Antitumor Activity of 1 M Tegafur–0.4 M 5-Chloro-2,4-dihydroxypyridine–1 M Potassium Oxonate (S-1) against Human Colon Carcinoma Orthotopically Implanted into Nude Rats

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ABSTRACT

The purpose of this study was to establish a nude rat orthotopic (organ-specific) human colorectal cancer model as an in vivo secondary screen for general evaluation of new anticancer agents against colorectal cancer and to evaluate practically the antitumor activity of 1 M tegafur–0.4 M 5-chloro-2,4-dihydroxypyridine–1 M potassium oxonate (S-1), a new p.o. fluoropyrimidine, in comparison to 1 M tegafur–4 M uracil ([UFT] effective on colorectal tumor in clinical). After implantation of KM12C, a human colorectal cancer cell line, into the subserosal layer of the colon as a single-cell suspension, extensive local tumor growth and invasion to both the mucosal and the serosal sides were observed in all rats. Metastatic foci were also formed in both lymph nodes and lungs following local tumor growth in all of them. Using this method, an equitoxic dose of S-1 (15 mg/kg/day) and UFT (30 mg/kg/day) was administered p.o. for 14 consecutive days from 7 days after tumor cell implantation. S-1 showed a higher tumor growth inhibition than UFT did (S-1, 57% (significantly different from the tumor weight of the untreated group at P < 0.05) and UFT, 18% (P > 0.05)). When both drugs were administered to nude rats bearing KM12C injected into the cecal wall for 28 consecutive days at equitoxic doses, the mean survival in the S-1 group was 16 days longer than that in the untreated group (P < 0.01) but that in the UFT group was only 8 days longer (P > 0.05). After the administration of an equitoxic dose of both drugs, S-1 gave the higher levels than UFT in various pharmacokinetic parameters as follows: area under the curve 0–24 h of 5-fluorouracil in plasma (3.5-fold), area under the curve 0–24 h of 5-fluorouracil incorporated into RNA in the tumor (1.3-fold), and thymidylate synthase inhibition rate (percentage) in the tumor (about 20%). Collectively, these findings suggested that this orthotopic human colorectal tumor model in nude rats is useful to evaluate the clinical therapeutic efficacy of drugs or therapies for colorectal cancer, and that S-1 had a higher therapeutic effect on human colorectal tumor than UFT did.

INTRODUCTION

Fluoropyrimidines have been widely used clinically in the treatment of solid tumors (1–3) since 5-FU(2) was first synthesized in 1957 (4). Because of both a good antitumor efficacy of 5-FU on gastrointestinal tract tumors as a single agent and a high incidence of gastrointestinal tract cancer cases in Japan, many attempts have been made to develop new superior 5-FU derivatives to the existing fluoropyrimidines. However, sufficient therapeutic efficacy of such drugs has not been obtained yet.

More recently we developed S-1, a new antitumor agent based on biochemical modulation of 5-FU, consisting of FT, CDHP, and Oxo in a molar ratio of 1:0.4:1 (Fig. 1). FT, which is a produrg of 5-FU, plays a role as an effector. Both CDHP and Oxo which do not have antitumor activity themselves play roles as modulators. CDHP competitively inhibits dihydroxyimidine dehydrogenase (EC 1.3.1.2), which degrades 5-FU, about 180 times more effectively than uracil in vitro (5), leading to the retention of a prolonged concentration of 5-FU in blood (6). Oxo competitively inhibits pyrimidine phosphoribosyl-transferase (EC 2.4.2.10), which converts 5-FU to 5-fluorouridine 5’-monophosphate in vitro (7). Oxo is mainly distributed in the gastrointestinal tract after p.o. administration to rats, leading to relief of gastrointestinal toxicity induced by 5-FU (7). S-1 showed a better therapeutic effect on various rat tumors and human xenografts than other p.o. fluoropyrimidines (6).

Most human tumor xenograft studies, including colorectal tumors, for the evaluation of antitumor effects of drugs utilized s.c. implantation systems for reasons of convenience and an allowance of direct quantitation of growth and therapeutic effects (8–10). However, those models have limitations for the study of the interaction of tumor cells with their relevant organ environment or organ distribution of drugs.

Recently, there has been increased interest in the use of in vivo models for the propagation of human tumors at organ-specific (orthotopic) sites in athymic nude mice, including renal cell carcinoma (11), colorectal carcinomas (12–15), lung carcinomas (16, 17), prostate cancer (18), breast cancer (19), and pancreatic carcinomas (20). According to the “seed and soil” hypothesis proposed by Paget (21), orthotopic implantation of tumor cells is essential for optimal growth and progression of tumors in vivo. However, whether such orthotopic human colorectal tumor models apply to the evaluation of new anticancer agents remains unknown.

