PSK as a Chemopreventive Agent

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Abstract
PSK, a protein-bound polysaccharide preparation obtained from cultured mycelia of the CM-101 strain of Coriolus versicolor belonging to basidioymycetes, is a biological response modifier capable of exhibiting diverse biological activities. This agent has been used clinically for the treatment of postoperative cancer patients in Japan by oral use. In this paper, chemopreventive aspects of PSK were reviewed. Oral administration of PSK reduced the incidence of tumor and/or prolonged the survival period in the following experiments: chemopreventive effect against carcinogenesis; gastrointestinal cancer induced by 1,2-dimethylhydrazine; rat hepatoma by 3'-methyl-4-dimethylaminobenzene; mouse thymic lymphoma by whole-body irradiation; mouse spontaneous mammary tumor; and so on. PSK did not interact and/or inhibit drug-metabolizing enzymes and had no effect on the Ames test. On the other hand, this agent scavenged active oxygen through the induction of manganese superoxide dismutase, prevented the increase in frequency of anticancer agent-induced sister chromatid exchange, and suppressed fetal deformation induced by transplacental injection of teratogen, suggesting an effect on the initiation or promotion process of carcinogenesis. Also, PSK regulated cytokine production and enhanced the antitumor activity of effector cells such as killer T-cells and natural killer cells, suggesting an effect on the growth process after the development of malignant cells. Thus, this agent seems to act at multiple steps during carcinogenesis rather than a particular step. The main mechanism may be an antitumorigenic effect attributed to radical trapping, preventive effects against chromosome injury, and immunomodulative effects attributed to the modulation of cytokine production and effector cell function. Because PSK has few side effects and may possibly be given orally for long periods, it was suggested that this agent may be a candidate for use as a chemopreventive agent.

Introduction
PSK (Krestin), a protein-bound polysaccharide derived from a basidiomycetes, has been widely used as an immunotherapeutic agent for cancer treatment in Japan (1). This preparation is obtained from cultured mycelia of the CM-101 strain of Coriolus versicolor (Fr.) Quel. by hot water extraction, followed by purification and dehydration. PSK is a brownish powder having a slight odor and no taste. It is practically insoluble in methanol, pyridine, chloroform, and n-hexane but is soluble in water. The pH of a PSK solution is about 7.

The average molecular weight of PSK is about 100,000 as determined by the ultracentrifugation method. It contains approximately 18–38% protein. The main fraction of the polysaccharide part of PSK is a β-glucan with main chain 1–4 bonds and branches at the 3 and 6 positions in a proportion of one per several residues. The protein consists predominantly of acidic amino acids such as aspartic acid and glutamic acid and neutral amino acids such as valine and leucine, with basic amino acids, including lysine and arginine, present in small amounts (1).

In fundamental studies, it has been demonstrated that PSK has not only antitumor activity mediated through the immune system but also diverse biologic activities such as the prevention of microbial infections and chemopreventive activity against carcinogenesis. In this paper, the characteristics of PSK as a chemopreventive agent are presented.

Chemopreventive Effect of PSK against Carcinogenesis
The effect of PSK on the process of carcinogenesis has been investigated in chemical carcinogen-induced, radiation-induced, and spontaneously developed animal cancer models: rat gastrointestinal cancer induced by 1,2-dimethylhydrazine; rat hepatoma by 3'-methyl-4-dimethylaminobenzene; mouse thymic lymphoma by whole-body irradiation; mouse spontaneous mammary tumor; and so on. PSK did not interact and/or inhibit drug-metabolizing enzymes and had no effect on the Ames test. On the other hand, this agent scavenged active oxygen through the induction of manganese superoxide dismutase, prevented the increase in frequency of anticancer agent-induced sister chromatid exchange, and suppressed fetal deformation induced by transplacental injection of teratogen, suggesting an effect on the initiation or promotion process of carcinogenesis. Also, PSK regulated cytokine production and enhanced the antitumor activity of effector cells such as killer T-cells and natural killer cells, suggesting an effect on the growth process after the development of malignant cells. Thus, this agent seems to act at multiple steps during carcinogenesis rather than a particular step. The main mechanism may be an antitumorigenic effect attributed to radical trapping, preventive effects against chromosome injury, and immunomodulative effects attributed to the modulation of cytokine production and effector cell function. Because PSK has few side effects and may possibly be given orally for long periods, it was suggested that this agent may be a candidate for use as a chemopreventive agent.

