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Am J Physiol Heart Circ Physiol 288:H1352-H1358, 2005. First published 14 October 2004;
doi:10.1152/ajpheart.00527.2003

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Submitted 6 June 2003; accepted in final form 11 October 2004

Lee, Tsung-Ming, Mei-Shu Lin, Tsai-Fwu Chou, and Nen-Chung Chang. Effect of simvastatin on left ventricular mass in hypercholesterolemic rabbits. *Am J Physiol Heart Circ Physiol* 288: H1352–H1358, 2005. First published October 14, 2004; doi:10.1152/ajpheart.00527.2003.—Epidemiological studies showed that hypercholesterolemia is associated with higher left ventricular mass. Endothelin signaling is activated in hyperlipidemic animals and may contribute to progressive ventricular hypertrophy. Simvastatin has been shown to inhibit endothelin-1. However, the behavior of simvastatin on ventricular hypertrophy in hyperlipidemic animals is not well understood. In this study, we evaluated the hemodynamic, biochemical, and morphological responses to simvastatin in cholesterol-fed (1%) rabbits. The left ventricular weight increased 8 wk after cholesterol feeding compared with that in normocholesterolemic rabbits. Simvastatin at a clinical therapeutic dose (1.2 $mg \cdot kg^{-1} \cdot day^{-1}$) significantly decreased left ventricular weight by 14% and left ventricular myocyte sizes by 14% as isolated by enzymatic dissociation. Hypercholesterolemia upregulated ventricular preproendothelin-1 mRNA as assessed by real-time quantitative RT-PCR and elevated production of cardiac endothelin-1 concentration. The increased endothelin-1 responses can be inhibited after simvastatin administration. Left ventricular mass indexed by body weight positively correlated with tissue endothelin-1 levels ($P = 0.0003$). In Langendorff-perfused rabbit hearts, hyperlipidemia led to significant QT prolongation compared with normocholesterolemia, which can be reversed by administering simvastatin. In contrast, simvastatin-induced beneficial effects were reversed by the addition of mevalonate. The addition of bosentan, a nonspecific endothelin receptor blocker, improved the response in hypercholesterolemic rabbits and did not have additional beneficial effects in simvastatin-treated rabbits. The results of the present study suggest that the antihypertropic and electrocardiographic effects of simvastatin at a clinical therapeutic dose are mediated through inhibition of tissue endothelin-1 expression, which is linked to mevalonate metabolism, and result in an amelioration of cardiomyocyte hypertrophy development by an atherogenic diet.

electrocardiogram; endothelin-1; hyperlipidemia; rabbit

WE (16) and others (33) have demonstrated that dyslipidemia is an independent determinant of increased left ventricular (LV) mass. Myocardial hypertrophy is an adaptation response of the heart to increased work load. However, increased LV mass is a risk factor of cardiac morbidity and mortality in the general population (4). Previous data have revealed that LV mass regression reduced cardiovascular complications (4). Thus reversal of LV mass is widely accepted as a desirable treatment goal. There is considerable evidence that electrophysiological changes are associated with the hypertrophied myocardium, such as an increased QT interval (29). QT interval prolongation, even within the normal range, has been associated with an increase in sudden death in patients (1) and in apparently healthy individuals (30). Agents with the regression of ventricular hypertrophy have been shown to normalize QT intervals (2).

Endothelin (ET)-1 levels have been shown to be increased in hypercholestolemic animals (9, 20) and patients (8). ET-1 has been shown to induce vasomotor dysregulation in hyperlipidemic pigs (20). In addition to its vasoactive properties, ET-1 also triggers hypertrophic signaling pathways by activation of extracellular signal-regulated kinase in myocardium (38), thereby implying a potential involvement of this peptide in the initiation and progression of ventricular hypertrophy. In vivo, gene knockout of the ET-1 gene has been reported to inhibit ventricular hypertrophy (5).

3-Hydroxy-3-methyglutaryl-CoA reductase inhibitor (statin) therapy has been shown to reduce cardiovascular morbidity and mortality, far surpassing the improvement of lipid profile. Statin therapy has many effects independent of changes in plasma cholesterol concentrations (18). Lipophilic statins have been shown to attenuate in vitro cellular hypertrophy (21, 27). Simvastatin has been shown to reduce in vivo ventricular hypertrophy at the established phase of LV hypertrophy (28); however, no data exist as to whether long-term use of simvastatin at a clinical therapeutic dose can attenuate cellular hypertrophy at the early development of LV hypertrophy. Previous studies have shown that different stages of ventricular hypertrophy may be differentially regulated (24). To our knowledge, no study has yet specifically examined the effect of hyperlipidemia on the development of ventricular hypertrophy, the effect of simvastatin in hypercholesterolemic animals, and whether the observed effect could be due to the attenuated formation of ET-1. In addition, we also explored the downstream functional significance of reduced ventricular hypertrophy by assessing the effect of QT interval in rabbits, a species widely used to determine the potential effects of new antiarrhythmic agents intended for use in humans.

