Effects of simvastatin on cardiac neural and electrophysiologic remodeling in rabbits with hypercholesterolemia

Yen-Bin Liu, MD, PhD,*† Yuan-Teh Lee, MD, PhD,* Hui-Nam Pak, MD, PhD,† Shien-Fong Lin, PhD,†§ Michael C. Fishbein, MD,‡ Lan S. Chen, MD,¶ C. Noel Bairey Merz, MD,† Peng-Sheng Chen, MD, FHRS†§

*From the *Division of Cardiology, Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University School of Medicine, †Division of Cardiology, Department of Medicine, Cedars-Sinai Research Institute, Cedars-Sinai Medical Center and David Geffen School of Medicine, UCLA, Los Angeles, California, ‡Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, UCLA, Los Angeles, California, ¶Department of Neurology, and §Krannert Institute of Cardiology, Division of Cardiology, Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana.*

BACKGROUND Significant cardiac neural and electrophysiologic remodeling occurs with hypercholesterolemia (HC). Whether simvastatin can reverse HC-induced remodeling is unclear.

OBJECTIVE The purpose of this study was to determine the mechanisms underlying the antiarrhythmic effects of statins.

METHODS Rabbits (N = 38) were fed HC chow (HC), standard chow (Control), HC chow followed by standard chow (Withdrawal), or HC chow and simvastatin (Statin) for 8 weeks. The hearts then were Langendorff-perfused for electrophysiologic studies. Nerves were identified by immunostaining of growth-associated protein-43 (GAP43) and tyrosine hydroxylase (TH). Action potential duration (APD) restitution in normal hearts with (N = 5) and without (N = 5) simvastatin therapy also was studied.

RESULTS Serum cholesterol levels (mg/dL) were 1,855 ± 533 in HC, 50 ± 21 in Control, 570 ± 115 in Withdrawal, and 873 ± 112 in Statin groups ($P < .001$). Compared with HC (16,700 ± 5,342; 12,200 ± 3,878 $\mu m^2/mm^2$), the Statin group had significantly reduced GAP43-positive (10,289 ± 3,393 $\mu m^2/mm^2$, $P = .03$) and TH-positive (7,685 ± 2,959 $\mu m^2/mm^2$, $P = .04$) nerve density, respectively. APD was longer in HC rabbits than in controls (192 ± 20 ms vs 174 ± 17 ms; $P < .03$). Withdrawal and Statin groups had less APD prolongation than HC group. Statin group has less repolarization heterogeneity than HC group ($P < .01$). Statin therapy flattened the slope of APD restitution in normal hearts. Ventricular fibrillation was either induced or occurred spontaneously in 79% of hearts in HC, 20% in Control, and 66% in Withdrawal groups. However, there was no VF in hearts of Statin group ($P < .001$).

CONCLUSION Simvastatin significantly reduced vulnerability to ventricular fibrillation via the mechanism of reduction of HC-induced neural and electrophysiologic remodeling.

KEYWORDS Arrhythmia; Statin; Lipids; Nervous system; Pathology

(Heart Rhythm 2009;6:69–75)

Introduction

Hypercholesterolemia (HC) in rabbits produces significant cardiac proarrhythmic neural and electrical remodeling.[1–4] Dyslipidemia increases the incidence of ventricular tachycardia/ventricular fibrillation (VT/VF) after acute myocardial infarction.[5] Lipid-lowering therapy using statins reduces VT/VF in patients with an implantable cardioverter-defibrillator (ICD).[6,7] Furthermore, statin use was associated with a significant reduction in sudden cardiac death in addition to preventing VT/VF.[7,8] Many other studies also found that statin use improved surrogate markers of arrhythmic risk in a population at certain risk for arrhythmic events.[9,10] These findings support the notion that statin use is antiarrhythmic. However, the mechanisms by which statins are antiarrhythmic remain unclear. Our previous study indicated that HC in rabbits can induce significant proarrhythmic neural and electrophysiologic remodeling.[1] Therefore, we hypothesize that statin is antiarrhythmic because it reverses the proarrhythmic remodeling induced by HC. The purpose of the present study was to test this hypothesis.

Methods

All animal study protocols were approved by the Animal Care and Use Committee and conformed to the guidelines of the American Heart Association. Three-month-old female New Zealand white rabbits were used for the study.

This study was supported by National Science Foundation (Taiwan) Grant NSC 93-2314-B-002-204 and NSC 94-2314-B-002-204 to Dr. Liu; a Piansky endowment to Dr. Fishbein; an endowment from The Women's Guild of Cedars-Sinai Medical Center, Los Angeles, California, to Dr. Bairey Merz; a Pauline and Harold Price endowment to Dr. Chen; a grant from Merck Inc.; NIH Grants P50 HL52319, HL66389, and HL71140; and the Ralph M. Parsons Foundation, Los Angeles, California. Merck Inc. donated the simvastatin and provided a research grant that partially supported this study. **Address reprint requests and correspondence:** Dr. Peng-Sheng Chen, Krannert Institute of Cardiology, 1801 North Capitol Avenue, E475, Indianapolis, Indiana 46202. E-mail address: chenpp@iupui.edu. (Received May 11, 2008; accepted October 1, 2008.)

Total serum cholesterol and triglyceride levels were determined before the animals were sacrificed.

