Article

Enhancement of Antitumor Effect of Tegafur/Uracil (UFT) plus Leucovorin by Combined Treatment with Protein-Bound Polysaccharide, PSK, in Mouse Models

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We evaluated the antitumor effect of combined therapy with tegafur/uracil (UFT) plus leucovorin (LV) (UFT/LV) and protein-bound polysaccharide, PSK, in three mouse models of transplantable tumors. UFT/LV showed antitumor effect against Meth A sarcoma, and the antitumor effect was enhanced when PSK given concomitantly. UFT/LV showed antitumor effect to Lewis lung carcinoma and PSK alone also showed antitumor effect at high dose, but a combination of UFT/LV and PSK resulted in no enhanced antitumor effect. Colon 26 carcinoma was weakly responsive to UFT/LV, and no enhancement of antitumor effect was found even PSK was used in combination. In conclusion, while the effect of PSK varies depending on tumor, combined use of UFT/LV and PSK may be expected to augment the antitumor effect. *Cellular & Molecular Immunology*. 2007;4(4): 295-299.

Key Words: UFT, leucovorin, PSK, Meth A, Lewis lung carcinoma, colon 26, antitumor effect

Introduction

The results of anticancer treatment has improved steadily over the years due to the development of therapies utilizing biochemical modulation (1, 2), novel chemotherapeutic agents (3) and molecular targeted therapies (4), as well as combination of these therapies. Tegafur/uracil (UFT) (5, 6) is an anticancer agent developed based on the principle of biochemical modulation. This agent contains a mixture of tegafur (FT), a prodrug of 5-fluorouracil (5-FU), and uracil, a competitive inhibitor of dihydropyrimidine dehydrogenase, in a molar ratio of 1:4. As a result, the 5-FU concentration in tumor tissue is specifically elevated compared to normal tissue (7), showing selective antitumor effect. This agent is used for the treatment of a variety of cancers including gastrointestinal and lung cancers. Furthermore, as an oral agent, UFT is convenient to use for the patients.

Recently, UFT plus leucovorin (LV) therapy (UFT/LV) has been focused from the viewpoint of double biochemical modulation aiming to enhance the effect of UFT. The effectiveness of this therapy for advanced or recurrent colorectal cancer and the usefulness as postoperative adjuvant therapy has been evaluated overseas. Studies have reported that UFT/LV has similar survival advantage as 5-FU/LV, one of the standard treatments for colorectal cancer, with fewer adverse reactions such as decreased white blood cell count, decreased neutrophils, decreased platelets and stomatitis/mucosal inflammation compared to 5-FU/LV therapy (1, 2, 8). Furthermore, trials of combination with oxaliplatin or irinotecan have been conducted attempting to improve the therapeutic effect (9). However, enhancement of antitumor effect is accompanied by an increase of adverse drug reactions, resulting in deterioration of patients' quality of life.

The response to chemotherapeutic agents varies from patient to patient. As factors influencing response to chemotherapy, other than the sensitivity of cancer cells to drugs and tissue penetration of drugs, the performance status (10), immunocompetence (11) and nutritional status (12) of the host have been reported. On the other hand chemotherapy has been reported to suppress immunocompetence (13, 14) and nutritional status (12) of the host. The protein-bound polysaccharide, PSK, is extracted and purified from the mycelial culture of the basidiomycete *Coriolus versicolor*. It is a biological response modifier (BRM) that possesses diverse biological activities including immunostimulatory actions (15, 16), induction of tumor apoptosis (17) and

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Abbreviations: PSK, protein-bound polysaccharide Kureha; C26, colon 26; LLC, Lewis lung carcinoma; UFT, tegafur/uracil; 5-FU, 5-fluorouracil; FT, tegafur; LV, Leucovorin.

suppression of angiogenesis (18). In Japan, PSK has been used clinically for over 30 years as an oral anticancer agent in surgical patients with gastric cancer and colorectal cancer and in patients with small cell lung cancer, with almost no severe adverse drug reactions. Randomized controlled studies have proven that combined use of PSK with chemotherapeutic agents prolongs the overall survival and also the disease-free survival period (19-22). Therefore, combined use of PSK with chemotherapeutic agents is considered to be a useful treatment modality. From this viewpoint, many basic studies on the combined use of PSK with chemotherapeutic agents have been reported (23-29). However, the effect of combined UFT/LV and PSK therapy has not been documented. We performed a basic investigation on the antitumor effect of combined UFT/LV and PSK therapy using three mouse models of transplantable tumors.

