Pharmacological activities of ginsenoside Rg5 (Review)

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Abstract. Ginseng, a perennial plant belonging to genus *Panax*, has been widely used in traditional herbal medicine in East Asia and North America. Ginsenosides are the most important pharmacological component of ginseng. Variabilities in attached positions, inner and outer residues and types of sugar moieties may be associated with the specific pharmacological activities of each ginsenoside. Ginsenoside Rg5 (Rg5) is a minor ginsenoside synthesized during ginseng steaming treatment that exhibits superior pharmaceutical activity compared with major ginsenosides. With high safety and various biological functions, Rg5 may act as a potential therapeutic candidate for diverse diseases. To date, there have been no systematic studies on the activity of Rg5. Therefore, in this review, all available literature was reviewed and discussed to facilitate further research on Rg5.

Contents

1. Introduction

Ginseng, a perennial plant belonging to genus *Panax*, has been widely used in traditional herbal medicine in east Asia and North America for millennia to reinforce immunity, provide nutrition and reduce fatigue (1,2). Ginsenosides are unique triterpenoid saponins predominantly extracted from *Panax ginseng* C.A. Meyer that act as the main bioactive constituents of ginseng (3,4). To date, >100 ginsenosides have been extracted from the roots, leaves, stems, fruits and flower heads of ginseng (3). All ginsenosides share a common four-ring hydrophobic structure, but differ in the number and types of glycosyl (5). Ginsenosides are classified into 20(S)-protopanaxadiol, 20(S)-protopanaxatriol saponins and oleanoic acid ginsenosides (6,7). The variability of attached positions, inner and outer residues, and types of sugar moieties may be associated with the specific pharmacological activities of different ginsenosides (4). Glycosylated major ginsenosides, such as Rb1, Rb2, Rc, Rd, Re and Rg1, constitute >80% of the total ginsenosides in various parts of ginseng (8). Deglycosylated minor ginsenosides, which have fewer sugar moieties attached on aglycon, are absent or present in smaller amounts in wild ginseng (9,10).

Ginsenoside Rg5 (Rg5) is a minor ginsenoside synthesized during ginseng steaming treatment; the structural formula is displayed in Fig. 1 (11). It is obtained by the deglycosylation of ginsenoside Rb1 and dehydration of carbon at position 20 of ginsenoside Rg3, and exhibits superior pharmaceutical effect compared with major ginsenosides (12,13). In previous studies, Rg5 was found to exert multiple pharmacological effects, such as antitumor, anti-inflammatory, antidiabetic, anti-osteoarthritis (OA), neuroprotective and cardioprotective properties (14-20). With high safety and various biological functions, Rg5 has the potential to act as a potential therapeutic candidate for diverse diseases. The present article reviewed the Rg5 literature and summarized its pharmacological activities.

2. Pharmacological activities of Rg5

Anticancer effects. Cancer is a group of life-threatening diseases that are characterized by abnormal proliferation of cells with potential to invade and spread to surrounding and distant tissues. Conventional therapies for cancer include surgical resection, radiotherapy and chemotherapy (21,22). Chemotherapy serves an important role in the treatment of malignant tumors (23). However, the severe side effects of traditional chemotherapy, such as myelosuppression and immune suppression, hinder the therapeutic effects (24). The use of ginsenosides as alternative antitumor agents has gained increasing attention (25,26). Several studies have reported

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the antitumor effects on human gastric and breast cancer of Rg5, which are mainly associated with promoting apoptosis, autophagy and cell cycle arrest (15-18).

Breast cancer is a major health risk for the adult female population. The mechanism underlying the effects of Rg5 on breast cancer has been investigated *in vivo* and *in vitro*. Kim and Kim (15) demonstrated that Rg5 promoted breast cancer cell apoptosis via downregulation of the Bax/Bcl-2 pathway. It was also demonstrated that Rg5 inhibited breast cancer cell proliferation by arresting the cell cycle at the G0/G1 phase by promoting the expression of p53, $p21^{WAF1/CIP1}$ and $p15^{INK4B}$ and inhibiting the expression levels of cyclin D1, cyclin E2 and CDK4 (15). Moreover, it was discovered that Rg5 exhibited improved pro-apoptotic effects on human breast cancer cell lines compared with ginsenoside Rg3 (15). Zou and Liu (16) reported that Rg5 exhibited antiproliferative effects against breast cancer cells by activating the AMPK pathway. More recently, Liu *et al* (14) discovered that Rg5 induced breast cancer cell apoptosis and autophagy by inhibiting the PI3K/Akt/mTOR signaling pathway. In addition, Kim *et al* (13) demonstrated that Rg5 inhibited the growth of tumors in breast cancer mouse models by promoting tumor cell autophagy and apoptosis without damaging the normal functions of major organs and immune cells, which indicated that Rg5 exerts effects against breast cancer *in vivo* (17).