METHODS

Experimental animals. Male New Zealand White rabbits weighing ~2 kg were randomly assigned to normolipidemic and hyperlipidemic groups. Hyperlipidemic rabbits received a 1% cholesterol diet for 8 wk. Cholesterol-fed rabbits were randomly allocated to three groups with oral doses of simvastatin (1.2 $mg \cdot kg^{-1} \cdot day^{-1}$, Merck, Sharp & Dome; Whitehouse Station, NJ), mevalonate (50 $mg \cdot kg^{-1} \cdot day^{-1}$, Sigma Chemical; St. Louis, MO), or a combination of simvastatin and mevalonate beginning from the first day of cholesterol feeding and

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continuing for 8 wk until necropsy. The fourth group of cholesterol-fed rabbits was left untreated (placebo group). In addition, aged-matched controls received standard lab chow (normolipidemic control group) and were allowed free access to water. Finally, to further confirm the role of chronic ET activation in the progression of ventricular hypertrophy, we performed an additional experiment with four groups to randomize the hyperlipidemic rabbits ($n = 8$ rabbits/group) fed with 1% cholesterol as described above: placebo, simvastatin (1.2 $mg \cdot kg^{-1} \cdot day^{-1}$), bosentan, and a combination of both. The bosentan-treated groups received bosentan (10 $mg \cdot kg^{-1} \cdot day^{-1}$, Actelion Pharmaceuticals; Allschwil, Switzerland), a nonspecific ET receptor blocker. The therapeutic efficacy of this dose has been previously demonstrated without hypotensive effects (23). The drugs were dissolved in drinking water, and the concentration was adjusted for the daily water intake and body weight to obtain the target dosage. In each treated group, drugs were withdrawn about 24 h before the experiments were performed to eliminate their pharmacological actions. All the procedures were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Pub. No. 85-23, Revised 1996).

Electrocardiographic measurement in Langerdorff-perfused rabbit hearts. After an 8-wk period, rabbits were anesthetized with intravenous ketamine (40 mg/kg). Using a 2-Fr micromanometer-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. When hemodynamic analyses were completed, the heart was rapidly excised and suspended for retrograde perfusion with a Langendorff apparatus. Each heart was perfused with a noncirculating modified Tyrode solution containing (in mM) 117.0 NaCl, 23.0 $NaHCO_3$, 4.6 KCl, 0.8 NaH_2PO_4, 1.0 $MgCl_2$, 2.0 $CaCl_2$, and 5.5 glucose equilibrated at 37°C and oxygenated with a 95% O_2-5% CO_2 gas mixture. The perfusion medium was maintained at a constant temperature of 37°C with a peristaltic pump at a constant flow of 40 ml/min. Epicardial electrograms were recorded by an atraumatic unipolar electrode placed on the epicardial surface of the right atrium and the anterior LV wall 2 mm below the circumflex artery. Atrial and ventricular epicardial electrocardiograms were continuously displayed on a Gould recorder at 100 mm/s chart speed and a HP monitor (model 54503A, Hewlett-Packard) at 100 mm/s sweep speed. If ST segment elevation (indicating myocardial injury) was observed, the heart was not used. The hearts were observed for 20 min to allow stabilization of contraction and rhythm.

All electrocardiograms were visually analyzed by an experienced cardiologist without knowledge of information to the nearest 2 ms with the aid of a caliper and a magnifying device with a grid as described previously (17). Intervals preceded by premature beats were not measured. The QT interval was averaged with each RR interval over three cardiac cycles. We considered that the ventricular repolarization was complete when the ECG signal returned to the isoelectrical line. The QT interval was corrected for heart rate (QT_c) using the standard Bazett formula (3) as follows: $QT_c = QT/(RR\ interval)^{1/2}$.

The coefficient variability for duplicate QT interval measurements was 2.0% (5.2 ms); the variability of the intraobserver variability of the QT interval by randomly inserting 30 electrocardiograms was 1.8% (4.5 ms).