Materials and Methods

Animals

Five-week-old female BALB/c and male C57BL/6N mice were purchased from Charles River Japan (Kanagawa). After acclimatization for one week, the six week-old mice were used in experiments. The mice were housed in an animal facility controlled at room temperature of 25 ± 2°C, humidity of 55 ± 10%, and lighting cycle of 12 hours per day. The animals were fed an animal diet CE-2 (Oriental Yeast, Tokyo) and sterilized tap water ad libitum. Nine or ten animals were used in each group. Each experiment was repeated at least once. The study protocol was reviewed by the Committee of Ethics on Animal Experiments of the animal facility and the experiments were conducted in accordance with the guidelines prepared by the Committee.

Tumors

Mouse Meth A sarcoma (Meth A), mouse Lewis lung carcinoma (LLC) and mouse colon 26 carcinoma (C26) passaged and maintained in our laboratory were used. Meth A was passaged by intraperitoneal injection into BALB/c mice. Peritoneal fluid was harvested 7 to 10 days after passage and centrifuged at 1,000 rpm for 5 min. After discarding the supernatant, the cell pellet was resuspended in RPMI medium containing 10% fetal calf serum. The cell density was adjusted to 1×10^7 cells/ml, and 0.1 ml was transplanted subcutaneously in the right axillary region of the mouse. LLC was passaged subcutaneously in C57BL/6N mice and C26 was passaged by subcutaneous injection into BALB/c mice. On days 10-12 after passage, the tumor nodule was dissected and the non-necrotized tissue was minced with scissors and forceps. Then the tissue fragments were stirred for approximately 1 h in RPMI 1640 medium containing 2 mg/ml of collagenase type I (Sigma-Aldrich, Tokyo) and 20% fetal calf serum. Tissue aggregates were removed using a stainless mesh to obtain a single-cell suspension. Subsequent procedures were as for Meth A.

Drugs

UFT was supplied by Taiho Pharmaceutical Co. Ltd. (Tokyo) and LV was supplied by Wyeth K. K (Tokyo). Before administration, the two compounds were dissolved separately in 0.5% hydroxypropyl methylcellulose. A volume of 0.1 ml/10 g body weight was administered orally to each mouse from day 1 after tumor transplantation once daily for 9 consecutive days. UFT was administered simultaneously with LV. Based on the result of our preliminary experiment that determined the minimum toxic dose in tumor-bearing mice, 60 mg/kg of UFT which is equivalent to 20 mg/kg of FT was used in this study. It was reported that 5.56 mg/kg of *l*-LV and above or 6.7 mg of *d, l*-LV and above significantly enhanced antitumor effect of UFT dose and dosing schedule - independently (30, 31). The dose range of LV examined was from 5.56 to 22.24 mg/kg. PSK was supplied by Kureha Corporation (Tokyo). Before use, the required quantity of PSK was dissolved in physiological saline, and each dose of PSK was administered intraperitoneally into mice three times a week from day 1 after tumor transplantation. The control group was administered physiological saline. The dose of PSK and the dosing schedule were as reported previously (32). In our preliminary experiment, subcutaneously transplanted Meth A was completely rejected by the administration of 50 mg/kg or 100 mg/kg of PSK, the growth of LLC was inhibited by the administration of 100 mg/kg of PSK, and the growth of C26 was not inhibited by the administration of 100 mg/kg of PSK (data not shown). Therefore 5 mg/kg and 10 mg/kg of PSK were used in the study using Meth A, and 50 mg /kg and/or 100 mg/kg of PSK were used in the study of LLC and C26.

Evaluation of antitumor effect

Changes in tumor size over time after tumor transplantation were assessed. The length and width of the tumor were measured using calipers. Tumor volume was calculated by the following formula: $(\text{width})^2 \times \text{length} / 2$. In addition, the mice were observed everyday after tumor transplantation for tumor-related death, and survival curves were constructed.

