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Ginsenosides Derived from Asian (*Panax ginseng*), American Ginseng (*Panax quinquefolius*) and Potential Cytoactivity

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ABSTRACT

Ginseng is a slow-growing, deciduous perennial plant that belongs to the Araliaceae family and the *Panax* genus. There are a variety of species but the two main types are *Panax ginseng* C.A Meyer (Asian ginseng) and *Panax quinquefolius* (American ginseng). Asian ginseng is further subdivided by drying method of the root into either red or white ginseng. Traditionally, both Asian and American ginsengs have been used for a wide array of preventive and therapeutic purposes. Ginsenosides or dammarane triterpenoids are plant secondary metabolites and are thought to be the major active constituents of the *Panax* species. Ginsenosides are primarily classified into two groups, which are the 20(S)-protopanaxadiol (PD) and the 20(S)-protopanaxatriol (PT), which is based on their chemical structural differences. Differences in ginsenosides chemical structure are due to the type, position, and the number of sugar moieties attached by glycosidic bonds. Both Asian and American ginsengs generally contain a similar ginsenosides profile but vary in terms of amount of individual compounds. Rare ginsenosides which may not naturally be present in ginseng extracts can be obtained via processing methods such as steaming, microbial or enzymatic transformation. The detection and generation of rare ginsenosides can produce ginsenosides such as Rg3, Rh2, IH-901 (K), 25-OH-PD, 25-OCH3-PD among others and this has increased the interest into the biological activity of ginseng. This review focuses on the recent developments in ginseng research.

Keywords: *Panax ginseng*, *Panax quinquefolius*, ginsenosides, dammarane triterpenoids, apoptosis, adipocytes
Abbreviations: AMP kinase, 5' adenosine monophosphate-activated protein kinase; **ELISA**, Enzyme-linked immunosorbent assay; **ESI**, Electrospray ionization; **GLUT4**, Glucose transporter type 4; **HPLC**, High-performance liquid chromatography; **HPTLC**, High performance thin-layer chromatography; **NIRS**, Near infrared spectroscopy; **PD**, Protopanaxadiol; **PPARγ**, Peroxisome proliferator activated receptors gamma; **PT**, Protopanaxatriol

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INTRODUCTION

Ginseng has a long history of usage and recent literature suggests that it has broad effects and multiple plausible mechanisms of action. Ginseng research, in general, is hampered by the structural complexity of the bioactive dammarane triterpenoids also known as ginsenosides, which can make specific determinants of bioactivity difficult. Furthermore, the combination of ginsenosides in extracts renders chemical and biological classification extremely difficult and variable. Ginseng is likely the most researched natural health product and a number of reviews have been written (Shibata *et al*. 1985; Kitts and Popovich 2003; Popovich and Kitts 2003; Popovich and Kitts 2006); however, recently there has been an increased interest into specific rare ginsenosides present or metabolites produced from lactic acid bacterial fermentation and utilization of enzymes to produce specific ginsenosides. This review will mainly focus on the recent literature which includes the characterization of rare types of ginsenosides, detection of ginsenosides, and the effect of ginsenosides on cultured adipocyte regulation.

GINSENG AND GINSENOSIDE CLASSIFICATIONS

Asian ginseng (*Panax ginseng* C.A. Meyer) has traditionally been the main source of ginseng root utilized in traditional medicines and it is subdivided into two main categories based on the drying conditions used to preserve the root. Ginseng root which is typically air or oven dried is known as white ginseng. If ginseng root is steamed prior to oven drying a second type of ginseng is produced, known as red ginseng and is also referred to as Korean ginseng. The red color of this type of ginseng is a result of caramelization of sugars present in the root. American ginseng (*Panax quinquefolius* L.), a plant native to North America and has

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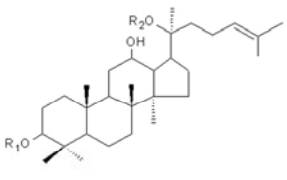
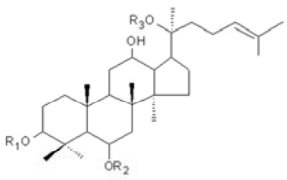
Invited Mini-Review



20 (S) Protopanaxadiol

	R1	R2
Rb1	O-G-G	O-G-G
Rb2	O-G-G	O-G-Ap
Rb3	O-G-G	O-G-X
Rc	O-G-G	O-G-Af
Rd	O-G-G	O-G
Rg3	O-G-G	O-H
Rh2	O-G-G	O-H
Aglycone (PD)	O-H	O-H



