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Significant prolongation of disease-free period gained by oral polysaccharide K (PSK) administration after curative surgical operation of colorectal cancer

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Summary. To examine the clinical efficacy and the mechanism of action of polysaccharide K (PSK), a protein-bound polysaccharide extracted from a Basidiomycetes fungus, a randomized double-blind trial was performed by administering PSK to 56 patients and a placebo to another group of 55 patients after surgical operations on their colorectal cancers. The rate of patients in remission (or disease-free) was significantly higher in the PSK group than in the placebo group; the difference between both groups was statistically significant at $P < 0.05$ by the log-rank test. The survival rate of patients was also significantly ($P < 0.05$) higher in the PSK group than in the control group. The most significant laboratory finding was that polymorphonuclear leukocytes from PSK-treated patients showed remarkable enhancement in their activities, such as random and/or chemotactic locomotion, and phagocytic activity, when compared with those in the control group. In conclusion, PSK was useful as a maintenance therapy for patients after their curative surgical operations for colorectal cancer. The beneficial effects were probably due to the activation of leukocyte functions as one of the many biological-response-modifying (activities induced by PSK).

Introduction

During the last decade or so, immunotherapy as one of the multidisciplinary treatments for advanced cancer has gained momentum in its rapid progress through the development of immunostimulators, which potentiate host defense mechanisms [17, 18, 21, 22, 33]. Among the many immunostimulators developed thus far, OK-432 (a streptococcal preparation) [8, 13, 28, 34, 36, 39] and polysaccharide K (PSK, a plant-derived polysaccharide) [5, 14, 25, 32], have been used most often in Japan. PSK especially is an interesting drug because its oral administration induces the activation of the host immune system to show anti-tumor activity [1, 10, 27, 41].

For a long time in Japan it has been believed beneficial, though without solid evidence, that cancer patients orally take the boiled extract of polyporaceae species. Technical and theoretical progress in modern medicine has shed light on the old approach for this incurable illness. Thus, a partially purified protein-bound polysaccharide extracted from a fungus called *Coriolus versicolor* (strain 101) of Basidiomycetes has been shown to have immunomodulatory activities, and its clinical usefulness has been established in various clinical trials in the early 1970s, resulting in the approval of the Japanese Health and Welfare Department for its usage in the treatment of certain types of cancer.

There have been a number of reports that show the clinical usefulness of PSK when used in combination with other chemotherapeutic agents for treating patients with cancer in various organs such as the stomach [5, 14, 20, 24, 25], uterus [30] or lung [4]. For example, Ito et al. [5] have reported that a group of gastric cancer patients at stage III treated with mitomycin C and PSK showed a longer survival time than those treated with mitomycin C alone. However, there have been few reports available that study the clinical effect of PSK alone on any type of cancer, especially colorectal cancer, as will be described in this paper. Although PSK has been shown to have various immunostimulating activities in vivo as well as in vitro, the exact mechanism of its action has not been well understood. Therefore, an attempt was also made to analyze the underlying mechanisms of the anti-cancer activity of PSK in this study.

Materials and methods

PSK. A boiled aqueous extract of the cultured mycelia of *Coriolus vesiocolor*, a Basidiomycetes fungus (Fig. 1), was precipitated by ammonium sulfate and the desalted powder was designated as PSK (protein-bound polysaccharide Kreha, (reviewed in [35]). This compound con-

