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Antiedema effects of Siberian ginseng in humans and its molecular mechanism of lymphatic vascular function in vitro

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ABSTRACT

The lymphatic system in the skin plays a major role in tissue fluid homeostasis, in the afferent phase of the immune response, and in tumor metastasis. Although lymphangiogenic factors involved in embryonic development and the metastatic spread of tumor cells have been well studied, little is known about small-molecule compounds that activate lymphatic function, especially under physiological conditions. We hypothesized that the identification of a lymphatic-activating compound could provide a method for improving edema. Here, we show that Siberian ginseng (*Eleutherococcus senticosus*) and its component eleutheroside E induce phosphorylation of the endothelial-specific receptor Tie2 in vitro. The activation of Tie2 on lymphatic endothelial cells (LECs) is known to stabilize lymphatic vessels, so we examined the effects of Siberian ginseng on LECs. We found that Siberian ginseng induces the migration and cord formation of LECs. Permeability assays demonstrated that it stabilizes LECs by promoting the intercellular localization of vascular endothelial cadherin, which is an endothelium-specific cell-cell adhesion molecule involved in endothelial barrier function, and it induces the phosphorylation of endothelial nitric oxide synthase by LECs. These effects appear to be mediated by the activation of Tie2 in LECs. Finally, we investigated whether the consumption of Siberian ginseng powder improves edema in a 2-way, randomized, crossover study in 50 healthy female volunteers. Edema of the lower limbs was significantly attenuated at 2 and 4 hours after ingestion as compared with the control group. Thus, we demonstrate that Siberian ginseng exerts its potent antiedema activity mainly by promoting lymphatic function.

Abbreviations: Ang1, angiopoietin-1; EBM, endothelial basal medium; eNOS, endothelial nitric oxide synthase; FITC, fluorescein isothiocyanate; HUVECs, human umbilical vein endothelial cells; LECs, lymphatic endothelial cells; NO, nitric oxide; SG, Siberian ginseng; VE, vascular endothelial; VEGF, vascular endothelial growth factor.

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1. Introduction

The lymphatic vascular system consists of a network of endothelium-lined vessels. Lymphatic capillaries in the skin are thin-walled vessels approximately 30-80 μm in diameter composed of a single layer of oak-leaf–shaped lymphatic endothelial cells (LECs) that differ in many respects from blood vascular endothelial cells [1,2]. The roles of the lymphatic system include the transport of interstitial fluid and the transfer of lymphocytes and antigen-presenting cells to lymph nodes [3]. *Lymphedema* has been defined as an abnormal accumulation of protein-rich fluid in soft tissues resulting from dysfunction of the lymphatic system, specifically, an imbalance between lymph formation and its absorption into the initial lymphatics. Despite the importance of lymphatic function in various pathologies such as edema, the mechanisms involved are not fully understood. Recently, it was established that angiopoietin-1 (Ang1) promotes lymphatic vessel formation and function by activating the receptor tyrosine kinase Tie2 on LECs [4,5]. Furthermore, we have suggested that Ang1, or small molecules that directly activate Tie2, may have potential for the treatment of lymphatic dysfunction during acute skin inflammation and edema formation induced by ultraviolet B irradiation [6]. The activation of Ang1/Tie2 signaling attenuates inflammation by promoting lymphatic integrity as well as by inhibiting blood vascular hyperpermeability in inflamed tissue [7]. Therefore, small-molecule dietary components or herbs that can activate Tie2 may have potential therapeutic value for improving lymphatic vessel function.

Elleutherococcus senticosus, known as Siberian ginseng (SG), is a medicinal herb that belongs to the family Araliaceae. It is a powerful tonic herb with a broad range of reported health benefits, including anti-inflammatory [8], antifatigue [9], antisteatosis [10], antiosteoporosis [11], and neuroprotective effects [10]. Recently, Yoon et al [12] reported that SG extracts have an antimetastatic activity via the activation of macrophages and natural killer cells. Several components of SG have been identified, including triterpenoid saponins, lignans, coumarins, and flavones, among which phenolic compounds such as eleutheroside E are generally considered to be the most active [13]. Although the health effects of SG, like those of *Panax ginseng* or *Panax notoginseng*, may be attributed to a general improvement of body circulation, probably via vasodilatory effects elicited by its active ingredients, there has been no direct in vitro study of SG or its extracts to evaluate their effects on vascular function. Therefore, the aim of this study was to test the hypothesis that a small-molecule compound that activates Tie2 in vitro could have a potential to improve edema in humans and to elucidate its molecular mechanism in lymphatic vascular function. For this purpose, we identified a Tie2 activating herb/compound and characterized its effects on LECs in vitro.

2. Methods and materials

2.1. Reagents

Siberian ginseng powder was obtained from Matsuura-yakugyo (Nagoya, Japan) and was extracted with hot water. We confirmed that the content of eleutheroside E in the powder was more than 1%. Ang1 (R&D Systems, Minneapolis, MN, USA) and eleutheroside E (PhytoLab, Vestenbergsgreuth, Germany) were also purchased.

2.2. Cells

Human dermal LECs were isolated from neonatal human foreskins by immunomagnetic purification as described previously [14]. Lineage-specific differentiation was confirmed by real-time reverse transcriptase polymerase chain reaction using the lymphatic vascular markers Prox1, LYVE-1, and podoplanin, as well as by immunostaining for Prox1 and podoplanin. Human umbilical vein endothelial cells (HUVECs) were purchased from PromoCell (Lonza, Verviers, Belgium). Cells were cultured in endothelial basal medium (EBM) 2 (Lonza, Basal, Switzerland) with supplements provided by the suppliers for up to 11 passages.

2.3. Immunoblotting

For Western blot analysis of Tie2, p-Tie2, NOS3 (endothelial nitric oxide synthase [eNOS]), and p-eNOS, cells were cultured with or without Ang1 (500 ng/mL), SG (1-10 μg/mL), or eleutheroside E (0.01-1 μg/mL) for 15 minutes, followed by homogenization in lysis buffer (EMD Millipore, Billerica, MA, USA). Protein concentrations were determined using a BCA kit (Pierce Biotechnology, Rockford, IL, USA). Aliquots of cellular proteins were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (ATTO, Tokyo, Japan) and transferred to Immobilon-P membranes (Millipore, Darmstadt, Germany). The membranes were stained with antibodies against Tie2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), p-Tie2 (Cell Signaling Technology, Danvers, MA, USA), eNOS (Santa Cruz Biotechnology, Santa Cruz, CA, USA), or p-eNOS (Cell Signaling Technology, Danvers, MA, USA), and detection of specific proteins was carried out by enhanced chemiluminescence.

2.4. Permeability assay

Lymphatic endothelial cells were grown to confluence on the fibronectin-coated surface of tissue culture inserts of 0.4-μm pore size (Transwell; Corning, Lowell, MA, USA), after which the medium was changed to serum-free EBM2 for 24 hours. Ang1 (500 ng/mL) or SG (1 μg/mL) was placed in the upper chambers for 6 hours. Fluorescein isothiocyanate (FITC)–dextran was then added to the upper chamber. After incubation at room temperature for 15 minutes, the concentration of FITC-dextran in the lower chamber was determined at 492 nm using a Fluoreskan Ascent spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.5. Immunohistochemistry

Cells were grown to confluence on fibronectin-coated culture slides (BD Bioscience, Bedford, MA, USA) and exposed to Ang1 (500 ng/mL) or SG (1 μg/mL) as described for the permeability assays. After fixation with 4% paraformaldehyde at room temperature for 15 minutes, cells were permeabilized with 0.2%