Pharmacokinetics, Biodistribution, and Dosimetry of Specific and Control Radiolabeled Monoclonal Antibodies in Patients with Primary Head and Neck Squamous Cell Carcinoma

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ABSTRACT

The pharmacokinetics, biodistribution, and dosimetry of an IgG1 radiolabeled anti-mucin mAb (HMFG1) and an isotype-matched control (4D513) were studied in 29 patients with primary head and neck squamous cell carcinoma. Patients were given injections at 3 fixed time points prior to surgery, i.e., 24 (n = 12), 48 (n = 9), or 72 (n = 8) h. They were subsequently classified into two groups based on their immunohistochimical positivity for polymorphic epithelial mucin. Fourteen patients (48%) were positive, 5 of which were studied with both antibodies; and 15 patients were negative (52%), 7 of which were studied with both antibodies. There was no significant difference in serum pharmacokinetics and cumulative urinary clearance of the two antibodies. There was no significant difference in overall normal tissue uptake of specific and control antibody; however, when each component of the normal tissue category was analyzed individually, there was a significantly increased uptake of HMFG1 in mucosa as compared to control antibody. Immunohistochimical studies revealed positive staining of mucosa with HMFG1.

There was significantly increased uptake of specific antibody in antigen-positive tumors as compared to uptake of control antibody (P < 0.02). A tendency for less label loss over time from positive tumors as compared to control was documented. Absolute antibody uptake and tumor/normal tissue ratios demonstrated significant overlap in individual patients from each category depending on the specific ratio (e.g., tumor/adipose tissue) or time point studied; hence arbitrary cutoff values could not be recommended as indicators of specific uptake. Specificity and localization indices were the most reproducible indicators of specific localization.

Areas under the curve were calculated for all tissues, and local dosimetry for the two ß-emitting isotopes 131I and 90Y is presented. The D50 values for antigen-positive tumors were 2.9 cGy/mCi for 131I and 9.0 cGy/mCi injected for 90Y. For antigen-negative tumors these values were 2.9 cGy/mCi for 131I and 9.0 cGy/mCi injected for 90Y. For antigen-negative tumors these values were significantly lower at 0.83 and 2.4 cGy/mCi of 131I and 90Y, respectively. Bone marrow D50 was calculated to be 0.87 cGy/mCi of 131I-HMFG1 injected.

Because the purpose of our ongoing research is to assess the therapeutic potential of the combination of radiolabeled antibody and external radiotherapy, detailed dose calculation to local dose-limiting tissues is required. D50 to mucosa was calculated to be 1.1 and 3.8 cGy/mCi of injected 131I and 90Y, respectively.

We conclude that a 9–10-Gy dose increment may be achieved in two administrations of 150 mCi of 131I-HMFG1 during a course of external radiotherapy. This may lead to improved control of local disease in patients with head and neck cancer.

INTRODUCTION

Squamous cell carcinoma represents the predominant histological type among tumors of the head and neck. They account for approximately 5% of all malignant neoplasms in Europe and the United States (1). An estimated 378,500 new cases were diagnosed in 1982 worldwide and more than 500,000 new cases were projected to occur in 1992 (2). The early stages are treated with single modality surgery or radiotherapy, advanced stages with combined surgery and radiation therapy. The role of chemotherapy is not yet established (3). Sixty % of patients have a stage III–IV tumor at presentation. Despite nonuniformity in treatment options and varied prognoses for the many possible sites of a head and neck squamous cell carcinoma, the overall 5-year disease-free survival for these patients is around 30–40%. Local recurrence is the main reason for treatment failure (50–60%) and death, with about 10% of patients dying of distant metastases (4).

Head and neck cancer is a radiosensitive tumor with a relatively steep dose-response curve. There is empirical and preclinical evidence indicating that, were it possible to achieve a 10–20% increase of the currently applied dose of radiotherapy, a significant number of patients who fail treatment due to lymph node micrometastases and/or postsurgical microscopic local tumor remnants may be cured (5). However, any increase in this dose via external beam radiotherapy alone will result in a disproportionate increase in local normal tissue damage (5).

