Oral 5-FU is a More Effective Antimetastatic Agent than UFT

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Abstract. 5-Fluorouracil (5-FU), a pyrimidine analog, is widely used to treat gastrointestinal and other cancers. In the present study, we compared the efficacy of oral 5-FU and the 5-FU prodrug, uracil plus tegafur (UFT), on liver metastasis in a highly metastatic mouse model. Genetic labeling of the tumor with green fluorescent protein (GFP) along with inexpensive video detectors, positioned external to the mouse, allowed the real-time monitoring of details of tumor growth, metastatic spread and drug response in this mouse model. 5-FU at 10 and 20 mg/kg significantly prolonged the survival time of treated animals compared with untreated controls (p=0.003 for 5-FU, 10 mg/kg; p=0.016 for 5-FU, 20 mg/kg). In contrast, UFT only showed a trend to increase survival (p=0.078). 5-FU at 10 mg/kg substantially prolonged the survival time compared to UFT (p=0.012). 5-FU (10 mg/kg) was also more effective in prolonging survival than Furtulon (5'-deoxy-5-fluorouridine, another 5-FU prodrug) (p=0.042). All control and UFT-treated animals died by day 45. In contrast, at 45 days, 5 out of 8 animals were alive in the 5-FU 10 mg/kg group, which was found to be the best treatment regimen in this study. 5-FU (10 mg/kg)-treated animals had a median survival time of 53 days compared to 26.5 in controls and 33.5 days for UFT. These results suggest the potential clinical superiority of oral 5-FU compared to an antimetastatic agent (21). UFT is an oral antineoplastic drug combining uracil and tegafur (a prodrug of 5-fluorouracil) in a 4:1 molar ratio. It is used as postsurgical adjuvant treatment in Japan (28). The present report compares oral 5-FU with oral UFT as well as Furtulon (5'-deoxy-5-fluorouridine), another 5-FU prodrug (29-32), in the highly liver metastatic mouse model using GFP whole-body imaging to visualize tumor and metastatic growth.

Materials and Methods

Animals. Male athymic CD-1 nude mice between 5 and 6 weeks of age were used in this study. The animals were bred and maintained in a HEPA filtered environment with cages, food and bedding sterilized by autoclaving. All animal studies were conducted in accordance with the principles and procedures outlined in the National Institute of Health Guide for the Care and Use of Animals under Assurance Number A3873-1.

Surgical orthotopic implantation (SOI) (24). Two tumor fragments (1 mm³) from a liver-metastatic AC3488UM GFP tumor (25,26) from a single animal were implanted by SOI into the liver and peritoneal cavity (6). 5-FU is converted to two activated forms: 5-fluorouridine triphosphate (FUTP) and 5-fluoro-2'-deoxyuridine monophosphate (FdUMP) (15). FUTP and FdUMP act at both RNA and DNA levels (17,18).

5-FU can also be administered orally (5,8,10,12,14) as well as by vascular routes (6,7). The effectiveness of oral 5-FU in suppressing liver metastasis was previously assessed in a highly-metastatic mouse model in our laboratory (21). Doses of 20 and 25 mg/kg oral 5-FU significantly suppressed primary and metastatic tumor growth (p=0.012). These inhibitory effects were more dramatic in suppressing liver metastasis (p=0.0). The efficacy of 5-FU was visualized by whole-body fluorescence imaging of the green fluorescent protein (GFP)-expressing tumor and its subsequent metastases (22,23). Toxicity was observed only in the 30 mg/kg dose. Furthermore, we showed that the non-toxic doses of 5-FU significantly prolonged survival in these animals. These data suggested the important clinical potential of oral 5-FU as an antimetastatic agent (21).

UFT is an oral antineoplastic drug combining uracil and tegafur (a prodrug of 5-fluorouracil) in a 4:1 molar ratio. It is used as postsurgical adjuvant treatment in Japan (28). The present report compares oral 5-FU with oral UFT as well as Furtulon (5'-deoxy-5-fluorouridine), another 5-FU prodrug (29-32), in the highly liver metastatic mouse model using GFP whole-body imaging to visualize tumor and metastatic growth.

Metastatic colon cancer is the third leading cause of cancer death in the United States (1). Fluorouracil (5-FU), a pyrimidine analog, is widely used for treatment of colorectal cancers, other gastrointestinal cancer, as well as non-gastrointestinal cancers (2-21). The efficacy of 5-FU can be enhanced if it is administered with agents such as leucovorin (4,5,7) or oxaliplatin (4) or by regional administration to the liver and peritoneal cavity (6). 5-FU is converted to two activated forms: 5-fluorouridine triphosphate (FUTP) and 5-fluoro-2'-deoxyuridine monophosphate (FdUMP) (15). FUTP and FdUMP act at both RNA and DNA levels (17,18).

