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Lysophosphatidylcholine activates the Akt pathway to upregulate extracellular matrix protein production in human aortic valve cells

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ABSTRACT

Background: Overproduction of extracellular matrix (ECM) protein by aortic valve interstitial cells (AVICs) plays an important role in valvular sclerosis (thickening) associated with the early pathobiology of aortic stenosis. Accumulation of oxidized low-density lipoprotein (oxLDL) is observed in sclerotic aortic valve and may have a mechanistic role in valvular disease progression. Lysophosphatidylcholine (LysoPC) is a component of oxLDL and has multiple biological activities. This study was to test the hypothesis that oxLDL and LysoPC upregulate ECM protein production in human AVICs.

Methods and results: AVICs were isolated from normal human aortic valves. Cells were treated with oxLDL (40 μg/mL) or LysoPC (40 μmol/L). Immunoblotting was applied to analyze ECM proteins (collagens I and III and biglycan) in cell lysate and Picrosirius red staining was used to examine collagen deposition. Both oxLDL and LysoPC upregulated the production of biglycan and collagen I. The upregulation of ECM proteins by LysoPC was preceded by the phosphorylation of Akt and ERK1/2. Inhibition of Akt markedly reduced the effect of LysoPC on ECM protein production and collagen deposition. However, inhibition of ERK1/2 had no effect.

Conclusions: LysoPC upregulates the production of biglycan and collagen I in human AVICs and may mediate the effect of oxLDL on ECM protein production. The Akt pathway appears to be critical in mediating the effect of LysoPC. oxLDL accumulation and generation of LysoPC in the aortic valve tissue may contribute to the mechanism of valvular sclerosis associated with the development and progression of aortic stenosis.

Introduction

Aortic stenosis involves valvular sclerosis and calcification.[1] Aortic valve sclerosis (AVS), defined as leaflet thickening without obstruction of left ventricular outflow, occurs in a large number of people aged ≥65 y and is an early sign of the development of aortic stenosis.[1] Although moderate AVS does not cause left ventricular outflow obstruction, it is associated with an increased risk of cardiovascular death and myocardial infarction.[2] Thus, suppression of AVS progression will reduce

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the risk of associated cardiovascular conditions and prevent the development of severe aortic stenosis. Understanding the cellular and molecular mechanisms of AVS is required for pharmacologic suppression of the development and progression of AVS.

Increasing evidence shows that AVS is an active process that has a similarity to the pathobiological process of atherosclerosis, including lipoprotein deposition, chronic inflammation, and extensive remodeling of extracellular matrix (ECM).[3,4] Oxidized low-density lipoprotein (oxLDL) has the proinflammatory and growth-stimulating properties[5] and is involved in the pathobiology of atherosclerosis.[6] Several studies have found oxLDL accumulation in sclerotic aortic valves.[7] Our previous studies found that oxLDL upregulates the expression of bone morphogenetic protein-2 (BMP-2) in human aortic valve interstitial cells (AVICs)[8,9] and human coronary artery endothelial cells.[10] Furthermore, oxLDL synergizes with lipopolysaccharide to elicit augmented AVIC osteogenic responses.[11] It appears that oxLDL has an impact on human AVIC pro-osteogenic activity. Currently, the effect of oxLDL and its components on the production of ECM proteins by aortic valve cells is unclear although overproduction of ECM proteins contributes to the pathobiology of AVS.

Lysophosphatidylcholine (LysoPC) is the main phospholipid component of oxLDL. It is a hydrolysis product of phosphatidylcholine (PC) in oxLDL.[12] In vertebrate plasma, increased levels of LysoPC have been found in atherosclerotic vascular tissue. LysoPC can induce the pro-osteogenic responses in vascular smooth muscle cells and AVICs.[13,14] Thus, it possesses a similar activity as oxLDL.

Growing evidence shows that the accumulation of ECM proteins is a characteristic change in AVS.[15] Human AVICs, primarily fibroblast-like cells, and myofibroblasts are a population of resident cells in the aortic valve tissue and actively synthesize ECM proteins, including biglycan (BGN; a stationary component of the ECM), collagen I, collagen III, and collagen IV.[15,16] Currently, the effect of oxLDL and LysoPC on the production of ECM proteins in human AVICs is unclear.