Plasma and tissue levels of ET-1 and lipid profiles. Because of a local release of ET-1, blood samples from the aortic root and the tissue from the LV were obtained for measurements of systemic and local ET-1 levels at the end of the study. At the completion of the electrocardiogram, the heart was then rapidly divided into right and left atria, right ventricles, and LVs. Each tissue was then weighed individually. The basal one-third of the LV was cut into slices, one of which was embedded in OCT compound (Tissue Tek), frozen in liquid nitrogen, and stored at −70°C until use for molecular biological analyses. For the measurement of cardiac ET-1 levels, the apical two-thirds of the LV were immediately homogenized with a Polytron homogenizer for Triton X-100, boiled for 7 min, and centrifuged at 20,000 *g* for 30 min at 4°C. After measurement of the protein concentration, the supernatant was stored at −70°C until use. The plasma ET-1 concentration was measured by collecting 4 ml of blood in test tubes containing 2% ethylenediaminetetraacetic acid (80 μl/ml blood). Blood samples were immediately centrifuged at 3,000 *g* for 10 min, and the plasma was stored at −70°C until further analysis. ET-1 was measured by immunoassay (R&D Systems; Minneapolis, MN). Plasma (1 ml) was acidified with 3 ml of 4% acetic acid, and ET-1 was extracted with a Sep-Pak C-18 cartridge. The detection limit was 1 pg/ml for ET-1. Intra- and interassay coefficients of variation were 4.5% and 6.6%, respectively. Total serum cholesterol and triglyceride levels were measured by standard enzymatic techniques (18).

Real-time RT-PCR. Real-time quantitative RT-PCR was performed with a TaqMan system (Prism 7700 Sequence Detection System, PE Biosystems) as described previously (19). For ET-1, the primers were (forward) 5′-TGCTCCTGCTCCTCGCTGAT-3′ and (reverse) 5′-AAGAGCGAGTGAGAGAGTGA-3′ (corresponding to nucleotide sequences 270–289 and 786–767 of the rabbit prepro-ET-1 gene, GenBank Accession No. X59931). For GAPDH, the primers were (forward) 5′-CTTCACCACCATGGAGAAGGC-3′ and (reverse) 5′-GGCATGGACTGTGGTCATGAG-3′. For quantification, ET-1 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.

Cell isolation. Because cardiac hypertrophy is a combination of reactive fibrosis and myocyte hypertrophy, we measured cardiomyocyte sizes from the LV in addition to using myocardial weight to avoid interference of nonmyocytes. Myocytes were enzymatically isolated according to previously described techniques (19). Briefly, the rabbits were heparinized, and the heart was excised and perfused at a constant flow of 8 ml/min by a modified Langendorff technique at 37°C with a nominally Ca^{2+}-free, oxygenated Tyrode solution (pH 7.4) containing (in mM) 137 NaCl, 5.4 KCl, 1.1 $MgCl_2$, 11 dextrose, and 10 HEPES. After 5 min of equilibration, the perfusion was changed to the same solution containing 0.34 mg/ml collagenase (type II, Sigma Chemical). After 8–10 min of digestion, the residual enzyme-containing solution was cleaned by 5-min perfusion with 0.2 mM Ca^{2+}-Tyrode solution. The heart was then removed from the cannula, and the LV was mechanically dispersed. Random high-power fields of the rodlike relaxed myocytes with clear striations were selected in phase-contrast viewing conditions to eliminate selection bias. At least 50 cells from each section were selected for measurement of cell area, length, and width ratio, and the mean value was used as the individual value.

Statistical analysis. Results are presented as means ± SD. Data were analyzed with SPSS version 10.0. Two-way ANOVA was used to search for possible effects of simvastatin and mevalonate 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 simvastatin and mevalonate effects was incorporated into the model. Correlation between the ratio of LV mass/body weight and ET-1 levels or the QT measures was assessed by Pearson's correlation coefficient. The significant level was assumed at value of $P < 0.05$.

RESULTS

After 8 wk, the average plasma cholesterol levels were significantly elevated to 27-fold compared with those in the normal diet-fed rabbits ($P < 0.0001$; Table 1). This dose of simvastatin did not normalize the increased level of serum

Table 1. *Hemodynamics, cardiac morphology, and lipid profiles*

Parameters	Control	Hyperlipidemia			
		Placebo	Simvastatin treatment	Mevalonate treatment	Simvastatin + mevalonate treatment
n	10	10	10	10	10
Body weight, kg	2.5±0.1	2.4±0.1	2.4±0.1	2.4±0.1	2.4±0.2
HR, beats/min	124±13	125±12	124±14	124±7	117±9
LVESP, mmHg	123±7	121±10	123±10	123±13	121±9
LVEDP, mmHg	10±2	11±2	10±2	9±2	10±2
LV weight/body weight, g/kg	1.64±0.08	2.00±0.17*	1.73±0.10†	1.91±0.15*	1.96±0.16*
Plasma cholesterol, mg/dl	54±15	1,460±366*	699±106†	1,446±374*	1,457±314*
Plasma triglyceride, mg/dl	52±12	500±118*	292±67†	511±108*	473±160*

Values are means ± SD; *n,* no. of rabbits; HR, heart rate; LVESP, left ventricular (LV) end-systolic pressure; LVEDP, LV end-diastolic pressure. *$P < 0.05$ compared with control; †$P < 0.05$ compared with placebo.

cholesterol. Blood pressure and heart rate did not differ among the groups.

