Review

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Immunomodulatory activities of five clinically used Chinese herbal polysaccharides

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Abstract

Polysaccharide is a natural macromolecular compound with complex, important and multifaceted biological activities. Some of polysaccharides have been marketed in China as drugs or healthy products.

More studies confirm that the active ingredient of many traditional Chinese medicine exist in the form of polysaccharides. They play a role in disease therapy by activating immune cells and the complement system; regulating the cytokines expression; promoting the production of antibodies; inhibiting tumor cell proliferation and inducing tumor cell apoptosis; inhibiting virus entering cells and replication; increasing activity of antioxidant enzyme; scavenging free radicals; and inhibiting lipid peroxidation.

In this review, we focus on the immunomodulatory effects and its possible mechanism of polysaccharides from Chinese herbal polysaccharides products, including Lentinan, *Astragalus* polysaccharide, *Polyporus* polysaccharide and *Achyranthes bidentata* polysaccharide. The immunomodulatory activities of polysaccharides were categorized in the paper into general immunoregulatory activity, anti-tumor, anti-infections, anti-inflammatory, anti-oxidative, anti-mutagenic and radioprotective, anti-complementary, anti-adhesive, and anti-allergy since all the activities are related to modulate immune responses by the polysaccharides. Also the challenges in the research of polysaccharides will be discussed.

Key words:
Chinese herbal medicine;
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Introduction

Polysaccharides are natural macromolecular compounds. They constitute an important component of living organisms, and play an important role in the control of cell division, regulating cell growth and maintaining normal metabolism of living organisms. Furthermore, they possess important biological activities such as anti-tumor, anti-viral, anti-bacterial, anti-oxidant, anti-inflammatory, and immunomodulation [1-6].

Polysaccharides are divided into plant, animal and microbial polysaccharides. To date, nearly 300 types of polysaccharides are isolated from natural products, and numerous studies confirm that the active ingredient of many traditional Chinese medicines exist in the form of polysaccharides [7, 8]. Because of their broad spectrum of therapeutic properties and relatively low toxicity, they have attracted a great deal of biomedical attention and become one of the hotspots in life science research. Up to now, a few polysaccharides including Lentinan, *Astragalus* polysaccharide, *Polyporus* polysaccharide and *Achyranthes bidentata* polysaccharide have been licensed in clinic application in China.

In this paper, we summarize the progress on immunopharmacological research of polysaccharides which are extracted from some Chinese herbs and marketed in China for clinical application, including Lentinan, *Ganoderma lucidum* polysaccharide, *Astragalus* polysaccharide, *Ginseng* polysaccharide, and *Glycyrrhiza* polysaccharide. The difficulties and challenges existed in the research of polysaccharides will be also discussed. The immunomodulatory activities of polysaccharides were categorized in the paper into general immunoregulatory activity, anti-tumor, anti-infections, anti-inflammatory, anti-oxidative, anti-mutagenic and radioprotective, anti-complementary, anti-adhesive, and anti-allergy since all the activities are related to modulate immune responses by the polysaccharides. Also the challenges in the research of polysaccharides will be discussed.

Immunomodulatory activities of polysaccharides from *Lentinus edodes* mushroom: lentinan

Lentinan, a (1,3)-β-D-glucan, isolated and purified from *Lentinus edodes* (also called shiitake), has been licensed as an over-the-counter (OTC) dietary supplements and immuno-stimulatory drug.

Anti-tumor activities

Combination of lentinan, S-1 (an oral anti-neoplastic agent) and paclitaxel (PTX) could be effective and safe for advanced gastric cancer [9]. Another study suggested that combination of lentinan and S-1 showed an effective therapeutic action against advanced oral squamous cell carcinoma (OSCC) by down-regulated levels of thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD), and orotate phosphoribosyl transferase (OPRT) [10]. Some researchers have investigated the initiatory anti-tumor mechanisms of lentinan. On the one side, the potential mechanisms were that lentinan could decrease tissue glutathione S-transferase II and III (GST-II and GST-III) contents, recruited effector T cells, suppressed tumor cell proliferation [11, 12]. On the other side, lentinan had a stimulatory effect on innate and adaptive immunity against tumor by increasing concentration of TNF-α, IL-12 and IFN-γ, and also an increased number of effector T cells, such as $CD4^+$ and $CD8^+$ cells in the spleen and peripheral blood [13]. A study offered that lentinan increased $CD4^+IFN\text{-}\gamma^+$ T cells whereas decreased $CD4^+IL\text{-}4^+$ T cells or $CD4^+IL\text{-}6^+$ T cells in peripheral blood; these phenomenon suggested that lentinan might improve the balance between Th1 and Th2 [14]. Taken together, lentinan alone or combined use with other chemotherapeutic agents possessed anti-tumor, immunomodulatory activities and had a life prolonging effect on various malignancies.