We hypothesized that oxLDL is capable of elevating human AVIC activity to overproduce ECM proteins. The purpose of this study was to determine (1) the effect of oxLDL on ECM protein production in human AVICs, (2) whether LysoPC may mimic the effect of oxLDL in human AVICs, (3) whether oxLDL and LysoPC influence AVIC production of ECM proteins by inducing myofibroblastic transition, and (4) what signaling mechanism upregulates ECM protein production in human AVICs.

Materials and methods

Materials

Antibody against BGN was purchased from R&D System (Minneapolis, MN). Antibodies against collagen I and collagen III were purchased from Abcam (Cambridge, MA). Antibodies against α-smooth muscle actin (α-SMA), phosphorylated Akt (Ser473 or Thr 308), total Akt, phosphorylated ERK1/2, and total ERK1/2 were purchased from Cell Signaling Technology (Beverly, MA). Antibody against β-actin was purchased from Santa Cruz Biotechnology Inc (Santa Cruz, CA). Medium 199 was purchased from Lonza (Walkersville, MD). ERK1/2 inhibitor PD98059 was purchased from EMD Millipore (Billerica, MA). MK2206, a specific Akt inhibitor, was purchased from Selleckchem (Houston, TX). OxLDL was purchased from Alfa Aesar (Lancashire, UK). LysoPC and all other chemicals and reagents were purchased from Sigma–Aldrich Chemical (St Louis, MO).

Cell isolation and culture

This study was approved by the Colorado Multiple Institutional Review Board of the University of Colorado Denver. Normal aortic valve leaflets were collected from the explanted hearts of six heart transplant recipients (four males and two females; mean age, 59 ± 8.1 y) with cardiomyopathy. The valve leaflets were thin and had no histologic abnormality. All patients gave informed consent for the use of their aortic valves for this study.

AVICs were isolated and cultured using the previously described method[17] with modifications.[18] Briefly, valve leaflets were subjected to an initial digestion with a high concentration of collagenase (2.5 mg/mL) to remove endothelial cells. Then, the remaining tissue was treated with a low concentration of collagenase (0.8 mg/mL) to free the interstitial cells. Cells were collected by centrifugation and cultured in M199 growth medium containing penicillin G, streptomycin, amphotericin B, and 10% fetal bovine serum. AVIC isolates obtained using this protocol are a lack of endothelial cells as verified by von Willebrand factor staining. Each isolate from a separate donor is used as a cell line. Thus, six normal AVIC cell lines were used in this study. Cells of passages 3-6 were treated when they reached 80%-90% confluence.

To determine the effect of oxLDL on the production of α-SMA, BGN, and collagens I and III, cells were treated with oxLDL (40 μg/mL) for 24-72 h. LysoPC from egg yolk was dissolved in ethanol to make a stock solution. To determine the effect of LysoPC on the production of these proteins, cells were treated with LysoPC (20 or 40 μmol/L; 7.1 or 14.2 μg/mL; final concentration of ethanol being 0.12% or 0.24%) for 24 or 48 h. Protein levels of α-SMA, BGN, collagen I, and collagen III in cell lysates were assessed by immunoblotting. Cells treated with 40 μmol/L of LysoPC for 1-8 h were used to analyze the phosphorylation of ERK1/2 and Akt. To determine the role of the ERK1/2 and Akt pathways in modulation of ECM protein production, cells were treated with ERK1/2 inhibitor (PD98059, 25 μmol/L) or Akt inhibitor (MK2206, 5.0 μmol/L) 1 h before the exposure to LysoPC. In additional experiments, AVICs were treated with LysoPC (40 μmol/L) for 7 d with or without Akt inhibitor. Picrosirius red (PSR) staining was applied to evaluate the role of the Akt pathway in mediating collagen deposition.

Immunoblotting

Immunoblotting was applied to analyze the levels of α-SMA, BGN, collagen I, collagen III, phosphorylation of ERK1/2, total ERK1/2, phosphorylation of Akt, and total Akt, with β-actin as the loading control. Cells were lysed in a sample buffer (100 mmol/L Tris-HCl, pH 6.8, 2% sodium dodecyl sulfate, 0.02% bromophenol blue, and 10% glycerol). Protein samples were separated on gradient (4%-20%) mini-gels and