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Combined therapeutic effects of an immunomodulator, PSK, and chemotherapy with carboquone on rat bladder carcinoma

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Summary. *Responses of bladder cancer in ACI rats to combination therapy with an immunomodulator, PSK, and an alkylating agent, carboquone, are reported. PSK is a protein-bound polysaccharide isolated from Basidiomycetes, and carboquone is the alkylating agent 2,5-bis(1-aziridinyl)-3-(2-hydroxy-1-methanoxyethyl)-6-methyl-p-benzoquinone carbornate, molecular weight 3,214.*

The immunomodulator, PSK, was shown to enhance the effectiveness of the chemotherapeutic agent, carboquone. The therapeutic effect of combination tratment was monitored by measuring growth rates of tumors transplanted SC and by measuring decreases in metastatic spread to lungs in tumor-bearing animals. Effects of PSK on host immunity were monitored by measuring serum levels of immunosuppressive substance.

Introduction

Surgery, radiotherapy, chemotherapy, and immunotherapy have all been used in the management of malignant tumors. Bladder cancer is immunologically the most extensively studied urologic tumor. Various manipulations of immune systems in tumor-bearing hosts have been used to restrict or alter bladder tumor growth. Nonspecific active immunotherapy has received the most attention, because it is felt that the general immune status of the tumor-bearing host is usually depressed and may be further depressed by the use of chemotherapeutic agents. Therefore, many animal studies and some human studies of bladder cancer have used agents such as *Bacillus Calmette-Guérin* (BCG), *C. parvum, Streptomyces* (OK432), levamisole, thymosin, and poly I : C in efforts to enhance host immune systems.

In animal studies BCG has been used extensively, but with mixed results [1, 5, 14, 19, 32]. Differences in responses may well be due to type, route of administration, and dose schedule. In human studies BCG has been used successfully in the treatment of patients with superficial bladder cancer [4, 15, 18, 19]. One study [11] indicates that the streptococcal OK-432 might also be beneficial, but in other studies *C. parvum* [29] and poly I:C [8] were not effective in reducing the recurrence rate of superficial bladder cancer. Interferon has also been used as a preparation for intralesional injection [9] and shows promise, but more studies need to be done.

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The mechanism by which most immunomodulators exert an antitumor effect remains speculative. BCG, for example, elicits a host response, which is mediated by tissue and circulating macrophages. Macrophages may then exert a direct antitumor effect or indirect tumor cell destruction by the recruitment of cytotoxic lymphocytes. BCG may also modulate suppressor cells and induce interferon, which in turn may activate NK cells [3].

Since one of the common side effects of chemotherapy is suppresion of the antitumor immunity of the host, it is important to counteract this suppression to achieve an optimum chemotherapeutic effect. Various immunochemotherapeutic regimens have been reported [10, 16, 20, 24, 33, 34], all designed with the aim of enhancing host antitumor immunity while reducing the immunosuppressive action of the chemotherapeutic agent. PSK has been the immunomodulator in some of these studies [16, 20, 34] and is the agent used in the studies described below.

Materials and methods

PSK is a protein-bound polysaccharide lectin (molecular weight 100,000) isolated from *Basidiomycetes* [2, 26, 27, 35] and obtained from Kureha Chemical Company, Japan. Carboquone (CQ) is an alkylating chemotherapeutic agent (Sankyo Co, Japan). ACI male rats 8–10 weeks old and weighing 200 g were used as hosts for bladder tumor BC-47 [7]. The solid tumor was removed from a host rat, trypsinized into a single cell suspension [36], and inoculated SC into the backs of experimental animals (10 animals/group) on day 0. PSK suspension was administered PO by catheter from day 1 through day 30 at a dose of 1 g/kg. CQ was administered IP on days 1, 4, 8, and 12 at a dose of 0.5 mg/kg. Tumor sizes (cm^3) were measured on days 7, 14, 21, and 28. Groups consisted of control (no drug), PSK alone, CQ alone, and PSK + CQ. The effect of treatment in each group was monitored by noting survival time in days, changes in body weight (weekly) and serum concentration of immunosuppressive substance [30] on day 28, measured by single radial immunodiffusoin. The amount of immunosuppressive substance. Increase in life span for each treatment group was determined by noting the time in days for 60% of animals to die from tumor burden in relation to the untreated control group.