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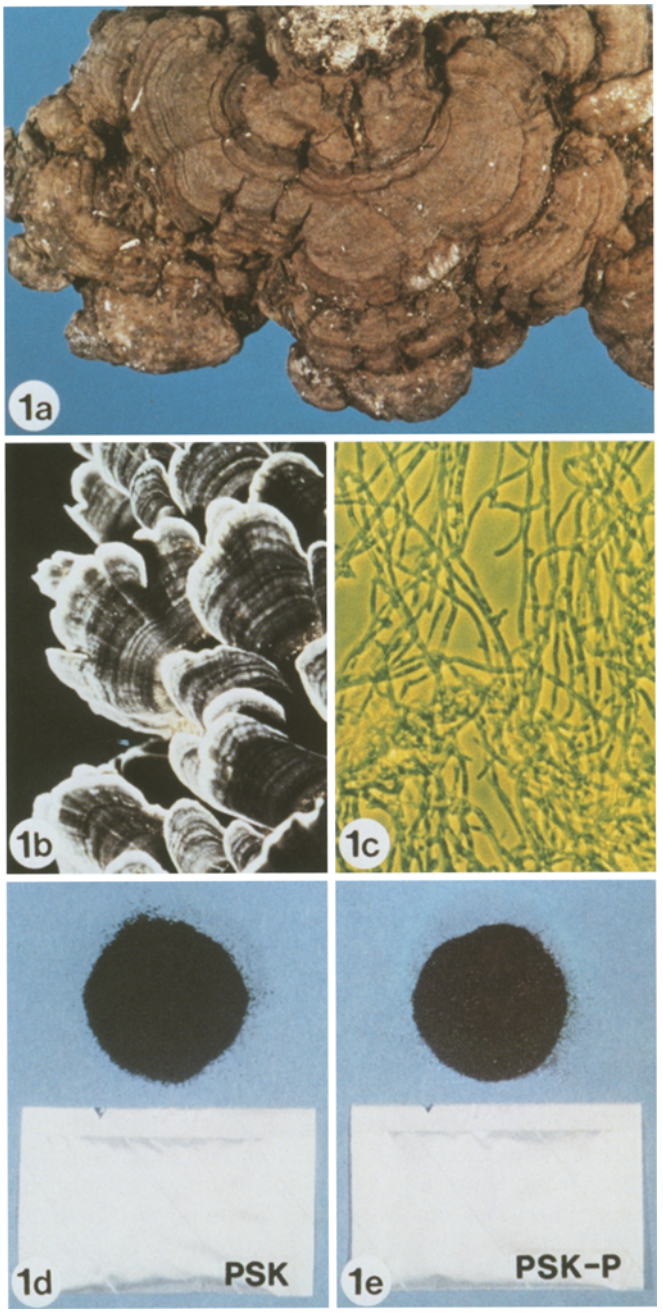

Fig. 1 a–e. Preparations of polysaccharide K (*PSK*) and PSK placebo (*PSK-P*). **a** The wild-growing polyporasceae, which has been used as a medicine for various illnesses including malignancy in China as well as in Japan. **b** A Basidiomycetes, *Coriolus versicolor* (strain 101) with its cultured spores (**c**). **d, e** PSK as a partially purified preparation from the boiled aqueous extract of the Basidiomycetes and the placebo (PSK-P) as a similar brownish powder

tains about 15% protein and its average relative molecular mass is approximately 100000. The sugar portion consists of five kinds of sugar, mainly glucose, and a major protein has a straight-chain structure with (β-1-4)-glucan branching at the 6 or 3 position. The protein consists of 19 amino acids, mainly aspartic acid, glutamic acid, and leucine. Because Sephadex G-100 gel filtration shows multiple peaks, naturally PSK is not represented as a single entity rather as a mixture of substances. Since PSK is a brown tasteless powder easily soluble in water, the placebo in the present trial was also made as a brown powder (Fig. 1 d and Fig. 1 e). Each batch of PSK is produced in a modern pharmaceutical factory under a highly quality-controlled system. The reproducibility of different batches is always assured by an assay in which the antitumor effect of a tested batch on experimental tumors in vivo (sarcoma 180) in ICR mice is equivalent to that of an internal standard.

Patients. The anti-cancer effect of PSK was evaluated in patients with advanced colorectal cancer in a randomized double-blind trial. The placebo for PSK (PSK-P) was a brown powder resembling PSK, composed of mannitol and caramel, 79% and 21% respectively. Either PSK or a placebo was randomly administered to a total of 120 patients. A total of 61 patients were entered in the PSK group: 5 died of causes unrelated to cancer (myocardial infarction, diabetes, hypertension, traffic accident, and cerebral infarction), 2 went to other hospitals to be treated with anti-cancer chemotherapy and 5 took PSK only for the first 2 weeks and never came back to the clinic; they were, therefore dropped from the final analysis, leaving 56 patients for evaluation. Among the 59 patients entered in the PSK-P group, 6 patients died of causes unrelated to cancer (cerebral hemorrhage, myocardial infarction, traffic accident, cerebral infarction, and acute heart failure) and 4 patients took PSK-P only for the first 2 weeks and never came back to hospital again. Thus, 55 patients were evaluated in the placebo group. As shown in Table 1, these two groups of patients were almost equivalent in terms of age, surgical operative methods employed, histological diagnosis, and cancer stages, which were established by Japanese Research Society for Cancer of the Colon and Rectum [7]. Stages III and IV of the macroscopical classification of colorectal cancer corresponded to Dukes C (Dukes' classification). The microscopical cancer stage was determined by a single pathologist. Other factors, such as choice of anesthetics, duration of anesthesia and operation, number and amount of blood transfusions, etc. were not significantly different between both groups.

Administration schedule of PSK and placebo. The first administration of PSK or placebo (PSK-P) started 10–15 days after surgical operations. A dose of 3 g was taken orally daily until 2 months after surgery, then 2 g daily until 24 months and 1 g daily thereafter. When distant metastasis or recurrence was detected in any patient during the follow-up period after surgery, the patient was immediately removed from this oral drug administration protocol and was put under a new treatment protocol including reoperation and immunochemotherapy, which was determined to be the best for that patient at that time. This protocol was adapted from a similar one employed in our previous trial of PSK on gastric cancer [11].

