General review of polysaccharopeptides (PSP) from C. versicolor: Pharmacological and clinical studies

Review Article

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Keywords: Coriolus Versicolor (Yunzhi); polysaccharopeptide (PSP); immunomodulation; anti-tumor

Abbreviations: biological response modifiers (BRM); Coriolus versicolor (CV); cyclophosphamide (CPA); human immunodeficiency virus (HIV); Interleukine-2 (IL-2); intraperitoneal (i.p.); National Center for Complementary and Alternative Medicine (NCCAM); Natural Killer Cells (NK cells); Office of Dietary Supplements (ODS); polysaccharopeptide (PSP); polysaccharopeptide Krestin (PSK)

Received: 20 December 2007; Revised: 30 January 2008
Accepted: 5 February 2008; electronically published: February 2008

Summary

In China, C. versicolor is named Yun Zhi (meaning “cloud-like mushroom”). C. versicolor is mainly used as an adjuvant in the treatment of cancer. The active principle derived from C. versicolor belongs to a new class of elements called biological response modifiers (BRM) which are defined as agents capable of stimulating the immune system and thereby, they express various therapeutic effects. The best know commercial polysaccharopeptide preparations of C. versicolor are polysaccharopeptide Krestin (PSK) and polysaccharopeptide (PSP). One of the most important functions of PSP and PSK is their immunomodulatory and anti-cancer actions. The present paper reviewed and summarized the pharmacological and clinical properties, as well as its background of Coriolus versicolor or PSP.

I. Introduction

Mushrooms have an established history of use in traditional oriental therapies. In Asian cultures, mushrooms are combined with herbal mixtures to treat cancer. The mushroom Coriolus Versicolor (C. versicolor) is a macrofungi belonging to the Basidiomycetes class, which encompasses about 20,000 and 25,000 known species (Gregory and Hirst, 1957; Hyde and Adams, 1960). In China, C. versicolor is named Yun Zhi (meaning “cloud-like mushroom”). Researches have found that this mushroom has antimicrobial, antiviral and anti-tumor properties (Jong and Birmingham, 1993; Ulrike et al, 2005). Nowadays C. versicolor is mainly used as an adjuvant in the treatment of cancer (Tsang et al, 2003; Hattori et al, 2004). It has been demonstrated that extracts obtained from this mushroom are likely to show stimulatory effects on the immune system and to inhibit the growth of cancer cells. Because of these properties, Yun Zhi is called a biological response modifier (BRM) (Leung et al, 2006).

According to the record of Ben Cao Gang Mu (本草綱目) written during the Ming Dynasty (1368-1644), there were over 120 strain of C. versicolor (Hyde and Adams, 1960). Recent literatures report that more than 270 medicinal fungi are used in traditional Chinese medicine for their preventive and/or curative effects (Ding, 1987; Ying et al, 1987). In the clinical practice of traditional Chinese medicine, C. versicolor is recommended for various types of cancers, chronic hepatitis, and infections of the upper respiratory, urinary, and digestive tracts (Jong and Yang, 1999; Li, 2003).

In Asia, C. versicolor extract is available as a health supplement and can be purchased without a prescription. In both China and Japan, health authorities regard C. versicolor extract as a valuable adjuvant for combination chemotherapy or radiotherapy in the treatment of various cancers (Mizuno, 1999; Yan et al, 2000; Chu et al, 2002). The present paper reviews and summarizes the pharmacological and clinical properties, as well as its background of Coriolus versicolor or PSP.
II. Composition of C. versicolor

The composition of the polysaccharopeptide is depending on the source of the material and the method of extraction used. For example, polysaccharopeptide Krestin (PSK) is obtained from the extraction of C. versicolor (CM-101) strains, while polysaccharopeptide (PSP) obtained from the extraction of C. versicolor (Cov-1) strains (Chu et al., 2002; Zhou et al., 2007).

The active principle derived from C. versicolor belongs to a new class of elements called BRM. BRM are defined as agents capable of stimulating the immune system and thereby, they express various therapeutic effects (Leung et al., 2006). Polysaccharides linked to a small protein (or peptide) are at the base of this immunomodulatory activity. This “polysaccharide-peptide” is termed polysaccharopeptide, or PSP in its abbreviated form. The strain used for PSP production is called Cov-1 and was obtained through careful selection of over 80 wild strains collected from various areas in China.

III. The difference between PSP and PSK

The best known commercial polysaccharopeptide preparations of C. versicolor are PSK and PSP. Both products are obtained from extraction of C. versicolor mycelia (Figure 1). PSK is a Japanese product, while PSP is Chinese product which was first isolated in 1986 (Yang and Van, 1986). Both products have similar physiological activities but are structurally different. PSK is produced from CM-101 strain of C. versicolor, the extraction is done by salting out with ammonium sulfate from the hot water extract. PSP is produced from Cov-1 strain of C. versicolor (Figure 2), the extraction is done by using alcoholic precipitation from the hot water extract.

