confounding factors, such as induction of regional ischemia and systemic neuro-hormonal changes in triggering cardiac arrhythmias, we performed the in situ perfused heart model as described previously (21). After isolation, each heart was perfused with a noncircuiting modified Tyrode solution. Epicardial electrograms were recorded by an atrumatic unipolar electrode, placed on the epicardial surface of the right ventricle and anterior LV wall 2 mm below the ligation site of the left anterior descending artery. The hearts were observed for 20 min to allow stabilization of contraction and rhythm. The protocol for pacing was modified from that of Nguyen et al. (26). Stimulation intensity was twice the threshold, and stimulus length was 5 ms. Induction of ventricular arrhythmias was then attempted by ventricular stimulation at a basic cycle length of 150 ms (S\(_1\)) with single (S\(_2\)), double (S\(_3\)), and triple (S\(_4\)) extrastimuli delivered after eight paced beats. The end point of ventricular pacing was induction of ventricular tachyarrhythmia. A preparation was considered noninducible when pacing produced either no ventricular premature contraction or only self-termi-nating salvos of fewer than six beats. Ventricular tachyarrhythmias, including ventricular tachycardia and ventricular fibrillation, were considered nonsustained when lasting ≤15 beats and sustained when lasting >15 beats. An arrhythmia scoring system was used: 0, non-inducible preparations; 1, nonsustained tachyarrhythmias induced with three extrastimuli; 2, sustained tachyarrhythmias induced with three extrastimuli; 3, nonsustained tachyarrhythmias induced with two extrastimuli; 4, sustained tachyarrhythmias induced with two extrastimuli; 5, nonsustained tachyarrhythmias induced with one extrastimulus; 6, sustained tachyarrhythmias induced with one extrastimulus; and 7, tachyarrhythmias induced during the 20 paced beats at a basic cycle length of 100 ms. If the heart stopped before the pacing, the arrhythmia score assigned to that heart was 8. When multiple forms of arrhythmias occurred in one heart, the highest score was used. The experimental protocols were typically completed within 10 min.

Immunohistochemical analysis of ET-1. In addition to endothelial cells, various cells, including cardiomyocytes, have the ability to synthesize ET-1 (25). To investigate the spatial distribution of ET-1, immunohistochemical staining was performed on LV muscle at the border zone and nonischemic areas as previously described (21). Immunostaining with ET-1 antibodies was performed by using a standard immunoperoxidase technique (N-Histofine Simple Stain Rat MAX PO kit, Nichirei, Tokyo, Japan). The antibody used had been tested for specificity in the rat. Negative controls were performed by omitting the primary antibody. Because of the wide variability of structural composition of border zone regions, which resulted in intercellular connection ranging from total disruption in fully scarred regions to negligible alterations with normal appearing myocytes, we selected samples for analysis that were composed of cardiomyocytes separated by diffuse interstitial fibrosis.

Real-time RT-PCR. To further confirm the degree of ventricular hypertrophy, mRNA levels of ANP were measured by real-time quantitative RT-PCR from samples obtained from the border zone with the TaqMan system (Prism 7700 Sequence Detection System, PE Biosystems) as previously described (21). For ANP, the primers were 5'-GCCCTTGGCTGTTGTCA and 5’-TGGAGCTCCAGGAGG-TATT. For glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), the primers were 5’-CTTCACCATGGAGAAGGC and 5’-GCGATGACTTGTTGCTAGAG. For quantification, ANP expression was normalized to the expressed housekeeping gene GAPDH. Reaction conditions were programmed on a computer linked to the detector for 40 cycles of the amplification step.

Plasma and tissue levels of ET-1 and plasma cholesterol levels. Because of a local release of ET-1, blood samples from the aortic root and the tissue at the border zone and remote interventricular zone were obtained for measurements of systemic and local ET-1 levels at the end of the study. Plasma and tissue ET-1 concentrations were measured as previously described (21). Cholesterol was measured in plasma by an automated method.