Preventive Effect of PSK against Chemical Carcinogen-induced Carcinogenesis
Gastrointestinal cancer induced by s.c. injection of DMH2 in rats or mice exhibits patho-

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2 The abbreviations used are: DMH, 1,2-dimethylhydrazine; 3-MDAB, 3-methyl-4-dimethylaminobenzene; MNNG, N-methyl-n'-nitrosoguanidine; IL, interleukin; 5-AC, 5-azacytidine; CY, cyclophosphamide; MnSOD, manganese superoxide dismutase; SCE, sister chromatid exchange.

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1 To whom requests for reprints should be addressed, at Sapporo Cancer Seminar Foundation, Odori Nishi-6, Chuo-ku, Sapporo 060, Japan.
logic findings similar to those of colorectal cancer in humans and is well known as an excellent experimental model of carcino genesis. Sakita et al. investigated the effect of PSK on the development of gastrointestinal cancer in DMH-treated Wistar rats given 2% PSK-containing chow for 45 weeks, starting from the first day of DMH injection. The incidence of histopathologically confirmed cancer in weeks 25 and 35 of DMH treatment was significantly lower in the D and E groups (6). Concerning carcinogenesis models other than those described above, chemopreventive effects of PSK have been observed in the following systems (Table 1): rat gastric cancer induced by MNNG or the related compound N-ethyl-n'-nitrosouguainidine in drinking water (3, 7); rat esophageal cancer induced by N-n-butylnitrosourethane in drinking water (8); hamster hepatoma induced by i.v. injection of colloidal thorium dioxide (thorotrust; Ref. 9); rabbit lung cancer induced by transtracheal application of n-methyl-N-nitrosourethane or MNNG (10, 11); rat mammary tumor induced by i.v. injection of 7,12-dimethylbenzanthracene (DMBA, 12); rat bladder cancer induced by N-n-butyl-nitrosourea in drinking water (13); neurogenic tumor induced by i.v. injection of ethyl nitrosourea in pregnant rats (14); and fibrosarcomas induced by s.c. injection of methylcholanthrene in mice (15) or rats (16).

**Preventive Effect of PSK against Radiation-induced Carcinogenesis.** Repeated whole-body irradiation induces lymphoma in the thymus of mice (17). Kurishita et al. (18) investigated the effect of PSK on the development of thymic lymphoma in C57BL/6 mice produced by exposure to irradiation at the magnitude of 1.7 Gy,

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* t.t. transtrachely.
starting at 35 days postpartum and administered once a week for 4 weeks. The incidence of thymic lymphoma in the tenth month after irradiation was significantly inhibited in the group given chow containing 2% P5K. In the analysis of thymic lymphocyte subsets within 1 month after the irradiation, CD4- and CD8-positive cells decreased, and CD4-positive and CD8-negative cells increased by the administration of P5K. These results were interpreted as an indication of promotion of thymus cell differentiation by P5K.

**Preventive Effect of PSK against Spontaneously Developed Carcinogenesis.** Female C3H/He Ouj mice, produced by the Jackson Laboratory in the United States, is an appropriate strain for the prediction of spontaneous mammary tumors. Fujii et al. (19) investigated the effect of PSK on the development of mammary tumors in C3H/He Ouj mice by giving them chow containing 2% P5K for 1 year starting at the age of 6 weeks. Although there was little difference between control and PSK-treated groups with respect to the time of palpable tumor formation, the incidence of tumors 1 year after starting the experiment was significantly reduced in the PSK-treated groups. Furthermore, the mean number of tumors per mouse (multiplicity) was significantly less in the PSK-treated group. There was no significant difference between test and control groups in body weight, feed consumption, hematological tests, or blood biochemistry during the experimental period. These results were interpreted as an effect of PSK in regulating host defense mechanisms.

**Mechanisms of Action of PSK**

The mechanism by which PSK produces a cancer chemopreventive effect includes (a) a mechanism related to an antitumorigenic effect, (b) a radical trapping effect, (c) a protective effect against chromatic injuries, and (d) an immunomodulating effect. PSK seems to act at multiple steps during carcinogenesis rather than a particular step.

**Immunomodulation Effect**

**Effects on Cytokine Production and Effector Cell Functions.** PSK acts on monocytes, macrophages, and T-cells. It regulates the production of cytokines and enhances the antitumor activity of natural killer cells, lymphokine-activated killer cells, killer T-cells, and macrophages, among others. The immunomodulating effect of PSK seems to play an important role in the growth process after the development of malignant cells.