PSP and PSK are light or dark brown powders that are soluble and stable in hot water. They are chemically similar and posse similar physiological activity profiles, PSK and PSP differ mainly in the presence of fucose in PSK and rhamnose and arabinose in PSP (Table 1).

IV. Safety studies

The toxicological experiments conducted with a variety of animals (dogs, monkey and guinea pigs) had shown negative results for acute, genetic, reproductive and chronic toxicity.

A. Acute toxicity

The LD₅₀ value of PSP is 26-300.36mg/kg for mice administered by intraperitoneous injection. The highest daily tolerant dose was over 18-20g/kg for mice (Jin, 1999; Ze et al., 2003). According to the "Procedures for Toxicological Assessment on Food Safety Acute Toxicity Test (GB15193.3-94)”, PSP is considered to be non-toxic (Procedures for Toxicological Assessment on Food Safety Acute Toxicity Test GB15193.3-94).

B. Long-term toxicity

Subchronic and chronic toxicity studies were done by Jian et al who continuously administrated 4 oral doses (0, 1.5, 3.0, and 6.0 mg/kg) to 80 rats for 62 days. The results showed no toxic symptoms or death. Neither were there any obvious toxic changes in blood and serum biochemistry (Jian, 1999). Rats and monkeys were administrated with PSP by oral at a dose of higher than 200 and 100 times of human dose separately daily for 6 months, no abnormal changes in development, hematology, blood chemistry, and electrocardiography were observed (Zou et al., 2003).

Figure 1. Trametes (Coriolus) versicolor (http://www.alohamedicinals.com/chapt3_c.pdf).
Figure 2. Typical partial structures of polysaccharide portions of the polysaccharide peptide (PSP) of COV-1 strain of Coriolus versicolor (Zhou and Yang, 1999).

Table 1. The differences of PSP and PSK

<table>
<thead>
<tr>
<th>Source</th>
<th>PSP</th>
<th>PSK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Produced in</td>
<td>China</td>
<td>Japan</td>
</tr>
<tr>
<td>Appeared on the market in</td>
<td>1987</td>
<td>1977</td>
</tr>
<tr>
<td>Extract method</td>
<td>Recovered by alcoholic precipitation from hot water extract</td>
<td>Recovered from hot water extracts of the biomass by salting out with ammonium sulfate</td>
</tr>
<tr>
<td>Physicochemical properties</td>
<td>Brown in color; soluble in water; insoluble in organic solvents; stable to heat; mean MW=100kDa</td>
<td>Brown in color; soluble in water; insoluble in organic solvents; stable to heat; mean MW=100kDa</td>
</tr>
</tbody>
</table>
| Chemical composition          | \((1\rightarrow3)\beta\text{-}glucan branched at 4\text{'
and 6\text{' positions} | 18\%--38\% w/w protein \((1\rightarrow3)\beta\text{-}glucan branched at 4\text{'}
and 6\text{' positions} |
| Biological properties         | Rhamnose, arabinose | Fucose |
| References                    | Sakagami and Takeda, 1993; Ng et al, 1999; Kidd, 2000; Cui and Yusuf, 2003 | \textbf{In vitro and in vivo} immunorestorative and anti-tumor activities |

Accumulating evidence suggested that the polysaccharopeptides were nontoxic even when administered at several times the therapeutically effective dosage and over extended periods. Extended use of PSP at 100-fold the normal clinical dose had not induced acute and chronic toxicity in animals (Ng et al, 1997).

C. Genetic toxicity (Zhong et al, 1999)

Mutagenicity of PSP was assessed with the Ames test and with the chromosomal aberration test of bone marrow cells in mice. It was concluded that PSP showed no evidence of mutagenic or cytogenetic activity.

Cytotoxicity tests of PSP with V79 Chinese hamster cells \textit{in vitro} also showed no toxic effects against the V79 cell line.

\textit{In vivo} micronucleus tests to assess the cytogenotoxicity on mammalian somatic cells, the results indicated that PSP showed no evidence of mutagenic potential.

The results of chromosomal aberration tests and cytogenetic lesions in mice showed that the number of chromosomes had not changed in PSP treated groups even at the high dose rate 126 mg/kg.

D. Reproductive test

The possible effects on male and female reproductive physiology and embryonic development were also examined. Results from these studies suggested that PSP could not cause sperm aberration at a dosage 100 times higher that the usual clinical dose (Qian et al, 1993). The lack of deleterious effects on ovarian follicular development, ovulation, pregnancy and embryo development in mice was also demonstrated (Ng et al, 1997). Polysaccharopeptides appear to be safe during pregnancy. No adverse effects of PSP had been observed in female reproductive and embryonic development in mice (Ng et al, 1997).
V. Pharmacological actions
A. Immunomodulatory effects

The immunological activities of PSP and PSK have been extensively investigated both in vitro and in vivo. PSP strengthening the immunological functions was studied. Its anti-tumor effect seemed to be mediated more through immunomodulatory regulation rather than by direct cytotoxicity as was the case for most anticancer drugs currently used. Numerous reports had demonstrated the ability of PSK and PSP to active cellular and humoral components of the host immune system. In addition, these polysaccharides had been shown to inhibit the growth of tumor cell lines and to have in vivo anti-tumor activity (Tzianabos, 2000).

