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Biological activities and chemistry of saponins from *Panax ginseng* C. A. Meyer

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Abstract

The roots of *Panax ginseng* C. A. Meyer, known as Korean ginseng have been a valuable and important folk medicine in the East Asian countries, such as China, Korea and Japan for about 2000 years. Panax is derived from a word "panacea", which means cure-all diseases and longevity as well as physical strength and resistance. As the use of traditional Chinese herbs as a food supply becomes more and more popular in the western countries, sales of *Panax ginseng* are increasing in North America and Europe as well as other parts of the world. Active constituents found in most ginseng species include ginsenosides, polysaccharides, peptides, polyacetylenic alcohols and fatty acids. Major active components in *Panax ginseng* are the ginsenosides, a group of saponins with triterpenoid dammarane structure. More than 30 ginsenosides have been isolated, and known compounds are identified but new compounds were elucidated. Pharmacological effects of ginseng have been demonstrated in cancer, diabetes mellitus, cardiovascular system, immune system and central nervous system including anti-stress and anti-oxidant activity. We have focused this review on the effect of ginseng on diabetes, anticancer activity and cardiovascular system and chemical structures of ginsenosides. In addition, our recent biological study on 20(S)-ginsenoside Rg_3 is also touched upon as follows. Multidrug resistance (MDR) has been a major problem in cancer chemotherapy. In this study *in vitro* and *in vivo* modulations of MDR by 20(S)-ginsenoside Rg_3 (Rg_3), a saponin characteristic of red ginseng, was investigated. In flow cytometric analysis using rhodamine 123 as an artificial substrate, Rg_3 promoted accumulation of rhodamine 123 in drug-resistant human fibrocarcinoma KBV 20C cells in a dose-dependent manner, but it had no effect on parental KB cells. Additionally Rg_3 inhibited [^{3}H]-vinblastine efflux and reversed MDR to DOX (doxorubicin), COL, VCR (vincristine) and VP-16 in KBV20C cells. Reverse transcriptase-polymerase chain reaction and immuno-blot analysis after exposure of KBV20C cells to Rg_3 showed that inhibition of drug efflux by Rg_3 was due to neither repression of *MDR1* gene expression nor P-glycoprotein (Pgp) level. Photo-affinity labeling study with [^{3}H]-azidopine, however, revealed that Rg_3 competed with [^{3}H]-azidopine for binding to the Pgp demonstrating that G-Rg_3 competed with anticancer drug for binding to Pgp thereby blocking drug efflux. Furthermore, Rg_3 increased life span in mice implanted with DOX-resistant murine leukemia P388 cells *in vivo* and inhibited body weight increase significantly. Further clinical trial of Rg_3 in reversal of Pgp-associated MDR is highly feasible.

Introduction

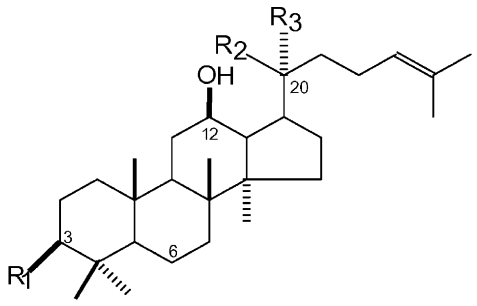
The root of *Panax ginseng* is one of traditional and folk medicines which has been used for many therapeutic purposes in the oriental countries such as Korea, China and Japan for thousands of years. The source plant of ginseng is *Panax ginseng* C. A. Meyer (Araliaceae), a herb with fleshy roots which grows wild in cool and shady forests extending from Korea and North eastern China to far eastern Siberia. However, because wild ginseng is relatively rare and very expensive, it has been cultivated in Korea, China and Japan. Most of commercially available ginseng is the root of *Panax ginseng* cultivated in Korea, the northeast district of China and Japan. The medical effects described in the related reference books categorize ginseng as a "mild" medication, a tonic that strengthens, invigorates a weakened body. The mainly physical ailments for which ginseng is said to be effective include headache, fatigue, dizziness, nausea, asthma, hemorrhage and impotence. It is further said to generally strengthen the viscera, improve resistance to external disease- causing agents, and improve the general physical conditions and mental capacity. Modern scientific data is substantiating many of these claims. Eminent scholars from around the world have focused their research their research into a question: which substances in ginseng manifest such miraculous effects ? The research has made much progress, but still insufficient to fully uncover the mystery of ginseng. In a noteworthy development in the 1960s, Sibata group identified the structures of saponin and called them as ginsenosides (Shibata et al., 1963a, b). Latest surveys show that saponins account for about 3–4 % of Korean ginseng, and more than 30 kinds of ginsenosides have been found in it, double the number in ginseng of other countries (Park, 1996). Considering that each of these ginsenosides has different pharmacological activities, it becomes apparent that Korean ginseng might have a pharmacological effectiveness superior to those of any other. In this paper, we review recent studies on the effects of ginsenosides on various diseases, particularly on diabetes, hypertension and cancer. Ginseng improves glucose homeostasis and insulin sensitivity. It exerts cytotoxic and anti-metastatic activities against various kinds of cancer cell lines, and induces differentiation or apoptosis of several cancer cells. Furthermore, ginsenoside has been reported to have an anti-neoplastic effect by enhancing immune function. An anti-hypertensive effect of ginsenoside is shown to occur by the enhanced synthesis and release of nitric oxide. In addition, ginsenoside exerts anti-atherosclerotic and anti-platelet effects. This review provides useful information on the pharmacological and clinical usage, and also suggests a method to conduct further study on ginseng to identify its potential effects.

Structural property of ginsenoside

The history of scientific research on ginseng dates back to 1854 when Garriques, an American scientist, isolated a saponin from ginseng. However, the actual introduction of ginseng to the West happened after World War II. The chemical structures of several ginseng saponins were characterized in the late 1960s and further accelerated scientific research on ginseng. Saponin components are triterpenoidal dammarane glycosides, named ginsenosides Rx according to their mobility on TLC plates, with polarity decreasing from index "a" to "h". Ginsenosides, known as saponins, are the major components of ginseng (Figures 1, 2, 3 and 4). Ginsenosides possess dammarane triterpenoidal skeleton with a modified side chain at C-20 (Shibata et al., 1995; Huang, 1999). They differ from one another by the type of sugar moieties, their number and their site of attachment. Among the saponins, the genuine sapogenins, protopanaxadiol and protopanaxatriol have been identified as dammar-24-ene-3β, 12β, 20(S)-triol and dammar-24-ene-3β, 6α 12β, 20(S)-tetrol, respectively (Shibata et al., 2001).

