Localization of Radioiodine Conjugated to the Monoclonal Antibody HMFG2 in Human Ovarian Carcinoma: Assessment of Intravenous and Intraperitoneal Routes of Administration

Bruce G. Ward,1 Stephen J. Mather, Laurie R. Hawkins, Mary E. Crowther, John H. Shepherd, Marie Granowska, Keith E. Britton, and Maurice L. Slevin

ABSTRACT

The localization of i.p. injected, radioiodine conjugated monoclonal antibody HMFG2 was studied in 18 patients with ovarian carcinoma. Patients were injected i.p. at time points up to 168 h before laparotomy, at which time tumor, ascites, normal tissue, and blood samples were removed and the contained radioactivity measured. In the first 10 patients, localization was compared with that of a simultaneously injected irrelevant (nonspecific) antibody (UJ13A) of the same immunoglobulin class and, in the subsequent 8 patients, with HMFG2 administered i.v. After i.p. injection, HMFG2-radioiodine was found in concentrations of 0.0001-0.0030% of the injected amount per gram in solid tumor, 0.0363-0.02560%/g in ascites, 0.0003-0.0017%/g in blood, and 0.001-0.0012%/g in normal tissue. Tumor:normal tissue ratios of 0.9-10.0 and tumor: blood ratios of 0.3-4.0 were seen up to 168 h after injection. Localization of the HMFG2 conjugate was consistently greater than that of the irrelevant antibody.

For solid tumor, the i.v. route of administration resulted in consistently higher absolute levels of HMFG2 conjugate uptake but tumor: blood and tumor: normal tissue ratios were similar. On the other hand the route of administration offered consistent advantages of 4- to 71-fold over the i.v. route when HMFG2 conjugate localization on ascites cells was examined. Ascites: normal tissue and ascites: blood ratios of up to 512 and 448, respectively, were achieved.

After i.p. injection, radioiodine was cleared from the body exponentially with a half-life of 50 h. Maximum circulating blood levels of 8.6 ± 2.8% injected activity were seen at 48 h and these then decreased with a t½ value of 38 h. Over 80% of injected activity was cleared in the urine as nonprotein bound iodine by 168 h.

INTRODUCTION

There have been many reports in the literature of the success of immunoscintigraphy in the detection of ovarian carcinoma (1-4). The requirements for antibody guided radiotherapy, however, are different from those of immunoscintigraphy, where small differences in antibody uptake between tumor and normal tissue can be enhanced by computer or subtraction techniques to aid diagnosis (5). For antibody guided therapy, large differences between tumor and normal tissue are required to obtain a therapeutic effect and to avoid radiation side effects (6).

The Medical Internal Radiation Dose Tables of Radioactive Decay (American Society of Nuclear Medicine, 1975) can be used to calculate minimum required uptake of antibody-isotope conjugate by tumor for therapy and maximum tolerated uptake by normal tissue. These calculations depend on a knowledge of the effective half-life of isotope, conjugated to antibody, in the tumor and normal tissue.

Results of studies using i.v. injection of monoclonal antibod-
jugates were assessed by a direct radiobinding assay which demonstrated
overlap of the counts of known standards. From the time of instillation, all urine passed by the patient was
saved, the volume measured 12 hourly and aliquots taken and stored
for counting. Blood samples were taken at 6 and 18 h after injection
and continued for 10 days after resumption of oral intake.

Results were expressed as the percentage of instilled antibody per
gram of tissue. In this way comparisons of HMFG2 uptake in normal
and malignant tissue could be made, as could comparisons of uptake
of HMFG2 and UJ13A. Each patient, therefore, served as her own
control.

All samples were assessed histopathologically to ensure tumor pres-
ence or absence as expected. All tumor samples were assessed for
HMFG2 antigen expression by the immunoperoxidase technique as
described elsewhere (14) and were positive. Antigen expression varied
from tumor to tumor and within the same tumor (14).

After 10 patients had been studied in this fashion it was apparent
that absolute levels of HMFG2-radioiodine conjugate uptake in tumor,
tumor-normal tissue ratios, and HMFG2-UJ13A ratios in tumor, were
similar to those reported previously for i.v. injection of antibody. In
order to test the hypothesis that i.p. administration could produce
increased tumor uptake, a direct comparison was made of the uptake
of HMFG2 after simultaneous i.p. and i.v. injection. The study was
conducted in the same way as previously described except that no
UJ13A was injected i.p. Instead the same quantity (0.5 mg) of radioi-
donated HMFG2 was injected i.v. in 5 ml normal saline.

For these studies, early time points (4, 18, and 36 h) were assessed
as it was felt that maximum i.p./l.v. advantage would be seen before
significant amounts of labeled antibody had entered the circulation
after i.p. injection.