Effects of therapy on lung metastatic growth of BC-47 were also evaluated. Male ACI rats 3 weeks old were divided

into groups of 10 and received no therapy (control), PSK alone, CQ alone, or PSK + CQ. Solid tissue (BC-47) was removed from a stock animal, trypsinized into single cells, and injected IV (2×10^6 cells/animal) on day 0. PSK was administered from day 1 through day 30 at a dose of 1 g/kg PO while CQ was administered IP on days 1 and 7 at 0.5 mg/kg. All groups were terminated on day 30. Effects of treatment on lung metastasis was evaluated by animal survival time in days and extent of lung metastasis in each animal at death or at termination of experiment on day 30.

Results

Effect of therapy on growth rates of tumors and on survival of animals

The slope of exponential growth of BC-47 injected into untreated ACI rats and those treated with PSK alone was 0.47 and 0.36 (cm^3/day), respectively, and this was not a significant difference (unpaired *t*-test, $P \leq 0.05$; Fig. 1). The slope of exponential tumor growth in untreated rats aind in rats treated with CQ alone was 0.47 and 0.14 (cm^3/day), respectively, and this difference was significant (unpaired *t*-test, $P = 0.001$; Fig. 1). When exponential growth rates of tumors in untreated animals and those treated with a combination of PSK and CQ were measured the slopes were 0.47 and 0.06 (cm^3/day7, respectively, a significant difference (unpaired *t*-test, $P = < 0.001$; Fig. 1). The difference in growth rate between tumors in animals treated with CQ alone and CQ + PSK (0.14 and 0.06, respectively) was not significant (unpaired *t*-test, $P = > 0.05$, Fig. 1).

The effect of therapy on animal survival is shown in Fig. 2. Increase in life span over untreated tumor-bearing control animals was 57%, 32%, and 82% for animals receiving CQ alone, PSK alone, or CQ + PSK, respectively. At 60 days after tumor inoculation in animals receiving CQ alone, PSK alone, and CQ + PSK, one, one, and three animals, respectively. were alive. All animals in the untreated group were dead at day 41 after tumor inoculation.

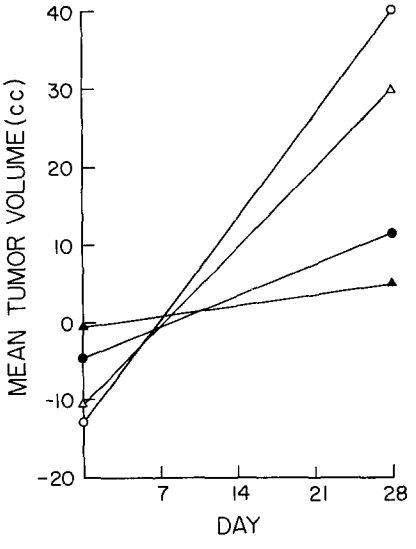

Fig. 1. Linear regression analysis of mean tumor growth in BC-47 bladder carcinoma. Each group contained 10 animals. Control group (○ no treatment), *r* value = 0.9806; PSK alone group (△), *r* value = 0.9337; CQ alone group (●), *r* value = 0.9053; PSK + CQ group (▲), *r* value = 0.9041. PSK dose = 1.0 g/kg PO daily; CQ dose = 0.5 mg/kg IP on days 1, 4, 8, and 12