The effects and associated mechanisms underlying Rg5 in the treatment of digestive system cancer have also been reported. Liu and Fan (18) investigated the anticancer activity of ginsenoside in human gastric cancer cell lines and suggested that Rg5 inhibited cell proliferation by inducing G2/M phase arrest, apoptosis and autophagy by activating reactive oxygen species (ROS)-mediated MAPK pathways. Moreover, in a human xenograft nude mouse model, Rg5 displayed significant effects against gastric cancer with few side effects (18). Zhang *et al* (27) revealed that Rg5 suppressed proliferation and promoted the apoptosis of human esophageal cancer cells, which was associated with inhibition of the PI3K/Akt signaling pathway. Wang *et al* (28) demonstrated that Rg5 bound to annexin A2, and inhibited the interaction between annexin A2 and NF-κB p50 subunit, which resulted in the promotion of caspase activation and NF-κB activity in human hepatoma HepG2 cells. Lee *et al* (29) reported that Rg5 arrested the cell cycle of human hepatoma SK-HEP-1 cells at the G1/S transition phase through cyclin E-dependent protein kinase activity by increasing the expression of $p21^{Cip/WAF1}$ and reducing cyclin E.

Additionally, Rg5 promotes apoptosis in retinoblastoma cells by inhibiting the Akt signaling pathway and thereby downregulating Bcl-2 expression (30). Rg5 also promotes human cervical cancer cell apoptosis in concentration- and time-dependent manners by inducing DNA double-strand breaks and fragments (11). Rg5 has also been demonstrated to effectively overcome ATP binding cassette subfamily B member 1 (ABCB1)-mediated drug resistance by inhibiting ABCB1 transporter and blocking the activation of Akt/nuclear-related factor 2 pathways without affecting the expression of ABCB1 transporter (31). Therefore, Rg5 may serve as a chemosensitizer for reversing multidrug resistance.

With the development of nanomedicine, the antitumor effects of Rg5 through biocompatible nanoscale drug delivery systems has also been investigated (32). Dong *et al* (32) used folic acid (FA)-modified bovine serum albumin (BSA) nanoparticles (FA-Rg5-BSA NPs) as carriers to entrap Rg5. It was discovered that the FA-Rg5-BSA NPs exhibited superior anticancer activity compared with Rg5 in MCF-7 cells with low cytotoxicity to L929 cells. The FA-Rg5-BSA NPs facilitated cellular uptake and induced apoptosis in MCF-7 cells. Furthermore, an *in vivo* antitumor study demonstrated that FA-Rg5-BSA NPs were more effective in reducing tumor growth than Rg5 and Rg5-BSA NPs in an MCF-7 xenograft mouse model. This *in vivo* real-time bioimaging study demonstrated that the FA-Rg5-BSA NPs had an advanced ability to accumulate in tumors. These results indicated that FA-Rg5-BSA NPs has the potential to serve as an attractive therapeutic strategy for the management of cancer (32).

Anti-inflammatory effects. Inflammation is defined as a defensive mechanism characterized by the release of proinflammatory cytokines and the transmigration of inflammatory cells to protect human bodies from harmful stimuli (19,20). Excessive or abnormal inflammatory responses have the potential to lead to various diseases, such as organ failure, central nerve system injury, tissue damage and even death (33). Thus, regulating the expression of inflammatory mediators should be beneficial for the treatment of inflammation-related diseases (13,34). A number of studies have reported that Rg5 exerted anti-inflammatory effects in inflammatory responses by inhibiting the production of proinflammatory cytokines, such as TNF-α and IL-1β by interfering NF-κB or the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome pathways (35-40).

