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Atorvastatin reduces cardiac and adipose tissue inflammation in rats with metabolic syndrome☆

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ABSTRACT

Background: Statins are strong inhibitors of cholesterol biosynthesis and help to prevent cardiovascular disease. They also exert additional pleiotropic effects that include an anti-inflammatory action and are independent of cholesterol, but the molecular mechanisms underlying these additional effects have remained unclear. We have now examined the effects of atorvastatin on cardiac and adipose tissue inflammation in DahlS.*Z-Lepr*^{fa}/*Lepr*^{fa} (DS/obese) rats, which we previously established as a model of metabolic syndrome (MetS).
Methods and results: DS/obese rats were treated with atorvastatin (6 or 20 mg kg^{-1} day^{-1}) from 9 to 13 weeks of age. Atorvastatin ameliorated cardiac fibrosis, diastolic dysfunction, oxidative stress, and inflammation as well as adipose tissue inflammation in these animals at both doses. The high dose of atorvastatin reduced adipocyte hypertrophy to a greater extent than did the low dose. Atorvastatin inhibited the up-regulation of peroxisome proliferator–activated receptor γ gene expression in adipose tissue as well as decreased the serum adiponectin concentration in DS/obese rats. It also activated AMP-activated protein kinase (AMPK) as well as inactivated nuclear factor–κB (NF-κB) in the heart of these animals. The down-regulation of AMPK and NF-κB activities in adipose tissue of DS/obese rats was attenuated and further enhanced, respectively, by atorvastatin treatment.
Conclusions: The present results suggest that the anti-inflammatory effects of atorvastatin on the heart and adipose tissue are attributable at least partly to increased AMPK activity and decreased NF-κB activity in this rat model of MetS.

1. Introduction

Metabolic syndrome (MetS) is a cluster of conditions that are associated with cardiovascular morbidity and mortality. Chronic inflammation is a common feature of MetS, with inflammatory signals emanating from visceral adipose tissue as the fat deposit expands as a result of chronic positive energy balance. Both adipocytes and macrophages within adipose tissue secrete numerous hormones and cytokines that contribute to the pathophysiology of MetS, and local inflammation within fat may trigger systemic inflammation and insulin resistance. The event that triggers such adipose tissue inflammation remains unclear, although fat-derived cytokines, such as leptin and interleukin-6, may participate directly in endothelial cell activation and inflammation in the cardiovascular system.

Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and thereby block cholesterol biosynthesis and help to prevent atherosclerotic cardiovascular disease, with their clinical benefit having been demonstrated in clinical trials such as the Heart Protection Study (HPS) [1] and West of Scotland Coronary Prevention Study (WOSCOPS) [2]. In addition to their cholesterol-lowering action, statins have been proposed to exert anti-oxidative and cardioprotective effects [3,4]. They also have anti-inflammatory effects [5] as well as activate AMP-activated protein kinase (AMPK) in vitro and in vivo [6]. Simvastatin exerts an anti-inflammatory effect through activation of the protein kinase Akt and subsequent generation of nitric oxide by endothelial nitric oxide synthase [7]. AMPK signaling inhibits inflammatory responses elicited by the nuclear factor–κB (NF-κB) system [8]. However, the molecular mechanisms underlying the anti-inflammatory actions of statins have remained largely unclear. Moreover, the effects of statins on

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glucose metabolism including insulin resistance have been unresolved [9]. Treatment with atorvastatin (25 or 50 mg kg^{-1} day^{-1}) for 3 weeks enhanced insulin sensitivity in a dose-dependent manner in Zucker fatty and lean rats [10]. Treatment with pravastatin (100 mg kg^{-1} day^{-1}) over 24 weeks had a moderate lipid-lowering effect as well as prevented the onset of diabetes mellitus and left ventricular (LV) diastolic dysfunction in association with amelioration of cardiac oxidative stress and normalization of adipokine profiles in a rat model of insulin resistance. In contrast, atorvastatin (100 mg kg^{-1} day^{-1}) had a pronounced lipid-lowering effect but did not retard the progression of insulin resistance or LV diastolic dysfunction in this model [11]. These findings thus suggest that the pleiotropic effects of statins might be dependent on the dose or duration of treatment in animal models of insulin resistance [12], with the mechanisms underlying such effects remaining unknown.

We have established the DahlS.*Z-Lepr*^{fa}/*Lepr*^{fa} (DS/obese) rat, derived from a cross between Dahl salt-sensitive and Zucker rats, as an animal model of MetS [13]. In addition to a MetS-like condition, which is characterized by adipocyte hypertrophy and adipose tissue inflammation, these rats develop salt-sensitive hypertension and LV diastolic dysfunction as well as LV hypertrophy and fibrosis, with these alterations being associated with increased cardiac oxidative stress and inflammation [14–17]. The aim of the present study was to examine and characterize the effects of atorvastatin at two different doses on cardiac and adipose tissue pathology and metabolism in DS/obese rats.

2. Methods

2.1. Animals

Male inbred DS/obese rats at the age of 9 weeks were randomly assigned to three groups: those treated with vehicle (MetS group) or with a low (6 mg kg^{-1}, ATV-L group) or high (20 mg kg^{-1}, ATV-H group) dose of atorvastatin. Age-matched male homozygous lean littermates of DS/obese rats (DahlS.*Z-Lepr*$^{+}$/*Lepr*$^{+}$, or DS/lean, rats) treated with vehicle served as control animals (CONT group). Atorvastatin (Pfizer, Peapack, NJ) or vehicle (0.5% methyl cellulose solution) was administered daily by oral gavage from 9 to 13 weeks of age. At 13 weeks, an oral glucose tolerance test (OGTT) and an insulin tolerance test (ITT) were conducted and organ harvesting was performed for analysis as described previously [17].