In the present study, the orthotopic human colorectal tumor model in athymic nude rats has been used to clarify the antitumor effect of S-1 in comparison to that of UFT, which is composed of FT and uracil in a molar ratio of 1:4 and widely used as a p.o. antitumor agent in Japan (22–25). In addition, 5-FU levels in plasma, 5-FU levels incorporated into the RNA fraction (F-RNA), and TSIR in both tumor and intestinal tissue were examined to determine the mode of action of CDHP and Oxo.

MATERIALS AND METHODS

Preparation of Drugs. FT, CDHP, and Oxo were synthesized in the Taiho Pharmaceutical Co. (Tokyo, Japan). Uracil was purchased from the Yamasa Co. (Chiba, Japan). [6-3H]FdUMP (551 GBq/mmol) was enzymatically synthesized from [6-3H]5-fluorodeoxyuridine (New England Nuclear, Boston, MA). All other chemicals used were the highest grade of standard commercial products. S-1 was prepared by mixing with FT, CDHP, and Oxo in a molar ratio of 1:0.4:1. UFT was prepared by mixing with FT and uracil in a molar ratio of 1:4. S-1 was dissolved in a 0.5% (w/v) HPMC solution. UFT was suspended in a 0.5% HPMC solution because of the insolubility of uracil. Since the active component in both S-1 and UFT is FT, the amount of only FT
in both drugs was mentioned as their dosage. The dose of each drug for in vivo treatment was set to the equitoxic level, i.e., S-1, 15 mg/kg/day, and UFT, 30 mg/kg/day, in a volume of 0.1 ml/kg and was administered once daily.

Animals. Female F344/N-nu rats were purchased from CLEA Japan, Inc. (Tokyo, Japan) and were free of known pathogens at the time of study. All procedures for in vivo experiments were performed in a specific pathogen-free barrier in our laboratory.

Tumor Cell Line and Culture. The human colorectal adenocarcinoma line KM12C (26) was provided by Dr. K. Morikawa (Iwamizawa Worker’s Compensation Hospital, Hokkaido, Japan). Tumor cells were maintained in Eagle’s MEM (ICN Biomedicals, Inc., Cleveland, OH) supplemented with 10% fetal bovine serum (Cell Culture Technology, Toronto, Ontario, Canada), 0.1 mm sodium pyruvate, 1% nonessential amino acids, 2 mML-glutamine, and 0.9% NaCl solution for injection. Only single-cell suspensions with greater than 95% viability (tested by trypan blue exclusion) were used for in vivo injection.

Intracolonic Implantation of Tumor Cells and Inhibition of Tumor Growth by Drugs. The technique performed was based on the method of Morikawa et al. (27). Namely, nude rats were anesthetized with diethyl ether, and the abdomen was prepared for sterile surgery. The colon was exteriorized, and KMI2C cells (1 X 106/0.025 ml) were injected into the colonic wall located at about 5 cm above the anus from the serosal side with a 30-gauge needle. The colon was returned to the abdominal cavity, and the wound was closed with metal clips. All rats survived after surgery. Rats were randomized into treatment or control groups (10 rats) based on body weight on 7 days after tumor cell injection. The drugs were administered p.o. for 14 consecutive days, from day 7 to day 20. The control group was given a 0.5% HPMC solution only, according to the same schedule. On day 21, body weight was determined just before they were killed with diethyl ether, and the tumors were removed and weighed. Tumor growth inhibition as an antitumor effect and body weight were confirmed by macroscopic necropsy (Fig. 2A), and then extensive local tumor growth and invasion both inward and outward were observed in all rats (Fig. 2, B-D). Metastases to mesenteric lymph nodes and lungs (Fig. 2E) following local tumor growth were found in all of them. Metastases to liver were not found at all. Metastatic tumors were histologically the same as the primary intracolonic tumor. The aspect of tumor growth and metastasis after the intracolonic injection of KM12C cells was similar to that after the intracolonic injection (data not shown).

In the treatment, the equitoxic dose was administered for 14 consecutive days. In fact, each drug brought the equivalent BWS on day 14. The mean tumor-inhibitory effect of S-1 was expressed as a percentage of the mean growth rate in control animals. For example, the mean tumor growth rate of 100% in control animals was equal to the mean tumor growth rate in treated animals.