It has been demonstrated that Rg5 exhibited protective effects on major organs through its anti-inflammatory mechanisms. Kim *et al* (35) suggested that Rg5 ameliorates lung inflammation in mice by blocking the binding of lipopolysaccharide (LPS) to Toll-like receptor (TLR)-4 on macrophages, which are associated with inhibition of NF-κB activation. Park *et al* (36) demonstrated that Rg5 exerted protective effects against cisplatin-induced renal damage by attenuating JNK/p53/caspase-3 cascade-mediated inflammation. Li *et al* (37) demonstrated that Rg5 attenuated renal dysfunction by reducing the expression of inflammatory mediators, including NF-κB, p65 and cyclooxygenase-2 (COX-2). Similarly, Lee (38) reported that Rg5 also decreased the expression of NF-κB, inducible nitric oxide (NO) synthase (iNOS) and COX-2 in HepG2 cells treated with TNF-α and thereby acted as a potential anti-inflammatory agent against hepatitis. The anti-inflammatory activity of Rg5 was increased when compared with ginsenoside Rb1, Rd and Rg3 and this increased bioactivity of Rg5 was hypothesized to be due to the higher lipophilicity compared with Rb1, Rd and Rg3 (38). Rg5 also ameliorated acetaminophen (APAP)-induced liver injury by suppressing APAP-induced expression of the inflammatory cytokines, TNF-α and IL-1β (39). Moreover, Rg5 demonstrated protective effects in high-fat diet/streptozotocin-induced diabetic nephropathy mice and improved renal injury by attenuating oxidative stress and inflammatory states by suppressing ROS-mediated activation of NLRP3 inflammasome, p38 MAPK and NF-κB signaling pathways in the kidneys of diabetic nephropathy mice (40).

