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Probucol mediates vascular remodeling after percutaneous transluminal angioplasty via down-regulation of the ERK1/2 signaling pathway

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Abstract

Although probucol is known to prevent restenosis by regulating vascular remodeling after percutaneous transluminal coronary angioplasty, the mechanisms remain unclear. The present study was designed to investigate whether probucol mediates vascular remodeling via the extracellular signal-regulated kinase 1/2 (ERK1/2) signalling pathway. A rabbit restenosis model was used, in which the New Zealand white rabbits received angioplasty with a 3.5 F angioplasty balloon catheter and the proliferation and migration of smooth muscle cells (SMCs) was induced by oxidized low-density lipoprotein (ox-LDL). We evaluated several vascular remodeling parameters and found that probucol prevented lumen restenosis and mediated expansive remodeling with a remodeling index greater than 1 and that the proliferation and migration of SMCs was inhibited. Based on Western blot analyses, probucol decreased the expression of phospho-mitogen-activated protein kinase kinases 1 (p-MEK1) and phospho-ERK1/2 and enhanced the expression of mitogen-activated protein (MAP) kinase phosphatase-1 (MKP-1) and caveolin-1. Cells treated with the MEK1 inhibitor PD98059 demonstrated a remarkable suppression of the effects of probucol. Furthermore, immunofluorescence analysis showed that probucol inhibited the activation of ERK1/2 by preventing its translocation to the nucleus. It was also found that c-myc expression in aortic tissue after angioplasty and the activator protein 1 (AP1) activity in SMCs induced by ox-LDL were decreased with probucol treatment. In conclusion, probucol mediated vascular remodeling to prevent restenosis after angioplasty by down-regulating the ERK1/2 signaling pathway.

Keywords: Probucol; Vascular remodeling; ERK1/2; VSMC

1. Introduction

Probucol, a powerful antioxidant, can significantly reduce coronary restenosis after percutaneous transluminal coronary angioplasty (Jackson and Pettersson, 2001) and may exert its anti-restenotic effects by improving vascular remodeling after angioplasty (Cote et al., 1999). However, the mechanisms by which probucol prevents coronary restenosis and regulates vascular remodeling are not well characterized.

Vascular remodeling plays an important role in restenosis after balloon angioplasty (Madrid et al., 1998; Wyttenbach et al., 2004). The remodeling of the vessel wall in response to injury involves the proliferation, migration and apoptosis of smooth muscle cells (SMCs) (Faxon et al., 1997), neointimal thickening, adventitial fibrosis (Pels et al., 1999), and the changes in the extracellular matrix. The hyperplasia and migration of SMCs are the major cause of coronary artery neointima formation and remodeling after percutaneous transluminal coronary angioplasty (Christen et al., 2001). It has been reported that extracellular signal-regulated kinase 1/2 (ERK1/2) is a signal transduction pathway associated with the proliferation of SMCs (Hirata et al., 2000). Furthermore, phospho-p42/p44 extracellular signal-regulated kinase activities have been suggested to contribute to coronary microvascular remodeling in rats (Kobayashi et al.,

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2001). Therefore, in the present study, we investigated the effect of probucol on the vascular remodeling after percutaneous transluminal angioplasty and explored whether the effects are related to the down-regulation of the ERK1/2 signal transduction pathway.

2. Materials and methods

2.1. Chemicals and drugs

Probucol and vitamin E (VitE) were purchased from Sigma (USA). Oxidized low-density lipoprotein (ox-LDL) was provided by the Department of Biochemistry, Peking University (China). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenylterazoliumbromide) and dimethylsulfoxide (DMSO) were products of AMERSCO (USA). ERK1/2, phospho-ERK1/2 (p-ERK1/2), mitogen-activated protein (MAP) kinase phosphatase-1 (MKP-1), caveolin-1, phospho-mitogen-activated protein kinase kinases 1 (p-MEK1) and c-myc antibodies were purchased from Santa Cruz Biotechnology (USA). The secondary antibody was purchased from DAKO (Denmark). MEK1 inhibitor PD98059 (2′-amino-3′-methoxyflavone, molecular formula: $C_{16}H_{13}NO_3$) was from Cell Signaling (USA). DMEM cell culture medium was obtained from GIBCO BRL (USA). Fetal calf serum was purchased from Intergen (USA).