2.2. Echocardiography and hemodynamics

Systolic blood pressure (SBP) was monitored once a week in conscious animals by the tail-cuff plethysmography (BP-98A; Softron, Tokyo, Japan). At the age of 13 weeks, rats were subjected to transthoracic echocardiography [18] and subsequent cardiac catheterization [19]. A more detailed description of methodology is provided elsewhere [15].

2.3. Histology and immunohistochemistry

LV and visceral (retroperitoneal) fat tissue were processed for histological analysis as described previously [16]. Immunohistochemical staining for the monocyte-macrophage marker CD68 in LV and adipose tissue sections was also performed and all images were analyzed as described previously [16].

2.4. Biochemistry

Blood specimen was collected and metabolic parameters were measured as described [20]. The homeostasis model assessment of insulin resistance (HOMA-IR) and of β-cell function (HOMA-β) was calculated as described previously [21].

2.5. Assay of superoxide production

Superoxide production in LV tissue sections was measured by dihydroethidium (Sigma, St. Louis, MO) staining as described [22]. A more detailed description of principle and methodology is provided elsewhere [23].

2.6. Quantitative RT-PCR

Quantitative reverse transcription (RT) and polymerase chain reaction (PCR) analysis of LV and visceral fat tissue was performed as described previously [16] with specific primers for cDNAs encoding atrial natriuretic peptide (ANP) [18], brain natriuretic peptide (BNP) [18], collagen type I and type III [24], connective tissue growth factor (CTGF) [25], transforming growth factor–β1 (TGF-β1) [18], the $p22^{phox}$ [26], $gp91^{phox}$ [26], and Rac1 [14] subunits of NADPH oxidase, monocyte chemoattractant protein–1 (MCP-1) [25], cyclooxygenase-2 (COX-2) [26], adiponectin, peroxisome proliferator–activated receptor γ (PPARγ), and sterol regulatory element–binding protein–1c (SREBP-1c) (Supplemental Table S1). Reagents for detection of human 18S rRNA (Applied Biosystems, Foster City, CA) were used to quantify rat 18S rRNA as an internal standard.

2.7. Immunoblot analysis

Total protein was isolated from LV and visceral fat tissue and quantitated with the use of the Bradford reagent (Bio-rad, Hercules, CA). A more detailed description of methodology is provided elsewhere [20]. The membrane was incubated with a 1:200 dilution of mouse monoclonal antibodies to PPARγ (Santa Cruz Biotechnology, Dallas, TX) or with 1:1000 dilutions of rabbit monoclonal antibodies to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Cell Signaling Technology, Beverly, MA). The other antibodies were described previously [20,27].

2.8. Statistics

Data are expressed as means ± SEM. Differences among groups of rats at the age of 13 weeks were assessed with one-way factorial analysis of variance (ANOVA) and Fisher's multiple-comparison test. The time courses of sequential data were compared among groups by two-way repeated-measures ANOVA. A P value < 0.05 was considered statistically significant.

3. Results

3.1. Physiology and cardiac morphology and function

Body weight, food intake, and SBP were increased in DS/obese rats compared with DS/lean rats at 9 weeks of age and thereafter and were not affected by atorvastatin (Fig. 1A–C). At 13 weeks, there were no significant differences in the ratios of heart or LV weight to tibial length (indices of cardiac and LV hypertrophy, respectively) as well as those of visceral (retroperitoneal, epididymal, and mesenteric) or subcutaneous (inguinal) fat weight to tibial length among the MetS, ATV-L, and ATV-H groups (Supplemental Table S2).

Echocardiography revealed that the thickness of the interventricular septum and LV posterior wall, LV mass, relative wall thickness, LV fractional shortening, and LV ejection fraction were all similar in the MetS, ATV-L, and ATV-H groups (Supplemental Table S3). The deceleration time, isovolumic relaxation time and tau were shortened and both LV end-diastolic pressure (LVEDP) and the ratio of LVEDP to LV end-diastolic dimension, an index of LV diastolic stiffness, were reduced in the ATV-L and ATV-H groups in comparison with the MetS group (Supplemental Table S3).

3.2. Lipid and glucose metabolism

Both low and high doses of atorvastatin lowered the serum concentrations of total cholesterol, LDL-cholesterol, triglyceride, and free fatty acids (FFAs), but not that of HDL-cholesterol (Table 1). No significant difference in the fasting serum concentration of glucose was detected among the three experimental groups (Table 1). The fasting insulin concentration in serum and HOMA-IR and HOMA-β indices were all reduced in the ATV-L and ATV-H groups in comparison with the MetS group (Table 1). OGTT and ITT curves showed that both the glucose intolerance and insulin resistance apparent in MetS rats were ameliorated by atorvastatin treatment (Fig. 1D, E).

3.3. Cardiomyocyte hypertrophy and cardiac fibrosis

Microscopic examination showed that the cross-sectional area of LV cardiomyocytes in MetS rats was unchanged by atorvastatin treatment at either dose (Supplemental Fig. S1A, B). However, the abundance of ANP and BNP mRNAs in the heart was down-regulated by atorvastatin at both doses (Supplemental Fig. S1C, D). Azan-Mallory staining showed that fibrosis in perivascular and interstitial regions of the LV myocardium was reduced in the ATV-L and ATV-H groups compared with the MetS group (Supplemental Fig. S1E–H). The abundance of collagen types I and III, CTGF, and TGF-β1 mRNAs in LV tissue was also decreased

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