Anecdotally, all forms of immunotherapy have at one time or another been tried for head and neck cancer. Chemoimmunotherapy, using Bacillus Calmette-Guérin and chemotherapeutic agents, was tried without success (6). Currently a variety of nonspecific immunotherapy protocols based on α-interferon or interleukin 2 administration have been tried (7). Apart from establishing an increase in tumor-infiltrating lymphocytes in the tumor, no complete remission has yet been documented. Regional administration of interleukin 2 has been used with equivocal results (8).

The existence of a variety of antigenic targets, e.g., EGFR2 has seen the initiation of in vivo experiments to assess the result of functional blockade of these receptors. Results, at least in animal models, are promising (9). Radioimmunotherapy of head and neck squamous cell carcinoma-derived xenografts has been successful with either 131I or 186Re as the therapeutic isotope (10, 11). At present a small number of antibodies exist that have been produced specifically against head and neck cancers (12–14). Of these, few have properties compatible with immunodiagnosis and immunotherapy. A number of antibodies raised against antigens from other cancers, either squamous or adenocarcinomas, cross-react with head and neck cancers (15). One such antibody that has also been extensively used in clinical trials is HMFG1, directed against P- and S1, localizability index; AUC, area under the curve; PDTRP, proline-aspartic acid-threonine-arginine-proline; FPLC, fast protein liquid chromatography; MIRD, medical internal radiation dose; RI, radioimmunotherapy; i.d., injected dose; CEA, carcinoembryonic antigen; RI, radioimmunotherapy index.

1 The abbreviations used are: EGFR, epidermal growth factor receptor; HMFG1, human milk fat globule 1, HMFG2, human milk fat globule 2; PEM, polymorphic epithelial mucin; SI, specificity index; LI, localization index; AUC, area under the curve; PDTRP, proline-aspartic acid-threonine-arginine-proline; FPLC, fast protein liquid chromatography; MIRD, medical internal radiation dose; RI, radioimmunotherapy; i.d., injected dose; CEA, carcinoembryonic antigen; RI, radioimmunotherapy index.

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2 The abbreviations used are: EGFr, epidermal growth factor receptor; HMFG1, human milk fat globule 1, HMFG2, human milk fat globule 2; PEM, polymorphic epithelial mucin; SI, specificity index; LI, localization index; AUC, area under the curve; PDTRP, proline-aspartic acid-threonine-arginine-proline; FPLC, fast protein liquid chromatography; MIRD, medical internal radiation dose; RI, radioimmunotherapy; i.d., injected dose; CEA, carcinoembryonic antigen; RI, radioimmunotherapy index.

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radiolabeled antibodies administered i.v. has shown that only 0.01–0.001% of injected dose/g of tissue binds the tumor. The majority of literature references quote a possible 10–20-Gy radiation dose to typical tumors studied, depending on isotope used, antibody biodistribution, and number of administrations (17, 18). This is not a toxic dose. Bone marrow sensitivity is the usual dose-limiting factor. Nevertheless, this amount of selectively targeted radiation to the tumor may be significant if used as a combination supplement during a routine external beam radiotherapy treatment course.

The concept of combining two distinct radiation modalities is not new. Apart from classical implant brachytherapy/external radiotherapy, e.g., for the treatment of floor of mouth lesions (19), i.e., radioactive colloid treatments together with external beam radiotherapy, either fractionated or single dose, have been tried (20). However, the nonselectivity of the colloid interstitial treatment has led to high local normal tissue toxicity.

This study is the precursor of a longer term project involving the assessment of the combination of radiolabeled antibodies and external radiotherapy for the treatment of head and neck squamous cell carcinoma and was undertaken with a view to derive dosimetric data for the HMFG1 radiolabeled antibody.

**Materials and Methods**

**mAbs**

HMFG1. This antibody, originally designated 1.10.F3, was raised against the human milk fat globule (21). HMFG1 (IgG1) was developed from a mouse receiving a further boost of HMFG and recognizes an antigenic determinant on the core protein of the HMFG designated PEM with a high molecular weight ($M_r > 400,000$) composed of tandem repeats of a PDTRP motif (22). Variation in glycosylation patterns of PEM are proposed to explain the difference in antigen positivity patterns noted for a variety of adenocarcinomas. This antibody reacts strongly with a wide range of human carcinomas.

4D513. This antibody was used as an isotype-matched negative control for HMFG1. 4D513 is an anti-idiotypic mAb, which recognizes an idiotype on an IgM immunoglobulin isolated from the serum of a patient with B-cell lymphoma (23). Its reactivity is strictly restricted to the immunizing immunoglobulin. It was kindly supplied by Unipath Ltd., Bedford, United Kingdom.

