Reduction of Hepatic Metastases in Rabbits by Administration of an Oily Anticancer Agent into the Portal Vein

Kenji Yamasaki, Toshimitsu Konno, Yoshimasa Miyauchi, and Hiroshi Maeda

First Department of Surgery [K. Y., T. K., Y. M.] and Department of Microbiology [H. M.], Kumamoto University Medical School, Kumamoto 860, Japan

ABSTRACT

We studied a prophylactic chemotherapy against hepatic metastases arising from the shedding of tumor cells into the portal circulation. The therapy was done with a lymphographic oily contrast medium, Lipiodol, and a high molecular weight anticancer agent named poly(styrene-maleic acid) copolymer conjugated neocarzinostatin (SMANCS), developed in our laboratory. SMANCS was dissolved in Lipiodol by sonication (SMANCS/Lipiodol, 1 mg of SMANCS in 1 ml of Lipiodol). Twelve rabbits were simply inoculated with the highly malignant carcinoma VX-2. Fifteen rabbits were given injections of SMANCS in glucose and Lipiodol into the portal vein and were subsequently inoculated with the tumor cells. Eighteen were given injections of SMANCS/Lipiodol and then the tumor cells. These rabbits were killed 12 days later. Thirty were given injections of the tumor cells alone and were allowed to survive. Sixteen were given injections of SMANCS/Lipiodol and then with the tumor cells; they were allowed to survive. Rabbits given injections of SMANCS/Lipiodol before tumor inoculation had significantly fewer (P < 0.001) metastases than those not treated or those given SMANCS in glucose and Lipiodol. Survival was significantly longer [P < 0.005; 36.0 ± 7.7 (SD) days] with SMANCS/Lipiodol before tumor inoculation than without treatment [23.5 ± 3.0 days]. SMANCS/Lipiodol has a prolonged anticancer effect because it remains in the portal vein and allows sustained drug release from the oil (Lipiodol) to aqueous spaces. Hepatic metastases might be prevented by portal administration of the appropriate oily anticancer agent.

INTRODUCTION

Hepatic metastasis is a major problem in patients with various kinds of cancers, especially gastrointestinal cancer. Various techniques and agents for the prevention of hepatic metastases have been evaluated for efficacy in combination and by different routes of administration (1, 2). Anticoagulants, anti-platelet agents, immunooactivators, cytotoxic chemotherapy, and radiation have been tested to see if they inhibit the formation of metastases (3–5). However, these methods have not proved to be satisfactory. Surgery itself may spread tumor cells, leading to the growth of hepatic metastases (6). There is no established prophylactic chemotherapy for patients with gastrointestinal cancer.

When Lipiodol, a lipid lymphographic contrast medium, is administered arterially, it can be used as a carrier for a lipophilic anticancer agent of high molecular weight, SMANCS(7, 8). Both SMANCS and Lipiodol are deposited selectively in the tumor tissue when injected into the artery supplying the tumor. Lipiodol also acts as a reservoir of the anticancer agent, thus prolonging drug release (9–11). Legar et al. (12) and Idezuki et al. (13) reported that hepatography done by injecting Lipiodol into the portal vein showed liver tumors as a negatively stained area. We were interested in their notion that Lipiodol remained in the portal vein for a long time. Here, SMANCS dissolved in Lipiodol (SMANCS/Lipiodol) was injected into the portal vein in rabbits to study the possible prevention of hepatic metastases.

MATERIALS AND METHODS

Drug. SMANCS was prepared at the Department of Microbiology, Kumamoto University Medical School, Kumamoto, Japan, or at the Kuraray Co., Ltd., Osaka, Japan. Details were described elsewhere (8). Two styrene-maleic acid copolymer groups (M, 1,500) were conjugated through amide linkage to the antitumor protein neocarzinostatin (M, 12,000); the product, SMANCS, had a molecular weight of about 15,000. SMANCS is soluble in lipid, some organic solvents and water. We dissolved 1 mg of SMANCS in 1 ml of Lipiodol by sonication (SMANCS/Lipiodol). In another experiment, 1 mg of SMANCS was dissolved in 1 ml of 5% glucose (SMANCS/glucose). Lipiodol, used in lymphography, is an oily contrast medium. It is a fatty acid ester containing 38% iodine (w/w) with a density of 1.38 (g/ml), and a product of the Laboratorie Guerbet, Paris, France, obtained through Kodama K.K., Tokyo, Japan.

