Pharmacokinetics of 5-Fluorouracil Following Hepatic Intra-arterial Infusion in a VX2 Hepatic Metastasis Model

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Background: Hepatic intra-arterial infusion chemotherapy of 5-fluorouracil (5-FU) or fluorodeoxyuridine (FUDR) has been a treatment option for liver metastasis from colorectal cancer. However, an optimal administration schedule of 5-FU is still controversial. This study was conducted to evaluate a suitable schedule from the viewpoint of 5-FU metabolites and related enzymes.

Methods: 5-FU was infused into the hepatic artery of rabbits having hepatic deposits of VX2 tumor cells in a daily dose of 1, 4, or 8 mg/kg using various schedules. 5-FU, Thymidylate synthase (TS), TS inhibition rate (TSIR), and the amount of fluoro-RNA (F-RNA) were measured.

Results: A high concentration of 5-FU was detected in the tumors of the group that was administered a dose of 8 mg/kg. TSIR in the tumor was about two-fold higher in the rabbits that were administered a total dose of 8 mg/kg than in those that were administered doses of 4 mg/kg or less. F-RNA, ranging from 27 to 36 ng/mg RNA, was detected in the tumor of the rabbits that were administered a total dose of 8 mg/kg. No difference was observed between the short period and the continuous administration schedules of rabbits that were administered a dose of 8 mg/kg of 5-FU. However, DNA synthesis inhibition in normal hepatic tissue was more dependent on the administration schedule than on the total dose of 5-FU because TSIR was significantly higher with shorter periods of drug administration.

Conclusion: Intermittent bolus administration of large doses of 5-FU might cause more severe hepatic impairment than continuous administration. These results suggest that hepatic intra-arterial infusion of 5-FU should be administered continuously for liver metastasis, although further experiments including a longer administration period of 5-FU are required.

Key words: 5-fluorouracil – hepatic intra-arterial infusion – liver metastasis

INTRODUCTION

Hepatic intra-arterial infusion chemotherapy of 5-fluorouracil (5-FU) or fluorodeoxyuridine (FUDR) have been extensively used for liver metastasis from colorectal cancer to achieve better anti-tumor effects and to minimize systemic adverse reactions (1). Both short-term and continuous intra-arterial infusions have been used, but no conclusions have been arrived at with regard to which method is better with respect to anti-tumor effects, and prevention of adverse reactions.

Various methods have been reported for the intravenous administration of 5-FU. In a randomized study for advanced colorectal cancer, the efficacy of 5-FU was greater with continuous intravenous infusion than with intermittent bolus infusion (2–5). The adverse reactions differ based on the method of administration. Mucosal disorders, such as the hand-foot syndrome are more often associated with the continuous infusion, and myelosuppression such as leucopenia, are more commonly associated with bolus administration (6).

In the present study, we investigate the anti-tumor effects, and adverse reactions according to the various intra-arterial administration schedules of 5-FU from the viewpoint of 5-FU metabolites, and 5-FU-related enzymes, in a hepatic metastasis model.

PATIENTS AND METHODS

ANIMALS AND PREPARATION OF THE METASTASIS MODEL

A suspension containing 10⁷ VX2 tumor cells was inoculated by subcapsular injection into the liver of white rabbits weigh-
ing 2.5 to 3.0 kg. The animals were examined on day 14 after the inoculation. A catheter was inserted via the gastric artery and connected to a pump for hepatic intra-arterial drug administration. Food and water were provided ad libitum.

**INTRA-ARTERIAL INFUSION OF 5-FU**

The rabbits were divided into groups of six. The control group was untreated, receiving only physiological saline for 24 h. A1 and A3 groups were administered doses of 5-FU 1 mg/kg over 24 h, for 1 day and 3 days, respectively. B1 and B2 groups were administered doses of 5-FU 4 mg/kg over 24 h, for 1 day and 2 days, respectively. C1 and C’1 groups were administered doses of 5-FU 8 mg/kg, for 1 day and 2 hours, respectively. In the C’1 group, physiological saline was infused continuously for 22 h following a 2 h infusion of 5-FU. Groups A1, B1, and C1 were used to compare the effect of the 5-FU dose. The A1 and A3, and B1 and B2 groups were used for comparison based on the administration period. The B2, C1, and C’1 groups, that were administered the same dose, were used to compare the administration methods (Fig. 1).

**MEASUREMENT OF ENZYME ACTIVITY**

Tumor, and normal hepatic tissue specimens were collected on completion of drug administration and immediately frozen at −80°C, until assay. Tissue concentrations of 5-FU, thymidylate synthase (TS) (total and free), and fluoro-RNA (F-RNA) were measured. The 5-FU concentration was measured using HPLC, the TS level (total and free) by radiobinding assay, and the F-RNA by the GC-MS method (7–9). The extent of TS inhibition rate (total TS – free TS/total TS) was calculated and expressed as a percentage. In the control group, the activity of the drug-metabolizing enzyme dihydropyrimidine dehydrogenase (DPD) in normal hepatic and tumor tissues was measured by the radio-HPLC method (10). Blood was collected from each animal just before the completion of drug administration, and the 5-FU serum concentration was measured by HPLC.