**Immunohistochemistry**

The following technique was found to be reliable and was used throughout this study. mAbs were used at 10 $\mu$g/ml PBS/0.05% Tween 20.

**Paraffin Sections.** Serial sections 5 $\mu$m thick were cut and picked up on slides coated with 0.1% poly-L-lysine, dried overnight at 37°C, and stored at room temperature until use. For immunostaining, they were dewaxed in four changes of xylene, hydrated through graded alcohols, and finally placed in 100% absolute alcohol (United Kingdom) and incubation was allowed to proceed for 30 min. After three washings in PBS, sections were drained but not rinsed and the first antibody was applied. Incubation proceeded for 30 min at room temperature. All incubations were performed in a humid atmosphere at room temperature. Slides were then washed with water, stained lightly with Mayer’s hemalum, differentiated with 1% acid alcohol, blued with Scott’s tap water, dehydrated through alcohols, cleared, and mounted in Histomount (National Diagnostics, NJ).

**Frozen Sections.** Sections 5 $\mu$m thick were cut and picked up on uncoated slides, air-dried overnight, and stored at –20°C until use. For immunostaining they were fixed in acetone/methanol (1:1) at 4°C for 5 min and then allowed to air-dry. Endogenous peroxidase was blocked in a solution containing 1 mM sodium azide, 1 mM D-glucose, and 1 unit/ml glucose oxidase (Sigma) in PBS. Slides were incubated in this solution for 1 h at room temperature and then washed for 5 min in PBS. Nonspecific reactions were blocked by a 5% solution of nonimmune serum of the species which provided the second antibody. Slides were incubated with this solution for 30 min. After this step the procedure was completed as described for paraffin sections.

**Radiolabeling** (1211, 1311)

The Iodo-Gen method was used for iodinations (24). Briefly, 1,3,4,6-tetrachloro-3a,6a-diphenylglycoril (Iodo-Gen; Pierce, United Kingdom) was used for the covalent labeling of HMFG1 and 4D513 with $^{1211}$ or $^{1311}$ (Amersham IMS 30 and IBS 30). A chloroform solution containing 5 mg/ml of Iodo-Gen was made. Aliquots of 100 $\mu$L (50 $\mu$g) in sterile NUNC tubes were left overnight in a fume hood to evaporate. These were subsequently stored at –20°C and used as required. New batches were made every 3 months.

Of the pH of the antibody solution was adjusted to 7.5 using 1 M Tris, pH 9.5. Protein was mixed with $^{1211}$ or $^{1311}$ in a tube containing 50 $\mu$L Iodo-Gen, at room temperature, rotating for 10 min. Free iodine was separated from labeled protein using a 20-ml Sephadex G-50 column (Pharmacia, Uppsala, Sweden). The column was eluted with PBS. Fractions of 2 $\mu$L were collected and the activity of each fraction was counted in a gamma counter. The fractions containing the protein peak were collected and filtered through a 0.22 $\mu$m Millipore filter (Millipore, Watford, United Kingdom). Labeling efficiency was calculated as the ratio of protein bound activity over activity loaded on the column and specific activity as the value of protein bound activity ($\mu$Ci) per total protein ($\mu$g). The purity and stability of the conjugates were tested by FPLC. All patients were given injections within 3 h of labeling with 160 $\mu$Ci $^{1211}$; patients studied with both labels received a further 210 $\mu$Ci $^{1311}$-labeled control antibody.

**Fast Protein Liquid Chromatography**

Radiolabeled fractions of injection samples were obtained by FPLC (Pharmacia). Superose 6 was used for the gel filtration. This column can achieve good resolution for proteins with molecular weights between 5,000 and 5,000,000. The flow rate was 0.5 ml/min and 0.5-ml fractions were collected in scintillation tubes. The volume of the samples loaded was 100 $\mu$L containing 2–5 $\mu$g of protein. The elution buffer was PBS containing 0.05% sodium azide. Tubes were subsequently measured in a gamma counter.

**Direct RIA**

This assay was used as a semiquantitative estimation of the immunoreactivity of HMFG1. Solid phase antigen was PDTRP conjugated to BSA. PDTRP is the amino acid motif that is tandemly repeated to form the core of PEM. Conjugation of PDTRP to BSA was achieved according to the method described by Gullick (25).