20 (S) Protopanaxatriol

	R1	R2	R3
Re	O-H	O-G-R	O-G
Rf	O-H	O-G-G	O-H
Rg1	O-H	O-G	O-G
Rg2	O-H	O-G-R	O-H
Rh1	O-H	O-G	O-H
Aglycone (PT)	O-H	O-H	O-H

Fig. 1 Chemical structure of ginsenosides classified as protopanaxadiol and protopanaxatriol groups. Abbreviations refer to the following: Ap = arabinopyranose, Af = arabinofuranose, G = glucopyranose-, R = rhamnopyranose, X= xylopyranose.

traditionally been used as an alternative to Asian ginseng for its purported cooling effects (yin) as opposed to Asian ginseng which has heating effects (yang). These heating and cooling effects are representative of the main principles of traditional Chinese medicine which aims to achieve the correct balance between yin and yang forces (Shi and Chu 1987). Most of the scientific literature generally focuses on white or red Asian ginseng and American ginseng. Differences in biological activity between these main types of ginseng have been reported and it is thought to be a result of differences in bioactive compounds (ginsenosides) attributed to each type.

Classification of the dammarane triterpenoids or ginsenosides generally falls into two main categories based on the attachment position of various water soluble sugar moieties to the non-polar aglycone. The amphiphilic nature ginsenosides is influenced by the polarity of the different sugar moieties attached to the ring structure. The first classification is known as the protopanaxadiol (PD) type and the second one is referred to as the protopanaxatriol (PT) (**Fig. 1**). The various combinations of sugar moieties are attached at position C-3 of the PD dammarane ring structure while PT has two attachment sites positions C-3 and C-6, respectively. These two types of ginsenoside make up the bulk of the reported ginsenosides. The PD type includes commonly reported ginsenosides Rb1, Rb2, Rc, Rd and rarer types known as Rg3 and Rh2 while the PT type includes Re, Rf, Rg1, Rg2 and Rh1. Ginsenosides were originally named for the migratory pattern when separated on a one dimensional thin layer chromatograph plate but this method of identification has been replaced by more robust methods of detection such as high performance liquid chromatography with an electrospray ionizing mass spectrometer (Popovich and Kitts 2004) (refer below).

Ginsenosides are secondary plant metabolites, and as other secondary metabolites are thought to be used as part of the defensive strategy of plants to thwart microorganisms and discourage herbivores (Wink 2008). Ginsenosides have been detected in all parts of the plant including the root, stems, leaves (Popovich and Kitts 2004) and flowers (Wang *et al*. 2009a). It is generally thought that the root contains higher amounts of ginsenosides compared to other parts of the plant.

There are compositional differences between the types of ginseng with respect to the 7 fingerprint ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rg1 and Rf) that are often measured to standardize ginseng extracts. With the exception of Rf, 6 ginsenosides overlap in Asian and American ginseng. In Asian and American ginseng, most abundant ginsenosides are Rb1, Rb2, Rc, Rd, Re and Rg1 (Ji *et al*. 2001). The amount of Rb1, Re and Rd in American are generally higher compared to Asian ginseng while the amount of Rg1, Rb2 and Rc are greater in Asian ginseng (Wang *et al*. 2007a), and a higher ratio of Rg1 to Rb1 compared to American ginseng (Chen *et al*. 2008). Ginsenoside Rf is thought to be found only in Asian ginseng and was considered a useful way to distinguish between Asian and American ginseng, however, there have been conflicting reports on whether Rf is indeed only found in Asian ginseng; this is mainly due to similar retention times and molecular weight to 24-(R)-pseudoginsenoside F11 in American ginseng (Chan *et al*. 2000). The presence of ginsenoside Rf in Asian ginseng and 24-(R)-pseudoginsenoside F11 in American ginseng can be used for identification of both species provided that LC-MS is used for the final detection (Chan *et al*. 2000; Li *et al*. 2000). A third type of ginsenoside has been detected in ginseng and is referred to as oleanane type but it is generally not quantified in most studies.