Skin tests. Before and 2 months after the surgery, skin reactivity was tested with various antigens and mitogens such as phytohemagglutinin protein (PHA-P), *p*-phenylenediamine (PPD), dinitrochlorobenzene (DNCB) and keyhole limpet hemocyanin (KLH).

Circulating T lymphocytes and responsiveness to PHA. T and B lymphocytes in peripheral blood were counted using a modification of Tachibana and Ishikawa's method [31]. The normal ranges of T and B lymphocytes in 50 healthy volunteers were $64.5 \pm 9.8\%$ and $35.1 \pm 8.9\%$ respectively. Blood mononuclear cells were stimulated with PHA-P and its stimulation index was determined as described previously [33].

Serum sampling. Approximately 10 ml blood was obtained aseptically by venipuncture. Blood was left to clot at room temperature for 1 h and serum was separated by centrifugation at 800 *g* for 10 min and stored at –70° C until use.

Table 1. Patients with advanced colorectal cancer entered in the trial[a]

Parameter	PSK group	PSK-P group
Number of patients	56	55
Age (years)		
Range	34–86	34–83
Mean	59.3	58.4
Surgical methods (no. cases)		
Right colectomy	13	13
Transverse colectomy	3	4
Descending colectomy	5	6
Sigmoid colectomy	15	13
Abodomino-perineal resection	20	19
Histological diagnosis		
Well differentiated adenocarcinoma	37	39
Moderately differentiated adenocarcinoma	8	7
Poorly differentiated adenocarcinoma	3	3
Mucinous carcinoma	2	1
Signet-ring-cell carcinoma	2	1
Squamous-cell carcinoma	1	2
Others	3	2
Macroscopical stages		
Stage III (Dukes C)		
Ho Po N_1 S_3 M_0	37	38
Stage IV (Dukes C)		
Ho Po N_2 S_3 M_0	13	13
Ho Po N_3 S_3 M_0	6	4
Pathological stages		
Stage III	36	34
Stage IV	20	21

PSK, polysaccharide K; PSK-P, placebo

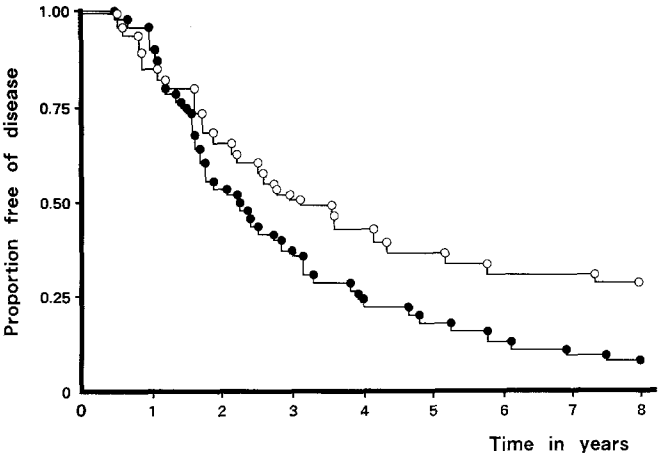

Fig. 2. Effects of oral administration of PSK on the disease-free intervals in colorectal cancer patients after curative surgery. More patients in the PSK-treated group were disease-free than in the control group (the PSK-P-treated). The difference between both groups is statistically significant at $P < 0.05$. ○–○ PSK group (56 cases); ●–● PSK-P group (55 cases)

Serum immunoglobulin. Immunoglobulin levels (IgG, IgA, IgM) were measured using Partigen plates purchased from (Behring Werke Ltd, Marburg). The level of IgE was assayed by a radioimmunosorbent by use of the Phadebas IgE test (Pharmacia Labs., Piscataway, NJ).

Serum complement assays. Total complement (C) activity was measured in 50% hemolytic units (CH_{50}) according to Mayer [19] and immune adherence hemagglutination (IA_{50}), using the method of Nishioka and Linscott [26]. The serum concentration of C1q and C5 was determined using the single radial immunodiffusion method [16]. The amount of C4 and C3 was measured using a Partigen plate (Behring Institute, Marburg).