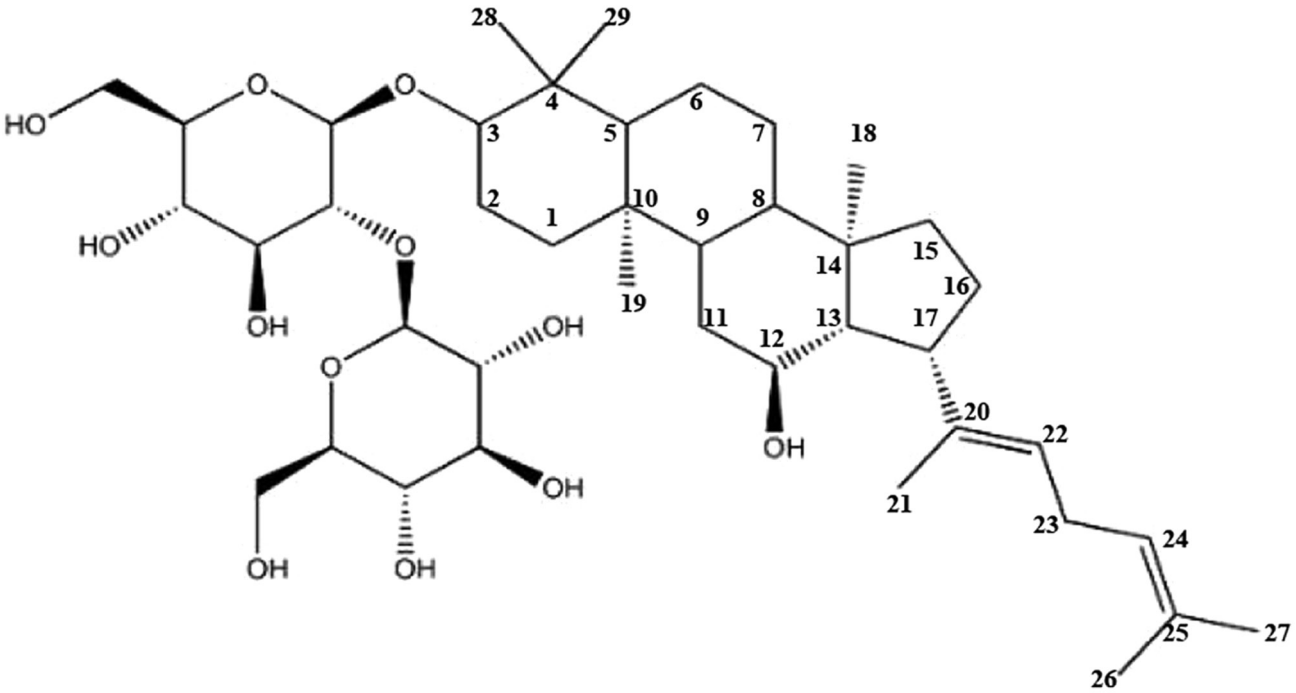

Figure 1. Structure of ginsenoside Rg5.

Sepsis is a systemic inflammatory response syndrome caused by the body's response to infection (41). High mobility group box 1 (HMGB1) is regarded as a crucial mediator of sepsis (42). The suppression of HMGB1-induced inflammatory reactions and maintenance of endothelial integrity have served as promising therapeutic strategies for the treatment of sepsis (43). Kim *et al* (44) demonstrated that Rg5 suppressed the release of HMGB1 in LPS-activated human umbilical vein endothelial cells (HUVECs). Moreover, Rg5 inhibited the adhesion and migration of leukocytes toward HUVECs. The aforementioned study indicated that Rg5 may be a potential therapeutic option for the treatment of severe vascular inflammatory diseases, such as sepsis and septic shock.

Rg5 has also demonstrated anti-inflammatory properties in dermal diseases (45,46). Shin *et al* (45) demonstrated the inhibitory effects of Rg5 and its metabolite ginsenoside Rh3 in oxazolone-induced mouse ear contact dermatitis by inhibiting the expression of COX-2, TNF-α and IL-1β produced by macrophage cells and IFN-γ produced by Thl cells. Ahn *et al* (46) discovered that Rg5 had anti-inflammatory effects in two atopic dermatitis-related cell lines. LPS-induced production of NO and ROS was downregulated by Rg5 in RAW264.7 cells, indicating that Rg5 has the ability to improve chronic inflammatory skin disease by blocking the NF-κB/p38/MAPK/STAT1 signaling pathways.

Anti-inflammatory effects of Rg5 in the central neural system have also been demonstrated. Chu *et al* (47) reported that Rg5 significantly suppressed the expression of pro-inflammation-related cytokines, including IL-1β, TNF-α, COX-2 and iNOS, and thereby attenuated neuroinflammatory responses in STZ-induced memory-impaired rats. Rg5 was also revealed to relieve cerebral ischemic injury by decreasing NF-κB transcriptional activity and the expression of proinflammatory cytokines, such as IL-1β, TNF-α and IL-6, by activating TLR4/MyD88 and sirtuin 1 signaling pathways, contributing to reductions in cerebral ischemic injury (48). In addition, Lee *et al* (49) demonstrated that Rg5 suppressed neuroinflammation induced by LPS in BV2 microglial cells by inhibiting the MAPK and PI3K/Akt pathways.

Neuroprotective effects. Neurodegenerative diseases have become another category of health-threatening diseases (50). Rg5 exerts beneficial effects on nervous system diseases, such as Alzheimer's disease (AD) and Huntington's disease (HD) (51-53). AD is a multifactorial neurodegenerative disease featuring extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles in the brain (51). Inhibition of cAMP response element-binding protein (CREB) and brain-derived neurotrophic factor (BDNF) has the potential to lead to memory deficits in patients with AD (52,53). Kim *et al* (54) revealed that Rg5 significantly reversed memory deficits induced by acetylcholinesterase using the passive avoidance, Y-maze and Morris water maze tasks in mice. The research revealed that treatment with Rg5 ameliorated the reduction of BDNF expression and CREB phosphorylation induced by scopolamine (54). Chu *et al* (47) demonstrated that Rg5 improves cognitive dysfunction in streptozotocin-induced AD rats by modulating the cholinergic system, decreasing Aβ deposition and promoting the expression levels of neurotrophic factors BDNF and insulin-like growth factor 1 (IGF-1). Moreover, Rg5 has significant ameliorative effects on STZ-induced neuroinflammatory responses (47). Choi *et al* (55) revealed that Rg5 suppressed thermal stress-induced cell cycle arrest at G1/S phase by activating p21 and poly(ADP-ribose) polymerase cleavage. CREB and BDNF were also increased by Rg5 in thermal stress-exposed HT22 cells (55). HD is an autosomal-dominant neurogenic disorder that leads to progressive nerve cell damage in the brain. Wu *et al* (56) demonstrated Rg5

Table I. Summary of the pharmacological activities of Rg5.