Morphometric studies. After 8 wk of a high cholesterol diet, the hyperlipidemic rabbits had a significantly higher ratio of LV weight to body weight than that of the control group (2.00 ± 0.17 vs. 1.64 ± 0.08 g/kg in controls, $P < 0.0001$). A significant reduction in LV mass occurred after simvastatin treatment by 14% ($P = 0.0003$). There was a significantly residual LV hypertrophy after simvastatin treatment, being 5% above that in control ($P = 0.04$).

Treatment of bosentan attenuated ventricular hypertrophy in hyperlipidemic rabbits by 14% compared with the placebo group, a figure similar to that in the simvastatin-treated group (14%; Fig. 1). However, the addition of bosentan did not further attenuate ventricular hypertrophy in simvastatin-treated rabbits.

To characterize the cardiac hypertrophy on a cellular level, we isolated cardiomyocytes in additional rabbit groups (Table 2). The cells in the hyperlipidemic group significantly increased by 25% compared with those from the same area of control hearts (4,277 ± 153 μm^2 in the hyperlipidemic group vs. 3,412 ± 182 μm^2, $P < 0.0001$). Simvastatin reduced cell areas by 14% compared with the hyperlipidemic group ($P < 0.0001$). The cell width and length of the simvastatin-treated myocytes were significantly smaller than those of the hyperlipidemic group (8% and 11%, both $P < 0.05$). Conversely, the rabbits treated with the combination of mevalonate and simvastatin developed significantly higher cardiomyocyte hypertrophy than the simvastatin-treated group alone (4,173 ± 128 μm^2 in the combination group vs. 3,672 ± 132 μm^2, $P < 0.0001$).

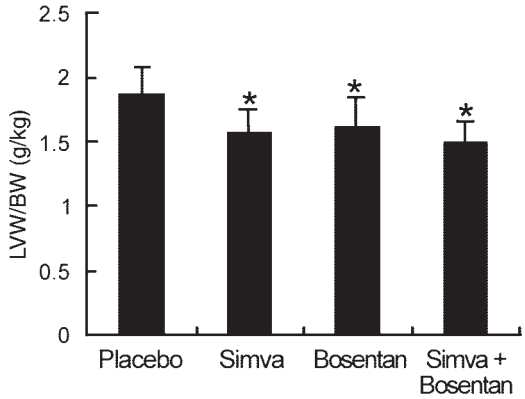

Fig. 1. Effects of simvastatin (Simva) and bosentan on ventricular mass indexed by body weight (BW) in hyperlipidemic rabbits. LVW, left ventricular (LV) weight. *$P < 0.05$ compared with placebo.

Circulating and myocardial ET-1 levels and prepro-ET-1 mRNA. Circulating ET-1 levels remained similar among the groups (Table 3). To investigate the possible role of cardiac ET-1 synthesis in the reduction of plasma ET-1 levels, we determined the ventricular ET-1 levels. LV ET-1 levels were significantly upregulated by 1.7-fold in the hyperlipidemic rabbits than in controls (6.3 ± 1.1 vs. 3.8 ± 1.6 pg/mg protein, $P = 0.0005$). LV ET-1 levels were significantly lower in simvastatin-treated rabbits than in hyperlipidemic rabbits ($P = 0.002$). The mRNA levels of prepro-ET-1 showed a 1.8 ± 0.2-fold upregulation in the hyperlipidemic rabbits than in control ($P < 0.0001$; Fig. 2). Thus the mRNA levels of prepro-ET-1 changed in parallel to the tissue peptide levels, implying that the production of prepro-ET-1 is a critical regulation step for its local activation. Mevalonate administration significantly increased both prepro-ET-1 mRNA and ET-1 peptides compared with rabbits treated with simvastatin alone, implicating that mevalonate was involved in the inhibitory effect of simvastatin on ET-1 levels.

QT interval in isolated Langendorff-perfused rabbit hearts. Figure 3 shows QT and QT_c intervals. Compared with controls in hyperlipidemic rabbits, there was a significant prolongation in QT_c intervals (Fig. 4), reflecting that the magnitude of QT prolongation was independent of heart rate. QT and QT_c intervals significantly decreased after simvastatin treatment (295 ± 20 ms in the hyperlipidemic group vs. 244 ± 35 ms for QT interval, $P = 0.002$; 426 ± 34 in the hyperlipidemic group vs. 352 ± 63 ms for QT_c interval, $P = 0.008$). Mevalonate administration significantly increased the QT interval in simvastatin-treated rabbits compared with rabbits treated with simvastatin alone.