Less abundant ginsenosides such as Rg3 and Rh2 were thought to be found only in red ginseng. Steaming process used to prepare red ginseng has been known to affect the ginsenosides profile, resulting in a decrease in ginsenoside Rb1, Rb2, Rb3, Rc, Rd, Re and Rg1 and an increase in characteristic ginsenosides Rg2, 20(R)-Rg2, Rg3, Rh1 and Rh2 (Wang *et al*. 2007a; Choi 2008). However, these compounds have also subsequently been reported to be produced as a result of the utilization of high temperature during the steaming process and extraction in American ginseng (Popovich and Kitts 2002). For example, Rg3 and Rh2 have been reported in American ginseng extracts that were subjected to elevated temperatures during extraction and were formed from deglycosylation of sugars present on Rb1 which resulted in increased amounts of structurally related molecules Rd, Rg3 and Rh2. Both Rg3 and Rh2 have been detected in the dried leaves of American ginseng (Popovich and Kitts 2004) and Asian ginseng (Huang *et al*. 2008) and *Panax notoginseng* (Dan *et al*. 2008).

Panax notoginseng (also known as Sanchi Ginseng) is another well-known *Panax* species that has ginsenosides Rg1 and Rb1 as the most abundant ginsenosides (Dan *et al*. 2008; Wang *et al*. 2009a; Chen *et al*. 2010). Interestingly, ginsenoside Rg3 seems to be readily present in the rhizome, root and flower bud of *P. notoginseng*, with up to 6.90 ± 0.36 mg/g in the flower bud (Dan *et al*. 2008). The unique notoginsenosides fingerprints that includes notoginsenoside R1, -R2, -R4, -Fa, -Q, -S, -Fc, -H, -A, -B, -C, -D, -E, -F, -G, -H, -I, -J have allowed *P. notoginseng* to be easily distinguished (Yoshikawa *et al*. 1997; Ng 2006; Dan *et al*. 2008) from American and Asian ginseng. *Panax vietnamensis* (also known as Vietnamese Ginseng) ginsenoside composition includes Rb1, Rb2, Rd, Rd. Re, Rh5, Rg1, Rh1, Rh4 and also contains vina-ginsenosides R1, R2, R10, R25, pseudo-ginsenoside RT4, and majonoside R2 (Tran *et al*. 2001).

DETECTION AND ANALYSIS

Generally, ginsenosides can be extracted with a variety of solvents but hot water, methanol and ethanol are typically used. Methanol extraction tends to extract a greater abundance of ginsenosides but has limited usage for food grade application, resulting in ethanol and water extraction being favored. Various methods such as high performance thin-layer chromatography (HPTLC)-densitometry (Chen and Staba 1980; Vanhaelen-Fastre *et al*. 2000), gas chromatography (GC) (Cui *et al*. 1993; Cui *et al*. 1997), high-performance liquid chromatography (HPLC) (Cui *et al*. 1993; Corbit *et al*. 2005; Christensen *et al*. 2006; Hu *et al*. 2009), nuclear magnetic resonance (NMR) (Lee *et al*. 2009b; Han *et al*. 2010), near infrared spectroscopy (NIRS) (Ren and Chen 1999; Guan *et al*. 2007a), ultra-performance liquid chromatography (Chan *et al*. 2007; Guan *et al*. 2007a, 2007b; Toh *et al*. 2010), have been reported for the analysis of ginsenosides. Among these methods, high-performance liquid chromatography (HPLC) coupled with photodiode array or UV detection is by far the most commonly employed methods (Shangguan *et al*. 2001; Li *et al*. 2004;

Table 1 Selective ginsenosides molecular weight and main ion fragments detected by MS-ESI analysis.

Ginsenoside	Empirical formula	Molecular weight (Da)	Main ion fragments, *m/z*	
			$[M-H]^-$	Others
Rg1	$C_{42}H_{72}O_{14}$	801	800.0	859.5, 1199.9
Re	$C_{48}H_{82}O_{18}$	947	946.0	975.7, 1021.0
Rf	$C_{42}H_{72}O_{14}$	801	800.1	845.4, 859.3. 860.4
Rb1	$C_{54}H_{92}O_{23}$	1109	1108.0	1137.3, 1249.8
Rc	$C_{53}H_{90}O_{22}$	1079	1077.9	1107.4, 1137.1
Rb2	$C_{53}H_{90}O_{22}$	1079	1077.8	1107.2, 1123.2, 1437.2
Rd	$C_{42}H_{72}O_{14}$	947	946	1021, 968.7, 1087.6
Rg3	$C_{42}H_{72}O_{13}$	785	784.8	843.5, 873.4, 1039.3
Rh2	$C_{36}H_{62}O_8$	623	622.1	681.6, 933.5,1243.6

ESI conditions: The molecular weights of ginsenosides standards were confirmed by ThermoFinnigan (Thermo Fisher Scientific, Waltham, MA, USA) LCQ-ESI quadrupole ion trap MS system with MS^n capabilities in negative mode. The standards were delivered to ESI-MS at an ion spray voltage of 4.5 kV, a capillary temperature of 250°C and a capillary voltage of 40 eV. A full scan mass spectrum over the range of 100-1500 *m/z* was recorded using Xcaliber 2.0 data processing software.