Statistical analysis

Differences in tumor volume were analyzed using two-way repeated measure ANOVA. Survival curves were analyzed using Log-rank test. $p < 0.05$ was considered statistically significant.

Results

Antitumor effect against Meth A

In our preliminary experiment, 16.68 mg/kg, 22.4 mg/kg or 27.8 mg/kg of LV plus 60 mg/kg of UFT showed similar antitumor effect against Meth A (data not shown). The dose of LV was used 22.4 mg/kg. Growth of Meth A was significantly inhibited by the administration of UFT 60 mg/kg plus LV 22.24 mg/kg (control *vs* UFT/LV, $p < 0.001$) (Figure 1). When this UFT/LV administration was combined with PSK 5 mg/kg (PSK5) or 10 mg/kg (PSK10), the tumor volume was further reduced (UFT/LV *vs* UFT/LV + PSK5, $p = 0.030$; UFT/LV *vs* UFT/LV + PSK10, $p < 0.001$) (Figure 1).

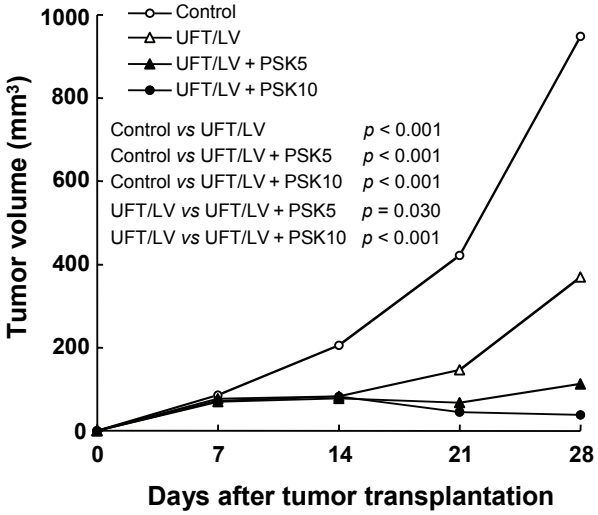

Figure 1. Antitumor effect of combined UFT/LV and PSK therapy against Meth A. Meth A tumors were transplanted into mice (10 per group). UFT/LV (60 mg/kg / 22.4 mg/kg) was administered orally from day 1 after transplantation for 9 days. PSK 5 or 10 mg/kg was administered intraperitoneally three times a week from day 1 to day 28 after tumor transplantation.

Antitumor effect against LLC

In our preliminary experiment, 5.56 mg/kg, or 16.68 mg/kg of LV plus 60 mg/kg of UFT showed similar antitumor effect against LLC (data not shown). The dose of LV was used as 5.56 mg/kg. Proliferation of LLC was rapid and most of mice in the control group died of tumor at week 4 after transplantation. Therefore tumor volume was measured up to 3 weeks after tumor transplantation. Tumor growth was inhibited by the administration of UFT 60 mg/kg plus LV 5.56 mg/kg (control *vs* UFT/LV, $p < 0.001$) (Figure 2). PSK 50 mg/kg did not inhibit tumor growth when given alone, and did not further reduce tumor volume when used in combination with UFT/LV compared to UFT/LV alone (Figure 2A). On the other hand, PSK 100 mg/kg inhibited tumor growth when used alone (control *vs* PSK; $p = 0.050$) (Figure 2B), but did not enhance the antitumor effect when used in combination with UFT/LV (Figure 2B). Life span was prolonged by the administration of UFT 60 mg/kg plus LV 5.56 mg/kg, but not by PSK 100 mg/kg alone (control *vs* UFT/LV, $p < 0.001$; control *vs* PSK, $p = 0.400$) (Figure 2C). When UFT/LV administration was combined with PSK 100 mg/kg, survival curve did not differ from that of UFT/LV administration (Figure 2C).

Antitumor effect against C26

From the results of Meth A and LLC, the dose of LV was used 16.68 mg/kg in the study of C26. Growth of C26 was inhibited by the administration of UFT 60 mg/kg plus LV 16.68 mg/kg compared with control (control *vs* UFT /LV, $p = 0.038$) (Figure 3). Even when PSK 100 mg/kg was given in combination with UFT/LV, the tumor volume did not differ from that of UFT/LV administration (Figure 3).