Correlation. The linear regression models showed a significant correlation between tissue ET-1 levels and the ratio of LV mass to body weight [LV mass-to-body weight ratio = 0.073 × tissue ET-1 levels (in pg/mg protein) + 1.469, $P = 0.0003$; Fig. 5]. The ratio of LV weight to body weight was not correlated with systolic blood pressure, plasma ET-1 levels, cholesterol levels, and triglyceride levels. In addition, there was a correlation between the ratio of LV weight to body weight and QT_c interval ($P = 0.005$).

Table 2. *Characteristics of isolated cardiomyocytes*

Parameters	Control	Hyperlipidemia			
		Placebo	Simvastatin treatment	Mevalonate treatment	Simvastatin + mevalonate treatment
n	4	4	4	4	4
Myocyte length, μm	146±8	172±9*	153±8†	169±8*	175±10*
Myocyte width, μm	22±2	25±2*	23±2†	26±1*	25±2*
Measured myocyte areas, μm^2	3,412±182	4,277±153*	3,672±132†	4,341±107*	4,173±128*

Values are means ± SD; *n,* no. of animals. *$P < 0.05$ compared with control; †$P < 0.05$ compared with placebo, mevalonate, and simvastatin + mevalonate.

DISCUSSION

The present study demonstrates three novel findings through combined use of molecular, biochemical, electrocardiographic, and morphological methods. First, hyperlipidemia, acting through the corresponding increase of ventricular prepro-ET-1 mRNA and tissue ET-1 levels, results in the development of ventricular hypertrophy, which can be prevented by administering ET receptor blockers. Second, simvastatin administration has beneficial effects on attenuated ventricular hypertrophy independent of blood pressure reduction at the development stage of LV hypertrophy by attenuation of tissue ET-1 levels. This suggestion is based on the observation that bosentan administration did not further reduce ventricular hypertrophy in simvastatin-treated rabbits, suggesting a common pathway between both agents. Finally, the inhibitory effect of simvastatin is specifically prevented by the addition of exogenous mevalonic acid, indicating that the inhibitory effects of the drug are due to blockade of the pathways leading to isoprenoid synthesis. These combined results indicate that simvastatin prevents progression of ventricular hypertrophy through attenuation of mevalonate-dependent tissue ET-1 levels.

Hyperlipidemia and LV mass. Our results show that hypercholesterolemia was associated with increased LV mass and that local activation of ET-1 may be related to ventricular hypertrophy progression irrespective of a change in plasma ET-1 levels. To the best of our knowledge, although several studies have shown that ET-1 levels have significantly increased release by vascular endothelium cells in hyperlipidemic rabbits (25, 37), no studies have addressed local ET-1 release by myocardium. We demonstrated that tissue ET-1 levels and tissue immunoreactivity are enhanced in the evolution of ventricular hypertrophy induced by hypercholesterolemia. Our results extend previous studies of the raised ET-1 immunoreactitivity from coronary vascular wall to myocardium.

Our results were compatible with a recent study (10) showing that simvastatin prevents cardiac hypertrophy induced by aortic banding. In the present study, we complemented these findings by showing attenuation of cardiomyocyte hypertrophy assessed by enzymatic dissociation. It appears from our study that the attenuated myocyte hypertrophy is related to a cholesterol-independent decreased cardiac ET-1 levels in response to simvastatin treatment. The addition of mevalonate to simvastatin-treated rabbits impaired not only their ability to attenuate cellular hypertrophy but also their ability to suppress ET-1 levels. Thus blocking the mevalonate pathway is a critical step in the mechanism of simvastatin-induced attenuation of ET-1 levels. Mevalonate is not only involved in the synthesis of cholesterol but is also the precursor for the synthesis of isoprenoids. Isoprenoids are essential for the function of signal transduction molecules of the Rho family (15). 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 (PPAR) (6). Activation of PPAR-γ is beneficial in preventing cardiac ET-1 secretion by inhibiting the activator protein-1 signaling pathway (6). Nishikimi et al. (26) have shown that statins can directly attenuate ET-induced cardiac hypertrophy through inhibition of the Rho pathway. In addition, decreases in regional ET-1 concentrations may result from decreases in ET-1 production. Because ET-1 is not stored intracellularly but is generated de novo and its production is controlled at the level of mRNA production (31), our results showed that simvastatin inhibits expression of prepro-ET-1 mRNA. Taken together, either inhibition of ET-1 secretion or synthesis after the administration of simvastatin reduces tissue ET-1 levels, which was supported by our immunohistochemical observations.