Christensen *et al*. 2006; Zhou *et al*. 2008a; Christensen and Jensen 2009; Hu *et al*. 2009; Lee *et al*. 2009b; Han *et al*. 2010). Most reported HPLC methods use a reverse phase C_{18} column with water or phosphate buffers and acetonitrile mixture as solvent system, run in either isocratic or gradient elution mode. Ginsenosides are however poor chromophores that require low-wavelength UV (198-205 nm) detection, resulting in high baseline noise and consequently, poor sensitivity. To counter this, HPLC is often coupled with various other detections techniques such as electrospray ionization (ESI) (Song *et al*. 2005; Zhang and Cheng 2006; Li *et al*. 2007; Zhang *et al*. 2007; Xia *et al*. 2008; Joo *et al*. 2010), evaporative light scattering detection (ELSD) (Kim *et al*. 2007; Wan *et al*. 2007; Zhao *et al*. 2009a), MS/MS (Ji *et al*. 2001; Zhang *et al*. 2003; Qian *et al*. 2005; Song *et al*. 2005; Qian *et al*. 2006). HPLC-ESI-MS is known to be a sensitive and fast technique that has been widely used for the qualitative studies on saponins, including ginsenosides (Popovich and Kitts 2004; Zhang *et al*. 2009b). **Table 1** shows the main ginsenosides molecular ion and fragments measured by ESI-MS in negative mode. Three pairs of ginsenosides isomers (Rg2 and Rg3, Rg1 and F11, Rd and Re) were reported to be separated and identified through accurate mass measurement of mass spectrometry (MS) and MS-MS (Song *et al*. 2005). Spectrum interpretation and accurate mass measurement have allowed the structures of aglycone and various sugars attached to ginsenosides to be determined. While the ESI-MS data accurately suggested co-eluted ginsenoside existed within the sample fraction, the interpretation of MS-MS spectrum and fragmentation pathways allowed the detection of isomers (Song *et al*. 2005).

MEASUREMENT IN BIOLOGICAL FLUIDS

Ginsenosides Rb1 and Rg1 have been detected in rat serum using an enzyme-linked immunosorbent assay (ELISA) with highly specific monoclonal antibodies (Bae *et al*. 2000; Hasegawa 2004; Chao *et al*. 2006). This methodology was shown to have good correlations for Rb1 ($\gamma = 0.997$) and Rg1 ($\gamma = 0.998$) between assay values from both ELISA and HPLC (Chao *et al*. 2006). Detection of ginsenosides transformed by intestinal microbiota (see below) was successfully detected by HPLC equipped with high-resolution Fourier transform ion cyclotron resonance mass spectrometer (FTICR-MS) (Kong *et al*. 2009). There are increasing number of reports on ginsenoside detection in rat plasma (Qian *et al*. 2005; Xie *et al*. 2005; Qian *et al*. 2006; Li *et al*. 2007; Xia *et al*. 2008; Joo *et al*. 2010), rat urine (Li *et al*. 2004), dog plasma (Wang *et al*. 2007b) and in clinical pharmacokinetic studies (Zhang *et al*. 2009a). LC/MS-MS was validated to be a rapid and sensitive technique for the determination of PD in human plasma and was successfully applied to a clinical pharmacokinetic study of PD in healthy volunteers with a detection limit of 0.05 ng/mL (Zhang *et al*. 2009a).

