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Effects of atorvastatin on the Th1/Th2 polarization of ongoing experimental autoimmune myocarditis in Lewis rats☆

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Abstract

Objective: Experimental autoimmune myocarditis (EAM) in rats is a T cell-mediated disorder and the involvement of Th1/Th2 unbalance has been demonstrated. This study was designed to test the hypothesis that 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitor, atorvastatin, affects T cell-mediated autoimmunity through modulating the balance of Th1/Th2 and reduces the severity of EAM.

Methods: Myocarditis was induced in 23 Lewis rats by injection of porcine cardiac myosin. High-dose (10 mg/kg/day) or low-dose (1 mg/kg/day) atorvastatin or vehicle was administered orally for 3 weeks to rats with EAM at the same time of immunization. Seven Lewis rats received neither immunization nor statins therapy were used as normal controls. On day 21 after immunization (the climax of inflammation), echocardiography was examined and the severity of myocarditis was evaluated by histopathological evaluation. The area ratio (affected/entire area percentage) of myocardial lesions was determined in histological sections. Heart weight/body weight ratio was determined and the serum lipid levels were measured. Levels of serum IFN-γ, IL-2, IL-4 and IL-10 were measured by ELISA.

Results: Cardiac function was improved in the two atorvastatin-treated groups compared to the untreated group. Heart weight/body weight ratio and the degree of inflammation were significantly lower in the two dosage statin-treated groups than that in the untreated one. Furthermore, treatment with atorvastatin decreased the expression levels of Th1 cytokine (IFN-γ and IL-2), and increased the expression levels of Th2 cytokine (IL-4 and IL-10). Atorvastatin attenuated the histopathological severity of myocarditis. Plasma lipid levels did not differ between the groups.

Conclusions: Atorvastatin ameliorates EAM by inhibiting T cell responses and suppressing Th1-type and inflammatory cytokines production and this activity is independent of cholesterol reduction, whereas Th2-type cytokines production was promoted. Atorvastatin may have beneficial effects on myocarditis by modulating the Th1/Th2 balance. These results demonstrate an important role of Th1/Th2 polarization in the pathogenesis of EAM and suggest that HMG-CoA reductase blockade may be a promising new strategy for the treatment of organ specific autoimmune diseases.

Keywords: EAM; Idiopathic dilated cardiomyopathy; HMG-CoA reductase inhibitor; Th1/Th2; Autoimmunity

1. Introduction

Myocarditis is the major cause of sudden unexpected death in patients less than 40 years of age and may account for up to 20% of mortality from cardiovascular causes. In 30% of cases, myocarditis can give rise to dilated cardiomyopathy with progression to heart failure, a major cause of morbidity and mortality among young adults, whose prognosis is poor with a 10-year survival rate of less than 40% [1]. Because the pathogenesis of myocarditis remains unclear, treatment is not directed at the disease itself but instead at managing the symptoms.

Experimental autoimmune myocarditis (EAM) induced in rats by injection of cardiac myosin is an animal model of human myocarditis and post-myocarditis dilated cardiomyopathy [2]. It has been reported that EAM in rats is a $CD4^+$ T cell-mediated disease and is thought to be related to Th1

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responses in the acute phase. Proinflammatory cytokines and Th1/Th2 imbalance play crucial roles in the induction of EAM and in the progression of myocardial injury in this disease [3].

Atorvastatin, a 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitor, may modulate Th1/Th2 imbalance and minimize proinflammatory cytokines [4]. Focusing on Th1/Th2 bias of peripheral lymphocytes and serum cytokine levels during disease progression, we tested the hypothesis that atorvastatin ameliorated EAM in rats attributing to the suppression of inflammatory cytokines and modulation of Th1/Th2 balance.

