Compound K derived from ginseng: neuroprotection and cognitive improvement

Jisun Oh and Jong-Sang Kim*

The evidence for the neuroprotective and cognitive effects of compound K, a metabolite biotransformed from ginsenosides Rb1, Rb2, and Rc, is reviewed here. Compound K is more bioavailable than other ginsenosides and therefore has greater potential to exert bioactive functions in the body. Although the capability of compound K to cross the blood–brain barrier is not clear, it has been reported to have neuroprotective and cognition enhancing effects and decrease inflammatory biomarkers in animal models of Alzheimer’s disease and cerebral ischemia. The plethora of potential health benefits of compound K warrants further research to evaluate its biochemical mechanisms and its ability to protect healthy populations from neurodegenerative diseases.

Introduction

Ginseng, the rhizome of plants of the Panax species (family Araliaceae), is widely consumed as a nutraceutical or functional food because of its beneficial effects in various diseases, including inflammation,1 cancer,2 diabetes,3 immune disorders,4 and neurodegeneration.5 Ginseng generally contains triterpene glycosides (saponins), sesquiterpenes, polyacetylenes, phenolic compounds, polysaccharides, peptidoglycans, fatty acids, and vitamins.6,7 The beneficial effects of ginseng are reported to be primarily attributable to the function of saponins, which are also known as ginsenosides.8–10 Approximately 150 different ginsenosides are classified into two groups, the dammarane group and the oleanane group, depending on the aglycone to which water soluble sugar moieties are attached.11 Based on their chemical structure, the dammarane group ginsenosides are further classified into two types: protopanaxadiol (PPD), including Ra1, Ra2, Ra3, Rb1, Rb2, Rb3, Re, Rd, Rg3, Rh2, F2, and compound K (CK; 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol), and protopanaxatriol (PPT), including Re, Rf, Rg1, Rg2, Rh1, and F1 (Fig. 1).12,13

To express bioactive functions, ginsenosides must be converted into aglycones by physical and biological treatments such as heat and enzymatic activity of intestinal microorganisms.10,14–16 For instance, the PPD-type ginsenoside Rb1 shows limited intestinal absorption and therefore lacks efficacy. However, Rb1 is disintegrated by gastric acid and/or intestinal microorganisms into smaller molecules, such as Rd, F2, and compound K, and further into PPD. Similarly, ginsenoside Rg1 is converted into Rh1 and F1, and further into PPT, which is better absorbed in the gastrointestinal (GI) tract and therefore more bioactive than parent compounds.

Compound K can be produced from PPD-type ginsenosides such as Rb1, Rb2, and Rg1, by heating,17 microbial conversion,18,19 or enzymatic hydrolysis.20 Recent studies reported the diverse biological functions of compound K. This review summarizes the structural and functional features of compound K, highlighting its neuroprotective and cognitive effects.

Overview of metabolism and pharmacokinetics of ginsenoside compound K

Compound K, 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol, was first isolated from soil bacteria and from the YSB-6-mediated hydrolysate of a mixture of ginsenosides Rb1, Rb2, and Rg1.21 Rb1 and Rb2 can be metabolized by intestinal bacteria to compound K22,23 via specific metabolic pathways (Fig. 2).18 Because compound K, but not Rb1, was found in plasma and urine after oral administration of Rb1,24 researchers have focused on the biological functions of compound K and the methodology for effective production of compound K from major ginsenosides.

Major ginsenosides can be biotransformed into compound K, an absorbable metabolite, by microorganisms or specific enzymes. Since Hasegawa and coworkers reported the conversion of ginsenosides Rb1, Rb2, and Rg1 into compound K by...
**Fig. 1** Chemical structures of ginsenosides.

