The Clinical Significance of Neoadjuvant Chemotherapy for Colonic Cancer
— Evaluation with Thymidylate Synthetase Inhibition Rates and Effects on Tissues —

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Summary

The usefulness of neoadjuvant chemotherapy of antitumor agent HCFU was evaluated in ten patients with colonic cancer using, as indices, TSIR and effects on tissues. There were significant differences in TSIR between parts of oral or anal to the incision and normal mucous membrane. As for effects on tissues, specimens from five patients were found in grade 0 and those from other five in grade II A. The TSIR of patients whose tissues showed negative effects was 37.0±6.68%, while the TSIR of patients with positive effects was 58.3±24.35% which was significantly higher than the former. The mean dose of the antitumor agent administered to patients with negative effects was 5.7±0.36 g, while that to patients with positive effects was 11.0±4.74 g which was significantly larger than the former. Although there were no correlations between TSIR and tissue effects, it was considered that the antitumor agent could be expected to be clinically effective in patients whose TSIR were higher than 50%, as the TSIR of patients with positive tissue effects were 58.3%. However, details must be studied on problems such as conditions in analyses, correlations between TSIR and tissue effects, evaluation of tissue effects, and correlations between preoperative antitumor treatments and postoperative outcomes. Furthermore, accumulation of cases with clinically effective TSIR and long-term follow up of patients are needed.

Introduction

Although clinical effects of preoperative chemotherapy have been studied in Japan since 1974[1,2], the analysis of thymidylate synthetase (TS), the key enzyme in the metabolism, has only recently been reported[3-9]. In the present study, the usefulness of preoperative, oral anti-tumor agents was evaluated with TS inhibition rates (TSIR) and tissue effects in patients with colonic cancer who were given 1-hexylcarbamoyl-5-fluorouracil (HCFU), a masked compound of 5-fluorouracil (5-FU).

Materials and methods

Ten patients with colonic cancer (average
age: 56.7 years old, 7 males and 3 females) who underwent surgery at our hospital were used as subject. Dose of HCFU administered preoperatively was 4.2 to 16.2 g (average 8.36 g), and periods from the discontinuation of the agent to collection of specimens were 4 to 42 hours (average 11.2 hours). Specimens were collected and frozen immediately after the resection of tumors. After vovine serum albumin folic acid and excess 3H-5'-fluorodeoxyuridine monophosphate (3H-FdUMP) were added to specimens, the mixture was incubated at 30°C for 20 minutes. Following centrifugation (3000 rpm. 15 min), radioactivity of the binding complex of 3H-FdUMP-TS-methyltetrahydrofolic acid in supernatant fluid was measured to calculate TSIR in logistics. Concentrations of 5-FU were analyzed with Gas Chromatographic Mass Fragmentographic Method (GCMF). Specimens were collected from oral side of the tumor, anal side, lymph nodes and normal mucous membrane.

Significant differences were determined in student t-test with a significance level of p<0.05.

Results
1. Clinicopathological background
In the classification of sites of tumors, there were two A, one T, two D, four S and one Ra, with average diameters of 52.4×39.8 cm. In macroscopic types, seven localized ulcer type and three of ulcerative infiltration type. Advancement of degree was found at stage I in three tumors, stage II in three, stage IIIa in one and stage IV in three. All tumors were well differentiated adenocarcinomas in terms of the histogical type.

2. Quantity of total TS and free TS at different parts of tumors
Quantity of total TS were 10.88±8.02 pmol/g at the part of tumor proximal to the incision, 9.42±5.27 pmol/g at the part proximal to the anus, 9.0±3.99 pmol/g at lymph nodes and 4.17±2.99 pmol/g at normal mucous membrane. There were significant differences between total TS at normal membrane and other sites. Quantity of free TS were, at respective sites, 5.78±5.48, 4.42±2.53, 5.12±2.94 and 2.78±2.04 pmol/g. There was no significant difference between sites.

3. TSIR and concentrations of 5-FU at different parts of tumors
The TSIR was 47.5±24.2 % at the part of tumor proximal to the incision, 47.8±20.8 % at the part proximal to the anus, 38.5±25.4 % at lymph nodes, and 25.5±21.1 % at normal mucous membrane. There were significant differences between parts of tumor proximal to the incision or the anus and normal mucous membrane.

Concentrations of 5-FU were, at respective sites, 0.33±0.51 μg/g, 0.37±0.63 μg/g, 0.11±0.82 μg/g and 0.16±0.27 μg/g.

There were large differences between individual patients (Fig. 1, 2).

4. Tissue effects
Effects on tissues were determined according to the Classification by Ooboshi and Shimomato11. Specimens from five patients were found to be at grade 0, and those from other five patients at grade II A. There was no clinicopathological characteristics among patients whose tissues showed effects.