Anti-bacterial activities

Some *in vivo* and *in vitro* studies demonstrated the effectiveness of lentinan administrated intra-peritoneally before infection of *Mycobacterium tuberculosis*. The results suggested lentinan could mobilize host defense potential and reduce mycobacterium infection [15]. Another research demonstrated that lentinan induced high level of alveolar macrophage activation manifested through enhanced bactericidal effect against *M.tuberculosis*, which correlated with the induction of reactive nitrogen intermediates, increased activity of lysosomal enzymes (acid phosphates), and with effective phagolysosomal fusion followed by destruction of *Mycobacterium* [16]. Kupfahl *et al* tested the effect of lentinan in the well-established model system of the murine *Listeria monocytogenes* infection. Pre-treatment of bone marrow macrophages and dendritic cells (DCs) with lentinan increased production of TNF-α and IL-12 after *L.monocytogenes* infection *in vitro*. Bone marrow macrophages also showed increased nitric oxide (NO) production and enhanced cytotoxic activity against *L.monocytogenes* after lentinan treatment. Furthermore, pre-treatment of mice with lentinan resulted in increase of TNF-α, IL-12 and IFN-γ and also an increased number of *L.monocytogenes* specific $CD8^+$ T cells in the spleen. The bacterial burden in spleen and liver of mice was significantly reduced during primary and secondary *Listeria* infection after lentinan pre-treatment of mice [13].

Anti-viral activities

A clinical study was carried out for observing the therapeutic effect of lentinan on condyloma acuminatum (CA). The results offered the group treated with lentinan plus CO_2 laser irradiation had a reducing recurrence rate compared with the group treated with laser irradiation alone. After treatment, the CD4/CD8 ratio and serum IL-2 in the test group raised whereas serum soluble IL-2 receptor (SIL-2R) lowered significantly as compared with before treatment. The results suggested that lentinan had a potential on modulating the cellular immune function of CA patients and reduced the recurrence rate of CA [17]. A placebo-controlled trial of phase I/II on lentinan treating HIV-positive patients was conducted in San Francisco General Hospital and the Community Research Initiative in New York. The results showed that a trial of lentinan in combination with didanosine (ddI) could increase CD4 cells over a twelve month period, in contrast to decrease in CD4 cells in patients on ddI alone [18].

Anti-parasitic activities

An experiment tested the effects of lentinan during blood stage infection by *Plasmodium yoelii* 17XL. The results showed pre-treatment of mice with lentinan significantly decreased the parasitoid and increased their survival after infection by enhancing Th1 immune response. Lentinan could result in enhanced expression of MHC-II, CD80/CD86, and toll-like receptors (TLR2/TLR4), and increased production of IL-12 in spleen DCs co-cultured with parasitized red blood cells (pRBCs). Moreover, the number of $CD4^+CD25^+$ regulatory T cells (Tregs) and the levels of IL-10 secreted by Tregs were reduced by pre-treatment with lentinan in the spleen of malaria-infected mice. Additionally, apoptosis of $CD4^+$ T cells in spleens was significantly reduced. These findings suggested that lentinan had prophylactic potential for the treatment of malaria [19].

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Immunomodulatory activities of *Ganoderma lucidum* polysaccharides (GLPS)

Ganoderma lucidum (lingzhi or reishi) is a basidiomycete white rot fungus which has been used for the prevention and treatment of a variety of diseases in East Asia for centuries [20, 21]. Polysaccharides isolated from *G.lucidum* are high molecular weight polysaccharides linked together by β-glycosidic linkages. There are more than 150 polysaccharides isolated from the fruit bodies of *G.lucidum* and the main active ingredients are shown to be (1,3)- and/or (1,6)-β-D-glucans [22]. Most have a molecular weight ranging from 4×10^5 to 1×10^6 in the primary structure.

Anti-tumor activities

A number of studies indicate that polysaccharides isolated from *G.lucidum* are main antitumor components *in vivo*. The *G.lucidum* polysaccharides (GLPS) exhibited significant anti-tumor effect in several tumor bearing animals. However, they neither induced tumor cells apoptosis nor inhibited their proliferation *in vitro* directly. It is reported that GLPS inhibited the growth of S-180 in a dose-dependent manner [23-25].

