Beneficial effects of protein-bound polysaccharide K plus tegafur/uracil in patients with stage II or III colorectal cancer: Analysis of immunological parameters

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Abstract. Protein-bound polysaccharide K (PSK) increased the 5-year disease-free survival rate and reduced the risk of recurrence in a randomised, controlled study for stage II and III colorectal cancer. In order to elucidate the disease-free survival benefits with PSK and what immunological markers could indicate a PSK responder, serial changes in immunological parameters were monitored in the study. PSK decreased the mean serum immunosuppressive acidic protein (IAP) level, and increased the mean population of natural killer (NK) cells compared with the controls. The 5-year disease-free and overall survival rate for patients with serum IAP values ≤500 μg ml^{-1}, which represents the normal value, were 75.5% (95% CI: 66.8-84.2%; p=0.016) and 85.1% (95% CI: 77.9-92.3%; p=0.032), respectively, in the PSK group compared with 57.5% (95% CI: 43.3-71.6%) and 70.2% (95% CI: 57.1-83.3%) in the control group. In patients with NK cell population ≥8% at 3 months after surgery, PSK conferred a significantly better (p=0.038) 5-year disease-free survival (86.7%; 95% CI: 74.5-98.8%) compared to the control group (60.0%; 95% CI: 29.6-90.4%). In the proportional hazards model, the presence of regional metastases (relative risk, 3.595; 95% CI: 1.518 to 8.518; p=0.004) and omission of PSK treatment (relative risk, 3.099; 95% CI: 1.202 to 7.990; p=0.019) were significant indicators of recurrence. PSK acts as an immunomodulatory activity and biochemical modulator in stage II or III colorectal cancer. Pre-operative serum IAP values ≤500 μg ml^{-1} and an NK cell population ≥8% at 3 months after surgery are possible PSK response predictors.

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Introduction

Colorectal cancer is one of the most common malignant diseases in developed countries. Although 70% of patients with this cancer are curable by surgery, about 50% of those who undergo surgery subsequently develop incurable recurrent disease (1). For that reason, adjuvant chemotherapies and immunotherapies that would eliminate microscopic disease and thereby prevent recurrent disease have been studied. Levamisole was introduced as an adjuvant, based on evidence of immune stimulation (2) and specific biochemical modulation of fluorouracil (5-FU) (3). 5-FU/levamisole became the standard adjuvant treatment for stage III colon cancer (4) and formed the control arm in many studies conducted in the 1990s. Currently, the standard adjuvant treatment for stage III colon cancer is 5-fluorouracil and leucovorin (5-FU/LV) for 6 to 8 months (5). Interestingly, adjuvant-active specific immunotherapy with an autologous tumour cell BCG vaccine in combination with surgical resection was shown to be more beneficial than surgery alone in treating stage II colon cancer in a randomised trial (6).

In an attempt to improve the therapeutic index of 5-FU and its tolerability, we performed a randomised, controlled trial of oral tegafur/uracil (UFT®; Taiho Pharmaceutical Co., Japan) plus protein-bound polysaccharide K (PSK) (Krestin®; Kureha Chemical Industry Co., Japan) for stage II and III colorectal cancer. Tegafur/uracil is an orally administered fluoropyrimidine which contains tegafur and uracil in a 1:4 molar ratio with good absorption in the small intestine. Tegafur is the orally bioavailable pro-drug form of 5-FU, which is gradually converted to fluorouracil in the liver by the cytochrome P-450 enzyme. Uracil enhances the serum concentration of fluorouracil by competitive inhibition of dihydropyrimidine dehydrogenase, the enzyme responsible for fluorouracil catabolism. There is a favourable report of a randomised controlled study that found UFT is useful as an adjuvant chemotherapy against stage I lung adenocarcinoma (7). PSK, which is extracted from the mycelia of *Coriolus versicolor*, has immunomodulatory activities, and documented

anticancer and antimetastatic activity *in vitro* and *in vivo* (8-15).