Polymorphonuclear leukocyte (PMN) preparation. PMN from either patients or healthy volunteers were separated by dextran sedimentation. Briefly, after the mononuclear cells had been removed by gradient centrifugation on the Ficoll-Conray solution, the PMN-rich cell suspension was mixed with a quarter of its volume of dextran (Wako-Junyaku, Tokyo; M_r 200000–300000) in 0.9% saline and the red cells were allowed to sediment for 60 min at room temperature. The PMN-rich buffy coat layer was centrifuged at 350 *g* for 5 min. After the supernatant had been aspirated and discarded, the contaminated red cells were lysed by treatment with 0.83% ammonium chloride in TRIS-HCl buffer for 10 min. The cells obtained were washed twice in phosphate-buffered saline and resuspended in tissue-culture medium. RPMI-1640 medium containing 100 U penicillin and 100 µg streptomycin was used for chemotaxis assays. Medium 199 without phenol red was used for chemiluminescence tests. The cell preparation usually contained 97%–98% granulocytes and 2%–3% mononuclear cells [3].

Chemotaxis assay. Chemotaxis assays were performed with the Millipore filter (pore size 3 µm) method in modified Boyden double chambers as described previously [37]. Assays were carried out in triplicate. The upper compartment of each chamber contained 5×10^5 PMN in 0.2 ml RPMI-1640 medium supplemented with 10% heat-inactivated human AB serum. The lower compartment of each chamber contained 0.2 ml RPMI-1640 medium supplemented with 10% bacterial factor. Bacterial factor was prepared by the filtration of culture supernatant of *Escherichia coli* [38]. The chambers were incubated for 90 min at 37° C in a 5% CO_2 humidified atmosphere; the filters were fixed in ethanol and stained with Mayer's hematoxylin. Five fields were selected at random under high-power light microscopy (200×) to count the number of migrating cells, by which the chemotactic activity was expressed.

Chemiluminescence. Chemiluminescence was measured according to the method of Easmon et al. [2]. Briefly, the reaction mixture consisted of 0.5 ml cell suspension [1×10^6/ml) and 0.4 ml medium 199 containing 8 µM luminol in a 3-ml polystyrene vial. This mixture was placed in the light-proof chamber of a luminometer 1250 (LKB, Wallac). A suspension (0.1 ml) of opsonized zymosan (Sigma, St. Louis) was added and the resulting output (mV) on a chart recorder (LKB, Bromma 2210) was continuously recorded.

Statistical analysis. Statistical analysis was performed using Student's *t*-test for paired and unpaired values or the χ^2-test for unpaired values as mentioned in the text and tables. Both the disease-free and the survival rate were calculated by the Kaplan-Meier method and the equalities of their distributions for the patient groups were tested by the log-rank test. Computations were carried out using the statistical package, BMDP IL, on an IBM system 4381 computer.

Results

PSK prolongs the disease-free interval and survival time after curative surgery in colorectal cancer patients

Over the last 13 years, a total of 111 patients have been analyzed in this double-blind randomized trial: 56 patients treated with PSK alone and 55 with placebo alone after respective curative surgical operations on colorectal cancer. As described in Materials and methods, both groups of patients were almost equivalent in terms of disease status as well as in other general conditions, as shown in Table 1. To determine the disease-free interval, each patient had to visit our clinic at least once every 1–3

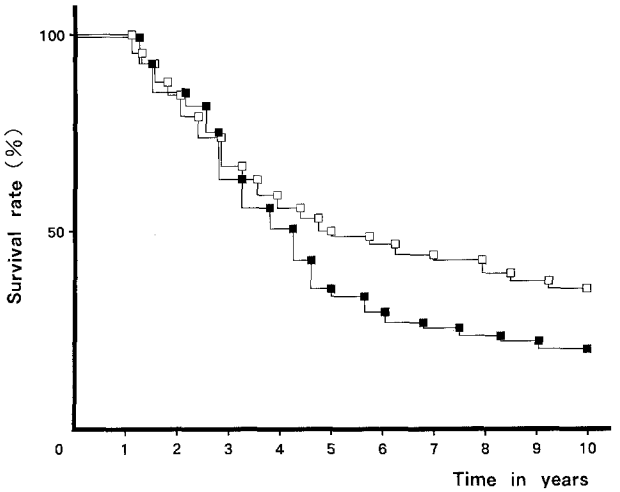

Fig. 3. Effects of oral administration of PSK on the survival rate of colorectal cancer patients after curative surgery. More patients in the PSK-treated group survived than in the control group. The difference was statistically significant at $P < 0.05$. □ – □ PSK group (56 cases); ■ – ■ PSK-P group (55 cases)

Table 2. Laboratory findings in patients with advanced colorectal cancer before and after the treatments

Parameter	PSK	PSK-P
Number of patients	56	55
Serum protein (g/100 ml)		
Before[a]	6.59 ± 0.26	6.48 ± 0.29
After[a]	7.18 ± 1.36	7.09 ± 1.35
Serum albumin (g/100 ml)		
Before	3.82 ± 0.34	3.86 ± 0.42
After	3.96 ± 0.23	3.85 ± 0.40
T cells		
Before	49.7 ± 11.4	49.0 ± 12.6
After	56.9 ± 10.8	53.4 ± 12.9
PHA stimulation index[b]		
Before	44.8 ± 17.8	44.6 ± 15.9
After	59.3 ± 20.4	55.2 ± 12.6

