

Long-term Postoperative Adjuvant Chemotherapy of UFT/LV Improves Survival in a Primary Tumor Resection-Pulmonary Metastasis Model

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Abstract. *Background: The optimum regimen and optimum duration of administration of postoperative chemotherapy would vary with the postoperative residual tumor volume. Whether or not prolonged administration of oral uracil/tegafur (UFT) with leucovorin (LV) would prolong the survival period was assessed experimentally. Materials and Methods: Murine models of pulmonary metastasis with different volumes of residual tumor after primary tumor resection were prepared, and the efficacy of 12-week and 4-week oral administration of UFT/LV as postoperative adjuvant chemotherapy was compared. Results: In the model with only a small volume of occult residual tumor after early resection of the primary tumor, the survival period in the 12-week UFT/LV group was significantly increased as compared with that in the untreated group, whereas no significant difference was noted between the 4-week UFT/LV group and the untreated group. Conclusion: Long-term administration of UFT/LV as postoperative adjuvant chemotherapy may be potentially beneficial.*

In recent years, the usefulness of postoperative adjuvant chemotherapy for colon cancer using oral drugs has been assessed. The National Surgical Adjuvant Breast and Bowel Project (NSABP) C-06 study, which compared orally administered uracil/tegafur (UFT) plus leucovorin (LV) with intravenously administered 5-fluorouracil (5-FU) plus LV in individuals with stage II/III colon cancer, demonstrated that both regimens were equivalent in terms of the overall survival rate and the disease-free survival rate at 5 years and since then, oral treatment with UFT/LV has been one of the standard regimens for postoperative adjuvant chemotherapy for colon cancer (1).

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At present, however, the optimum duration of treatment with oral fluoropyrimidines in postoperative adjuvant chemotherapy for colorectal cancer remains unclear. Some experimental studies have shown that the rate of cure of micrometastases, which are highly sensitive to cell cycle-specific antimetabolites, can be increased by postoperative chemotherapy and that postoperative chemotherapy becomes inefficient when the tumor volume becomes excessive (2, 3). It is therefore considered that the optimum regimen and optimum duration of administration of postoperative chemotherapy would vary with the postoperative residual tumor volume. The incidences of hematological toxicity, stomatitis and mucositis after oral administration of UFT/LV are significantly lower than those after intravenous injection of 5-FU/LV, and other side-effects of oral UFT/LV are also milder than those associated with intravenous injection of 5-FU/LV (4, 5), suggesting that oral UFT/LV may be administered for prolonged periods of time. Whether or not prolonged administration of oral UFT/LV would prolong the survival period was experimentally assessed in murine models of distant metastasis after resection of the primary tumor, with different volumes of residual tumor.

Materials and Methods

Animals, tumor implantation and primary tumor resection. Five-week-old male CDF₁ mice (Japan SLC Inc., Hamamatsu, Japan) were acclimatized for 8 days before the start of this study. They were housed in a room lit for 12 hours each day (from 8:00 am to 8:00 pm) and given food and water *ad libitum*. Colon 26 PMF-15 cells, which have metastatic ability to the lung, were maintained in RPMI-1640 medium supplemented with 5% fetal bovine serum and 25 mM HEPES (6). The tumor cells (5×10^5 cells/animal) were implanted into the hind foot pad of the CDF₁ mice. Twelve days (Experiment 1) or 7 days (Experiment 2) after implantation, the primary tumors were surgically removed and the animals were divided into three groups (10 animals/group) in each experiment on the basis of the body weight in such a manner that the average body weight of all the groups was similar.

Drug preparation and administration. UFT was prepared by carefully combining tegafur (Taiho Pharmaceutical Co. Ltd., Tokyo, Japan) with uracil (Taiho Pharmaceutical Co. Ltd.) at a molar ratio

of 1:4 and subsequently suspending the mixture in an aqueous 0.5% sodium carboxymethylcellulose solution. The appropriate quantity of LV (calcium leucovorin; Wako Pure Chemical Industries Ltd., Osaka, Japan), corrected for calcium and water content, was added to the UFT solution. The mixture was treated ultrasonically to yield the UFT/LV dosing solution.