Antigen was immobilized in 96-well immunoassay microtiter plates (Dynatech, Chantilly, VA). Antigen coupled to BSA was diluted at 5 $\mu$g/ml in bicarbonate buffer (pH 9.6) and 50 $\mu$L were transferred to each well of the microtiter plate. Some rows were left without antigen and some rows contained only BSA in order to assess the nonspecific binding of the reagents to the plates/BSA.

The radiolabeled antibodies were diluted to 200 nM in PBS/Tween 20 and 90 $\mu$L were transferred to each well, performing 1:3 dilutions down the plate. Plates were incubated with primary antibodies for 1 h at 37°C in a humid chamber. Blocking of nonspecific reactions was achieved by incubating the plates with 100 $\mu$L 2% BSA in PBS per well at 37°C for 30 min prior to incubation with primary antibody. After being washed as previously described, well were cut and transferred to tubes, and radioactivity was counted in a gamma counter with a standard of the first dilution in triplicate.

**Patients**

Written informed consent was obtained from all patients. The study protocol was approved by the Hammersmith Ethics Committee. From February 1992 to
October 1993 31 patients with primary head and neck cancer, 25 males and 6 females with a mean age of 62 ± 12 (SD) years (range, 28–89 years), received radionuclide labeled HMFG1 antibody i.v.

**Protocol of Biodistribution Study.** Patients were given injections at fixed time points (24, 48, or 72 h) prior to surgery. Immediate blood samples (5–6 samples in the first 30 min) after injection were drawn. A 3–4 ml blood sample at 1–2 h and a daily sample for the following 4 days were obtained. Samples were clearly and accurately labeled with the time and date of vesesection. A 24-h urine collection was initiated immediately in order to collect the total volume of urine excreted following the injection and was maintained for 96 h. Complete blood clearance data were obtained from 4 patients for the control antibody and 10 patients for HMFG1, while complete cumulative urinary excretion data were obtained from 13 patients for HMFG1 and 5 for 4D513. Tissue samples were obtained at surgery. These included tumor tissue from the primary, whereas tumor-invaded distal sites (e.g., lymph nodes) were obtained where available. Specimen size was 6 x 2 x 2 mm or more. No panendoscopy biopsies were included in these studies. Samples of normal tissues (skin, muscle, fat cartilage, lymph node, tonsil, etc.) were also obtained when and as available. Areas of normal tissues altered by electrocautery were avoided. All tumor samples were cut in half; one half was fixed and the other half was stored in liquid nitrogen. Normal tissue samples were fixed. Standards of the injection(s) material were made in triplicate and used to cross-correct for the dual label and to correct for isotope decay. The tissue, blood, and urine samples were weighed and then measured for activity together with the standards in a gamma counter. Results were expressed as a percentage of the injected dose per kg.

**Calculation of LI and SI**

These two indices given an impression of relative targeting efficiency of the specific antibody in relation to a nonspecific control. They can be derived for individual tumors where both antibodies (Ab) have been simultaneously injected and measured in the same tumor and normal tissue specimens. They are calculated as

\[
LI = \frac{\text{Specific Ab in tumor}}{\text{Specific Ab in blood}} \times \frac{\text{Control Ab in tumor}}{\text{Control Ab in blood}}
\]

(A)

\[
SI = \frac{\text{Specific Ab in tumor}}{\text{Specific Ab in normal tissue}} \times \frac{\text{Control Ab in tumor}}{\text{Control Ab in normal tissue}}
\]

(B)

Five tumors in the antigen-positive groups (two tumors at day 1 and three at day 3) and seven in the antigen-negative group were eligible for calculation of LI, inasmuch as they were simultaneously studied with both antibodies. SI could be calculated for all these patients except for one in the antigen negative group for whom only tumor and blood samples were available.

**Volume Measurements**

Human tumor dimension measurements (orthogonal diameters) were obtained for 20 of the 29 pathological tumor specimens. Tumor volume was calculated as

\[
V = \frac{4}{3} \pi \cdot d1 \cdot d2 \cdot d3
\]

(C)

where \(V\) is the tumor volume and \(d1, d2,\) and \(d3\) are the three orthogonal diameters \((H \times W \times L)\) of the measured tumor.