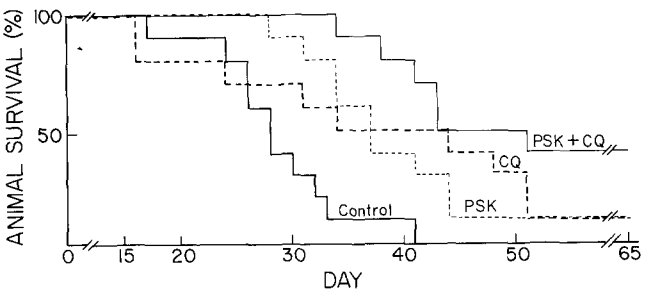

Fig. 2. Effect of PSK and CQ therapy on life span of rats hosting BC-47 bladder carcinoma. Tumor inoculation on day 0, 10 animals in each group. Control group (no treatment), *solid heavy line;* PSK alone group, *dashed light line;* CQ alone group, *dashed heavy line;* PSK + CQ group, *solid light line.* PSK dose = 1.0 g/kg PO daily; CQ dose = 0.5 mg/kg IP on days 1, 4, 8, and 12

Effect of therapy on tumor growth metastatic to lung

The effect on life span of animals injected with BC-47 IV is shown in Fig. 3. The lung metastatic tumor percentage take rate was 70, 60, 60, and 40 for control, PSK alone, CQ alone, and PSK + CQ groups, respectively. All animals with one or more metastatic nodules died by day 27. The experiment was terminated on day 30. Therapy did not increase survival in any group if only those animals with lung tumors are considered. However, the incidence of tumor growth in the PSK + CQ combination group was lower than in each of the other three groups (control, PSK alone, CQ alone).

Effect of therapy on host parameters

Each treatment group was evaluated during therapy by monitoring changes in body weight. All groups gained weight over the 4-week treatment period (Fig. 4). The correlation coefficients for the no treatment (control) and PSK alone groups indicated that group mean weight gains were a linear

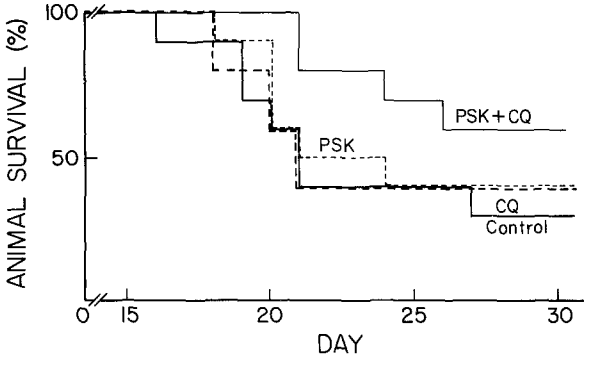

Fig. 3. Effect of PSK and CQ therapy on life span of rats injected IV with BC-47 bladder carcinoma; 2×10^6 viable cells inoculated on day 0. 10 animals in each group. Rats evaluated for lung lesions on day of death or at termination of experiment. Control group (no treatment), *solid heavy line;* PSK alone group, *dashed light line;* CQ alone group, *dashed heavy line;* PSK + CQ group, *solid light line.* PSK dose = 1.0 g/kg PO daily; CQ dose = 0.5 mg/kg IP on days 1 and 7

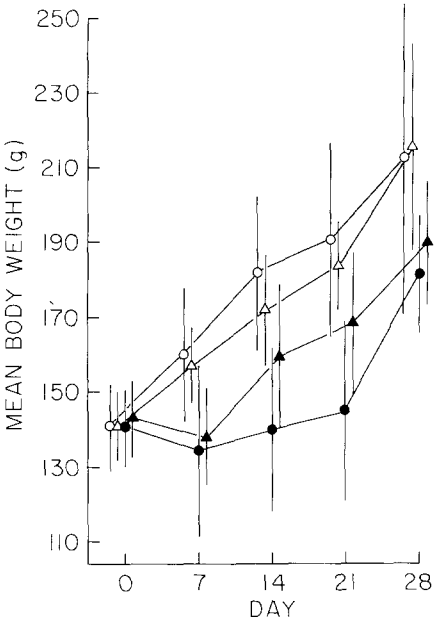

Fig. 4. Effect of PSK and CQ therapy on mean body weight (g) of rats hosting BC-47 bladder carcinoma. Each group contained 10 animals. O, control group, no treatment; △, PSK alone group; ●, CQ alone group; ▲, PSK + CQ group. PSK dose = 1.0 g/kg PO daily; CQ dose = 0.5 mg/kg IP on days 1, 4, 8, and 12

