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Abietic acid isolated from pine resin (Resina Pini) enhances angiogenesis in HUVECs and accelerates cutaneous wound healing in mice☆

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ABSTRACT

Ethnopharmacological relevance: Resin known as Resina Pini is listed in the Korean and Japanese pharmacopoeias and has been used for treating skin wounds and inflammation. Resin is composed of more than 50% abietic acid and 10% neutral substances.

Objective: In the present study, the wound-healing effects of abietic acid and the possible underlying mechanism of action were investigated in various in vitro and in vivo models.

Materials and methods: The effects of abietic acid on tube formation and migration were measured in human umbilical vein vascular endothelial cells (HUVECs). Protein expression of mitogen-activated protein kinase (MAPK) activation was evaluated via Western blotting analysis. The wound-healing effects of abietic acid were assessed using a mouse model of cutaneous wounds.

Results: The results showed that abietic acid enhanced cell migration and tube formation in HUVECs. Abietic acid induced significant angiogenic potential, which is associated with upregulation of extracellular signal-regulated kinase (ERK) and p38 expression. Additionally, 0.8 μM abietic acid-treated groups showed accelerated wound closure compared to the controls in a mouse model of cutaneous wounds.

Conclusion: The current data indicate that abietic acid treatment elevated cell migration and tube formation in HUVECs by the activation of ERK and p38 MAPKs. We suggest that abietic acid can be developed as a wound-healing agent.

1. Introduction

Wound healing is an important process that repairs and regenerates tissue structure and function that has been disrupted or wounded by physical, bacterial, chemical, or viral insults (Muralidhar et al., 2011; Hwang et al., 2016). In general, there are four major stages of wound healing: clot formation, inflammation, proliferation, and remodeling (Hosemann et al., 1991; Watelet et al., 2002; Kim et al., 2015a).

Wound healing processes include the stimulation of new blood vessel formation simultaneously, proliferation of cells such as fibroblasts and keratinocytes, and production of basement membrane zones and connective tissues (Bates and Jones, 2003; Hwang et al., 2004). The production of a number of growth factors in the wound area affects its healing processes by influencing the activation, recruitment, pro-

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liferation, differentiation, and migration of various cells (Tsuboi and Rifkin, 1990; Choi et al., 2015).

Angiogenesis plays an essential role in physiological and pathological processes such as wound healing, embryonic development, tumor growth, and chronic inflammation (Pandya et al., 2006; Ha et al., 2016). Angiogenesis is regulated by several growth factors, including basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF); these factors promote proliferation, migration, and other angiogenic activities of endothelial cells (Scharpfenecker et al., 2007). However, the side effects or toxic effects of the growth factors are of concern owing to their multidistribution and multiple functions (Fu et al., 2005; Choi et al., 2016). Consequently, the identification of non-toxic natural products with pro-angiogenic activity would be very useful for the development of a potential alternative agent for wound healing (Kim et al., 2011).

Resin, also known as Resina Pini, is a natural resin obtained from plants belonging to the Pinaceae family and it is commercially produced from 7 *Pinus* species including *P. palustris* Mill., *P. pinaster* Ait., *P. sylvestris* L., *P. laricio* Poiret, *P. longifolia* Roxb., *P. densiflora* Siebold et Zucc., and *P. thunbergii* Parlatore. Resin is listed in the Korean and Japanese pharmacopoeias and has been used for treating wounds in traditional Korean medicine (Simbirtsev et al., 2002; Sipponen and Lohi, 2003; Sipponen et al., 2007). Resin is composed of approximately 90% resin acids and 10% neutral substances. In particular, resin is composed of more than 50% abietic acid (Fig. 1(A)). Abietic acid has been reported to show anti-inflammatory, anti-allergic, phytoalexin-like, and anti-convulsant activities and inhibitory effects on melanoma cancer metastasis (González et al., 2010; Hsieh et al., 2015; Gao et al., 2016). During a search for angiogenic substances produced by natural products, we observed that pine resin and abietic acid stimulated the migration of human endothelial cells. In this study, we demonstrated that abietic acid stimulated angiogenic properties in vitro and enhanced cutaneous wound healing in mice.