Co-administration of GLPS potentiated the anti-tumor activity of cyclophosphamide in mice. Inhibitory rate was significantly higher than those in the groups treated with polysaccharides or cyclophosphamide alone [26]. Hu and Lin found that the GLPS at 50 and 100 mg/kg inhibited the growth of S-180 in BALB/c mice and Kunming mice, with an inhibitory rate of 37.8-78.1% [25]. GLPS was also able to prolong the life-span of Lewis carcinoma-implanted C57BL/6 mice and promote anti-tumor activities of cytotoxic drugs and chemical immunomodulators [27]. These results indicate that GLPS not only has anti-tumor activity, but also has synergic effect on the anti-tumor activity of cytotoxic drugs such as cyclophosphamide. The addition of GLPS to the cultures of S-180 or HL-60 tumor cells directly had no inhibitory effect against the proliferation and apoptosis of tumor cells, even at the very high concentration such as 400 mg/L of GLPS [23-25]. These results suggest that mechanisms other than direct cytotoxicity may be involved in the anti-tumor activity of GLPS. Besides of directly inhibiting cancer growth, GLPS could also produce anti-tumor activities through regulating function of immune system. A high molecular weight polysaccharide fraction, designated as F3, induced the maturation of DCs, enhanced mixed lymphocyte reaction, and stimulated the production of cytokines and chemokines *in vitro*. In addition, F3 increased the number of DCs as well as $CD4^+$ T, $CD8^+$ T, regulatory T, B, NK, and NKT cells in the spleen *in vivo*. The levels of multiple cytokines and chemokines in the blood of mice were also increased [28]. Another study showed that GLPS was able to stimulate the proliferation of enteric mucosal lymphocytes, and regulate the production of IL-2, IL-10 and TNF-α, which indicated that enteric mucosal immune responses might be one of the important pathways for the immunomodulatory activity of GLPS [29]. GLPS could induce macrophage or T lymphocyte to secrete TNF-α and IFN-γ, which are known to play an important role in suppressing tumor cells growth and inducing apoptosis of tumor cells, suggesting that the anti-tumor activity of GLPS was mainly through its immunoenhancing activity in the tumor-bearing animals [23, 24].

Immunoregulatory activities

It has been indicated that GLPS (in particular active β-D-glucans) could bind to lymphocyte surfaces through specific receptors or serum specific proteins, leading to alteration of the activities of macrophages, T-helper, NK cells, and other effector cells [30]. Li demonstrated that IL-1α and TNF-α production was significantly increased by mouse peritoneal macrophages treated with GLPS [31, 32]. Berovic *et al* also reported that the GLPS which were mainly composed of β-D-glucanes could induce TNF-α synthesis in primary cultures of human peripheral blood mononuclear cells (PBMC)[33]. Further studies also showed that the addition of GLPS (25-400 g/L) to the *in vitro* macrophages culture media resulted in a significantly increased TNF-α mRNA expression in a concentration-dependent manner. Researches indicated that the GLPS could induce TNF-α expression *in vivo* and *in vitro*, decrease the production of free radicals and increase the intracellular level of free calcium in the peritoneal macrophages [34, 35]. GLPS also increased the production of cAMP in a concentration- and time-dependent manner in murine peritoneal macrophages [36]. A recent study revealed that exposure of human neutrophils to GLPS time-dependently caused increases in protein kinase C (PKC), p38 mitogen-activated protein kinase (MAPK), hematopoietic cell kinase (HCK) and another tyrosine kinase Lyn activities, these maybe the action that corresponded to an enhanced unspecific immune function [37]. GLPS was shown to promote not only the maturation of cultured

murine bone marrow derived DC *in vitro*, but also the immune response initiation induced by DC [38]. Further data showed that GLPS was able to promote the cytotoxicity of specific cytotoxic T lymphocytes (CTL) induced by DC during the stage of antigen presentation mainly through IFN-γ and granzyme B pathways [39]. BN3A, BN3B, and BN3C, three kinds of *G.lucidum* polysaccharides significantly increased the lymphocyte proliferation induced by Con A and the IL-2 production in the normal mice, as well as in the aged mice *in vitro*. BN3A and BN3C also could antagonize the suppressive effect of hydrocortisone on the proliferation of mouse spleen cells [40]. Further study showed that GLPS increased the DNA synthesis of spleen cells in MLC through the enhancement of DNA polymerase induction in the young and aged mice [41]. It was found that GLPS not only increased the contents of nuclear DNA and RNA but also remarkably changed the cell ultra structure in the murine splenocytes [42]. It appears that the cytokines-modulating effect of GLPS would be tissue-specific. GLPS had potent healing effect on indomethacin-induced gastric lesions in the rat due partly to the suppression of gene expression of TNF-α [43]. Application of GLPS also significantly mitigated hepatic tumefaction, decreased ALT enzyme release, and NO production in serum or supernatant, improved the pathological changes of chronic and acute inflammation in the BCG-induced immune liver injury in mice. Moreover, the immunohistochemical result showed that GLPS inhibited iNOS protein expression in BCG-immune hepatic damage model [44]. Recently some studies demonstrated that GLPS injection could decrease the serum glucose level and the prevalence of diabetes in the multiple low dose streptozotocin induced autoimmune diabetes [45]. A polysaccharide with a molecular weight of 1.26×10^5, obtained from the sporoderm-broken spores of *G.lucidum* was found to have a strong suppressing effect on the antibody production and the Con A or LPS induced lymphocyte proliferation in mice [46].