function, whereas in the CQ alone and CQ + PSK groups they were nonlinear. These two groups experienced a net loss in mean group weights in weeks 1 and 2, the period during which therapy was given.

All treatment groups had serum immunosuppressive substance levels determined at week 4 to determine the effect of therapy on that aspect of the host imune system. The no therapy (control), PSK alone, and CQ alone groups all had significantly elevated levels (unpaired *t*-test, $P < 0.001$) compared either with the treatment group receiving CQ + PSK or with the non-tumor-bearing normal rat group (Fig. 5).

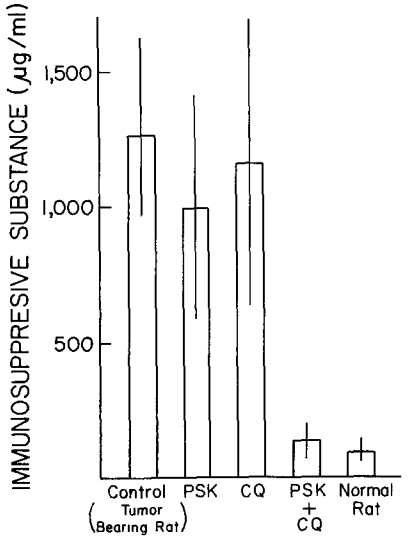

Fig. 5. Effect of PSK and CQ therapy on serum levels of immunosuppressive substance in rats hosting BC-47 bladder carcinoma. PSK = 1 g/kg PO daily; CQ = 0.5 mg/kg IP on days 1, 4, 8, and 12. Serum levels of immunosuppressive substance were measured on day 28. Tumor inoculation on day 0

There was no significant difference in immunosuppressive substance serum levels among the no treatment (control) group, the CQ alone group and the PSK alone group (unpaired *t*-test, $P > 0.05$), although the mean serum levels were lowest in the PSK alone group. There was no significant difference between the group receiving CQ + PSK and the non-tumor-bearing normal rat group (unpaired *t*-test, $P > .005$).

Discussion

Several studies using immunomodulators, such as PSK, in conjunction with various chemotherapeutic agents have been reported previously [2, 12, 28]. It is clear that the combined effects are dependent on the specific chemotherapeutic agents used and on the administrative routes and schedules. Combination therapy with PSK and CQ has been effective in animal tumors [13, 31] as well as in human tumors including lung carcinomas [17] and urologic carcinomas [21].

In this study, CQ significantly decreased the growth rate of the test tumor BC-47. However, three of 10 animals were dead at 4 weeks, all with lung metastases. CQ alone increased the life span of the tumor-bearing animals by 57% beyond the untreated (control) animal group. PSK alone was not an effective chemotherapeutic agent, since it did not significantly decrease the tumor growth rate. The life span of the PSK-treated group, however, was extended by 32% beyond that of the untreated control group.

Combination therapy of BC-47 with PSK and CQ demonstrated significant inhibition of tumor growth and an increase in life span of 82% over the not treatment (control) group. In the experiment in which BC-47 cells were injected IV and metastatic lesions to lungs were monitored, the groups receiving no therapy, PSK alone, and CQ alone had similar life spans and a similar incidence of lung lesions. Obvious prolongation of survival was evident in the group receiving combination PSK and CQ, With 60% of the animals having no tumor modules in either lung at 30 days, the last day of the experiment. Longer observation periods are needed to determine whether these animals were tumor-free or had undetectable small lesions at day 30.