Cell isolation. Because the infarct size measurement procedure does not permit quantitation of cardiomyocyte sizes, additional groups of rats were infarcted by using the same procedures and used for measurement of cell sizes at the end of the study. All procedures for cardiomyocyte enzymatical isolation were performed as described in detail in our previous reports (21), a more reliable method to quantify the cardiomyocyte size than analysis of tissue section (10). Myocytes were enzymatically isolated with collagenase (type II; Sigma Chemical) and protease (type XIV, Sigma). Random high-power fields of the rodlike relaxed myocytes with clear striations were selected to eliminate selection bias. At least 100 cells from each section were selected for measurement of cell length, width, and area, and the mean value was used as the individual value for each section. In the sham-operated group, cell width and length were measured from the LV free wall for comparisons.

Statistical analysis. Results were presented as means ± SD. A two-way ANOVA was used to search for possible effects of pravastatin and olmesartan on the measurements of hemodynamics, ET-1 levels, cholesterol levels, and myocyte sizes, and, if an F value was found to be significant, a two-tailed Student’s t-test for paired observation with Bonferroni’s correction was used to test differences. The interaction term of pravastatin and olmesartan effects was incorporated into the model. Correlation between the degree of attenuated cardiomyocyte hypertrophy and the dose of olmesartan was assessed by Pearson’s correlation coefficient. Electrophysiological data (sco-
Table 1. Cardiac morphology and hemodynamics

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<td>+dP/dt, mmHg/s</td>
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<td>-dP/dt, mmHg/s</td>
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<td>LVDW/BW, mg/g</td>
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Values are means ± SD. Abbreviations as in Table 1.

Cardiac morphometric and gene expression. Body weights were unchanged by infarction or treatment. When compared with sham-operated rats in the vehicle group, there was a significant increase in right ventricular weight-to-body weight ratio, and lung weight-to-body weight ratio. Four weeks after infarction, the infarcted area of the LV was very thin and was totally replaced by fully differentiated scar tissue. The weight of the LV inclusive of the septum remained similar 4 wk after coronary artery occlusion among the infarcted groups.

To characterize the cardiac hypertrophy on a cellular level, we isolated cardiomyocytes from different treated groups (Table 2). After infarction, cell area increased by 59% (P < 0.0001) at the border zone when compared with the sham-operated group. Pravastatin (5 mg·kg⁻¹·day⁻¹) alone significantly reduced cell areas (23%) compared with the vehicle group. Olmesartan alone significantly attenuated cardiomyocyte hypertrophy in a dose-dependent manner. However, compared with each agent alone, their combination decreased cell sizes to a greater extent (P < 0.05 for the interaction term). When compared with the group treated with pravastatin alone (3,502 ± 266 μm²), the magnitude of attenuated cardiomyocyte hypertrophy was significantly increased in combination therapy even at the nonpressor dose of olmesartan (0.01 mg/kg, 3,020 ± 368 μm², P < 0.05), suggesting that olmesartan provides an additional beneficial effect on attenuated cardiomyocyte hypertrophy independent of hemodynamic changes.