The effect of PSP on the phagocytic functions had been tested in normal ICR mice. It was determined that the carbon clearance rate of the groups given oral doses of 0.5-1.5g PSP/kg or intraperitoneal (i.p.) injections of 100, 200, 400mg/kg was similar to that of groups treated with acanthopanax (300mg/kg). Regardless of the route of administration, PSP increased the carbon clearance rate in mice and it suggested that PSP could increase the phagocytic function of normal animals (Yang et al, 1993). It was reported that PSP in different concentrations promoted the proliferation of T-lymphocytes both in human peripheral blood and mouse splenocytes (Li, 1999). PSP augmented T-helper cell (CD4⁺) activation, and also increased the ratio of CD4⁺/T suppressor (CD8⁺) production (Li, 1999). It appeared that the basic mechanisms for PSP to inhibit tumor cells included the activation of 1) macrophages, 2) natural killer cells and 3) T-helper cells (CD4⁺) which induce T-killer cells, antibody production, and interleukins (Li, 1999). Studies were conducted in vitro with numerous cell lines to investigate the immunomodulatory effects of PSP. It was recognized that Interleukine-2 (IL-2) plays a critical role in immune defense against tumors, because it is a potent inducer of activation of NK cells. In a study designed to evaluate the anti-tumor potential of IL-2 and PSP, it was demonstrated that the combination of the two agents had the most dramatic anti-tumor effect (Mao et al, 1996). Moreover, PSP could effectively stimulate the generation of Interferon-α (IFN-α) and markedly improve the production of IFN-γ (Yang et al, 1999). A study demonstrated that PSP did not exert a direct cytotoxic effect on tumor cell lines but rather stimulated macrophages, thus strengthening the hypothesis that C. versicolor anti-tumor effects were mediated by an immunomodulatory mechanism (Liu et al, 1999). In vivo studies had revealed that PSP usually had no significant immunological effects on a normal host, but could restore a depressed immunological responsiveness as seen in cancer or with chemotherapy (Chu et al, 2002).

PSP can also counteract the depressive effect of cyclophosphamide (CPA) on the white blood cell count and interleukin-2 production. CPA has become the leading drug in the clinical treatment of cancer, particularly for lymphomas, leukemia and solid tumors. This drug is cytotoxic, kills rapidly dividing neoplastic and normal cells, but has deleterious effect on the immune system. Researches demonstrated that PSP can antagonize the immunosuppression caused by such chemotherapeutic agents (Qian et al, 1997; Li, 1999). The administration of PSP (at a dosage of 2 grams/kg day) on cyclophosphamide-induced immunosuppressed rats demonstrated that the mushroom extract was effective in restoring their immune system. It did so by stimulating lymphocytes proliferation, NK cell functions, and the growth of spleen and thymus where lymphocytes mature and transit (Qian et al, 1997).

B. Anti-tumor effects in vivo

Many studies have been done since 1980’s to demonstrate the effectiveness of PSP, and its counterpart PSK (Table 2). The anti-tumor actions are predominantly considered to be host-mediated. The preliminary determination of anti-tumor activity is usually assessed by a bioassay system normally using Sarcoma 180 in mice and B16 melanoma-bearing mice through transplantable animal tumor (Yang et al, 2007). Several hundreds of polysaccharides or polysaccharide-protein complexes have been screened for their anti-tumor activity, and three of them, namely schizophyllan, lentilin and protein-bound polysaccharides (PSK and PSP), have been used clinically (Kobayashi et al, 1993). PSK has been successful in postoperative treatment of respectable cancer in humans, increasing survival rates (Ito et al, 2004). Protein-bound polysaccharide (PSK) prepared from the cultured mycelia of Coriolus versicolor in Japan demonstrated a significant anti-tumor effect against allogeneic tumors such as Sarcoma 180 and Ehrlich carcinoma of experimental animals by both intraperitoneal and oral administration (Taniguchi et al, 1984; Kobayashi et al, 1993). Researches indicated that PSK and PSP can suppress pulmonary metastasis from induced sarcomas, induced prostate cancer, lymphatic metastasis of mouse leukemia P388, and that both could prolong the survival period in spontaneous metastasis model (Xu, 1999).

Polysaccharopeptides from C. versicolor influence cancer metastasis in a number of ways: 1) by suppression of intravasation through the inhibition of tumor cells infiltration, 2) by suppression of tumor cell attachment to endothelial cells through the inhibition of tumor cell-induced platelet aggregation, 3) by suppression of tumour cell migration after extravasation through the inhibition of tumor cell mobility, and 4) by suppression of tumor growth after extravasation through the inhabitation of angiogenesis, the modulation of cytokine production and the augmentation of effector cell function (Liu et al, 2004). In addition to anti-cancer effect, animal study also demonstrated that PSP had central analgesic effect (Yin et al, 2002).

