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Compound K, an intestinal metabolite of ginsenosides, inhibits PDGF-BB-induced VSMC proliferation and migration through G1 arrest and attenuates neointimal hyperplasia after arterial injury

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ABSTRACT

Objective: Compound K (CK), an intestinal metabolite of ginsenosides, has pharmacological properties such as anti-angiogenesis, anti-inflammation, anti-platelet and anti-cancer activities. In the present study, we investigated the inhibitory effect of CK on vascular smooth muscle cell (VSMC) proliferation and migration *in vitro* and neointima formation in a rat carotid artery injury model.
Results: CK significantly inhibited both the proliferation and migration of PDGF-BB-stimulated VSMCs in a concentration-dependent manner. In accordance with these findings, CK blocked the PDGF-BB-induced progression of synchronized cells through the G0/G1 phase of the cell cycle. CK also decreased the expressions of cell cycle-related proteins, including cyclin-dependent kinase (CDK) 2, cyclin E, CDK4, cyclin D1, and proliferative cell nuclear antigen (PCNA) in response to PDGF. However, CK did not affect early signal transduction through PDGF-Rβ, Akt, ERK1/2 and PLC-γ1 phosphorylation. CK attenuated PDGF-BB-induced VSMC migration by inhibiting MMP-2 and MMP-9 expression. Furthermore, the CK-treated groups showed a significant reduction in neointima formation vs. the control group. Immunohistochemical staining demonstrated decreased expression of PCNA in the neointima of the CK-treated group.
Conclusion: Our findings demonstrated that CK was capable of suppressing the abnormal VSMC proliferation and migration. It suggested that CK can be a therapeutic agent to control pathologic cardiovascular conditions such as restenosis and atherosclerosis.

1. Introduction

Vascular smooth muscle cells (VSMCs) one of the important components of vascular walls and its proliferation and migration play a crucial role in atherosclerotic lesion formation [1,2]. The abnormal proliferation and migration of VSMCs are critical factors in neointimal formation and vascular lumen loss during atherosclerosis and restenosis [3,4]. All these events can be induced by cytokines and growth factors such as platelet-derived growth factor (PDGF). PDGF initiates a multitude of biological effects through the activation of intracellular signal transduction pathways that contribute to VSMC proliferation and migration [5]. Therefore, the inhibition of PDGF-mediated VSMC proliferation and migration may represent an important point of therapeutic intervention in atherosclerosis and restenosis after angioplasty.

The cell cycle, as a final common pathway of proliferative signaling cascade, is shared by various mitogenic stimuli [6]. The modulated expression of the cell cycle regulatory genes is one of the important mechanisms of cell growth inhibition [7]. Progression through several major checkpoints in the cell cycle is controlled by multiple protein kinases, each of which contains a regulatory cyclin component and a catalytic cyclin-dependent kinase (CDK) [8]. After a vascular injury, VSMCs are stimulated to

Abbreviations: CDK, cyclin-dependent kinase; DMEM, Dulbecco's Modified Eagle's Media; DMSO, dimethylsulfoxide; ERK, extracellular signal regulated kinase; FBS, fetal bovine serum; HEPES, N-[2-hydroxyethyl]piperazine-N-[2-ethanesulfonic acid]; MAPK, mitogen-activated protein kinase; PBS, phosphate buffered saline; PDGF, platelet-derived growth factor; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; VSMCs, vascular smooth muscle cells.

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divide in response to mitogens, and exiting the G1 phase and entering the S phase. Cyclin D1-CDK4 and cyclin E-CDK2 predominantly act in sequence during the G1/S transition and are required for cell cycle progression through this period [9]. Many pharmacological agents, including anti-proliferative agents of VSMC, as well as anti-thrombotic and anti-platelet agents, could alter one or more regulatory events in the cell cycle resulting in blockade of cell cycle progression, thereby suppressing cell proliferation [10].

Panax ginseng C.A. Meyer is a herbal root that has been used for more than 2000 years throughout Far Eastern countries including China, Japan, and Korea [11]. Its beneficial effects have been analyzed by extensive preclinical and epidemiological studies [12,13]. Compound K (CK; 20-O-β-(D-glucopyranosyl)-20(S)-protopanaxadiol) (Fig. 1A) formed from ginsenosides was isolated and purified after giving ginseng extract p.o. to human and rats [14]. CK showed to have diverse pharmacological activities such as anti-metastatic, anti-cancer, anti-proliferation and anti-inflammatory activities *in vitro* and *in vivo* [15–18]. However, the effect of CK on vascular diseases has not yet been extensively investigated.

Thus, in our current study, we have evaluated the anti-proliferative and anti-migratory activity of CK on PDGF-BB-stimulated VSMCs. In particular, the influences of CK on the PDGF-BB-inducible cell cycle progression as well as PDGF-BB-mediated early signal transduction, and cell cycle-regulatory proteins such as cyclins and CDKs, were also investigated. In addition, we investigated that CK significantly reduced neointimal formation in a rat carotid balloon injury model. Taken together, these data indicate that CK exhibits a potent anti-proliferative and anti-migratory effect on VSMCs *in vitro*, and prevents neointima formation *in vivo*.