Animals. Ninety New Zealand White rabbits about 3 months old, weighing 2 to 3 kg, were used. First, general anesthesia was induced with 30 mg/kg of sodium pentobarbital injected i.v., and laparotomy was done through a midline abdominal incision. Then, SMANCS/Lipiodol (0.4 ml/kg) was injected into the portal vein of the rabbits.

Inoculation of Tumor Cells. The VX-2 tumor line was maintained by us by successive transplantation into the liver of rabbits. The VX-2 tumor was removed from the liver, minced in Hanks’ solution, and filtered through four layers of gauze. The filtrate was adjusted to a concentration of 6–9 × 10⁶ carcinoma cells/ml. A total of 0.3 ml of a suspension containing about 2.5 × 10⁶ cells was injected into the superior mesenteric vein with a 23-gauge needle.

Experimental Groups and Treatment Protocol. Eight groups of rabbits were used. In group 1, SMANCS/Lipiodol (0.4 ml/kg) was injected into the mesenteric vein of ten rabbits that had not been inoculated, and one was killed at 5 min, 24 h, 48 h, 72 h, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, and 3 months later. The livers were removed. We did a low kVp X-ray examination using a Softex instrument and a histological study with oil red stain to identify Lipiodol. Rabbits in group 2 (n = 3) were given injections of SMANCS/Lipiodol (0.4 ml/kg) in the mesenteric vein. Rabbits in group 3 (n = 3) were given injections of SMANCS/glucose (0.4 ml/kg) and Lipiodol (0.4 ml/kg) together. Rabbits in groups 2 and 3 were killed at 5 and 24 h, and 3 days later, respectively, the liver, lung, and kidney were removed to evaluate the bioactivity of SMANCS at the above indicated times. The bioactivity of SMANCS, which was parallel to the antitumor activity, was determined by an antibacterial assay using Micrococcus luteus on a soft agar plate as described previously (14). Rabbits in group 4 (n = 12) were simply inoculated with the tumor. Rabbits in group 5 (n = 15) were given injections of SMANCS/glucose (0.4 ml/kg) and Lipiodol (0.4 ml/kg) and about 2 min later tumor was inoculated. Rabbits in group 6 (n = 18) were given injections of SMANCS/Lipiodol (0.4 ml/kg) and then inoculated with tumor cells about 2 min later. All rabbits in groups 4, 5, and 6 were killed 12 days after the inoculation. The livers were removed and the number of visible metastases on the surface of the liver was counted. Group 7 rabbits (n = 13) were treated as in group 4, and group 8 rabbits (n = 16) as in group 6. The animals in groups 7 and 8 were allowed to survive until death.

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2 To whom requests for reprints should be addressed.

3 The abbreviation used is: SMANCS, poly(styrene-maleic acid) copolymer conjugated neocarzinostatin.
Fig. 1. Low kVp X-ray film by Softex instrument 5 min (A), 72 h (B), 2 weeks (C), 3 months (D) after injection of SMANCS/Lipiodol (0.4 ml/kg) into the portal vein. Lipiodol seems to have filled the trunk and periphery of the portal vein 5 min after injection and then gradually decreasing in intensity (from A to D). See text for details.

RESULTS

The results of the X-ray examination of the liver in rabbits in group 1 are shown in Fig. 1. Lipiodol seemed to fill the entire liver, as well as the periphery of the portal vein 5 min after the injection (Fig. 1A); later, it gradually decreased in intensity, although the venous tree was still clearly stained. The injection appeared as fine granular spots 2 weeks later (Fig. 1C). Three months after injection, slight staining remained in peripheral areas of the liver (Fig. 1D). Histological studies with
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Table 1 Biological activity of SMANCS in the rabbit liver after injection into the portal vein

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Biological activity (µg/ml) at various time periods after injection</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td>Specimen</td>
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<tr>
<td>2</td>
<td>SMANCS/Lipiodol (0.4 ml/kg)</td>
<td>Liver</td>
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<td></td>
<td></td>
<td>Lung</td>
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<td>Kidney</td>
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<tr>
<td>3</td>
<td>SMANCS/glucose (0.4 ml/kg)</td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td>and Lipiodol (0.4 ml/kg)</td>
<td>Lung</td>
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<td></td>
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<td>Kidney</td>
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* Each group consisted of 3 rabbits.

Table 2 Incidence of hepatic metastases in experimental animal groups

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Treatment</th>
<th>Total no. of rabbits with following no. of hepatic metastasis nodules</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Inoculation with tumor, no drug</td>
<td>0 (0)* 0 (0) 5 (41.7) 7 (58.3)</td>
</tr>
<tr>
<td>5</td>
<td>SMANCS/glucose and Lipiodol → tumor inoculation</td>
<td>0 (0) 2 (13.3) 5 (33.3) 8 (53.3)</td>
</tr>
<tr>
<td>6</td>
<td>SMANCS/Lipiodol (oily anticancer agent) → tumor inoculation</td>
<td>8 (44.4) 6 (33.3) 4 (22.2) 0 (0)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentages of rabbits for the respective metastatic categories.