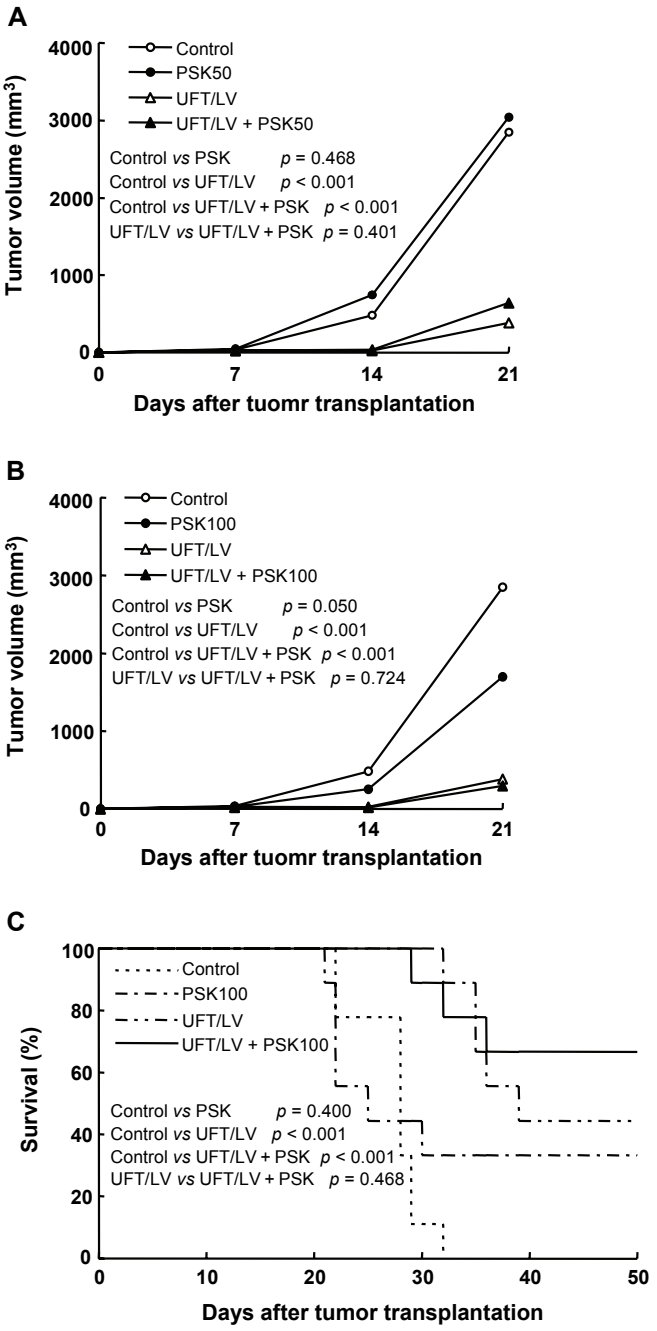

Figure 2. Antitumor effect of combined UFT/LV and PSK therapy against LLC. LLC tumors were transplanted into mice (9 per group). UFT/LV (60 mg/kg / 5.56 mg/kg) was administered orally from day 1 after transplantation for 9 days. PSK 50 mg/kg (A, tumor volume) or 100 mg/kg (B, tumor volume; C, survival curve) was administered intraperitoneally three times a week from day 1 to day 28 after tumor transplantation.

Discussion

Although the treatment result of anticancer chemotherapy has improved steadily over the years, the efficacy for advanced