In a randomised, controlled study of tegafur/uracil and PSK, we have determined that PSK as an adjunct to UFT reduces the rate of cancer recurrence by 43.6% and the rate of mortality by 40.2% in patients with stage II and III colorectal cancer. In cases of pathological stage III cancer, PSK reduces recurrence by 63.4% and mortality by 66.2%. Furthermore, PSK is a prognostic factor in Cox's proportional hazards model (16). Immunomodulatory activities of PSK, however, have not been well documented in human clinical trials, whereas prominent laboratory data and the survival benefits of PSK in human clinical trials are documented in previous studies (17,18) as well as this study.

In order to elucidate the disease-free survival benefits of PSK and which immunological markers could indicate the PSK responder, serial changes in immunological parameters were monitored in this study. We report the serial changes in immunological parameters and survival rates relative to patient immune status in the randomised, controlled trial of oral tegafur/uracil and PSK for stage II and III colorectal cancer in 5 years of follow-up.

Methods

The methods used in this study have been described elsewhere (16). The subjects were enrolled in the study between October 1994 and March 1997. All of the registered patients received bolus injections of 12 and 8 mg/m^2 mitomycin C (MMC®; Kyowa Hakko Inc., Japan) on post-operative days 1 and 2, respectively. The PSK group received oral PSK (3.0 g/day) and UFT (300 mg/day), starting 2 weeks after surgery and continuing for 2 years or until diagnosis of tumour recurrence. The controls received UFT alone.

Immunological parameters. To measure the immunological parameters, 20 ml of heparinized whole blood was collected from each patient before surgery, as well as 2 weeks and 1, 3, 6 and 12 months after surgery. Peripheral blood lymphocyte subsets were measured using two-colour flow cytometry with the following monoclonal antibodies: OKT4 (CD4) and OKT8 (CD8) (Ortho Diagnostics Inc., Raritan, NJ, USA); and Leu 7 (CD57), Leu 11 (CD16), and Leu15 (CD11b) (Becton-Dickinson Inc. and Coulter Inc., USA). $CD8^+CD11b^+$ cells were designated as suppressor T lymphocytes, $CD8^+CD11b^-$ cells as cytotoxic T lymphocytes, and $CD57^+CD16^+$ cells as NK cells. Serum IAP was measured by the single radial immunodiffusion method. The samples were analysed using enzyme-linked immunosorbent assay (ELISA) kits for the human activated form of tumour growth factor-ß$_1$ (TGF-ß$_1$; Genzyme Co. Ltd., Cambridge, MA, USA). The results are expressed as the mean ± standard error (mean ± SEM).

Statistical analyses. Statistical analyses were carried out using Statistical Analysis System software, version 8.2 (SAS; Cary, NC, USA). An intention-to-treat analysis was applied. Serial changes in the immunological parameters were analysed using ANOVA, and background factors were compared using the Chi-square or Mann-Whitney U test.

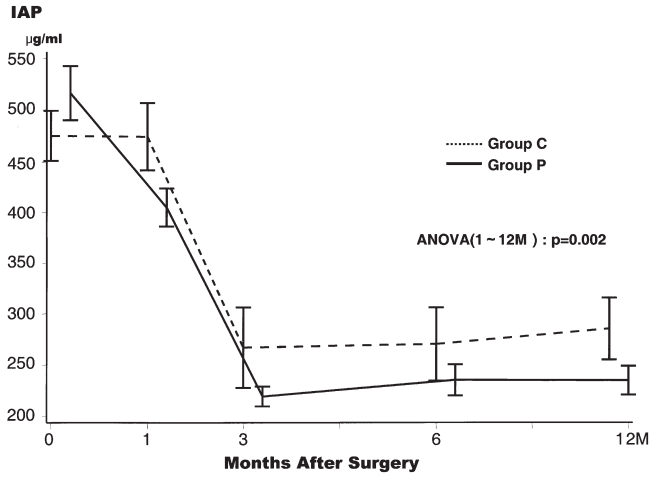

Figure 1. Serial changes in serum IAP values. The mean serum IAP values after surgery in the PSK group were significantly and serially lower than those in the control group ($p<0.01$).

