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REVIEW

A Review of Research on the Protein-Bound Polysaccharide (Polysaccharopeptide, PSP) from the Mushroom *Coriolus versicolor* (Basidiomycetes: Polyporaceae)

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ABSTRACT. 1. Protein-bound polysaccharides, designated as PSK and PSP, have been isolated from the CM-101 strain and the COV-1 strain, respectively, of the mushroom *Coriolus versicolor*. This article aims at summarizing existing research findings about PSP since information on PSK is well documented.

2. PSP possesses a molecular weight of approximately 100 kDa. Glutamic and aspartic acids are abundant in its polypeptide component, whereas its polysaccharide component is made up of monosaccharides with α-1,4 and β-1,3 glucosidic linkages. The presence of fucose in PSK and rhamnose and arabinose in PSP distinguishes the two protein-bound polysaccharides, which are otherwise chemically similar.

3. PSP is classified as a biological response modifier. It induces, in experimental animals, increased γ-interferon production, interleukin-2 production, and T-cell proliferation. It also counteracts the depressive effect of cyclophosphamide on white blood cell count, interleukin-2 production and delayed-type hypersensitivity reaction. Its antiproliferative activity against tumor cell lines and *in vivo* antitumor activity have been demonstrated. A small peptide with a molecular weight of 16–18 kDa originating from PSP has been produced with antiproliferative and antitumor activities.

4. PSP administered to patients with esophageal cancer, gastric cancer and lung cancer, and who are undergoing radiotherapy or chemotherapy, helps alleviate symptoms and prevents the decline in immune status. GEN PHARMAC 30;1:1–4, 1998. **© 1998 Elsevier Science Inc.**

KEY WORDS. Mushroom, polysaccharopeptide, *Coriolus versicolor*

Mushrooms are known for their nutritional and medicinal value (Breene, 1990) and also for the diversity of bioactive compounds they contain. The mushroom *Coriolus versicolor* (Yun Zhi) was recorded in the *Compendium of Materia Medica* by Li Shi Zhen during the Ming Dynasty in China, as being beneficial to health and able to bring longevity if consumed regularly. Various products derived from this mushroom and claimed to have medicinal value are commercially available. Among them, PSK (Sakagami *et al.*, 1991) and PSP are the most prominent. It is the intent of this article to summarize research data pertaining to PSP.

PSK (Sakagami *et al.*, 1991) and PSP are two chemically related products of the mushroom *Coriolus versicolor* isolated from deep-layer cultivated mycelia of the COV-1 and CM-101 strains, by Chinese and Japanese investigators, respectively. The similarities and differences of the two products have been pointed out by the Fungi Research Institute (1993a). Both possess a molecular weight of approximately 100 kDa and their polypeptide moieties are rich in aspartic acid and glutamic acid. Monosaccharides with α-1,4 and β-1,3 glucosidic linkages constitute the polysaccharide moieties of PSP and PSK: fucose is found in the latter, whereas arabinose and rhamnose occur in the former. Both PSP and PSK have been found to be immunoenhancing and effective against tumor cells.

The morphological characteristics of the fruiting bodies of the COV-1 strain of *C. versicolor* have been described (Fungi Research Institute, 1993b). The pileus is fan-shaped with a wavy margin and colored concentric zones. The cultivation conditions of the COV-1 strain have also been defined. The nitrogen source consists of peptone, yeast, maize pulp and bean cake powder, and the carbon source comprises glucose and starch. The temperature is 26±2°C, the pH is 5.0–7.0, the oscillation frequency is 220 rpm and the duration of cultivation is 2–3 days. The hyphae obtained after centrifugation at 3000 rpm for 15 min are inoculated into culture bottles with medium containing 78% sawdust, 18% bran, 1% sugar and 1% gypsum, and cultivated as 20–25°C for 3–4 weeks.

Yang and Zhou (1993) are credited with the structural elucidation of PSP. PSP has been subjected to infrared (IR) and nuclear magnetic resonance (NMR) spectroscopic studies. An IR spectrum with absorption peaks at 3400, 1650, 1050 and 893 cm^{-1} is indicative of the presence of an OH group, an NH_2 group, C—O—C and β-glycosidic linkage, respectively. The major absorption peak at 3.0–4.3 ppm in the NMR spectrum denotes the existence of polysaccharide. The proton resonance signals at 1.0–2.5 ppm and 5.38 ppm signify amino acid side chain and β-linked polysaccharide peptide, respectively. The absorption spectra of PSP and PSK over 230–700 nm exhibit a remarkable similarity to each other, whereas starch does not absorb at this range of wavelengths. Gel filtration of PSP on Sephadex G-100 yields a single peak containing both protein

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and carbohydrate. When taken together the above data infer that PSP is a polysaccharide bound to a polypeptide moiety. Analysis of the polysaccharide moiety using gas chromatography/mass spectroscopy reveals a predominance of 1→4, 1→2 and 1→3 glucose linkages together with small amounts of 1→3, 1→4 and 1→6 galactose, 1→3 and 1→6 mannose and 1→3 and 1→4 arabinose linkages.

