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Pterostilbene, a natural dimethylated analog of resveratrol, inhibits rat aortic vascular smooth muscle cell proliferation by blocking Akt-dependent pathway

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ABSTRACT

Vascular smooth muscle cells (VSMCs) are the main cellular component in the arterial wall, and abnormal proliferation of VSMCs plays a central role in the pathogenesis of atherosclerosis and restenosis after angioplasty, and possibly in the development of hypertension. Pterostilbene, a natural dimethylated analog of resveratrol, is known to have diverse pharmacological activities including anti-cancer, anti-inflammation and anti-oxidant activities. The present study was designed to investigate the effects of pterostilbene on platelet-derived growth factor (PDGF)-BB-induced VSMCs proliferation as well as the molecular mechanisms of the antiproliferative effects. The cell growth of VSMCs was determined by cell counting and [^{3}H]thymidine incorporation assays. Pterostilbene significantly inhibited the DNA synthesis and proliferation of PDGF-BB-stimulated VSMCs in a concentration-dependent manner. The inhibition percentages of pterostilbene at 1, 3 and 5 μM to VSMCs proliferation were 68.5, 80.7 and 94.6%, respectively. The DNA synthesis of pterostilbene at 1, 3 and 5 μM in VSMCs was inhibited by 47.4, 76.7 and 100%, respectively. Pterostilbene inhibited the PDGF-BB-stimulated phosphorylation of Akt kinase. However, pterostilbene did not change the expression of extracellular signal-related kinase (ERK) 1/2, PLCγ1, phosphatidylinositol (PI)3 kinase and PDGF-Rβ phosphorylation. In addition, pterostilbene down-regulated the cell cycle-related proteins including the expression of cyclin-dependent kinase (CDK) 2, cyclin E, CDK4, cyclin D1, retinoblastoma (Rb) proteins and proliferative cell nuclear antigen (PCNA). These findings suggest that the inhibition of pterostilbene to the cell proliferation and DNA synthesis of PDGF-BB-stimulated VSMCs may be mediated by the suppression of Akt kinase. Furthermore, pterostilbene may be a potential anti-proliferative agent for the treatment of atherosclerosis and angioplasty restenosis.

1. Introduction

Abnormal vascular smooth muscle cells (VSMCs) proliferation plays a fundamental role in the pathogenesis of vascular diseases, such as atherosclerosis, hypertension and restenosis (Ross, 1990; Schwartz, 1997). The release of growth factor is involved in the pathological processes of vascular lesions. Platelet-derived growth factor (PDGF)-BB secreted by injured endothelial cells and VSMCs as well as by platelets and macrophages, promotes the proliferation of fibroblast and VSMCs (Sachinidis et al., 1990). PDGF-BB initiated mitogenic signals through autophosphorylation of its respective PDGF beta-receptor on tyrosine residues, followed by downstream signal transduction and cell cycle progression (Ahn et al., 1999; Blenis, 1993). The binding of PDGF-BB to the PDGF receptor (PDGF-R) can activate three major signal transduction pathways, Akt, phospholipase C (PLC) γ1 and extracellular regulated kinase 1/2 (ERK1/2) (Claesson-Welsh, 1994; Heldin et al., 1998). Most of the growth factors such as insulin-like growth factor (IGF)-1 and PDGF bind to their respective receptors and then activate phosphatidylinositol (PI) 3 kinase. Akt, a downstream target of PI3 kinase, is overexpressed in gastric adenocarcinomas, breast cancer, hepatocarcinoma and prostate carcinoma, and its activation correlates to cancer progression (Sekine et al., 2007). Thus, the screening of Akt inhibitors may be a potential strategy for the development of anti-cancer agents (Mullany et al., 2007).

It has been suggested since the 1980s that the enhanced activity of PDGF-R plays an important role in cancers and leukemias (Levitzki, 2004; Pietras et al., 2003) and has been identified as a prime player in the onset of the atherosclerotic plaque (Ross and Glomset, 1976; Rutherford and Ross, 1976). Furthermore, it is well established that

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PDGF-R plays a key role in the process of restenosis subsequent to balloon angioplasty and by-pass operation. Thus, the field of pharmacological intervention in PDGF-R signaling is moving in parallel in two directions: anti-cancer therapy and anti-restenosis therapy. In cancers, PDGF and its receptors are one of a number of players with very few exceptions, whereas in restenosis PDGF-R signaling seems to be the major player. It seems therefore that PDGF-R kinase inhibitors will move faster as anti-restenosis agents than as anti-cancer agents. (Levitzki, 2004).

Pterostilbene (Fig. 1), a natural dimethylated analog of resveratrol from blueberries, is known to have diverse pharmacological activities such as anti-cancer, anti-inflammation and anti-oxidant activities (Remsberg et al., 2008). Pterostilbene has been suggested to possess anti-neoplastic activity as effective as resveratrol due to their close structural similarity (Rimando et al., 2004; Tolomeo et al., 2005). Resveratrol has been shown in numerous studies to exhibit beneficial effects in the control of atherosclerosis and heart disease (de la Lastra and Villegas, 2005; Fulda and Debatin, 2006). However, resveratrol has a low bioavailability to cells (Asensi et al., 2002). Thus, structural modifications of the resveratrol need to increase its bioavailability while preserving its beneficial activities (Ferrer et al., 2005). Structurally, pterostilbene has a better metabolic stability than resveratrol because it has only one hydroxyl group, while resveratrol has three. The dimethylether structure of pterostilbene was suggested to enhance its lipophilicity and increase membrane permeability, resulting in better pharmacokinetic profiles than resveratrol (Lin et al., 2009). However, a possible pharmacological mechanism of pterostilbene by which pterostilbene can cure the vascular diseases remains unknown.