Table 2. Characteristics of isolated cardiomyocytes at border zone

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<td>Myocyte length, μm</td>
<td>135±10</td>
<td>188±25</td>
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<td>137±26</td>
<td>154±25</td>
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<tr>
<td>Myocyte width, μm</td>
<td>21±4</td>
<td>23±3</td>
<td>21±5</td>
<td>21±3</td>
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<td>20±2</td>
<td>21±3</td>
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<td>Measured myocyte areas, μm²</td>
<td>2.847±257</td>
<td>4.527±241</td>
<td>3.784±346</td>
<td>3.462±312</td>
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<td>3.045±268</td>
<td>3.502±267</td>
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Values are means ± SD. Abbreviations as in Table 1.
Fig. 1. Top: representative sections with Masson’s trichrome staining (blue, magnification ×400) 4 wk postinfarct samples from remote zone. Collagen deposition within left ventricle (LV) is reduced after administration of either olmesartan (Olme), pravastatin (Prav), or both (Prav + Olme). A: sham. B: vehicle-treated infarcted rat. C: 0.01 mg/kg Olme. D: 0.1 mg/kg Olme. E: 1 mg/kg Olme. F: 2 mg/kg Olme. G: 5 mg/kg Prav. H: Prav + 0.01 mg/kg Olme. I: Prav + 0.1 mg/kg Olme. J: Prav + 1 mg/kg Olme. K: Prav + 2 mg/kg Olme. Line length corresponds to 50 μm. Bottom: LV collagen area fraction (%) at remote zone and border zone. Each column and bar represents means ± SD. *P < 0.05 compared with sham-operated group; †P < 0.05 compared with vehicle group; ‡P < 0.05 compared with Olme (0.01 mg/kg); §P < 0.05 compared with Prav + Olme (0.01 mg/kg).
At week 4 after infarction, collagen volume fraction increased by 164% (P < 0.0001) at the remote zone when compared with sham-operated group (Fig. 1). Pravastatin (5 mg·kg⁻¹·day⁻¹) alone significantly reduced collagen area fraction (29%) compared with the vehicle group at the infarct zone. Olmesartan alone significantly attenuated collagen area fraction in a dose-dependent manner. However, compared with each agent alone, their combination decreased collagen area fraction to a greater extent (P < 0.05 for the interaction term).

Regional myocardial expression of ANP was measured by competitive RT-PCR (Fig. 2). ANP was markedly increased at the border zone (15-fold, P < 0.0001) after myocardial infarction. Olmesartan reduced ANP expression at the border zone in a dose-dependent manner, consistent with the changes in cellular dimensions. Pravastatin also attenuated ANP expression at the border zone when compared with the vehicle-treated group (P = 0.03). The combination of olmesartan and pravastatin suppressed the upregulation of ANP to a greater extent than either intervention alone.

Electrophysiological stimulation. To further elucidate the physiological effect of attenuated cardiomyocyte hypertrophy, ventricular pacing was performed. Arrhythmia scores in sham-operated rats were very low (0.1 ± 0.3). In contrast, ventricular tachyarrhythmias consisting of ventricular tachycardia and ventricular fibrillation were inducible by programmed stimulation in infarcted rats (Fig. 3). Either pravastatin or olmesartan treatment decreased the inducibility of ventricular tachyarrhythmias. When compared with monotherapy, their combination more potently suppressed the inducibility of ventricular tachyarrhythmias.

Circulating and myocardial ET-1 levels. Circulating ET-1 levels remained similar in infarcted rats among the groups (Table 3). To investigate the possible role of cardiac ET-1 synthesis, we determined the ventricular ET-1 levels. Expression was region dependent with a significant increase at the border zone (7.85 ± 3.11 pg/mg tissue) compared with that in the interventricular septum (2.43 ± 1.12 pg/mg tissue, P < 0.0001) in the vehicle group. ET-1 levels at the border zone were significantly lower in pravastatin-treated rats than in vehicle-treated rats (2.72 ± 0.83 vs. 7.85 ± 3.11 pg/mg tissue, P < 0.0001). The suppressive effect of the combination of olmesartan (0.01, 0.1, 1, and 2 mg/kg) and pravastatin was similar to that of pravastatin alone in terms of tissue ET-1 levels at the border zone, implying that olmesartan was not related to tissue ET-1 levels. The finding was further confirmed by the data that the rats treated with olmesartan alone did not affect tissue ET-1 levels at the border zone when compared with vehicles.

Immunohistochemical analyses. To further confirm the changes in tissue ET-1 levels, we performed immunohistochemical analyses of ET-1. The infarcted myocardium revealed the presence of ET-1 immunoreactivity in myocardial tissue (Fig. 4). In the vehicle group, a marked increase in the intensity of ET-1 immunostaining was observed at the border zone compared with sham-operated rats, consistent with the results of tissue ET-1 levels. The intensity of the immunoreaction was reduced in pravastatin-treated groups compared with that in the vehicle group. However, olmesartan-treated groups showed similar intensity of ET-1 compared with the vehicle-treated group.