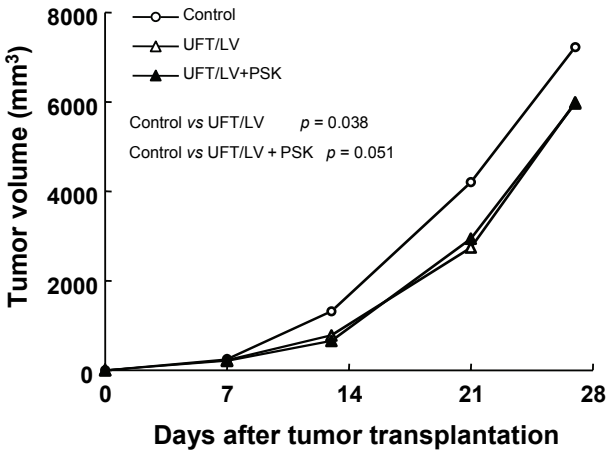

Figure 3. Antitumor effect of combined UFT/LV and PSK therapy against C26. C26 tumors were transplanted into mice (10 per group). UFT/LV (60 mg/kg / 16.68 mg/kg) was administered orally from day 1 after transplantation for 9 days. PSK 100 mg/kg was administered intraperitoneally three times a week from day 1 to day 28 after tumor transplantation.

cancer remains limited. Some possible reasons are serious adverse drug reactions, heterogeneity of cancer cells, and emergence of drug resistance due to mutation. On the other hand, while anticancer immunotherapy has few adverse reactions, the tumor-rejecting capacity of immunotherapy is restricted due to the limitation in number of effector cells including cytotoxic T lymphocytes. Hence, we believe that the optimal treatment is a combination of drugs that possess different mechanisms of action as mentioned above, and examined the effect of combined treatment with UFT/LV and PSK.

For Meth A, UFT/LV showed antitumor effect when used alone, and the antitumor effect was enhanced by combined use of PSK. In the case of LLC, UFT/LV was effective against the tumor and PSK alone also showed antitumor effect at high dose, but combined therapy of the two agents did not enhance the antitumor effect. On the contrary, UFT/LV showed weak antitumor effect against C26, and combined use with PSK also did not increase the antitumor effect. Thus the effect of combined therapy varies for different tumors. In the case of C26, UFT/LV was slightly effective when used at the dosing regimen in this study, resulting in rapid proliferation of the tumor, which probably did not allow PSK to exhibit its immunostimulatory effect. Further study to improve the dosing conditions of both drugs is required for C26. For Meth A and LLC, although UFT/LV effectively inhibited the growth of both tumors, the effect of combined therapy varied between tumors. A possible reason is that LLC grew at a higher speed than Meth A, and the effect of PSK was more difficult to manifest with the increased tumor mass. In addition, PSK exhibits actions other than the immunostimulatory effect as described below, and these actions may differ depending on the tumor.

Many reports have demonstrated that PSK enhances the antitumor effect when used in combined therapy with various anticancer agents, and the present study confirmed that combined use of UFT/LV also augments the antitumor effect against Meth A.

The mechanisms of action of combined UFT/LV and PSK therapy may be attributed to the immunostimulatory effects reported previously. Nakano et al. reported that while the delayed type hypersensitivity response to picryl chloride was suppressed by various chemotherapeutic agents, PSK treatment restored the response (16). Akiyama et al. also reported that combined use of cyclophosphamide with PSK augmented the delayed hypersensitivity footpad response to tumor cells (23). On the other hand, Zhang et al. observed that PSK enhanced the docetaxel-induced apoptosis of pancreas cancer cells probably *via* suppression of NFκB activation, suggesting a possibility that mechanisms other than immunostimulating effects may be involved (17). Wada et al. reported that PSK suppressed bFGF-induced angiogenesis (18). In the study of the mechanism of action of combination therapy with anti-vascular endothelial growth factor (VEGF) antibody and CPT-11, Wildiers et al. reported that anti-VEGF antibody normalized tumor vessels and increased the intratumoral CPT-11 concentration. It is possible PSK may also act *via* this mechanism (33). Further studies are required to elucidate the mechanism of action of PSK combined therapy.

In conclusion, we have shown that combined use of PSK enhances the antitumor effect of UFT/LV in a mouse Meth A sarcoma model. Although combined use of chemotherapeutic agent UFT/LV or further combination with oxaliplatin or irinotecan increases the antitumor effect, the adverse drug reactions are not negligible. But we reported previously that PSK attenuates the adverse reactions of chemotherapy in lung cancer (34), and the present study suggests that combination therapy of PSK and UFT/LV is a beneficial modality that improves the antitumor effect against some tumors.

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