Nakano et al. [22, 23] demonstrated that CQ administration had two measurable side effects on treated animals. First, delayed-type skin reactions were diminished and second, the animals had a decrease in body weight during therapy. When PSK was combined with CQ the skin reactions were restored to normal and weight loss was diminished. In the present studies, rats receiving CQ alone lost only 7% of the mean body weight during the 12 days of therapy and were gaining weight 7 days afterwards. Animals receiving PSK and CQ combination therapy lost 15% of the mean body weight during therapy but were also gaining weight 7 days after completion of CQ administration. For the group receiving the combination of CQ and PSK, the correlation coefficient of mean body weight changes during the 5-week experimental period was 0.7110, indicating the CQ administration caused a decrease in body weight. The addition of PSK in this group allowed an 18% mean increase in body weight at 4 weeks after inoculation. Animals in the control group had a 49% mean increase in body weight.

Immunosuppressive substance is known to increase in the serum of tumor-bearing animals [30]. Immunosuppressive substance inhibits several T-cell-mediated immune responses without affecting B-cell responses. It is a polypeptide with a molecular weight of less than 10,000, which suppresses

PHA-induced proliferation of lymphocytes in vitro and suppresses the in vivo induction of splenic plaque-forming cells in mice [25]. Glasgow et al. [6] suggested that immunosuppressive substance might be responsible for the failure of some cancer patients to mount an effective immune response against their tumors. In these experiments, all tumor-bearing groups had elevated serum levels of immunosuppressive substance. However, the serum concentration of immunosuppressive substance in the group receiving PSK alone was lower than the serum levels in either the no treatment (control) group or the group receiving CQ alone. A significant reduction (9.3-fold) in the serum concentration of immunosuppressive substance was evident in the group receiving combined CQ and PSK compared with the no treatment (control) group. The combination PSK and CQ group had serum levels only 1.5-fold above the serum levels of non-tumor-bearing ACI rats. These results suggest that although CQ is cytotoxic to tumor cells and causes slowing of tumor growth, the remaining tumor cells elicit production of immunosuppressive substance in the host and consequently decrease the life span of the tumor-bearing animals. These results suggest that combination therapy with PSK and CQ is more effective than CQ therapy alone. CQ alone acts as a cytotoxic chemotherapeutic agent. When PSK is added to the therapeutic regimen it decreases the immunosuppressive substance in the sera of the tumor-bearing animals to the concentration present in normal rat sera. In our experiments, there was a 72% increase in the life span of tumor-bearing animals receiving combination therapy over tumor-bearing animals receiving no therapy.

In summary, combination therapy of PSK and CQ was studied in ACI rats bearing rat bladder carcinoma BC-47. Comparison of the differences between treatment with CQ alone versus PSK plus CQ allows the following conclusions. First, there is no significant difference in tumor growth rates (Fig. 1); second, animals receiving the combination therapy had slightly longer survival times than animals receiving CQ alone (Fig. 2); third, the incidence of lung metastases in animals receiving combination therapy was 40%, versus 60% for animals receiving CQ alone; and fourth, the serum immunosuppressive substance concentrations in animals receiving combination therapy were significantly lower than concentrations in animals receiving CQ alone. It is suggestive that serum levels of immunosuppressive substance are elevated by the tumor burden, and that the combination of PSK plus CQ decreases serum levels to those found in normal, non-tumor-bearing rats. Whether immunosuppressive substance is a reflection of host immunity is not known. PSK may contribute slightly to the cytotoxicity of CQ. PSK seemed to be contributory to the prolongation of life in the aniaml receiving combination therapy.

