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Therapeutic effect of magnesium lithospermate B on neointimal formation after balloon-induced vascular injury

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Abstract

Vascular smooth muscle cell (VSMC) proliferation and migration in response to platelet-derived growth factor (PDGF) play an important role in the development of atherosclerosis and restenosis. Recent evidence indicates that PDGF increases intracellular levels of reactive oxygen species in VSMCs and that both PDGF-induced VSMC proliferation and migration are reactive oxygen species-dependent. Danshen is a representative oriental medicine used for the treatment of vascular disease. Previously, we reported that magnesium lithospermate B, an active component of Danshen, is a potent antioxidant. Thus we investigated the therapeutic potential of magnesium lithospermate B in neointimal formation after carotid artery injury in rats along with its effects on the PDGF signaling pathway for stimulating VSMC proliferation and migration *in vitro*. PDGF is dimeric glycoprotein composed of two A or two B chains. In this study, we used PDGF-BB, which is one of the isoforms of PDGF (i.e., PDGF-AA, PDGF-BB, and PDGF-AB). Our results demonstrated that magnesium lithospermate B directly scavenged reactive oxygen species in a xanthine/xanthine oxidase system and reduced PDGF-BB-induced intracellular reactive oxygen species generation in VSMCs. In a rat carotid artery balloon injury model, magnesium lithospermate B treatment (10 mg/kg/day, i.p) showed a significant effect on the prevention of neointimal formation compared with vehicle treatment. In cultured VSMCs, magnesium lithospermate B significantly attenuated PDGF-BB-induced cell proliferation and migration as measured by 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2-tetrazolium bromide (MTT) assay and transwell migration assays, respectively. Further, magnesium lithospermate B inhibited PDGF-BB-induced phosphorylation of phospatidylinositol 3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathways by scavenging reactive oxygen species. Together, these data indicated that magnesium lithospermate B, a potent reactive oxygen species scavenger, prevented both injury-induced neointimal formation *in vivo* and PDGF-BB-induced VSMC proliferation and migration *in vitro*, suggesting that magnesium lithospermate B may be a promising agent to prevent atherosclerosis and restenosis following angioplasty.

Keywords: Magnesium lithospermate B; Vascular smooth muscle cell; Platelet-derived growth factor; Reactive oxygen species; Neointimal formation

1. Introduction

Vascular smooth muscles in the tunica media are quiescent under the physiological conditions; however, following endothelial injury, they proliferate and migrate to the intima, leading to neointimal hyperplasia and resulting in atherosclerosis and

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restenosis (Schwartz et al., 1992; Schwartz, 1997; Dzau et al., 2002). These processes are triggered by multiple factors including cytokines and growth factors such as platelet-derived growth factor (PDGF), which is produced by platelets, vascular smooth muscle cells (VSMCs), and endothelial cells in the injured vascular wall (Heldin and Westermark, 1999; Leppanen et al., 2000; Miyauchi et al., 1998). The PDGF receptor is expressed at low levels in arteries in healthy adults, but its expression is upregulated during endothelial injury after angioplasty or in the early stage of atherosclerosis (Majesky et al., 1990).

Several studies have reported that reactive oxygen species generation is increased during restenosis after angioplasty (Sorescu et al., 2001; Souza et al., 2000; Szocs et al., 2002) and that antioxidants attenuate neointimal hyperplasia (Ghigliotti et al., 2001; Kappert et al., 2006; Nunes et al., 1997; Souza et al., 2000). Furthermore, recent evidence indicates that PDGF itself stimulates reactive oxygen species production in VSMC (Lyle and Griendling, 2006; Sorescu et al., 2001) and that the VSMC response to PDGF is reactive oxygen species-dependent (Sundaresan et al., 1995). These findings strongly suggest that reactive oxygen species may play a central role in VSMC proliferation and migration. Therefore, reduction of reactive oxygen species could be an effective therapeutic intervention in atherosclerosis and restenosis.