**Dosimetric Calculations**

Dosimetric calculations were made for the two isotopes available to us for treatment, \(^{32}\)P and \(^{124}\)I. The calculations are based on the MIRD formulations for internally administered isotopes (26). The tissues for which such calculations can be made in our case are the blood and in extrapolation the bone marrow and liver and the normal structures (muscle, fat, and mucosa). The equilibrium absorbed dose that is derived \(D_{eq}\) is expressed in GY/mCi of activity injected.

The following series of calculations have been performed for these isotopes. An antibody area under the curve (mAb AUC) for mucosa, muscle, fat, blood, and tumor was calculated. The area under the curve was derived from the geometric surface of the 0–3-day time points (one triangle and two trapezia) for each isotope (AUC\(_{0-3}\)) following decay correction for each time point as

\[
A_f = A_0 \cdot \exp \left( -\frac{t}{t_{1/2}} \right)
\]

\[D_{eq} = A_0 \cdot \exp \left( -\frac{t}{t_{1/2}} \right)
\]

where \(n\) is 1, 2, and 3 days, respectively, and \(t_{1/2}\) is the half-life of the isotope for which calculations are being made (expressed in days).

To calculate the AUC after the three studied time points (AUC\(_{0-3}\)), exponential decay was assumed using the formula

\[
A_{0-3} = A_{1-3} \cdot 1.44 \cdot A_3
\]

(E)

where \(A_3\) represents antibody uptake at 3 days. The effective half-life \(t_{1/2-3}\) was calculated as

\[
t_{1/2-3} = \frac{1}{t_{1/2-3}} = \frac{1}{t_{1/2-3}} + \frac{1}{t_{1/2-3}}
\]

(F)

Antibody half-life \(t_{1/2-3}\) was calculated for each tissue separately after fitting an exponential curve to the three existing time points.

The equilibrium absorbed dose \(D_{eq}\) for 1 g of tissue uniformly irradiated by the isotope is given by the MIRD equation

\[
D_{eq} = \frac{A_0}{S_{tissue}} \cdot \Delta_t
\]

(G)

where \(\Delta_t\) is the equilibrium dose constant for the isotope studied \((g \cdot GY/\mu Ci \cdot h)\).

Calculation of equilibrium absorbed dose to bone marrow was based on the assumptions that bone marrow is 2.2% of body weight (1500 g) and contains 33% of blood at any given moment, while equilibration between blood and bone marrow is taken as instantaneous. The equation for this tissue is

\[
D_{eq(marrow)} = 0.33 \times \frac{A_0}{S_{blood}} \cdot \Delta_{marrow}
\]

(H)

where \(S_{marrow}\) is the S value for marrow for reference man as obtained from the MIRD tables.

The equilibrium absorbed dose to the liver was calculated based on the assumption that 6% of the blood volume is contained in the liver at any one time and its extracellular space is not functionally part of the circulation. Irradiation is therefore assumed for both these organs to be primarily from the blood pool. The equation for this organ is

\[
D_{eq(liver)} = 0.06 \times \frac{A_0}{S_{blood}} \cdot \Delta_{liver}
\]

(I)

where \(S_{liver}\) is the S value for liver for reference man as obtained from the MIRD tables. These indirect calculations for these two organs (bone marrow and liver) were made only for \(^{124}\)I, because there is experimental evidence showing that \(^{80}\)Y accumulates in liver and bone matrix, therefore invalidating the above assumptions.

**Pharmacokinetics and Statistics**

The blood clearance half-lives were calculated by a least squares fit to the equation for exponential decay

\[
A = A_0 \cdot e^{-\lambda n}
\]

(J)

where \(\lambda = \ln 2/t_{1/2}\). This was done separately for each set of data obtained from individual patients. Table 2 represents the mean of the individual blood clearance half-life values obtained. The computer program used was P. Fit from Biosoft, Cambridge, United Kingdom. The statistical significance of the difference between means of the patient groups that emerged based on the above histological classification system was determined by Student's t test. \(P < 0.05\) was considered to be significant.