Immunomodulatory activities of *Astragalus* polysaccharides

Astragalus membranaceus (Huang Qi) is a common plant in China that has been widely utilized in traditional Chinese medicine as a tonic to enhance the body's natural defense functions. *Astragalus* polysaccharides (APS) extracted from this herb are widely used for their anti-inflammatory [47], anti-viral [48], anti-tumor [49, 50] and effective immunomodulatory functions [51-55].

Immunoregulatory activities

Recently, more attentions have been focused on the effective immunoregulatory functions of APS in humans and animals [51-55]. APS can stimulate proliferation of T cells [56] and promote the expression of surface antigens on lymphocytes [57, 58]. APS can also increase serum antibody titer and enhance secretion of a broad range of cytokines [59, 60]. A study investigated the regulatory effects of APS on maturation and function of cultured murine bone marrow (BM)-derived DCs. APS increased the expression of CD-11c and MHC class II molecules on DC surface, and inhibited the phagocytosis of DCs. In addition, APS-treated DCs secreted a higher level of IL-12 than untreated DCs [58]. Another study indicated that APS significantly induced NO production and inducible NO synthase (iNOS) transcription in mouse peritoneal macrophages and RAW 264.7 cell line, which was through activating nuclear factor-kappaB/Rel (NF-κB/Rel)[61]. Xu *et al* investigated the effects of APS on the phagocytosis of *Mycobacterium tuberculosis* by macrophages. Their results suggested that APS not only enhanced the phagocytotic activity of macrophages to *M.tuberculosis*, but also increased the secretion of cytokine IL-1, IL-6 and TNF-α [62]. Lee *et al* [63] also reported that APS appears to exert immune modulating effects by regulating the expression of cytokines, such as IL-1, IL-6 and iNOS, as well as the production of NO. Yang *et al* have shown that oral APS administration not only helps to enhance the immune adherence of erythrocytes, but also improves elimination of immune complexes [64]. APS treatment reduced cell accumulation, swelling and arthritic index of the joints and serum concentrations of TNF-α and IL-1β in a dose-dependent manner in adjuvant arthritic (AA) rats [5]. Shao *et al* reported that macrophages from C3H/HeJ mice (TLR4 mutation mice) are unable to respond to APS stimulation, suggesting the positive involvement of TLR4 in APS-mediated macrophage activation. Monoclonal antibodies against mouse TLR4 partially inhibit APS binding with macrophages, implying that there is a direct interaction of APS and TLR4 on the cell surface [56]. They also discovered that APS could activate mice B lymphocyte and macrophage. Weng *et al* demonstrated that APS could increase the release of IL-3, IL-4 and IL-6 in normal mice splenic cells, significantly promote the activity of NK cells and

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increased the release of IL-2, IL-3, IL-6 and IFN-γ in splenic cell of S-180 tumor-bearing mice after chemotherapy [65].

Anti-viral activities

It is showed that vaccination with a foot-and-mouth disease virus (FMDV) inactivated vaccine co-administrated with APS in BALB/c mice induced stronger phagocytic capacities in abdominal phagocytes, maturation of DCs, T lymphocyte proliferation, higher expression levels of cytokines, and increased antibody production [66]. The further study demonstrates that the appropriate dose of APS is able to elevate the proportions of some PBL subpopulations in FMDV vaccinated pigs at various periods after vaccination, and up-regulates specific antibody titers. The proliferative capacity of PBLs stimulated by Con A or LPS as well as the mRNA expression of IFN-γ and IL-6 are enhanced in pigs administered APS. These observations suggest that APS can be used as an immunomodulator for the FMDV vaccine and provide better protection against FMDV by stimulating both humoral and cellular immune responses [67]. In one report, Dang *et al* investigated the antiviral effects of emodin and APS in HBV transgenic mice and found that the combination of emodin and ASP suppressed HBV replication in HBV transgenic mice. Although lamivudine had a stronger direct inhibitory effect on HBV replication, emodin and APS showed no HBV recurrence 7 days after the last treatment, suggesting a long-lasting effect and may prove to be a potential therapeutic modality for hepatitis B infections [4].

Anti-bacterial activities

It's reported that, cultured 5637 cells and BALB/c mice treated with APS can fight against invading *E.coli*. The researchers monitored the TLR4 expression and bacterial colony numbers in order to determine the contribution of TLR4 to immune response. After 24 h incubation, only 5637 cells treated with 500 μg/mL APS expressed higher levels of TLR4 compared with the untreated group. However, after 48 h, all 5637 cells treated by APS showed higher levels of TLR4 expression than the control cells. The TLR4 expression in the bladder and macrophages mice that received APS was higher than that in the controls. Bacterial colonization in 5637 cells and the bladders of mice treated with APS was significantly reduced compared with the controls. These results demonstrate that with certain concentrations of APS, the expression levels of TLR4 are increased *in vivo* and *in vitro*. Further, up-regulation of TLR4 expression enhances innate immunity during mucosal bacterial infection [68].