Correlation. The dose effect of olmesartan on cardiomyocyte hypertrophy attenuation is presented in Fig. 5. The Pearson linear regression models showed that there was a significant correlation between the reduction of cardiomyocyte hypertrophy and the dose of olmesartan (0.01, 0.1, 1, and 2 mg/kg) in the combined therapy [reduction in cardiomyocyte hypertrophy (%) = 6.6 × the dose of olmesartan (mg·kg⁻¹·day⁻¹) + 19.1; r = 0.88, P = 0.046]. In the olmesartan alone, the reduction of cardiomyocyte hypertrophy was smaller than predicted from the combination groups at the same doses of olmesartan. Combination of olmesartan and pravastatin developed an additive effect in attenuated cardiomyocyte hypertrophy compared with those in the rats treated with olmesartan alone.

DISCUSSION

Our present work provided solid experimental evidence for the first time that combination therapy with ARB and pravastatin is more beneficial than each agent alone for attenuated cardiomyocyte hypertrophy and cardiac fibrosis and reduced...
Fig. 4. Immunohistochemical microscopy of endothelin-1 (ET-1; magnification ×200) at border zone (BZ). Positive staining for ET-1 (brown color) was distributed in the myocardium. Intensity of ET-1 in myocardium was significantly lower in Prav (G), Prav + Olme (0.01 mg/kg, H; 0.1 mg/kg, I; 1 mg/kg, J; and 2 mg/kg, K) groups than in groups treated with vehicle (B), Olme (0.01 mg/kg; C; 0.1 mg/kg, D; 1 mg/kg, E; and 2 mg/kg, F). Immunohistochemical microscopy of sham-operated group shown in A. Line length corresponds to 100 μm.

Table 3. Cholesterol levels and plasma, and tissue ET-1 concentrations

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<td>Plasma cholesterol, mg/dl</td>
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<td>Plasma ET-1, pg/ml</td>
<td>0.54 ± 0.26</td>
<td>0.74 ± 0.33</td>
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<td>0.52 ± 0.11</td>
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<td>Border LV ET-1, pg/mg tissue</td>
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<td>Remote LV ET-1, pg/mg tissue</td>
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Values are means ± SD. Abbreviations as in Table 1. ET-1, endothelin-1. *P < 0.05 compared with sham-operated group; †P < 0.05 compared with vehicle group.
arrhythmic score in a dose-dependent manner. This additive effect of olmesartan and pravastatin suggests that both agents improve ventricular remodeling by fundamentally different mechanisms.

Our conclusions are supported by the following evidence. 1) Either ARBs or statins can similarly improve ventricular remodeling after infarction as documented structurally, by reduction in myocyte sizes, molecularly, by ANP mRNA, and electrophysiologically, by attenuated inducibility of ventricular arrhythmias. Furthermore, with the highest dose of olmesartan (2 mg·kg⁻¹·day⁻¹) in combined therapy or alone, we observed a further reduction of cardiomyocyte hypertrophy and cardiac fibrosis when compared with lower doses of olmesartan, implying a dose-dependent effect of olmesartan. Furthermore, when compared with vehicles, the combination of olmesartan at the dose of 2 mg/kg and pravastatin reduced cardiomyocyte hypertrophy by 39%, which was greater than the percentage inhibition achieved when olmesartan (33%) and pravastatin (23%) were used alone, suggesting an additive effect between them on ventricular remodeling. 2) Different mechanisms are involved in the attenuation of ventricular remodeling between ARB and pravastatin. The synthesis of ET-1 in cardiomyocytes was reduced when they were exposed to pravastatin, both in the absence and presence of olmesartan, suggesting an ET-1-independent effect of olmesartan and an ET-1-dependent effect of pravastatin on cardiomyocyte remodeling. The capacity to inhibit angiotensin II activity is not the only factor responsible for the antihypertrophic effect in this model, and pravastatin has additional mechanisms of action at the myocardial level to inhibit ET-1 overexpression. The finding further supports the notion that combination therapy of pravastatin and olmesartan is additive in their effect on attenuated cardiomyocyte hypertrophy after infarction through different mechanisms.