**RESULTS**

During the radiolabeling procedure a specific activity of 2 \(\mu Ci/\mu g\) was aimed at. However, labeling efficiency varied unpredictably from 6% to 82%; therefore the policy that was adopted was to administer a standard radiation dose of 160 and 210 \(\mu Ci\) of \(^{125}\)I and \(^{131}\)I, respectively, to each patient. This meant that the protein quantity of the
Table 1 Anatomical origin and antigen status of the tumor samples studied

<table>
<thead>
<tr>
<th>Anatomical site of tumor in patients receiving HMFG1 in vivo</th>
<th>Total</th>
<th>Larynx</th>
<th>Tongue</th>
<th>Pyriform fossa</th>
<th>Skin</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEM antigen status of studied patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEM positive (&gt;50%)</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEM negative (&lt;50%)</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

*One patient with a laryngeal tumor and one with a tongue tumor had no evidence of tumor in the surgical specimens available for radioactivity counting.

**The anatomical locations of these tumors were 1 retromolar trigone, 1 maxillary sinus, and 1 gingiva.

Injection material was variable, ranging from 80 to 140 μg of each antibody per administration. A FPLC profile of the injection material demonstrated a single peak at the expected molecular weight for antibody and a smaller peak for the free isotope. The injection material contained less than 5% of free isotope in all cases. No apparent loss of immunoreactivity of the labeled specific antibody was observed, as demonstrated by radioimmunoassay.

**Patients.** Patients were given injections at 3 fixed time points prior to surgery, i.e., 24 (n = 13), 48 (n = 10), or 72 (n = 8) h. Two of these patients, one male and one female (one day 1 and one day 2), had no evidence of cancer in the surgical sample available for study and are not included in the calculations. Fourteen of these patients (day 1 n = 4, day 2 n = 4, day 3 n = 6) received a concurrent administration of a control antibody (4D513). The anatomical locations of these tumors are presented in Table 1.

There was no significant difference in patient age or anatomical location of tumor in the antigen-positive and -negative patient groups. However, all five female patients studied were in the positive group, an event probably attributable to chance.

**Histology Results.** It was apparent on hematoxylin and eosin staining that antigen positivity was associated with the more differentiated/keratinizing elements of tumor while the anaplastic lesions were largely negative. Table 1 represents the antigen profile of the patients studied. Of the 29 patients studied prospectively 18 (62%) were classified as having moderately differentiated, 4 (14%) well differentiated, and 7 (24%) poorly differentiated tumors. The patients were classified into two groups based on the immunohistochemical findings (Table 1). The tumor sections from patients categorized as being antigen positive had at least 80% of the section as studied at low power field infiltrated by tumor and at least 50% of cells positive for HMFG1 (Fig. 1). Fourteen patients were classified in this group. All other patients (n = 15) were classified as being antigen “negative.” It is obvious that the distinction is arbitrary and this scoring system is wide for the “negative” category, which comprises the few truly negative tumors but also tumors with antigen-positive elements (cells or islets) that do not meet the above criteria. Fig. 2 represents examples of tumors included in the “negative” group. It is interesting to note that all undifferentiated tumors fell into the “negative” category while all the well differentiated fell into the “positive.”

The majority of mucosa specimens studied showed varied levels of positivity for the antibody (HMFG1). It was noted that histologically normal mucosa in proximity to tumor tissue often exhibited more intense and widespread staining for the antibody compared to mucosa from the same patient but from a site remote to the tumor. Examples of these findings are presented in Fig. 3. It can also be noted that the more terminally differentiated layers of normal mucosa seemed to display greater positivity.

**Pharmacokinetics and Biodistribution of Antibodies.** The pharmacokinetics of the two antibodies is presented in Table 2. Clearance from the blood and urine for both antibodies was similar, with around 20% of label in the urine at 24 h, reaching a total of 35% by 96 h. Assuming excretion linearity, an excretion rate of 0.36% i.d./h was calculated for HMFG1 and 0.43% i.d./h for the control antibody (Fig. 4). This difference was not significant. The mean uptake of these two antibodies in tumor and mucosa over the 3 time points studied together with the relative circulating antibody levels at surgery are shown in Fig. 5 and Table 3.

