Efficacy of Combination Chemotherapy Using Oral Fluoropyrimidine S-1 with Oxaliplatin (SOX) against Colorectal Cancer In Vivo

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Abstract. Oxaliplatin is effective when used with 5-fluorouracil (5-FU) and leucovorin, or with capecitabine (COX) for the treatment of colorectal cancer. In this experiment, we investigated the optimal combination schedule and antitumor activity of oral S-1 with oxaliplatin combination therapy (SOX) against human colorectal cancer xenografts in vivo. Using human colon cancer COL-1-bearing nude mice, oxaliplatin was administered at a total dose of 8.3 mg/kg on day 1 alone, on day 8 alone, or in divided doses administered on days 1 and 8 with S-1 (6.9 mg/kg, days 1-14). The antitumor activity of SOX, administered according to the divided schedule was significantly superior to both monotherapies (p<0.01), and the toxicity was tolerable. However, administration on day 8 alone failed to significantly increase the antitumor activity, when compared with that of monotherapy, while administration on day 1 alone was toxic in this model. Next, the efficacy of SOX was compared with that of COX (360 mg/kg, days 1-14). The antitumor effect of SOX was significantly superior to that of COX (p<0.01), with an equivalent toxicity; moreover SOX suppressed COL-1 tumor growth for a longer period of time (2.2 times) than did COX. The antitumor activity of SOX against the 5-FU-resistant colorectal cancer cell line KM12C/5-FU was equivalent to that of COX. The evaluation of intermittent SOX administration in a clinical trial might be of critical value.

Colorectal cancer remains the third-leading cause of cancer-related death in Japan. Recently, new agents such as camptotecin (1), oxaliplatin (2, 3), and targeted monoclonal antibodies (bevacizumab, cetuximab, panitumumab) (4-6) have been approved for clinical use. However, 5-fluorouracil (5-FU)-based combination chemotherapy remains one of the most effective therapeutic agents for colorectal cancer. Oxaliplatin-containing regimens, such as FOLFOX4 or mFOLFOX6, are often used clinically; however, these regimens require a 48-hour period of continuous intravenous infusion (c.v.i.) of 5-FU or leucovorin. FOLFOX4 is a combination chemotherapy regimen involving the bolus administration of oxaliplatin in combination with the bolus administration of 5-FU and leucovorin, followed by a continuous 5-FU infusion; this regimen is reportedly active in patients who have been previously treated with 5-FU alone or in combination with leucovorin (3). However, the FOLFOX4 regimen requires at least three days of hospitalization because of the 48-hour c.v.i. and is unsatisfactory from the perspective of quality of life (QOL). Oral fluoropyrimidine-containing regimens are more convenient, and combination therapy using capecitabine and oxaliplatin (COX) has been used against colorectal cancer. The antitumor effects of COX are equivalent to these of FOLFOX, while the treatment regimen is more convenient than that for the FOLFOX regimen (7). However, the incidence of hand-foot syndrome is reported to be as high as 13% for grade 2 and 26% for grade 1, which is significantly higher than that for the FOLFOX regimen (8).

The oral fluoropyrimidine, S-1, is composed of 1 M tegafur (a masked form of 5-FU), 0.4 M 5-chloro-2,4-dihydroxypyrimidine [gimestat; a potent inhibitor of dihydropyrimidine dehydrogenase (DPD)], and 1 M potassium oxonate (which mainly inhibits the phosphorylation of 5-FU in the gastrointestinal (GI) tract) (9). S-1 has been shown to be...
Materials and Methods

**Chemicals.** Tegafur, gimestat and potassium oxonate were synthesized at Taiho Pharmaceutical Co., Ltd. (Tokyo, Japan). Capecitabine (N\(^4\)-pentyloxycarbonyl-5'-deoxy -5-fluorocytidine) was synthesized by KNC Laboratory, Ltd. (Kobe, Japan). Oxaliplatin (SP-4-2)-[(1R, 2R)-cyclohexane-1, 2-diamine-\(\kappa^N\)N', \(\kappa^N\)N'] [ethanedioato(2-)\(\kappa^O\), \(\kappa^O\)] platinum) was synthesized by SINOPHARM JIANGSU Co., Ltd. (Nanjing P.R., China). The glucose solution for injection (5%) was purchased from Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan). Hydroxypropyl methylcellulose (HPMC) was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). All other reagents were commercially available products of the highest grade.