**STATISTICAL ANALYSIS**

One-way, ANOVA, and Dunnett’s multiple method, were used for statistical analyses. P < 0.05 were considered statistically significant.

**Table 1.** The effect of the 5-FU dose and administration schedules on 5-FU levels and TS

<table>
<thead>
<tr>
<th>Group</th>
<th>5-FU Dose</th>
<th>Serum 5-FU (ng/ml)</th>
<th>Tissue 5-FU (ng/g)</th>
<th>Total TS (pmol/g)</th>
<th>TS Inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>Tumor</td>
<td>Liver</td>
<td>Tumor</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.9 ± 0.7</td>
<td>138.8 ± 23.9</td>
</tr>
<tr>
<td>A1</td>
<td>1 mg/kg/24 h</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td></td>
<td>5.4 ± 1.3</td>
</tr>
<tr>
<td>A3</td>
<td>3 mg/kg/72 h</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>4.5 ± 1.2</td>
<td>6.9 ± 1.3</td>
</tr>
<tr>
<td>B1</td>
<td>4 mg/kg/24 h</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>5.0 ± 2.4</td>
<td>6.0 ± 0.5</td>
</tr>
<tr>
<td>B2</td>
<td>8 mg/kg/48 h</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>20.8 ± 12.2</td>
<td>5.2 ± 2.2</td>
</tr>
<tr>
<td>C1</td>
<td>8 mg/kg/24 h</td>
<td>9.7 ± 7.7</td>
<td>4.7 ± 1.6</td>
<td>322.0 ± 290.6b</td>
<td>6.6 ± 1.6</td>
</tr>
<tr>
<td>C’1</td>
<td>8 mg/kg/2 h</td>
<td>20.3 ± 15.2c</td>
<td>6.2 ± 4.9</td>
<td>289.6 ± 150.8c</td>
<td>8.4 ± 1.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.01 C’1 vs. A1, A3, B1 and B2; 1P <0.01 C’1 vs. A1, A3, B1 and B2; †P <0.01 C’1 vs. A1, A3, B1 and B2; ‡P <0.05 vs. control; §P <0.01 C’1 vs. C1; ¶P < 0.05 vs. control; ‡‡P <0.05 C1 vs. A1 and B1.
RESULTS

5-FU CONCENTRATIONS (TABLE 1)
Serum 5-FU concentrations were low in the 1-day 5-FU administration groups (A1, B1, and C1) and in the different administration period groups (A1 and A3, B1 and B2), but were significantly higher value with shorter administration (C1 vs. A1, A3, B1, and B2, \( P < 0.01 \)). The 5-FU concentrations in normal hepatic tissue were not significantly different among all groups. The 5-FU concentrations in the tumor showed no significant difference based on the administration period, but showed a significant difference based on the 5-FU dose (C1 vs. A1 and B1, \( P < 0.01 \)), and administration method (C1 vs. B2, C’1 vs. B2, \( P < 0.01 \)).

TOTAL TS CONCENTRATION (TABLE 1)
In all the groups, the total TS concentration was significantly higher in the tumor than in the normal hepatic tissue. However, there was no significant difference in the total TS concentrations in both the tumor and normal hepatic tissue, among all groups.

TSIR (TABLE 1)
The TSIR in normal hepatic tissue in the control group was significantly lower than in the 5-FU administration groups (\( P < 0.05 \)). The values were similar among 1-day 5-FU administration groups (A1, B1 and C1). The values tended to increase with longer administration period (A1 vs. A3, B1 vs. B2), but the difference was not statistically significant. A comparison of the values among groups with different administration methods showed a significant difference between groups C’1 and C1 (\( P < 0.01 \)).

The TSIR in the tumor was significantly lower in the control group than in the 5-FU administration groups (\( P < 0.05 \)). The value was significantly higher in group C1 than in groups A1 and B1 (\( P < 0.05 \)). The difference was not statistically significant among groups with different administration periods (A1 vs. A3, and B1 vs. B2), and among groups with different administration methods (B2, C1, and, C’1).

F-RNA (Fig. 2)
In all the groups, the F-RNA concentrations in normal hepatic tissue were low or below the detection limit (4 ng/mg RNA). The F-RNA concentrations in the tumor were below the limit of detection in the A1 and B1 groups, but the C1 group showed a significantly elevated value of 32.0 ± 23.1 ng/mg RNA (C1 vs. A1, and B1, \( P < 0.01 \)) (Fig. 2-I). In group B2, the amount of F-RNA was 35.5 ± 27.1 ng/mg RNA, which was significantly higher than in group B1 (\( P < 0.01 \)) (Fig. 2-II). There was no significant difference among groups with different administration methods (B2, C1, and C’1) that received a dose of 8 mg/kg of 5-FU (Fig. 2-III).

DPD ACTIVITY
In the control group the DPD activity was significantly lower in the tumor (83.3 ± 55.0 pmol/min, per milligram of protein) than in normal hepatic tissue (585.5 ± 36.6 pmol/min per milligram of protein) (\( P < 0.01 \)).