<table>
<thead>
<tr>
<th>Ginsenoside</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rb1</td>
<td>-O-Glc-Glc</td>
<td>-H</td>
<td>-O-Glc-Glc</td>
</tr>
<tr>
<td>Rb2</td>
<td>-O-Glc-Glc</td>
<td>-H</td>
<td>-O-Glc-Ara(p)</td>
</tr>
<tr>
<td>Rc</td>
<td>-O-Glc-Glc</td>
<td>-H</td>
<td>-O-Glc-Ara(f)</td>
</tr>
<tr>
<td>Rd</td>
<td>-O-Glc-Glc</td>
<td>-H</td>
<td>O-Glc</td>
</tr>
<tr>
<td>Rg3</td>
<td>-O-Glc-Glc</td>
<td>-H</td>
<td>-OH</td>
</tr>
<tr>
<td>F2</td>
<td>-O-Glc-Glc</td>
<td>-H</td>
<td>-O-Glc</td>
</tr>
<tr>
<td>Rh2</td>
<td>-O-Glc-Glc</td>
<td>-H</td>
<td>-OH</td>
</tr>
<tr>
<td>Compound K</td>
<td>-OH</td>
<td>-H</td>
<td>-O-Glc</td>
</tr>
<tr>
<td>PPD</td>
<td>-OH</td>
<td>-H</td>
<td>-OH</td>
</tr>
</tbody>
</table>

**PPD-type**

<table>
<thead>
<tr>
<th>Ginsenoside</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Re</td>
<td>-OH</td>
<td>-O-Glc-Rha</td>
<td>-O-Glc</td>
</tr>
<tr>
<td>Rf</td>
<td>-OH</td>
<td>-O-Glc-Glc</td>
<td>-OH</td>
</tr>
<tr>
<td>Rg1</td>
<td>-OH</td>
<td>-O-Glc</td>
<td>-O-Glc</td>
</tr>
<tr>
<td>Rg2</td>
<td>-OH</td>
<td>-O-Glc-Rha</td>
<td>-OH</td>
</tr>
<tr>
<td>Rh1</td>
<td>-OH</td>
<td>-O-Glc</td>
<td>-OH</td>
</tr>
<tr>
<td>F1</td>
<td>-OH</td>
<td>-OH</td>
<td>-O-Glc</td>
</tr>
<tr>
<td>PPT</td>
<td>-OH</td>
<td>-OH</td>
<td>-OH</td>
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</tbody>
</table>

**PPT-type**

Glc, glucose  
Ara(p), arabinose in pyranose form  
Ara(f), arabinose in furanose form  
Rha, rhamnose

**Fig. 2** Biotransformation of major PPD-type ginsenosides to compound K.
intestinal microbiota, various methods for microbial conversion have been demonstrated (Table 1). A number of studies indicated that the intestinal bacteria isolated from human feces or the fungi derived from soil around ginseng roots as well as some food-derived microorganisms hydrolyzed ginsenosides to produce compound K. Enzymatic transformation is mostly conducted by β-glucosidase. Ginsenoside-hydrolyzing β-glucosidase was first purified from *Fusobacterium* K-60, a human fecal bacterium. This enzyme was characterized as a 320 kDa protein composed of four identical subunits, which hydrolyzed the ginsenoside-β-glucoside linkages of Rb1 to produce compound K. An enzyme with improved specificity and efficiency was later purified from microorganisms found in ginseng field soil. To shorten the purification process and obtain high product yields, thermostable recombinant β-glucosidases have been cloned using thermophilic microorganisms. Because the recombinant β-glucosidases effectively transform major ginsenosides into absorbable forms with low cost, recombinant enzymes are used for the practical preparation of compound K.

Orally administered ginsenosides undergo extensive bio-transformation in the GI tract and are deglycosylated into more bioactive molecules than the parent compounds by intestinal bacteria; however, the absorptive mechanism of ginsenosides in the GI tract is not fully understood. Because ginsenosides are hydrophilic, they are not likely to be well absorbed in the intestine. In fact, some studies have indicated that the bioavailability of unmodified ginsenosides and their metabolites is low. While the plasma level of Rb1 remained relatively constant for three days, the plasma Rg1 level was not detectable in the blood and intestinal contents of germ-free rats that were orally administered Rb1, whereas compound K was detected in the blood and intestinal contents of gnotobiotic rats fed Rb1. This suggests that intestinal microbiota played a crucial role in metabolizing Rb1 to absorbable forms, including compound K.