[a] Means before the curative surgery and 2 months after the surgery
[b] PHA, phytohemagglutinin

Table 3. Effect of PSK and PSK-P on skin tests of the patients with advanced colorectal cancer

Group	Skin tests[a]							
	Before operation				2 months after operation			
	PHA-P	PPD	DNCB	KLH	PHA-P	PPD	DNCB	KLH
PSK ($n = 56$)	20	25	23	27	40[b]	42[b]	39	38
PSK-P ($n = 55$)	22	26	24	26	29	33	38	37

[a] The number of patients with positive skin reaction of 49 patients tested in each group. PHA-P, phytohemagglutinin protein; PPD, *p*-phenylenediamine; DNCB, dinitrochlorobenzene; KLH, keyhole limpet hemocyanin
[b] Statistically significant at $P < 0.05$

months. At every visit, in addition to general physical and laboratory examinations, various tests were performed to examine immunological parameters and tumor markers. If there was the slightest sign of abnormality, an extensive search for possible metastasis or recurrence of tumor at the primary lesion was made by echocardiography, computed tomography and/or scintigrams. When metastasis or recurrence was discovered by these examinations, the patient was removed from this trial and immediately underwent a treatment protocol that was considered the best for the patient at that time. The disease-free interval was thus determined as the period between the time of curative surgical operation and first detection of recurrence.

As shown in Fig. 2, oral administration of PSK significantly prolonged the disease-free interval when compared with that of patients given only a placebo (PSK-P). Thus, the difference between the patient groups was statistically significant at $P < 0.05$. Figure 3 shows the survival curve for both the groups. The difference in survival rate between the PSK group and the PSK-P group was statistically significant at $P < 0.05$.

General laboratory findings

As shown in Table 2, all of the parameters: total serum protein, serum albumin, number of circulating T cells, and PHA stimulation index (blastoid transformation), increased when the values before surgery were compared with those 2 months after surgery in both PSK-treated and PSK-P-treated groups. Although all of these values were slightly higher in the PSK-treated group than in the controls 2 months after the treatments, they were statistically not significant.

Skin reactivity

Positive delayed-type skin reactions to PHA-P, PPD, DNCB, and KLH were observed in 20/56 (36%), 25/56 (45%), 23/56 (41%) and 27/56 (48%) of the PSK-treated patients respectively before surgery. The average reactivity to the three antigens was 44.7%, which was not significantly different from the reactivity (46.1%) in the control group before surgery. Two months after the start of oral administration of either PSK or PSK-P, all of these skin reactivities increased significantly, the average reactivities being 70.8% for the PSK group and 65.5% for the PSK-P group respectively. The increase in skin reactivities to PHA-P and PPD were significantly higher in the PSK-treated group than in the control group, while those to DNCB and KLH were not different between both groups after treatments. These data are all summarized in Table 3.

Serum complement

Immunochemical assays of the total serum complement showed that CH_{50} and IA_{50} values 2 months after the treatments were not significantly different from those before the treatments, in either the PSK-treated or the PSK-P-treated groups. Furthermore, complement components such as C1q and C5 were not significantly different between both groups, though the C4 and C3 values did in-

Table 4. Levels of serum complement and its components in patients with colorectal cancer treated with PSK or PSK-P

Complement activity[a]	PSK group ($n = 40$) before/after operation[b]	PSK-P group ($n = 40$) before/after operation
CH_{50} (U/ml)	35.6 ± 13.2/40.3 ± 7.2	36.4 ± 12.5/37.8 ± 7.3
IA_{50} (U/ml)	2400 ± 1200/2600 ± 900	2300 ± 1100/2400 ± 1000
Clq (U/ml)	0.16 ± 0.13/0.17 ± 0.12	0.17 ± 0.14/0.18 ± 0.12
C4 (mg/ml)	0.535 ± 0.19/0.638 ± 0.25	0.543 ± 0.17/0.540 ± 0.29
C3 (mg/ml)	1.332 ± 0.52/1.63 ± 0.36	1.357 ± 0.65/1.405 ± 0.43
C5 (mg/ml)	0.111 ± 0.014/0.124 ± 0.016	0.093 ± 0.09/0.104 ± 0.62

[a] CH_{50}, 50% hemolytic activity; IA_{50}, immune adherence hemagglutination

[b] Before surgical operation/2 months after the operation

Table 5. In vitro motility and chemiluminescence of polymorphonuclear leukocytes (PMN) from the patients with advanced colorectal cancer, before or after the treatments

