Radioimmunodetection of Hepatic Metastases from Human Colon Cancer in Nude Mice with a Gamma-detecting Probe

Michel Rivoire, Kazuhiko Yoshida, Chaitanya Divgi, Sydney Welt, Alfred Cohen, and Elin R. Sigurdson


Abstract

The utility of a gamma detecting probe (GDP) in the detection of experimental hepatic metastases in nude mice using radiolabeled monoclonal antibody (mAb) was assessed. Twelve mice with established hepatic metastases from the HT-29 LMM cell line, 5 mice with s.c. tumors in the left flank and 6 non-tumor-bearing control mice were given i.v. injections of 40 μCi/4 μg of 125I-labeled mAb HT-29-15. Six tumor-bearing mice were given i.v. injections of an isotype-matched control mAb (BL-3).

Using the GDP, measurements were obtained daily over the region of the heart, the region of the liver (i.e., the right flank), and the s.c. tumor when applicable. Intraoperative measurements were obtained at laparotomy on days 5 and 7. Subsequently, the metastases, normal liver, and blood were resected and the radioactivity/g tissue was measured in a gamma well counter.

External right flank/heart ratios were significantly higher in the tumor-bearing group than in controls. External measurements allowed detection of small tumors weighing only 161 ± 87 (SD) mg and occupying 11.5 ± 4% of the entire liver weight. Metastases counted intraoperatively with the GDP measured 1 to 7 mm in greatest diameter. The mean metastasis/heart GDP ratio was 1.7 ± 0.4:1. Tumors weighing as little as 51 ± 42 mg could be identified.

These experimental results confirm the usefulness of the GDP for the detection of small hepatic metastases from colon cancer and illustrate important features of probe measurement of radiolabeled mAb uptake.

Introduction

The prevalence of colon cancer continues to rise. Approximately 145,000 people each year develop colorectal cancer in the United States (1). Liver metastases represent the primary cause of therapeutic failure; in patients dying of large bowel cancer 48% will have liver involvement (2). Finlay and Mc Ardle (3) reported the development of hepatic metastases in 30% of patients undergoing curative resection for colorectal cancer. A majority of these metastases occurred within 1 year of operation. Thus it is essential, prior to treatment of liver metastases, to stage accurately their number, size, and location as part of the objective assessment of the proposed therapy.

The imaging methods currently used are computerized tomography, magnetic resonance imaging, and intraoperative ultrasonography. Computerized tomography accurately demonstrates the number and location of liver metastases in only 43% of patients (4). The results of magnetic resonance imaging for tumors between 1.5 and 2 cm in diameter are still controversial (5). Intraoperative hepatic ultrasonography was shown to be the most sensitive and accurate screening method for tumors 1 cm in diameter (6). However, the success of this technique is highly operator dependent.

Tumor localization by external radionuclide imaging after injection of monoclonal or polyclonal antibodies has been shown by many investigators (7-10). In order to improve the sensitivity of detection, a hand-held gamma detecting probe has been developed (11). It can be placed directly over the organ of interest during the course of an operation. Experimental studies in nude mice bearing s.c. xenografts (12) and preliminary results of intraoperative use for both primary and recurrent colorectal carcinomas have been encouraging (13-15).

In order to determine the clinical value of the GDP for localization of small (less than 1 cm) hepatic metastases, a study was undertaken in nude mice bearing experimental liver metastases from a human colon carcinoma cell line. The ability of the GDP to identify small hepatic metastases after radiolabeled mAb injection was assessed. In vivo (external and intraoperative) measurements with the GDP were compared to in vitro gamma well measurements. The minimum size of metastases detectable by external and by intraoperative measurements in nude mice was determined.

Materials and Methods

Mice. Specific-pathogen-free athymic female BALB/c mice 3 to 4 weeks of age were obtained from Walker Laboratory (Rye, NY). They were kept under sterile conditions in a laminar flow room in cages with filter bonnets and were fed sterilized mouse diet and water.

Tumor Cell Line. The human colon cancer cell line HT-29 LMM, a metastatic variant of HT-29 colon cancer cell line, was generously provided by Dr. I. J. Fidler (M. D. Anderson Hospital and Tumor Institute, Houston, TX). The HT-29 LMM cell line was grown under humidified conditions in presence of 5% carbon dioxide in Dulbecco's modified minimum essential medium (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (GIBCO).

Establishment of Hepatic Metastases and s.c. Tumors. Tumor cells grown in tissue culture were harvested by 5-min treatment with 0.05% trypsin and 0.02% EDTA and suspended in PBS. The mice were anesthetized with pentobarbital (12 μg i.p./g body weight), and a small left subcostal incision was made to expose the spleen. HT-29 LMM cells (2 × 10⁷) in 50 μl of PBS were injected beneath the splenic capsule using a 30-gauge needle. For s.c. tumors, an injection of 10⁷ HT-29 LMM cells in 250 μl of PBS was used.