The UFT/LV was administered orally for 4 weeks or 12 weeks from the day after the resection of the primary tumor in cycles of once a day for 5 consecutive days followed by a 2-day drug-free interval. The dose of UFT used in the murine models was 22 mg/kg/day (on tegafur basis), at which animals did not show weight loss even with prolonged administration (7). With regard to the usual clinical doses of UFT/LV in the Japanese population, LV is administered at a dose of 75 mg/day, while UFT is administered at a dose of 400-500 mg/day, showing that the dose of LV is approximately 1/6th to 1/5th of that of UFT. Based on this, the dose of LV was determined to be 4 mg/kg/day in the present experiments. The drugs were administered in a volume of 1 ml per 100 g of body weight. The animals that received no drug administration were allocated to the untreated group as controls.

Evaluation of the efficacy and toxicity. The day of start of treatment was designated as the day of start of observation (day 1) and the survival or death of the animals was confirmed daily during the study period until the 140th day of observation (day 140). The body weight was measured twice a week in the surviving animals and the body weights of the deceased animals were recorded after they were found dead. The deceased animals were autopsied immediately after they were found dead and the surviving animals were autopsied on day 140.

Statistical analysis. Significant differences in survival curves were analyzed by the log-rank test, and differences at $p < 0.05$ (two-sided) were considered to be statistically significant.

Results

Experiment 1 (resection of the primary tumor 12 days after tumor implantation, Figures 1 and 2). In the untreated group, 3 mice started to show swelling at the site of resection on days 20-23 and tachypnea, abnormal body hair, decrease of body temperature and dyspnea on day 21. All the animals in this group died by day 37. The median survival period was 28.5 days (mean±SD, 29.8±4.9 days). In the 4-week UFT/LV group, 4 mice started to show swelling at the site of resection on days 15-36 and tachypnea, abnormal body hair and decrease of body temperature on day 22. All the animals in this group died by day 59. The median survival period was 40.5 days (mean±SD, 41.3±9.4 days). In the 12-week UFT/LV group, 5 mice started to show swelling at the site of resection on days 19-40 and tachypnea, abnormal body hair, decrease of body temperature and dyspnea on day 18. All the animals in this group died by day 59. The median survival period was 43.5 days (mean±SD, 42.4±9.8 days).

There was almost no difference in body weight change among the three groups. All the deceased animals in every group showed weight loss associated with worsening of the general condition and metastasized nodules were confirmed on the surface of the lung excised at autopsy.

The survival period was significantly increased in the 12-week UFT/LV group ($p=0.0005$) and 4-week UFT/LV group ($p=0.0008$) as compared with that in the untreated group, whereas the difference in survival between the 12-week and 4-week UFT/LV groups was not significant ($p=0.6893$). None of the animals showed cure and all the animals died during the observation period.

Experiment 2 (resection of the primary tumor 7 days after tumor implantation, Figures 3 and 4). In the untreated group, 2 mice started to show swelling at the site of resection on days 22 and 26, respectively and tachypnea, abnormal body hair, decrease of body temperature, tendency to adopt the recumbent or prone position on day 11. Five mice, *i.e.* half of the group, died by day 47 and the remaining 5 survived until day 140. The median survival period was 93.5 days (mean±SD, 85.2±58.4 days). In the 4-week UFT/LV group, 2 mice started to show swelling at the site of resection on days 11 and 32, respectively and tachypnea, abnormal body hair and decrease of body temperature on day 14. Three mice died by day 58 and 7 survived until day 140. The median survival period was 140.0 days (mean±SD, 109.1±50.8 days).

There was almost no difference in body weight change among the three groups. All the deceased animals in both groups showed weight loss associated with worsening of the general condition. In the deceased animals, metastasized nodules were confirmed on the surface of the lung excised at autopsy, while none of the surviving animals showed abnormal findings. In the 12-week UFT/LV group, none of the animals had died by day 140 and no abnormal findings were revealed on autopsy.

The survival period in the 12-week UFT/LV group was significantly ($p=0.0115$) increased as compared with that in the untreated group, whereas no significant ($p=0.3136$) difference was noted between the 4-week UFT/LV group and the untreated group. The difference between the 12-week UFT/LV group and the 4-week UFT/LV group tended toward a significant difference ($p=0.0671$).