Ginsenosides of red and white ginsengs

The root of *Panax ginseng* is steamed and dried to prepare red ginseng, while the peeled roots dried without steaming are designated as white ginseng. The commercially available ginseng roots are classified into two forms, red and white ginsengs. It was reported that all of the saponins found in white ginseng were isolated in similar yields from red ginseng, while some partly deglycosylated saponins such as ginsenosides Rh_1, Rh_2 and Rg_3 are obtained from red ginseng as artifacts produced



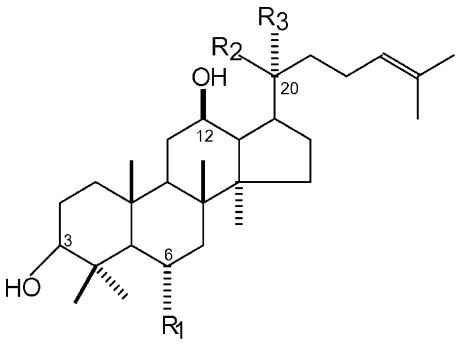
	R_1	R_2	R_3
20(S)-protopanaxadiol	OH	OH	CH_3
Ginsenoside Rb_1	O-glc(2→1)glc	O-glc(6→1)glc	CH_3
Ginsenoside Rb_2	O-glc(2→1)glc	O-glc(6→1)arap	CH_3
Ginsenoside Rc	O-glc(2→1)glc	O-glc(6→1)araf	CH_3
Ginsenoside Rd	O-glc(2→1)glc	O-glc	CH_3
20(S)-Ginsenoside Rg_3	O-glc(2→1)glc	OH	CH_3
20(R)-Ginsenoside Rg_3	O-glc(2→1)glc	CH_3	OH
Ginsenoside Rs_3	O-glc(2→1)glc(6)Ac	OH	CH_3
Compound K	OH	O-glc	CH_3
Ginsenoside Rh_2	O-glc	OH	CH_3

glc :β-D-glucopyranosyl arap : α-L-arabinopyranosyl

araf : α-L-arabinofuranosyl Ac :acetyl

Figure 1. Chemical structures of protopanaxadiol ginsenosides.

during steaming (Kitagawa et al., 1983). In addition, two minor saponins were also isolated only from red ginseng, two being designated as ginsenosides Rs_1 and Rs_2 (Kasai et al., 1983). Stepwise deglycosylated compounds such as compound K and 20(S)-protopanaxatriol can be generated through metabolic transformation by human intestinal bacteria (Hasegawa et al., 1996). Ginsenoside Rg_2 is converted into 20(S)-protopanaxatriol via ginsenoside Rh_1. The binding of the sugar has been shown to have influence on biological activity. Rh_1 and Rh_2 are structurally similar, but have different activity. Ginsenoside Rh_2 decreased growth of B16-BL6 melanoma cells, and stimulated melanogenesis and cell-to-cell adhesiveness. However, Rh_1 had no effect on cell growth and cell-to-cell adhesiveness, but stimulated melanogenesis (Odashima et al., 1985). Although both Rh_2 and Rh_3 induced differentiation of promyelocytic leukemia HL-60 cells into morphological and functional granulocytes, the potency of Rh_2 was higher (Kim et al., 1998). Another factor that contributes to structural difference between ginsenosides is stereochemistry at C-20. Although both 20(S)- and 20(R)-ginsenoside Rg_2 inhibit acetylcholine-evoked secretion of catecholamines from cultured bovine adrenal chromaffin cells, the 20(S) isomer shows a greater inhibitory effect (Kudo et al., 1998). For new dammarane glycosides named ginsenosides Rg_5, Rh_4, Rs_3 and Rf_2 have been recently isolated from Korean red ginseng and their chemical structures have been elucidated by chemical and spectroscopic methods, as 3-O-[β-D-glucopyranosyl (1 → 2)−β-D-glucopyranosyl]dammar-20 (22), 24-diene-3β,12β-diol (ginsenoside Rg_5), 6-O-β-D-glucopyranosyl-dammar-20(22), 24-diene-3β,

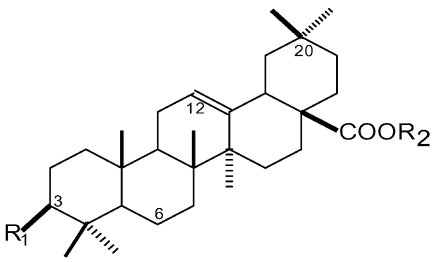


	R_1	R_2	R_3
20(S)-protopanaxatriol	OH	OH	CH_3
Ginsenoside Re	O-glc(2→1)rha	O-glc	CH_3
Ginsenoside Rg_1	O-glc	O-glc	CH_3
20(S)-Ginsenoside Rg_2	O-glc(2→1)rha	OH	CH_3
20(R)-Ginsenoside Rg_2	O-glc(2→1)rha	CH_3	OH
20(S)-Ginsenoside Rh_1	O-glc	OH	CH_3

glc :β-D-glucopyranosyl rha : α-L-rhamnopyranosyl

Figure 2. Chemical structures of protopanaxatriol ginsenosides.

6α,12β-triol (ginsenoside Rh_4), 3-O-[6″-O-acetyl-β-D-glucopyranosyl(1 → 2)−β-D-glucopyranosyl] 20(S)-protopanax adiol(ginsenoside Rs_3) and 6-O-[α-L-rhamnopyranosyl(1 → 2)−β-D-glucopyranosyl]dammarane-3β,6α,12β,20(R),25-pentol (ginsenoside Rf_2) (Park et al., 1998) (Figures 1 and 4).



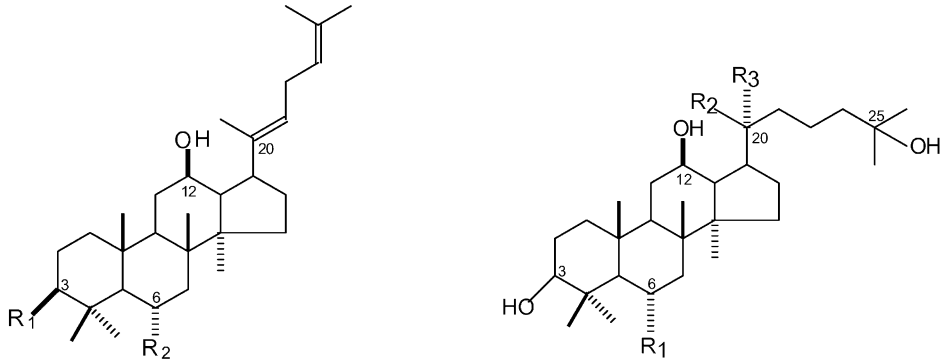
	R_1	R_2
Ginsenoside Ro	O-glcUA(2→1)rha	O-glc

glcUA : β-D-glucuronic acid rha : α-L-rhamnopyranosyl

Figure 3. Chemical structures of oleanane ginsenoside.