The dry roots of *Salvia miltiorrhiza* (Danshen) are a representative oriental medicine used for the treatment of coronary heart disease, cerebrovascular disease, hepatitis, liver cirrhosis, chronic renal failure, dysmenorrhea, and insomnia (Cheng, 2007; Zhou et al., 2005). The chemical constituents of Danshen have been studied since the early 1930s. Early studies focused mainly on lipophilic compounds, such as tanshinones, whereas recent studies have focused more on hydrophilic compounds. Indeed, at least 50 compounds have been isolated and identified from the aqueous extracts of Danshen (Liu et al., 2006; Wu et al., 2000; Zhou et al., 2005). Lithospermic acid B, the tetramer of caffeic acid, is the most abundant component in aqueous extracts of the *Salvia* species and is present mainly as a magnesium salt (Fig. 1). Magnesium lithospermate B is known to have antioxidative (Huang and Zhang, 1992; Wu et al., 2000) and antifibrotic (Jung et al., 2002; Shigematsu et al., 1994) effects. In addition, numerous studies have reported that magnesium lithospermate B has renoprotective (Yokozawa et al., 1997; Yokozawa et al., 1992) and myocardial salvage (Fung et al., 1993) effects. Previously, we reported an effective method for isolating magnesium lithospermate B from Danshen (Jung et al., 2002) and found that magnesium lithospermate B, a potent antioxidant, had a protective effect on diabetes-induced renal disease (Lee et al., 2003).

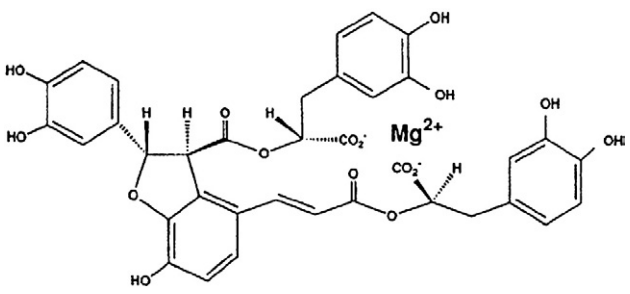

Fig. 1. Chemical structure of magnesium lithospermate B.

Based on the above considerations, we investigated the therapeutic potential of magnesium lithospermate B in injury-induced neointimal hyperplasia in rat carotid arteries as well as its effects on PDGF-induced VSMC proliferation and migration in culture in order to gain insight into the intracellular mechanism(s) of actions of magnesium lithospermate B.

2. Materials and methods

2.1. Materials

Magnesium lithospermate B was isolated from dried *S. miltiorrhiza* roots as previously described (Jung et al., 2002). Chemically PDGF is dimeric glycoprotein composed of A or B chains, which are able to form three isoforms: PDGF-AA, PDGF-BB, and PDGF-AB. In this study, we used recombinant rat PDGF-BB purchased from R&D System. Total Akt, phospho-Akt (Ser473) (p-Akt), total p44/p42 MAPK (ERK 1/2), and phospho-p44/p42 MAPK (Thr202/Tyr204) (p-ERK1/2) antibodies were purchased from Cell Signaling Technology. Proliferating cell nuclear antigen (PCNA) antibody was purchased from Santa Cruz. 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2-tetrazolium bromide (MTT), xanthine, xanthine oxidase, lucigenin, wortmannin, a phospatidylinositol 3-kinase (PI3K) inhibitor, U0126 (1,4-diamino-2,3-dicyano-1, 4-bis[2-aminophenylthio] butadiene), an ERK1/2 inhibitor, and *N*-acetylcysteine were purchased from Sigma.

2.2. VSMC isolation and culture

Rat VSMCs were isolated from the thoracic aorta of Sprague–Dawley rats (250–300 g; ORIENT-Charles River Technology, Seoul, Korea) as described previously (Lee et al., 2006). More than 95% of the cells were positive for α-actin and exhibited the typical hill-and-valley morphology of VSMCs. In this study, cell passages between four and eight were used. VSMCs were grown in Dulbecco-modified Eagle's medium (DMEM) (Sigma Chem, St. Louise, MO) supplemented with 10% fetal bovine serum (FBS) to subconfluence and synchronized by serum-deprivation (0.1% FBS) for 24 h. Synchronized cells were treated with magnesium lithospermate B or other agents for 24 h prior to PDGF-BB stimulation. PDGF-BB (10 ng/ml) was applied to VSMCs for 6 h in the migration assay, 48 h for the MTT assay and evaluation of PCNA expression, and 10 min for analysis of Akt and ERK activation.

2.3. Reactive oxygen species assay

First, the direct reactive oxygen species scavenging effect of magnesium lithospermate B was examined in the test tube without cells. Different concentrations of magnesium lithospermate B