A, Anti-inflammation

First author, year	Model	Effects	(Refs.)
Zhu *et al*, 2020	DN mice	Rg5 attenuates oxidative stress and inflammatory states in HFD/STZ-induced DN mice by inactivating p38 MAPK and NF-κB signaling pathways	(40)
Kim *et al*, 2019	HUVECs	Rg5/Rk1 reduces the secretion of HMGB1, and the adhesion and migration of leukocytes toward HUVECs	(44)
Kim *et al*, 2019	Male C57BL/6 mice	Rg5 reduces CLP-induced mortality and pulmonary injury	(44)
Yang *et al*, 2017	I/R rats	Rg5 reduces TNF-α, IL-6, and IL-1β tissue levels in I/R rats	(62)
Wang *et al*, 2018	Male ICR mice	Rg5 protects against oxidative/nitrative stress injury, inflammation and apoptosis in APAP-induced hepatotoxicity	(28)
Li *et al*, 2016	Male ICR mice	Rg5 attenuates oxidative stress, suppresses inflammation and inhibits apoptosis in cisplatin-treated kidney cells	(37)
Ahn *et al*, 2016	HaCaT cells	Rg5/Rk1 suppresses NF-κB/p38 MAPK/STAT1 signaling	(46)
Ahn *et al*, 2016	RAW264.7 cells	Rg5/Rk1 suppresses NF-κB/p38 MAPK/STAT1 signaling	(46)
Park *et al*, 2015	LLC-PK1 cells	Rg5 ameliorates renal cell damage by inhibiting inflammation and preventing apoptosis	(36)
Lee *et al*, 2013	BV2 microglial cells	Rg5 exhibits anti-inflammatory effects in LPS-stimulated microglia	(49)
Kim *et al*, 2017	Male C57BL/6 mice	Rg5 ameliorates lung inflammation via downregulation of NF-κB activation by inhibiting binding of LPS to TLR4 on macrophages	(13)
Shin *et al*, 2006	Female ICR mice	Rg5 improves chronic dermatitis or psoriasis in oxazolone-induced ICR mice via downregulation of IL-lβ, TNF-α and IFN-γ production	(45)
Chu *et al*, 2014	Male Wistar rats	Rg5 induces amelioration of STZ-induced neuroinflammatory responses	(47)

B, Neuroprotection

First author, year	Model	Effects	(Refs.)
Shao *et al*, 2018	Male Kunming mice, male Wistar rats	Rg5 exerts sedative and hypnotic effects by affecting GABA and serotonin signaling	(57)
Choi *et al*, 1994	HT22 cells	Rg5 inhibits thermal stress-induced apoptosis in HT22 cells	(55)
Chu *et al*, 2014	Wistar rats	Rg5 alleviates cognitive dysfunction in STZ-induced AD rats by regulating cholinergic signaling, attenuating Aβ deposition and increasing neurotrophic factor expression	(47)
Kim *et al*, 2013	Male ICR mice	Rg5/Rh3 protects against memory deficits by inhibiting AChE activity, and increasing BDNF expression and CREB activation	(54)
Wu *et al*, 2009	YAC128 mice	Rg5 protects striatal neurons via inhibition of Ca^{2+} signaling	(56)

Table I. Continued.