Metabolism of ginseng saponins by human intestinal bacteria

Crude drugs in herbal prescriptions contain many β-glycosides. Orally administered β-glycosides must meet gastric juice, digestive and bacterial enzymes in the gastrointestinal tract. Therefore, plant β-glycosides may act as natural prodrugs (Kobashi, 1998), which can be transformed to active metabolites after oral administration and finally induce biological activities. The decomposed products taken from the stomach of rats given a gastric bolus of ginsenoside Rb_2 differed from those produced by 0.1 N HCl (Han et al., 1982), and Rb_2 was found to be hardly decomposed by gastric juice with the exception of slight oxygenation (Karikura et al., 1991a, b). Certain ginsenosides such as Rb_1 and Rg_1 are poorly absorbed after injection (Odani et al., 1983). The metabolism of ginsenosides by human intestinal bacteria was examined. The oligosaccharides connected to the C-3 or C-20 hydroxy group of the aglycone are cleaved stepwise from the terminal sugar by bacterial hydrolysis. The



	R_1	R_2
Ginsenoside Rh_3	O-glc	H
Ginsenoside Rh_4	OH	O-glc
Ginsenoside Rg_5	O-glc(2→1)glc	H

glc :β-D-glucopyranosyl

	R_1	R_2	R_3
Ginsenoside Rf_2	O-glc(2→1)rha	CH_3	OH

glc :β-D-glucopyranosyl rha :α-L-rhamnopyranosyl

Figure 4. Chemical structures of ginsenosides with modified side chain.

main metabolic pathways are supposed as shown in Figure 5. Protopanaxadiol type saponins (ginsenosides Rb_1, Rb_2, Rc and Rd) were hydrolyzed to compound K(C-K) by intestinal flora (Karikura et al., 1991a, b) and the bacteria capable of hydrolyzing Rb_1 to C-K was identified as *Prevotella oris*. Whereas, protopanaxatriol type saponins (ginsenosides Re and Rg_1) were hydrolyzed to 20(S)-protopanaxatriol. C-K was shown to increase the cytotoxicity of antineoplastic drugs (Hasegawa et al., 1995) and to induce apoptosis in B16-BL6 melanoma cells (Wakabayashi et al., 1998). And also, the antiproliferative activity of C-K against tumor cells is primarily due to the induction of apoptosis *via* promotion of caspase-3 activity and regulation of apoptosis related proteins, which may lead to the anti-tumor activity (Suda et al., 2000). Moreover, in parallel with the antimetastatic effect of oral administration of C-K after the i.v. injection of colon 26-L5 cells, administration of C-K before tumor inoculation exerted a dose-dependent inhibition of liver metastasis (Hasegawa et al., 2000a). The pharmacokinetics of C-K and protopanxatriol were investigated. C-K was selectively accumulated into the liver and mostly excreted as bile, but about 24% of C-K was esterified with fatty acids in the liver and accumulated in the liver longer than C-K. In case of protopanaxatriol, it was absorbed from the small intestine into the mesenteric lymphatics followed by the esterification with fatty acids and its spreading to the organ and excretion as bile (Hasegawa et al., 2000b). The fatty acid esterification of intestinal bacterial metabolites of ginsenosides was found to potentiate the antitumor activity of the parental metabolites through delay of the clearance of C-K from the liver and immunostimulation of lymphocyte-mediated tumor cytotoxicity by the esterified form (Hasegawa et al., 2000c, 2002). These results definitely indicate that the pharmacological studies accompanied by metabolic elucidation of ginsenosides and other biologically active compounds lead to a better understanding of the real active ingredients in the body.

Reduction of blood glucose level and improvement of diabetes treatment by ginsenosides

Diabetes, affecting almost 3% of the world population, is one of the major global health problems and there is especially high number of incidence in

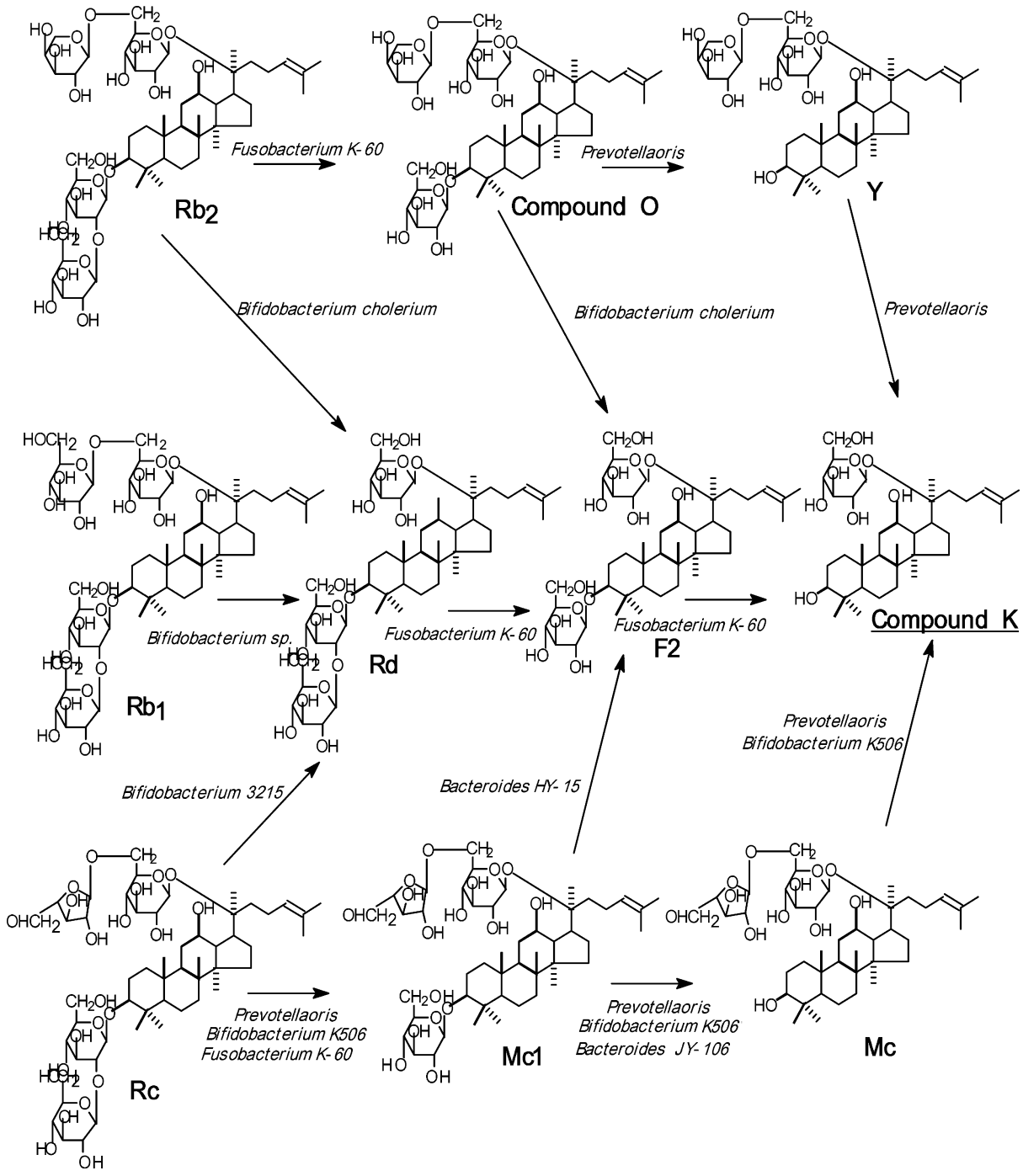


Figure 5. Metabolic pathway of ginsenosides Rb_1, Rb_2, Rc and Rd to compound K by intestinal bacterial flora after oral administraton.

elderly population. It has been shown that the root of *Panax ginseng* and other ginseng species possess anti-hyperglycemic activity *in vitro* (Kimura et al., 1980, 1981a, b) and *in vivo* (Kimura et al., 1981, 1981a, b, 1999; Yokozawa et al., 1985). More than 90% of patients with diabetes have type 2 diabetes,

which is related to aging and diet. Although type 2 diabetes is more common and has serious complications, even reducing life expectancy by 8–10 years (Astrup and Finer, 2000), most *in vivo* animal studies using ginseng have been conducted in type 1 rather than type 2 diabetes models. Here, we would like to focus on the effects of ginseng on type 2 diabetes.