**Tumor xenografts.** The human colorectal cancer cell line COL-1 (17) was obtained from the Central Institute for Experimental Animals (Kawasaki, Japan). A 5-FU-resistant human colorectal cancer cell line, KM12C/5-FU, was established in our laboratory, as described previously (18).

**Antitumor activity in vivo.** Four-week-old male BALB/c nude mice were purchased from CLEA Japan Inc. (Tokyo, Japan) and were housed under specific pathogen-free conditions; food and water were provided ad libitum. After the animals had been in quarantine for one week, they were implanted subcutaneously with a human colorectal tumor cell line, the volume of which was approximately 8 mm\(^3\). To evaluate the antitumor activity, the mice were grouped according to the tumor volume once the mean tumor volume reached about 150 to 200 mm\(^3\) (day 0). Each group consisted of 7 to 9 mice.

S-1 was prepared by mixing tegafur, gimestat, and potassium oxonate at a molar ratio of 1:0.4:1 in 0.5% HPMC. S-1 was administered orally at the reported effective dose of 6.9 mg/kg (10) once daily, for 14 consecutive days. Capecitabine, which was suspended in 0.5% HPMC, was administered orally at the reported effective dose of 360 mg/kg (19) once daily, for 14 consecutive days. Oxaliplatin was dissolved in 5% glucose solution and was administered intravenously.

Firstly, oxaliplatin was administered at 8.3 mg/kg (on day 1 alone or day 8 alone) or at 4.2 mg/kg (on days 1 and 8) in combination with S-1 (6.9 mg/kg), to COL-1-bearing nude mice, to determine the optimal schedule and dose of oxaliplatin to be used in combination with S-1. The total dose of oxaliplatin was estimated in a pilot experiment (data not shown).

The tumor diameters were measured twice a week until day 22, and the tumor volume was estimated as 0.5 × length × width\(^2\). The relative tumor volume (RTV) was calculated using the following formula: \(\text{RTV} = \text{tumor volume on measured day}/\text{tumor volume on day 0}\). On day 15, the tumor growth inhibition ratio (TGI) was calculated using the following formula: \(\text{TGI} = [1 – (\text{mean tumor volume of treated group})/(\text{mean tumor volume of control group})] \times 100.\)

The growth delay period (GDP), which indicates the difference in the period during which the RTV grew to 4 (corresponding to 50% of the size of the control tumors at the endpoint on day 22), was determined according to a previously reported procedure (20). Toxicity was defined as a 20% or more body weight loss or toxic death.

**Statistical analysis.** The significance of the differences in the mean RTV between the treated and control groups on day 15 was analyzed using the Aspin-Welch two-tailed \(t\)-test. The combinational effect of S-1 and oxaliplatin was analyzed according to the closed testing procedure using the Aspin-Welch two-tailed \(t\)-test (21); the analyses were performed using the EXSAS, Ver. 7.11 software (Arm Systex Co., Ltd., Osaka, Japan).

Results

**Determination of the optimal schedule and maximal tolerated dose (MTD) of oxaliplatin.** S-1 alone showed significant antitumor activity against COL-1 tumor, but oxaliplatin alone failed to show significant antitumor activity. As five out of seven mice treated with oxaliplatin at a dose of 8.3 mg/kg on day 1 had died before day 15, day 1 administration was thought to be toxic in this model. As the mice treated with oxaliplatin at a dose of 8.3 mg/kg on day 8 failed to exhibit a significant increase in the antitumor activity of S-1, day 8 administration was thought not to be useful. In contrast, oxaliplatin administered in a divided dose of 4.2 mg/kg on days 1 and 8 significantly increased the antitumor activity, compared with each monotherapy, without the death of the mice (Table I).