Estimation of Free and Protein Bound Iodine in Biological Fluid. To
determine that the data obtained from these i.v. studies referred to
antibody radioiodine conjugates and not to free iodine, the proportion
of each was estimated by TCA precipitation. To a 1-ml sample of the
fluid to be tested (e.g., serum, ascites, and urine) was added 1 ml of
10% TCA (Sigma; Poole, UK) in phosphate buffered saline. After
precipitation, the sample was centrifuged and the supernatant and protein precipitate were counted separately.

RESULTS

Localization of HMFG2 after i.p. Injection in Patients with
Ovarian Cancer. After i.p. injection, the HMFG2 radioiodine
conjugate was found to localize heterogeneously in these ovar-

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Table 1 Characteristics of patients in this study

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Study type</th>
<th>Histopathology*</th>
<th>Stage</th>
<th>Time studied (h)</th>
<th>Operation</th>
<th>Disease status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HMFG2/UJ13A</td>
<td>Mod. diff. serous</td>
<td>III</td>
<td>24</td>
<td>Primary debulking</td>
<td>Bulky disease and ascites</td>
</tr>
<tr>
<td>2</td>
<td>HMFG2/UJ13A</td>
<td>Mod. diff. serous</td>
<td>III</td>
<td>24</td>
<td>Primary debulking</td>
<td>Bulky disease</td>
</tr>
<tr>
<td>3</td>
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<td>Mod. diff. serous</td>
<td>III</td>
<td>24</td>
<td>Primary debulking</td>
<td>Bulky disease</td>
</tr>
<tr>
<td>4</td>
<td>HMFG2/UJ13A</td>
<td>Mod. diff. serous</td>
<td>III</td>
<td>72</td>
<td>Primary debulking</td>
<td>Bulky disease</td>
</tr>
<tr>
<td>5</td>
<td>HMFG2/UJ13A</td>
<td>Mod. diff. serous</td>
<td>III</td>
<td>72</td>
<td>Second look laparotomy</td>
<td>Residual disease, &lt;5 mm</td>
</tr>
<tr>
<td>6</td>
<td>HMFG2/UJ13A</td>
<td>Poorly diff. serous</td>
<td>III</td>
<td>72</td>
<td>Primary debulking</td>
<td>Bulky disease and ascites</td>
</tr>
<tr>
<td>7</td>
<td>HMFG2/UJ13A</td>
<td>Poorly diff. serous</td>
<td>III</td>
<td>72</td>
<td>Second look laparotomy</td>
<td>No residual disease</td>
</tr>
<tr>
<td>8</td>
<td>HMFG2/UJ13A</td>
<td>Poorly diff. serous</td>
<td>III</td>
<td>168</td>
<td>Second look laparotomy</td>
<td>Bulky disease</td>
</tr>
<tr>
<td>9</td>
<td>HMFG2/UJ13A</td>
<td>Mod. diff. serous</td>
<td>III</td>
<td>168</td>
<td>Primary debulking</td>
<td>Bulky disease</td>
</tr>
<tr>
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<td>HMFG2/UJ13A</td>
<td>Mod. diff. serous</td>
<td>III</td>
<td>4</td>
<td>Second look laparotomy</td>
<td>Bulky disease</td>
</tr>
<tr>
<td>11</td>
<td>IV/IP HMFG2</td>
<td>Mod. diff. serous</td>
<td>IV</td>
<td>4</td>
<td>Primary debulking</td>
<td>Bulky disease and ascites</td>
</tr>
<tr>
<td>12</td>
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<td>Mod. diff. serous</td>
<td>IV</td>
<td>4</td>
<td>Second look laparotomy</td>
<td>Residual disease, &lt;5 mm</td>
</tr>
<tr>
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<td>IV/IP HMFG2</td>
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<td>III</td>
<td>4</td>
<td>Paracentesis</td>
<td>Ascites</td>
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<td>IV</td>
<td>18</td>
<td>Primary debulking</td>
<td>Bulky disease and ascites</td>
</tr>
<tr>
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<td>Mod. diff. serous</td>
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<td>18</td>
<td>Primary debulking</td>
<td>Bulky disease and ascites</td>
</tr>
<tr>
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<td>Mod. diff. serous</td>
<td>III</td>
<td>36</td>
<td>Primary debulking</td>
<td>Bulky disease and ascites</td>
</tr>
<tr>
<td>17</td>
<td>IV/IP HMFG2</td>
<td>Mod. diff. serous</td>
<td>III</td>
<td>36</td>
<td>Second look laparotomy</td>
<td>Residual disease, &lt;5 mm</td>
</tr>
</tbody>
</table>