The root of *Panax ginseng* has been used to improve glucose homeostasis and insulin sensitivity (Sonnenborn and Proppert, 1990) and further clinically to treat type 2 diabetes (Bensky et al., 1993; Huang, 1999). It has been observed that blood glucose level falls significantly in genetically obese diabetic mice after treatment with a single 90 mg/kg of ginseng root extract at an intraperitoneal (i.p.) dose (Kimura et al., 1999). It has also been demonstrated that 3 g of American ginseng root given 40 min before the test meal significantly lowers blood glucose level in non-diabetic subjects and type 2 diabetic patients (Vuksan et al., 2000). Oral administration of *Panax ginseng* root to diabetic KKAy mice for 4 weeks reduced blood glucose levels similar to that of an insulin sensitizer (rosiglitazone)-treated group (Chung et al., 2001). Moreover, ginseng therapy for type 2 diabetes elevates mood, improves psychophysical performance, and reduces fasting blood glucose and body weight. A 200 mg dose of ginseng improves glycated hemoglobin, serum lipid, amino-terminal propeptide concentration, and physical activity. These observations suggest that ginseng is beneficial for the people with type 2 diabetes and to prevent development of diabetes in non-diabetic subjects.

The main component of *Panax ginseng* is ginsenosides (ginseng saponins). Ginsenoside Rb_2 was found to be the most effective component of ginsenosides for streptozotocin-diabetic rats (Yokozawa et al., 1985). Rats treated with ginsenoside Rb_2 showed a significant decrease in blood glucose level with increased activity of glucokinase and decreased activity of glucose 6-phosphatase. Recently, anti-hyperglycemic and antiobese effects of *Panax ginseng* berry extract have been demonstrated, and its major constituent, ginsenoside Re has been observed (Attele et al., 2002). Treatment of the berry extract by daily i.p. injection for 12 days in obese diabetic C56BL/6J mice reduced glucose to levels similar to normal control value; after treatment the mice also has significantly improved glucose tolerance. The improvement of blood glucose level in the extract-treated *ob*/*ob* mice is associated with a significant reduction in serum insulin level in fed and fasting mice. A hyperinsulinemic–euglycemic clamp study reveals more than two-fold increase in the rate of insulin-stimulated glucose disposal in treated *ob*/*ob* mice. In addition, the extract-treated *ob*/*ob* mice lost a significant amount of weight, which was associated with a significant reduction in food intake and very significant increase in energy expenditure and body temperature.

Anticancer activities of ginsenosides

The main weapons in the war against cancer have been early detection and surgical removal, radiotherapy, chemotherapy, and attempts to develop gene therapy. However, the results are less than ideal, and the strategy is now changing from therapeutic approaches to prevention of cancer by identifying effective natural products as chemopreventive agents. One promising candidate with cancer-preventive effects is *Panax ginseng*. Its usefulness in cancer has been shown by extensive preclinical and epidemiological studies. Here, we describe the anti-carcinogenic effect of ginseng based on its diverse mechanisms.

Effect on tumor cell cytotoxicity and differentiation

Saponin and non-saponin compounds have been reported to show cytotoxic activities against various kinds of cancer cell lines in culture. Major active components are ginsenoside Rh_2, a peculiar component of red ginseng, and also polyacetylenes, panaxydol, panaxynol and panaxytriol. Ginseng was found to have the ability to induce neoplastic cells into normal cells. Ginsenoside Rh_2 inhibited the growth and colony forming ability of Morris hepatoma cells in soft agar suspension culture, and stimulated serum protein synthesis of these cells, thus converting the cell characteristics both functionally and morphologically to those resembling original normal liver cells, a process known as "redifferentiation or reverse transformation" (Odashima et al., 1989). Similarly, ginsenosides Rh_1 and Rh_2 have been shown to cause differentiation of F9 teratocarcinoma stem cells via binding to a steroid receptor (Lee et al., 1993). Therefore, recent studies on therapy for various

types of cancers have focused on drugs, which induce differentiation of maturation-resistant cells causing the disease. Furthermore, ginsenoside Rh_2 has been found to significantly induce B16 cell differentiation and to increase melanin synthesis in B16 cells (Xia and Han, 1996). The cytotoxicity of natural glycosides from ginseng, semisynthetic analogues of the genus *Betula* was evaluated to elucidate structure–activity relationships. Cytotoxic effects of the dammarane glucosides were inversely proportional both to the number of sugars attached to the aglycones and to the number of hydroxyl groups of the aglycones. The type of side chain and the configuration of the hydroxyl group at C-3 in aglycones was not found to have a significant influence on the cytotoxicity (Atopkina et al., 1999). Ginsenosides Rg_3 and Rh_2 has been found to inhibit the proliferation of prostate cancer cells, which may be associated with modulation of three modules of MAP kinases (Kim et al., 2004). In addition, 20(S)-protopanaxadiol and ginsenoside Rh_2 reduce cell proliferation and increase sub-G1 cells in two cultured intestinal cell lines, Int-407 and Caco-2, indicating a specific structure–function relationshilp for bioactive ginsenosides in two contrasting intestinal cell types (Popovich et al., 2004).