DISCUSSION
Various methods, including continuous and bolus intravenous injection, and short-term and continuous intra-arterial infusions, have been reported for the administration of 5-FU to patients with liver metastases from colorectal cancer. No conclusions have been arrived at with regard to which method is the most suitable. In randomized comparisons of continuous intravenous and hepatic intra-arterial infusions of 5-FU or fluorodeoxyuridine (FUDR) for liver metastases, the efficacy of the intra-arterial infusion was significantly higher than that of continuous intravenous infusion (11–14). These results are
not surprising because the blood supply to liver metastasis is mainly provided by the hepatic artery when the tumor is as small as 2 mm (15). Systemic adverse reactions should also be less with intra-arterial infusion than with intravenous infusion.

FUDR is more extensively used for hepatic-arterial infusion chemotherapy in western countries because the first-pass extraction of FUDR is greater than 5-FU. 5-FU was used in the present study because FUDR cannot be used in Japan due to National Health Insurance restrictions. In Japan a regimen for hepatic intra-arterial infusion of 5-FU at 1000 mg/m², once a week for 5 h, has been extensively used for outpatients with liver metastases from colorectal cancer (16). Further clinical trials are required to determine the most appropriate method from the viewpoint of the dynamics of 5-FU metabolic enzymes, and the pharmacokinetics of 5-FU, because according to their method 5-FU is administered for only 5 h of the 168 h in a week according to their method.

Other studies have shown higher concentrations of 5-FU and fluorodeoxyuridine monophosphate (FdUMP), and higher total TS and TSIR in liver metastases than in normal hepatic tissue after a bolus injection of 5-FU at 500 mg/m² (2,3). Inaba et al. showed in an in vitro study that RNA impairment via FUTP occurs in the presence of high 5-FU concentrations for a short period, whereas DNA synthesis inhibition appears via FdUMP in response to low concentrations for a long time (17). However, the pharmacodynamics and pharmacokinetics of hepatic intra-arterial infusion have not been adequately studied. In the present study, the TSIR in the tumor was about two-fold greater in the animals given a total 5-FU dose of 8 mg/kg than in those receiving a total dose of ≤4 mg/kg. There was no difference that could be attributed to the administration method in the higher-dose groups, it appears that DNA synthesis inhibition is dependent on the total dose of 5-FU also in the intra-arterial infusion.

Weekly 5-FU administration has been reported to increase the total TS level in colon 26 tumor model (18). In the present study, no significant difference was observed in the total TS level of the tumor, between the 5-FU administration groups and the control group. It was assumed that a much longer 5-FU administration period was necessary for significant TS induction. The maximal duration of hepatic intra-arterial infusion of 5-FU was set at 3 days, because infusion of 5-FU for 4 days or longer was intolerable for rabbits.

The amount of F-RNA in the tumor was low or below the detection limit in the groups given a total 5-FU dose of ≤4 mg/kg, but it was in the 27 to 36 ng/mg range in the 8 mg/kg groups. There was no significant difference that could be attributed to the administration methods in the animals that were administered a dose of 8 mg/kg. Therefore, the extent of RNA impairment is similarly dependent on the total dose of 5-FU. It has been reported that the TSIR, and amount of F-RNA in the tumor, decrease in 5-FU-resistant strains (19), therefore, these measures are considered appropriate for evaluation of the anti-tumor effects of 5-FU.

It has also been reported that TS inhibition in normal tissue is a determinant of the adverse reactions to 5-FU (20). The present results showed that DNA synthesis inhibition in normal hepatic tissue is more dependent on the period of administration, than on the total drug dose. Therefore, it was assumed that in normal hepatic tissue the intermittent administration of large doses of 5-FU causes more severe hepatic disorders than continuous administration.

In the control group, the DPD activity in the tumor was about one sixth of that in normal hepatic tissue. Catabolism by DPD is generally considered to account for 80% to 90% of the metabolism of 5-FU. A 5-FU dose administered by a hepatic intra-arterial infusion is incorporated directly into each tissue, and is catabolized by DPD. In groups that were administered doses of ≤4 mg/kg of 5-FU a day, the drug could not be detected either in normal hepatic tissue or in the tumor. However, higher concentrations of the drug were detected in the tumors in the groups given a total dose of 8 mg/kg. These results suggest that the activity of DPD in the tissue might play an important role in the efficacy of 5-FU. When 5-FU is administered, it is important to use a dose that will withstand DPD activity in the tumor tissue.

At present, the range of chemotherapeutic agents is widening with the launch of new drugs such as CPT-11 and oxaliplatin. However, 5-FU or FUDR continues to be the basic chemotherapeutic agent for colorectal cancer, especially in patients with liver metastases. Therefore, it is extremely important to define the most appropriate dose and administration schedule for hepatic intra-arterial infusion of 5-FU, on the basis of its pharmacologic properties. The present study suggests that hepatic intra-arterial infusion of 5-FU should be administered continuously for liver metastasis, although further experiments including longer administration period of 5-FU are required.

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References


