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Chronic Monotherapy With Rosuvastatin Prevents Progressive Left Ventricular Dysfunction and Remodeling in Dogs With Heart Failure

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Objectives This study examined the effects of long-term monotherapy with rosuvastatin (RSV) on the progression of left ventricular (LV) dysfunction and remodeling in dogs with heart failure (HF).

Background 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors or "statins" possess other noncholesterol-lowering properties that include inhibiting proinflammatory cytokines, attenuating LV hypertrophy, and stimulating the release of bone marrow-derived stem cells (BMSCs).

Methods Twenty-one dogs with microembolization-induced HF were randomized to 3 months oral monotherapy with low-dose (LD) RSV (0.5 mg/kg once daily, n = 7), high-dose (HD) RSV (3.0 mg/kg once daily, n = 7), or to no therapy (control group, n = 7). The change (Δ) from pre- to post-therapy (treatment effect) in LV end-diastolic volume (EDV) and end-systolic volume (ESV) and ejection fraction (EF) was measured. Protein level of tumor necrosis factor (TNF)-α in LV tissue and the number of circulating Sca-1–positive BMSCs was also determined. Blood and LV tissue from 6 normal dogs was obtained and used for comparison.

Results There were no differences in ΔEDV, ΔESV, and ΔEF between control group and LD RSV. In contrast, ΔEDV and ΔESV were significantly lower, and ΔEF was significantly higher in HD RSV compared with control group. High-dose, but not LD, RSV also normalized protein levels of TNF-α and was associated with a significant increase in the number of circulating BMSCs.

Conclusions In dogs with HF, chronic therapy with HD RSV prevents progressive LV dysfunction and dilation. This benefit may be partly derived from normalization of TNF-α expression and partly from increased mobilization of BMSCs. (J Am Coll Cardiol 2007;50:551–7) © 2007 by the American College of Cardiology Foundation

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, commonly referred to as statins, modulate the activity of the enzyme that catalyzes the rate-limiting step in cholesterol biosynthesis (1). In recent years, evidence from several large clinical trials has shown that statins reduce morbidity and mortality in patients with virtually all stages of cardiovascular disease (CVD) (2). As a result, statins are currently recommended as a treatment of choice in the primary and secondary prevention of atherosclerotic CVD (3).

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Statins have also been shown to possess a host of other noncholesterol-lowering properties including ability to reduce systemic inflammation (4), improve endothelial function (5), stimulate angiogenesis (6), and mobilize bone marrow-derived stem cells (BMSCs) (7–9), all of which can contribute to the improvement of left ventricular (LV) function and prevention or attenuation of progressive LV remodeling in heart failure (HF). These properties constitute a rationale for the chronic use of this class of drugs in the prevention and treatment of HF. To date, only a few experimental (10–12) and observational (13–15) studies have reported a beneficial effect of statins in HF.

Rosuvastatin (RSV), a relatively new molecule, has emerged as an effective HMG-CoA reductase inhibitor with respect to lipid-lowering activity in low- to high-risk patients, showing a safety and tolerability profile similar to commonly used doses of other statins (16). In the present study, we investigated the effects of early long-term mono-

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Abbreviations and Acronyms

BMSC = bone marrow-derived stem cell
CVD = cardiovascular disease
ECM = extracellular matrix
EDV = end-diastolic volume
EF = ejection fraction
EPC = endothelial progenitor cell
ESV = end-systolic volume
HD = high-dose
HF = heart failure
HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A
LD = low-dose
LV = left ventricular/ventricle
MCSA = myocyte cross-sectional area
MI = myocardial infarction
MMP = matrix metalloproteinase
ODD = oxygen diffusion distance
RSV = rosuvastatin
TNF = tumor necrosis factor
VFIF = volume fraction of interstitial fibrosis
VFRF = volume fraction of replacement fibrosis

therapy with RSV on LV function and remodeling in dogs with intracoronary microembolization-induced HF.