Anti-metastatic effects

Generally, primary tumor is not fatal. Instead, most cancer patients succumb to metastases – multiple, widespread tumor colonies established by malignant cells that detach themselves from the original tumor and travel through the body often to distant sites. Some invading cells penetrate into a body cavity or the blood, lymph or spinal fluid and then are released (Friedberg, 1986). Ginseng saponin has recently received a great deal of attention to the effects to inhibit invasion and metastasis of cancer cells. 20(R)- and 20(S)-ginsenoside Rg_3 was found to possess an ability to inhibit the lung metastasis of tumor cells such as B16-BL-6 melanoma and colon 26M3.1, when they were orally administered at a dose of 100–1000 μg/mouse, and the mechanism of their anti-metastatic effect is supposed to be related to inhibition of the adhesion and invasion of tumor cells, and also to anti-angiogenesis activity (Mochizuki et al., 1995). Glucocorticoid receptor-induced down-regulation of matrix metalloproteinase (MMP)-9 by panaxadiol (PD) and panaxatriol (PT) appear to be associated with the reduced invasive capacity of HT1080, a highly metastatic human fibrosarcoma cell line (Park et al., 1999). Multiple administration of ginsenoside Rb_2 after the intravenous inoculation of B16-BL6 melanoma cells resulted in a significant inhibition of tumor-associated angiogenesis responsible for the inhibition of lung tumor metastasis (Sato et al., 1994). Ginsenoside Rb_2 has been reported to inhibit invasion to the basement membrane via MMP-2 suppression in some endometrial cancers such as HHUA and HEC-1-A cells, and is supposed to be used as a medicine for inhibition of secondary spreading of uterine endometrial cancers (Fujimoto et al., 2001). It was shown that ginsenoside Rg_3 inhibited experimental pulmonary metastasis by highly metastatic mouse melanoma B16FE7 cells, which was mediated by inhibiting the 1-oleoyl-lysophosphatidic acid (LPA)-triggered rise of intracellular Ca^{+2} (Shinkai et al., 1996). While these results suggest the usefulness of ginsenoside Rg_3 in preventing cancer spread, further studies on *in vivo* will be necessary to clarify anti-metastatic effects of ginseng.

Anticarcinogenic activities and synergistic effects in combination with chemical therapeutic agents

Several investigations were carried out to evaluate the inhibitory or preventive effects of ginseng on carcinogenesis induced by various chemical carcinogens. The prolonged administration of Korean red ginseng extract inhibited the incidence and the proliferation of tumors induced by 7,12-dimethylbenz(a)anthracene (DMBA), urethane, and aflatoxin B1 (Yun et al., 1983). The chemopreventive potential of ginseng was evaluated using DMBA-induced skin tumorigenesis (papillomagenesis) in male Swiss albino mice, and there was a marked reduction not only in tumor incidence but also in cumulative tumor frequency at the initiation phase of tumorigenesis, while a little reduction at the promotional stage, suggesting the anti-carcinogenic activities of ginseng (Kumar, 1993). Ginsenosides Rg_3 and Rg_5 were found to show statistically significant reduction of lung tumor incidence in the newly established 9 weeks' medium-term anti-carcinogenicity test model of lung tumors in mice, and ginsenoside Rh_2 showed a tendency of decreasing the incidence, indicating

them to be active anti-carcinogenic compounds (Yun et al., 2001). Subsequent studies have confirmed that *Panax ginseng* C. A. Meyer cultivated in Korea is a non-organ specific cancer preventive against human cancers and the anticarcinogenicity or human cancer preventive effects is due to ginsenosides Rg_3, Rg_5 and Rh_2 (Yun, 2003). The inhibitory effects of ginseng on the development of 1,2-dimethylhydrazine (DMH)-induced aberrant crypt foci (ACF) in the colon were investigated in rats. Dietary administration of red ginseng in combination with DMH suppresses colon carcinogenesis in rats associated partly with inhibition of cell proliferation, acting on ACF in the colonic mucosa (Fukushima et al., 2001). In addition, anti-carcinogenic effect of red ginseng on the development of liver cancer induced by diethylnitrosamine (DEN) in rats was identified in preventive and curative groups (Wu et al., 2001). More recently, it has been reported that less glycosylated protopanaxadiol derivatives are effective in cancer prevention and some oleanane-type pentacyclic triterpenoid compounds show anti-carcinogenic activities in two-stage anti-cancer promotion experiments *in vitro* and *in vivo* (Shibata, 2001). Ginsenoside Rh_2 has been described to have diverse effects on the expression of the transformed phenotype in BALB/c3T3 cells, and augments the metastatic potential in an experimental metastasis assay (Tatsuka et al., 2001). The estrogenic potential of American ginseng extract to induce the expression of pS2, an estrogen-regulated gene, was evaluated in breast cancer cell lines MCF-7, T-47D and BT-20. It is found that American ginseng exhibits estrogen-like effects on estrogen receptor-positive breast cancer cells by inducing pS2 expression, suggesting that it may play a protective role against breast cancer (Duda et al., 1996). It is further supported by the fact that American ginseng inhibits breast cancer cell growth by transcriptional activation of the p21 gene, a universal cell cycle inhibitor, independent of p53 (Duda et al., 2001). It is also reported that concurrent use of American ginseng extract and breast cancer therapeutic agents results in a significant suppression of cancer cell growth, suggesting its synergistic effects on breast cancer therapeutics (Duda et al., 1999). Compound K, an intestinal bacterial metabolite derived from protopanaxadiol saponin, has been found to show anti-inflammatory effects by inhibiting TPA-induced COX-2 expression, which may contribute to its antitumor-promoting effects on mouse skin carcinogenesis (Lee et al., 2005).

Antitumor activities

It has been reported that an inhibitory effect of oral administration of ginsenoside Rh_2 on tumor growth in nude mice bearing human ovarian cancer cells (HRA), resulting in a remarkable retardation of the tumor growth. In particular, tumor growth in mice treated with 15, 30 and 120 μM of ginsenoside Rh_2 was significantly inhibited, compared to that in CDDP (*cis*-diaminedichloroplatinum) (II)-treated mice as well as in untreated mice (Tode et al., 1993). Further investigation showed that p.o. but not i.p. treatment with ginsenoside Rh_2 resulted in induction of apoptosis in the tumor in addition to augmentation of the natural killer activity in spleen cells from tumor-bearing nude mice, suggesting that an evaluation of the treatment of recurrent or refractory ovarian tumors is warranted (Nakata et al., 1998).

Induction of apoptosis

Apoptosis is responsible for pathological mechanism related to human diseases such as cancer, autoimmune disease, viral infection and neurodegenerative disorder (Thompson, 1995). Ginsenoside Rh_2 has been shown to arrest cell cycle at the G1 phase and to prolong the S phase (Fujikawa-yammamoto et al., 1987; Lee et al., 1996). It was reported that ginsenoside Rh_2 induced apoptosis through protein kinase C in human neuroblastoma SK-N-BE and rat glioma C6Bu-1 cells (Kim et al., 1999a–d). In addition, ginsenoside Rh_2 has been shown to induce apoptosis independently of Bcl-2, Bcl-x_L, or Bax in C6Bu-1 cells (Kim et al., 1999a–d). In a parallel study, it was found that ginsenoside Rh_2-induced cell death was mediated by the generated reactive oxygen species and activation of caspase pathway in a Bcl-x_L-independent manner. These reports demonstrate that the induction of apoptosis by ginseng can be one of its anti-carcinogenic mechanisms. In structure and apoptosis relationship, the presence of sugars in protopanaxadiol and protopanxatriol aglycone structures has been found to reduce the potency to induce apoptosis in the human leukemia (THP-1)

cell line and alternately alter membrane integrity (Popovich et al., 2002).

