Antimetastatic Intraoperative Chemotherapy of Human Colon Tumors in the Livers of Nude Mice

Babak Rashidi, Zili An, Fang-Xian Sun, A. R. Moossa, and Robert M. Hoffman


ABSTRACT

We have developed a new antimetastatic chemotherapeutic strategy for combination with hepatic resection of human colon cancers in a high-metastasis nude mouse model. The new procedure involves i.p. administration of 5-fluorouracil (5-FU) 2 h before hepatic resection of the human colon tumors, with therapy continued postoperatively for 4 consecutive days. We termed this strategy neo-neoadjuvant chemotherapy. The regime significantly prolonged animal survival compared with preoperative 5-FU neoadjuvant therapy, 5-FU postoperative adjuvant therapy, surgery alone, 5-FU without surgery, or the untreated control. The median survival of neo-neoadjuvant i.p. 5-FU-treated group was 81 days, compared with 27 days for the control group (P < 0.009). The median survival of animals in the neoadjuvant group was 37 days (P < 0.021 compared with the control group). There was also a significant difference between the median survival of neo-neoadjuvant, and the neoadjuvant group (P < 0.031). When all animals in the control group had died, 70% of animals with neo-neoadjuvant and 60% of animals with neoadjuvant 5-FU were still alive (P < 0.003 and P < 0.011, respectively). When all animals with neoadjuvant 5-FU treatment had died, 70% of animals with neo-neoadjuvant treatment were still alive (P < 0.003). Survival of all other treatment groups, including 5-FU without surgery, surgery alone, and adjuvant postoperative chemotherapy, was not significantly different from the untreated control group. Two animals in the neoadjuvant group were free of tumors when sacrificed at days 154 and 165 post surgery. Whereas 100% of animals in the control, 90% in the 5-FU alone, 70% in the surgery alone, 60% in the 5-FU adjuvant, and 40% in the neoadjuvant groups had metastases in the lymph nodes draining the liver, only 10% of animals in the neo-neoadjuvant group had metastases. These data suggest that the neo-neoadjuvant therapy increased survival by preventing metastasis of cancer cells not removed in the liver resection procedure. The results of this study indicate that the neo-neoadjuvant treatment strategy for resection of colon cancer liver metastasis should be explored clinically.

INTRODUCTION

Each year, 150,000 new colon cancer cases are diagnosed in the United States. Approximately 40–50% of these patients have liver metastases either at the time of first diagnosis or after radical resection of the primary tumor (1). Hepatic resection has been widely accepted as the only curative treatment for colon cancer liver metastasis (1, 2). The survival of these patients is directly related to tumor involvement of lymph nodes draining the liver (1). However, up to 60% of patients with colon cancer liver metastases have recurrences after a median of only 9–12 months (3–6). In more than half of these cases, the liver is the first site of recurrence (7–12). The results of systemic chemotherapy for metastatic colon cancer have not shown significant survival benefits (13). It was thought that delivery of a higher concentration of chemotherapeutic agents to liver metastases via the hepatic artery after a curative resection could improve the survival of patients with colon cancer liver metastases. Postoperative adjuvant intra-arterial chemotherapy has not significantly prolonged survival (14, 15) in some cases but has in others (16, 17). Intra-arterial chemotherapy also is associated with considerable complications and high cost (14, 18, 19).

The portal vein supplies blood to hepatic micrometastases smaller than 0.5 mm (20). In an attempt to eradicate micrometastases after curative liver resection, postoperative adjuvant portal infusion of chemotherapeutic agents was used. These studies were discontinued because of high rates of complications (6, 21).

Adjuvant, postoperative i.p. chemotherapy also has not significantly improved the survival of patients with colon cancer liver metastases (9, 22).

5-FU remains the most frequently used chemotherapeutic agent for treatment of colon cancer. Unfortunately, systemic adjuvant postoperative 5-FU chemotherapy does not significantly improve the survival of patients with colon cancer liver metastases (9, 13–15, 18, 19, 21–26). New therapeutic strategies are, therefore, urgently needed.

The search for new anticancer agents and treatment modalities has been impeded by the limited availability of clinically accurate mouse models, specifically, highly metastatic models. Toward this goal, over the past 12 years in our laboratory, we have established clinically representative metastatic mouse models of human cancer with a novel method of SOI of intact tumor tissue fragments (27–32). We recently developed the
Table 1  Antimetastatic intraperitoneal 5-FU chemotherapy in nude mice for human hepatic colon tumors