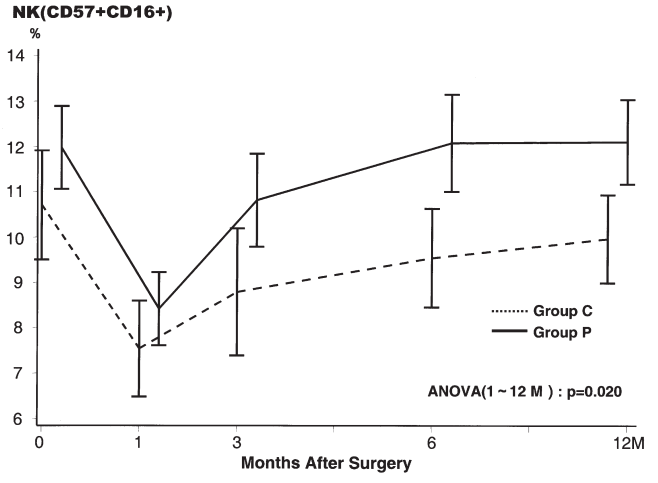

Figure 2. Serial changes in the population of natural killer cells. The serial mean NK cell population in the PSK group was significantly higher than that in the control group after surgery ($p=0.02$).

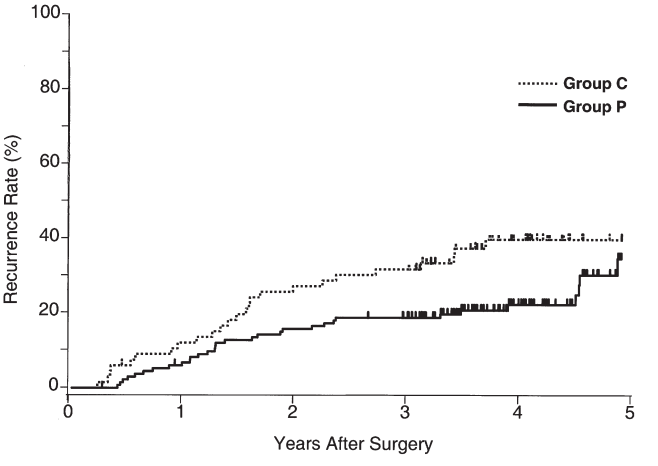

Figure 3. The recurrence rate at 5-year follow-up. A total of 57 patients experienced cancer recurrence, including 25 in the control group and 32 in the PSK group ($p=0.017$).

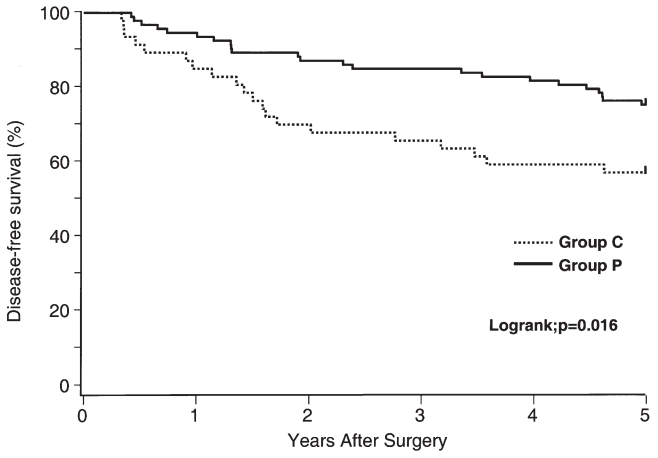

Figure 4. The 5-year disease-free survival rates of patients with serum IAP values ≤500 μg ml^{-1}. The 5-year disease-free survival rate of patients with serum IAP values ≤500 μg ml^{-1} was 75.5% (95% CI: 66.8-84.2%) in the PSK group and 57.5% (95% CI: 43.3-71.6%) in the control group (p=0.016).