5. Tissue effects and differences in TSIR and dose of HCFU

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While the TSIR of patients with negative tissue effects was $37.0 \pm 6.68\%$, that of patients with positive tissue effects was $58.3 \pm 24.35\%$, which was significantly higher than the former. The mean dose of HCFU administered to patients with negative effects was $5.7 \pm 0.36\, \text{g}$, while that to patients with positive tissue effects was $11.0 \pm 4.74\, \text{g}$, which was significantly higher than the former (Fig. 3, 4).

Discussion

Neoadjuvant chemotherapy is aimed to reduce the size of tumors by administering medications that are delivered to lesions via blood vessels before they are destroyed. The clinical significance of neoadjuvant chemotherapy is
that it allows easier surgical maneuvering because of reduced size of tumors, suppression of mechanical metastasis during surgery, suppression hematogenous micrometastasis and determination of anti-tumor agents sensitivity. The principal anti-tumor effect of 5-FU class of agents is inhibition of DNA synthesis. Although correlation of TSIR with antitumor effects has been studied\(^3\)-\(^6\), consistent results have not necessarily been obtained. This stems from the problems such as conditions in analyses, correlation between TSIR and tissue effects, evaluation of tissue effects, and correlation from preoperative treatments to postoperative outcomes and these problems must be further examined.

Regarding the analysis of TSIR, Kameyama et al\(^7\) studied effects of times from collection of specimens to freezing. As time passed, FdUMP binding TS was measured as free TS, resulting in lower TSIR. After 30 minutes, there were notable decreases in TSIR. Thus, they stated that a constant time should have been established between collection of specimens and freezing. Therefore, specimens in the present study were collected and frozen immediately after tumors were resected in order to set a constant condition.

Dose of anti-cancer agents should be 15 to 30 g or more, if direct effects are to be expected, and it is thought that there are correlations between tissue effects and dose of agents\(^3\)-\(^9\). However, there are various inconsistent results in studies on TSIR and tissue effects, which are pointed out to be probably due to dose of agents, duration of administration, intervals between administration and collection of specimens, all of which affect the pharmacodynamics of 5-FU, velocity of absorption and disappearance and distribution of agents into tissues\(^1\)-\(^3\). Kotake et al\(^3\) found in studies on tissue effects of Tegafur\(^\circledR\) suppository used as induction therapy that 12 out of 23 cases demonstrated tissue effects of grade 1, and tissues were affected by concentrations of the agent in tissues. The 5-FU concentration was an important factor as an index that the agent reached tissues, although there were large differences in concentrations among individual cases. In the comparison of TSIR between grade 0-group and grade 1-group, the former was 18.2±12.2 % and the latter 30.0±17.4 %. The latter was significantly higher than the former, suggesting tissue effects correlate with the TSIR and the correlation could be an index of efficacy of drugs. In other studies, however, TSIR were found not to correlate with tissue degeneration\(^8\)-\(^10\). More cases are needed for further studies. Namatame et al\(^8\) reported that administration of 5-FU 400 mg for five days produced TSIR at 46.7±7.11 %, which was the clinically effective inhibition rate. Usuiyama\(^4\) found the TSIR of a group of negative tissue effects was 41.6±2.2 %, while that of a group of positive tissue effects was 55.5±2.1 %, suggesting clinical effects could be expected when the TSIR was 50 % or higher. In the present study, the TSIR of a group of positive tissue effects was 58.3 %, suggesting clinically effective TSIR would be 50 % or higher, although there was no correlation between TSIR and tissue effects.

As to criteria of evaluation of tissue effects, it is reported colonic cancer which tends to necrosis may require a new classification of degeneration of cancer cells\(^11\), as effects of grade 2 are sometimes found in patients who do not receive anti-cancer agents. Tissue effects according to the conventional criteria alone are insufficient for evaluation, and it is believed that more accurate evaluation of efficacy can be
made with the new criteria including TSIR and dose.

Hayashida et al. studied the relationship between chemotherapy and prognosis and found the five-year survival rate of patients who received FT-207® was somewhat better, but not significantly, than that of patients who did not receive the agent. However, the recurrence rate in no cancer group was significantly lower in patients who received the agent than in those who did not. Shinohara et al. examined prognosis and FdUMP level, and found the FdUMP level of a group of bad prognosis, i.e. death or survived with recurrence, was $2.1 \pm 1.1 \text{pmol/g}$, while the FdUMP level of a group of good prognosis, i.e. survived without recurrence was $4.9 \pm 2.1 \text{pmol/g}$, which was significantly higher than the former. The authors suggested that FdUMP level was useful as an index of prognosis.

Cases of clinically effective TSIR should be accumulated more, and studied in further detail on them as well as long-term prognosis are needed.

Conclusion

1. The TSIR was highest at the part of tumors proximal to the anus. There was a significant difference in TSIR between tumors and normal mucous membrane, but there was no correlation between TSIR and dose of HCFU.

2. Tissue effects correlate with dose of agent more than TSIR. In a group of positive tissue effects, the TSIR was $58.3 \pm 24.35 \%$ and dose of HCFU given was $11.0 \pm 4.74 \text{g}$.

References