Anti-tumor activities

The aim of one study was to determine whether polysaccharopeptide (PSP) and APS can be combined together as a new complex prescription (PSP+APS) for aiding adriamycin (AMD) chemotherapy. Ehrlich's ascites carcinoma (EAC) was used to establish a solid tumor model in Kunming mice. Immunocytochemical and immunohistochemical analysis were employed to detect the immunoregulatory and anti-tumor effects of EAC bearing mice after 30 days of administration with PSP and APS. PSP and PSP+APS could significantly increase the percentage of $CD3^+$ and $CD4^+$ T-lymphocytes, the ratio of $CD4^+/CD8^+$, and the expression of IL-2/IL-2R in spleen tumor tissue, but led to a diminution of Bcl-2 and CDK4 in tumor tissue compared with those of control group. In addition, PSP+APS could restore the immunological effects against AMD-induced immunosuppression, such as the subset of leukomonocytes, the expression of IL-2/IL-2R in the spleen, and the thymus index. These findings suggest that the immunomodulatory and anti-cancer effects of this new formula (PSP+APS) were better than those of PSP alone, and also could resist immunosuppression induced by AMD [50].

Anti-inflammotory acitivities

In addition, APS might be used for prevention and treatment of intestinal inflammation. A study showed that the effects of APS on LPS-induced MAPK signaling and pro-inflammatory gene expression in IEC-6 (intestinal epithelial cell) lines were investigated. APS was found to inhibit the production of both TNF-α and IL-8 in LPS-induced IEC-6 cells in a concentration-dependent manner, and excessive production of TNF-α and IL-8 was observed to induce tissue injury, septic shock and inflammatory intestinal disease [69]. Some findings establish the use of APS to modulate the innate immune response of the urinary tract through TLR4 expression regulation as an alternative option for urinary tract infection treatment [68].

Immunomodulatory activities of *Radix Glycyrrhizae* polysaccharides

Radix Glycyrrhizae polysaccharides (GPS), one of the main active ingredients of *Radix Glycyrrhizae*, are attributed to many healing properties of the herb. It is composed of rhamnose,

glucose, arabinose and galactose. Of all monosaccharide composition, glucose is identified as the largest chemical component in the polysaccharides [70].

Immunoregulatory activities

It had been reported that GPS have many immunomodulatory activities. GPS promoted serum hemolysin IgM and IgG production, increased antibody-producing cells level, improved the thymus and spleen index, increased the phagocytosis of macrophages, induced macrophages to secret cytokines (IL-1, IL-6 and IL-12), enhanced both NK and antibody-dependent cell-mediated cytotoxicity (ADCC) activities, behaved as a mitogen of B lymphocytes, and induced the release of IFN from spleen cells [61, 71, 72]. One study was undertaken to discuss the preliminary immunoregulatory mechanism of GPS by cytochemistry, quantitative analyses, and intracellular enzyme measurement of macrophages. Acid phosphatase (ACPase), adenosine triphosphatase (ATPase), acid α-naphthyl acetate esterase (ANAE) and succinate dehydrogenase (SDH) in macrophages were stained with different methods. The results indicated that GPS increased the production of ACPase, ATPase, ANAE and SDH; the activities of lysozyme (LSZ) and superoxide dismutase (SOD) of macrophages were also induced by GPS [73]. The data suggested that one of the regulatory effects of GPS was to modulate the level of intracellular enzymes, which played significant role in energy metabolism, immunomodulatory effect, anti-bacterial, anti-tumor activities, clearing exogenous foreign bodies and endogenous residues and so on.

We investigated the effect of GPS on murine monocyte-derived DCs and the signaling pathways involved in this process. Our results indicated that treatment of DCs with GPS resulted in the enhanced expression of cell surface molecules CD80, CD86 and MHC I-A/I-E. Production of IL-12 by DCs was also be increased by GPS in a time-dependent manner. The endocytosis of DCs to FITC-dextran was suppressed. In addition, GPS-treated DCs enhanced allogenic $CD3^+$T proliferation and increased secretion of IFN-γ. Furthermore, using TLR4, NF-κB, p38 MAPK and JNK inhibitors partly inhibited the effect of GPS to DCs. These results suggested that GPS induced maturation and function of DCs *in vitro*, which were partly regulated via the TLR4 related signaling pathway. On the other hand, we also investigated the effects of GPS on Treg cells and Th1/Th2 cytokines in hepatocarcinoma bearing mice. Results showed that GPS could inhibit tumor progression. In the lymph node of tumor microenvironment and spleen, the proportion of Treg cells was significantly higher in the tumor bearing mice, and GPS administration could not only remarkably down-regulate the proportion of Treg cells, but also significantly decrease the IL-10 mRNA expression in the lymph node. In addition, GPS treatment could decrease IL-10 and TGF-β expression, but increase IL-2 and IL-12p70 expression in periphery (paper not published).