References

1. Adolphs HD, Thiele J, Kiel H (1979) Effect of intralesional and systemic BCG application or a combined cyclophosphamide /BCG treatment on experimental bladder cancer. Urol Res 7: 71
2. Akiyama J, Kawamura T, Coiohda E, Yamada Y, Hosokawa M, Kodama T, Kobayashi H (1977) Immunochemotherapy of transplanted KMT-17 tumor in WKA rats by combination of cyclophosphamide and immunostimulatory protein-bound polysaccharide isolated from *Basidiomycetes*. Cancer Res 37: 3042–3045
3. Brosman SA (1983) BCG in the management of superficial bladder cancer. Urology 23: 82
4. Camacho F, Pinsky C, Kerr D, Whitmore W, Oettgen H (1980) Treatment of superficial bladder cancer with intravesical BCG. (Abstract) Presented at the conference on immunotherapy of cancer: Present status of trials in man, National Cancer Institute, Bethesda, Md, April 28–30
5. De Kernion JB, Ramming KP, Fraser K (1978) A bladder tumor model response to immunotherapy. Natl Cancer Inst Monogr 49: 333
6. Glasgow AH, Menzoian JO, Nimberg RB, Saporoschetz I, Cooperband SR, Schmid K, Mannick JA (1974) Association of anergy with an immunosuppressive peptide in the serum of cancer patients. N Engl J Med 291: 1263
7. Hashimoto Y, Kitazawa HS (1979) Antigen characteristics of nitrosamine-induced urinary bladder cancer in rats. Gann 70: 305
8. Herr HW, Kemery N, Yagoda A, Whitmore WF (1978) Poly (I:C) immunotherapy in patients with papillomas or superficial carcinomas of the bladder. Natl Cancer Inst Monogr 49: 325
9. Ikic D, Maricic Z, Oresic V, Rode B, Nola P, Smudj K, Knezevic M, Jusic D, Soos E (1981) Application of human leucocyte interferon in patients with urinary bladder paillomatosis, breast cancer, and melanoma. Lancet I: 1022
10. Inokuche K, Kamishiro R (1980) Chemotherapy of gastric cancer. Surgical Therapy 42: 40
11. Kagawa S, Ogura K, Kurokawa K, Uyama K (1979) Immunological evaluation of a streptococcal preparation (OK-432) in treatment of bladder carcinoma. J Urol 122: 467
12. Kaneko S, Abe H, Aida T, Tsuru M, Kodama T, Kobayashi H (1980) Experimental study of immunochemotherapy of brain tumors – Experimental brain tumor model and immunochemotherapy by a combination of PSK And ACNU. Neurol Med-Chir (Tokyo) 20 : 997
13. Kondo T, Sakamoto J, Ichihashi H, Kamei H (1981) Effect of alternating administration of PS-K and Carbazilquinone in tumor-bearing mice. Gann 72: 293
14. Lamm DL, Yee GN, Reichert DF, Radwin HM (1979) Levamisole immunotherapy of experimental transitional cell carcinoma. Invest Urol 16: 286
15. Lamm DL, Thor DE, Harris SC, Stogdill VD, Radwin HW (1980) Intravesical and percutaneous BCG immunotherapy of recurrent superficial bladder cancer. (Abstract) Presented at the Conference on Immunotherapy of Cancer: Present status of trials in man. National Cancer Institute, Bethesda, Md, April 28–30
16. Matushima T, Katoh O, Yagi S, Okimoto N, Niki Y, Mizoguchi D, Kibayashi T, Tano Y, Soejima R, Naoe H (1980) Effect of immunotherapy with PSK and OK-432 on survival of non-operated lung cancer patients. Jpn J Cancer Chemother 7: 1998
17. Mohri A, Dobashi T, Yamagata Y, Fujiwara T, Matsumoto H, Wakamoto A (1979) Clinical trials of the combination therapy of PSK with Carboquone toward lung cancer. Basic Pharmacology and Therapeutics 7: 2072
18. Morales A (1978) Adjuvant immunotherapy in superficial bladder cancer. Natl Cancer Inst Monogr 49 : 315
19. Morales A, Eidinger D, Bruce AW (1976) Intracavitary *Bacillus Calmette-Guérin* in the treatment of superficial bladder tumors. J Urol 116: 180
20. Nagao T, Komatsuda M, Watanabe K, Nozaki H, Yamaguchi K, Arimori S (1980) Immunotherapy of acute leukemia and malignant lymphoma with PSK. Jpn J Cancer Chemother 7: 1473
21. Nakagami JY, Nemoto S, Chin Y, Kawai H, Matsumoto K, Tannowa K (1982) Studies on multimodal treatment against bladder cancer. II: The relationship between the changes of immune parameters and the maintenance therapy (PSK + CQ). J Jpn Soc Cancer Ther 17: 1145
22. Nakano Y, Taguchi T (1975) Immunochemotherapy for cancer using protein polysaccharide isolated from Basidiomycetes. Jpn J Cancer Chemother 2: 13
23. Nakano Y, Taguchi T, Kobayashi Y, Saito K, Hayashi S, Iijima H, Yoshikuni C, Hotta T (1978) Reduction of side effects of antitumor agents by PSK. Proc Jpn Cancer Assoc, 37th Annual Meeting, p 155