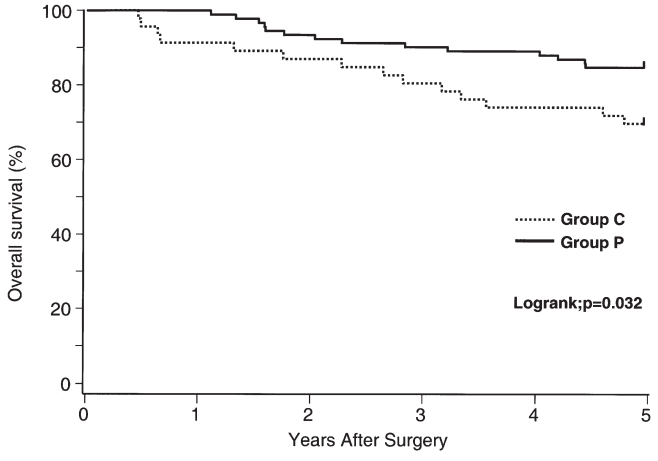

Figure 5. The 5-year overall survival rates of patients with serum IAP values ≤500 μg ml^{-1}. The 5-year overall survival rate for patients with serum IAP values ≤500 μg ml^{-1} was 85.1% (95% CI: 77.9-92.3%) in the PSK group compared with 70.2% (95% CI: 57.1-83.3%) in the control group (p=0.032).

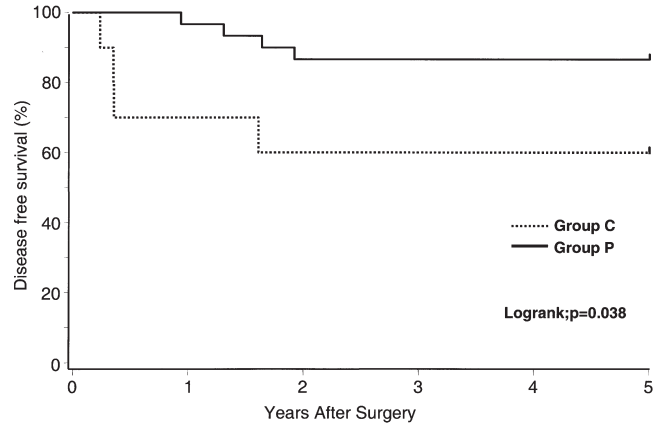

Figure 6. The 5-year disease-free survival rates of patients with NK cell population ≥8% at 3 months after surgery. PSK conferred a significantly better (p=0.038) 5-year disease-free survival rate (86.7%; 95% CI: 74.5-98.8%) compared with the control group (60.0%; 95% CI: 29.6-90.4%).

Disease-free and overall survival curves were generated by the Kaplan-Meier method, and the log-rank test was used to compare the curves. The relative risk and 95% confidence intervals (CIs) were calculated, to measure the degree of association between treatment groups. Proportional hazards regression analysis was applied to estimate the percentage of reduction in the risk of recurrence. Multivariate analyses using Cox's proportional hazards model were applied to estimate the simultaneous effects of prognostic factors on survival. Variables were retained in the model if the associated two-tailed p-values were ≤0.05.

Results

Patient characteristics and treatment compliance. We enrolled 207 patients and randomly assigned them to either the PSK group (139 patients) or control group (68 patients). Two patients (1.4%) in the PSK group were ineligible: one had macroscopic, non-curative tumours, and the other had multiple cancers. Consequently, 205 patients were analysed (137 PSK and 68 controls). All of the patients were closely monitored throughout follow-up, and no patients were lost during that time.

Changes in immunological parameters. The mean serum IAP values at 1, 3, 6, and 12 months after surgery were decreased, compared with the pre-operative values in both groups. Furthermore, the mean serum IAP values after surgery in the PSK group were significantly and serially lower ($p<0.01$) than those in the control group (Fig. 1). The mean serum TGF-ß$_1$ values at 1, 3, 6, and 12 months after surgery were similar to the pre-operative values, and no differences were observed between the two groups.

There were no significant serial changes in mean numbers of peripheral blood lymphocytes in the two groups. The mean population of $CD57^+CD16^+$ NK cells in both groups decreased significantly at 1 and 2 months after surgery compared with the pre-operative values. Furthermore, the serial mean NK cell population in the PSK group was significantly higher (p=0.02) than those in the control group after surgery (Fig. 2). There was a decrease in the mean population of $CD8^+CD11b^+$ cells suppressor T cells in the PSK group 6 months after surgery compared with the corresponding pre-operative values. The mean population of $CD8^+CD11b^-$ cytotoxic T cells in the PSK group at 1, 3, 6, and 12 months post-surgery were significantly higher than the pre-operative values, while there were no significant changes in the control group with respect to this cell population.