Methods

Experimental model. The canine model of chronic HF used in this study was previously described in detail (17). In this preparation, LV dysfunction is produced by multiple intracoronary microembolizations that result in loss of viable myocardium. The model manifests many of the sequelae of HF observed in humans including marked and progressive depression of LV systolic and diastolic function, reduced cardiac output, and increased LV filling pressures. In the present study, 21 healthy mongrel dogs, weighing between 20 to 30 kg, underwent serial coronary microembolizations to produce HF. Embolizations were performed 1 to 3 weeks apart and were discontinued when LV ejection fraction (EF), determined angiographically, was between 30% and 40%. All the procedures were performed during cardiac catheterization under general anesthesia and sterile conditions. Animals were sedated with intravenous oxymorphone hydrochloride (0.22 mg/kg) and diazepam (0.17 mg/kg), and a plane of anesthesia was maintained with 1% to 2% isofluorane. The study was approved by Henry Ford Health System Institutional Animal Care and Use Committee and conformed to the National Institute of Health "Guide and Care for Use of Laboratory Animals" and the "Position of the American Heart Association on Research Animal Use."

Study protocol. Two weeks after the last embolization, dogs underwent a prerandomization left and right cardiac catheterization. One day later, dogs were randomized to 3 months oral monotherapy with low-dose (LD) RSV (0.5 mg/kg once daily, n = 7), high-dose (HD) RSV (3.0 mg/kg once daily, n = 7), or no therapy at all (control group, n = 7). At the end of the follow-up period, a final left and right cardiac catheterization was performed. At the end of the cardiac catheterization and while under general anesthesia, the chest was opened and the heart rapidly removed for histological and biochemical examination. Whole blood and LV tissue were obtained from all HF dogs as well as from 6 normal dogs for comparisons with control and RSV-treated dogs. Blood samples were used to measure total cholesterol and triglyceride.

Hemodynamic and angiographic measurements. Hemodynamic and angiographic measurements were made at baseline, before any microembolization, at the time of randomization, before initiation of therapy (pretreatment), and at the end of 3 months of therapy (post-treatment). Aortic and LV pressures were measured with catheter-tip micromanometers (Millar Instruments, Houston, Texas). Left ventriculograms were obtained with the dog placed on its right side and recorded on 35-mm cine film at 30 frame/s during the injection of 20 ml of contrast material (RENO-M-60, Squibb, Princeton, New Jersey). Correction for image magnification was made with a radiopaque calibrated grid placed at the level of the LV. Left ventricular end-systolic volume (ESV) and end-diastolic volume (EDV) were calculated from LV silhouettes using the area-length method, and EF was calculated as previously described (18). Stroke volume was calculated as the difference between LV EDV and ESV. Cardiac output was calculated as the product of stroke volume and heart rate. Extrasystolic and postextrasystolic beats were excluded from any analysis.

Histomorphometric measurements. From each heart, including hearts from normal dogs, 3 transverse slices (approximately 3-mm thick), 1 each from basal, middle, and apical thirds of the LV, were obtained. From each slice, transmural tissue blocks were obtained and embedded in paraffin blocks. Transmural tissue blocks were also obtained from the free wall segment of the slice, mounted on cork using Tissue-Tek embedding medium, and rapidly frozen in isopentane precooled in liquid nitrogen and stored at −70°C until used. The volume fraction of replacement fibrosis (VFRF); volume fraction of interstitial fibrosis (VFIF); myocyte cross-sectional area (MCSA), a measure of cardiomyocyte hypertrophy; capillary density; and oxygen diffusion distance (ODD) were measured as previously described (19).

Expression of tumor necrosis factor-α (TNF-α), matrix metalloproteinase (MMP)-2, and circulating BMSC counts. All tissue and blood samples were submitted for analysis without treatment regimen identifiers. Protein levels of TNF-α and MMP-2 were measured in LV homogenate by Western blots. Primary antibodies specific to each protein were diluted based on the supplier's instructions. In all instances, the antibody was present in excess over the antigen, and the density of each protein band was in the linear scale. Band intensity was quantified in densitometric units. Circulating, Sca-1–positive BMSCs were isolated from whole blood. Sca-1–positive BMSC cells include mesenchymal, multipotent adult progenitor, and Hoechst side population cells (20). Samples were centrifuged over a Ficoll-Hypaque gradient, and isolated cells were stained with primary and secondary antibodies containing immunofluorescence (SC-8266, Santa Cruz Biotechnology, Inc., Santa Cruz, California). Sca-1–positive BMSCs were counted using a hemocytometer coupled to fluorescent microscopy.