Group	Chemotaxis[a]	Random locomotion[b] (cm^2)	Chemiluminescence[c] (mV)
PSK ($n = 56$)			
Before	52.3 ± 21.5	1.99 ± 1.15	37.5 ± 1.4
After[d]	85.3 ± 16.9[e]	3.76 ± 1.26[e]	59.8 ± 2.2[e]
PSK-P ($n = 55$)			
Before	51.8 ± 22.4	1.77 ± 1.18	38.5 ± 1.9
After	57.9 ± 19.2	2.34 ± 1.03	43.8 ± 1.8
Healthy volunteers (n=50)	102.5 ± 4.1	2.89 ± 1.23	49.3 ± 2.5

[a] The values represent the total number of migrated cells in five high-power fields ± SD

[b] The values represent the mean locomotion area (cm^2) of neutrophils in duplicate

[c] The results were expressed as the mean ± SE of three experiments

[d] Before surgical operation and 2 months after the operation

[e] Statistically significant at $P < 0.05$

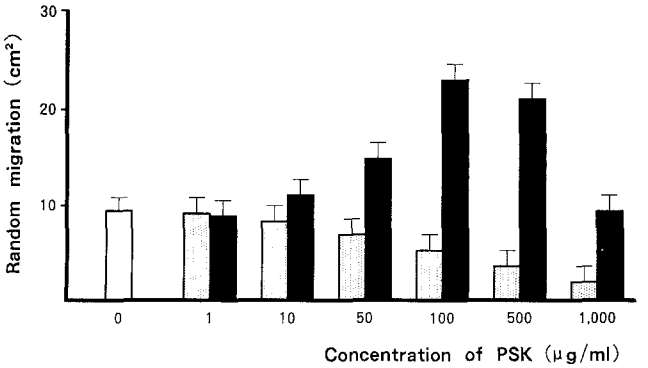

Fig. 4. Effect of PSK treatment on random migration of polymorphonuclear leukocytes (PMN) in vitro. Although PSK was inhibitory to migration, human serum treated with PSK generated a migration-enhancing factor (see text) □ PSK only; ■ PSK with serum

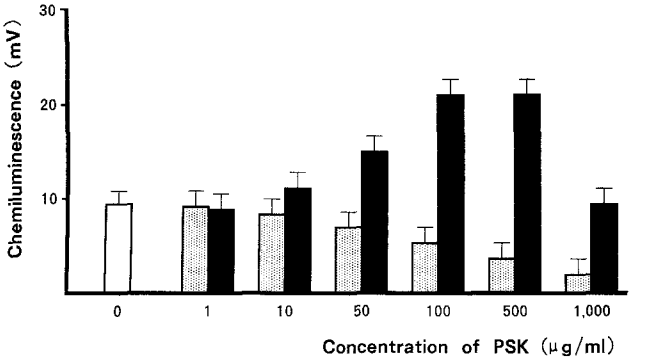

Fig. 5. Effect of PSK on zymosan-induced chemiluminescence of PMN. In a similar fashion to its effect on the migration of PMN, PSK could generate from serum an enhancing factor for phagocytosis (see text). □ PSK only; ■ PSK with serum

crease significantly more in the PSK group than in the controls after the treatments. These data are summarized in Table 4. Serum immunoglobulin levels, such as those of IgG, IgA, IgM, and IgE, did not change significantly after PSK treatment.

Leukocyte motility and chemiluminescence

The chemotactic responsiveness of polymorphonuclear cells (PMN) to a bacterial chemotactic factor in the PSK-treated group recovered from 52.4 ± 22.5 to 84.3 ± 16.8, though the values were still lower than those in healthy volunteers (Table 5). In contrast, no such improvement was observed in the PSK-P group. Furthermore, the in vitro random mobility (mean locomotion area) of PMN in the PSK-treated group increased significantly from 1.98 ± 1.14 cm^2 to 3.15 ± 1.28 cm^2, which was even higher than in the healthy controls. Although not statistically significant, there was also some increment of the values in the control PSK-P group, probably reflecting the improved general physical conditions of patients after surgery. These results may indicate that PSK has some pharmacological activity that conspicuously stimulates the motility of PMN.

Furthermore, the phagocytic activity of PMN, measured by chemiluminescence as described in Materials and methods, increased significantly in the patients treated with PSK from 37.6 ± 1.4 mV to 59.8 ± 2.1 mV. No significant change was observed in the control group.