COMPOUND K

Oral administration of ginseng or ginsenosides can result in biotransformation in the large intestine by the microflora; these microflora include *Bacteroides*, *Fusobactrium*, *Eubacterium* and *Provetella* species of bacteria (Lee *et al*. 2009a). Intestinal bacterial have been reported to hydrolyze fingerprint ginsenosides such as Rb1, Rc, Rd into more active deglycosylated products that tend to show more bioactivity in a variety of animal and cultured cell experiments. Different species of bacteria may be able to selectively cleave sugar moieties attached to either PD or PT aglycone. As a result there is slight variability in the literature with regard to the specific products that are produced by bacterial biotransformation. It is generally thought that the intestinal bacteria can transform ginsenoside Rb1 into Rg3, Rh2, PD and compound K (**Fig. 2**). Compound K is one of the major metabolites produced (Bae *et al*. 2000, 2002; Hasegawa 2004; Zhang *et al*. 2009a) and has been proposed as the most bioavailable metabolite produced from colonic fermentation (Hasegawa 2004). Many different schemes have been reported to produce compound K. Compound K has been produced utilizing fungal biotransformation with *Paecilomyces bainier* sp. 229 (Zhou *et al*. 2008b). Thermostable β-glycosides from *Sulfolobus solfataricus* has been reported to be able to hydrolyze ginsenosides and biotransform them into compound K at an optimal pH of 5.5 from a ginseng extract (Noh *et al*. 2009). Conversion of both Rb1 and Rb2 to Ginsenoside Rd is one route to the production of compound K (Noh *et al*. 2009). Breakdown products of PD-type ginsenosides utilizing crude enzyme preparation of lactase, and β-galactosidase from *Aspergillus oryzae* and cellulase from *Trichoderma viride* were found to produce ginsenoside Rd, and intermediate ginsenoside product F2 (**Fig. 2**) and compound K (Ko *et al*. 2007). Lactase from *Penicillium* sp produced Rh1 from PT-type of ginsenosides while it produced Rd, Rg3 and compound K from the PD-type of ginsenosides (Ko *et al*. 2007). Ginsenoside Rg3 has also been reported to be produced from a crude ginseng extract with the commercial enzyme cellulase-12T (Bioland) (Chang *et al*. 2009). Selective transformation of ginsenoside Rg3 to Rh2 has been reported using β-glucosidase from *Fusarium proliferatum* (Su *et al*. 2009). A variety of other metabolites have reported to be produced based on whether the source ginsenosides was either a PD or PT type of ginsenoside and have been effectively reviewed with an absorption scheme proposed (Hasegawa 2004). These metabolites are thought to be preferentially absorbed and esterified with either stearic, oleic or palmitic fatty acids in the liver (Hasegawa 2004).

An 80 kDa enzyme termed ginsenosides type I was isolated and described from *Aspergillus* sp. g48p strain and is reported to selectively hydrolyze sugar moieties glucose, arabinose, xylose on region R1 (**Fig. 1**) of dammarane ring structure of PD-type ginsenosides such as Rb1, Rb2, Rb3, Rc, Rd but not PT-type to produce F2, compound K and Rh2 (Yu *et al*. 2007). A second ginsenosidase type II further isolated from the same *Aspergillus* strain removes glyco-

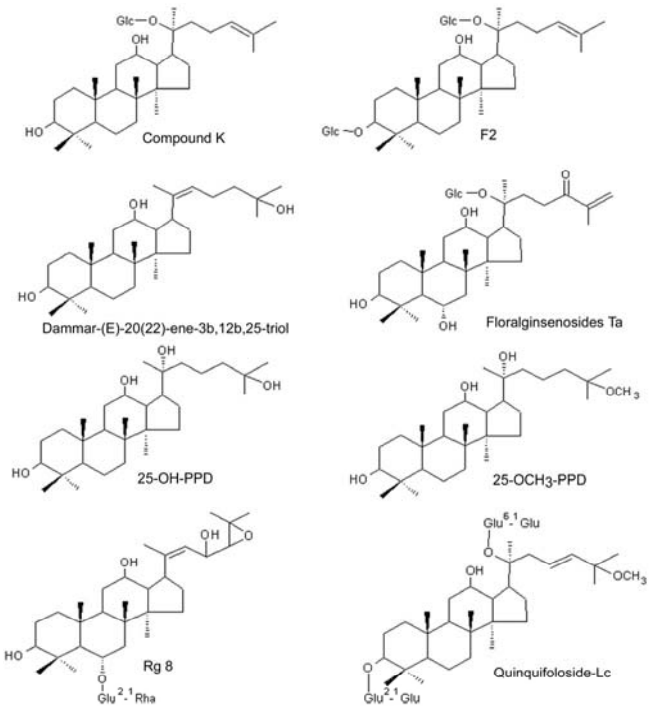


Fig. 2 The chemical structure of rare ginsenosides. Based on Dou *et al*. 2006; Qiu *et al*. 2009; Tao *et al*. 2009; Tung *et al*. 2010; Wang *et al*. 2009b.