Monoclonal Antibodies. HT-29-15 is a murine IgG1 mAb developed by Dr. J. Sakamoto in Memorial Sloan-Kettering Cancer Center (16). HT-29-15 was purified with protein A-Sepharose chromatography (Pharmacia, Piscataway, NJ). It was shown to react with a cell surface glycoprotein (M, 200,000).

BL-3 was kindly provided by Dr. J. Schlom (National Cancer Institute, NIH, Bethesda, MD). This mAb reacts with a human B-cell lymphoma and was used as an isotype-matched control. It was found to be unreactive with HT-29 LMM cell line.

Radiolabeling. mAbs were labeled with sodium [125I]iodide (Du Pont-New England Nuclear, Boston, MA) by a modified chloramine-T method (17). The radiolabeled mAbs were purified by exclusion chromatography on a Sephadex G-25 column (Pharmacia). The percentage of labeling was between 60 and 80%. The fraction corresponding to the radiolabeled mAbs typically had more than 95% of radioactive iodine bound to protein, as determined by trichloroacetic acid precipitation. Immunoreactivity was tested on each batch by a modification of the method described by Lindmo et al. (18) and was between 30 and 55% for HT-29-15 and <10% for BL-3.

Gamma-detecting Probe. The portable GDP Neoprobe 1000 (Neoprobe Corporation, Columbus, OH) includes a sensitive γ-ray detector and a microcomputer control unit. The probe contains a solid state γ-ray detector.

1 The abbreviations used are: GDP, gamma detecting probe; mAb, monoclonal antibody; PBS, phosphate-buffered saline.
ray detector made of cadmium telluride which is approximately 12 mm in diameter and 1.5 mm thick associated with a preamplifier. During a measurement, γ-rays striking the CdTe detector produce tiny electrical signals that are boosted by the preamplifier and transformed in the microcomputer as a digital display and an audible tone. Measurements can be made for fractions of 1 to 100 s.

Radioiodinated mAb Injection. Approximately 4 weeks after splenic injection of malignant cells, 12 mice with established hepatic metastases (5 of them additionally bearing s.c. tumors) were given i.v. injections of 40 μCi/μg of 125I-labeled HT-29-15. A control group of 6 non-tumor-bearing mice was given injections under the same conditions. Another control group of 6 tumor-bearing mice was given injections of the 125I-labeled control mAb (BL-3).

GDP Measurements. External GDP measurements consisted of sets of counts obtained for 10 s. The measurements were recorded daily, for both groups, over the region of the heart, the region of the liver, i.e., the right flank, and the s.c. tumor when applicable. Right flank/heart and s.c. tumor/heart ratios were calculated daily for all tumor-bearing and control animals.

Intraoperative GDP measurements were made on days 5 (7 mice) and 7 (5 mice) during laparotomy. Counts of 10 s were carried out for 1 to 3 small isolated metastases in each mouse. The metastasis/heart ratios were calculated.

Biodistribution. Following intraoperative measurements, the animals were sacrificed and tissues and organs of interest including metastases, blood, normal liver, s.c. tumor, and uninvolved organs were removed and weighed. Tissue radioactive counts were obtained in a gamma well counter and metastases/blood, metastases-normal liver, and normal liver/blood gamma well ratios were calculated.

Results

In Mice Receiving 125I-HT 29-15

s.c. Tumor Measurements. GDP measurements over s.c. tumors and the region of heart were obtained daily until the animals were sacrificed on days 5 and 7. The s.c. tumor/heart ratios increased from 1.6:1 on day 1 to 4.7:1 on days 6 and 7 (Table 1). These results compared well with the tumor/blood in vitro ratios measured on days 5 and 7 (1.8:1 to 3.8:1).

External Hepatic Metastases Measurements. GDP measurements over the right flank were obtained daily in both tumor-bearing and control groups. These external measurements varied depending on percent hepatic replacement by tumor. The mice were classified into two subgroups (Table 2). In the first subgroup (n = 7), the total metastasis weight, 161 ± 87 (SD) mg, represented 11.5 ± 4% of the liver weight. In these mice the number of macroscopic metastases varied between 1 and 5. In the second subgroup (n = 5), the total metastasis weight, 497 ± 153 mg, represented 34.6 ± 8% of the liver weight, and the number of metastases was greater than 5. The right flank/heart count ratios were significantly higher in both subgroups compared to the control group (P < 0.001 for the <15% tumor burden subgroup versus control mice, P < 0.0001 for the >15% tumor burden subgroup versus control mice, P < 0.01 for the <15% tumor burden subgroup versus control mAb, P < 0.0001 for the >15% tumor burden subgroup versus control mAb). In the greater than 15% subgroup the mean daily right flank/heart ratios were always greater than 1.72:1.