2. Methods

Thirty male Lewis rats, 6–8 weeks of age, were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. and maintained in our animal facilities. Atorvastatin calcium tablets were kindly provided by Pfizer Chemical Industries, Ltd. Throughout the studies, the protocol for these experiments was approved by the special committee on Animal Welfare of Harbin Medical University and all animals were treated humanly in accordance with the guidelines for the Care and Use of Laboratory Animals published by the U.S. National Institutions of Health (NIH Publication No. 85-23, revised 1996). EAM was induced in susceptible rats as previously described [3]. Twenty-three Lewis rats were immunized twice, with a 7-day interval between injections (on day 0 and day 7, respectively), by foot pad subcutaneous injection of 2 mg (11.7 mg/ml) of purified porcine cardiac myosin (Sigma Chemicals) emulsified with an equal volume of complete Freund's adjuvant (Sigma Chemicals) supplemented with *Mycobacterium tuberculosis* H37RA (Difco) at a concentration of 10 mg/ml. Statins therapy starts at the same time of immunization. Rats successfully induced with EAM were divided into three groups. High-dose atorvastatin (10 mg/kg/day, group H, $n = 9$), low-dose atorvastatin (1 mg/kg/day, group L, $n = 8$), or vehicle (group N, $n = 6$) was administrated orally by gastric gavage for 3 weeks from day 0 to day 21 after immunization. The other seven Lewis rats, received neither immunization nor statins therapy, were used as normal controls (group C, $n = 7$).

2.1. Echocardiography

Transthoracic echocardiography was performed on day 21 by using HP Sonos 2500 (Hewlett-Packard Co.) with a 10-MHz imaging linear scan probe transducer. We determined left ventricular end-diastolic diameter (LVEDd) and the left ventricular end-systolic diameter (LVEDs), and fractional shortening in the M-mode recordings. The diastolic interventricular septum thickness (IVSTd) and diastolic posterior wall thickness (PWTd) were measured at the time of LVEDd measurement.

2.2. Histopathology

Macroscopic findings were classified into five grades: 0, no inflammation; 1, presence of a small discolored focus; 2, presence of multiple small discolored foci; 3, diffuse discolored areas not exceeding a total of one-third of the cardiac surface; and 4, diffuse discolored areas totaling more than one-third of the cardiac surface. After heart weight was measured, the ratio of heart weight/body weight was evaluated. Then heart was fixed with perfusion of 3.8% formaldehyde, embedded in paraffin, sectioned into 4 μm slices, and stained with hematoxylin–eosin staining for histological examination. Microscopic scores of the severity of inflammation were graded into four grades: 0, no inflammation; 1, presence of a few small lesions, not exceeding 0.25 mm^2 in size; 2, presence of multiple small lesions or a few moderately sized lesions, not exceeding 6.25 mm^2; and 3, the presence of multiple moderately sized lesions or more, larger lesions. Areas of entire heart and region with infiltration by inflammatory cells and myocardial necrosis were determined with the NIH IMAGE 1.63 and the area ratio (affected area/entire area, as a percentage) was calculated. All histological/macroscopic evaluations were done in a double-blind manner by two professional staff.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Serum concentrations of interferon (IFN)-γ, interleukin (IL)-2, (IL)-4, and IL-10 were determined with an ELISA kit (BioSource International, Camarillo, CA) according to the manufacturer's instructions. The ratio of IFN-γ/IL-4 was used as indicator for Th1/Th2 polarization.

2.4. Plasma lipid levels

The lipid content of plasma was quantified enzymatically, using Boehringer Mannheim kits (Meylan, France), for total cholesterol (TC) and triglyceride (TG) and HDL-C levels in the four groups on day 21.

2.5. Statistical analysis

All data are expressed as mean ± S.E.M. Mean difference among four groups was tested by one-way ANOVA followed by Scheffe's modified F-test for multiple comparisons. The relationship of histopathological severity with Th1/Th2 cytokines level was evaluated by linear regression analysis. We used SPSS 11.0 to perform the statistical analysis. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Improvement of cardiac function by atorvastatin

No rat died prior to day 21. All rats of group N showed typical features of heart failure including massive pericardial effusion and pleural effusion. On day 21, the hearts of group N rats were enlarged and contained large grayish areas with