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Atorvastatin attenuates post-implant tissue degeneration of cardiac prosthetic valve bovine pericardial tissue in a subcutaneous animal model

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Abstract

Aims: The aim of our study was to examine the effects of statin therapy (atorvastatin) on post-implant structural changes of bovine pericardial tissue in a subcutaneous animal model.
Methods and results: Sixty male C57BL/6 mice underwent subcutaneous dorsal implantation of bovine pericardial fragments. Animals were randomized to treatment with atorvastatin (50 mg/kg) (statin group — SG) or to vehicle (control group — CG). After 1.5 months, all fragments were explanted and submitted to histopathological assessment (semi-quantitative analysis) to elucidate extent of inflammatory infiltrate, signs of tissue injury, or presence of microcalcification. Calcium determination of the implanted pericardial tissue was also performed by inductively coupled plasma mass spectrometry (ICP-MS) assessment.

ICP-MS analysis showed that pericardial fragments in SG had significantly ($p<0.01$) less calcium content than CG (625±142 vs. 962±590 μg/g, respectively). Light microscopy showed marked inflammatory infiltrates and tissue injury of pericardial specimens in CG animals, whereas SG animals maintained a better preserved original pericardial structure.
Conclusions: Our findings indicate that atorvastatin significantly attenuates the post-implant structural degeneration of artificial valve bovine pericardial tissue in a subcutaneous animal model. Further observations are mandatory to assess the effects of statins on the implanted bioprosthetic valve tissue in the blood circulation.

Keywords: Heart valve-bioprosthesis; Statin; Pericardium; Calcification

1. Introduction

Since the first implants of heterologous tissue valve prostheses in the sixties, improvements of tissue valve design and manufacturing have been outstanding. Biological prostheses today offer a reliable and effective

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solution for valve replacement to treat cardiac native valve dysfunction without the need for anticoagulation and better hemodynamics as compared to mechanical substitutes. Postoperative tissue degeneration, however, remains a critical issue and represents the most frequent cause of reoperation [1–15]. Several agents or new tissue-processing techniques have been variably applied during tissue valve manufacturing and proposed to reduce or prevent tissue degeneration, but no postoperative therapy has been specifically and prospectively investigated to counteract biological tissue derangement, calcification and inflammation. Similarities of the mechanisms and risk factors among atherosclerosis, native valve calcification, and postoperative tissue valve degeneration have recently been proposed [1–4]. This appealing concept might shed new light in the prevention and treatment of the post-operative failure of tissue valves. A few clinical studies have shown that the use of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase inhibitors, commonly known as statins, have reduced calcific degeneration of native aortic valves [16–20]. A few retrospective clinical series have also addressed the potential relationship between HMG-CoA inhibitors and tissue prosthetic valves, documenting a beneficial role of statins with regard to progression of bioprosthetic valve degeneration both in the presence of a hyperlipidic state or independently from serum cholesterol levels [1,21–23]. No study, however, has been designed, to our knowledge, to prospectively analyze the effects of HMG-CoA reductase inhibitors on the structural changes of the biological tissue of cardiac valve substitutes. Therefore, the aim of our investigation was to test, in a subcutaneous animal model, the effects of a specific statin therapy, namely atorvastatin (AT), on post-implant tissue changes of a commonly used bovine pericardial tissue.

2. Materials and methods

All animals were used in accordance with the 1996 NRC Guide for the Care and use of Laboratory Animals. The study was approved by the Laboratory Institutional Research and Ethics Committees of the University of Parma, School of Medicine.

2.1. Animals and study protocol

Sixty male C57BL/6 mice, 9 weeks old (range 110 to 120 days), were obtained from Charles River Laboratories Italia S.p.A. (Lecco, Italy). On day 0, two 8-mm discs of glutaraldehyde-fixed bovine pericardium (Perimount Valve, Edwards Lifesciences, Irvine, CA) were aseptically prepared and rinsed according to the manufacturer's recommendations and implanted into two specular (symmetrical) dorsal subcutaneous pockets in all the animals. The mice were anesthetized by an intraperitoneal tribromoethanol (800 μL, with a 70 mM solution) injection and subsequently a skin incision (about 2 cm) was made in order to insert the two fragments of pericardium in the back of each animal. On day 1 after surgery, mice were randomly assigned to treatment with AT (50 mg/kg, statin group — SG) or vehicle by gavage (control group — CG) for 30 days. This dose of AT in mice has been shown to correspond to a daily dose of 80 mg of atorvastatin in humans [24,25].

All animals were on normal diets (standard mice diet according to the Randoin–Causeret, Laboratory Piccioni, Gessate, Milan, Italy).

On day 45, all the animals were sacrificed by a lethal intraperitoneal dose of tribromoethanol (2.400 μL, 70 mM solution) and the pouches of bovine pericardium were retrieved. Each of these fragments was cut into two parts, one of which was immediately fixed in a 10% formalin buffered solution for 24 h. The other sides of the pouches were used for specific calcium content assessment.

2.2. Histomorphometric assessment

After the fixation process, each sample was embedded in a paraffin tissue block from which 5-μm thick histological sections were obtained. Following standard procedures, two of these sections were stained with both hematoxylin-eosin and the Von Kossa method for calcium. The remaining unstained sections were used for immunohistochemical assessment of the inflammatory infiltrate. The following primary antibodies were tested using the manufacturers' protocols: anti-CD3 (Novocastra, Newcastle, UK, dilution 1:100), anti-CD20 (DakoCytomation, Glostrup, Denmark, 1:50) anti-CD68 (DakoCytomation, 1:50), anti-CD138 (Novocastra, 1:50) and anti-Myeloperoxidase (DakoCytomation, 1:300). The negative control procedure omitted the primary antibody.

A semiquantitative grading was used in order to assess, along the pericardial fragments, the inflammatory infiltrate (0 = absent, 1 = slight, 2 = moderate and 3 = severe), necrosis (0 = absent, 1 = focal, 2 = diffuse) and microcalcifications (0 = absent, 1 = focal, 2 = diffuse). In addition, the proportions of the various inflammatory elements were quantitatively evaluated on the histological sections tested with anti-CD3, CD20, CD68, CD138 and myeloperoxidase primary antibodies. This morphometric measurement was carried out using a light microscope (Olympus BX 51, Tokyo, JPN, magnification x 1000) and by counting the total number of brown-stained cells in 30 histological fields (1.2 mm^2). Finally, the percentages of the different subsets were calculated by dividing the number of the single cell types by the total number of immunostained elements and then multiplying that value by 100.

Pathologists were blinded to the kind of treatment the animals received along the study course.

2.3. Tissue analysis of mineral content

The explanted valve tissues were dried at 100 °C for 2 h and then were digested by hyperpuric nitric acid for 30 min