* Mod. diff., moderately differentiated.
ian cancers. This variability was seen between patients (range, 0.001–0.003%/g) and between solid tumor nodules of the same patient (range, 0.00001–0.0200%/g). Within the same patient, maximum localization of the conjugate was seen in tumor nodules which were papillary and which had free access to the peritoneal cavity. Minimal uptake was seen in solid tumor masses, particularly those with most antigen positive cells buried within the tumor mass. This heterogeneity made it extremely difficult to dissect temporal trends from these studies. Solid tumor: blood ratios of 0.3–4.0 were seen, but did not appear to increase with time after injection, while solid tumor: normal tissue ratios of 0.9–10.0 were seen and did appear to increase with time. When individual tumor nodule size was assessed with regard to antibody conjugate uptake there appeared to be no relation. Tumor nodules ranged in size from 0.02 to 2.70 g (Fig. 1).

When uptake by ascites cells were assessed, a considerably greater degree of localization was apparent. Uptake of up to 0.9218%/g was seen, in all instances uptake by ascites cells was greater than the maximum seen for solid tumor. In one instance, an ascites: blood ratio of 448 was seen 4 h after injection of the antibody conjugate, this ratio had decreased to 121 by 36 h. Ascites: normal tissue ratios of >100 were seen at each time point out to 36 h.

These data are summarized in Tables 2 and 3.

In one patient (No. 14), an attempt was made to increase the tumor cell content of an ascites cell pellet by density gradient centrifugation. This allowed the removal of the contained erythrocytes and doubled the activity per gram of the cell pellet. No attempt was made to remove other cell types such as mesothelial cells, macrophages, or lymphocytes found in ascites, from the cell pellet as the manipulations required were thought to make any result obtained unreliable.

Comparison of Uptake of Specific (HMFG2) Antibody Conjugate with Nonspecific (UJ13A) Control. With the major exception of the three patients injected 72 h prior to laparotomy, preferential uptake of the HMFG2 conjugate into tumor tissue, when compared with the UJ13A conjugate, was seen. HMFG2: UJ13A ratios up to 4 were recorded. Of the three patients injected at 72 h, two were injected with the same preparation on the same day, however, the third was injected 2 months later.

No consistent difference in conjugate localization was seen when blood and normal tissue were examined (Table 2).

Comparison of the i.p. and i.v. Routes of Administration. After simultaneous i.v. and i.p. injection of HMFG2 radioiodine conjugate, consistently higher levels were seen in blood, normal tissue, and solid tumor after i.v. than i.p. injection; i.p. : i.v. ratios of 0.07–0.55 in solid tumor were seen. However, for ascites cells, considerably greater localization was seen after i.p. than i.v. injection, i.p.: i.v. ratios of 4–71 being recorded (Table 3).

Pharmacokinetics. After i.p. instillation, serum levels of isotope rose to peak at 8.6 ± 2.0% of the injected amount of 48 h. Assuming a mean blood volume in women of 4500 ml, the maximal blood activity was approximately 0.002%/ml whole blood. These data, collected on all patients up to surgery, accord well with the data presented in Tables 2 and 3 where the blood levels quoted are only from those patients undergoing surgery at that time point. The clearance half-life was measured as approximately 38 h.

Whole body activity declined exponentially with time. The whole body half-life was 50 h when calculated from Fig. 2. It must be noted that the data on whole body activity was collected on only two patients up to 168 h, so data for the later time points (>48 h) are less reliable than the earlier ones. Clearance half-life from blood was calculated as 38 h.

Most of the radioiodine was lost in the urine, with 80% of the injected dose excreted by this means by 168 h.

When the protein fraction of samples of peritoneal fluid, serum, and urine was precipitated with TCA, over 90% of the iodine precipitated from the peritoneal fluid, 80% from the serum (taken at all time points to 168 h), but less than 10% from the urine. Therefore, excretion of radioiodine in these patients occurred to a large extent in the urine as nonprotein conjugated iodine.

**DISCUSSION**

In a series of 18 patients with ovarian carcinoma, localization of i.p. injected HMFG2 in tumor tissue was shown to be approximately 0.001–0.003% of the injected amount per gram tumor tissue. A consistent advantage of HMFG2 localization...
in tumor over normal tissue was seen and a specific-nonspecific antibody localization advantage was seen in tumor tissue.

Injection i.v. was consistently associated with higher levels of antibody uptake in tumor tissue than i.p. injection. The absolute levels of antibody localized in tumor tissue after i.v. injection are similar to those previously published for these and other antibodies (7–10).

Three patients, studied at the 72-h time point, showed no specific localization of HMFG2. Specific and nonspecific antibody uptake into tumor tissue was of the same order as that seen at other time points for nonspecific uptake. Unfortunately in these patients, no assessment of antibody immunoreactivity was performed, as all previous assessments had shown no loss of activity by the method of iodination used. Thus, loss of HMFG2 specific activity at iodination may be the reason for these data; however, it is difficult to understand why this should not have occurred on other occasions.