NO has important roles in the nervous, immune and vascular systems [74]. It reacts with a large number of biological molecules, contributing to its signalling effects [75]. The synthesis of NO by activated macrophages is an important cytotoxic/cytostatic mechanism of non-specific immunity [76]. Studies demonstrated that GPS significantly induced NO production and iNOS transcription in peritoneal macrophages. Moreover, iNOS mRNA expression was strongly induced by GPS. Macrophages simultaneously treated with GPS plus LPS/IFN-γ increased NO and iNOS production as compared to that of GPS treatments alone. The production of NO and iNOS pre-treated with LPS followed by GPS was higher than that of treatment with GPS and LPS simultaneously. Results revealed that GPS might provide a second triggering signal for the expression of iNOS mRNA. Thus, the iNOS-mediated NO synthesis in response to GPS might be one of the mechanisms whereby this herbal medicine elicits its therapeutic effects [77].

Anti-oxidant activities

Some research investigated the anti-oxidant activities of GPS in rats fed high-fat diet. The results of the experiment showed a statistically significant decrease in serum anti-oxidant enzyme activities in high-fat group. Administration of GPS dose-dependently enhanced immune and anti-oxidant enzyme activities in the GPS-treated mice compared to the high-fat model mice. It is concluded that GPS treatment could reduce oxidative stress in high-fat mice [6].

Anti-complementary activities

Two anti-complementary polysaccharide fractions (GR-2IIa and GR-2IIb) were isolated from the roots of *Glycyrrhiza urulensis* Fisch by researchers. Each of them has five anti-complementary polysaccharides (GR-2IIa-1-5 and GR-2IIb-1-5); likewise, GR-2IIc gave two anti-complementary and mitogenic polysaccharides

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(GR-2IIc-1-2A and -2IIc-2) by gel filtration and HPLC. GR-2IIc-1-2A showed the most potent anti-complementary activity. The large fractions from GR-2IIa and -2IIc showed more potent anti-complementary activities than the original polysaccharide fractions, whereas the intermediate fractions and oligogalacturonides were inactive. The large fraction from GR-2IIc had more potent mitogenic acitivity than GR-2IIc, whereas the intermediate fraction and oligogalacturonides from GR-2IIc were inactive [78].

Anti-adhesive activities

One research investigated the effect of GPS on inhibiting adhesion of *Helicobacter pylori* to human gastric mucosa. *In vitro* cytotoxicity against *H.pylori* was investigated by agar diffusion assay; anti-adhesive properties of aqueous extract, raw polysaccharides and purified polysaccharide fractions were investigated by means of an *in situ* adhesion assay with FITC-labelled bacteria on tissue slides of human stomach resectates. The result showed aqueous extract of *Glycyrrhiza glabra* significantly inhibited the adhesion of *H.pylori* to human stomach tissue. This effect was related to the polysaccharides isolated from the extract, with one purified acidic fraction (0.25 SPB) as main active polymer. Purified polysaccharides did not exhibit direct cytotoxic effects against Helicobacter pylori and did not influence hemagglutination. Additionally raw polysaccharides from *Glycyrrhiza glabra* were shown to have strong anti-adhesive effects against *Porphyromonas gingivalis*. The research showed that aqueous extracts and polysaccharides from the roots of *Glycyrrhiza glabra* were strong anti-adhesive systems, which might be used as potent tools for a further development of cytoprotective preparations with anti-infectious potential [79].

Immunomodulatory activities of *Ginseng* polysaccharides

Ginseng is a slow-growing, deciduous, perennial plant of the Araliaceae family which includes *Panax ginseng* (Renshen, Chinese or Korean ginseng), *Panax japonicus* (Japanese ginseng) and *Panax quinquefolius* (Xiyangshen, American ginseng). *Ginseng* is one of the most well-known herbal medicines widely used in East Asia as a tonic, restorative and anti-aging agent in traditional Chinese medicine for more than 3,000 years. The extracts of *Ginseng* contain numerous active ingredients, in which polysaccharides are one of the most important bioactive components.