In vitro antitumour study, the C. versicolor extract was also found to tumour-selectively and dose-dependently inhibit the proliferation of lymphoma and leukemia cells possibly via an apoptosis-dependent pathway (Lau et al, 2004; Zhou et al, 2007). However, both immune property and anticancer potency of polysaccharopeptide-Coriolus versicolor were affected by the fermentation duration of the fungi (Lee et al, 2006).
Table 2. Some animal anti-tumor studies.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Test article</th>
<th>Administration</th>
<th>Dosage</th>
<th>Duration</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>PSP</td>
<td>By oral</td>
<td>1-2g/kg/day</td>
<td>15-20 days</td>
<td>Inhibited growth of human lung adenocarcinoma by 50-70%</td>
<td>Zeng et al, 1993</td>
</tr>
<tr>
<td>Nude mice</td>
<td>PSP</td>
<td>Intraperitoneal</td>
<td>50mg/kg/day</td>
<td>3 weeks</td>
<td>Inhibited the growth of Lewis lung cancer by nearly 45%</td>
<td>Wang et al, 1993</td>
</tr>
<tr>
<td>Mice</td>
<td>PSP</td>
<td>Intraperitoneal</td>
<td>2 weeks</td>
<td></td>
<td>Decreased the incidence of tumor growth, tumor size in control group about 3-5 times bigger than the PSP group</td>
<td>Dong et al, 1996</td>
</tr>
<tr>
<td>Rats</td>
<td>PSK</td>
<td>oral</td>
<td>150mg/kg</td>
<td>3 weeks</td>
<td>Prolonged the survival period of mammary tumor-bearing rats</td>
<td>Fujii et al, 1995</td>
</tr>
<tr>
<td>Mice</td>
<td>PSP</td>
<td>Oral</td>
<td>2, 5 g/kg daily</td>
<td>4 weeks</td>
<td>Tumor inhibition rate of 77 and 63% in treated groups</td>
<td>Zeng et al, 1999</td>
</tr>
<tr>
<td>Mice</td>
<td>CVP¹</td>
<td>Intraperitoneal</td>
<td>5-20mg/kg</td>
<td>3-15 days</td>
<td>Exerted inhibitory effects on experimental and clinical tumors</td>
<td>Wei et al, 1996</td>
</tr>
<tr>
<td>Rats</td>
<td>PSP</td>
<td>Oral</td>
<td>1.2g/day</td>
<td>21 days</td>
<td>Oral PSP did not prevent CTX²-induced cytopenia in rats</td>
<td>Kanoh et al, 1994</td>
</tr>
</tbody>
</table>

¹ Coriolus versicolor polysaccharides
² Cyclophosphamide

When PSK, obtained from cultured mycelia of Coriolus versicolor, was administered in mice, in vivo tumor-induced angiogenesis was suppressed (Mao et al, 2001).

However, some animal experiments could not demonstrate a specific effect of polysaccharopeptides extracted from the mushroom C. versicolor. For example, for a long-term control of brain tumors, intraperitoneal injection of 2mg of PSP with radiotherapy did not increase radiation efficacy (Mao et al, 1996). Another study investigated the effect of low-dose administration of IL-2 or PSP alone in a herpes virus type-2 transformed murine tumor in mice. The results indicated that IL-2 or PSP alone could slow down tumor progression, but the combination of the two modalities had no synergistic effects on tumor growth (Liu, 2001). These results warranted further investigation to determine if PSP could be effectively used for all types of tumors, or cancers, in which immunosuppression was a prominent feature.

In general, it seemed that oral administration of PSP reduced the incidence of tumor or prolonged the survival period, following chemical carcinogen-induced, radiation-induced and spontaneously developed animal cancer models. Coriolus versicolor (CV) regulated cytokine production and possessed both anti-tumor and immunopotentiating activities (Ho and Lau, 2004). The main mechanism might be an anti-teratogenic effect attributed to free radical trapping and prevention of chromosome injury, coupled to an immunomodulating effect linked to the modulation of cytokines production and effect cell function. Table 3 summaries the possible mechanisms of anticancer effects of PSP (Hobbs, 2004).

C. Antimicrobial effects

Besides its documented anti-cancer effects, polysaccharopeptides from C. versicolor could be useful for other conditions as well.

PSK has been demonstrated to have antiviral activities against ectromelia virus (Taniguchi et al, 1984) and cytomegalovirus infections (90) and human immunodeficiency virus (HIV) (Zou and Zhu, 2003).

In China, C. versicolor is also considered useful in the treatment of hepatitis. Preliminary studies had indicated that PSP was effective in protecting liver from hepatotoxins in laboratory animals (Song and Liang, 2000; Zou et al, 2003). As with antiviral action, the multivalent manner in which PSP acts to protect liver cells and detoxification mechanisms, demonstrates the potential usefulness of C. versicolor extracts to prevent damages from reactive compounds which could be carcinogenic agents (Jong and Yang, 1999; Yeung, 1999).
Table 3. Mechanisms of anticancer effects of extracts of C. versicolor.

