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American ginseng inhibits vascular smooth muscle cell proliferation via suppressing Jak/Stat pathway

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Abstract

Ethnopharmacological relevance—Ginseng, a folk medicine which has been used for thousands of years in Asia, has been promoted for the treatment or prevention of health problems including cardiovascular disease. However, the molecular mechanism of ginseng-induced cardiovascular protection is unclear. Thus, we investigated signaling mechanism by which American ginseng inhibits vascular smooth muscle cell (VSMC) proliferation, a key feature of diverse vascular disease.

Materials and methods: A standardized crude extract of American ginseng was supplied by the National Research Council of Canada, Institute for National Measurement Standards. Rat aortic smooth muscle cells (RASMCs) were exposed to fetal bovine serum (FBS), platelet derived growth factor (PDGF), insulin, or angiotensin II (Ang II) to induce proliferation that was examined by measuring DNA synthesis and cell numbers. Western blot was applied to determine the activations of Jak, Stat, Akt, and ERK.

Results—American ginseng inhibited RASMC proliferation induced by FBS, PDGF, insulin or Ang II. American ginseng slightly increased both basal and FBS-, PDGF- or Ang II-induced

activities of Akt and ERK in RASMCs; however, it dramatically inhibited the activation of Jak2 and Stat3.

Conclusion—These results demonstrate that American ginseng inhibits VSMC proliferation through suppressing the Jak/Stat pathway.

Keywords

Panax quinquefolius; American ginseng; Jak; Stat; Vascular smooth muscle cells; Proliferation

1. Introduction

The public enthusiasm for ginseng has been inspired by the growing evidence of ginseng-induced cardiovascular protection, despite the emerging concerns on potential adverse interactions of ginseng with cardiovascular drugs and ginseng-induced cardiovascular dysfunction (Gillis, 1997; Izzo et al., 2005; Lu et al., 2009; Lee et al., 2010). It is clear that the major active components in ginseng are ginsenosides and the minor components include amino acids, peptides, and minerals. Approximately 40 ginsenosides have been identified, and they are able to cause either beneficial or detrimental cardiovascular actions via modulating multiple signaling cascades. In fact, the content of various ginseng roots and the root extracts of the same species of *Panax* could differ depending on the method of extraction, geographic locations of cultivation, or the season of collection. Therefore, if ginseng was beneficial for the treatment of cardiovascular disease, the therapeutic efficacy of ginseng is likely achieved by a net effect orchestrated by multiple components of ginseng. Indeed, whole ginseng extract appears to give a better protection against radiation-induced DNA damage than isolated ginsenoside fractions (Lee et al., 2005). However, the nature of ginseng-mediated cardiovascular protection is still enigma while the molecular mechanism by which ginseng exerts cardiovascular protection remains unresolved.

Abnormal growth of VSMCs is a key feature of diverse vascular complications and dysfunctions that are the major contributors to cardiovascular disease, the leading cause of death and disability in USA (Rosamond et al., 2008). In an effort to understand the putative beneficial effect of ginseng for the treatment of cardiovascular disease, it has been demonstrated that crude extracts of *Panax ginseng* or *Panax notoginseng* induce VSMC apoptosis and inhibit VSMC proliferation, thereby attenuating vascular lesion formation (Wu et al., 2001; Xu et al., 2011; Yu et al., 2011; Zhang et al., 2012). Increasing nitrogen monoxide (NO) formation or inactivating ERK is proposed to be critical for ginseng-induced growth inhibition in VSMCs, while up-regulating expression of p53, Bax, and caspase 3 is postulated to be crucial for ginseng-induced VSMC apoptosis. Moreover, several ginsenosides such as Rg1, Rb1, Rb3, and Rd have been shown to inhibit VSMC proliferation via regulating multiple signaling cascades (Zhang and Wang, 2006; Cai et al., 2009; Wang et al., 2010; Li et al., 2011). Inactivating ERK cascade is likely a common mechanism of the Rg1-, Rb1-, or Rb3-mediated growth inhibition, while suppressing voltage-independent Ca^{2+} entry is linked with the growth inhibitory effect of Rd. Of note, it is not ginsenoside Rb1, Rc, or Re, but Rg3 that increases NO production in vasculature (Kim et al., 2003). However, a recent study has demonstrated that Rg3 exerts vascular toxicity leading to vascular smooth muscle dysfunction by disturbing Ca^{2+} influx (Lee et al.,

2010). These results clearly demonstrate that ginsenosides individually activate multiple signaling cascades resulting in either beneficial or detrimental consequences in the vasculature. It is our view that the putative therapeutic efficacy of ginseng on cardiovascular disease is attributed to a fraction of ginseng bioactive components rather than any single ginsenoside. By exploring the common mechanisms by which crude extracts of different ginsengs protect against vascular dysfunction, we may pave a way to understand ginseng-induced cardiovascular protection.