Inhibitory activities of 20(S)-ginsenoside Rg_3 on multi-drug resistance (MDR)

One of the major side effects to the effective treatment of human malignancies is the acquisition of broad anti-cancer drug resistance by tumor cells. This phenomenon has been termed "multi-drug resistance (MDR)". Therefore, development of new modulator for inhibition of drug resistance is required. MDR inhibitory activity was determined by measuring cytotoxicity to MDR cells using multi-drug resistant human fibrocarcinoma KB V20C, which is resistant to 20 nM of vincristine and expresses high level of *mdr1* gene. 20(S)-Ginsenoside Rg_3 (Rg_3), saponin of red ginseng, was found to have the most potent inhibitory activity on MDR and its ID_{50} (dose for 50% inhibition) was 8.2×10^{-5} M. In cytotoxicity assay, ginsenoside Rg_3 did not affect the growth of normal cells (Park et al., 1996). More recently, it was shown that the mechanism of Pgp (P-glycoprotein) inhibition and *in vivo* efficacy by Rg_3 has been elucidated as follows by us (Kim et al., 2003).

Inhibition of rhodamine 123 efflux by 20(S)-ginsenoside Rg_3

Rhodamine 123 acts as a good substrate for MDR-associated Pgp and agents that block Pgp have been found to increase the retention of rhodamine 123 in MDR cells. Therefore, drug-sensitive and -resistant cells were exposed to rhodamine 123 at the various concentrations of verapamil or Rg_3 for 30 min and retention of rhodamine 123 was determined. Treatment of KB cells with Rg_3 did not affect retention of rhodamine 123, but treatment of KBV20C cells significantly increased the accumulation of rhodamine 123 in a dose-dependent manner (Figure 6). Rg_3 did not increase efflux of rhodamine 123 effectively at lower doses, however Rg_3 was more potent than verapamil in modulating MDR function at the higher concentrations than 200 μM of Rg_3, as reflected by higher rhodamine 123 retention in the resistant cells. Since incubation of 320 μM of Rg_3 for 30 min with KBV20C cells significantly increased efflux of rhodamine 123 without losing cells integrity in PBS buffer, subsequent time course experiment used 320 μM of Rg_3. These results clearly demonstrated that Rg_3 reversed MDR in KBV20C cells specifically and effectively in a dose dependent manner.

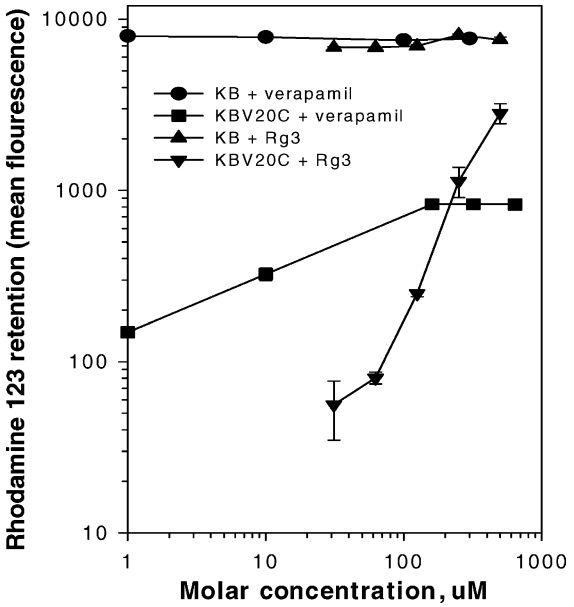


Figure 6. Increase of rhodamine 123 retention by Rg_3. Rhodamine 123 retention in resistant KBV20C but not in parent KB cells. KB or KBV20C cells were incubated at 37 °C for 24 h, and were resuspended in fresh media containing rhodamine 123, and further incubated at 37 °C for 30 min. After washing, the cells were incubated again in fresh media containing Rg_3, or verapamil at the indicated concentrations at 37 °C for 30 min. Cells were then removed and re-suspended in PBS, and analyzed by flow cytometry. The median fluorescence was used as a quantitative measure of intracellular fluorochrome accumulation and hence an indicator for Pgp inhibition. The vertical bar represents mean ± SD of the two experiments in triplicate. If vertical bars are not apparent, the size of the SD was close to zero. The average fluorescence values in KB and KBV20C without Rg_3 or verapamil were 6777 and 44.5, respectively.

Inhibition of [^{3}H]-vinblastine efflux by Rg_3

To ascertain further that Rg_3 can enhance the accumulation of chemotherapeutic agents, KB or KBV20C cells were incubated with [^{3}H]-vinblastine for the indicated time in the presence of either verapamil or Rg_3, and the accumulated [^{3}H]-vinblastine in KB or KBV20C cells was subsequently counted. In KB cells, Rg_3 or verapamil did not exhibit any difference from the group without any treatment in [^{3}H]-vinblastine accumulation

(data not shown). In KBV20C cells, 80 μM of Rg_3 had *a* marginal effect in reversing MDR, however at 320 μM [^{3}H]-vinblastine accumulation was enhanced by approximately four-fold after 30 min incubation at 37 °C. In comparison, verapamil at 100 μM augmented drug accumulation by two-fold demonstrating that 320 μM of Rg_3 inhibited drug efflux more potently than 100 μM of verapamil (Figure 7). This result confirmed that Rg_3 is an effective MDR modulator. Also potentiation of the accumulation of drug in MDR cells, but not in drug-sensitive cells, indicate that the MDR reversing effects of Rg_3 were not due to the toxicity of Rg_3 itself.

Inhibition of [^{3}H]-azidopine binding to Pgp by Rg_3

Since Rg_3 *did not* affect *MDR1* gene expression, MDR reversal by Rg_3 might be due to inhibition of drug binding to Pgp by Rg_3. To demonstrate competitive inhibition of drug binding by Rg_3, photoaffinity labeling of Pgp with [^{3}H]- azidopine was used. Treatment of 100 μM of Rg_3 completely inhibited binding of [^{3}H]- azidopine to Pgp demonstrating that Rg_3 competes with [^{3}H]- azidopine for binding to Pgp (Figure 8).