When PPD-type ginsenosides, including Rb1, Rb2, Rb3, Rg1, Rg3, Rh2, and Rs1, were incubated with human intestinal microflora, compound K was the major metabolite produced by the action of the intestinal microflora, including *Bifidobacterium* H-1, *Bifidobacterium* K-110 and -506, *Bacteroides* JY-6, *Eubacterium* A-44, *Fusobacterium* K-60, and *Prevotella oris*. In addition, recent studies showed that either prebiotic fibers or Western diet enhanced the formation and absorption of compound K probably through modulating intestinal microbiota.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Classification</th>
<th>Source</th>
<th>Metabolizing pathways</th>
<th>Processing condition</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacteroides</em> sp., <em>Eubacterium</em> sp., and <em>Bifidobacterium</em> sp.</td>
<td>Human feces</td>
<td>Human feces</td>
<td>Bb – Rd or Mb – CK</td>
<td>37 °C, pH 7.0</td>
<td>Bae et al. 25</td>
</tr>
<tr>
<td><em>Esteya vermicola</em> Nematodes in forest soil</td>
<td>Soil around ginseng roots</td>
<td>Soil around ginseng roots</td>
<td>Rd or gypenoside → F2 → CK</td>
<td>50 °C, pH 5.0</td>
<td>Hou et al. 81</td>
</tr>
<tr>
<td><em>Paecilomyces bainieri</em> Soil around ginseng roots</td>
<td>Soil around ginseng roots</td>
<td>Soil around ginseng roots</td>
<td>CK - → F2 or F2 → CK</td>
<td>30 °C, pH 7.0</td>
<td>Zhou et al. 82,83</td>
</tr>
<tr>
<td><em>Fusarium sacchari</em> Soil around ginseng roots</td>
<td>Soil around ginseng roots</td>
<td>Soil around ginseng roots</td>
<td>Rb1 → Rd or saponins → F2 → CK</td>
<td>30 °C, pH 7.0</td>
<td>Han et al. 84</td>
</tr>
<tr>
<td><em>Cladosporium cladosporioides</em> Soil around ginseng roots</td>
<td>Soil around ginseng roots</td>
<td>Soil around ginseng roots</td>
<td>CK → F2 or F2 → CK</td>
<td>30 °C, pH 7.0</td>
<td>Wu et al. 85</td>
</tr>
<tr>
<td><em>Acremonium strictum</em> Soil around ginseng roots</td>
<td>Soil around ginseng roots</td>
<td>Soil around ginseng roots</td>
<td>Rd or gypenoside → F2 or F2 → CK</td>
<td>30 °C, pH 7.0</td>
<td>Chen et al. 86,87</td>
</tr>
<tr>
<td><em>Aspergillus niger</em> Soil around ginseng roots</td>
<td>Soil around ginseng roots</td>
<td>Soil around ginseng roots</td>
<td>CK → F2 or F2 → CK</td>
<td>30 °C, pH 7.0</td>
<td>Liu et al. 89</td>
</tr>
<tr>
<td><em>Leuconostoc citreum</em> Kimchi</td>
<td>Kimchi</td>
<td>Kimchi</td>
<td>Rd or gypenoside → F2 or F2 → CK</td>
<td>30 °C, pH 7.0</td>
<td>Quan et al. 90</td>
</tr>
<tr>
<td><em>Leuconostoc mesenteroides</em> DC102 Kimchi</td>
<td>Kimchi</td>
<td>Kimchi</td>
<td>Rd or gypenoside → F2 or F2 → CK</td>
<td>30 °C, pH 7.0</td>
<td>Quan et al. 91</td>
</tr>
<tr>
<td><em>Lactobacillus paralimentarius</em> LH4 Kimchi</td>
<td>Kimchi</td>
<td>Kimchi</td>
<td>Rd or gypenoside → F2 or F2 → CK</td>
<td>30 °C, pH 7.0</td>
<td>Quan et al. 91</td>
</tr>
</tbody>
</table>

It has been reported that ginsenosides could be absorbed via a sodium-dependent glucose co-transporter 1 in the gut, although it was not clear whether compound K was absorbed by the same mechanism. In fact, compound K is likely absorbed by passive diffusion to a certain extent when consid-
erating the moderate capability of compound K to permeate the cell membrane in Caco2 cells without a directional effect.40