sides containing glucose from Rb1, arabinose-pyranose (Rb2), xylose (Rb3) arabinose-furanose (Rc) at region R3 (**Fig. 1**) of PD-type to yield ginsenosides Rd and Rg3 (Yu *et al*. 2009). A β-glucosidase termed G-II extracted from a fungus *Cladosporium fulvum* has been reported to selectively cleave the glycoside bond at R3 of ginsenoside Rb1 to produce Rd at an optimal pH and temperature of 5.5 and 45°C, respectively (Zhao *et al*. 2009b).

COMPOUND K AND CYTOACTIVITY

Compound K produced by microbial transformation of commercial obtained ginsenoside extract with *Aspergillus niger* were found to inhibit the growth of a variety of cultured cells including melanoma (B16-B6, LC_{50} 12.7 μM), hepatocarcinoma (Hep-G2, LC_{50} 11.4 μM), myeloid leukemia (K562, LC_{50} 8.5 μM) and lung carcinoma (95-D, LC_{50} 9.7 μM) cells (Zhou *et al*. 2006).

Compound K (IH-901) is thought to the major biotransformation product of PD ginsenosides that can be detected in the urine of both rats and humans (Bae *et al*. 2000, 2002). The effect of compound *in vivo* has not been fully elucidated. In cultured hepatocellular cells (SMMC7721), compound K reduced cell growth that was dependent on both concentration and incubation time assessed by the MTT viability assay (Ming *et al*. 2007). Treatment induced apoptotic cell death with a cell cycle arrest at the G0/G1 phase at concentrations between 25-75 μM and up-regulated cytochrome c, p53 and Bax expression and down-regulated procaspase-3 expression, all of which are involve in the apoptotic cell death pathway (Ming *et al*. 2007). In human malignant astrocytoma cells, 13 ginsenosides and metabolites were screened and compound K and Rh2 were identified to induced apoptotic cell death by activating the caspases, p38, and MAPK while not affecting the growth of primary astrocytes (Choi and Choi 2009). The effects were magnified when combined with a Fas ligand which is part of the TNF/NGF receptor family (Choi and Choi 2009).

NEWLY CHARACTERIZED GINSENOSIDES

A variety of newly isolated and characterized ginsenosides have been reported recently (**Table 2**). A panaxadiol type (dammar-(E)-20(22)-ene-3β,12β,25-triol) has been isolated from Asian ginseng utilizing acid hydrolysis for 8 h at 80°C with 10% HCl in 50% ethanol (Tao *et al*. 2009). Three newly characterized ginsenoside-like saponins were extracted from the flower buds of Asian ginseng and are referred to as floralginsenosides Ta, Tb, and Tc (Tung *et al*. 2010) (**Fig. 2**). The cytotoxicity of these compounds were tested on human leukemia cells (HL-60) and floraginsenosides Ta was found to have an LC_{50} of 36.3 μM assessed by the MTT viability assay and induced apoptosis where as Tb and Tc did not show cytotoxicity (Tung *et al*. 2010). Extraction and characterization of the leaves of American ginseng yield a new compound named quinquifoloside-Lc (**Fig. 2**) (Qiu *et al*. 2009). This new compound showed some cytotoxicity with an LC_{50} value determined to be 93.8 μmol/L in estrogen dependent cultured breast cancer cells (MCF-7) but it was not as cytotoxic as ginsenoside Rh2 (LC_{50}, 20 μmol/L) (Qiu *et al*. 2009). A further new type of dammarane triterpenoid from the American ginseng root was characterized by infra red (IR), MS, 13C-NMR and was named as ginsenoside Rg8 and was detected along with two previously identified ginsenosides (20E)-F4 and F1 which have been detected in American ginseng root for the first time (Dou *et al*. 2006).

Two new ginsenosides 25-OH-PPD and 25-OCH_3-PPD were identified from Asian ginseng and cytotoxicity established in cultured pancreatic cells (Panc-1, HPAC) by the MTT assay (Wang *et al*. 2009b). The LC_{50} of 25-OH-PPD was determined to be 21.2 μM in Panc-1 cells and 22.5 μM in HPAC whereas the 25-OCH_3-PPD was more potent with LC_{50} values of 7.8 and 5.8 μM in the respective cells lines (Wang *et al*. 2009b). Both compounds showed an ability to arrest the cell cycle at the G1 phase (Wang *et al*. 2009b). It is likely that as more attention is paid to complete identification of compounds present in ginseng and ginseng plant parts that further new compounds will be characterized and bioactivity assessed.

