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Original article

Atorvastatin attenuates transplant-associated coronary arteriosclerosis in a murine model of cardiac transplantation

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Abstract

Accelerated coronary arteriosclerosis remains a major problem for the long-term survival of cardiac transplant recipients. However, the pathogenesis of transplant vasculopathy is poorly understood and there is no effective therapy. HMG-CoA reductase inhibitors, or statins, are widely prescribed to lower plasma cholesterol level. Accumulating evidence indicates that statins have various effects on vascular cells which are independent of their lipid-lowering effect. We investigated whether orally administered atorvastatin, one of the most potent statins, inhibits the development of intima hyperplasia in a mouse model of cardiac transplantation. Cardiac allografts from DBA mice were transplanted heterotopically into B10.D2 mice. Mice were administered either vehicle or atorvastatin everyday by gavage. Morphometrical analysis revealed that atorvastatin significantly reduced the development of coronary arteriosclerosis on the cardiac allografts harvested at one month. Immunohistochemical analysis revealed that atorvastatin attenuated infiltration of inflammatory cells with reduced expression of TGF-β and adhesion molecules. These results suggest that atorvastatin may be effective in preventing transplant-associated arteriosclerosis along with other immunosuppressive agents.

Keywords: Transplantation; Arteriosclerosis; Statin

1. Introduction

Although recent advances in immunosuppressive therapy have contributed to the dramatically enhanced early survival of cardiac transplant recipients [1], accelerated coronary arteriosclerosis has emerged as the major problem facing long-term recipient survival. Histopathological examination of the arteriosclerotic lesions in the long-term cardiac allografts reveals diffuse concentric lesions which are distinct from the common types of atherosclerotic lesions. The pathogenesis of graft vasculopathy is poorly understood and there is no effective therapy.

Statins are widely used to lower cholesterol levels, since they inhibit HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase that catalyzes the committed step in cholesterol biosynthesis [2]. Large trials have demonstrated that statins reduce the mortality and the incidence of myocardial or cerebral infarction [3–5]. Of note is the clinical evidence that the beneficial effects of statins can be observed even in patients with average cholesterol levels [6–8]. Experimental studies have reported lipid-independent effects by statins, including improvement of endothelial function and inhibition of inflammation [9]. It was reported that the administration of statins may inhibit inflammatory response [10] and proliferation of smooth muscle cells [11].

Here, we explored therapeutic potential of atorvastatin to prevent neointimal hyperplasia occurring in the coronary arteries of cardiac allografts. Our findings demonstrate that atorvastatin,

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a widely prescribed drug without severe adverse effects, may serve as a prophylactic treatment of transplant vasculopathy.

2. Materials and methods

2.1. Mice

DBA/2 (H-2^d) and B10.D2 (H-2^d) mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). DBA/2 and B10.D2 mice share major histocompatibility antigens, but differ in minor antigens. DBA/2 cardiac allografts transplanted into B10.D2 recipient mice without immunosuppressants develop severe vasculopathy which mimics those observed in human cardiac allografts [12,13]. Adult, male, 6- to 8-week old mice were used throughout the study. All mice were kept in microisolator cages on a 12-h day/night cycle and fed on regular chow. All procedures involving experimental animals were carried out in accordance with protocols approved in the local institutional guidelines for animal care of The University of Tokyo and complied with the "*Guide for the Care and Use of Laboratory Animals*" (NIH publication no. 86-23, revised 1985).

2.2. Heterotopic cardiac transplantation

Cardiac transplantation was performed according to the method of Corry and co-workers [14]. In brief, donors and recipients were anesthetized with 4% chloral hydrate at 0.01 ml/g body weight. Donor hearts were perfused with chilled, heparinized saline via the inferior vena cava. The aorta and pulmonary artery of the donor hearts were anastomosed to the abdominal aorta and inferior vena cava of the recipient mice using a microsurgical technique. Vehicle (0.5% carboxymethyl cellulose) or atorvastatin (10 mg/kg/day) was administered to the recipient mice everyday by gavage starting five days before the transplantation.