The result of the bioactivity of SMANCS is shown in Table 1. The bioactivity of SMANCS in the liver in rabbits given injections of SMANCS/Lipiodol (group 2) was found to be very high even after 3 days of the injection.

In all of the rabbits in groups 4 and 5, hepatic metastases were observed macroscopically 12 days after inoculation (Fig. 3). The distribution of metastases as multiple nodules or foci 12 days after the inoculation is shown in Table 2. The difference between group 4 and group 5 was not significant. In 8 of the rabbits of group 6, metastases were not found; in the remaining 10, they were found, but there were significantly fewer nodules than in groups 4 or 5 on 12 days after inoculation (P < 0.001).

Rabbits treated with SMANCS/Lipiodol (group 8) which is equivalent to group 6 lived longer than the rabbits not treated (group 7; equivalent to group 4). Survival periods are shown in Fig. 4. The mean survival in group 7 was 23.5 ± 3.0 (SD) days and that in group 8 was 36.0 ± 7.7 days. The difference is significant (P < 0.005; Fig. 4). All rabbits in groups 7 and 8 died of hepatic metastases. All rabbits in group 7 were dead within 30 days; their livers had diffuse, uncountable metastases. Twelve of the 16 rabbits in group 8 survived more than 30 days and in their livers, only a few nodular metastases were found (Figs. 4 and 5). There was no operative mortality in any group.

DISCUSSION

SMANCS/Lipiodol seems to inhibit VX-2 tumor metastasis because the antitumor effect is long-lasting, since it remains in the portal vein and venules a long time after administration (Table 1; Refs. 8–11). Biologically active SMANCS dissolved in Lipiodol diffuses out to the surrounding tissue. While SMANCS remains in the Lipiodol, it is likely to be protected from hydrolytic enzyme in the aqueous environment of the tissue by this oily substance. This would potentially allow for a long-lasting anticancer effect. Complete embolization due to SMANCS/Lipiodol has not occurred, so acute and adverse side effects are improbable (Fig. 2).

There are several advantages to this method: (a) deposition of SMANCS/Lipiodol was in the hepatic branches of portal vein, where metastatic tumor cells would lodge at first; (b) administration need not be frequent, and the dose required was much lower than the 50% lethal dose; therefore side effects should be local and minimal. (c) the anticancer effect in the
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Fig. 4. Survival of rabbits inoculated with VX-2 tumor (group 7, n = 13) and of those given SMANCS/Lipiodol and then tumor inoculation (group 8, n = 16). Mean survival of group 7 was 23.5 ± 3.0, and that of group 8 was 36.0 ± 7.7 days. Bars, SD. Details in text.

Fig. 5. Livers of rabbits removed at autopsy. A, group 7, without chemotherapy; liver occupied by diffuse numerous metastases; B, group 8, treated with a single injection of SMANCS/Lipiodol, and surviving for more than 30 days; liver contains only a few nodular metastases.

portal venous system should be pronounced and long-lasting; (d) the drug is eventually eliminated through the bile and urine (11, 13); (e) the procedure is simple and readily applied to patients; (f) the same method might be used with another drugs with a different antitumor spectrum, or with a combination of other anticancer agents.

The acute toxicity of Lipiodol (50% lethal dose i.v.) in dogs is 1.58 ml/kg of body weight (15). That of SMANCS in rats is 1.2 mg/kg of body weight (8). Idezuki et al. (13) used a mean Lipiodol dose of 0.5 ml/kg in dogs and 0.14 ml/kg in humans, injecting it into the portal vein. Histological studies of the liver did not show any permanent changes, nor were there adverse effects on liver function. We did not find any drastic, permanent changes in most rabbits, either. However, in a few, slight atrophy in the periphery of the liver was seen. This is probably caused by the cytotoxicity of SMANCS/Lipiodol.

We believe that micrometastatic cancer nodules (nonvascularized tumors less than 2 mm across) in the liver are fed not only by hepatoarterial blood but also by portal blood (16, 17). Administration of oily anticancer agents into the portal vein before or during surgery seems to be effective against such micrometastases.

This study provides the first experimental evidence that a highly malignant tumor, VX-2, when introduced into the liver, can be suppressed for its metastasis by an earlier single portal injection of a drug, prolonging survival.

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