C, Cardioprotection

First author, year	Model	Effects	(Refs.)
Yang *et al*, 2017	Male ICR mice	Rg5 protects mitochondrial morphological and functional integrity by regulating HK-II and Drp1 translocation via Akt activation	(62)
Cho *et al*, 2015	HUVECs	Rg5 promotes angiogenesis and vasorelaxation by activating signal transduction pathways downstream of IGF-1R	(61)
Cho *et al*, 2015	C57BL/6J mice	Rg5 promotes angiogenesis and vasorelaxation by activating signal transduction pathways downstream of IGF-1R	(61)

D, Anti-osteoarthritis/anti-osteoporosis

First author, year	Model	Effects	(Refs.)
Zhang, 2017	Male Wistar rats	Rg5 prevents destruction of articular cartilage via inhibition of chondrocyte apoptosis and matrix damage in osteoarthritis rats	(66)
Siddiqi *et al*, 2014	MC3T3-E1	Rg5/Rk1 promotes the function of MC3T3-E1 cells via BMP-2/Runx2 signaling	(68)

E, Antidiabetes/anti-obesity

First author, year	Model	Effects	(Refs.)
Ponnuraj *et al*, 2014	3T3-L1 cells	Rg5/Rk1 ameliorates insulin sensitivity in 3T3-L1 cells via CHOP signaling	(69)
Xiao *et al*, 2017	3T3-L1 cells	Rg5 inhibits succinate-associated lipolysis via reducing cellular energy charge, and effectively prevented insulin resistance by reducing lipid deposits	(70)
Xiao *et al*, 2017	Male ICR mice	Rg5 inhibits succinate-associated lipolysis and prevents insulin resistance by reducing lipid deposition	(70)
Xiao *et al*, 2017	Male C57BL/6J mice	Rg5 reduces succinate accumulation and inhibits hepatic cAMP accumulation	(70)
Yesmin Simu *et al*, 2017	3T3-L1 cells	Rg5/Rk1 exhibits anti-adipogenic activity via downregulation of STAT3/PPARγ/CEBPα signaling	(77)

Rg5, ginsenoside Rg5; DN, diabetic nephropathy; HFD, high-fat diet; STZ, streptozotocin; HUVEC, human umbilical vein endothelial cell; HMGB1, high mobility group box protein 1; CLP, cecal ligation and puncture; I/R, ischemia/reperfusion; APAP, acetaminophen; LPS, lipopolysaccharide; TLR, Toll-like receptor; GABA, γ-aminobutyric acid; AD, Alzheimer's disease; Aβ, β-amyloid; AChE, acetylcholinesterase; BDNF, brain-derived neurotrophic factor; CREB, cAMP response element-binding protein; IGF-1R, insulin-like growth factor-1 receptor; HK-II, hexokinase-II; Drp1, dynamin-related protein 1; BMP-2, bone morphogenic protein-2; Runx2, Runt-related transcription factor 2; PPARγ, peroxisome proliferator-activated receptor γ; CEBPα, CCAAT/enhancer-binding protein α.γ.

