British Journal of Pharmacology (2007) 152, 172–174
 0007–1188/07 $30.00
www.brjpharmacol.org

COMMENTARY

A ginseng-derived oestrogen receptor β (ERβ) agonist, Rb1 ginsenoside, attenuates capillary morphogenesis

A Papapetropoulos

Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Patras, Greece

Ginseng extracts contain a variety of active ingredients and have been shown to promote or inhibit angiogenesis, depending on the presence of different ginsenosides that exert opposing effects on blood vessel growth. Leung *et al.* in this issue of the *British Journal of Pharmacology* report that Rb1, a ginsenoside that constitutes only 0.37–0.5% of ginseng extracts (depending on manufacturing and processing methods), blocks tube-like network formation by endothelial cells *in vitro*. At the molecular level, Rb1 binds to the oestrogen receptors and stimulates the transcription of pigment epithelium-derived factor that, in turn, inhibits matrix-driven capillary morphogenesis.
British Journal of Pharmacology (2007) **152,** 172–174; 10.1038/sj.bjp.0707360; published online 2 July 2007

Keywords: ginsenoside Rb1; angiogenesis; ginseng; oestrogen receptors; pigment epithelium-derived factor

Abbreviations: ER, oestrogen receptor; PEDF, pigment epithelium-derived factor; VEGF, vascular endothelial growth factor

Introduction

Ginseng, the root of *Panax ginseng* and related species, has been a key component of traditional medicine in the Far East for over a thousand years. The genus name *Panax* means 'cure all' in Greek; it, thus, comes as no surprise that ginseng has been described as beneficial in many different ailments (Huang, 1999; Kiefer and Pantuso, 2003; Ng, 2006). Perhaps the most studied biological actions of ginseng extracts and constituents are those relating to its inhibitory effects on solid tumour growth (Yun, 2001). The main active ingredients in ginseng-based herbal preparations are thought to be the ginsenosides, comprising 3–6% of ginseng extracts (Huang, 1999).

Angiogenesis

Angiogenesis is the process of new blood vessel formation from pre-existing structures. During sprouting angiogenesis, endothelial cells (ECs) degrade their surrounding extracellular matrix, proliferate, migrate towards gradients of angiogenic growth factors and assemble into vascular structures, which are structurally and functionally supported by mural cells (Folkman and Shing, 1992; Carmeliet, 2000). As many solid tumours fail to grow beyond a certain size in the absence of increased blood supply, angiogenesis inhibitors have attracted a lot of attention as anti-tumour agents. A vascular endothelial growth factor (VEGF)-neutralizing antibody (bevacuzimab) was the first agent of this class to be approved for clinical use against colon cancer in 2004 (Ferrara *et al.*, 2005). More angiogenesis inhibitors are currently being developed for several types of cancer (Herbst, 2006).

Ginsenosides altering angiogenic responses

Many natural products contained in the diet or in botanical remedies are thought to be capable of preventing or delaying the onset of cancer. Thus, there is great interest in the identification of the chemical entities that are responsible for the beneficial effects of such nutraceuticals. *Panax ginseng* presumably inhibits tumour growth by affecting both cancer cells and their blood supply (Yun, 2001; Sengupta *et al.*, 2004). Using purified active ingredients from ginseng, researchers have identified so far three distinct ginseng saponins capable of affecting neovascularization and angiogenesis-related properties of ECs. The ginsengoside Rg1 stimulates vascularization of a synthetic scaffold implant (Sengupta *et al.*, 2004), while 20(*R*)-ginsenoside Rg3 inhibits bFGF-induced neovascularization *in vivo* (Yue *et al.*, 2006).

Correspondence: Dr A Papapetropoulos, Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Patras 26504, Greece.
E-mail: apapapet@upatras.gr
Received 23 March 2007; accepted 14 May 2007; published online 2 July 2007

On the other hand, the properties of Rb1 have been less clearly defined. Sengupta *et al.* (2004) reported that Rb1 stimulated EC proliferation via a partially nitric oxide-mediated mechanism. However, Rb1 also strongly inhibited hepatocyte growth factor-stimulated migration of ECs. The latter property could explain the limited vessel ingrowth in polyether polyurethane sponge implants in mice observed after Rb1 administration. In contrast, Rb1 was reported to stimulate angiogenesis during healing of burn wounds (Kimura *et al.*, 2006). In this case, Rb1 increased neovessel formation and promoted VEGF and interleukin-1β formation from the burn wound after topical application. A hypothesis that could reconcile the pro- and anti-angiogenic properties of Rb1 is that this ginsenoside targets a variety of molecules involved in cell signalling with opposing effects on blood vessel formation. Thus, the effects observed after Rb1 treatment will depend on the concentration of Rb1 used and the target molecules (receptors and enzymes) expressed under the experimental conditions studied. As mediators and signalling pathways driving vessel formation in various settings are different, neovascularization during developmental angiogenesis, tumour angiogenesis, ischaemia-induced angiogenesis, or inflammation-related angiogenesis could be differentially affected by Rb1.