2. Materials and methods

2.1. Materials and reagents

CK (BioMechatronic Co., Ltd, Seoul, Korea) was dissolved in 0.05% DMSO and further diluted in DMEM without FBS. Rat aortic VSMCs were obtained from BioBud Co., Ltd. (Seoul, Korea). The purity of the VSMCs cultures was >95%, as confirmed by Western blotting staining of α-smooth muscle actin. Cell culture materials were purchased from Gibco BRL (Gaithersburg, MD, USA). ERK1/2, Akt and PLC-γ1 antibodies were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). PDGF-Rβ antibody was obtained from Upstate Biotechnology (Lake Placid, NY, USA). Cyclin D1, cyclin E, CDK2, CDK4 and α-actin antibodies were from Santa Cruz (Santa Cruz, MA, USA). All other chemicals were of analytical grade.

2.2. Cell culture

VSMCs were cultured in DMEM supplement with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, 8 mM HEPES and 2 mM L-glutamine (all obtained from Gibco-BRL) at 37 °C in a humidified 5% CO_2 incubator. Passages 5–9 of the VSMCs were used in this study.

2.3. Cell proliferation assay

The proliferation of VSMC was measured by direct counting and by the nonradioactive colorimetric WST-1 assay (premix WST-1, Takara, Japan). For direct cell counting, VSMCs were seeded into

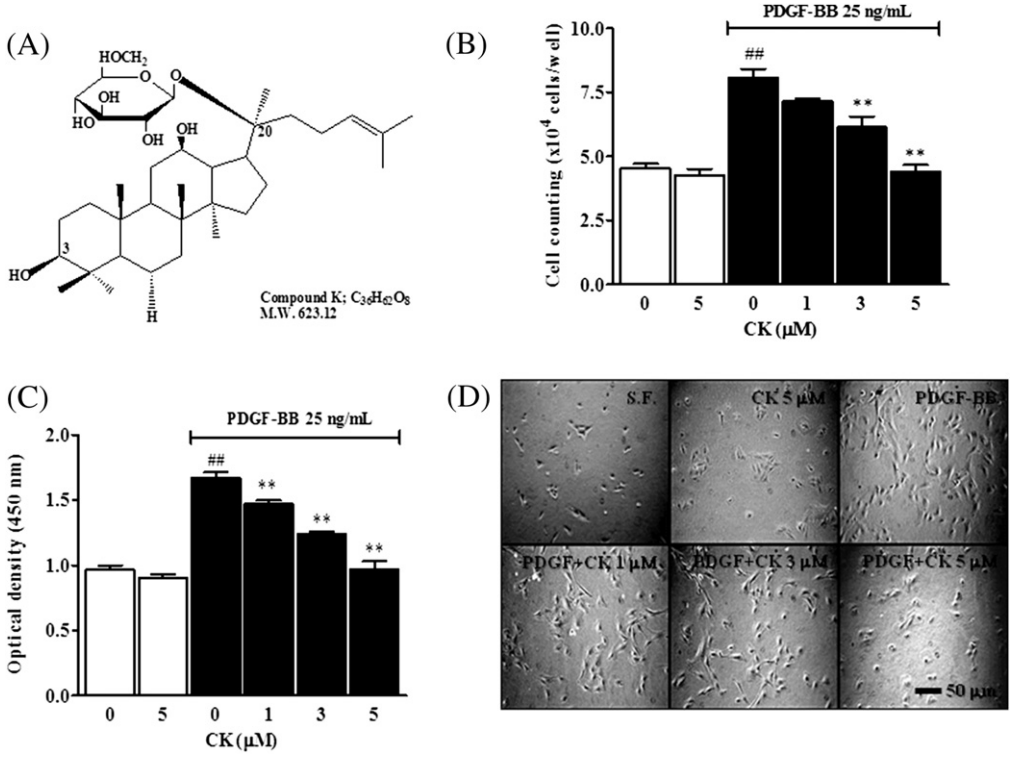

Fig. 1. Effects of CK on PDGF-BB-induced VSMCs proliferation. (A) Chemical structure of [20-*O*-β-(D-glucopyranosyl)-20(S)-protopanaxadiol] (CK). (B) VSMCs pre-cultured in the serum-free medium in the presence or absence of CK (0–5 μM) for 24 h, and then stimulated with 25 ng/mL PDGF-BB for a further 24 h. The cells were trypsinized and counted using a hematocyometer. (C) WST-1 reagent was added at 22 h and further incubated for 2 h. The absorbance was then determined by ELISA at a wavelength of 450 nm. (D) Morphology of cells cultured under different conditions was observed by microscopy. Magnification × 40. Data are expressed as means ± S.E.M. from four different sets of experiments ($n = 4$). $^{\#\#}P < 0.01$ vs. untreated control; $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. PDGF-BB alone.