Here, we demonstrate for the first time that American ginseng crude extract inhibits VSMC proliferation via selectively suppressing Jak/Stat pathway. Our findings suggest that suppressing Jak/Stat signaling in VSMCs is critical for the ginseng-mediated vaso-protective effect.

2. Materials and methods

2.1. American ginseng extract

A standardized American ginseng extract was supplied by the National Research Council of Canada, Institute for National Measurement Standards (NRCC-INMS) and solutions of American ginseng extract, at different concentrations, were freshly prepared before each experiment as previously described (Ichikawa et al., 2009; Li et al., 2010). The ginseng extract was produced by an aqueous ethanol extract of 4 year old ground roots of *P. quinquefolius* that was spray dried on a maltodextrin support. The total ginsenoside content (means $\pm$ 1 sd, $n=6$) as measured by LC–MS using authentic standards is $10.5\pm0.2\%$ (w/w) measured as the sum of Rg1 (3.5 ± 0.1 mg/g), Re (21.2 ± 0.4 mg/g), Rb1 (45.1 ± 1.6 mg/g), Rc (15.5 ± 0.7 mg/g), Rb2 (2.2 ± 0.1 mg/g) and Rd (17.8 ± 0.4 mg/g). Further information on the production and processing of the extract including voucher specimens were detailed in our previous reports (Ichikawa et al., 2009; Li et al., 2010).

2.2. Cell culture

VSMCs were isolated from thoracic aorta of adult Sprague Dawley rats and cultured in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% fetal bovine serum as previously described (Cui et al., 2000). Cell viability was measured using the Cytotoxicity Detection Kit ^{PLUS} (Roche Applied Science) (Li et al., 2010). Cell numbers were determined relatively by measuring LDH activity of whole cell lysates. DNA synthesis was determined by [³H] thymidine uptake (Cui et al., 2000).

2.3. Western blot

Western blot analysis was performed as previously described (Ichikawa et al., 2009). Primary antibodies of anti-Stat3, anti-ERK1,2, anti-Akt, anti-phospho-Stat3 (Tyr705), anti-phospho-ERK1,2 (Thr202/204), and anti-phospho-Akt (Ser473) were purchased from Cell Signaling. Primary antibody of anti-Jak2 was purchased from Invitrogen, and anti-phospho-Jak2 (Tyr1007/ 1008) was from Upstate.

2.4. Statistics

Values are expressed as mean $\pm$ SEM. The data were analyzed using ANOVA with the LSD test unless specified. Values of $p < 0.05$ were considered to be statistically significant.

3. Results and discussion

In the present study, we explored the molecular mechanism of ginseng-induced vaso-protective effect in RASMCs utilizing a standardized American ginseng crude extract.

To exclude any cytotoxic effect of the American ginseng extract, we carefully determined its cytotoxicity in RASMCs. In quiescent RASMCs that were induced by serum starvation for 24 h, the American ginseng extract did not exhibit any cytotoxic effects below the concentration of 200 μ g/ml (data not shown). The observed cytotoxic effects of the American ginseng extract (>200 μ g/ml) are consistent with the previous report that crude extract of *Panax notoginseng* (200, 400, 800 μ g/ml) induced VSMC apoptosis (Xu et al., 2011). Thus, we used the American ginseng extract at a non-cytotoxic dose of 50 μ g/ml to exclude any potential vascular toxicity of American ginseng in our study.