Meanwhile, there are a few pharmacokinetic studies of compound K. The pharmacokinetics of Rb1 and compound K were compared after the intake of Korean red ginseng extract in ten healthy human subjects.41 The mean maximum plasma concentrations (C_max) of compound K and Rb1 were 8.35 ± 3.19 and 3.94 ± 1.97 ng mL⁻¹ respectively which represents an approximately 2-fold higher plasma concentration in the compound K group than in the Rb1 group. These data suggest that compound K is better absorbed than Rb1. The mean time to reach C_max (T_max) of compound K (12.20 ± 1.81 h) was longer than that of Rb1 (8.70 ± 2.63 h), which supported the idea that intestinal microflora metabolized Rb1 to form compound K. The mean area under the plasma concentration–time curve extrapolated to infinity (AUC_H) of compound K and Rb1 was 123.9 ± 57.5 and 307.7 ± 145.6 ng h mL⁻¹ respectively, suggesting that the plasma half-life (t_1/2) of Rb1 was longer than that of compound K. This study demonstrated the significant differences in the pharmacokinetic characteristics of compound K and its parent compound, Rb1.

Jin and coworkers compared the pharmacokinetic profiles of compound K after administering fermented or non-fermented ginseng extracts to healthy subjects.42 The average C_max(s) of compound K (±SD) for the fermented ginseng-fed group and the non-fermented ginseng-fed group was 325.00 ± 91.97 and 13.88 ± 7.24 ng mL⁻¹ respectively. The T_max of compound K was 3.29 ± 1.00 h for the fermented ginseng-treated group and 12.04 ± 4.96 h for the non-fermented ginseng-treated group. The area under the plasma concentration–time curve from time zero to the time of the last concentration (AUC-last) of compound K was 2083.00 ± 524.68 ng h mL⁻¹ for the fermented ginseng-treated group and 134.05 ± 63.10 ng h mL⁻¹ for the non-fermented ginseng-treated group. The study confirmed that the AUC-last of fermented ginseng was larger than ginseng extract (GE) and red ginseng extract (RE).

There are several ways to enhance the bioavailability of compound K and other ginsenosides, such as the use of nanocarriers, emulsification of ginsenosides, and inhibition of the P-glycoprotein efflux system. In particular, P-glycoprotein is related to multi-drug resistance, which is one of the major hurdles to successful chemotherapy. A specific ginsenoside (Rh2) has been reported to inhibit permeability glycoprotein (also known as P-gp or MDR1) and thereby enhance the efficacy of anti-cancer drugs.43

**Neuroprotective effect**

Oxidative stress is a critical factor in the pathophysiology of neurodegenerative disorders.44 The central nervous system (CNS) is typically vulnerable to oxidative stress as it consumes a high amount of oxygen and has a relatively lower level of the endogenous antioxidant defense system such as antioxidant enzymes than other organs. Several studies indicated the antioxidant potential of ginseng and ginsenosides through the scavenging of free radicals45,46 and the reduction of ROS generation through modulation of the endogenous antioxidant defense system (Fig. 3).47

Multiple studies indicated that compound K did not display radical scavenging and xanthine oxidase-inhibitory activities;48-50 however, it induced antioxidant enzymes in a nuclear factor (erythroid-derived 2)-like 2 (Nrf2)-dependent manner and thereby attenuated cell death and mitochondrial damage in a mammalian neuronal cell line.51 In addition, compound K was found to exert a protective effect from scopolamine-induced memory deficits in mice through induction of Nrf2-mediated antioxidant enzymes without affecting acetylcholine esterase (AChE) activity.51

Park and coworkers reported that compound K upregulated heme oxygenase 1 (HO-1) expression and inhibited ROS production in activated microglial cells; this may be related to the anti-inflammatory activity of compound K.52 This study also showed that compound K administration reduced microglial activation in a cerebral ischemic model. Since neurodegenerative disorders are often accompanied by activation of glial cells, compound K may be potentially used as a preventive or therapeutic agent for the treatment of neurodegenerative diseases.