Intraoperative Metastasis Measurements. For intraoperative GDP measurements, small, isolated, superficial metastases of 1 to 7 mm in greatest diameter were studied. Under anesthesia, GDP measurements were obtained on days 5 and 7 over 1 to 3 metastases in each animal. The metastasis/heart ratios were calculated for each metastasis measured (Table 3). The mean size of metastases was 4.7 ± 1.5 mm, or 51 ± 42 mg. The ratios obtained for all metastases were always greater than 1 (1.7 ± 0.4:1 versus 0.8 ± 0.0:1 for the control mAb P < 0.0001).

Biodistribution Study. On days 5 and 7, after intraoperative measurements had been taken, metastases, s.c. tumor (when applicable), blood, normal liver, and uninvolved organs were weighed and then counted in a gamma well counter. The metastasis/blood ratios were 2.6 ± 0.8:1 and 3.1 ± 0.8:1 on days 5 and 7, respectively. Normal liver/blood and metastasis/normal liver ratios were stable between days 5 and 7 (0.2 ± 0.0:1 and 10.7 ± 0.3:1, respectively).

In Mice Receiving 125I-BL-3

No clear distinction between radioactive uptake and external/ intraoperative GDP measurements could be made (Table 4). In vitro well counting did not show any mAb localization in tumor.

Discussion

Recent experimental studies using s.c. xenografts in nude mice have shown that the GDP can detect small tumors with radioactivity from radioiodinated mAb uptake at levels too low to be visualized with a scintillation camera (13). Intraoperative results in humans with the GDP have suggested that this device could be of value in determining appropriate margins for local resection as well as in detecting the presence of regional met-

| Table 1 Daily s.c. tumor/heart GDP ratios compared with s.c. tumor/blood gamma well ratios

<table>
<thead>
<tr>
<th>Days after injection</th>
<th>Tumor/heart (in vivo)</th>
<th>Tumor/blood (in vitro)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.2 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.1 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.6 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.7 ± 0.8</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>6</td>
<td>4.7 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4.7 ± 0.7</td>
<td>3.8 ± 2.0</td>
</tr>
</tbody>
</table>

| Table 2 Classification of tumor-bearing mice according to the total metastasis weight, the percentage of liver replacement, and the number of macroscopic metastases

<table>
<thead>
<tr>
<th>No. of mice</th>
<th>Wt (mg)</th>
<th>% of liver wt</th>
<th>No. of metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>161 ± 87</td>
<td>11.5 ± 4</td>
<td>1–5</td>
</tr>
<tr>
<td>5</td>
<td>497 ± 153</td>
<td>34.6 ± 8</td>
<td>&gt;5</td>
</tr>
</tbody>
</table>

| Table 3 Intraoperative GDP measurements: size, weight, and metastases/heart GDP ratios compared to metastases/blood gamma well ratios

<table>
<thead>
<tr>
<th>Days</th>
<th>Size (mm)</th>
<th>Wt (mg)</th>
<th>Metastases/heart (in vivo)</th>
<th>Metastases/blood (in vitro)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4.7 ± 1.0</td>
<td>46 ± 24</td>
<td>1.7 ± 0.3</td>
<td>2.6 ± 0.8</td>
</tr>
<tr>
<td>7</td>
<td>4.6 ± 1.5</td>
<td>56 ± 50</td>
<td>1.6 ± 0.4</td>
<td>3.1 ± 0.8</td>
</tr>
</tbody>
</table>

| Table 4 Tumor/heart GDP ratios in the ≤15% tumor burden subgroup, >15% tumor burden subgroup, the control mice group, and control mAb group. Results are the mean ± SD. (P < 0.001 for the ≤15% tumor burden subgroup versus control mice, P < 0.0001 for the >15% tumor burden subgroup versus control mice, P < 0.001 for the ≤15% tumor burden subgroup versus control mAb). In the >15% tumor burden subgroup versus control mAb) |