In the present study, we sought to elucidate the anti-proliferative activity and the machinery target of pterostilbene in PDGF-BB-stimulated signaling pathway. Our findings provide evidence that pterostilbene can inhibit VSMCs proliferation and cell cycle progression via the cell cycle-related proteins by regulating Akt kinase in VSMCs.

2. Materials and methods

2.1. Materials

The cell culture materials and FBS were obtained from Gibco-BRL (Gaithersburg, MD, USA). [^{3}H]Thymidine was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Pterostilbene was purchased from Sigma Chemical Co. (St. Louis, MO, USA), dissolved in dimethyl sulfoxide (DMSO) and added to Dulbecco's modified Eagle's medium (DMEM) with a maximum final concentration of 0.05 %. Pre-exposure of VSMCs to DMSO 0.05% did not change their cell viability and cell proliferation, compared to control VSMCs. (data not shown) PDGF-BB was acquired from Upstate Biotechnology (Lake Placid, NY, USA). phospho-ERK1/2, phospho-Akt, phospho-PLCγ1, phospho-PDGF-Rβ, ERK1/2, Akt, PLCγ1, PDGF-Rβ and phospho-pRb antibodies were supplied by Cell Signaling Technology Inc. (Beverly, MA, USA). PCNA, cyclin D1, cyclin E, CDK2 and CDK4 antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). All other chemicals were of analytical grade.

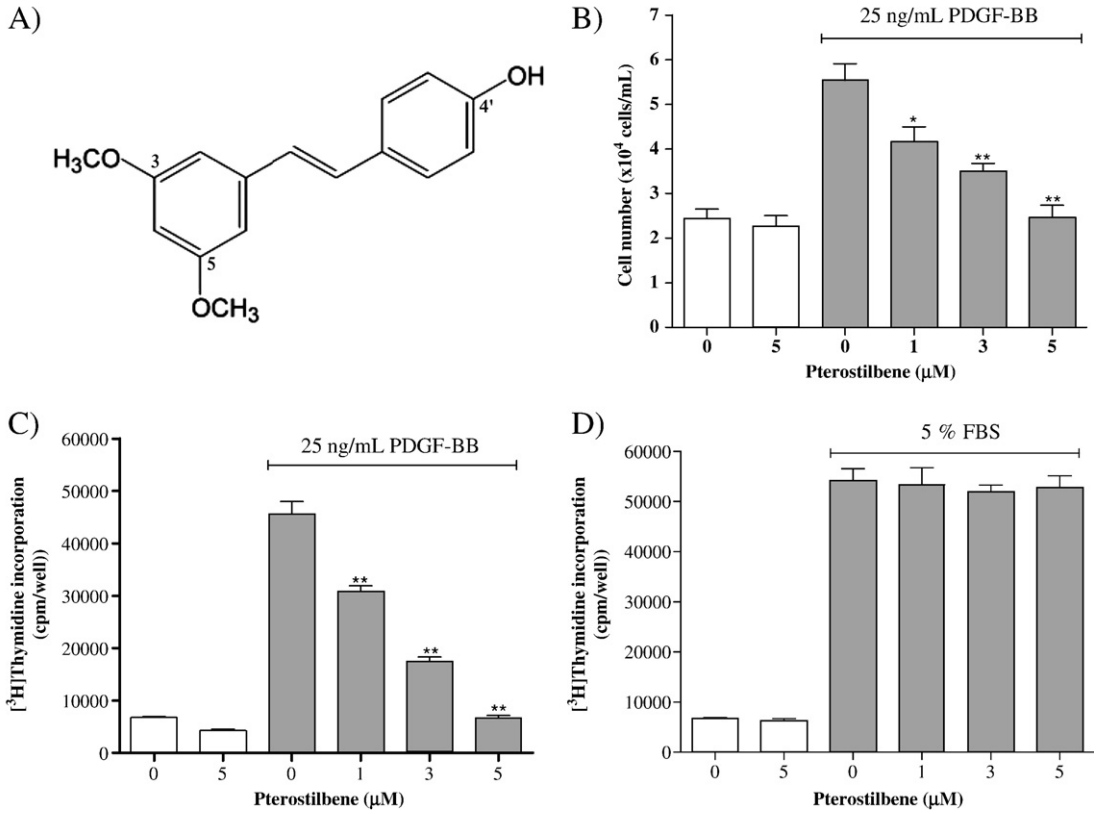

Fig. 1. Inhibitory effects of pterostilbene on PDGF-BB-stimulated proliferation and DNA synthesis of rat aortic VSMCs. (A) Chemical structure of trans-3,5-dimethoxy-4′-hydroxystilbene (pterostilbene). (B) Effect of pterostilbene on the number of PDGF-BB-stimulated VSMCs. The VSMCs were pre-cultured in the serum-free medium in the presence or absence of pterostilbene (1–5 μM) for 24 h, and then stimulated by 25 ng/mL PDGF-BB for 24 h. The cells were trypsinized, and counted using a hemocytometer. The VSMCs were cultured in serum-starved medium in the presence or absence of pterostilbene (1–5 μM) for 24 h, and then stimulated with (C) 25 ng/mL PDGF-BB or (D) 5% FBS for 20 h before 1 μCi/mL [^{3}H]thymidine was added to the medium. The labeling reaction was quenched and quantified using a liquid scintillation counter, 4 h later. The data were reported as the mean ± S.E.M. from four different sets of experiments. *P<0.05 and **P<0.01 vs. only PDGF-BB-stimulated VSMCs.