In the present issue of the *British Journal of Pharmacology*, Leung *et al.* (2007) report on the ability of ginsenoside Rb1 to inhibit tube-like network formation on Matrigel. It should be pointed out that Sengupta *et al.* (2004), using similar concentrations of Rb1, showed an increase in FGF-2 + TNF-α and VEGF + TNF-α-stimulated tubulogenesis in fibrin gels and matrix-driven network formation on the Matrigel. The reason for the discrepancy between the study appearing in this issue of *British Journal of Pharmacology* and the earlier report is unclear. The authors of the study in this issue are proposing that the observed inhibitory effects of Rb1 on capillary morphogenesis are mediated through an increase in the production of pigment epithelium-derived factor (PEDF). Although the receptor for PEDF on ECs remains largely unknown, this factor has been shown to inhibit EC migration (Duh *et al.*, 2002) and to stimulate apoptosis by activating the FAS-FasL death pathway (Volpert *et al.*, 2002). Moreover, PEDF blocks the production of angiogenic growth factors from tumour cells and stimulates γ-secretase-dependent cleavage of VEGFR1, acting as an inhibitor of VEGF signalling (Volpert *et al.*, 2002; Takenaka *et al.*, 2005; Cai *et al.*, 2006). The inhibitory effects of Rb1 observed by the authors with respect to Matrigel-driven network formation might be related to the induction of apoptosis or the blockade of cell motility that affects tube-like network formation on Matrigel.

In their publication, Leung *et al.* (2007) propose that Rb1 exerts its effects on PEDF expression by activating the ERβ (Figure 1). It has been previously proposed that Rb1 stimulates ER without physically interacting with them (Cho *et al.*, 2004). These conclusions were based on the observation that high concentrations of Rb1 (in the μM range) stimulated gene expression driven by an oestrogen responsive element, while Rb1 failed to inhibit specific binding of [^{3}H]17-oestradiol to ERs in whole cell-binding assays. It should, however, be kept in mind that the cells used by Cho *et al.* (2004), MCF-7, express high levels of the ERα. In the article by Leung *et al.* (2007) published in this issue of *British Journal of Pharmacology*, they show that Rb1 binds to the ERβ ($K_D = 70$ nM), but not the ERα as it replaces a

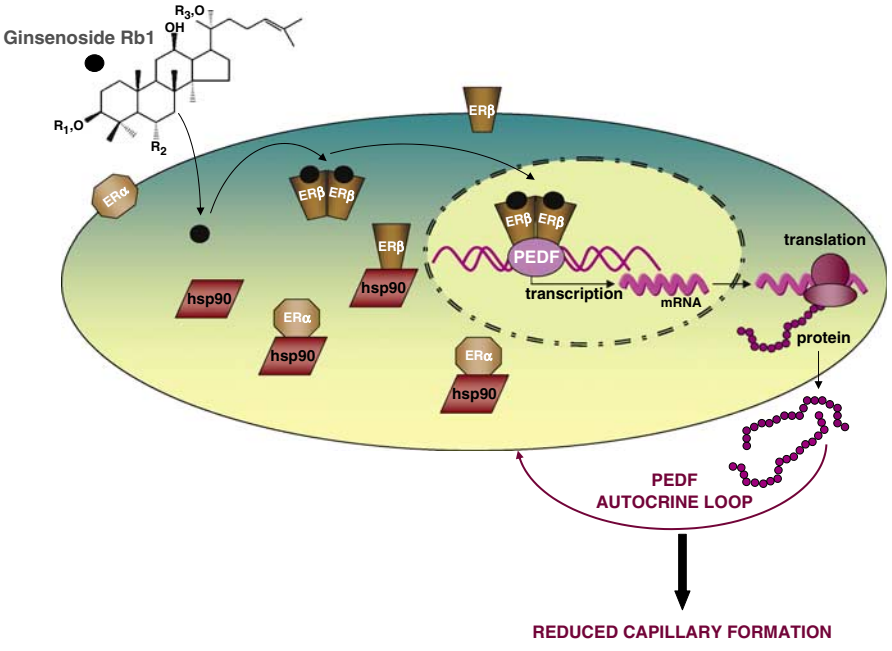

Figure 1 Schematic representation of the proposed mechanism of action for ginsenoside Rb1. R1 is -Glc^{2}–^{1}Glc, R2 is -H and R3 -Glc^{6}–^{1}Glc. (Glc: β-D-glucopyranosyl). Both types of oestrogen receptor, ERα and ERβ, are expressed in endothelial cells. However, the ERα have been shown to promote angiogenesis (Johns *et al.*, 1996), while the ERβ have been proposed to inhibit angiogenesis (Hartman *et al.*, 2006). ER, oestrogen receptor.

fluorescent oestrogen ligand from the former type of ER. The finding that Rb1 is ERβ selective has been independently reported from a different laboratory (Cvoro *et al*., 2007).