In vivo *efficacy*

For *in vivo* evaluation of Rg_3, a standard P388 murine leukemia model was used. In a preliminary experiment, DOX (doxorubicin) alone or a combination of a wide range of doses (2.5, 10, 40, 80 mg/kg) of the Rg_3 with 4 mg/kg of DOX was administered to mice (6/group) implanted with P388/DOX tumors. The result demonstrated that administration of a combination of 2.5 mg/kg of Rg_3 and 4 mg/kg of DOX did not result in significant increase in life span of mice *or* mice weight, and administration of 80 mg/kg of Rg_3, by itself, did not produce any significant difference in life span compared with the vehicle control. In addition, combination treatment with 4 mg/kg DOX and 80 mg/kg Rg_3 produced no significant increase in life span of mice than combination treatment with 4 mg/kg DOX and 40 mg/kg Rg_3 (data not shown). Therefore mice were treated with a combination of 4 mg/kg DOX plus 10 or 40 mg/kg Rg_3. When mice were treated with a combination of 4 mg/kg DOX plus 10 mg/kg of the Rg_3, *a* significant increase *in* life-span ($p < 0.01$) was observed. Increasing the dose of Rg_3 upto

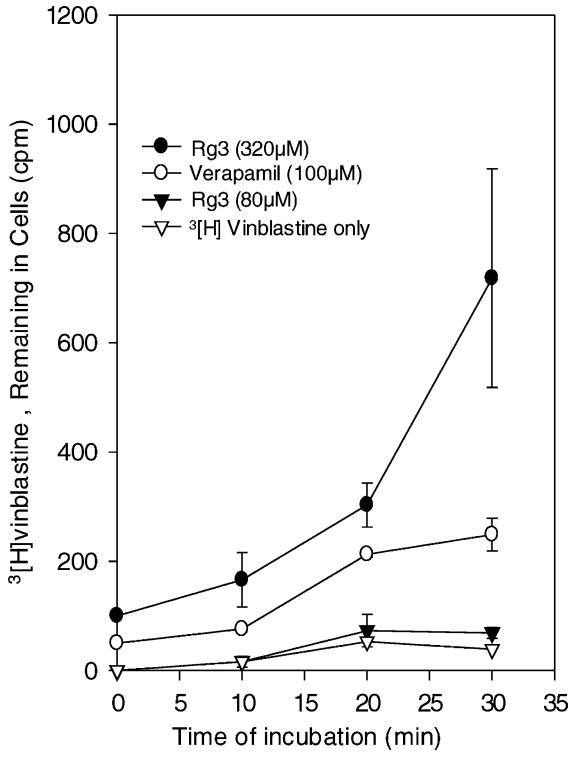


Figure 7. Increase of [^{3}H]-vinblastine accumulation by Rg_3. [^{3}H]-vinblastine accumulation in KBV20C cells was determined after incubation with 26 μM [^{3}H]-vinblastine and the appropriate modulator for the indicated time. After the indicated time, the cells were washed with ice-cold PBS twice, solubilized in 0.2% Triton X-100 in 10 mM phosphate buffer (pH 7.4), harvested, and then counted. The vertical bar represents mean ± SD of triplicate determinations.

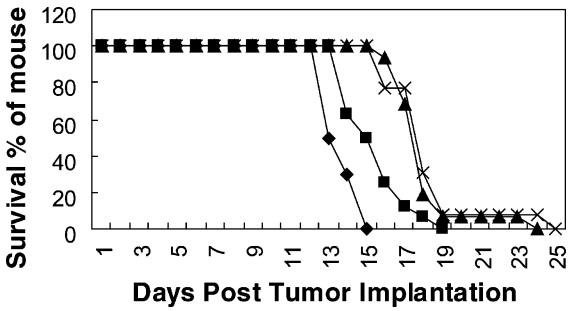


Figure 8. Inhibition of [^{3}H]-azidopine labeling of P-glycoprotein by Rg_3. To determine inhibition of [^{3}H]-azidopine binding to Pgp, resistant KBV20C plasma membrane proteins were incubated with 0.2 μM [^{3}H]-azidopine in the absence or presence of Rg_3 or verapamil at the indicated concentrations and cross-linked by UV irradiation. Photolabeled membrane proteins were analyzed by SDS-PAGE and visualized by autoradiography.

40 mg/kg did not extend life span any further (Figure 9). These results suggest that *the* increase of DOX cytotoxicity by Rg_3 *in vivo* is modulated through the interaction of the Rg_3 with Pgp. Further clinical trial in reversal of Pgp-associated MDR is highly feasible.

In addition, it is also reported that quasipanaxatriol, 20(S)-protopanaxatriol, ginsenoside Rh_2 and compound K, metabolic final product of protopanaxadiol saponin, greatly enhanced the cytotoxicity of the anti-cancer drugs in P388/ADM cells (adriamycin-resistant P388 leukemia cells) (Hasegawa et al., 1995). The reversal of daunomycin resistance in P388/ADM by quasipanaxatriol with double bond introduced at C-20 of protopanaxatriol was found to show the effective accumulation of the drugs mediated by the daunomycin-efflux blockage. Furthermore, a recent similar result showed that protopanaxatriol ginsenoside has a chemosensitizing effect on Pgp-mediated MDR cells by increasing the intracellular accumulation of drugs through direct interaction with Pgp at the azidopine site (Choi et al., 2003). These results suggest that further clinical trial of ginseng components in reversal of Pgp-associated MDR is highly feasible.

Antihypertensive effects of ginsenosides

High blood pressure is associated with decreased life expectancy and increased risk of stroke, coronary heart disease and other end-organ disease such as renal failure. Ginseng contains active compounds normalizing blood pressure. The effect of a certain drug on the blood pressure can be analyzed by investigating the effect of the drug on the smooth muscle of blood vessel. It is well established that blood vessel smooth muscle tone is regulated by the available intracellular Ca^{2+} concentration, which in turn is profoundly influenced by interaction of the cellular membrane and sarcoplasmic reticulum in the smooth muscle. It is found that both protopanxatriol and protopanaxadiol saponins inhibit Ca^{2+} binding to the cellular membrane, but protopanaxatriol is approximately 180% more potent than protopanaxadiol ginsenosides (Lee, 1980). It was reported that ginseng induced no significant change in blood pressure in those subjects with normal blood pressure, but had a normalizing effect on the subjects with abnormal blood pressure (Yammamoto, 1992). It has recently been reported that vasodilation and protective effect of ginsenoside Rg_1 against free radical injury might be related to enhanced synthesis and release of NO (Nitric Oxide), demonstrating the usefulness of ginseng in treatment of pulmonary and systemic hypertension (Gillis et al., 1993). The detailed mechanism and evidence of anti-hypertensive effect of ginseng are as follows.

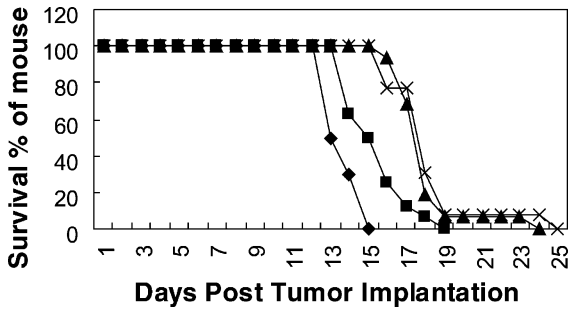


Figure 9. Increase of the survival of mice implanted intraperitoneally into P388/DOX murine leukemia tumor cells by Rg_3 in combination with DOX. Multidrug resistant P388/DOX cells (2×10^6 cells/0.1 ml) were inoculated intraperitoneally into BDF1 female mice. Compounds were administered intraperitoneally at every 3 days after tumor implantation. The results represent the survival percentage versus days after tumor implantation. Control, ◆; DOX 4 mg/kg, ■; DOX 4 mg/kg + Rg_3 10 mg/kg, ▲; DOX 4 mg/kg + Rg_3 40 mg/kg, ×.