24. Niimoto M, Toue T, Tani T, Nagusa H, Nakano A, Koh T, Oride M, Takiyama T, Nishiwatari K, Hattori T (1979) Adjuvant immunochemotherapy toward gastric cancer. Operation 33: 39
25. Nimberg RB, Glasglow AH, Menzoian JO, Cosntantian MB, Cooperband SR, Mannick JA, Schmid K (1975) Isolation of an immunosuppressive peptide fraction from the serum of cancer patients. Cancer Res 35: 1489
26. Nomoto K, Yoshikumi C, Matsunaga K, Fujii T, Takeya K (1975) Restoration of antibody-forming capacities by PS-K in tumor-bearing mice. Gann 66: 365
27. Oguchi Y, Matsuda Y, Yoshimura M, Toyoda N, Matsunaga K, Fujii T, Yoshida T, Yoshimura C, Hotta T, Yanagiasawa M, Tsura S, Nomoto L (1981) Study on the population of lymphatic cells in tumor-bearing mouse with or without PSK. Proc Jpn Cancer Assoc 40: 140
28. Oh-hashi F, Kataoka T, Tsukagoshi S (1978) Effect of combined use of anticancer drugs with polysaccharide preparation, Krestin, on mouse leukemia. Gann 69: 255
29. Purves EC, Snell M, Cope WA, Addison JE, Copland RFP, Berenbaum MC (1979) Subcutaneous *Corynebacterium parvum* in bladder cancer. Br J Urol 51: 278
30. Sakita M, Imai H, Kasuga M, Kageyama N, Imai S, Tamai M, Fujita Y, Majima S (1983) Effect of a protein-bound polysaccharide preparation, PS-K, on dimethyl hydrazine induction of intestinal tumors in rats. Gann 74: 351
31. Shinkai K, Sasagawa K, Arakawa M (1977) Combination effect with Carboquone and PSK on Ehrlich ascites carcinoma. Jpn J Cancer Chemother 4: 1329
32. Soloway MS (1978) Effectiveness of long-term chemotherapy and/or BCG on murine bladder cancer. Natl Cancer Inst Monogr 49: 327
33. Sugimachi K, Inokuchi K, Oudaira Y, Yaita A, Nakamura T (1980) Adjuvant long-term immunochemotherapy for squamous cell carcinoma of the thoracic esophagus. Am Surg 46: 551
34. Taguchi T (1979) Clinical studies on PSK: Combination therapy of PSK with surgery and chemotherapy. Recent Results Cancer Res 68: 236
35. Tsukagoshi S, Ohashi F (1974) Protein-bound polysaccharide, PS-K, effective against mouse bladder sarcoma-180 and rat ascites hepatoma AH-13 by oral use. Gann 65: 557
36. Usui S, Urano M, Koike S, Kobayashi Y (1976) Effect of PSK, a protein polysaccharide, onpulmonary metastases of a C3M mouse squamous cell carcinoma. J Natl Cancer Isnt 56: 185

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