Direct effects of PSK on the in vitro motility of PMN

In order to analyze the mechanism by which oral administration of PSK could increase the mobility of PMN, we examined a possible direct effect of PSK on the in vitro migration of PMN obtained from normal volunteers. Because the serum level of PSK in the treated patients may be approximately 50–100 μg/ml when administered orally, 1×10^7 cells/ml of normal PMN were incubated with PSK at concentrations between 1 μg/ml and 1000 μg/ml at 37° C for 30 min. After washing with medium, the random mobility of PMN was assayed. As shown in Fig. 4, the cellular motility decreased significantly in a dose-dependent man-

Table 6. Effects of heating, dialysis and anti-(complement C5) serum on a serum factor to enhance the zymosan-induced chemiluminescence

Material	Chemiluminescence (mV)				
	Treated			C5-rich fraction from Sephadex G-200	
	with PSK (50 μg/ml)	Heating at 56° C for 30 min	Dialysis	Untreated	Anti--C5 serum
Serum	67.3 ± 2.5	69.2 ± 3.4	67.4 ± 2.9	45.5 ± 1.6	34.3 ± 1.2
PBS	26.4 ± 2.1	28.5 ± 2.9	N. T.	28.4 ± 2.1	N. T.

Table 7. Side-effects of PSK administration

Side-effect	Frequency in patients (%)	
	PSK ($n = 56$)	PSK-P ($n = 55$)
Pigmentation of nails	8 (14.2)[a]	0 (0)
Cough on administration	7 (12.5)[a]	3 (5.4)
Diarrhea	3 (6.1)	3 (5.4)
Constipation	4 (7.1)	1 (2.0)
Nausea	2 (4.1)	1 (2.0)
Vomiting	2 (4.1)	1 (2.0)
Appetite loss	3 (6.1)	2 (4.1)
Fever	0	0
Leukopenia	0	0

[a] Statistically significant ($P = 0.001$)

ner when incubated with PSK at a concentration higher than 50 μg/ml. Interestingly, however, the random movement of the cells increased significantly when they were incubated in the serum from normal individuals that had been pretreated in vitro with PSK. This increase indicates the generation of a serum factor to stimulate leukocyte locomotion when serum was treated in vitro with PSK. As shown in Fig. 4. there was an optimal concentration for PSK to generate such a factor, since 100 μg/ml PSK induced the highest activity while neither 10 μg/ml nor 1000 μg/ml showed the activity.

Effects of PSK on leukocyte chemiluminescence

A sample of 1×10^7 cells/ml PMN was incubated with various concentrations of PSK at 37° C for 30 min in the manner described above. Zymosan-induced chemiluminescence of these cells was suppressed significantly in a dose-dependent manner when the cells were pre-treated with PSK at a concentration higher than 10 μg/ml, as shown in Fig. 5. As in the case of PMN motility, the serum treated with PSK contained a factor to activate the zymosan-induced chemiluminescence. The generation of such a factor from serum occurred optimally at 50 μg/ml PSK, while 1000 μg/ml PSK had rather inhibitory effects (Fig. 5). The results indicate that the phagocytic ability of PMN, measured by chemiluminescence, was activated by a serum factor generated by the in vitro treatment with an optimal concentration of PSK.

Characterization of a phagocytosis-activating factor in serum

The next series of experiments were performed in order to characterize a chemiluminescence-enhancing (or phagocytosis-activating) factor that was generated from serum incubated with PSK. When serum was incubated with 50 μg/ml PSK, the serum had the ability to enhance the zymosan-induced chemiluminescence of PMN, from 26.4 mV in the untreated group to 67.3 mV in the treated. The activity was unchanged after either heating at 56° C for 30 min or dialyzing against buffer solution as shown in Table 6. These results suggested that the factor might be related to a complement component. The C5-rich fraction was collected by Sephadex G-200 gel filtration of the serum. The fraction was then incubated with 50 μg/ml PSK. As a result, a significant amount of chemiluminescence-enhancing factor (45.5 ± 1.6 mV) was induced. The activity was significantly diminished when the factor was incubated with anti-C5 goat antiserum and removed by centrifugation at 10000 rpm for 30 min. These results strongly suggested that PSK activated the complement system, especially C5, to generate a factor that enhanced the phagocytic activity (the zymosan-induced chemiluminescence) of PMN.

Side-effects of PSK treatments

As shown in Table 7, pigmentation of the nails and coughing during drug administration increased significantly in the PSK group. Although an increased incidence was also observed for diarrhea and constipation, there was no statistically significant difference between the groups. No other significant side-effects were seen. It would certainly help to reduce the incidence of coughing if the drug were formulated in tablets instead of in powder form as it is currently supplied.