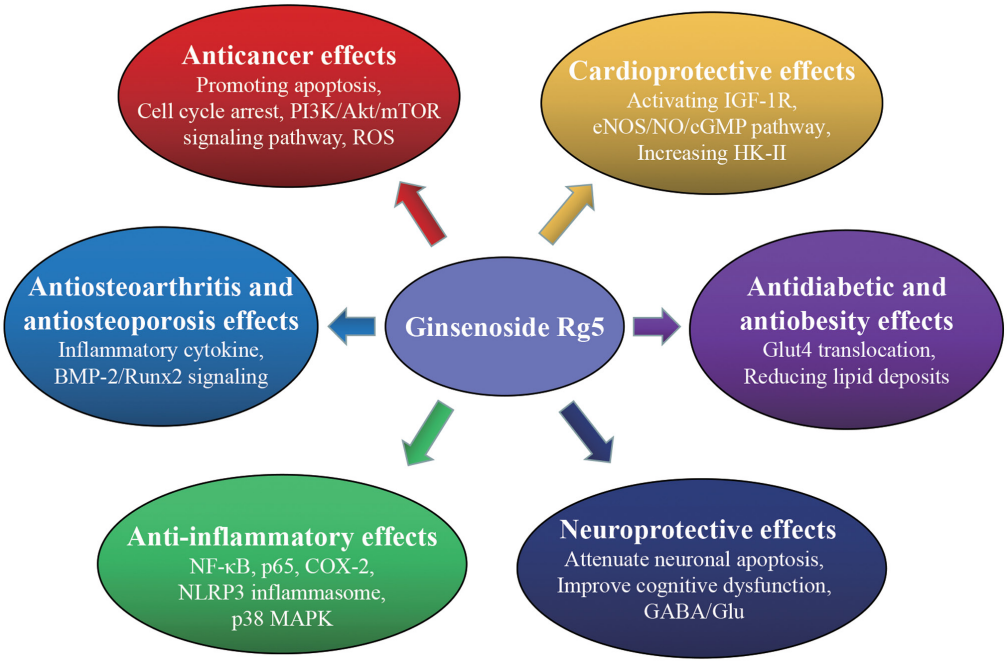

Figure 2. Potential mechanisms of pharmacological activities of ginsenoside Rg5. ROS, reactive oxygen species; IGF-1R, insulin-like growth factor; COX-2, cyclooxygenase-2; BMP-2, bone morphogenetic protein 2; Runx2, runt-related transcription factor 2; Glut4, glucose transporter 4; NO, nitric oxide; eNOS, endothelial NO synthase; HK-II, hexokinase-II; GABA, γ-aminobutyric acid; Glu, glutamate.

attenuates neuronal apoptosis by inhibiting glutamate-induced increases of Ca^{2+} concentrations in cultured medium spiny neurons, which indicated Rg5 has the potential to be useful for HD therapy. Wu *et al* (56) further discovered that the inhibitory activity of Rg5 on glutamate-induced Ca^{2+} responses was similar to ginsenoside Rc and far greater than ginsenoside Re.

In addition, an *in vivo* study revealed that Rg5 may regulate nerve transmission by affecting neurotransmitter and neuroregulatory receptors (57). Glutamate (Glu) is known as a major excitatory neurotransmitter, whereas γ-aminobutyric acid (GABA) is well-known as a major inhibitory neurotransmitter in the CNS (58,59). Shao *et al* (57) suggested that Rg5 downregulates the GABA/Glu ratio, and augments the expression of $GABA_A$ and $GABA_B$ receptors. Serotonin (5-HT) is a neurotransmitter involved in sleep-wake cycle regulation (60). Shao *et al* (57) demonstrated that 5-hydroxytryptophan, together with a precursor of 5-HT, promotes the sleep effects of Rg5 in mice and Rg5 augments the expression of 5-HT1A. The results indicated that Rg5 exhibited hypotensive and sedative activities by modulating GABA and serotonin signaling in the nervous system, thus ameliorating sleep in mice models (57).

Cardioprotective effects. Studies into the therapeutic effects of Rg5 on cardiovascular diseases have also been reported. Cho *et al* (61) demonstrated that Rg5 regulates neovascularization and vasorelaxation by activating IGF-1 receptor (IGF-1R). The angiogenic activity of Rg5 is highly associated with a specific increase in IGF-1R phosphorylation and the subsequent activation of multiple angiogenic signals (61). Furthermore, the vasodilative activity of Rg5 is mediated by the endothelial NOS/NO/cGMP pathway (61). These findings offer a mechanistic explanation of the beneficial effects of Rg5 on neovascularization and endothelial function under pathological conditions. Yang *et al* (62) reported that Rg5 increased cardiomyocyte resistance to ischemic injury by regulating the translocation of two important enzymes, hexokinase-II (HK-II) and dynamin-related protein 1 (Drp1). Drp1 and HK-II exert opposite effects on mitochondrial function in cardiomyocytes by competing for binding to mitochondria (62). Rg5 protects mitochondrial morphological and functional integrity by suppressing Drp1 activation and increasing HK-II binding to cardiomyocyte mitochondria through Akt activation (62). These results provide a rationale for utilizing Rg5 for treating cardiovascular diseases.

Anti-OA and anti-osteoporosis (OP) effects. OA and OP are common bone diseases in middle-aged and elderly populations. OA involves a series of complicated processes characterized by the destruction of chondrocytes and remodeling of subchondral bones, resulting in progressive joint degeneration (63). The regulation of inflammatory cytokine networks by ginsenosides has attracted increased attention for the treatment of OA (64,65). Zhang (66) revealed that Rg5 prevented articular cartilage degradation and inhibited synovium disintegration in OA rat models. The level of OA-related enzyme metalloproteinase-13 decreased to 45% compared with controls; tissue inhibitors of metalloproteinase-1 increased by 67% after treatment with Rg5. The levels of inflammatory mediators, such as IL-1β, TNF-α, NO and iNOS, decreased by 67, 54, 32 and 49%, respectively, after 1 month of treatment with Rg5 (66). The expression of bone morphogenetic protein-2 (BMP-2) and TGF-β1 increased to 67 and 52%, respectively, after treatment with Rg5. Therefore, Zhang (66) considered Rg5 useful for OA therapy.