Immunoregulatory activities

Ginseng polysaccharides have been shown to have multiple immunomodulatory biological activities. Studies demonstrated that *Ginsan*, an immunomodulatory polysaccharide from *Panax ginseng*, induced DCs maturation [80], increased the production of cytokines by macrophages [81], elevated the number of bone marrow (BM) cells [82] and enhanced humoral antibody response [83, 84]. To examine the maturation-inducing activity of *Ginsan* on DCs, Mi-Hyoung Kim *et al* measured the surface expression levels of the maturation markers MHC class II and CD86 on DCs. The results suggested that Ginsan profoundly enhanced the expression of CD86 on DC surfaces, whereas it increased that of MHC class II only marginally. In addition, *Ginsan*-treated DCs significantly stimulated proliferation of allogeneic $CD4^+$ T lymphocytes. In their study, Ginsan profoundly enhanced the production of IL-12, IL-10 and TNF-α by DCs in a concentration-dependent manner. The results demonstrated that ginsan might modulate DCs function by altering cytokine levels [80]. In addition, *Ginsan* significantly enhanced viability and proliferation of spleen cells, increased the surface expression of CD25 and CD69 as well as production of IL-2 from spleen cells. Compared to $CD4^+$ and $CD8^+$ T cells, the proliferation of $CD19^+$ B lymphocytes are increased, which suggests that *Ginsan* could be used to enhance humoral immunity. In addition, *Ginsan* enhanced viability by decreasing the percentage of late apoptotic cells. Further studies indicated that spontaneous cell death of spleen cells and the protective effects of *Ginsan* were closely related to the mitochondrial membrane potential [85]. Another studies showed that, before oral Salmonella antigen, *Ginsan* treatment significantly increased the antibody production both in secretory and serum. The expression of cyclooxygenase (COX) and chemokine (C-C motif) ligand 3 (CCL3) mRNA in the Peyer's patches were increased too. In addition, more DCs were found in the Peyer's patch and mostly migrated into the subepithelial dome region. The expression of CCL3 was reduced by COX inhibitors which antagonized both the migration of DCs and the humoral immune response against oral Salmonella antigen. The results indicated that *Ginsan* enhanced antibody response to orally introduced antigens by modulating the COX expression in the Peyer's patches and enhanced COX expression increased DCs migration to the Peyer's patch via CCL3 [84]. Du *et al* investigated the synergistic effect of

pidotimod and red ginseng acidic polysaccharide (RGAP) from *Panax ginseng* C.A. Meyer on humoral immune response challenged by LPS and sheep red blood cells (SRBC) in immunosuppressed mice. The results indicated that combined treatment with pidotimod and RGAP significantly increased the number of plaque-forming cells in spleen in response to LPS and SRBC, and the IgG levels in serum in secondary responses to SRBC in co-treated mice [83].

Anti-adhesive activities

Bacterial adhesion to host cells is essential to the initiation of pathogenic diseases. PG-F2 (12KDa) and PG-HMW (80KDa) from *Panax ginseng* are pectin-type polysaccharides. They demonstrated strong anti-adhesive activities against oral and skin pathogens to host cell lines in a dose-dependent manner [86]. Further studies demonstrated that PG-F2 had anti-adhesive effects against *Actinobacillus actinomycetemcomitans*, *Propioni-bacterium acnes*, and *Staphylococcus aureus*, but showed no inhibitory effects on *Lactobacillus acidophilus*, *Escherichia coli*, and *Staphylococcus epidermidis*. Results suggested that PG-F2 may exert a selective anti-adhesive effect against pathogenic bacteria, while having no effects on beneficial and commensal bacteria [87].

Anti-mutagenic and radioprotective activities

A study on assessing the effect of *Ginsan* before as well as after 1.5 Gy of gamma-irradiation on frequency of micronucleated polychromatic erythrocytes (MNPCE) in the bone marrow of C57BL/6 male mice showed that *Ginsan* applied 30 min before or 15 min after irradiation reduced MNPCE in a dose-dependent manner [88]. *Ginsan* also restored the reduced level of IFN-γ [89] and modulated the anti-oxidant defense systems [90] in irradiated splenocytes. Further studies demonstrated that pretreatment with *Ginsan* significantly increased the viability of bone marrow cells (BMs) against gamma radiation. *Ginsan*-treated BMs had a significantly higher amount of IL-12, and the expression of MHC class II molecules was also increased. Meanwhile, *Ginsan*-treated BMs showed significantly higher levels of allogeneic $CD4^+$ T lymphocyte proliferation. *In vivo* studies showed *Ginsan*-treated mice had a larger number of BMs after gamma radiation than the control mice, and the BMs of *Ginsan*-treated mice were successfully cultured into DCs. Therefore, *Ginsan* might be a good candidate radioprotective agent for BMs [82]. Han *et al* also showed that *Ginsan* not only effectively increased the acitivities of SOD and glutathione peroxidase (GPx), but also normalized the expression of heme oxygenase-1 (HO) and non-protein thiol (NP-SH) induced by irradiation. In addition, *Ginsan* was able to restore broken cytokine balance by irradiation through increasing T helper type-1 cytokines such as IL-12 and IFN-γ [90].