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all candidate nude mice. After proper exposure of the colon following a lower midline abdominal incision, the serosa of the colon was removed and two 1 mm³-pieces of tumor fragments per mouse were implanted. An 8-0 surgical suture was used to penetrate these small tumor pieces and suture them on the wall of the intestine. The intestine was then returned to the abdominal cavity. The incision in the abdominal wall was closed with a 6-0 surgical suture in one layer. The animals were kept under isoflurane anesthesia during surgery. All procedures of the operation described above were performed with a 7 × magnification microscope (Olympus). The animals were kept in a barrier facility under HEPA filtration.

Whole-body optical imaging of green fluorescent protein-expressing tumor and metastasis (22, 23, 27). A Leica stereo fluorescence microscope model L22 equipped with a mercury lamp power supply was used. Selective excitation of GFP was produced through a D425/60 band-pass filter and 470 DCXR dichroic mirror. Emitted fluorescence was collected through a long-pass filter GG475 (Chroma Technology, Brattleboro, VT, USA) on a Hamamatsu C5810 3-chip cooled color CCD camera (Hamamatsu Photonics Systems, Bridgewater, NJ, USA). Experiments were controlled and images were processed for contrast and brightness and analyzed using the Image Pro Plus 3.1 software (Media Cybernetics, Silver Spring, Maryland, USA). High-resolution images were captured directly on the computer or continuously through video output on a high-resolution Sony VCR.

Analysis of metastases. The time course of tumor progression was tracked by GFP fluorescence whole-body imaging after transplantation. Periodically, the tumor-bearing mice were examined using the fluorescence light box. The time of tumor occurrence in different organs and the numbers of metastases were recorded (22, 23, 27).

Study design. The orthotopically-transplanted animals used for the study were divided into 5 groups of 8 mice each for treatment purposes 3 days after surgery. Groups for each of the cohort conditions were randomly chosen. Groups 2-4 received 5, 10 and 20 mg/kg of 5-FU, respectively. Group 5 received UFT 65 mg/kg (20 mg/kg as Tegafur) and Group 6 received Furtulon 185 mg/kg. Group 1 served as the negative control and did not receive any treatment. 5-FU and UFT were diluted in water and administered orally by gavage. Dosing was performed every day for three weeks. GFP in vivo imaging was performed for each mouse twice a week.

Results

The survival time of the mice in each of the treated groups was compared to that of the untreated control by the log rank test. The results showed that 5-FU at 10 mg/kg significantly prolonged the survival time of the treated animals compared to UFT or untreated controls (Figure 1). The mean survival time for each group is listed in Table I. Treatment with 5-FU at 10 mg/kg resulted in a median survival of 53 days compared to 26.5 days for control, 33.5 days for the 5-FU prodrug UFT and 35.5 days for Furtulon.

<table>
<thead>
<tr>
<th>Group</th>
<th>Median survival time (days)</th>
<th>p-value vs. control</th>
<th>p-value vs. UFT</th>
<th>p-value vs. Furtulon</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU 5 mg/kg</td>
<td>31.0</td>
<td>0.147</td>
<td>0.459</td>
<td>0.219</td>
</tr>
<tr>
<td>5-FU 10 mg/kg</td>
<td>53.0</td>
<td>0.003</td>
<td>0.012</td>
<td>0.042</td>
</tr>
<tr>
<td>5-FU 20 mg/kg</td>
<td>41.0</td>
<td>0.016</td>
<td>0.077</td>
<td>0.285</td>
</tr>
<tr>
<td>UFT 65 mg/kg (20 mg/kg as Tegafur)</td>
<td>33.5</td>
<td>0.078</td>
<td>–</td>
<td>0.260</td>
</tr>
<tr>
<td>Furtulon 185 mg/kg</td>
<td>35.5</td>
<td>0.009</td>
<td>0.260</td>
<td>–</td>
</tr>
<tr>
<td>Untreated control</td>
<td>26.5</td>
<td>–</td>
<td>0.078</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Please see text for details of treatment and the liver-metastatic mouse model used.

Statistically significant differences in survival were observed in the following groups compared with the control: (p = 0.003 for 5-FU, 10 mg/kg; p = 0.016 for 5-FU, 20 mg/kg; p = 0.009 for Furtulon, 185 mg/kg) (Table I and Figure 1). UFT at 65 mg/kg showed a trend to increase survival (p = 0.078). 5-FU at 10 mg/kg was found to be the best treatment regimen in this study with a median survival time of 53 days compared to 26.5 for controls and 33.5 days for UFT. As can be seen in Figure 1, over 35% of the animals in the control group died between day 17 and day 24 of the study. In contrast, none of the animals died in the 5-FU 10 mg/kg group, UFT 65 mg/kg group or Furtulon 185 mg/kg group during this period. Only one mouse died in the 5-FU 20 mg/kg group during this period of time. All control and UFT-treated animals died by day 45. In contrast, at 45 days, 5 out of 8 animals were alive in the 5-FU 10 mg/kg group. Four mice in the treated groups (2 mice in the 5-FU 5 mg/kg group, 1 mouse in the 5-FU 10 mg/kg group and 1 mouse in the 5-FU 20 mg/kg group) survived until they were sacrificed on day 80 after SOI (Figure 1).