- Inhibition of DNA of tumour cells
- Enhancement of cytokine production
- Antitumour activity in wide range of animal systems
- Tumour cell killing effect
- Inhibition of carcinogen-induced cancers in rats
- Antioxidant effects in tumour-bearing rats
- Induction of apoptosis
- Antiproliferative effect on many cancer cell lines
- Anti-invasion effects
- Angiogenesis effects
- Tumouricidal and cytotoxicity effects
- Antimetastic activity
- Immunoprotective effects during radiation and chemotherapy

D. Anti-nociceptive effects

In an animal study, two different mice pain models were used to investigate the anti-nociceptive effects of PSP. The results demonstrated that PSP could induce hyperalgesia. PSP-induced hyperalgesia was related to the activation of peritoneal macrophages and mast cells and, hence, increased the release of inflammatory mediators (Chan and Yeung, 2006).

VI. Clinical studies

The therapeutic use of PSP or PSK as an adjuvant therapy in cancer treatment has been substantiated by numerous clinical trials. Table 6 summarizes the methodological parameters and the results of some of these trials. As an adjuvant in the treatment of many types of cancers, Yun Zhi (PSP) was subjected to Phase I, II and III clinical trials in Shanghai, China. The results showed that addition of PSP to radiotherapeutic or chemotherapeutic protocols can greatly improve the quality of life of cancer patients and reduce chemotherapy-induced side effects (Zhong et al, 2001), because PSP alleviated weakness, anorexia, vomiting, dryness of throat, spontaneous sweat and pain symptoms. In addition to symptoms improvement, polysaccharopeptides from Yun Zhi also have an impact on the survival rate of different types of cancer. PSK used in Japanese trials significantly extended the survival rate at five years for stomach, colorectal, esophagus, nasopharynx, breast and lung cancers. Study also showed that PSP has effect of cancer prevention and development (Zhou et al, 2001). The following were the clinical trial results selected from research papers conducted in Japan (Table 4, 5).

A. Breast cancer

As early as 1970, breast cancer patients received long term combination chemotherapy along with C. versicolor extract immunotherapy. Addition of the extract to the regimen significantly extended the survival rate. In a large trial done in 1995 in Japan, the survival rate at 10 years was 81% for the PSK plus chemotherapy group which the rate fell to 64% for the group that used chemotherapy alone. The study concluded that immuno-chemotherapy with mushroom extract could improve the prognosis of patients with resectable breast cancer with vascular invasion. Mild and well tolerated side effects such as leukenopia and nausea were observed in 5 out 227 patients (Iino et al, 1995).

Table 4. Randomized controlled trials for breast cancer.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Stage</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>914 cases Standard or Radical mastectomy</td>
<td>IIA, IIB, III</td>
<td>1. Patients(ER+ tumours) chemo +/- tamoxifen 2. Patients (ER- tumour) Chemo +/- PSK</td>
<td>Longer overall survival for patients in Stage IIA T2N1 cancer ER- and node-negative treated with chemo + PSK compared with other ER-subgroups without PSK. Risk ratio lower in the chemo+ PSK group. Overall and diseasefree survival rates not significant for all groups.</td>
<td>Toi et al, 1992</td>
</tr>
<tr>
<td>227 cases operable breast cancer with v+ and/or n+ involvement</td>
<td></td>
<td>Chemo (n=77) Chemo +LMS (n=76) Chemo, +, PSK (n=74)</td>
<td></td>
<td>Iino et al, 1995;</td>
</tr>
<tr>
<td>134 cases Typed as HLAA, HLA-B and HLA-C Operable with v+ and/or n+</td>
<td></td>
<td>Previously randomised into two groups(ref 23): 1. Chemo 2. Chemo +PSK Each group stratified by HLA type B40+ or B40-.</td>
<td>Years for chemo + PSK group: HLA-B40+ : 100%; HLA-B40- : 76% and 55%, respectively. Significant difference at p =0.05.</td>
<td>Yokoe et al, 1997</td>
</tr>
</tbody>
</table>
Table 5. Selected randomized controlled trials for colorectal cancer.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Stage (II/III)</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>55 cases</td>
<td>Advance</td>
<td>1. Surgery + placebo</td>
<td>8-yrs survival rate significant in the PSK group (p&lt;0.05); Disease-free interval (p&lt;0.05)</td>
<td>Torisu et al, 1990</td>
</tr>
<tr>
<td>56 controls</td>
<td></td>
<td>2. Surgery + PSK (3g/day for 2 months; 2g/day for 24 months; 1gm/day thereafter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multicentre</td>
<td></td>
<td>1. Chemo</td>
<td>Disease-free interval and survival significantly better for PSK in the colon group (p&lt;0.05 in both)</td>
<td>Mitomi et al, 1992</td>
</tr>
<tr>
<td>221 cases</td>
<td></td>
<td>2. Chemo + PSK (3g/day for 3 years)</td>
<td>Overall survival rate higher in the PSK group but not significant (p=0.21). 3-year disease-free survival rate significantly higher in the PSK group (p=0.02). Stage III, Patients 3-year overall and disease-free survival rates in the PSK significant (p=0.02; p=0.01) 5-year overall survival rate significantly higher in the PSK group (p&lt;0.016; p&lt;0.056 respectively).</td>
<td>Ohwada et al, 2003</td>
</tr>
<tr>
<td>227 controls</td>
<td></td>
<td>1. Chemo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 207</td>
<td>Primary</td>
<td>2. Chemo + PSK (3g per day for &gt;2 yrs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>134 cases</td>
<td>(II/III)</td>
<td>All patients received Mitomycin-C post-surgery.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>67 controls</td>
<td></td>
<td>1. Chemo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 withdrew</td>
<td></td>
<td>2. Chemo + PSK (3g/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 205</td>
<td>Primary</td>
<td>Both treatments for 2 yrs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>137 cases</td>
<td>(II/III)</td>
<td>All patients received chemo after surgery for 3-4 weeks, then 10 courses of treatment.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>68 controls</td>
<td></td>
<td>1. PSK 4 weeks then 4 weeks chemo.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon cancer</td>
<td>Dukes A:7%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with lymph node</td>
<td>B:45.5%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>metastasis</td>
<td>C:47.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 441</td>
<td></td>
<td>2. 4 weeks rest then 4 weeks chemo.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>220 cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>221 controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>202 primary colon</td>
<td>Primary</td>
<td>99 underwent adjuvant treatment with PSK and 5-FU (PSK group), 103 were treated with 5-FU alone (5-FU group).</td>
<td>The presence of diffuse nuclear accumulation-type β-catenin activation identifies patients with colon cancer who respond better to immunotherapy with polysaccharide K.</td>
<td>Yamashita et al, 2007</td>
</tr>
<tr>
<td>cancer patients</td>
<td>colon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-FU + PSK.99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-FU alone:103</td>
<td>cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5-FU = 5-fluorouracil

