Low-dose Chemotherapy with Leucovorin Plus 5-Fluorouracil for Colorectal Cancer Can Maintain Host Immunity

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Abstract. Background: Anticancer drugs may frequently show host immunosuppression. Low-dose chemotherapy has been used for unresectable cancer as a tumor dormancy therapy, and it has been reported that the patients treated this way demonstrated favorable survival without toxicity. In this study, host immunity before and after a low-dose leucovorin plus 5-fluorouracil regimen (low-dose LV/5-FU) and S-1 plus irinotecan regimen (S-1/CPT-11) was compared to assess whether low-dose chemotherapy can maintain host immunity. Patients and Methods: Fourteen patients with recurrent or metastatic colorectal cancer underwent low-dose LV/5-FU, or S-1/CPT-11 treatment. The host immunity (cytokine production of the peripheral blood mono-nuclear cells (PBMC), serum soluble interleukin-2 receptor (sIL-2R) levels and phenotypic analyses of the PBMC) was measured before and after the first chemotherapy treatment. Results: An increase of sIL-2R and CD4+CD25+ T cells with S-1/CPT-11 treatment, and a decrease with low-dose LV/5-FU treatment were observed and these changes in the first course were significantly different (p=0.0722 for the sIL-2R, p=0.0187 for the CD4+CD25+ T cells). Conclusion: The current study indicated that there is no change or an improvement in host immunity with the low-dose LV/5-FU treatment as compared with the S-1/CPT-11 treatment. Low-dose LV/5-FU treatment should be considered for metastatic colorectal cancer in order to maintain a host immunity during chemotherapy.

Abbreviations: 5-FU, 5-fluorouracil; LV, leucovorin; CPT-11, irinotecan; PS, performance status; ADR, adverse drug reaction; PBMC, peripheral blood mono-nuclear cell; PHA, phytohemagglutinin; Con-A, Concanavalin-A; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; mAb, monoclonal antibody; CDDP, cisplatin.

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In recent years, chemotherapy for unresectable and metastatic colorectal cancer has been progressing. Recent additions to the chemotherapy armoury for this disease have begun to prolong median survival times (1-3). In trials in which patients were exposed to oxaliplatin, irinotecan (CPT-11), and 5-fluorouracil/leucovorin (5-FU/LV), or capcitabine during the course of their disease, median survival reached 20 months (4). The current standard first-line regimens for metastatic colorectal cancer are FOLFOX (infusional 5-FU/LV with oxaliplatin) and FOLFIRI (infusional 5-FU/LV with CPT-11) (5). The addition of bevacizumab to a two-drug regimen (CPT-11 with 5-FU/LV) prolongs median survival to 20 months, but with increased toxicity (6).

Current cancer chemotherapy usually entails the administration of the maximum tolerable dose of anticancer drugs so as to maximize the direct antitumor effect of the drugs (7). However anticancer drugs may have highly suppressive effects on the host immunity (8-11). Low-dose chemotherapy has been used for unresectable cancer as a "tumor dormancy" therapy (12). The use of low-dose LV/5-FU and low-dose CPT-11/CDDP for recurrent or metastatic colorectal cancer has been reported to show favorable survival without toxicity (13, 14). Although the response rate was low, low-dose LV/5-FU did not have severe adverse effects and so long-term treatment was possible, so that longer median survival time was achieved. On the other hand, treatment with S-1 which is a new oral futafur with 5-chloro-2,4-dihydroxypyridine and potassium oxonate plus CPT-11 is expected to show the similar results to FOLFOX or FOLFIRI (15). In this study, the host immunity before and after administration of both regimens was compared, to assess whether low-dose chemotherapy could maintain host immunity.

Patients and Methods

Patients. Fourteen patients with recurrent or metastatic colorectal cancer underwent low-dose LV/5-FU, or S-1/CPT-11 treatment between April 2003 and June 2004. The patient characteristics are summarized in Table I. Eastern Cooperative Oncology Group (ECOG) performance status (PS) of each group was one or less. Although an adverse drug reaction (ADR) (16) was observed in...
Isolation of PBMC and production of cytokines. Gumma, Japan). The cell suspensions recovered at the interface heparinized venous blood over Ficoll-Conray gradients (IBL, obtained by centrifugation (400 g for 30 minutes at 18°C) of three patients with low-dose LV/5-FU and four with S-1/CPT-11, all the toxicities were less than grade 2.