Data from animal xenograft experiments suggest that, after i.v. injection, specific uptake of antibody is inversely proportional to tumor mass (22). When tumor mass and antibody uptake were correlated in this study, no such relationship could be demonstrated. The proportion of antibody localized in a gram of tumor tissue was not dependent on the size of the tumor nodule from which it came.

To date, two other studies have been published for comparison with these data on i.p. injection. Larson et al. (23) investigated two patients with pseudomyxoma peritonei from gastrointestinal cancer after i.p. injection of the monoclonal antibody B72.3 labeled with $^{131}$I and 0.02–0.92% injected dose per gram of tumor was recovered at surgery. Paganelli et al. (24), investigated the i.v. and i.p. routes of administration of labeled monoclonal antibodies in patients with peritoneal deposits from colon cancer. Although absolute tumor levels were not reported, the statement was made that no concentration advantage was shown for the i.p. over i.v. administration although tumor:nontumor ratios were higher when the antibody was given i.p.

It is therefore clear that i.p. instillation of specific monoclonal antibody has not led to the major concentration advantages in solid tumor which were expected.

For antigen positive ascites, however, there was demonstrated a 4- to 71-fold advantage of i.p. instillation over i.v. In addition, in individual patients the absolute levels of antibody uptake ranged from 0.0121 to 0.9218% g ascites cells. This variation may be due to variations in the HMFG2 antigen positive cancer cell content in the ascites cell pellet and the presence of greater or lesser amounts of HMFG2-antigen negative, normal cells. The major observation made was that in these patients the localization of HMFG2 onto ascites cells was significantly greater than in solid tumor and that ascites cells:normal tissue ratios were 192–4553.

These data are generally consistent with those derived in nude mice studies using s.c. and i.p. xenografts of human ovarian cancer (19). Using these models, it was shown that concentration of radiisotope-antibody conjugate in solid tumors was proportional to concentration of conjugate in blood, regardless of route of administration. However, for ascites, a consistent concentration advantage was seen when the conjugate was given i.p. That study suggested that direct access to solid tumor was not available from the peritoneal cavity and that absorption into the vascular compartment was required for distribution to tumor. In these patient studies, however, the highest absolute concentration and best tumor: blood ratio was seen at 4 h, before there had been significant absorption into the vascular compartment. These levels were low relative to i.v. injection, and may represent antibody bound peripherally to antigen directly exposed to the peritoneal cavity.

This study was designed to assess if sufficient discrimination between antibody uptake in normal tissue and tumor and between tumor and blood could be achieved by the i.p. route of administration to allow antibody guided $^{131}$I regional irradiation therapy as proposed by several groups (25–27). It is known that the dose to any target tissue is $K \times t_o \times C$, where $K$ is a constant dependent on the isotope and target tissue, $t_o$ is the effective half-life of that isotope in the target tissue, and $C$ is the concentration of isotope in the tissue. The $t_o$ value in tumor has been assessed by Larson et al. (23) as 120 h; $t_o$ in blood has...
been measured in this study as 38 h. Then,
\[
\frac{D_t}{D_b} = \frac{120}{38} \times \frac{C_x}{C_y} \times \frac{K_t}{K_b}
\]
where \(D_t\) is dose to tumor and \(D_b\) is dose to blood.

For efficacy of tumor cell death, the dose to tumor value needs to be \(\geq 5000\) cGy and for limitation of toxicity the dose to blood value must be \(< 200\) cGy (6). Then the minimum requirements (tumor:blood ratio) for safe, effective therapy are
\[
\frac{5000}{200} = \frac{120}{38} \times \frac{C_x}{C_y} \approx \frac{K_t}{K_b}
\]
i.e.,
\[
\frac{C_x}{C_y} = \frac{5000}{38} \times \frac{120}{200} = 8
\]

In this study a localization advantage of \(> 8\) was not observed for solid tumor; however, when ascites and blood concentrations of isotope were examined, their ratio consistently surpassed this figure. It is acknowledged that these calculations are greatly simplified and represent the minimum requirements for therapy. The use of more sophisticated microdosimetry calculations is unlikely to be more encouraging, as the presence of viable, antigen negative tumor cells in any tumor cell population means that a tumoricidal dose of irradiation has to be delivered to the whole tumor and not just to the antigen positive cells. The overall tumor concentration of isotope is therefore important. The influence of alternative radioisotopes on these calculations is not yet known.

On the basis of these data it is predicted that the use of injected \(^{131}I\)HMFG2 guided radiotherapy will not be effective in treating solid tumor deposits in ovarian cancer, but may be extremely effective in treating recurrent ascites.

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