**Effects on Cytokine Production.** Hirose et al. (20) found that PSK would induce monokines such as IL-1α, IL-1β, IL-6, IL-8, tumor necrosis factor α, and monocyte chemotactic and activating factor, when cultured in vitro with peripheral blood mononuclear cells of healthy humans. Ueda et al. (21) and Abe et al. (22) reported that PSK would enhance the production of IL-2 and γ-interferon in human peripheral blood mononuclear cells and their T-cell-rich fractions. Saji et al. found that gene expression of IL-1β, IL-6, and tumor necrosis factor α was induced in the spleen of healthy mice after the administration p.o. of PSK (23).

**Effects on Effector Cells.** It has been reported that PSK enhanced the in vitro induction of killer T-cells and lymphokine-activated killer cells when cultured with lymphocytes and tumor cells (24–27) and that administration of PSK augmented natural killer cell activity in mouse spleen, killer T-cell activity and complement-dependent cell-mediated cytotoxicity in the spleen of mice immunized with tumor cells, and cytostatic activity of peritoneal neutrophils (25, 28–30).

Yefenof et al. (31) investigated the effects of PSK on the radiation-induced leukemia/thymic lymphoma model. PSK enhanced the T-cell response to viral-infected cells in vitro and attenuated the suppressor activity of mouse spleen cells abnormally enhanced by the inoculation of the virus in vitro. The coexistent administration of PSK was necessary throughout the incubation period for the effect to be manifested.

**Effects on Immunosuppressive Substances.** Reduction of immunosuppressive substances caused by cancer is one of the characteristic effects of PSK (23, 32). Increase of nonspecific immunosuppressive substances in the body fluid of cancer-bearing hosts is involved in the mechanism of cancer-induced immunosuppression. In addition, normal augmentation of suppressor cells. PSK reduced the level of immunosuppressive substances or immunosuppressive activities in the sera of tumor-bearing hosts.

Sakita et al. (4) investigated the antagonistic effects of PSK on immunosuppressive substances in DMH-induced gastrointestinal tumor models of rats. A marked inhibitory activity of serum phytohemagglutinin-induced blastogenesis was observed in rats 35 weeks after treatment with DMH. This inhibitory activity was significantly reduced in rats treated with PSK. The serum level of immunosuppressive substance exhibited a similar tendency and showed significantly lower values in weeks 25 and 35 in animals given PSK. The authors interpreted the chemopreventive effect of PSK as being due to the reduction of immunosuppressive substances in serum.

**Antitumorogenese**

When carcinogenic substances are administered to pregnant animals, they induce not only transplacental carcinogenesis but also deformities in fetuses. Some carcinogens induce both fetal deformation and cancer when they are administered to pregnant animals at different gestation periods. These two, fetal deformation and cancer, share some characteristics. PSK has been shown to exhibit antitumorogenic effects, supporting the preventive effect of PSK against carcinogenesis to an extent.

Kurishita (33) investigated the effect of PSK on 5-AC-induced teratogenesis (33). The digital defects induced by 5-AC, such as syndactyly and brachydactyly, were significantly suppressed by the s.c. administration of PSK. The effect of PSK was significant when given 24 h prior to or 1 h after 5-AC treatment, suggesting a time dependency for expression of the activity. Tanaka (34) investigated the suppressive effect of PSK on 5-AC-induced teratogenesis in a murine system. External deformities, external encephalopathy, cleft palate, and ophthalmic abnormalities in day 13 fetuses were significantly suppressed when PSK was given to pregnant mice treated with 5-AC on day 7.5 of gestation. In addition, PSK suppressed MNNG-induced fetal cleft palate (35, 36).

PSK is effective against CY, chlorambucil, and radiation-induced teratogenesis. Tongoe (37) investigated the
effect of PSK on CY-induced teratogenesis and found that an increase in the incidence of absorbed fetuses and a decrease in body weight caused by CY treatment were reduced by administration p.o. of PSK, starting from 50 days prior to mating (37). Chloramphenicol-induced fetal polydactyly and radiation-induced fetal ophthalmic malformation were significantly reduced by the administration of PSK (36, 38).

**Radical Trapping Effect of PSK.** While the involvement of active oxygen in carcinogenesis has not yet been elucidated completely, the radical trapping effect of PSK may be an important mechanism of its chemopreventive action.

Nakamura and Nakajima (39) and Iwagaki et al. (40) investigated the direct radical trapping effect of PSK in a cell-free system. They confirmed that superoxide and hydroxy radicals generated by enzymatic or nonenzymatic reactions were dose-dependently trapped by PSK using colormetry and electron spin resonance. As for in vivo studies, Yoshikawa et al. reported that administration p.o. of PSK suppressed the generation of lipid peroxides in the liver of rats treated with carbon tetrachloride (41).