Yang *et al.* (1993b) classified PSP as a new biological response modifier which is defined as an agent capable of modifying the host's biological response by stimulating the immune system and thereby eliciting various therapeutic effects (Tomada *et al.*, 1987). PSP exerts immunomodulatory and antitumor activities (Yang *et al.*, 1992). Oral administration of PSP at 1.5 mg/kg to normal ICR mice brought about an elevated production of interleukin-2. ConA-stimulated proliferation of mouse T cells was enhanced by incubation in the presence of PSP at and above 100 μg/ml. Interferon-α production by human white blood cells was also augmented by PSP. PSP could also partially offset the decrease in white blood cells and interleukin-4 production induced by cyclophosphamide injection in mice, and reverse the inhibition of the delayed-type hypersensitivity reaction produced by cyclophosphamide. In addition, it prevented thymus involution and increased production of IgG and complement C_3 in sarcoma-bearing mice (Yang *et al.*, 1993a).

Zeng *et al.* (1993) reported that oral administration of PSP at 1–2 g/kg per day for 15–20 days to nude mice inhibited growth of human lung adenocarcinoma by 50–70%. Wang *et al.* (1993) found that PSP administered intraperitoneally (IP) at 50 mg/kg per day for about 3 weeks produced approximately 45% inhibition of the growth of Lewis lung cancer. A reduced inhibition of liver cancer was observed after treatment with PSP. Yang *et al.* (1993b) noted an inhibitory effect of PSP on incorporation of ^{3}H-uridine and ^{3}H-thymidine into nucleic acids in Ehrlich ascites tumor cells. PSP exerted a greater inhibitory effect than PSK on P388 leukemia cells. The antiproliferative potencies of PSP and PSK against human gastric cancer, lung cancer, lymphoma and mononuclear leukemia cell lines were similar. Intraperitoneal or oral administration of PSP likewise inhibited the growth of sarcoma 180 cells in mice, with the former route of administration being more effective. Yang *et al.* (1993c) purified a small peptide with a molecular weight of 16–18 kDa from a crude preparation of PSP using reverse-phase HPLC. The peptide, designated PCV, suppressed ^{3}H-thymidine incorporation into human leukemia HL-60 cells, colon cancer LS174 T cells, human hepatoma SMMU-7721 cells and human gastric cancer SCG-7901 cells. It also reduced tumor growth in mice in which myeloma cells, leukemia cells or hepatoma cells had been implanted or inoculated. The survival rate of the tumor-bearing mice was higher after PSP treatment. No lesions were produced in the vital organs after prolonged treatment (2 months) with therapeutically effective doses (40 mg/kg) of PCV. In contrast, necrotic changes were detected in tumor cells. PCV treatment increased white blood cell count and serum IgG in mice. An increase in $CD4^+$, $CD8^+$ β lymphocytes and neutrophils also occurred.

Xu *et al.* (1993) observed that PSP restored the immune status in cyclophosphamide-treated rats as witnessed in serum IgG titer, lymphocyte proliferation and NK cell function. Yu *et al.* (1993) noticed an increase in percentage of acidic α-naphthol-acetate-esterase-positive T cells in rats, indicating an increase in immune function.

Liu *et al.* (1993) did not, however, detect tumoricidal activity when five tumor cell lines, including P388D1 (mouse monocyte-macrophage), B16 (mouse melanoma), S180 (mouse sarcoma), PU5-1.8 (mouse monocyte-macrophage) and JAR (human placental choriocarcinoma) were cultured in the presence of 2.5–10 μg/ml of PSP. Nevertheless, elevated production of reactive nitrogen intermediates, superoxide anions and tumor necrosis factor was noted in peritoneal macrophages from inbred C57 mice administered PSP in their drinking water for 2 weeks. Northern blot analysis also revealed that PSP stimulated transcription of the tumor necrosis gene in peritoneal macrophages, illustrating the immunostimulatory action of PSP.