ET-1 has been reported to accelerate the hypertrophy of myocytes (32). ET-1 elicits complex and highly regulated cascade of signal transduction. ET-1 activates at least three classes of signaling pathways in the cardiomyocytes, i.e., protein kinase C, mitogen-activated protein kinase, and the phosphatidylinositol 3-kinase/Akt pathway (32). ET receptor blockers suppressed the increase in protein synthesis and the activation of these signaling pathways in in vitro and in vivo studies (32). It is likely that ET-1-induced hypertrophic growth is, at least in part, mediated through the ET_A receptor (32). Our results were compatible with the notion that blocking the growth-promoting effect of ET-1 at the ET receptor level is effective in preventing ongoing cardiac hypertrophy.

Table 3. *Cholesterol and plasma and tissue ET-1 concentrations*

Parameters	Control	Hyperlipidemia			
		Placebo	Simvastatin treatment	Mevalonate treatment	Simvastatin + mevalonate treatment
Plasma ET-1, pg/ml	0.50±0.19	0.67±0.22	0.54±0.21	0.71±0.23	0.61±0.22
Tissue LV ET-1, pg/mg protein	3.77±1.56	6.34±1.09*	4.56±1.06†	5.98±0.86*	6.71±1.16*

Values are means ± SD. ET-1, endothelin-1. *$P < 0.05$ compared with control; †$P < 0.05$ compared with placebo, mevalonate, and simvastatin + mevalonate.

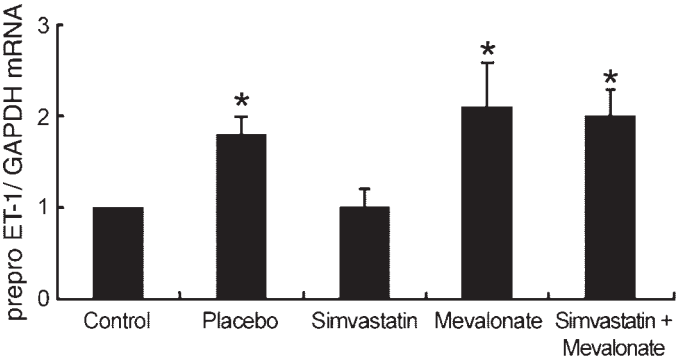

Fig. 2. LV prepro-endothelin (ET)-1 mRNA levels of control normolipidemic, placebo-treated hyperlipidemic, simvastatin-treated (1.2 $mg \cdot kg^{-1} \cdot day^{-1}$) hyperlipidemic, mevalonate-treated (50 $mg \cdot kg^{-1} \cdot day^{-1}$) hyperlipidemic, and combination-treated hyperlipidemic rabbits. Each mRNA was corrected for an mRNA level of GAPDH. Each column and bar represent the mean ± SD. $*P < 0.05$ compared with control and simvastatin-treated groups.

Other mechanisms. Our results are consistent with cardioprotection of simvastatin by chronic inhibition of ET-1 levels. However, there are possible other candidates modulating the antihypertrophic effects of simvastatin, such as angiotensin II and free radicals. The renin-angiotensin system is intimately linked to the endothelin axis. 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 (12). Angiotensin II has been shown to induce ET-1 synthesis in cardiomyocytes in vitro (12). Ishiye et al. (11) have shown in an in vivo study that the increase in ventricular ET-1 content can be inhibited by specific blockade of angiotensin type 1 receptors. Simvastatin has been shown to inhibit the activity of angiotensin II (27), thus accounting for the downregulation effect on ET-1 protein expression. In addition, blockade of free radicals alleviated the development of cardiac hypertrophy