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Therapeutic regimens listed in order of administration of treatment modalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>Saline, 0.5 ml, once a day for 5 days; beginning 3 days after tumor implantation.</td>
</tr>
<tr>
<td>5-FU alone</td>
<td>10</td>
<td>20 mg/kg in 0.5 ml once a day for 5 days; beginning 3 days after tumor implantation.</td>
</tr>
<tr>
<td>Liver resection alone</td>
<td>10</td>
<td>Curative liver resection 3 days after tumor implantation.</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>10</td>
<td>Curative liver resection 3 days after tumor implantation + 2 days rest, then first dose of 5-FU (20 mg/kg in 0.5 ml) once a day for 5 days.</td>
</tr>
<tr>
<td>Neoadjuvant</td>
<td>10</td>
<td>Three days after tumor implantation, 5-FU (20 mg/kg in 0.5 ml) once a day for 5 days. Then 2 days rest and subsequent curative liver resection.</td>
</tr>
<tr>
<td>Neo-neoadjuvant</td>
<td>10</td>
<td>5-FU (20 mg/kg in 0.5 ml) beginning 2 h prior to curative liver resection, 3 days after tumor implantation. Then, four more daily doses beginning from first postoperative day.</td>
</tr>
</tbody>
</table>

RESULTS

Survival of Animals.  Animals with hepatic colon tumors treated with intraoperative neo-neoadjuvant i.p. 5-FU during curative hepatectomy survived much longer than all other treatment or control groups (Fig. 1). The median survival of the neo-neoadjuvant i.p. 5-FU group was 81 days, compared with 24 days for the control group (P < 0.009). The median survival of animals in the neoadjuvant group was 37 days (P < 0.021 compared with the control group). There also was a significant difference between the median survival of 81 days for neo-

MATERIALS AND METHODS

Animals. Athymic nu/nu BALB/c mice (Charles River Laboratories, Wilmington, MA) of both sexes, 6–7 weeks of age, were used in the study. The animals were maintained in a specific pathogen-free environment in compliance with United States Public Health Service guidelines governing the care and maintenance of experimental animals. All animal studies were conducted in accordance with the principles and procedures outlined in the NIH Guide for the Care and Use of Animals under assurance number A3873-1. Mice were fed with an autoclaved rodent laboratory diet (Teklad LM-485; Western Research Products, Orange, CA).

Human Colon Cancer. We previously established the highly metastatic AC3488 human colon cancer SOI model in nude mice (32). The tumor results in the death of all of the implanted animals from metastases within 30–35 days. The original fresh specimen was obtained from a resected liver metastasis of a patient with colon cancer at the Department of Surgery, School of Medicine, University of California, San Diego, CA (32).

Tumor Implantation on Nude Mice Livers. In the present study, AC3488 human colon tumor tissue fragments were implanted directly on the left lobe of the liver of 60 animals to simulate liver metastasis. Prior to intrahepatic transplantation, liver metastases of AC3488 (~2 cm in size) that originated from SOI to the colons of nude mice were harvested and carefully inspected under a dissecting microscope (×5) to remove necrotic tissue. The harvested tumor tissues were then divided equally into small pieces of 1 mm³ each. Tumor tissue fragments were mixed thoroughly before the implantation procedure to ensure that each mouse received equally viable tissues. The left lobe of the liver of each mouse was isolated via a left subcostal incision using isoflurane anesthesia. A small cut was then made on the glissonian capsule. Two pieces of the above tumor tissue fragments were inserted into the incision on the left lobe and then fixed in place using an 8-0 nylon suture. The abdomen was closed with a 6-0 silk suture using different instruments than those used for tumor implantation to prevent any spreading of the tumor in the incision site.

Curative Partial Hepatectomy. Hepatectomy was performed 3 days post transplantation. Mice were anesthetized with isoflurane inhalation and put in a supine position. The abdomen was sterilized with iodine and alcohol swabs. To prevent any residual tumor growth, the left subcostal incision site, which was used for tumor implantation, was completely excised. Through this abdominal wall opening, the tumor-bearing left lobe of the liver was identified, and its biliary-vascular bundle was ligated with a 6-0 nylon suture. The entire left lobe was then resected. The incision line was at least 1 cm distant from the liver metastasis. The resected lobe corresponded to ~20% of the total liver. The abdomen was then closed with a 6-0 silk suture using different instruments than those used for the resection to prevent any spreading of the tumor at the incision site.

Chemotherapeutic Agent. One cycle of i.p. 5-FU at 20 mg/kg, given in 0.5 ml once a day for 5 days, was used in this study. 5-FU was obtained from the Calbiochem-Novabiochem Corporation (La Jolla, CA).

Study Design. Sixty mice, all surgically implanted with AC3488 on the left lobe of the liver, simulating liver metastases, were divided into six groups. Each group contained 10 mice (Table 1).

Statistical Analysis. The incidence of survival at defined time points and the incidence of metastasis was analyzed using Fisher’s exact test. The median survival was analyzed using Wilcoxon’s rank-sum test.