B. Colorectal cancer

C. versicolor extract was assessed for its potential anticancer activity in patients with advanced colorectal cancer (stages III and IV) (Torisu et al, 1990). PSK was given at 3 grams/day for two months after surgery, followed by 2 grams/day until the end of the second year and 1 gram/day thereafter. This study found that the leukocyte activity of the PSK group was remarkably enhanced. Notably, polymorphonuclear leukocytes from PSK-treated patients showed enhancement in their activities, such as random and/or chemotactic locomotion, and phagocytic activity, when compared with those of the control group. The survival rate of the PSK group reached 40%, a net improvement over the 25% rate registered for the placebo group. From this study, it was concluded that PSK could be useful as a maintenance therapy for patients after their curative surgical operations for colorectal cancer.

Sakamoto and colleagues performed in 2006 a meta-analysis of colorectal cancer, which involved 1094 patients in three clinical trials. The aim of the meta-analysis was to evaluate the effect of PSK as adjuvant immunochemotherapy for patients with curatively resectable colorectal cancer. The results suggested that adjuvant immunochemotherapy with PSK can improve both survival and disease-free survival of patients with curatively resectable colorectal cancer.

C. Gastric and esophageal cancer

Gastric cancer is a major cause of mortality in Japan and China and, for that reason, has been the object of many clinical trials with polysaccharopeptides from C. versicolor. Trials done in the 1970s and 80s had evidenced a better survival rate at two or three years. More recent clinical studies, done in the 1980s and 90s, established that PSK could improve the survival rate at five years and beyond in stomach cancer patients, including some patients with advanced Stage III and IV cancer with metastasis (Kidd, 2000).
Table 6. Assessment of methodological parameters on clinical trials of PSP or PSK.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Jadad Score (max = 5)</th>
<th>Study design</th>
<th>Sample size</th>
<th>Inclusion/Exclusion criteria</th>
<th>Treatment schedule</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitomi et al., 1993</td>
<td>2</td>
<td>Randomized control, follow-up study</td>
<td>448 colorectal cancer patients treated with chemotherapy</td>
<td>Patients younger than 75 years of age with stage III or IV colorectal cancer and consented form obtained were included. Patients with WBC&lt;4000/mm³, PLT&lt;100,000/mm³, TP&lt;6.0g/dl, Alb&lt;3.0g/dl, A/G&lt;1, SGOT/SGPT&gt;100U, urine protein (+), Crea&gt;1.5mg/dl were excluded.</td>
<td>227/221 patients were randomly assigned to control (chemotherapy for 6 months) and treatment (chemotherapy 6 months plus PSK 3g/day for 3 years)</td>
<td>The disease-free survival and the survival of the PSK group were better than those of the control group (Disease-free survival: p=0.0214, Survival: p=0.0272)</td>
</tr>
<tr>
<td>Nakazato et al., 1989</td>
<td>3</td>
<td>Randomized control, follow-up trial</td>
<td>262 gastric cancer patients with radical surgery</td>
<td>Patients with age&lt;75 years, PPD(+), diagnose confirmed by pathology were included. Patients who underwent any radiotherapy, chemotheraphy, or immunotherapy or having multiple cancers, or any abnormal hematological findings were excluded.</td>
<td>129/124 were randomly assigned to control (chemotherapy) and treatment (chemotherapy plus PSK 3g/day for 4 weeks)</td>
<td>The disease-free survival curves and overall survival curves of PSK treatment group were significant (p=0.018 and p=0.045) better than those of control group.</td>
</tr>
<tr>
<td>Ichihashi et al., 1987</td>
<td>3</td>
<td>Randomized control follow-up study</td>
<td>168 patients with stomach cancer</td>
<td>Inclusions included: age &lt;75 years, without multiple cancers, normal hematological findings and diagnosis confirmed by post-operation pathology</td>
<td>49/47/28 patients were randomly assigned to receive chemotherapy (CQ), PSK (3g/50kg) plus CQ for 13 months and no CQ and PSK treatment (as control).</td>
<td>Overall 7 year survival rate in PSK group higher than chemotherapy group but no significant difference.</td>
</tr>
<tr>
<td>Guo, 2000</td>
<td>3</td>
<td>Double-blind placebo-controlled randomized study</td>
<td>34 patients with non-small cell lung cancer</td>
<td>Completed conventional treatment for advanced NSCLC</td>