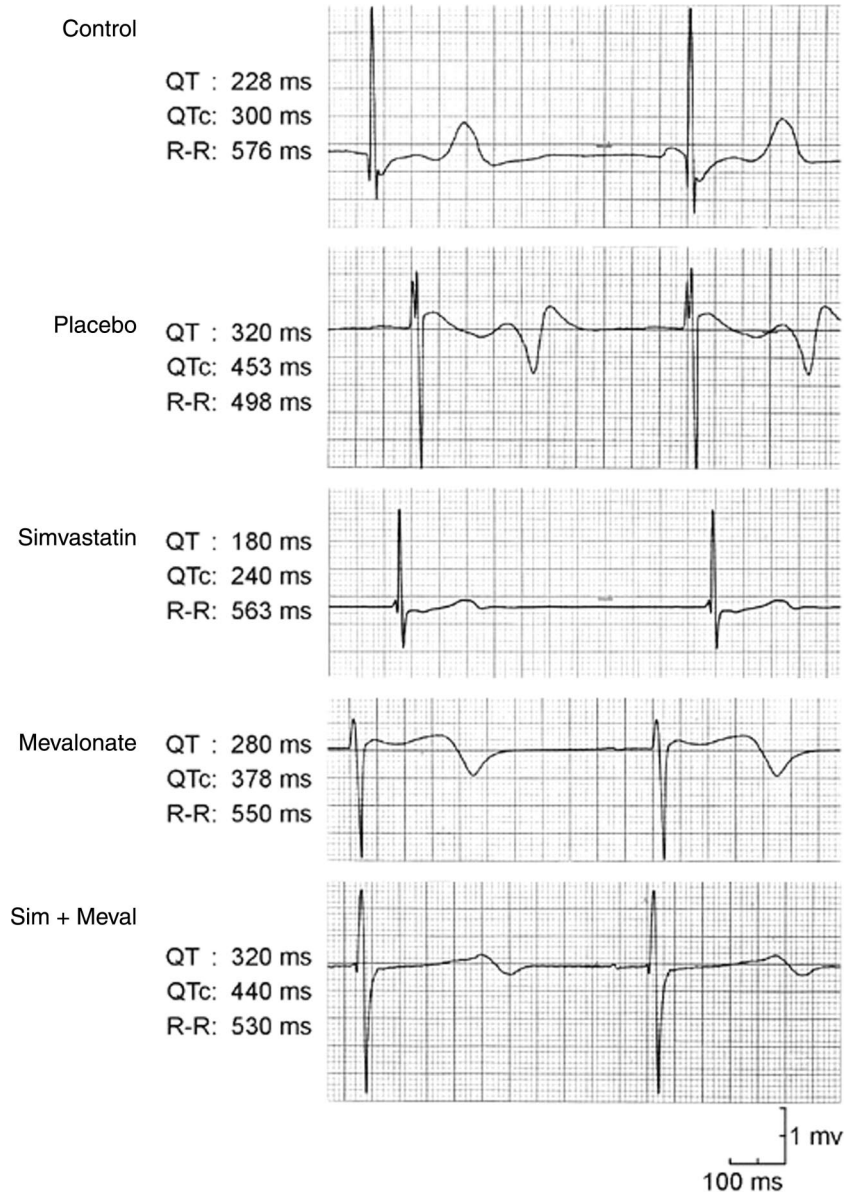

Fig. 3. Effect of hyperlipidemia and treatment on QT and QT_c intervals in spontaneously beating isolated Langendorff-perfused rabbit hearts. Note that hyperlipidemia caused a 153-ms increase in the QT_c interval compared with the control rabbit. Simvastatin (Sim) administration decreased the QT_c interval by 213 ms. Meval, mevalonate.

(16). Previous studies have demonstrated that statins decrease myocardial oxidative stress by inhibiting Rac-induced NAD(P)H oxidase activity (16, 34). Increased production of free radicals may induce cardiac hypertrophy via activation of mitogen-activated protein kinases (34). Thus simvastatin may attenuate cardiac hypertrophy by attenuated production of free radicals.

QT intervals. Because the electrocardiogram remains the clinical means of assessing the antiarrhythmic effect, we determined the antitropic effects of simvastatin on QT interval in isolated, Langendorff-perfused rabbit hearts. LV hypertrophy is associated with structural, biochemical, and electrophysiological abnormalities. The results were consistent with our previous studies showing an increase in QT interval observed in hypertrophic patients with aortic stenosis (36). Attenuated ventricular hypertrophy after simvastatin administration has benefits in electrocardiographic QT intervals. These cellular alterations may provide a basis for attenuated malignant ventricular arrhythmias. Because the drugs were discontinued 24 h before death, the QT changes observed in the simvastatin-treated rabbits would seem to be mediated by a reduction in LV mass rather than a direct pharmacological action of simvastatin. Myocyte hypertrophy may cause a lengthening of action potential duration via downregulation of the transient outward current, a prominent current in rabbit ventricular epicardium (7), as well as the delayed and background rectifier current (13, 14). The differential distribution in the density and regulation of functional ion channels within the myocardium in response to hypertrophy may exaggerate the prolongation of action potential duration (35). In addition, endogenous ET-1 may possess direct arrhythmogenic properties. ET-1 has been shown to directly prolong the action potential associated with a lengthening of the QT interval (35), which was consistent with the electrocardiographic effect of simvastatin by attenuation of ET-1 levels. Taken together, regardless of the relative importance of each of these factors, all of the changes caused by simvastatin are compatible with our understanding of beneficial effects on reduction of the QT interval.