<table>
<thead>
<tr>
<th>Days</th>
<th>Tumor burden (≤15%)</th>
<th>Tumor burden (&gt;15%)</th>
<th>Control mice (no tumor)</th>
<th>Control mAb (BL-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0 ± 0.23</td>
<td>1.93 ± 0.18</td>
<td>0.88 ± 0.10</td>
<td>0.95 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>1.08 ± 0.17</td>
<td>2.37 ± 0.24</td>
<td>0.88 ± 0.04</td>
<td>0.91 ± 0.13</td>
</tr>
<tr>
<td>3</td>
<td>0.96 ± 0.28</td>
<td>2.00 ± 0.39</td>
<td>0.88 ± 0.04</td>
<td>0.91 ± 0.12</td>
</tr>
<tr>
<td>4</td>
<td>1.19 ± 0.24</td>
<td>1.72 ± 0.31</td>
<td>0.78 ± 0.04</td>
<td>0.92 ± 0.12</td>
</tr>
<tr>
<td>5</td>
<td>1.02 ± 0.10</td>
<td>2.04 ± 0.41</td>
<td>0.64 ± 0.05</td>
<td>0.87 ± 0.18</td>
</tr>
<tr>
<td>6</td>
<td>1.11 ± 0.23</td>
<td>1.88 ± 0.32</td>
<td>0.79 ± 0.06</td>
<td>0.82 ± 0.09</td>
</tr>
<tr>
<td>7</td>
<td>1.12 ± 0.04</td>
<td>2.69 ± 0.85</td>
<td>0.82 ± 0.02</td>
<td>0.83 ± 0.06</td>
</tr>
</tbody>
</table>

878s

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astatic nodes, hepatic metastases, or occult recurrence (15).

We chose an animal model of nude mice bearing experimental hepatic metastases from a human colon cancer cell line to determine the sensitivity of the GDP for small hepatic metastases. Cardiac counts truly represent blood pool and were used as background. Representation of background by distal extremity counts results in spuriously low values and consequent falsely elevated ratios; usage of cardiac blood pool more closely approximates the clinical condition. In our study the contralateral flank could not be used because it was the site of tumor implantation.

External right flank/heart GDP ratios confirmed the ability of the GDP to detect liver metastases weighing less than 160 mg. Using intraoperative GDP measurements, superficial liver metastases as small as 50 mg were detected. External counts were made over skin (approximately 2 mm thick) without knowing whether the metastases were directly under the counting surface or 1 to 2 cm distant from it (i.e., in the left part of the liver). Despite this, it was possible to differentiate tumor-bearing from non-tumor-bearing animals receiving specific mAb using right flank GDP measurements. Furthermore these results correlated with the total liver metastases weight and percentage of liver replacement and therefore could be used to predict the tumor burden.

During the intraoperative measurements, the GDP was as close as possible to very small, superficial metastases. While the mean greatest diameter of 4.6 ± 1.5 mm is important, the shape of the metastases was not spheroidal (they were usually very thin). Thus the mean weight was thought to be a more accurate parameter. The 1.7:1 ratio obtained with intraoperative GDP measurements is significant especially considering that the diameter of the CdTe gamma detector is 12 mm (i.e., much greater than the diameter of all the metastases). There was thus an important overlap with the normal liver during the metastases counts. Because the in vitro normal liver/blood ratio was 0.2 ± 0.0:1 this overlap will naturally reduce the GDP measurements of the metastases.

The in vitro metastasis/normal liver ratio was 10.7 ± 0.3:1. It would have been useful to determine the metastasis/normal liver GDP ratio. Precise normal liver GDP measurements were impossible in most mice because of the small surface of normal liver available. Additionally, because the mouse liver is very thin, normal liver GDP counts would more closely approximate cardiac background counts.

Reliable detection of hepatic metastases in the mouse model has been shown to be possible even with tumor burden as low as 160 mg representing 11.5% of total liver mass. This has important bearing on the clinical situation; while this model closely approximates the human condition, absolute quantification of tumor burden is not strictly extrapolable given the vastly different dimensions of human liver metastases. The model can also be used to determine specificity of radiolabeled mAb localization; it is useful to differentiate between radiolabeled mAbs of varying specificity/affinity and provide important control data not readily available through clinical studies. Gamma camera imaging is not feasible in this situation; the GDP will thus play an important role in the screening of such antibodies. We have seen that isotype-matched mAb did not show any specific localization, determined by the GDP and verified by in vitro counting.

This model is important because it helps determine the relative contribution of radiolabeled mAbs by both tumor and surrounding normal tissue. Moreover, the validity of intraoperative GDP measurements in the radioimmunodetection of very small (usually occult in human situation) hepatic metastases has been demonstrated.

In summary, the intraoperative use of a GDP after radiolabeled mAb injection shows promise as a clinical tool for diagnosis of occult tumors from colorectal cancer. The results of the present study show the ability of the GDP to detect small liver metastases in nude mice with both external and intraoperative measurements. Future studies will evaluate the ability of this device to detect deeper metastases (perhaps utilizing 131I) in order to determine the extent of its utility in the intraoperative management of patients with colorectal cancer. This model will also be used to determine the extraction efficiency by the liver after portal or hepatic artery injection of mAbs.

References

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