ADIPOCYTE REGULATION

As obesity levels in the developed world and especially in North America have increased substantially in the past few decades (Flynn *et al*. 2006), adipocyte regulation by natural products such as ginseng has become a burgeoning research topic area. The molecular regulation of adipocyte is now thought to contribute to a number of factors that have been either linked to the development of the metabolic syndrome

Table 2 Summary of rare ginsenosides extraction and possible cytotoxicity.

Ginsenosides	Source	Extraction mode	Biological effect	Reference
dammar-(E)-20(22)-ene-3β,12β,25-triol)	Asian ginseng	Acid hydrolysis HCl (10%), ethanol (50%), 80°C, 8 h	Not yet characterized	Tao *et al*. 2009
floralginsenosides Ta	Asian ginseng flowers	Hot methanol	Cytotoxic effect on HL-60 cells LC_{50} 36.3 μM	Tung *et al*. 2010
quinquifoloside-Lc	American ginseng leaves	Ethanol (60%)	Cytotoxic effect on MCF-7 cells, LC_{50} 93.8 μmol/L	Qiu *et al*. 2009
Rg8	American ginseng	Ethanol (70%)	Not yet characterized	Dou *et al*. 2006
25-OH-PPD	Asian ginseng fruit	Ethanol (75%)	Cytotoxic effect on Panc-1 cells LC_{50} 21.2 μM, 22.5 μM HPAC cells	Wang *et al*. 2007b, 2009b
25-OCH_3-PPD	Notoginseng leaves	Ethanol (75%)	Cytotoxic effect on Panc-1 cells LC_{50} 7.8 μM, 5.8 μM HPAC cells	Zhao *et al*. 2007; Wang *et al*. 2009b

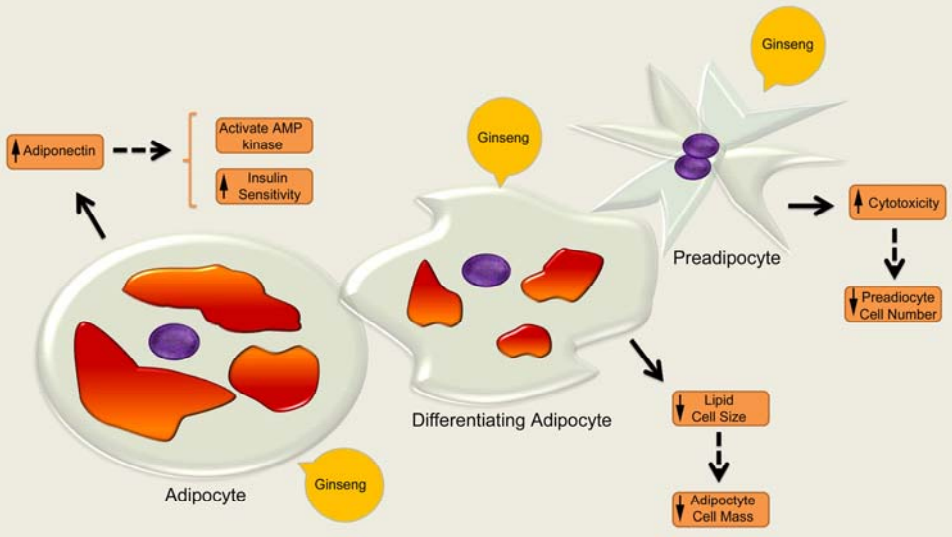


Fig. 3 American ginseng regulation on cultured 3T3-L1 preadipocyte, differentiating adipocyte and mature adipocyte. Black arrows are summarized from (Yeo *et al*. 2010), dotted arrow represent potential outcomes *in vivo*. Purple circles represent the nucleus and red color inside the cells represents the lipid content.