2.3. Histological examination

Ten mice (vehicle-treated group, $n = 5$; atorvastatin-treated group, $n = 5$) were sacrificed at 30 days after transplantation for histological examination. After perfusion with heparinized saline followed by 10% neutralized buffered formalin at 130 cmH_2O, cardiac allografts were removed, fixed in 10% neutralized buffered formalin and embedded in paraffin. Serial sections (5 μm) were deparaffinized, and stained with hematoxylin and eosin (H&E) or with elastic van Gieson for histological evaluation of cellular rejection and graft vasculopathy.

2.4. Morphometric analysis

Morphometric analysis of the graft coronary arteries was performed as previously described elsewhere [15,16]. Transverse sections were made every 100 μm. Serial sections (5 μm) were deparaffinized and stained with elastic van Gieson. Middle-sized coronary arteries were analyzed ($n = 10$ arteries for each graft). The image was digitized using a Fujix Digital Camera (HC-300/OL, Fuji film Co., Tokyo) on a PROVIS AX80 microscope (Olympus, Tokyo). The lumen, internal elastic lamina, and external elastic lamina were traced using an image analysis software (Image-Pro Plus Version 4.5, Media Cybernetics, USA). The intimal area was determined by subtracting the area of the lumen from the area enclosed by the internal elastic lamina. The medial area was determined by subtracting the area enclosed by the internal elastic lamina from the area enclosed by the external elastic lamina. The intima/media ratio was averaged for each graft.

2.5. Immunohistochemical analysis

Immunohistochemistry was performed as previously described [17]. Paraffin-embedded sections (5 μm thick) were incubated with primary antibodies against α-smooth muscle actin (SMA, clone 1A4, Sigma), CD31 (clone MEC13.3, BD Biosciences Pharmingen, San Diego, CA), CD45 (clone 30F11.1, BD Biosciences Pharmingen), macrophage (clone F4/80, Serotec Ltd, Oxfordshire, UK), intercellular adhesion molecule (ICAM)-1 (CALTAG Laboratories, Burlingame, CA), or transforming growth factor (TGF)-β1 (Santa Cruz Biotechnology, Santa Cruz, CA). Antibody distribution was visualized by avidin–biotin complex technique and Vector Red substrate (Vector). Sections were counterstained with hematoxylin.

2.6. Statistical analysis

All data were expressed as the mean value ± S.E.M. Mean values were statistically compared by Student's t test. A P value of <0.05 was considered to be significant ($n = 5$ for each group).

3. Results

3.1. Inhibition of neointimal hyperplasia by atorvastatin

Cardiac allografts from DBA/2 mice were heterotopically transplanted into B10.D2 mice. The recipient mice were treated with either vehicle (0.5% carboxymethyl cellulose) or atorvastatin (10 mg/kg/day) everyday by gavage. At 30 days, subepicardial large arteries and intramyocardial arteries showed significant luminal narrowing due to neointimal hyperplasia. Neointima in the coronary artery was mainly consisted of α-SMA positive cells (Fig. 1). CD45-positive hematopoietic cells were also present in neointima and media in both groups. The luminal side was coated by endothelial cells in both groups as determined by anti-CD31 immunostaining. There was no apparent difference between the two groups in the cellular composition of neointima. The degree of neointimal hyperplasia was evaluated in middle-sized coronary arteries. Morphometric analysis revealed that atorvastatin significantly attenuated neointimal thickening (intima/media ratio: vehicle, 1.61 ± 0.28; atorvastatin, 0.88 ± 0.23, $P < 0.01$) (Fig. 2).

3.2. Anti-inflammatory effects of atorvastatin

To investigate the mechanism by which atorvastatin attenuates transplant-associated arteriosclerosis in murine model of