**Effect of pravastatin and olmesartan on cardiac hypertrophy.** Our observations showed that reduced pravastatin-related ET-1 levels, in concert with blockade of angiotensin II, may play crucial roles in attenuation of myocyte hypertrophy. Angiotensin II and ET-1 constitute a complex positive circuit acting on cardiomyocytes in an autocrine/paracrine fashion. In vitro studies have demonstrated cross talk between the angiotensin system and ET-1 system (8). Angiotensin II has been shown to induce ET-1 synthesis in cardiomyocytes in vitro (8). Ishii et al. (13) have shown in an in vivo study that the increase in ventricular ET-1 content can be inhibited by specific blockade of angiotensin AT₁ receptors. Cardiac ET-1 may induce the hypertrophic effect of endogenous angiotensin II in the model of volume overload cardiac hypertrophy. However, Loennechen et al. (24) have shown that blockade of angiotensin AT₁ receptors by administering losartan did not affect the ET-1 expression. This could have implications for the potential interaction of drugs that interrupt the renin-angiotensin system and endothelin receptor, which can be blocked by different agents. The hypothesis was compatible with our observation that the combination of olmesartan and pravastatin may exert differential pharmacological action from either agent alone.

We (21) have shown that the tissue ET-1 level at the border zone was increased after infarction, which can be reduced by administering pravastatin. The molecular mechanism of non-cholesterol effects of statins is the inhibition of the isoprenoid intermediates of the cholesterol pathway. Isoprenoids are essential for the function of signal transduction molecules of the Rho family (19). Regulation of Rho activity by statins is separate from that of statins on lipids. Inhibition of Rho signaling by statins can activate peroxisome proliferator-activated receptors (7), which in turn suppress ET-1 secretion (33). Besides, decreases in regional ET-1 concentrations may result from decreases in ET-1 production and/or increased ET-1 clearance. The production of ET-1 begins with the cleavage of the translational product prepro-ET-1. We have previously shown that pravastatin inhibits prepro-ET-1 mRNA at the transcription levels (21). Thus decreases in ET-1 production might play a major role in regional ET-1 changes.

**Arrhythmias.** Our results showed that favorable ventricular remodeling after drug administration has benefits not only in anatomical structures but also in arrhythmia susceptibility. In infarcted groups administered either one or both drugs, hypertrophied cardiomyocyte sizes and cardiac fibrosis significantly attenuated compared with those in the vehicle-treated group. At the same time, the incidence of ventricular tachyarrhythmias was significantly reduced compared with the vehicle. It is suggested that the enhanced arrhythmogenesis was reversed by the attenuation of cardiomyocyte hypertrophy and cardiac fibrosis. The infarct size was similar among the groups, suggesting that suppression of arrhythmia was not the result of differences in infarct areas. These cellular alterations are important because the border zone is a region where malignant arrhythmia origins (1). The hypertrophic growth of the surviving myocytes may create a shift in the sympathovagal balance toward a sympathetic prevalence that leaves the myocardium in greater jeopardy for the development of life-threatening arrhythmias (18). Besides, action potential dispersion and the conduction alterations were more prominent in hypertrophied than in normal tissue during ischemia (2). Finally, antiarrhythmic mechanism may be the result of reducing fibrosis in the viable portion of the heart after drug administration. We have shown in this study that either ARB or pravastatin treatment can attenuate cardiac fibrosis, thereby reducing the risk of isolated regional slowing of conduction and reentrant arrhythmias. Taken together, regardless of the relative importance of each of these factors, all of the changes caused by either pravastatin, olmesartan, or combination are compatible with
our understanding of beneficial effects on induction of ventricular arrhythmias.