insulin resistance and diabetes (Gil-Campos *et al*. 2004) or the maintenance of health. The effect of specific ginsenosides on the adipocyte regulation and the promotion or inhibition of adipogenesis is currently unclear. The 3T3-L1 murine fibroblast cell line is typically utilized to study *in vitro* adipocyte metabolism as it differentiates from a fibroblast into an adipocyte-like cell. Ginsenoside Rb1 treatment (10 μM) of differentiating 3T3-L1 fibroblast (3T3-L1 cells) increased adipogenesis or lipid acquisition measured by Oil-Red-O staining of cells in a dose-dependent manner (Shang *et al*. 2007). Rh1 treatment further increased both peroxisome proliferator activated receptors gamma (PPARγ), C/EBPα quantified by RT-PCR and up-regulated GLUT4 (glucose transporter type 4) (Shang *et al*. 2007). Expression of both PPARγ and C/EBPα are important for cellular differentiation. Ginsenoside Rb2 has also been reported to have an influence on cultured 3T3-L1 cells (Kim *et al*. 2009). Under high cholesterol condition and elevated serum conditions of the culture media, Rb2 prevented lipid acquisition and decreased triglyceride levels while stimulating the expression of leptin mRNA (Kim *et al*. 2009). Ginsenoside Rh2 has been reported to possess anti-obesity like properties in 3T3-L1 cells (Hwang *et al*. 2007). Ginsenoside Rh2 effectively inhibited the differentiation of preadipocyte-like fibroblasts into adipocytes by PPARγ inhibition and reduced the lipid acquisition at concentrations of 20 and 40 μM (Hwang *et al*. 2007). AMP kinase (5' adenosine monophosphate-activated protein kinase) which is involved in cellular energy homeostasis was also up-regulated after Rh2 treatments. As with ginsenoside Rh2, Rg3 has similarly been reported by the same research group to inhibit both PPARγ and activating AMP kinase. Both PPARγ inhibition and AMP kinase activation are desirable traits and are thought to decrease potential risk factors of metabolic diseases such as diabetes. Protopanaxatriol (PT) was reported to activate PPARγ dose-dependently in 3T3-L1 cells and this activity was similar to the thiazolidinedione troglitazone a PPARγ agonist (Han *et al*. 2006); at concentration of between 1– 25 μM treatment increased the GLUT4 expression (Han *et al*. 2006).

Ginsenoside Rg3 and Re at concentrations between 1 – 10 μM were reported to increase glucose uptake in 3T3-L1 cells by approximately 10 and 12% and this increase was likely through increase in GLUT4 expression (Lee *et al*. 2011). Similarly compound K and Rg1 both increase glucose transport in 3T3-L1 cells by increasing the expression of GLUT4 but not GLUT1 and compound K was reported to suppress lipid accumulation while Rg1 increase it (Huang *et al*. 2010).

In our laboratory we have attempted to establish a baseline response of ginsenosides contained in extracts derived from American ginseng on 3T3-L1 differentiation and adiponectin expression (Yeo *et al*. 2011). An extract that contained Rg1 (347.3 ± 99.7 μg/g, dry weight), Re (8280.4 ± 792.3 μg/g), Rb1 (1585.8 ± 86.8 μg/g), Rc (32.9 ± 8 μg/g), Rb2 (62.6 ± 10.6 μg/g) and Rd (90.4 ± 3.2 μg/g) dose-dependently reduce the growth of 3T3-L1 fibroblasts with an LC_{50} value determined to be 40.3 ± 5 μg/mL (Yeo *et al*. 2011). When the ginseng extract was included along with the differentiation hormone cocktail required for 3T3-L1 cells differentiation, it significantly reduced lipid acquisition by 13% and 22% when treated at concentrations of 20.2 μg/mL and 40.3 μg/mL compared to untreated differentiating control cells. Furthermore, this extract increased adiponectin release measured by western blot. *In vivo*, decreased adiponectin has been observed in the obese and is related to insulin resistance (Abbasi *et al*. 2004; Antuna-Puente *et al*. 2008). **Fig. 3** shows a representation of the potential interaction of ginsenosides with the preadipocyte, differentiating adipocyte and the mature adipocyte.

CONCLUSION

The complexity of the triterpenoids contained in ginseng both from American and Asian ginseng sources allow for an interesting field of research. Novel metabolites produced by colonic fermentation with intestinal microbiota or biotransformation of ginseng with other microorganism or enzymes have expanded the number of ginsenosides. As new a more sensitive analytical detection techniques are focused on ginseng more novel compounds will be uncovered. It is likely that these new metabolites or compounds would convey greater or different bioactive response compared to the typical ginsenosides contained in a fingerprint analysis. As ginseng research progresses, the determination of biological activity such as the influence on adipocyte function becomes increasingly important to the overall understanding of impact of these roots with a long history of use.

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