Chemotherapy regimens. The low-dose LV/5-FU treatment consisted of LV at 20 mg/m² immediately followed by 5-FU at 370 mg/m² by intravenous infusion for two hours daily for five consecutive days. The S-1/CPT-11 treatment was a dose of 40 mg S-1 twice daily for 21 consecutive days, with a 7-day rest period and 80 mg CPT-11 infused intravenously over 90 min on day 1 and day 15.

Sampling technique. Blood samples were collected before and after the first chemotherapy cycle. The cytokines except sIL-2R, were obtained from stimulated peripheral blood mono-nuclear cells (PBMC) while, serum was used for the sIL-2R assessment. The CD4/8 and Th1/2 ratios, and the CD4⁺CD25⁺ T cells were determined by flow cytometric analysis using monoclonal antibodies (mAbs) to CD4, CD8, interleukin-4 (IL-4), interferon-γ (IFN-γ), and CD25. The changes in these parameters in both groups was statistically analyzed by repeated analysis of variance (ANOVA), p<0.1 was considered significant.

Isolation of PBMC and production of cytokines. The PBMC were obtained by centrifugation (400 g for 30 minutes at 18°C) of heparinized venous blood over Ficoll-Conray gradients (IBL, Gumma, Japan). The cell suspensions recovered at the interface were washed and resuspended at a density of 1x10⁶ cells in a total volume of 1 ml of complete medium (RPMI 1640 supplemented with penicillin (200 IU/ml), streptomycin (100 µg/ml), L-glutamine (2 mM), and 10% heat-inactivated fetal calf serum). To assess cytokine production, the PBMC were stimulated in vitro with phytohemagglutinin (PHA) (20 µg/ml) and Concanavalin-A (Con-A) (7 µg/ml) for 24 h at 37°C in 5% CO₂. The supernatants were collected and immediately frozen for subsequent determination of cytokine concentration. The concentrations of human cytokines were determined using an enzyme-linked immunosorbent assay (ELISA). The cytokines IL-10, IL-6, IL-12p70, IFN-γ, tumor necrosis factor-α (TNF-α) and sIL-2R were assessed using commercial ELISA Kits (IL-10 and IFN-γ, BioSource Europe; IL-6, Fuji Rebio; IL-12p70, R & D systems; TNF-α, JIMRO and sIL-2R, EURO/DPC) according to the manufacturer’s instructions.

Flow cytometric analysis. Flow cytometric analysis of the PBMC was performed using a FACSorter (Becton Dickinson, Mountain View, CA, USA), using the Diva software (BD Biosciences, Mountain View, CA, USA). The area of positivity was determined using an isotype-matched control mAb. Ten thousand events for each sample were acquired.

| Table I. Characteristics in patients treated with LV/5-FU or S-1/CPT-11. |
|-----------------------------|-----------------------------|
|                            | LV/5-FU (n=7)              | S-1/CPT-11 (n=7)          |
| Age                        | 61 (50-73)                 | 68 (61-73)                |
| Gender (M:F)               | 3:4                        | 2:5                       |
| PS (0:1)                   | 5:2                        | 5:2                       |
| ADR (-:+)                  | 4:3                        | 3:4                       |

PS: performance status (ECOG), ADR: adverse drug reaction.

Phenotypic analyses of the PBMC. Although there was no significant difference in the CD4/8 ratio (Figure 1G), with S-1/CPT-11 treatment the Th1/2 ratio tended to decrease, while in contrast, tended to increase in LV/5-FU treatment, but the difference was not significant (Figure 1H). The CD4⁺CD25⁺ T cells were increased by S-1/CPT-11 treatment, but decreased by LV/5-FU treatment and this change was significantly different (p=0.0187) (Figure 1I).

Discussion

High-dose chemotherapy has not always been superior to low-dose chemotherapy in tumor treatment (17-23). Since low-dose chemotherapy is not as immunosuppressive as high-dose, it results in increased host anti-tumor immunity which aids in tumor eradication (21-23).