2. Materials and methods

2.1. Chemicals, reagents, and plant sample

Endothelial cell basal medium-2 (EGM-2) was purchased from Lonza Inc. (Walkersville, MD, USA). Pine resin (500 g) was purchased from KANTO CHEMICAL CO., INC. (Tokyo, Japan) in March 2016. The material was confirmed by one of the authors (K. H. Kim). A voucher specimen (SKKU-RS-2016-03) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea. The standard abietic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies for p38, phosphorylated-p38, ERK and phosphorylated-ERK, as well as all secondary antibodies were obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA). U0126 and p38 kinase inhibitor SB203580 were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA).

2.2. Quantitative analysis of abietic acid by LC-MS

The detection of abietic acid was analyzed by LC-MS, Agilent 1200 Series analytical system equipped with a photodiode array (PDA) detector combined with a 6130 Series ESI mass spectrometer. Briefly, rosin was purchased from KANTO CHEMICAL CO., INC. (Tokyo, Japan). The rosin (5.0 mg) was dissolved in MeOH (500 μL) and filtered through a 0.50 μm syringe filter. The filtered sample was analyzed using a Kinetex C18 column (2.1×100 mm, 5 μm; Phenomenex, Torrance, CA, USA) set at 25 °C. The mobile phase was a gradient program from mixtures of 0.1% formic acid in water (A) and MeOH (B), which was as follows: 0–30 min from 10% to 100% B; 30–40 min at 100% B; followed by a rapid drop to 10% B at 41 min, and then isocratic condition with 10% B to 52 min. The flow rate was set at 0.3 mL/min, and the injection volume was 10 μL. Abietic acid was detected at 30.2 min of retention time. Calibration curves and linear regression equations were generated for the external standard,

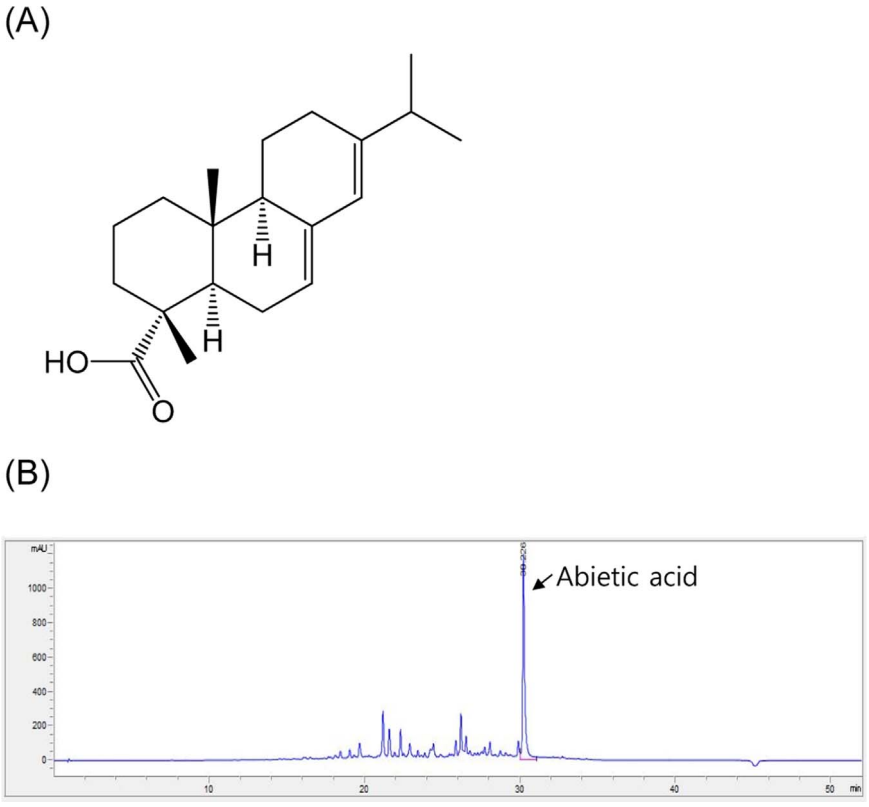

Fig. 1. Quantitative analysis of abietic acid by LC-MS. (A) Chemical structure of abietic acid. (B) UV chromatogram of LC/MS (detection wavelength was set as 254 nm) of resin extract.