2.2. Animal models

Thirty-five male New Zealand white rabbits weighing 1.5–2.0 kg were fed with 2% (weight/weight) cholesterol-enriched chow (Lau et al., 2003) except that eight rabbits were assigned to a control group and received normal chow. Prior to angioplasty, the rabbits were anesthetized with pentobarbital (30 mg/kg) and received heparin sulfate 300 U/kg through the marginal ear vein. After the left femoral artery was exposed, a 3.5 F angioplasty balloon catheter was introduced through the left femoral artery and advanced into the thoracic aorta. The balloon was inflated to 4–6 bars pressure with air and withdrawn slowly to the femoral artery three times in order to remove the endothelium. The left femoral artery was then ligated, and rabbits continued to receive the 2% cholesterol-containing diet for 4 weeks. Twenty-four rabbits survived the procedure. The survivors were then randomly divided into the following groups: (1) percutaneous transluminal angioplasty group (PTA group, $n=8$, without drug administration.); (2) probucol group ($n=8$); (3) vitamin E group ($n=8$). Penicillin (15,000 U) was administrated intramuscularly for 3 days after surgery. The percutaneous transluminal angioplasty was performed with a 3.5 F angioplasty balloon catheter 4 weeks after endothelial denudation. Then the rabbits were fed with normal chow. The rabbits were sacrificed 2 weeks after percutaneous transluminal angioplasty. The injured thoracic artery was rapidly removed for organ chamber experiments, morphometric analysis and other experiments. The normal control rabbits were anesthetized with pentobarbital (30 mg/kg), the left femoral arteries were exposed and ligated without endothelium injury and received penicillin (15,000 U) intramuscularly for 3 days. Four weeks later, the right femoral artery was done as before. The normal control rabbits were fed with a normal chow throughout. The Medical Laboratory Animal Management Committee of Xiangya School of Medicine, Central South University approved all animal procedures. Probucol 1% (weight/weight) and vitamin E (500 mg/kg/day) were mixed with chow respectively. Drug administration started 1 week before percutaneous transluminal angioplasty.

2.3. Cell culture

Human vascular smooth muscle cells (American Type Culture Collection) were cultured in DMEM supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin at 37 °C with 95% humidity and 5% CO_2. Vascular smooth muscle cells (VSMCs) were induced by ox-LDL (50 mg/l) (Yan et al., 2002; Batt et al., 2004) and treated with probucol (10 μM–80 μM) or vitamin E (50 μM) (Fukuzawa et al., 2004; Mamputu et al., 2006).

2.4. Determination of vascular restenosis and remodeling

After the thoracic arteries were removed, some arterial segments were fixed in 10% buffered formalin and embedded in paraffin. Hematoxylin/eosin and elastic fiber staining were performed. The following parameters of the lesion site were measured by a computerized digitizer system (SPOT Version 3, Diagnostic Instrument, Inc., USA): the bore and the outside diameter of arteries, lumen area, neointimal area, and the areas circumscribed by the internal elastic lamina and external elastic lamina. The area circumscribed by the external elastic lamina of the reference site which is the arterial segment located above the lesion site was also measured, and the remodeling index was defined by the ratio of the area circumscribed by the external elastic lamina of the lesion site to the same area of the reference site (Lafont et al., 1995). Expansive remodeling was defined by a remodeling index >1 and constrictive remodeling by a remodeling index <1.

2.5. MTT assay

The MTT assay was used to assess the effect of probucol on cell viability. VSMCs were seeded to 96-well plates with 1×10^4 cells/well. After incubated with ox-LDL in the absence or presence of probucol (10 μM–80 μM) for 48 h, the cells were treated with 10 μl MTT (5 mg/ml) and incubated for 4 h at 37 °C. DMSO was then added to each well and mixed thoroughly to dissolve the dark blue crystals at room temperature. Finally, the density of cells was read by using a Dynatech EL309 Microelisa reader, with a wavelength of 570 nm and a reference wavelength of 450 nm. Control wells contained the culture medium only.

2.6. Flow cytometry analysis

When VSMCs were grown to 80% confluence in 100 ml tissue culture bottle treated with ox-LDL and with or without probucol (20 μM) for 2 days, cells were washed three times with D'Hank's and harvested by trypsinization. Then cells were