Discussion

PSK certainly affects various aspects of the immune response. Thus, it had previously been found that this drug had some kind of inhibitory activity against tumor cell motility while it showed chemotactic activity for macrophages [9]. Furthermore, it was shown that PSK activated the alternative as well as the classical pathway of the complement cascade [12]. As far as clinical efficacy is concerned, it has been reported that a significant prolongation of the disease-free period was observed in the PSK-treated group in a randomized clinical trial on patients surgically operated for stage III gastric cancer [11]. Encouraged by these results, we have performed a randomized trial of PSK on patients with colorectal cancer, as described in this paper, in an attempt to find both the best therapeutic benefit

for the individual patient and the best objective protocol for the evaluation of a drug. The number of patients entered in this trial was not large because of the strict conditions set for the selection of patients. As mentioned in Results, the patients had been requested to visit our outpatient clinic at least once every 1–3 months in order for us to follow their physical conditions in detail. As soon as any sign of recurrence was observed, the patient was removed from the trial and was put immediately under the multidisciplinary therapy that was determined to be the best for that patient. This approach to clinical trials satisfactorily removed our reluctance to carry out a generally accepted randomized trial. More important, we believe that this procedure may be one of the best approaches to evaluate the clinical effects of anti-cancer drugs. As a result, the present study has shown that the group of patients treated with PSK alone enjoyed significant prolongation of the average disease-free period when compared with those treated with a placebo (Fig. 2). A survival period instead of a disease-free period has been utilized as the evaluation method for many randomized trials performed previously. However, the survival period may not be a good indicator for evaluation of a single anti-cancer drug, since it is certainly affected by various other therapies, such as chemotherapy, surgery and irradiation, which are usually employed after a period of randomized trials with a specific drug and therefore obscure the specific effects of the drug in question. In the present study, in any event, we have also examined the survival rate of patients in both the PSK and the PSK-P groups (Fig. 3). The difference was less conspicuous in the survival rate than in the disease-free period, although it showed a statistically significant difference at the same level of $P < 0.05$. From these considerations, we would like to advocate the use of the disease-free period as a good and solid endpoint for this type of clinical trial instead of the use of the survival rate.

It is certainly difficult to deduce the exact mechanism of the beneficial effect of PSK from the present study, because an enormous number of factors may influence the length of the disease-free period in individual patients. Therefore, we have started to analyze its mechanism on the basis of some of our previous findings. First, the skin reactivity to all of the agents tested increased after surgical operation regardless of PSK administration (Table 2). This finding may reflect the general improvement of the physical conditions of patients after curative surgical operations. In addition, this finding probably points to the anamnestic response and/or sensitization because of antigenic exposure at the first skin test before the operation. Although skin responsiveness to PPD and PHA-P was significantly higher in the PSK-treated group than in the placebo controls, this increased incidence may be due to a nonspecific stimulation of immunoreactivity, though the exact mechanism is unknown. A similar phenomenon was previously observed when PSK was administered to older people [11].

The present study also confirmed that random as well as chemotactic mobility of inflammatory cells was significantly enhanced in the PSK-treated group (Table 3). In general, the prognosis of a patient was better when the cellular infiltration around tumor sites was stronger [6, 15, 23, 29, 40]. In addition, the stronger the in vitro motility of inflammatory cells from patients, the stronger their cytotoxicity. The present data taken together with previous observations may well indicate the underlying mechanisms for the prolongation of the disease-free (remission) period when cancer patients are treated with PSK after surgical operations. The present results also show that PSK itself did not enhance the random mobility of neutrophils but that mobility increased to its highest level when 100 μg/ml PSK was added together with serum, indicating the existence of an optimal dose of the drug for this activity. Previously, it has also been observed that an optimal dose of PSK could induce the chemotaxis of macrophages. Phagocytic activity assayed by chemiluminescence was also enhanced by serum treated with PSK. In view of the results shown in Table 5, these activities may be due to C5a generated from serum treated with PSK.

Based upon the available data mentioned above, a plausible explanation for the mechanism of action may be as follows. PSK, an orally administered immunostimulant, is absorbed in the intestine, and enters general circulation where it activates the complement system to generate C5a which in turn, activates leukocytes for the enhanced mobilities and phagocytic activities. These activated leukocytes therefore attack the resurgent cancer cells at primary or metastatic sites of tumors, inducing a delay in the possible recurrence of the disease, recognizable as the prolongation of the disease-free interval in a double-blind randomized trial as described above. Naturally, this conjecture may represent only one aspect of a more complicated mechanism of action of PSK, because it has been shown to have additional immunomodulatory activities. From this reasoning, we believe that the oral administration of PSK is effective as a maintenance therapy for patients with colorectal cancer after therapeutic surgery. We are now investigating the best combination of PSK with other anticancer agents, for the treatment of not only colorectal cancer, but also various other cancers including mammary tumors and thyroid cancers.

In any event, the present study indicates that PSK may be useful in treating colorectal cancer without causing observable adverse reactions.

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