Chen *et al.* (1993) reported that PCV induced tumor regression in some liver cancer patients. Liu *et al.* (1993) conducted a clinical trial of PSP on patients with lung cancer, gastric cancer and esophageal cancer. Most of the patients were beyond the early stage of the disease. The lung cancer and gastric cancer patients had undergone operation and often chemotherapy as well, whereas the esophageal cancer patients had received radiotherapy. The results were encouraging. There were marked alleviations of the symptoms. A large percentage of patients had put on ≥1 kg of body weight, and a smaller percentage lost ≥1 kg of body weight. The activity of NK cells, production of interleukin-2, $CD4^+/CD8^+$ ratio and white blood cell count increased as a consequence of PSP treatment. Gao (1993) performed a similar study and obtained comparable results. Liu (1993) examined the effect of combined treatment of esophageal cancer with radiotherapy and PSP and found that the decline in the $CD4^+/CD8^+$ ratio following radiotherapy was prevented by PSP treatment. The 1-year survival rate was increased from 50% in patients receiving radiotherapy only to 70% in patients subjected to both radiotherapy and PSP treatment. Wu *et al.* (1993) also studied the beneficial influence of PSP treatment given concurrently to esophageal patients undergoing radiotherapy. The treatment regimen was similar to that used by Liu *et al.* (1993); that is, 0.34 g PSP per capsule, three capsules each time, three times a day for 1–2 months. In general, results similar to those of Liu *et al.* (1993) were obtained, with the finding regarding the $CD4^+/CD8^+$ ratio being the only discordant data.

In gastric cancer patients receiving chemotherapy, Shi *et al.* (1993) demonstrated that simultaneous PSP treatment increased NK cell activity, $CD4^+$ and $CD4^+/CD8^+$ ratio. Xie (1993) showed that PSP, administered to gastric cancer patients operated on to remove their cancers, and receiving chemotherapy, increased red cell immunity as evidenced in the elevated erythrocyte-tumor-cell rosette rate. The NK cell activity and serum interleukin-2 level were heightened. Xu (1993) similarly reported that PSP treatment applied during chemotherapy of gastric cancer brought about an alleviation of symptoms arising from chemotherapy and a strengthening of the immune function, including NK cell activity, interleukin-2 level and $CD4^+/CD8^+$ ratio. Shi *et al.* (1993) furnished further corroborative data.

Liao and Zhao (1993) undertook a clinical trial of PSP on lung cancer patients, most of whom had passed the early stage of the disease. The patients were on chemotherapy. PSP treatment ensued with an alleviation of symptoms, a stabilization of or an increase in body weight, and an increase in white blood cell count, blood platelet count, hemoglobin level, interleukin-2 level and NK cell activity. Ke (1993) briefly reported the efficacy of PSP in boosting immune function as reflected in NK cell activity and number of lymphocytes and in minimizing the side effects of chemotherapy in lung cancer patients.

PSP given to breast cancer patients receiving chemotherapy caused an increase in appetite and prevented a fall in white blood cell count and platelet count without impairing liver or kidney function (Shiu *et al.*, 1993). PSP has been shown to exert a protective effect against paracetamol-induced hepatotoxicity in the rat (Yeung *et al.*, 1994). It is devoid of teratogenic effects in mice (Ng and

Chan, 1997) and rats (Qian *et al.*, 1993), but exerts analgesic action in mice (Ng and Chan, 1997; Qian *et al.*, 1993).

In summary, PSP has been shown to manifest immunomodulatory and antitumor activity in both experimental animals and cancer patients. Its ability to diminish side effects of radiotherapy and chemotherapy make its inclusion as an adjunct for cancer treatment worthy of consideration. Recently, peptide-bound and protein-bound polysaccharides with immunomodulatory and antitumor activities have also been purified from other mushrooms, for example, *Tricholoma* species (Wang *et al.*, 1995). From the culture filtrates of *Tricholoma lobayense*, a protein-bound polysaccharide with a molecular weight of 154 kDa was isolated. It inhibited the growth of sarcoma 180 cells that had been implanted in mice, restored the phagocytic function of peritoneal exudate cells and mitogenic activity of T cells in tumor-bearing mice and induced gene expression of some immunomodulatory cytokines in mice (Liu *et al.*, 1995, 1996a,b). From a submerged mycelial culture of another *Tricholoma* species, a peptide-bound polysaccharide with a molecular weight of 17 kDa, which exhibited more potent immunomodulatory and antitumor activities than PSP, was purified (Wang *et al.*, 1995). Another peptide-bound polysaccharide with a molecular weight of 15.5 kDa was prepared from the cultured mycelia of *Tricholoma mongolicum* (Wang *et al.*, 1995). It possessed the properties of activating macrophages, stimulating macrophage antigen-presenting activity, which in turn enhanced T-cell proliferation, and inhibited the growth of sarcoma 180 cells implanted into mice. Wang *et al.* (1996a) found that both mouse lymphocytes and macrophages were activated by preparations of polysaccharopeptide from cultured mycelia and culture medium of *C. versicolor*. Glucans including lentinan and schizophyllan and those extracted from other fungi such as *Ganoderma lucidum* and *Volvariella volvacea* are also known to possess antitumor and immunomodulatory activities. It is hoped that these polysaccharides and protein-bound and peptide-bound polysaccharides can be developed into clinically useful drugs. Evidence has been accumulating that mushrooms have nutritional as well as medicinal value.

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