**Absolute Antibody Uptake in Tumors.** The mean uptake values for HMFG1 and 4D513 in (a) all tumors irrespective of antigen status and day of injection, (b) the antigen-positive and antigen-negative tumors, and (c) the local normal tissues are presented in Table 4. The mean uptake in all tumors studied with HMFG1 was significantly different from the uptake of 4D513 (P < 0.02). Uptake in antigen-positive tumors for HMFG1 as compared to 4D513 for the five patients studied with both labels was different at P < 0.02. When all the positive patients were compared to all the control patients the difference was significant at P < 0.001. The mean uptake of HMFG1 in positive tumors was significantly more (P < 0.002) than the mean uptake of the same antibody in the corresponding antigen-negative tumors. No difference in blood at surgery levels were found for any antibody in any category. No statistical difference was found in the tumor uptake of HMFG1 versus 4D513 in the antigen-negative tumors. No difference was found in the intercontrol comparison of
uptake and blood levels of 4D513 in the cohort of antigen-positive patients versus antigen-negative patients either in tumor or in blood at surgery values.

**Tumor Volume/Mass Calculations.** The mean volumes and masses for the human tumors are presented in Table 5. The specific gravity of tumor tissue has been calculated for a representative squamous cell carcinoma xenograft H.Ep-2 derived from a laryngeal carcinoma.

LI and SI. Localization indices for the positive tumors were 1.35, 1.36 for the day 1 tumors and 3.99, 5.43, and 1.93 for the day 3 tumors. The mean LI was 2.8 ± 1.8. The mean localization index for the seven antigen-negative tumors was 0.97 ± 0.09. The difference was statistically significant at P < 0.02. This index expresses the relative amounts of specific antibody to control antibody in each of the studied tumors, following correction for the variation in circulating activity in blood at the time of surgery.

The specificity indices for the five patients that were antigen positive were 1.30, 1.18 for day 1 and 2.9, 2.12, and 4.12 for day 3. The mean SI for these five tumors was 2.3 ± 1.2 (n = 5) while the mean SI for the antigen-negative tumors was 0.9 ± 0.2 (n = 6; normal tissue other than blood at surgery existed for 6 of the 7 patients in this category) which was different with a value of P < 0.02. This index expresses the relative amounts of specific antibody to control antibody in each of the studied tumors, following correction for the nonspecific uptake in normal tissue studied.

**Tumor/Normal Tissue Ratios.** The mean normal tissue uptakes were 1.8 ± 1.2% i.d./kg (n = 28) for HMFG1 and 1.3 ± 1.1% i.d./kg (n = 11) for the control antibody, while uptake in the individual components of the "normal tissue" category is presented in Table 4. The mean uptake of HMFG1 in mucosa was significantly more than that of 4D513 (P < 0.05). The difference in uptake in tumor compared to mucosa for HMFG1 for all patients was significant (P < 0.02). For the patients in the antigen-positive group this difference was highly significant at P < 0.001. The mean tumor/mucosa ratio was 2.4 ± 0.7 (n = 11). For the patients in the antigen-negative group the mean tumor/mucosa ratio was 1.0 ± 0.5 (n = 12). The tumor/mucosa ratio for control antibody was 1.2 ± 0.7 (n = 9). No significant differences in mean tumor uptake to mean normal tissue uptake existed for the control antibody, and no significant difference existed in the uptake of specific antibody in normal tissues to that of control.

The mean tumor/normal tissue ratio for the specific antibody was 3.6 ± 1.1 (n = 14) while that for the control was 1.8 ± 1.0 (n = 11) which was significantly different at P < 0.001.

**Tumor and Local Tissue Radiation Dose**

The relative dosimetry for the specific antibody in positive tumors is presented in Table 5.

The equilibrium absorbed dose (D_{eq}) to the tumor from the control antibody and the HMFG1 antibody in negative tumors was: 4D513, 0.83 cGy/mCi of 131I injected and 2.8 cGy/mCi of 90Y injected; HMFG1 in negative tumors, 0.84 cGy/mCi of 131I injected and 3.4 cGy/mCi of 90Y injected.

**Bone Marrow and Liver Dosimetry.** The calculated value for AUC for any based on the antibody half-lives (t_{1/2} and t_{1/2}p) was 35% i.d./kg-days which was remarkably similar to the one calculated from the blood at surgery data in Fig. 5 (calculation of surface area of three trapezia and exponential decay based on a half-life calculated from an exponential fitted to these data led to a value of 40% i.d./kg-days). Assuming initial uptake of 20% i.d./kg in blood (~5 kg) and solving Equations H and I, the calculated doses to bone marrow and liver for 131I were 0.87 cGy/mCi and 0.27 cGy/mCi injected, respectively. Assuming the data from Fig. 5 a similar dose of 1.2 cGy/mCi to the bone marrow is derived for 131I.