Consistent with the well-documented growth inhibitory effect of different ginsengs on VSMCs, we observed that American ginseng crude extract at non-cytotoxic doses (0–50 μ g/ml) inhibited the basal as well as FBS-, PDGF-, insulin-, and AngII-induced RASMC proliferation (Fig. 1). The American ginseng extract dose-dependently inhibited FBS-, PDGF-, insulin-, and AngII-induced cell number increases of RASMCs (Fig. 2B). The percentage decreases by American ginseng crude extract at doses of 10, 25, and 50 μ g/ml are as follows: FBS, $95.39 \pm 7.05\%$ ($p < 0.05$, $n = 6$), $92.24 \pm 4.14\%$ ($p > 0.05$, $n = 6$), and $81.88 \pm 4.11\%$ ($p < 0.05$, $n = 6$); PDGF, $92.91 \pm 6.27\%$ ($p > 0.05$, $n = 6$), $72.74 \pm 10.99\%$ ($p < 0.05$, $n = 6$), and $52.16 \pm 3.74\%$ ($p < 0.05$, $n = 6$); Insulin, $93.42 \pm 3.34\%$, $79.25 \pm 3.99\%$, and $57.71 \pm 10.21\%$ ($p < 0.05$, $n = 6$); AngII, $92.70 \pm 11.31\%$, $86.14 \pm 5.89\%$, and $60.76 \pm 6.64\%$ ($p < 0.05$, $n = 6$). Thus, like other ginsengs, American ginseng exerts a growth inhibitory effect on VSMCs, suggesting that inhibiting VSMC growth may be a common cellular mechanism by which ginsengs protect against vascular injury or disease progression. At a molecular level, however, unlike the previous report that *Panax notoginseng* crude extract and ginsenosides Rg1 and Rb1 suppress ERK and Akt activation (Zhang and Wang, 2006; Li et al., 2011; Zhang et al., 2012), we found that American ginseng crude extract slightly increased both basal and FBS-, PDGF- or Ang II-induced activities of Akt and ERK in RASMCs (Fig. 2). Because of the well-established mediator role of ERK and Akt in VSMC proliferation (Muto et al., 2007), it is likely that American ginseng inhibits VSMC growth via a mechanism independent of activating ERK and Akt cascades. Since Jak2/Stat3 pathway plays a mediator role in VSMC growth and vascular lesion formation (Shibata et al., 2003; Kirabo et al., 2011), we were wondering whether American ginseng crude extract could inactivate the Jak2/Stat3 pathway thereby inhibiting VSMC growth. Notably, we observed that American ginseng crude extract dramatically suppressed the activation of Jak2/Stat3 pathway in RASMCs (Fig. 2). These results reveal that American ginseng inhibits VSMC growth at least partly via suppressing the Jak2/Stat3 pathway.

It is worthy to note that American ginseng crude extract preferentially suppresses pro-inflammatory Stat cascade contributing to resolution of inflammation (Ichikawa et al., 2009). Considering the crucial role of chronic inflammation and VSMC proliferation in diverse vascular diseases, our findings highlight a notion that the unique inhibitory effect of American ginseng on Jak/Stat signaling might represent a common molecular mechanism of ginseng-mediated vaso-protection. On the other hand, total saponins of *Panax ginseng* have been demonstrated to augment the erythroid differentiation of hematopoietic stem and progenitor cells via enhancing erythropoietin (Epo)/Epo receptor-mediated activation of Jak2/Stat5 signaling (Chen et al., 2009). Although it is unlikely that American ginseng exerts a similar effect in VSMCs since the activation of EpoR by Epo hardly affected Jak2/Stat5 signaling in RASMCs (Ammarguella et al., 2001), cell or tissue type specific actions of American ginseng extract need to be carefully dissected. Further characterization of American ginseng-mediated suppression of Jak/Stat pathway in pathological vascular remodeling and dysfunction will untwist the molecular basis by which ginseng acts as a folk medicine or perhaps as a drug for the treatment of vascular diseases.

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Fig. 1.

Effect of American ginseng on RASMC proliferation. (A) RASMCs were cultured in serum-free (SF) DMEM for 24 h to induce a quiescent status, and then treated with or without American ginseng extract (Am. g.) as indicated for additional 24 h (left panel) or 48 h (right panel). * $p < 0.05$; vs. non-treated group (Am. g. SF-), $n = 6$. # $p < 0.05$; Am. g. (+) vs. Am. g. (-) in each group, $n = 6$. (B) Quiescent RASMCs were treated with or without American ginseng extract as indicated for 48 h. * $p < 0.05$ vs. Vehicle (Am. g. G, 0), $n = 6$. Concentrations of stimuli: FBS (2%), PDGF (20 ng/ml), Insulin (Insu, 100 nM), Ang II (0.1 µM).

Fig. 2.

Effect of American ginseng on the activation of growth-promoting signaling in RASMCs. (A) RASMCs were cultured in serum-free (SF) DMEM for 24 h to induce a quiescent status, and then treated with or without American ginseng extract (50 $\mu\text{g/ml}$) as indicated for additional 10 min. (B) Quiescent RASMCs were treated with or without American ginseng extract (50 $\mu\text{g/ml}$) as indicated for 5 min. Concentrations of stimuli: FBS (2%), PDGF (20 ng/ml), Ang II (0.1 μM). Results are representatives of four independent experiments.