RESULTS

Survival of Animals. Animals with hepatic colon tumors treated with intraoperative neo-neoadjuvant i.p. 5-FU during curative hepatectomy survived much longer than all other treatment or control groups (Fig. 1). The median survival of the neo-neoadjuvant i.p. 5-FU group was 81 days, compared with 27 days for the control group (P < 0.009). The median survival of animals in the neoadjuvant group was 37 days (P < 0.021 compared with the control group). There also was a significant difference between the median survival of 81 days for neo-
neoadjuvant therapy and 37 days for neoadjuvant therapy ($P < 0.031$). Although survival of the surgery-alone, 5-FU-alone, and adjuvant postoperative 5-FU groups was longer than the control group, this was not statistically significant (Fig. 1). When all animals in the control group had died, 70% of the animals receiving neo-neoadjuvant 5-FU and 60% of the animals with receiving neoadjuvant 5-FU were still alive ($P < 0.003$ and $P < 0.011$, respectively). When all animals receiving neoadjuvant 5-FU treatment had died, 70% of the animals receiving neo-neoadjuvant treatment were still alive ($P < 0.003$). Two animals receiving neo-neoadjuvant treatment did not show any sign of neoplastic disease when they were sacrificed at days 154 and 165 post tumor implantation. All other animals in all groups had recurrences of tumor in the liver. The liver and lymph node metastatic rates in the different treatment groups are shown in Table 2.

**DISCUSSION**

Adjuvant, postoperative chemotherapeutic regimes have not significantly improved survival of patients with metastatic colon cancer (4, 6, 13–15, 18, 19, 21). Up to 60% of patients with resected colon cancer liver metastases have recurrences, with a median time before recurrence of only 9–12 months (3–6). Recurrences are mostly intra-abdominal, with the liver as the first relapsing site (3–10). The recurrences in the residual liver after a curative liver resection are believed to be due to microscopic residual foci of tumor or the spreading of malignant cells by surgical manipulation or both (7).

It is known that the portal vein supplies microscopic liver metastases of $<0.5$ mm in diameter (20). Pharmacokinetic and phase I clinical studies have indicated that i.p. administration of
5-FU achieves drug concentrations in the peritoneal cavity between 300- and 2200-fold higher than in the systemic circulation (23, 34, 35). Anatomical considerations and experimental data suggest that a major mechanism of clearance of compounds placed into the peritoneal cavity is by way of the portal circulation (36). i.p. infusion of 5-FU results in a portal vein concentration ~4-fold higher than that in the systemic circulation (34, 37). The delivery of the drug to the liver by way of the portal circulation essentially equals the amount of drug entering the liver during intrahepatic artery infusion (34, 37).

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The two sites most frequently involved with recurrences after a curative liver resection are the residual liver and i.p. organs (1–5). i.p. 5-FU was effective in one study for patients with three or less liver metastases (9). However, i.p. 5-FU did not significantly improve the survival of patients with liver metastases in another study (22).

It is also known that liver metastases >0.5 mm in diameter are supplied by the arterial circulation (38, 39). Intra-arterial chemotherapy as an adjuvant regime has not significantly improved the survival of patients after curative resection of colon cancer liver metastases in some studies (14, 15, 18, 19). However, recent reports have indicated that the combination of adjuvant hepatic artery infusion and systemic chemotherapy increased the disease-free and overall survival of patients compared with surgery alone or adjuvant systemic chemotherapy alone (16, 17). However, the adjuvant regime can have serious complications and high cost (14, 18, 19).

Intraportal injection of chemotherapeutic agents has been used as an adjuvant, postoperative regime to attempt to improve survival after curative resection of colon cancer liver metastases. Unfortunately, these studies were discontinued because of high rates of complications (6, 21). Standard postoperative systemic adjuvant chemotherapy also did not improve the survival of patients with resectable colon cancer liver metastases (9, 13). However, nonresectable colon cancer liver metastases can become operable after long-term preoperative, neoadjuvant chemotherapy (40).

The timing of the initiation of chemotherapy in this study is important for two reasons: (a) the effect of growth factors, which stimulate tumor growth, released early after liver resec-

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver metastases</th>
<th>Lymph node metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>5-FU alone</td>
<td>10/10</td>
<td>9/10</td>
</tr>
<tr>
<td>Liver resection alone</td>
<td>10/10</td>
<td>7/10</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>10/10</td>
<td>6/10</td>
</tr>
<tr>
<td>Neoadjuvant</td>
<td>10/10</td>
<td>4/10</td>
</tr>
<tr>
<td>Neo-neoadjuvant</td>
<td>8/10</td>
<td>1/10</td>
</tr>
</tbody>
</table>

*Number of animals involved with metastases/total number of animals in each group.

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