Conclusions and future studies

In summary, ginsenoside-Rb1 was shown to inhibit capillary morphogenesis through activation of ERβ and increased production of PEDF. For Rb1 to be considered as a putative anti-tumour agent, the current observations would need to be confirmed *in vivo*, and evidence for the effectiveness of Rb1 in blocking tumour vascularization and size should be provided. It would also be interesting to further test Rb1 in other disorders characterized by excessive angiogenesis (that is, psoriasis, arthritis and diabetic retinopathy) using appropriate models. The search for additional molecules (receptors or enzymes) that serve as targets for Rb1 in ECs should continue to better characterize its pharmacological profile. Nature has provided us in the past with a range of chemical entities that are remarkably effective in treating human disease. Whether ginseng contains such a 'panax' anti-angiogenic agent remains to be proven.

References

Cai J, Jiang WG, Grant MB, Boulton M (2006). Pigment epithelium-derived factor inhibits angiogenesis via regulated intracellular proteolysis of vascular endothelial growth factor receptor 1. *J Biol Chem* **281**: 3604–3613.

Carmeliet P (2000). Mechanisms of angiogenesis and arteriogenesis. *Nature Med* **6**: 389–395.

Cho J, Park W, Lee S, Ahn W, Lee Y (2004). Ginsenoside-Rb1 from *Panax ginseng* C. A. Meyer activates estrogen receptor-{alpha} and -{beta}, independent of ligand binding. *J Clin Endocrinol Metab* **89**: 3510–3515.

Cvoro A, Paruthiyil S, Jones JO, Tzagarakis-Foster C, Clegg NJ, Tatomer D *et al*. (2007). Selective activation of estrogen receptor-{beta} transcriptional pathways by an herbal extract. *Endocrinology* **148**: 538–547.

Duh EJ, Yang HS, Suzuma I, Miyagi M, Youngman E, Mori K *et al*. (2002). Pigment epithelium-derived factor suppresses ischemia-induced retinal neovascularization and VEGF-induced migration and growth. *Invest Ophthalmol Vis Sci* **43**: 821–829.

Ferrara N, Hillan KJ, Novotny W (2005). Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy. *Biochem Biophys Res Commun* **333**: 328–335.

Folkman J, Shing Y (1992). Angiogenesis. *J Biol Chem* **267**: 10931–10934.

Hartman J, Lindberg K, Morani A, Inzunza J, Strom A, Gustafsson J (2006). Estrogen receptor beta inhibits angiogenesis and growth of T47D breast cancer xenografts. *Cancer Res* **66**: 11207–11213.

Herbst RS (2006). Therapeutic options to target angiogenesis in human malignancies. *Expert Opin Emerg Drugs* **11**: 635–650.

Huang KC (1999). *The Pharmacology of Chinese Herbs*. CRC Press: Boca Raton.

Johns A, Freay AD, Fraser W, Korach KS, Rubanyi GM (1996). Disruption of estrogen receptor gene prevents 17 beta-estradiol-induced angiogenesis in transgenic mice. *Endocrinology* **137**: 4511–4513.

Kiefer D, Pantuso T (2003). Panax ginseng. *Am Fam Physician* **68**: 1539–1542.

Kimura Y, Sumiyoshi M, Kawahira K, Sakanaka M (2006). Effects of ginseng saponins isolated from Red Ginseng roots on burn wound healing in mice. *Br J Phamacol* **148**: 860–870.

Leung KW, Cheung LWT, Pon YL, Wong RNS, Mak NK, Fan TPD *et al*. (2007). Ginsenoside-Rb1 inhibits tube-like structure formation of endothelial cells by regulating pigment epithelium-derived factor through estrogen receptor beta. *Br J Pharmacol* **152**: 207–215 (this issue).

Ng TB (2006). Pharmacological activity of sanchi ginseng (*Panax notoginseng*). *J Pharm Pharmacol* **58**: 1007–1019.

Sengupta S, Toh S-A, Sellers LA, Skepper JN, Koolwijk P, Leung HW *et al*. (2004). Modulating angiogenesis: the yin and the yang in ginseng. *Circulation* **110**: 1219–1225.

Takenaka K, Yamagishi S, Jinnouchi Y, Nakamura K, Matsui T, Imaizumi T (2005). Pigment epithelium-derived factor (PEDF)-induced apoptosis and inhibition of vascular endothelial growth factor (VEGF) expression in MG63 human osteosarcoma cells. *Life Sci* **77**: 3231–3241.

Volpert OV, Zaichuk T, Zhou W, Reiher F, Ferguson TA, Stuart PM *et al*. (2002). Inducer-stimulated Fas targets activated endothelium for destruction by anti-angiogenic thrombospondin-1 and pigment epithelium-derived factor. *Nature Med* **8**: 349–357.

Yue PYK, Wong DYL, Wu PK, Leung PY, Mak NK, Yeung HW *et al*. (2006). The angiosuppressive effects of 20(R)- ginsenoside Rg3. *Biochem Pharmacol* **72**: 437–445.

Yun T-K (2001). Panax ginseng – a non-organ-specific cancer preventive? *Lancet Oncol* **2**: 49–55.