When the survival efficacy of 5-FU at 10 mg/kg was compared with UFT, the difference showed a significant p-value of 0.012 (Table I). When 5-FU 10 mg/kg was compared with Furtulon for survival, the difference also was significant (p = 0.042).

Figure 2 indicates that metastasis quantitated by whole-body GFP imaging increased more rapidly in the control than in any of the treated groups. Figures 3A-E are a series of real-time, whole-body GFP images that show the
Figure 1. Survival rate is compared for the different doses of 5-FU and prodrugs of 5-FU with the untreated control group. For the duration of the study (80 days), 5-FU at 10 and 20 mg/kg and Furtulon at 185 mg/kg significantly prolonged the survival time of treated animals compared with the untreated control \( (p=0.003 \text{ for } 5\text{-FU} \text{ 10 mg/kg}; \ p=0.016 \text{ for } 5\text{-FU} \text{ 20 mg/kg}; \ p=0.009 \text{ for Furtulon 185 mg/kg}). \) Median survival time: Untreated control 26.5 days; 5-FU 10 mg/kg for 53.0 days, 5-FU 20 mg/kg for 41.0 days and Furtulon 185 mg/kg for 35.5 days. Each group started with eight animals.

Figure 2. Liver metastasis was observed in all groups by whole-body GFP imaging. See Materials and Methods for details. Liver metastasis was less extensive in all treated groups compared to the control group. A statistically significant difference \( (p<0.05) \) was observed in the 5-FU 10 mg/kg-treated group compared to control.
Figure 3-A. Untreated Control

Figure 3-B. 5-FU 10 mg/kg

White arrow: primary and extentional tumor
Red arrow: metastasis (mainly in liver)
Figure 3-C. 5-FU 20 mg/kg

Figure 3-D. UFT 65 mg/kg (20 mg/kg as Tegafur)

Figure 3-C. Real-time whole-body images of AC3488-GFP-tumor progression. A series of external fluorescence images visualizes primary (white arrow) and metastatic growth (red arrow, mainly in liver) in a single mouse treated with 20 mg/kg 5-FU. Images were obtained from day 7 (day 5 of initial treatment) to day 28 (5 days after treatment) post surgical orthotopic implantation (SOI). The primary and metastatic areas are smaller compared to untreated animals (refer to Figure 3-A).

Figure 3-D. Real-time whole-body images of AC3488-GFP-tumor progression. A series of external fluorescence images visualizes primary (white arrow) and metastatic growth (red arrow, mainly in liver) in a single mouse treated with 65 mg/kg UFT (20 mg/kg as Tegafur). Images were obtained from day 7 (day 5 of initial treatment) to day 28 (5 days after treatment) post surgical orthotopic implantation (SOI). The primary and metastatic areas are smaller compared to untreated animals (refer to Figure 3-A).
real-time progression of primary and metastatic tumor growth in representative mice from the control and all treated groups. These images demonstrate that the metastatic areas grew slower in the treated groups versus the control group (Figure 3B-E vs. Figure 3A). In the control group, metastasis was observed in the liver beginning from day 7 after SOI. The sizes of the metastases increased over time. The tumor and metastatic area (mainly in liver) grew more rapidly in the control group than in any of the treated groups as visualized by whole body imaging (Figure 3A-E).

Conclusion

Visualizing cancer cells that stably express GFP in vivo (1, 21-23, 27) is far more sensitive and rapid than the traditional cumbersome procedures of histopathological examination or immuno-histochemistry, which require sacrifice of the animal. GFP expression in the tumor cells is stable over an indefinite period of time, allowing the quantitative imaging of tumor growth and metastasis. The very bright GFP fluorescence enables internal tumor and metastasis to be externally observed in critical organs.
such as the liver. No contrast agents, substrates, radioactive materials, anesthesia, or treatment need to be administered to the animals; just blue light illumination is necessary (21-23,27).

The data from the current study, which determined survival as well as real-time metastatic progression by whole-body GFP imaging, indicated that oral 5-FU (10 mg/kg) results in greatly increased survival and in suppression of primary and metastatic tumor growth in the liver (Figures 1-3) and is consistent with our previous study (21). This dose of 5-FU, as well as 185 mg/kg Furtulon prolonged survival without causing any observable toxicity in the nude mice (data not shown). Most importantly, 5-FU at 10 mg/kg had significantly better survival efficacy than UFT ($p=0.012$). 5-FU (10 mg/kg) treatment also resulted in a significant increase in survival compared to Furtulon ($p=0.042$). These data suggest the high clinical potential for oral 5-FU as an anti-liver-metastatic drug and the lack of clinical need for the complex prodrug UFT.

References


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