Endothelium-dependent vasorelaxation

Endothelium plays an important role in regulating vascular tone by releasing several vasoactive autacoids including prostacyclin, endothelium-derived relaxing factor (EDRF) and endothelium-derived hyperpolarizing factor (EDHF) (Vane et al., 1990). EDRF has been identified as NO, which is produced from L-arginine by NOS (nitric oxide synthase) (Palmer et al., 1988). NO relaxes blood vessel mostly by stimulating soluble guanylyl cyclase, which leads to an increased production of cGMP in vascular smooth muscle (Rapoport and Murad, 1983). Ginsenosides have been found to lower blood pressure in a dose-dependent manner in rats at doses of 10–100 mg/kg, which is mediated by release of endothelium-derived NO, enhancing the accumulation of cGMP (Kim et al., 1994). Recently, it is also reported that ginseng can improve the vascular endothelial dysfunction in patients with hyper-

tension possibly through increasing synthesis of NO. Protopanaxatriol and its purified ginsenosides Rg_1 and Re caused endothelium-dependent relaxation, which is associated with the formation of cGMP. In contrast, protopanaxadiol or ginsenosides Rg_1 and Re did not affect vascular tone or production of cGMP in rat aorta. Moreover, ginsenosides Rg_1 and Re were less effective endothelium-dependent vasodilators than total ginsenosides and protopanaxatriol (Kang et al., 1995). Ginsenoside Rg_3 is one of protopanaxadiol group of red ginseng, steamed and dried ginseng. Present findings indicate that ginsenoside Rg_3 effectively stimulates the NO formation in endothelial cells, which accounts for the endothelim-dependent relaxation and production of cGMP in the rat aorta. The ginsenoside Rg_3-induced endothelium-dependent relaxation was markedly inhibited by a non-selective K^+ channel blocker, but not by an ATP-sensitive K^+ channel blocker (Kim et al., 1999a–d). These findings show that ginsenoside Rg_3 activates tetraethylammonium-sensitive K^+ channels in endothelial cells, which presumably leads to an influx of Ca^{2+} and the subsequent activation of the endothelial NOS (eNOS) (Kim et al., 1999a–d). In order to investigate the effect of red ginseng on the blood pressure, the change of blood pressure and heart rate after intravenous injection of red ginseng was studied in the conscious normotensive and one-kidney, one-clip Goldblatt hypertensive rats. Experimental evidence indicates that the NO-releasing effect of red ginseng is, like other NO donors, partly contributed to hypotensive effects (Jeon et al., 2000). Clinical study has been performed to estimate the effect of Korean red ginseng on vascular endothelial cell dysfunction in patients with hypertension. To assess the function of the vascular endothelial cell, changes of forearm blood flow to infusion of acetylcholine, sodium nitroprusside and bradykinin in incremental doses were measured by venous occlusion plethysmography. In ginseng-treated hypertensive group, forearm blood flows at the highest dose of acetylcholine and bradykinin were significantly higher than those of the non-treated hypertensive group. It was found that Korean red ginseng could improve the vascular endothelial dysfunction in patients with hypertension possibly through increasing the NO synthesis (Sung et al., 2000). These results support that ginseng shows pharmacological activities on circulatory diseases including hypertension. In another study, ginsenosides Rb_1 and Re has been found to decrease cardiac contraction in adult rat ventricular myocytes, which may be mediated in part through increased NO production (Scott et al., 2001). Based upon the previously published research data, several reviews underscore the potential benefit effects of ginseng on cardiovascular diseases and emphasizes the necessity for more rigorous systemic investigation (Zhou et al., 2004). When electrophysiological effects of ginsenosides were examined on action potential and membrane currents recorded from isolated guinea pig ventricular myocytes, inhibition of L-type Ca^{+2} current and enhancement of the delayed rectifier K^+ current appear to be associated with ginsenoside Re (Bai et al., 2003). Effect of notoginsenoside R1, active component of *Panax notoginseng*, was found to increase the fibrinolytic potential in cultured human pulmonary artery smooth muscle cells by increasing the production of tissue-type plasminogen activator, suggesting the effect of notoginsenoside R1 in the treatment of cardiovascular diseases (Zhang et al., 1997).

Antiatherosclerosis and antiplatelet effects

Endothelial cell damage is considered to be the initial step in the genesis of thrombosis and arteriosclerosis, the common precursors of cardiovascular disorders. Platelet hyperfunction such as enhanced platelet aggregation associated with overproduction of thromboxane A_2 (TXA_2), a potent platelet aggregative and vasoconstrictive substance, has been frequently encountered in patients with cardiovascular thrombotic diseases. Prostaglandin I_2 (PGI_2), a potent anti-platelet aggregative and vasodilatatory substance produced in vascular walls, is also reported to less synthesize in patients with atherosclerotic changes than normal subjects. Panaxynol was found to markedly inhibit the aggregation of platelets induced by collagen, arachidonic acid and platelet activating factor (PAF), while ginsenosides had no significant effect on the aggregation. It is suggested that panaxynol is the most potent anti-platelet agent in ginseng and its mechanism of action is chiefly due to the inhibition of thromboxane formation (Teng et al., 1989). In addition, it was

reported that panaxynol inhibited the aggregation, release and thromboxane formation in rabbit platelets, while ginsenosides Ro, Rg_1 and Rg_2 suppressed only the release (Kubo et al., 1990). Continually, it was also demonstrated that ginsenoside Rg_1 inhibited platelet activation induced by TXA_2 through inhibition of TXA_2-induced Ca^{2+} mobilization, and ginsenoside Rg_3 induced TXA_2-induced platelet aggregation. Ginsenoside Rc was shown to stimulate *in vitro* PGI_2 formation by cultured rat vascular smooth muscle cells through enhanced gene expression of cyclooxygenase (Hirai, 1999). These results suggest that ginsenosides Rg_1 and Rg_3, which have anti-platelet and anti-atherosclerotic effects, may have clinical potential for the prevention and the treatment of certain thrombotic and atherosclerotic disorders. Moreover, American ginseng extract was proved to be associated with the inhibition of thrombin-induced endothelin release due to NO release (Yuan et al., 1999). This result suggests that American ginseng may play a therapeutic role in facilitating the hemodynamic balance of vascular endothelial cells.

Conclusions

For a long time, ginseng has been one of the highly valued herbs in the East. Recently, many clinical trials using ginseng have been undertaken in the West as well as in the East area. Here, we have summarized diverse pharmacological and physiological effect of ginsenosids on the various diseases such as diabetes, cancer and cardiovascular diseases. However, although there have been lots of evidence suggesting that ginseng can be useful for the treatment of various diseases, we still have a long way to go. First of all, a lot of experiments reported have been performed in animal models instead of human beings. Therefore, a large-scale, controlled clinical study is needed to validate these results and apply to human beings. Secondly, there is still little evidence how ginsenosides and so on can be effective in the molecular levels. Understanding of molecular regulation of ginsenosides will be necessary to apply ginseng clinically, and also to find out new therapeutic effect of ginseng.

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