Clinical implications. Our results confirmed and extended the beneficial effect of combination therapy of statins and ARBs on treating atherosclerotic plaque progression and postinfarction ventricular remodeling. Koh et al. (15) have shown that combination therapy of ramipril and simvastatin improved endothelial function greater than either treatment alone in patients with type 2 diabetes mellitus. The combination benefit of the statin and angiotensin-converting enzyme inhibitors was further assessed in the Simvastatin/Enalapril Coronary Atherosclerosis Trial (16). The main finding was that lipid-lowering therapy for 3–5 yr with simvastatin resulted in significant slowing of coronary atherosclerosis in normolipidemic coronary artery disease patients. Furthermore, valsartan together with a low dose of fluvastatin has been shown to effectively attenuate neointimal formation in vascular smooth muscle cells (12). Our results extended the protective effects of combination therapy from vessels to myocardium. Indeed, our results were consistent with the clinical finding of the GREek Atorvastatin and CHD Evaluation (GREACE) Study (35), showing the benefits of combination therapy of statins and angiotensin-converting enzyme inhibitors in patients with established coronary artery disease. In this high-risk cohort, combination therapy reduced the risk of mortality, myocardial infarction, and stroke. Furthermore, similar to our findings, clinical benefit could not be attributed to a reduction in blood pressure because angiotensin-converting enzyme inhibitor-treated patients had a similar systolic and diastolic blood pressure as the nonangiotensin-converting enzyme inhibitor-treated patients.

Because the inducibility of ventricular tachyarrhythmia by programmed electrical stimulation is a well-established marker of an increased risk of ventricular tachyarrhythmia (6), this beneficial effect of combination therapy may have important clinical implications regardless of the underlying mechanisms. Although caution should be taken in extrapolating to humans the results obtained with experimental animals, the attenuation of ET-1 and angiotensin II is central to myocardial arrhythmogenicity in the diseased state, and a therapeutic strategy may be to correct the process of ventricular remodeling. Unlike antiarrhythmic therapy directly targeting membrane currents, pravastatin and/or olmesartan may prevent the generation of arrhythmogenic cells undergoing hypertrophy and fibrosis. Furthermore, previous studies have demonstrated that regional myocyte hypertrophy parallels regional myocardial dysfunction during postinfarction remodeling (17). Thus attenuation of ventricular remodeling prevents the transition for compensatory dilation to ventricular dysfunction and failure (23). Our findings support the rationale that early treatment with a combination of ARBs and statins may provide an optimal endothelial protection after large infarction.

Study limitations. There are some limitations in the present study that have to be acknowledged. Only rats with large infarcts (>30% of the LV) were examined, raising the possibility that the reaction of the surviving myocytes may be different with smaller infarcts. Sakai et al. (31) have shown that there was a good correlation between the extent of infarct size and ET-1 levels. Because an activation of the ET-1 system is a prerequisite for the pravastatin effect, our finding cannot necessarily be extrapolated to animals with small to moderate infarction. Besides, only one time point after infarction was studied. The immunohistochemical and electrophysiological studies were not performed until 4 wk after infarction. Future research should identify early events, such as myocardial inflammation, and trace their progression after the administration of drugs.

In conclusion, our present work provided the first evidence that both ARBs and statins use different actions to exert a similar effect on attenuation of cardiac hypertrophy and cardiac fibrosis in a manner independent of their antihypertensive and hypolipidemic effects. The combination therapy of ARB and statin is associated with an additive beneficial effect on ventricular remodeling than each alone. Because cardiac hypertrophy is hard to normalize by either ARBs or statins alone, the present study proposes a good indication for combination therapy with ARB and statin against cardiac remodeling after myocardial infarction. Characterization of the cardioprotective mechanisms provides a rationale for the future design of combination drugs in the secondary prevention of coronary artery disease.

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REFERENCES

9. Fears R, Richards DH, and Ferres H. The effect of compain, a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme-A reductase activity, on...