Clinical implications. In this model, hypercholesterolemic rabbits not only developed vascular atherosclerotic lesions but also ventricular hypertrophy. Although our previous studies have shown in humans that hyperlipidemia was associated with increased LV mass (16), it is difficult to determine the direct effects of hyperlipidemia on cardiac hypertrophy because there are many confounding factors in interpreting LV mass in clinical settings. In the present study, therefore, we used the well-known hyperlipidemic model to investigate the direct effect of hyperlipidemia on myocyte hypertrophy.

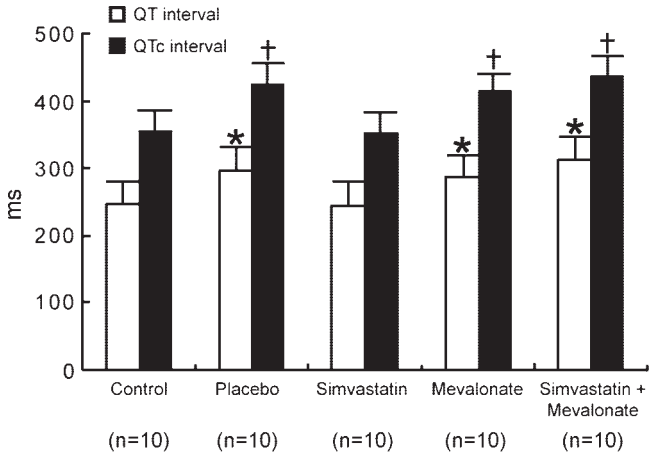

Fig. 4. QT and QT_c intervals. Data are expressed as means ± SD. $*P < 0.05$ compared with the normocholesterolemic control and hypercholesterolemic simvastatin-treated groups; $\dagger P < 0.05$ compared with the normocholesterolemic control and hypercholesterolemic simvastatin-treated groups.

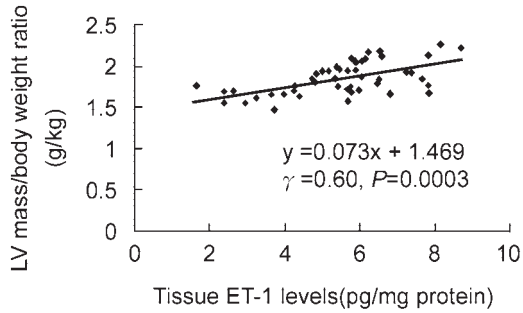

Fig. 5. ET-1 concentrations of the LV in relation to LV mass/BW. A significant correlation was found between the 2 variables ($r = 0.60$, $P = 0.0003$).

In the present study, we demonstrated that simvastatin administration attenuates the signaling of hypertrophy in hyperlipidemia rabbits, at least in part by modulating the activities of ET-1 pathways. The dose of simvastatin (1.2 $mg \cdot kg^{-1} \cdot day^{-1}$) in this study is considered safe and inferior to the previously used dose of 3.6 $mg \cdot kg^{-1} \cdot day^{-1}$, which was shown to induce regression of cardiac hypertrophy in load-induced hypertrophy (22). The used dose is similar to the conventional dose of simvastatin used in humans (up to 80 $mg \cdot kg^{-1} \cdot day^{-1}$), and thus this beneficial effect of simvastatin therapy may have important clinical implications.

In conclusion, the present study demonstrated that ventricular prepro-ET-1 mRNA is quantitatively increased by the high-cholesterol diet, resulting in the corresponding increase of tissue ET-1 levels, which resulted in the development of ventricular hypertrophy as confirmed by the administration of bosentan. Long-term cholesterol-lowering treatment with simvastatin starting at an early age retards the progression of LV hypertrophy probably through attenuation of ET-1 levels independent of lipid changes. These findings may be important in LV mass-related risk stratification of hyperlipidemic patients. Optimal treatment of hyperlipidemia should not only focus on adequate reduction of cholesterol but also on concomitant reduction of LV mass, which may help us understand the complexity of the interactions of these drugs with the heart. The long-term clinical benefit of the LV mass reduction obtained after chronic treatment with simvastatin has to be elucidated in clinical studies.

ACKNOWLEDGMENTS

Bosentan was a generous gift from Actelion Pharmaceuticals (Allschwil, Switzerland).

GRANTS

This study was supported by National Science Council Grants NSC90-2314-B-002-201 and NSC91-2314-B-384-009 (Taiwan, Republic of China) and Chi-Mei Medical Center Grants CMFHT 9201, CMFHR9303, CMFHR9307, and Chi-TMU9305.

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