<td>Patients received 28-day administration of PSP</td>
<td>After 28-day treatment, there was a significant improvement in blood leukocyte and neutrophil counts, serum IgG and IgM, and percent of body fat among the PSP, but not the control, patients (p&lt;0.05)</td>
</tr>
<tr>
<td>Ze et al, 2003</td>
<td>3</td>
<td>Randomized controlled trial</td>
<td>201 patients with stage II or III colorectal cancer</td>
<td>Age &lt; 75 year and historical confirmed colorectal cancer. Exclude underwent any radiotherapy, chemotheraphy or immunotherapy.</td>
<td>137/68 patients treated with 3g PSK plus 300mg tegafur/uracil or 300mg tegafur/uracil alone for 2 years</td>
<td>The three-year, disease-free survival rate was 80.6% in PSK group (p=0.02) compared with 68.7% in the control group.</td>
</tr>
<tr>
<td>Ebihara and Minamishima, 1984</td>
<td>2</td>
<td>Randomized controlled trial</td>
<td>25 advanced malignant cancer patients</td>
<td>Advanced malignant cancer patients without immunotherapy</td>
<td>Treatment group orally given PS 5g each time 3 times a day for 2 months plus symptoms control treatment Control group only gave symptoms control treatment</td>
<td>Quality of life and symptoms were improved in PSP-treated group compared with control group (p&lt;0.01 and p&lt;0.05)</td>
</tr>
</tbody>
</table>
In a randomized clinical trial including 579 patients with gastric cancer receiving chemotherapy, C. versicolor extract was administrated orally as an adjuvant to surgery in a combination therapy to part of the group, at a daily dose of 3 grams for 1 year. Results from this study showed a significant increase in the 5-year survival rate for the PSK-treated group when compared with the other groups (Niimoto et al, 1988).

In an additional study involving more than 260 patients who underwent surgery for stomach cancer at 46 hospitals in Japan, those who received PSK along with chemotherapy experienced a higher 5-year disease-free rate and a better 5-year survival rate than subjects who underwent chemotherapy alone (73% vs. 60%). The two regimens had slight toxic effects, consisting of nausea, leucopenia, and liver function impairment but no characteristic toxic effects were linked to PSK administration (Nakazato et al, 1994).

Trials with PSP indicated that C. versicolor extract has the potential to alleviate the side effects normally associated with chemotherapy (lassitude, inappetence, spontaneous perspiration, etc.) in patients with stomach cancer classified as stage I to IV (Zhang et al, 1999). Moreover, an increase in the immunological functions and a concomitant decrease of the adverse hematological side effects of chemotherapeutical drugs was demonstrated for stomach cancer patients following the administration of PSP (1 gram three times a day for 8 weeks) (Wu et al, 1999).

Results from a prospective multi-centre study including 158 esophageal cancer patients, indicated that those who received PSK (3 grams/day for three months after surgery) had a significantly better survival rate at five years (55% and 58%) compared with those without PSK supplementation (26% and 31%) (Ogoshi et al, 1995a). Another study reported the results of 174 patients who underwent esophagectomy and were then assigned to receive radiotherapy or chemotherapy with or without PSK. There was a tendency for longer survival on PSK, but statistical significance was not reached. However, regression analysis indicated that C. versicolor extract might have a beneficial effect on esophageal carcinoma when combined with radiotherapy and chemotherapy (Ogoshi et al, 1995b).

Better survival rates were also achieved with PSP. A hundred patients with esophageal carcinoma were randomly divided into two groups: one group was treated with radiotherapy alone while other one received radiotherapy plus PSP (3 grams daily for a total dose of 190 grams during the period of radiation time). The results demonstrated that patients treated with radiotherapy plus PSP had higher one, three and five survival rates (67%, 38% and 19% respectively) versus the control group (47%, 21% and 14%) (Yao, 1999). Addition of PSP to the regimen improved the relief of major symptoms commonly associated with esophageal cancer, such as change of weight, alteration in the hemogram profile and in immunological functions. The relief of these symptoms was quantified to reach 61% in the PSP-treated group, while it was 31% in the control group (Wu and Wang, 1999).