Anti-allergy activities

Ginseng polysaccharides also had the pharmaceutical activities of anti-allergy. A study tested the effect and mechanism of *Ginsan* against allergic reaction in an ovalbumin (OVA)-induced murine asthmatic model. *Ginsan* treatment reduced airway hyperresponsiveness, remodeling and eosinophilia. The IL-5 level in the supernatant of cultured splenocytes was also decreased. Moreover, *Ginsan* treatment up-regulated the mRNA and protein expression of COX-1 and COX-2 in the lung, and inhibited the allergic reaction aggravated by indomethacin, a COX inhibitor [91].

Current challenges in polysaccharides research

Though great progresses have been made on the research and application of polysaccharides extracted from Chinese herbal medicine, there are still some challenges in this field.

Structure identification

Polysaccharides from Chinese herbal medicine are complex polymers with specific spatial structure. Their molecular weights are from tens of thousands to tens of millions. The structural units of polysaccharides are single sugar, which are connected with glycoside bond. The common glycoside bonds are the α-1,4-, β-1,4- and α-1,6-bonds. Structural units can be connected into straight chain or form branch chain. Straight chain are generally connected by α-1,4 - and β-1,4-glycoside bond, while the connection point of branch chain is often α-1,6- glycoside bond. Researchers found that there existed close relationship between biological activity of polysaccharides and their structures, including sugar unit, glycoside bond, molecular weight, conformation and so on [7, 92, 93]. The activity disappeared immediately if the conformations of polysaccharides changed, for example, from order into disorder. A few researchers thought that the biological activity of polysaccharides might be directly related to a certain kind of conformation, also called the active site of polysaccharides [94, 95]. Although it is difficult to correlate the structure and antitumor activity of complex

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polysaccharides, obvious variations of antitumor polysaccharides are also noted [1, 96-98]. For example, it has been reported that most of the *Ganoderma* polysaccharides show the same basic β-glucan structure with different types of glycoside linkages, and therefore the structural features such as β-1,3-linkages in the main chain of the glucan and further β-1,6-branch points are needed for immuno-modulating and antitumor activities [99]. But so far, this conformation is still not precisely defined in massive polysaccharides. Because of the structural complex of polysaccharides, the studies of structure function relationships of polysaccharides have always concentrated on the β-glucan mainly from fungi polysaccharides from plants, such as Lentinan, *Ganoderma lucidum* polysaccharide [100, 101]. Thus more chemical structure research on polysaccharides is needed.

Isolation and purification

Polysaccharide molecules from Chinese herbal medicine are polar compounds, and they are usually isolated by water with different temperature, alkaline solvent, enzyme, and ultrasonic wave or microwave [102-104]. After isolation, some impurities such as proteins, pigments, oligosaccharides in polysaccharides must be removed. At present, the purification methods include organic solvent, dialysis, DEAE cellulose, chromatography system and so on [105-107]. While the new technologies and methods continue to emerge, isolation and purification of polysaccharides are still companied with cumbersome steps, low efficiency, and low output. Moreover, polysaccharide contents in some natural plants are pretty low, which add difficulties to separation process.

Quality control

Currently most isolated Chinese herbal polysaccharides are not optimized, mixed with proteins and pigments, and also different extraction and separation might cause the difference in structure and activity of polysaccharides. Thus it is hard to ensure the quality of polysaccharide products. Though many of the polysaccharides from Chinese herbal medicine have been used in clinic, they still show poor reproducibility in efficacy because of the inconsistence in the preparation of the products and complex structure. Therefore, quality control of polysaccharides has become an important part in polysaccharide drug research and development [108].

Mechanism exploration

Though most studies are focusing on the pharmacological effects of polysaccharides, there is little systemic and in-depth discussion in their mechanism study. There are still some shortages in the mechanism exploration, such as the exact relationship between structure and function, active sites of polysaccharides, the exact receptor on cells recognized by polysaccharides, the signaling pathway affected by polysaccharides and so on. In addition, the polysaccharide modifications also need to be paid more attention. The evidence showed that taking appropriate methods such as degradation, sulfation, sulfonation, acetylation, alkylation to change the molecular weight and substituent type of polysaccharides can increase the activity of polysaccharides and/or reduce side effects [109-112], which might provides new insights for the development of new polysaccharide drugs.

Conclusions

Chinese herbal polysaccharides, an important class of natural active substances, are rich in resources. Numerous pharmacological effects of polysaccharides laid a solid foundation for further clinical application. They therefore attract more attention of life science researchers and biopharmaceutical companies. At present, Lentinan, *Polyporus* polysaccharide, *Astragalus* polysaccharide, *Achyranthes bidentata* polysaccharide, *etc.* are used clinically. With more favorable conditions for polysaccharides research, such as the improvement on isolation and analysis technique, it is believed that further progress may be made for successfully application of polysaccharides from Chinese herbal medicine.

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