Survival rates. A total of 57 patients experienced cancer recurrence, including 25 patients in the control group, and 32 in the PSK group (p=0.017) (Fig. 3).

The patients were stratified with pre-operative serum IAP values of 500 μg ml^{-1}, which represents the normal value. Among the patients with serum IAP values ≤500 μg ml^{-1}, there were no striking differences between the groups in terms of patient factors and histopathological characteristics, such as depth of invasion, grade of nodal metastasis, pTNM stage classification, curability, histological differentiation, and lymphatic and vessel permeation, with the exception of

Table I. Characteristics of patients with pre-operative mean serum IAP levels ≤500 μg ml^{-1}.

Characteristic	Category	Control group n=47	PSK group n=94	p-value	Test
Age (years)	25-49	8 (17.0%)	6 (6.4%)	0.017	U
	50-59	13 (27.7%)	22 (23.4%)		
	60-69	20 (42.6%)	40 (42.6%)		
	70-75	6 (12.8%)	26 (27.7%)		
Sex	Male	27 (57.4%)	46 (48.9%)	0.439	χ^2
	Female	20 (42.6%)	48 (51.1%)		
Location	Colon	21 (44.7%)	56 (59.6%)	0.135	χ^2
	Rectum	26 (55.3%)	38 (40.4%)		
Tumour size (cm)	≤3.9	12 (25.5%)	20 (21.3%)	0.516	U
	4.0-5.9	22 (46.8%)	44 (46.8%)		
	≥6.0	13 (27.7%)	30 (31.9%)		
Histopathological grade	G1	26 (55.3%)	34 (36.2%)	0.082	χ^2
	G2	21 (44.7%)	59 (62.8%)		
	G3	0 (0.0%)	1 (1.1%)		
Primary tumour	T1	2 (4.3%)	4 (4.3%)	0.630	U
	T2	6 (12.8%)	8 (8.5%)		
	T3	34 (72.3%)	80 (85.1%)		
	T4	5 (10.6%)	2 (2.1%)		
Regional lymph nodes	N0	25 (53.2%)	55 (58.5%)	0.265	U
	N1	9 (19.1%)	26 (27.7%)		
	N2	13 (27.7%)	13 (13.8%)		
TNM stage	I	5 (10.6%)	11 (11.7%)	0.573	U
	II	20 (42.6%)	44 (46.8%)		
	III	22(46.8%)	39 (41.5%)		
Lymphatic invasion	L0	19 (40.4%)	28 (29.8%)	0.283	χ^2
	L1	28 (59.6%)	66 (70.2%)		
Venous invasion	V0	25 (53.2%)	58 (61.7%)	0.350	U
	V1	21 (44.7%)	34 (36.2%)		
	V2	1 (2.1%)	2 (2.1%)		
Residual tumour	R0	47 (100%)	93 (98.9%)	1.000	χ^2
	R1	0 (0.0%)	1 (1.1%)		
	R2	0 (0.0%)	0 (0.0%)		
IAP (mg/ml)		365.3±11.8	360.6±9.0	0.754	t
Lymphocyte (%)		31.0±2.3	30.0±1.1	0.664	t
TGF-ß		10.6± 3.9	9.0±1.0	0.700	t
Ts ($CD8^+CD11b^+$) cells		0.7±0.2	0.9±0.1	0.576	t
Tc ($CD8^+CD11b^-$) cells		19.2±1.3	19.0±0.8	0.893	t
NK ($CD57^+CD16^+$) cells		10.5±1.4	12.0±0.9	0.375	t
CEA ng/ml		4.3±1.7	4.1±0.9	0.897	t

IAP, immunosuppressive acidic protein; CEA, carcinoembryonic antigen; TGF-ß, transforming growth factor. [a]TNM cancer staging according to AJCC 6th edition.

Table II. Characteristics of patients with NK cell population ≥8% at 3 months post surgery.