Animals and diets

The rabbits were fed according to the following dietary protocols:

- Control group: Ten rabbits were fed with standard chow (Purina 5321) for 8 weeks.
- HC group: Fifteen rabbits were fed high fat and cholesterol chow (Purina 5321 + 0.5% cholesterol + 5% coconut oil) for 8 weeks.
- Withdrawal group: Six rabbits were fed with high fat and cholesterol chow for 6 weeks, followed by standard chow for 2 weeks.
- Statin group: Seven rabbits were fed with high fat and cholesterol chow and simvastatin 20 mg/day for 8 weeks.

To demonstrate the effects of statin on action potential duration (APD) restitution in rabbits without HC, two additional groups were included:

- Control group: Five rabbits fed with standard chow for 8 weeks.
- Statin group: Five rabbits fed with standard chow and simvastatin 20 mg/day for 8 weeks.

Immunocytochemical study

Ventricular tissues of rabbit hearts were fixed in 4% formalin for 1 hour, followed by 70% alcohol, and cross-sectioned from apex to base. Three sections of each heart were used for immunocytochemical studies. Details of the staining techniques have been reported elsewhere.[1,11] In brief, anti–growth-associated protein-43 (GAP43) and anti-tyrosine hydroxylase (TH) antibodies (monoclonal mouse anti-GAP43 and anti-TH, respectively, 1:50 dilution; Chemicon International, Inc., Billerica, MA, USA) were used for immunocytochemical staining. GAP43 is a marker for nerve sprouting.[12] TH is a marker of sympathetic nerves.[13] Nerve density was determined by computerized morphometry (Image-Pro Plus 4.0, Media Cybernetics, Inc., Bethesda, MD, USA).[1] The nerve density was the nerve area divided by the total area examined ($\mu m^2/mm^2$). Each slide was examined under a microscope with 20× objectives. For data analyses, the slides were divided into four quadrants. and one microscopic field with the highest nerve density from each quadrant was selected for nerve counting. Because three sections were taken for each heart, a total of 12 microscopic fields were counted. The average nerve density of all selected fields was used to represent the nerve density of that heart.

Isolated rabbit heart preparation and electrophysiologic study

The hearts were quickly removed during anesthesia and Langendorff-perfused with 37°C Tyrode's solution of the following composition (in mM): 125 NaCl, 4.5 KCl, 0.25 $MgCl_2$, 24 $NaHCO_3$, 1.8 NaH_2PO_4, 1.8 $CaCl_2$, and 5.5 glucose, equilibrated with 95% O_2 and 5% CO_2,pH 7.4. Coronary perfusion pressure was regulated between 80 and 95 mmHg. Pseudo-electrocardiogram (ECG) registered with a widely spaced bipole was used to monitor heart rhythm. Electrical stimuli of 2-ms duration and two to three times the diastolic threshold were delivered through a catheter in the right ventricle. Ventricular vulnerability was tested with single and double extrastimuli given after eight S_1 at a pacing cycle length (PCL) of 400 ms. Premature coupling intervals initially were paced at 380 ms, then shortened successively until the effective refractory period was reached or fibrillation was induced. After baseline studies, 0.1 μM isoproterenol and repeated programmed stimulations were given to induce arrhythmia.

Optical mapping studies were performed using a setup similar to that reported in a previous study.[14] The tissues were stained with 0.5 μM di-4-ANEPPS (Molecular Probes, Eugene, OR, USA). An electromechanical uncoupler (cytochalasin D 5 μM; Sigma Inc., St. Louis, MO, USA) was used to inhibit motion. Laser light of 532-nm wavelength (Verdi, Coherent Inc., Santa Clara, CA, USA) illuminated the tissues, and epifluorescence was collected through a long-pass filter with a cutoff wavelength of 600 nm (R60, Nikon, Melville, NY, USA) and a high-speed charge-coupled device (CCD) camera (420 frames/s, model CA D1-0128T, Dalsa Inc., Waterloo, Ontario, Canada). APD was measured at PCL of 400, 300, and 200 ms. One hundred points over the ventricular anterior wall were selected for APD analysis. A computer algorithm automatically determined APD_{80}. The standard deviation (SD) and the difference (between the longest and shortest APD_{80}) of APD_{80} were used to represent APD dispersion. APD restitution was determined by a dynamic pacing protocol.[15] The ventricles initially were paced at a constant cycle length of 400 ms. PCL was shortened successively in steps of 20 ms for PCL >200 ms and in steps of 10 ms for PCL <200 ms until PCL = 100 ms or loss of 1:1 ventricular capture. Restitution curves were constructed from APD_{80} and the preceding diastolic intervals at nine evenly spaced sites on the left ventricular anterior wall using data obtained during pacing. The maximal slope of APD restitution curve was determined after curve fitting by first-order exponential fitting with Origin software (MicroCal, Northampton, MA, USA). Conduction velocity (CV) along three different directions from the S1 pacing site at PCL of 300 ms was measured. The mean of the three values was used as the CV (cm/s) for that heart.[4,14]

Statistical analysis

Values are expressed as mean ± SD. Analysis of variance for repeated measurements with Newman-Keuls test was used to determinate the significance of between-group comparisons. $P \leq .05$ was considered significant.

Results

Table 1 summarizes the serum cholesterol and triglyceride concentrations in the first four groups. Serum cholesterol levels were significantly higher in the HC group than in the Control group. Statin treatment resulted in a 53% reduction in serum cholesterol levels compared with the HC group