**Comparison of increased antitumor activity of SOX and COX.** The antitumor activity of oxaliplatin administered in divided doses of 4.2 mg/kg on days 1 and 8 in combination with an effective dosage of S-1 (6.9 mg/kg) or capecitabine (360 mg/kg), was compared in COL-1-bearing nude mice in vivo. The COL-1 tumor volume change, after treatment with SOX, and with COX is shown in Figure 1. On day 15, the growth-inhibitory activity of SOX was significantly superior to that of COX, with a tolerable toxicity (Table II). After treatment, the tumor volume was measured twice a week continuously until day 22. The period required for the RTV to reach 4 (GDP) was 10.3 days for the SOX group and 4.9 days for the COX group. From these results, SOX was expected to suppress tumor growth more effectively for longer periods than COX in COL-1-bearing nude mice (Figure 1 and Table II).
Antitumor activity toward 5-FU-resistant tumors. The tumor volume change in the 5-FU-resistant colorectal cancer KM12C/5-FU, after treatment with SOX or COX, as mentioned above, is shown in Figure 2. The antitumor activity and toxicity (body weight change) on mice following treatment with SOX was equivalent to that of COX (Table III).

Discussion

We evaluated a SOX-containing administration schedule for oxaliplatin. The divided administration of oxaliplatin was optimal for increasing the antitumor activity, while obtaining a lower toxicity compared with other schedules. With this schedule, SOX had a significantly higher antitumor activity against COL-1-bearing nude mice than did COX and an equivalent activity in a KM12C/5-FU-bearing colorectal cancer mouse model. SOX has not been shown to be inferior to COX in terms of efficacy as a first-line treatment against metastatic colorectal cancer, not accompanied by hand-foot syndrome and in terms of hospitalization (22). In all previous clinical studies, oxaliplatin has been administered on the first day of treatment (23, 24); however, we found that the antitumor activity of the divided administration of oxaliplatin was equivalent to day 1 administration without any increase in toxicity. The mechanisms of the drug interactions have not yet been clarified, but the divided administration did not

Table I. Optimal schedule of oxaliplatin in combination with S-1 against human colorectal cancer, COL-1-bearing nude mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Schedule</th>
<th>Toxic death (%)</th>
<th>Body weight change (mean±SD)</th>
<th>Tumor volume (mm³, mean±SD) (%)</th>
<th>TGI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–13.9±3.5</td>
<td>884.1±132.6</td>
<td>–</td>
</tr>
<tr>
<td>S-1 alone</td>
<td>6.9</td>
<td>Days 1-14</td>
<td>0</td>
<td>–17.1±4.6</td>
<td>572.3±154.0</td>
<td>36.9%</td>
</tr>
<tr>
<td>Oxaliplatin alone</td>
<td>8.3</td>
<td>Day 1 alone</td>
<td>0</td>
<td>–8.2±6.8</td>
<td>641.8±201.5</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>8.3</td>
<td>Day 8 alone</td>
<td>0</td>
<td>–12.8±1.9</td>
<td>820.0±304.6</td>
<td>28.6%</td>
</tr>
<tr>
<td></td>
<td>4.2</td>
<td>Days 1, 8</td>
<td>0</td>
<td>–13.8±3.3</td>
<td>669.2±103.8</td>
<td>NS</td>
</tr>
<tr>
<td>SOX</td>
<td>6.9+8.3</td>
<td>Days 1-14+1</td>
<td>5/7</td>
<td>–15.7±6.6</td>
<td>419.4±164.5</td>
<td>NE</td>
</tr>
<tr>
<td></td>
<td>6.9+8.3</td>
<td>Days 1-14+8</td>
<td>0</td>
<td>–18.0±1.3</td>
<td>505.4±81.1</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>6.9+4.2</td>
<td>Days 1-14+1, 8</td>
<td>0</td>
<td>–22.5±4.2</td>
<td>394.5±68.8</td>
<td>55.1%</td>
</tr>
</tbody>
</table>

SOX: S-1+oxaliplatin; TGI: tumor growth inhibition; NE, not evaluable; NS, not significant vs. Control group; *p<0.01 vs. control group, by Welch t-test, #p<0.05 vs. S-1 alone group.