In this report, LV/5-FU as a low-dose chemotherapy and S-1/CPT-11 as a high dose therapy were compared in terms of their influence on host immunity over one treatment cycle. Two parameters, the sIL-2R and CD4⁺CD25⁺ T cells did show differences in reaction between the two regimens. An increase of sIL-2R by the S-1/CPT-11 treatment, and a decrease by LV/5-FU treatment were observed. Given that sIL-2R is produced by activated T and B cells (24-26), the serum level is an indicator of lymphocyte activation (24). The sIL-2R level has been shown to be elevated in patients with malignant lymphoma, hairy cell leukemia, adult T cell leukemia, autoimmune diseases, hepatitis B and C and solid tumors (27-37). Additionally, elevated serum sIL-2R levels were observed in pediatric oncology patients with febril neutropenia (38) and sIL-2R have been reported to correlate to nutritional insufficiencies and poor prognosis in patients with cancer cachexia (39). Among many suppressive factors, sIL-2R might be one of the important effectors in developing immune suppression in the patients with advanced malignant diseases (39-41). Thus an

Cytokine production of the PBMC and serum sIL-2R levels. With S-1/CPT-11 treatment the Th2 cytokine, IL-10 tended to increase, whereas it tended to decrease with LV/5-FU treatment however, the difference was not significant. The Th1 cytokines, IL-12 and IFN-γ levels did not differ significantly between the S-1/CPT-11 and LV/5-FU treatments after the first chemotherapy cycle. The changes in the pro-inflammatory cytokines, IL-6 and TNF-α were also not significantly different (Figure 1A-E). An increase of sIL-2R by S-1/CPT-11 treatment, and a decrease by LV/5-FU treatment were observed. This change in the first time chemotherapy was significantly different (p=0.0722) (Figure 1F).

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increase of sIL-2R by the S-1/CPT-11 treatment indicates immune suppression induced by this regimen. In addition, the LV/5-FU treatment could reduce immune suppression.

Similarly the CD4+CD25+ T cells were increased by S-1/CPT-11 treatment, and it decreased by LV/5-FU treatment. Sakaguchi et al. were the first to promote interest in "regulatory" T cells (T_reg cells) by identifying a population of CD4+ T cells highly expressing CD25 and which prevented autoimmunity in a murine model (42, 43). Numerous reports in the following years have revealed major aspects of T_reg cell biology, characterizing different T cell subpopulations with regulatory properties including naturally occurring CD4+CD25^{high} T cells and induced T_reg cells, such as Tr1 and TH3 cells, as well as CD4+CD25^{high} T cells developed in the periphery by conversion of CD4+CD25^{-} T cells. All these different T-cell populations with regulatory function coexist and contribute to immune suppression (44-47). Woo et al. have reported increased percentages of CD4+CD25^{high} T_reg cells in tumor infiltrating lymphocytes in non-small cell lung cancer and ovarian cancer (48). A larger study concluded that the prevalence of CD4+CD25^{high} T_reg cells is increased not only in the tumor microenvironment of patients with invasive breast or pancreas carcinoma but also in the peripheral blood, suggesting that the increase of T_reg cells is a generalized phenomenon (49). In patients with gastrointestinal malignancies, the relative increase of T_reg cells might actually be explained by a significant reduction of CD4+CD25^{-} T cells. Interestingly, in patients with gastric carcinoma, poor prognosis and decreased survival rates were closely
correlated with higher $T_{reg}$ cell frequencies (50, 51). Curiel et al. demonstrated that CD4+CD25high $T_{reg}$ cells suppress tumor-specific T-cell immunity, contribute to tumor growth, and accumulate during progression in ovarian cancer (52). Because CD4+CD25high $T_{reg}$ cells and the CD4+CD25+ $T$ cells that were analyzed in this study are not exactly the same cells, further investigation is needed. Our data suggested that S-1/CPT-11 treatment can reduce tumor immunity while LV/5-FU can maintain it. S-1 or CPT-11 alone would not influence anti-tumor immunity (53, 54).

The current study indicated that there was either no effect or an improvement to host immunity given by low-dose LV/5-FU treatment as compared to S-1/CPT-11 treatment. The low-dose LV/5-FU treatment should be considered for metastatic colorectal cancer from a point of view of maintaining a host immunity during chemotherapy.

References


27. Pui CH, Ip SH, Kung P, Dodge RK, Berard CW, Crist WM and Murphy SB: High serum interleukin-2 receptor levels are


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