On the other hand, there are also reports that the radical trapping effect of PSK may be attributed to the induction of MnSOD present in mitochondria. Hirose et al. (42) investigated the effect of PSK on MnSOD induction and found that PSK induced MnSOD gene expression of healthy human peripheral blood mononuclear cells *in vitro* and that administration p.o. of PSK also induced the same gene expression in mouse spleen cells. The induction of this enzyme has been confirmed by protein levels as well. It has been suggested that the induction of MnSOD by PSK exerts a protective effect against injury to normal cells caused by treatment with quinone-type anticancer drugs or irradiation.

**Protection against Chromatid Injury.** Although the mechanism of generation and biological significance of SCE has not yet been proved completely, it has been suggested that PSK may inhibit DNA changes in the initiation or progression processes of carcinogenesis by suppressing the incidence of mutation-induced SCE.

Hasegawa et al. (43) reported suppression by PSK of SCE frequency induced by anticancer agents. When mitomycin C was injected i.v. into C57BL/6 mice, the incidence of SCE frequency in bone marrow cells was markedly increased. This increase was significantly prevented by the i.p. administration of PSK immediately after treatment with mitomycin C. The suppressive effect of PSK against SCE has been observed in CY or nimustine chloride-treated mice and X-ray-irradiated mice (44). Torigoe et al. (45, 46) also found that CY-induced SCE in mouse bone marrow and spermatogonium cells was prevented by the administration of PSK.

**Clinical Studies**

The life-prolonging effect of PSK against gastric cancer, colorectal cancer, and lung cancer was demonstrated in strictly controlled randomized clinical studies mainly when it was combined with chemotherapy after surgery (47–53), although there has been no report that refers to chemoprevention directly. Representative results of the controlled clinical studies are described below.

Nakazato et al. (47) evaluated the efficacy of PSK for adjuvant immunochemotherapy in gastric cancer patients who had undergone radical gastrectomy. In a randomized controlled trial in collaboration with 46 institutions in the Chubu district of Japan, 262 patients were allocated to Group P (5-fluorouracil, 150 mg/day, and PSK, 3 g/day, p.o.) and Group C (5-fluorouracil, 150 mg/day alone), using the central registration system. Two hundred fifty-three of those patients (97.0%) satisfied the eligibility criteria. The distribution in these two groups of the degree of serous infiltration and lymph node metastases was well balanced at any level by the use of minimization methods. They reported that Group P did significantly better than Group C with respect to disease-free period ($P = 0.044$) and overall survival period ($P = 0.033$) after 5 years' follow-up (54).

Motomi et al. (48) studied the effect of PSK on the survival of curatively resected colorectal cancer patients. In a randomized controlled trial in collaboration with 35 institutions in Kanagawa prefecture in Japan, 462 patients were allocated into Group P (mitomycin C i.v. on the day of and the day after surgery, followed by 5-fluorouracil, 200 mg/day, for 6 months and PSK, 3 g/day, for over 3 years, p.o.) and Group C (mitomycin C i.v. on the day of and the day after surgery, followed by 5-fluorouracil, 200 mg/day, for 6 months p.o.). Four hundred forty-eight (97.0%) of those patients satisfied the eligibility criteria. They reported that the disease-free survival curve and overall survival curve of Group P were significantly better than those of Group C after 4 years of follow-up ($P = 0.013$).

In the clinical history of PSK, side effects caused by PSK were experienced in 114 patients of a total of 11,300 cancer patients (1.01%), and the number of occurrences was 126 up to 1980 (55). The main side effects consisted of symptoms of gastrointestinal upset such as diarrhea and nausea.

**Summary**

PSK exhibits preventive activity against carcinogenesis in many chemical carcinogen-induced cancers, in radiation-induced lymphoma, and in spontaneously developed mammary tumor models. The principal mechanism may be an antiteratogenic effect attributed to radical trapping, preventive effects against chromosome injury, and immunomodulatory effects attributed to the modulation of cytokine production and effector cell function.

PSK has been used clinically as an anticancer agent in Japan. This agent is considered to be a candidate for use as a chemopreventive agent because it has few side effects and may possibly be given orally for long periods. Recently, cancer chemoprevention has been in the spotlight in Europe and the United States. Among the immunomodulators that have been used so far (56–58), PSK may have considerable preventive effects. In the future, further development of PSK as an immunomodulator will require more detailed analysis of its mechanism of action on the immune system.

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