Table II. Antitumor activity of S-1+oxaliplatin (SOX) and capecitabine+oxaliplatin (COX) against COL-1 in vivo.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Schedule</th>
<th>Toxic death (%)</th>
<th>Body weight change (mean±SD)</th>
<th>ΔBWC (%)</th>
<th>Tumor volume (mm³, mean and SD) (%)</th>
<th>TGI (%)</th>
<th>GDP (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>0/8</td>
<td>–12.6±3.6</td>
<td>–</td>
<td>739.9±196.7</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>SOX</td>
<td>6.9+4.2</td>
<td>Days 1-14+1, 8</td>
<td>0/8</td>
<td>–19.8±2.6*</td>
<td>–7.3</td>
<td>380.1±73.1*</td>
<td>34.9</td>
<td>10.3</td>
</tr>
<tr>
<td>COX</td>
<td>360+4.2</td>
<td>Days 1-14+1, 8</td>
<td>0/8</td>
<td>–19.2±3.5*</td>
<td>–6.6</td>
<td>500.5±100.6*</td>
<td>25.8</td>
<td>4.7</td>
</tr>
</tbody>
</table>

ΔBWC: Body weight change of treated group –control group; TGI: tumor growth inhibition; GDP: growth delay period; *p<0.01 vs. Control group by Welch t-test, #p<0.01 vs. COX group.

Table III. Antitumor activity against KM12C/5-FU in vivo.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Schedule</th>
<th>Toxic death (%)</th>
<th>Body weight change (mean±SD)</th>
<th>Tumor volume (mm³, mean±SD) (%)</th>
<th>TGI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>0/9</td>
<td>–8.6±6.0</td>
<td>1778.0±538.3</td>
<td>–</td>
</tr>
<tr>
<td>SOX</td>
<td>6.9+4.2</td>
<td>Days 1-14+1, 8</td>
<td>0/9</td>
<td>–15.7±6.1*</td>
<td>1225.5±379.8**</td>
<td>30.2</td>
</tr>
<tr>
<td>COX</td>
<td>360+4.2</td>
<td>Days 1-14+1, 8</td>
<td>0/9</td>
<td>–17.9±5.0**</td>
<td>1092.7±293.5**</td>
<td>36.2</td>
</tr>
</tbody>
</table>

SOX: S-1+oxaliplatin; COX: capecitabine+oxaliplatin; TGI: tumor growth inhibition; *p<0.05 and **p<0.01 vs. Control group, by Welch t-test.
It is worth considering the divided administration of oxaliplatin in clinical trials. Several drugs (irinotecan, gefitinib, gemcitabine, and docetaxel) reportedly increase the antitumor activity of 5-FU or its derivatives through the down regulation of thymidylate thymidylate synthase (TS) (25-28), and oxaliplatin reportedly down regulates TS in a similar manner (29). The antitumor activity of 5-FU originates from nucleotide imbalances through the inhibition of TS, RNA dysfunction, and the false incorporation of fluorodeoxyuridine triphosphate (FdUTP) originating from fluorodeoxyuridine monophosphate (FdUMP) into DNA. Generally, FdUTP is metabolized to FdUMP by dUTPase; however, in the presence of oxaliplatin, which inhibits dUTPase, the antitumor activity of 5-FU is potentiated (30). Both S-1 and capecitabine are metabolized to 5-FU and exhibit a common antitumor activity. However, unlike capecitabine, tegafur is metabolized not only to 5-FU, but also to γ-hydroxybutyric acid and γ-butyrolactone, which reportedly inhibit angiogenesis (31). Furthermore, S-1 reportedly induces the anti-angiogenesis factor thrombospondin-1 to a greater extent than capecitabine (32, 33). These activities may contribute to potent antitumor effects of S-1 via pathways not related to 5-FU, unlike capecitabine.

In conclusion, SOX may be useful against colorectal cancer in a manner equivalent to that of FOLFOX or COX but with a greater convenience and at a lower cost, and the intermittent administration of oxaliplatin may further accelerate the effects of SOX.

References


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