Characteristic	Category	Control group n=10	PSK group n=30	p-value	Test
Age (years)	25-49	0 (0.0%)	1 (3.3%)	0.346	U
	50-59	4 (40.0%)	7 (23.3%)		
	60-69	4 (40.0%)	11 (36.7%)		
	70-75	2 (20.0%)	11 (36.7%)		
Sex	Male	7 (70.0%)	17 (56.7%)	0.709	χ^2
	Female	3 (30.0%)	13 (43.3%)		
Location	Colon	3 (30.0%)	22 (73.3%)	0.038	χ^2
	Rectum	7 (70.0%)	8 (26.7%)		
Tumour size (cm)	≤3.9	3 (30.0%)	6 (20.0%)	1.000	U
	4.0-5.9	4 (40.0%)	18 (60.0%)		
	≥6.0	3 (30.0%)	6 (20.0%)		
Primary tumour	T1	0 (0.0%)	1 (3.3%)	0.267	U
	T2	4 (40.0%)	0 (0.0%)		
	T3	4 (40.0%)	29 (96.7%)		
	T4	2 (20.0%)	0 (0.0%)		
Histopathological grade	G1	7 (70.0%)	9 (30.0%)	0.071	χ^2
	G2	3 (30.0%)	18 (60.0%)		
	G3	0 (0.0%)	3 (10.0%)		
Regional lymph nodes	N0	6 (60.0%)	23 (76.7%)	0.188	U
	N1	1 (10.0%)	6 (20.0%)		
	N2	3 (30.0%)	1 (3.3%)		
TNM stage	I	4 (40.0%)	1 (3.3%)	0.531	U
	II	2 (20.0%)	22 (73.3%)		
	III	4 (40.0%)	7 (23.3%)		
Lymphatic invasion	L0	4 (40.0%)	10 (33.3%)	1.000	χ^2
	L1	6 (60.0%)	20 (66.7%)		
Venous invasion	V0	5 (50.0%)	24 (80.0%)	0.152	χ^2
	V1	5 (50.0%)	6 (20.0%)		
	V2	0 (0.0%)	0 (0.0%)		
IAP value	>500 μg/ml	4 (40.0%)	9 (30.0%)	0.846	χ^2
	≤500 μg/ml	6 (60.0%)	21 (70.0%)		
IAP (mg/ml)		495.1±60.7	520.9±62.8	0.823	t
Lymphocyte (%)		30.6±4.2	26.0±1.7	0.242	t
TGF-ß		4.1±0.0	8.8±1.8	-	t
Ts ($CD8^+CD11b^+$) cells		0.5±0.3	0.7±0.2	0.582	t
Tc ($CD8^+CD11b^-$) cells		16.3±1.9	18.1±1.3	0.499	t
NK ($CD57^+CD16^+$) cells		14.3±1.8	13.4±1.3	0.756	t
CEA (ng/ml)		6.7±3.3	2.7±0.8	0.268	t

Table III. Relative risks of recurrence according to proportional hazards regression model.

Characteristic	Category	Hazard ratio	95% CI	p-value
Sex	Male/female	1.320	0.569-3.006	0.518
Age (years)	≥60			
	≤59	1.912	0.793-4.613	0.149
IAP value	>500 μg/ml			
	≤500 μg/ml	2.299	0.856-6.175	0.099
Location	Colon			
	Rectum	0.756	0.320-1.787	0.524
Tumour size (cm)	≤3.9			
	4.0-5.9	0.430	0.155-1.192	0.105
	≥6.0	0.469	0.154-1.427	0.182
Primary tumour	T1+2			
	T3+4	8.432	0.894-79.500	0.063
Histopathological grade	G1			
	G2, G3	2.132	0.880-5.177	0.093
Regional lymph nodes	N0			
	N1, 2, 3	3.595	1.518-8.518	0.004
Ts ($CD8^+CD11b^+$) cells		1.023	0.706-1.418	0.906
Tc ($CD8^+CD11b^-$) cells		1.053	0.995-1.114	0.074
NK ($CD57^+CD16^+$) cells		1.016	0.963-1.072	0.562
CEA		1.021	0.983-1.061	0.285
Group	PSK			
	Control	3.099	1.202-7.990	0.019

the age of the patients. Patient age was higher in the PSK group than in the control group. No marked differences in immunological factors, such as lymphocyte population, TGF-ß1, $CD8^+CD11b^+$ cells, $CD8^+CD11b^-$ cells, and $CD57^+CD16^+$ cells, were observed between the groups (Table I).