Aggregation of β-amyloid peptides (Aβ) is a known cause of neurofibrillar tangles, neuronal loss, and memory loss leading to Alzheimer’s disease (AD).53 Neural cells, including neurons and astrocytes, are cellular sources of Aβ production in the brain.54 Since astrocytes are at least five-fold more numerous than neurons and activated astrocytes can generate Aβ, astrocytic production of Aβ may significantly contribute to neuronal viability and further AD progression.55 One study indicated that compound K facilitated Aβ clearance by enhancing autophagy through activation of mTOR and its downstream molecules in astrocytes56 (Fig. 3). Compound K, but not Rb1, also increased spontaneous gamma aminobutyric acid (GABA) release into hippocampal CA3 pyramidal neurons through a presynaptic Ca²⁺-dependent mechanism,57 suggesting that compound K induced the modulation of hippocampal neuronal activity.

Inflammation is a major part of the pathology of neurodegenerative diseases and mounting evidence suggests that microglia are a key causative factor in this process.58 Alzheimer’s disease is reported to be associated with the toxic effects of microglial activation.59 Compound K is known to have various immunopharmacological activities, including anti-inflammatory and anti-tumor effects.60,61 Recent studies indicated that compound K derived from ginseng could exert neuroprotective activity through suppression of microglial activation. In addition, compound K was found to inhibit tumor necrosis factor (TNF)-α induced phosphorylation of IκB kinase and the subsequent phosphorylation and degradation of IκB.62,63 Compound K also inhibited the TNF-α induced phosphorylation of MKK4 and the subsequent activation of the JNK-AP-1 pathway. Collectively, compound K and other ginsenosides appeared to exert their anti-inflammatory effects through the inhibition of NF-κB and JNK signaling pathways (Fig. 4).
It was also reported that the compound K-rich fraction of ginseng suppressed lipopolysaccharide (LPS)-induced nitric oxide (NO) release in macrophages. More specifically, it blocked LPS-mediated mRNA expression of interferon-β and inducible NO synthase. Moreover, the compound K-rich fraction diminished the translocation and activation of interferon regulatory factor 3 (IRF3) and NF-κB (p50 and p65). This extract also inhibited the upregulation of NF-κB-linked luciferase activity promoted by phorbol-12-myristate-13 acetate as well as myeloid differentiation factor 88 (MyD88), TIR domain-containing adaptor inducing IFN-beta (TRIF), an inhibitor of NF-κB (IkBα) kinase (IKKβ), and IRF3-mediated luciferase activity induced by TRIF and TANK-binding kinase 1 (TBK1). Finally, the compound K-rich fraction downregulated the NF-κB pathway by blocking IKKβ and the IRF3 pathway by inhibiting TBK1, as evidenced by reporter gene assays, immunoblotting analysis, and an Akt/IKKβ/TBK1 overexpression strategy (Fig. 4).

Another study reported that compound K had an affinity to the glucocorticoid receptor (GR) and exhibited strong anti-inflammatory activities through MyD88-dependent mechanisms. The exposure of macrophages to compound K led to significant inhibition of zymosan-mediated secretion of TNF-α, interleukin (IL)-6, and IL-12 p40, and the activation of ERK1/2 and p38. Compound K also markedly suppressed the superoxide generation, NADPH oxidase activities, and Ser345-p47phox phosphorylation induced by zymosan in macrophages. The inhibitory effects of compound K in zymosan-induced inflammation and reactive oxygen species (ROS) generation by macrophages are likely to be mediated by Dectin-1, a signaling non-toll-like receptor (TLR) pattern-recognition receptor, as inhibition of Dectin-1 profoundly attenuated the effect of compound K. In addition, the administration of compound K effectively protected cells from zymosan-induced injury by suppressing the production of systemic inflammatory cytokines in the animal model. These findings suggest that compound K could be a novel neuroprotective agent through the alleviation of excessive ROS production and lethal inflammation. However, there is no direct evidence that compound K prevents neurodegenerative diseases, including Alzheimer’s disease, or alleviates the related symptoms in humans through anti-inflammatory activity. Therefore, further study is required to confirm its anti-inflammation-mediated neuroprotective effect.