RESULTS

Growth and Metastasis of KM12C After Implantation into the Colon or Cecum. Fig. 2 shows the local tumor growth and systemic metastasis after the intracolonic injection of KM12C cells. Four days after implantation, the first tumor mass within the bowel wall was confirmed by macroscopic necropsy (Fig. 2A), and then extensive local tumor growth and invasion both inward and outward were observed in all rats (Fig. 2, B–D). Metastases to mesenteric lymph nodes and lungs (Fig. 2E) following local tumor growth were found in all of them. Metastases to liver were not found at all. Metastatic tumors were histologically the same as the primary intracolonic tumor. The aspect of tumor growth and metastasis after the intracolonic injection of KM12C cells was similar to that after the intracolonic injection (data not shown).

The mean survival period of rats after the injection of KM12C cells into either the colon or cecum was 90.7 days (SD = 22.7, n = 3) or 55.0 days (SD = 4.8, n = 4), respectively. Necropsy revealed that the causes of death in these animals were bowel obstruction or a pulmonary death due to lung involvement.

Growth Inhibitory Effect of S-1 on KM12C Implanted into the Colon. Table 1 shows the antitumor activity of S-1 on the intracolonic KM12C tumor in comparison to that of UFT. In the treatment, the equitoxic dose was administered for 14 consecutive days. In fact, each drug brought the equivalent BWS on day 14. The mean tumor weight after S-1 treatment was less than one half of that in the control group, and this difference was statistically significant (P < 0.05). In addition, no lymph node metastases were observed in any of the 10 rats in the S-1 group. On the other hand, UFT did not effectively suppress tumor growth.

Elongation of the Life Span of Nude Rats Bearing KM12C Implanted into the Cecum by S-1. Table 2 shows the elongation of the life span of the intracolonic KM12C-bearing rats by S-1 in comparison to that by UFT. In the treatment, the equitoxic dose was administered for 28 consecutive days, when each drug brought approxi-
Table 1 Inhibitory effect of S-1 on the growth of KM12C human colon adenocarcinoma implanted into colon in F344/N-nu rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/day)</th>
<th>Body weight (g)</th>
<th>BWS (%)</th>
<th>Tumor weight (g)</th>
<th>Tumor growth inhibition (%)</th>
<th>Lymph node metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>136 ± 15c</td>
<td>0.36 ± 0.27c</td>
<td>57/10</td>
<td>2/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-1</td>
<td>15</td>
<td>129 ± 6</td>
<td>0.15 ± 0.07d</td>
<td>18/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UFT</td>
<td>30</td>
<td>129 ± 12</td>
<td>0.29 ± 0.23</td>
<td>18/10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a On day 21.
*b Mean ± SD.
*d Significantly different from the control at P < 0.05.

Table 2 Effect of S-1 on survival of F344/N-nu rats bearing KM12C human colon adenocarcinoma implanted into the cecum

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/day)</th>
<th>Body weight (g)</th>
<th>BWS (%)</th>
<th>Survival period (days)</th>
<th>Increase of life span (%)</th>
<th>Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>173 ± 13</td>
<td>73 ± 7</td>
<td>7/7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-1</td>
<td>15</td>
<td>158 ± 10</td>
<td>9</td>
<td>89 ± 8</td>
<td>22</td>
<td>6/6</td>
</tr>
<tr>
<td>UFT</td>
<td>30</td>
<td>161 ± 5</td>
<td>7</td>
<td>81 ± 10</td>
<td>11</td>
<td>7/7</td>
</tr>
</tbody>
</table>

*a On day 28.
*b Significantly different from the control at P < 0.05.

timately equivalent BWS. The mean survival period in the S-1 group was 16 days longer than that in the control group (P < 0.01), whereas that of the UFT group was only 8 days longer (P > 0.05).

5-FU Levels in Plasma after Administration of S-1. Fig. 3 shows 5-FU levels in plasma at various time points after the administration of the equitoxic dose of both drugs. The metabolic parameters of the S-1 group resulted in higher 5-FU levels than those of the UFT group as follows: AUC₀₋₂₄: S-1, 2.89 µg/mL; UFT, 0.83 µg/mL; Cmax: S-1, 0.92 µg/mL; UFT, 0.17 µg/mL.

F-RNA Levels in Tumor and Normal Colonic Tissue after Administration of S-1. Fig. 4 shows the F-RNA levels in tumor tissue implanted into the colon at various time points after the administration of the equitoxic dose of both drugs. The metabolic parameters of the S-1 group revealed a higher F-RNA level in tumor tissue than in the UFT group as follows: AUC₀₋₂₄: S-1, 2.80 µg/g tissue; UFT, 2.11 µg/g tissue; Cmax: S-1, 0.16 µg/g tissue; UFT, 0.12 µg/g tissue. Moreover, the F-RNA level in normal colonic tissue was also determined in the same animals in the S-1 group (Fig. 4). The AUC₀₋₂₄ value of F-RNA in tumor tissue was 1.3 times as much as that in normal colonic tissue (1.58 µg/g tissue).