Discussion

Patients diagnosed as having pathological stage I, II or III disease after curative resection of colorectal cancer include those with true stage IV disease with metastases of various numbers of tumor cells. These patients are at high risk of recurrence and more efficacious postoperative adjuvant chemotherapy appears to be necessary in these cases (8, 9). Schabel reported that the sensitivity of micrometastases to chemotherapy was related to the proliferative state of the tumor cells (2). In other words, when the tumor is small, tumor cells show active metabolism to undergo active cell division. The sensitivity of the cells to cell cycle-specific drugs (such as antimetabolites) and to cell cycle stage-

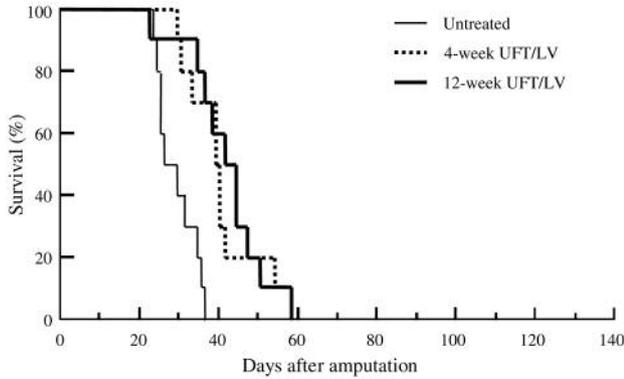


Figure 1. Survival effects of UFT/LV in Experiment 1. Colon 26 PMF-15 cells (5×10^5 cells/mouse) were implanted into the hind foot pad of CDF₁ mice. Twelve days after implantation, the primary tumor was surgically removed. UFT/LV was orally administered once daily for 4 weeks or 12 weeks from the day after resection on 5 days of the week (10 animals/group). Twelve-week UFT/LV group vs. untreated group, $p=0.0005$; 4-week UFT/LV group vs. untreated group, $p=0.0008$; 12-week UFT/LV group vs. 4-week UFT/LV group, $p=0.6893$ (log-rank test).

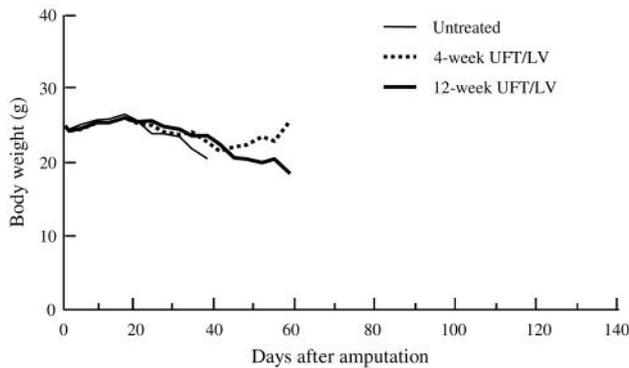


Figure 2. Body weight changes of CDF₁ mice in Experiment 1.

specific drugs (such as S-stage-specific antimetabolites and M-stage-specific mitotic inhibitors) is considerably increased at this stage. According to Schabel, these cells must be treated with effective concentrations of cell cycle-specific drugs for a long time, while the cells are sensitive during the stage of active division, to achieve effective cytotoxicity.

Zoetmulder and Zwaveling (10), in a murine model of metastases of Lewis lung carcinoma, compared groups subjected to primary tumor resection alone, chemotherapy alone without primary tumor resection, or chemotherapy after primary tumor resection. The survival rate was significantly improved in the group treated by primary tumor resection combined with chemotherapy, although the duration of chemotherapy was not taken into consideration for the analysis.

In the present model, with a large volume of residual tumor after primary tumor resection, all the animals died at a relatively early stage in the untreated group and the survival