OP systemically decreases bone mass and strength, and is characterized by the disturbance of osteoblast activity (67). Siddiqi *et al* (68) demonstrated that Rg5/Rk1 stimulates osteoblast cell growth and promotes the expression of

osteoblastic markers, such as alkaline phosphatase activity and type I collagen content, BMP-2 and calcium deposition in dose-dependent manners. Moreover, Rg5/Rk1 also stimulates the mRNA expression of Runt-related transcription factor 2 (Runx2) and osteocalcin (68). These results indicate that Rg5/Rk1 has the potential to prevent OP by stimulating osteoblast proliferation and differentiation via the BMP-2/Runx2 signaling pathway.

Antidiabetic and anti-obesity effects. The antidiabetic effect of Rg5 can be attributed to the amelioration of insulin resistance and reduction of glucagon response (67-72). A high level of insulin is required to regulate blood glucose under insulin resistance conditions. During endoplasmic reticulum stress, the Rk1/Rg5 ginsenoside complex was found to improve insulin sensitivity and increase glucose uptake to exert protective effects in 3T3-L1 cells through CHOP-mediated glucose transporter 4 translocation (69). Furthermore, Xiao *et al* (71) discovered that Rg5 inhibited succinate-associated lipolysis by reducing cellular energy charge and effectively prevented insulin resistance in muscle by reducing lipid deposits. The inhibitory effects of Rg5 in hepatic glucagon response have also been demonstrated (70). Xiao *et al* (70) revealed that Rg5 decreased succinate accumulation by suppressing hepatic fatty acid oxidation and cAMP accumulation by blocking succinate/hypoxia-inducible factor-1α expression, leading to an attenuated hepatic glucagon response.

Ginsenosides have also been widely reported to have an anti-obesity effect (73-76) and the anti-obesity effect of Rg5 has been reported *in vitro*. Yesmin Simu *et al* (77) demonstrated that Rg5/Rk1 inhibited lipid droplet accumulation and decreased triglyceride content in 3T3-L1 adipocyte cells. The expression levels of STAT3, peroxisome proliferator-activated receptor (PPAR)γ, CCAAT/enhancer-binding protein (CEBP)α and adaptor protein complex were also reduced in dose-dependent manners after treatment with Rg5/Rk1. Furthermore, Yesmin Simu *et al* (77) reported no significant cytotoxicity effects on 3T3-L1 cells up to 100 μg/ml. Their results indicated that Rg5 may have therapeutic potential for treating obesity via the STAT3/PPARγ/CEBPα signaling pathway.

3. Conclusion

The current review summarized the pharmacological effects of Rg5. In general, Rg5 has substantial potential activity for use as a broad-spectrum anticancer and anti-inflammatory drug. Rg5 has been reported to exert several positive effects on the nervous system, which potentiate the clinical applications of Rg5 in the treatment of neurodegenerative diseases. Additional studies have investigated other pharmacological properties, such as cardioprotective, anti-OA, anti-OP, antidiabetic and anti-obesity effects. The biological activities of Rg5 have been widely investigated and the mechanisms underlying the actions of Rg5 based on the existing studies are summarized in Table I and Fig. 2. These optimized therapies should also be evaluated for their efficacies *in vivo*. It may be possible to develop novel Rg5 analogues with improved efficacy, pharmacokinetics and bioavailability profiles. Evidence of Rg5 efficacy has yet to be demonstrated in humans. There is a significant need to perform larger cohort clinical studies to confirm Rg5 efficacy for improved applications in the clinic. With this considered, further investigations in clinical trials are highly recommended to provide more reliable evidence for the clinical efficacy of Rg5.

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Availability of data and materials

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Authors' contributions

MYL and FL wrote the manuscript. YLG and JNY collected the references and produced the figure. HJL designed, interpreted and funded the study and revised the manuscript. WQY and JGL revised the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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