TSIR in Tumor and Normal Colonic Tissue after Administration of S-1. Fig. 5 shows the TSIR in tumor tissue implanted into the colon at various time points after the administration of the equitoxic dose of both drugs. From 4 to 24 h after drug administration, TSIR in the S-1 group was about 20% higher than that in the UFT group.
Moreover, TSIR in normal colonic tissue was also determined in the same animals in the S-1 group (Fig. 5). From 2 to 8 h, TSIR in tumor tissue was 20–36% higher than that in the normal tissue.

DISCUSSION

Paget (21) originally proposed that human tumor cell populations require organ site-specific interaction for optimal maintenance and progression. This concept has been widely supported by numerous studies (11–20) including colorectal carcinoma (12–15).

Recently, we have established the colorectal tumor models by the implantation of the human colorectal adenocarcinoma line KM12C into the colon or cecum in nude rats. Since intracolonic tumors were much closer to clinical tumors than s.c. tumors from the view of the histology of tumor growth or metastasis (Fig. 2), this system was applicable to the evaluation of the tumor growth inhibitory effect by drugs (Table 1). The preparatory experiment to confirm the survival period of rats after the injection of KM12C cells into either the colon or cecum revealed that the deviation of survival time of intracally implanted rats (SD, 4.8 days) was smaller than that of intracolonic implanted rats (SD, 22.7 days); therefore, the intracalce implant system was used for the evaluation of the elongation of life span by drugs (Table 2). This system, however, did not seem to be applicable to the evaluation of the tumor growth inhibitory effect, since intracalceal tumor growth was too fast to be suppressed by drugs (data not shown).

Liver metastases, which we failed to produce in nude rats, are the most frequent occurrence in colorectal tumor patients. Morodomi et al. (32) have performed orthotopic implantation in nude mice and nude rats using the human colon carcinoma KM12SM, which is a subline of KM12C (27). They found liver metastases in 0 of 11 nude rats and found them in 57% of nude mice (12/21). Where metastases are formed may be due to a combination between host species and a feature of tissue distribution, i.e., distribution into normal tissue or tumor tissue in the intestinal tract, of the drug can be clarified. These features indicate that these orthotopic colorectal tumor models are useful in evaluating the clinical therapeutic efficacy of drugs or therapies on colorectal cancer.

Recently, a long-term continuous venous infusion of 5-FU has been used as an optimal schedule of 5-FU compared with a bolus injection of 5-FU (33–36), the prolonged retention of 5-FU in blood leading to a higher therapeutic effect. In this manner of treatment, the dose-limiting factor is not myelosuppression but gastrointestinal disorders such as diarrhea and mucositis (37, 38).

In the present study, S-1 inhibited tumor growth on KM12C implanted into the colon more than UFT at the equitoxic dose (Table 1). Moreover, S-1 showed a significant elongation of the life span of KM12C-bearing nude rats with implants in the cecum, although UFT did not (Table 2). The high antitumor activity of S-1 on orthotopic colorectal tumor was in accordance with the higher level of 5-FU in plasma (Fig. 3) and F-RNA (Fig. 4) and TSIR (Fig. 5) in the tumor. It is considered that these effects were due to CDHP, which is a 180 times more potent inhibitor of 5-FU degradation than uracil (5) to make the level of 5-FU in blood prolonged like continuous venous infusion of 5-FU (6).

It is interesting that the levels of F-RNA and TSIR in normal colonic tissue were lower than those in tumor tissue (Figs. 4 and 5). Shirasaka et al. (7) reported that Oxo was mainly distributed in the gastrointestinal tract after p.o. administration to Yoshida sarcoma-bearing rats, and that it inhibited the formation of 5-fluorouridine 5'-monophosphate and F-RNA from 5-FU in the small intestine and markedly reduced injury of the gastrointestinal tract and severe diarrhea without influencing the antitumor effect of UFT. These findings suggest that Oxo could selectively decrease the anabolism and the cytotoxicity of 5-FU in normal tissue of the intestine. Additional biochemical features of Oxo are under investigation in our laboratory. Collectively, these findings indicate that CDHP and Oxo gave S-1 a higher antitumor effect than UFT on orthotopic KM12C colorectal tumors. S-1 is expected to have a high therapeutic efficacy for the treatment of colorectal tumors clinically.

REFERENCES


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