The 5-year disease-free survival rate of patients with serum IAP values ≤500 μg ml^{-1} was 75.5% (95% CI: 66.8-84.2%) in the PSK group and 57.5% (95% CI: 43.3-71.6%) in the control group (p=0.016) (Fig. 4). The mean disease-free survival time of the PSK group was 52.4 months (95% CI: 49.1-55.8 months) and that of the control group was 43.1 months (95% CI: 36.6-49.6 months) (p=0.012). In contrast, the disease-free survival rate for patients with serum IAP values >500 μg ml^{-1} was 67.4% (95% CI: 53.4-81.4%) in the PSK group and 61.9% (95% CI: 41.1-82.7%) in the control group (p=0.783). The 5-year overall survival rate for patients with serum IAP values ≤500 μg ml^{-1} was 85.1% (95% CI: 77.9-92.3%) in the PSK group compared with 70.2% (95% CI: 57.1-83.3%) in the control group (p=0.032) (Fig. 5). The mean overall survival time of patients was 55.7 months (95% CI: 53.3-58.1 months) in the PSK group and 50.5 months (95% CI: 45.3-55.6 months) in the control group (p=0.068). PSK conferred no significant overall survival benefits on patients with serum IAP values >500 μg ml^{-1} (PSK vs. control, 74.4% vs. 76.2%; p=0.855).

The population of NK cells was decreased significantly 1 month after surgery, and recovered 3 months later. The cases were stratified into two groups, higher NK cell population (40 patients) and lower NK cell population (29 patients), based on whether the population of NK cells 3 months after surgery was ≥8%. There were no striking differences between the groups in terms of stratification factors, histopathological characteristics and immunological parameters, with the exception of tumour location (Table II). In patients with NK cell population ≥8% at 3 months after surgery, PSK conferred a significantly better (p=0.038) 5-year disease-free survival rate (86.7%; 95% CI: 74.5-98.8%) compared to the control group (60.0%; 95% CI: 29.6-90.4%) (Fig. 6). The mean disease-free survival time of the PSK group was 54.3 months (95% CI: 48.8-59.9 months) vs. 39.1 months (95%

CI: 19.4-58.7 months) in the control group (p=0.121). No striking differences were observed in patients with NK cell population <8% at 3 months after surgery (PSK vs. control, 63.2% vs. 60.0%; p=0.896). PSK prevented cancer recurrence in the patients with NK cell population ≥8% at 3 months after surgery (p=0.04; odds ratio, 0.15; 95% CI: 0.03-0.78), and there were no significant differences between the groups with regard to liver, lung, peritoneal, and lymph node metastases. However, the 5-year overall survival rate was not significant (PSK vs. control, 90.0% vs. 70.0%, p=0.092).

Relative risks of recurrence according to the proportional hazards regression model. According to the proportional hazards regression model, the presence of regional metastases (relative risk, 3.595; 95% CI: 1.518 to 8.518; p=0.004) and the treatment that omitted PSK (relative risk, 3.099; 95% CI: 1.202 to 7.990; p=0.019) were significant indicators of cancer recurrence. Pathological primary tumours (relative risk, 8.432; 95% CI: 0.894-79.500; p=0.063) and $CD8^+CD11b^-$ cytotoxic T cells (relative risk, 1.053; 95% CI: 0.995-1.114; p=0.074) tended to be indicators of recurrence. The pre-operative serum IAP values, carcinoembryonic antigen (CEA) values, and other lymphocyte populations were not significant indicators of recurrence (Table III).

Discussion

PSK serially decreased the serum IAP values and increased the population of NK cells compared to the controls in curatively resected stage II and III cancer. Indeed, PSK had an apparent beneficial effect on the disease-free survival rate in patients with pre-operative serum IAP values ≤500 μg ml^{-1} and patients with NK cell population ≥8% at 3 months after surgery. Thus, the immunological and survival data of the randomised, controlled clinical study support the laboratory data that PSK has immunomodulatory and biochemical modulatory activities, and a role in the restoration of host immunity that has been impaired by the tumour and/or antitumour chemotherapeutic agents (19,20).