**Cognitive effect**

There is increasing evidence that ginseng or ginsenosides have beneficial effects on cognitive function including memory and
behavior. Specific pharmacological effects of ginsenosides on particular CNS targets were mainly attributable to cognitive enhancement. Molecular targets in the CNS for compound K include GABA receptors, serotonin receptors, Ca2+ channels, MAP kinase pathways, and the Nrf2 signaling pathway.

Patch clamp experiment in which acutely isolated rat hippocampal CA3 pyramidal neurons were used suggested that compound K stimulates spontaneous GABA release by increasing the Ca2+ concentration in the synaptic terminal. However, the mechanism of how GABA release is associated with hippocampal function and learning and memory processes is not clearly elucidated.57 Consistent with the above observation, Rb1, a precursor of compound K, was found to have anxiolytic-like effects in a mouse model.67

While a cure for Alzheimer’s disease (AD) is not available so far, the most common prescription drugs are choline esterase inhibitors including Aricept, Exelon, and Razadyne. Research suggests that potential therapeutic schemes for AD include reduction of generation or aggregation of β amyloid peptide (Aβ), enhancement of Aβ removal from the cells, interruption of tau hyperphosphorylation and the use of more effective antioxidant and anti-inflammatory drugs. A few studies exhibited that ginsenosides had protective and trophic effects against AD.68 Our recent study showed that compound K attenuated memory deficit induced by scopolamine in the mouse model.51 Considering that compound K did not inhibit AChE activity and failed to ameliorate scopolamine-induced memory deficit in Nrf2-null mice, it is most likely that compound K causes cognitive improvement through Nrf2-mediated induction of antioxidant enzymes in brain tissues such as the hippocampus.51,69

Considering that Rb1 administration ameliorated learning and memory impairment in a scopolamine-induced memory deficit animal model,70,71 but did not change the electrophysiological properties of hippocampal neurons,72 the memory enhancing effect of Rb1 administration is likely to be attributable to its metabolite, compound K.

Hippocampal neurogenesis occurs in the dentate gyrus and is correlated with learning and memory.73 Chronic exposure to cyclophosphamide, a chemotherapeutic agent, can impair the cognitive performance, including learning and memory abilities.74–77 Hou and coworkers found that cyclophos-
phamide treatment induced cognitive impairment that was concomitant with disruption in hippocampal neurogenesis in adult mice.\textsuperscript{78} More importantly, compound K ameliorated cyclophosphamide-induced cognitive impairment and neural stem cell depletion. Considering that compound K induced DNA repair and suppressed UV radiation-induced apoptosis, it is likely that the compound K attenuated the inhibition of hippocampal neurogenesis by preventing cyclophosphamide-induced oxidative damage and cell death.

Conclusion

Although there are many research reports regarding the bioactive function of ginsenosides and ginseng, studies on the neuroprotective effect and effects on the cognitive function of compound K are limited. It is generally agreed that compound K is more bioavailable than the parent ginsenosides, including Rb1, Rb2, and Rc, and is the major contributing factor to the health benefits of ginseng. However, as most studies were conducted using disease-associated models, such as Alzheimer’s disease and ischemic stroke, the results cannot be directly translated to the healthy normal population. Furthermore, it is not clear whether compound K can cross the blood–brain barrier and exert any action on cognitive function in humans, even though the compound was reported to facilitate GABA release in the hippocampus and exhibit a protective effect against scopolamine-induced hippocampal damage in a mouse model. The possible mechanisms of action of compound K in neuroprotection and cognitive improvement include attenuation of ROS levels in neural cells through induction of antioxidant enzymes, regulation of NO, GABA, and serotonin receptors, Ca\textsuperscript{2+} channel modulation, regulation of the MAPK pathway, and inhibition of inflammation.

Although ginseng and ginsenosides were shown to have neuroprotective and cognitive enhancing effects, further research is required to establish whether compound K is the major component of ginseng responsible for cognitive improvement in humans.

Conflict of interest statement

There is no potential conflict of interest or competing interest.

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