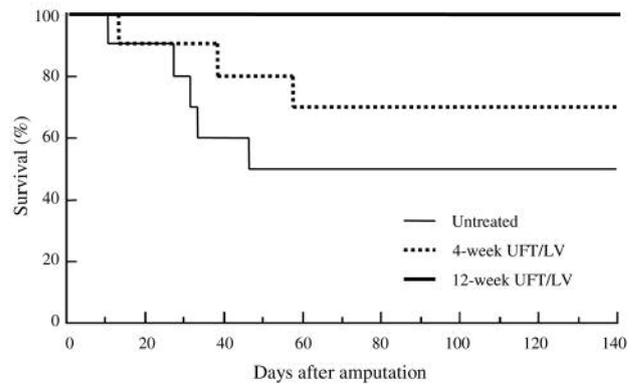


Figure 3. Survival effects of UFT/LV in Experiment 2. Colon 26 PMF-15 cells (5×10^5 cells/mouse) were implanted into the hind foot pad of CDF₁ mice. Seven days after implantation, the primary tumor was surgically removed. UFT/LV was orally administered once daily for 4 weeks or 12 weeks from the day after resection on 5 days of the week (10 animals/group). Twelve-week UFT/LV group vs. untreated group, $p=0.0115$; 4-week UFT/LV group vs. untreated group, $p=0.3136$; 12-week UFT/LV group vs. 4-week UFT/LV group, $p=0.0671$ (log-rank test).

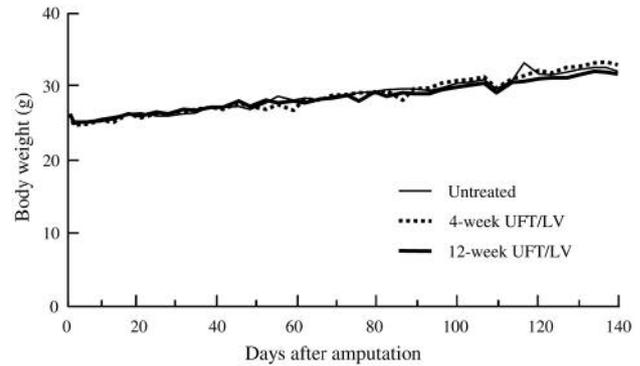


Figure 4. Body weight changes of CDF₁ mice in Experiment 2.

period in the UFT/LV groups was significantly increased as compared with that in the untreated group (Experiment 1). This result showed the promise of UFT/LV therapy as postoperative adjuvant chemotherapy. However, the condition was not cured in any of the cases and no survival prolongation was observed with long-term as compared to short-term UFT/LV administration. It was therefore considered that more potent chemotherapeutic regimens might be needed. In the model with only a small volume of residual tumor after primary tumor resection, half of the animals even in the untreated group showed cure and the survival period was significantly increased in the 12-week UFT/LV group as compared with that in the untreated group (Experiment 2). However, no such survival prolongation as compared with the untreated group was observed in the 4-week UFT/LV group. It was therefore considered that more potent chemotherapeutic regimens might be needed when the treatment duration needs to be short. There

was no additional weight loss in the long-term UFT/LV administration group as compared with the short-term administration or untreated groups in both experiments.

Browder *et al.* have indicated that in murine models of tumors, chemotherapy-resistant cell proliferation can be efficiently controlled by promoting sustained apoptosis of endothelial cells within the tumor bed by frequent administration of low doses of cytotoxic anticancer drugs at short intervals, based on the concept that the tumor-associated endothelial cells could recover during a drug-free interval to promote regrowth of the tumor cells (11). Klement *et al.* have also indicated that in murine models of tumors the blood supply to the tumors could be decreased and tumor growth could be suppressed by long-term administration of a low dose of cytotoxic anticancer drugs (12). Such regimens of administering low-dose cytotoxic anticancer drugs at short intervals of administration for prolonged periods of time was referred to as “metronomic” dosing regimens by Hanahan *et al.* (13). Based on the report that UFT has only slight or mild adverse effects, so that it can, therefore, be administered for prolonged periods of time and that its metabolites exert an inhibitory effect against angiogenesis (14), Kerbel *et al.* listed UFT as a cytotoxic anticancer drug that is highly suitable for use in metronomic chemotherapy (15, 16).

The results of the present experiments suggested that in the model with only a small number of residual tumor cells after early resection of the primary tumor, the residual tumors not only showed high sensitivity to UFT/LV, but the drug also exerted a suppressive effect on tumor growth *via* its inhibitory action against tumor angiogenesis during prolonged administration, thereby promoting prolongation of survival and cure. Although the contribution of the inhibitory action against angiogenesis to the antitumor effect of UFT has not yet been clinically clarified, oral UFT or UFT/LV may be expected to be efficient for postoperative adjuvant chemotherapy when administered for prolonged periods of time. It would be highly desirable to confirm the efficacy of long-term, *i.e.* over 6 months, postoperative adjuvant chemotherapy of UFT/LV by clinical studies.

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