Serum IAP and TGF-ß1 are noteworthy immunosuppressive factors. IAP, which is isolated from the ascitic fluids of cancer patients, suppresses both phytohaemagglutinin-induced lymphocyte blast formation and the mixed lymphocyte reaction *in vitro* (21). Decreased serum IAP values may reflect a reversion of the immunosuppressive state caused by the cancer, thereby restoring T-cell responses. Indeed, PSK serially decreased the serum IAP values, which also represents restoration of the immunosuppressive state. PSK improves the impaired anti-tumour $CD4^+$ T-cell response, mainly through the suppression of TGF-ß1 production and restoration of interferon (IFN)-γ production (14). In this study, however, we were unable to confirm that PSK suppresses serum TGF-ß1.

We did not evaluate lymphocyte function in this study. PSK activates NK cells in a manner that is independent of IFN and the interleukin (IL)-2/IL-2 receptor system, and activates lymphokine activated killer (LAK) cells *in vivo* and *in vitro* (9,13-15). Genetic studies have revealed increased expression of key immune cytokines in response to treatment with PSK (8,9,22,23). NK cells are involved in limiting the growth and metastasis of many different tumours (13,24). PSK reduced cancer recurrence, particularly that of lung metastasis (16). In patients with NK cell population ≥8% at 3 months after surgery, PSK conferred a significantly better disease-free survival rate (p=0.04) and prevented cancer recurrence (p=0.04; odds ratio, 0.15; 95% CI: 0.03-0.78) compared to the control group. The disease-free survival benefits and reduced recurrence suggest that the activities of PSK-induced NK cells are more enhanced than those of spontaneously increased NK cells.

PSK also functions as a specific biochemical modulator of antitumour agents, such as mitomycin C, 5-fluorouracil, cyclophosphamide, bleomycin, CPT-11, cisplatin, and docetaxel. PSK causes tumour cell apoptosis and cell differentiation, and suppresses tumour cell invasiveness, either independently or synergistically with other anticancer agents (9,22,25-27). PSK up-regulates the genes for IL-1, IL-6, and IL-8 in peripheral mononuclear cells and the genes for TNF and macrophage chemotactic factors in tumour cells (8,9) and induces apoptosis (28). In addition, PSK induces differentiation genes, and produces leukaemic cell differentiation *in vitro* (11). Furthermore, PSK suppresses tumour cell invasiveness via the down-regulation of several invasion-related factors, which include TGF-ß1, urokinase plasminogen activator, and matrix metalloproteinases (MMP)-2 and -9 (29). In this study of patients with stage III disease, the control group had dramatically lower disease-free and overall survival rates than the PSK group. It seems likely that PSK acts as a biochemical modulator of UFT, and suppresses tumour cell invasiveness.

To predict responses to chemotherapy or immunochemotherapy for individual cancer patients before treatment would ensure a better outcome and improve the quality of life for patients. Serum IAP values, the purified protein derivative (PPD) skin test, the granulocyte/lymphocyte ratio, pre-operative serum CEA values, and pre-operative values of serum sialyic acid and HLA-A2 antigen (30-33) have been reported as good predictors of response to PSK therapy. Serum IAP values above the threshold value of 580 μg ml^{-1}, which was designated using Cox's proportional hazards model, have been reported as a good indicator in gastric cancer patients that received PSK plus 5-FU (30). In contrast, the pre-operative serum IAP value of 500 μg ml^{-1} may be a good predictive factor for PSK responsiveness in patients with stage II or III colorectal cancer. However, in Cox's proportional hazards regression model, the pre-operative serum IAP values, CEA, and respective lymphocyte population were not significant predictors. Therefore, it remains to be determined as to which colorectal cancer patients would derive sufficient benefit from immunochemotherapy.

Further studies on the mechanisms behind anticancer, immunostimulatory, and biological response modifying effects of PSK are warranted. With modern cellular and molecular biology techniques, it is possible to achieve a better understanding of the specific molecular effects of PSK on tumour cells and leukocytes. This information may facilitate the development of novel strategies for the treatment of malignancies, including the use of adjuvant immunotherapies in combination with surgery, chemotherapy, and radiotherapy (18).

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