A Meta-analysis study was performed to evaluate the effect of immunochemotherapy on survival in patients with curative resections of gastric cancer. The meta-analysis included 8,009 patients from eight randomized controlled trials after central randomization. The results suggest that adjuvant immunochemotherapy with PSK improves the survival of patients after curative gastric cancer resection (Oba et al, 2007).

**D. Leukemia**

A study with PSK as an adjuvant to chemotherapy done in the early 1980’s (with 28 patients) found that remission and survival were significantly prolonged for patients who received PSK plus chemotherapy over those who received chemotherapy alone (Nagao et al, 1981). In another multi-center trial including 67 patients in remission of acute non-lymphocytic leukemia (ANLL) in Japan, patients who received a maintenance chemotherapy plus immunotherapy with PSK tended to have longer survival over the group that received chemotherapy alone but without significance. However, analysis of the data of patients who had maintained complete remission for more than 270 days revealed that immunotherapy had a suggestive beneficial effect (p=0.105), prolonging the 50% remission period by 418 days (885 vs. 467 days). It was concluded that PSK may help in the treatment of adult ANLL when used for maintenance therapy in combination with chemotherapy, especially in patients with a good prognosis.

**E. Lung cancer**

A clinical trial conducted in Japan was done with patients having different lung cancers at stages I-III; a group was selected to receive 3 grams of PSK daily after cessation of radiation therapy. PSK was given in repeating cycles of two weeks on and two weeks off. After 5 years, 27% of the patients treated with PSK were alive compared to 7% for those not given the mushroom extract (Hayakawa et al, 1993). The study also demonstrated that patients with Stage III disease who received PSK had a better prognosis than those with stages I and II without PSK. A study investigated the effects of PSK as an accessory treatment for lung cancer (Ke et al, 1999), 30 patients were administered the mushroom extract while they received chemotherapy. The symptoms (side effects) improvement for PSP-treated patients was over 87%, while it was 47% for the control group.

Thirty-four patients, with no significant difference in their baseline demographic, clinical or tumor characteristics, or previous treatment regimes (p>0.05), were recruited into each of the PSP and control arms. After 28-day treatment, there was a significant improvement in blood leukocyte and neutrophil counts, serum IgG and IgM, and percent of body fat among the PSP, but not the control patients (p<0.05) (Tsang et al, 2003).

**VII. Discussion and Conclusion**

Since 1970s numerous mushroom fungi have been increasingly used as a source of medicinal compounds and

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therapeutic adjutants or health food supplements. PSP has been previously shown to have immuno-stimulatory, antitumour and analgesic effects (Zhong et al., 2001) in animal models. When used as an adjunct in cancer chemotherapy in clinical trials carried out in China, PSP improved the quality of life in the patients by improving appetite and alleviating symptoms associated with cancer chemotherapy. Cancer chemotherapy and radiotherapy are standard treatments of cancer in the past 30 years, often associated with side effects such as immuno-suppression, poor appetite, and vomiting, which seriously affected the therapy. Studies in Japan and China have suggested mushroom proteoglycans may provide a possible solution, with Polysaccharide Krestin and PSP being systemically studied as adjunct to cancer chemotherapy (Kidd, 2000). Nowadays the biological activities of PSP and PSK have received much attention in biomedical sciences. One of the most important functions of PSP and PSK is their immunomodulation and anti-cancer actions. Various experimental evidences demonstrated that the anti-tumor action of mushroom polysaccharides is due to the enhancement and potentiation of cell-mediated immune system through the regulation of immunomodulatory cytokines and activation of the complement system and Natural Killer Cells (NK cells) (Ohwada et al., 2006). However the mechanism of anti-tumor actions of PSP and PSK from most fungi is still not clear. It is accepted that anti-tumor polysaccharides enhance various immune responses, and act as biological response modifiers (Ohwada et al., 2006). PSK/PSP are nonspecific immunopotentiators and exert immunomodulatory actions by promoting the proliferation of T-lymphocytes, the activation of macrophages, natural killer cells, and T-helper cells, thereby inducing the production of antibody and interleukins (Mao et al., 1996; Li, 1999). PSK also has favorable effect on the activation of leucocyte chemotactic locomotion and phagocytic activity (Torisu et al., 1990). However further studies on the mechanisms behind anticancer, immunostimulatory, and biological response modifying effects of PSK or PSP are needed.

Acknowledgments
This project was made possible by Grant Number 1 P50 AT002779-01 from the National Center for Complementary and Alternative Medicine (NCCAM) and the Office of Dietary Supplements (ODS). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NCCAM, ODS or the National Institute of Health.

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From left to right: King-Fai Cheng, Ping-Chung Leung
Cheng and Leung: General review of polysaccharopeptides (PSP) from C. versicolor