Calculations were not performed for 90Y because it has been shown that this form of indirect extrapolation of bone marrow and liver dosimetry is very inaccurate (27). Maximum injectable dose of 90Y was derived indirectly from the empirical data obtained in Maraveyas et al. (28) for an 90Y-isothiocyanatobenzyldiethylethylenetriaminepentaacetic acid radioimmunoconjugate injected i.p. into patients with ovarian cancer. Cumulative activity in the circulation was calculated from the data in that paper and the value obtained (24% i.d./kg-days) divided by the cumulative activity obtained in the present study. The constant obtained (~0.68) was used to multiply the injectable dose as determined in the aforementioned study which was 18–19 mCi/m2. These values obviously depend on the assumption that the circulating cumulative activity does reflect the dose that the marrow receives even if this is not from the blood pool only, a prerequisite for MIRD formulae to be effective.

**Dose Rate.** Dose rate was calculated for each 24-h period from the data in Table 5. Dose rates of 2.75 cGy/h for the 24–48 h peak, dropping to 0.6 cGy/h 1 week later, were calculated. A mean tumor dose rate of 1.9 cGy/h was calculated for the dose of 150 mCi over a period equivalent to one effective half-life (144 cGy/76 h) of 131I-HMFG1.

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3 Unpublished data.
DISCUSSION

Studies using radiolabeled antibodies for solid tumor therapy have demonstrated sporadic tumor responses in humans. These responses have usually been short lived or incomplete. It appears unlikely that radiolabeled antibodies alone will cure solid cancers; combined modality therapy therefore is of potential clinical interest. RIT is generally viewed as a systemic therapy for cancer; however, our proposed strategy is to utilize the relatively small dose that can be delivered with RIT as a radiation increment to external beam radiotherapy, with a view to improving locoregional control. This approach is primarily suitable for tumors where inadequate local control is the major reason for treatment failure. Head and neck squamous cell carcinoma typifies one such cancer. From the results presented it became obvious that absolute amounts of radioactivity per g of tissue were within the usual range of 0.001–0.01% i.d./g. The highest levels in tumor were found at day 1 postinjection and a subsequent decline in levels was documented with a decay-corrected half-life of 123 h. At no point in this study did absolute antibody uptake in any biopsy specimen systematically exceed that in the blood. It became obvious that a longer period from injection to surgery than 72 h should be studied for this phenomenon to become apparent. Despite the persistent high circulating levels, all tissues including tumor seemed to exhibit a decline in absolute uptake over time.

While it has been unequivocally shown in the murine models that antigen positivity is a vital property for successful tumor targeting (29), the same does not seem to be as clearly apparent in humans. Whereas some observers (30, 31) have demonstrated a clear advantage of antigen-positive tumors to bind the specific antibody, other observers (32) have not seen a great effect. However, the histological criteria are often not well described or loosely defined. Boxer et al. (33) is a good example of a study seeking correlation of well defined histological and immunohistochemical criteria to tumor uptake of anti-CEA antibody in 56 patients with colorectal cancer. No correlation was found. However, there are substantial differences between their study and that presented in this paper. Fixed time points were not studied: all patients were positive with at least one cellular compartment (membrane or cytoplasm) being intensely positive, in effect reporting on variation within a single group. No control was used, mean injection to resection time was 140 h, and finally the CEA glycoprotein is probably subject to a different metabolic fate in vivo than the PEM glycoprotein (34, 35).

Our biodistribution results reveal that the subpopulation of patients that is deemed to be strongly positive to the antibody, with the aforementioned criteria, demonstrates a significantly increased antibody uptake than either the patients who were "negative" for HMFG1 or to the control antibody. This is not due to differences in blood levels, because blood clearance is the same for both specific and control antibody. Tumor/normal tissue ratios (all tissues) are of the order of 3.5 for the specific antibody, which is significantly higher than the control antibody. The overall indices (SI and LI) for all 5 positive patients studied with both antibodies is significantly higher than that for the antigen-negative tumors,
indicating the specific nature of the HMFG1 uptake and, although not enough positive patients were studied with both antibodies at days 1 and 3 to show the apparent increase in indices over time statistically, that this tendency is clearly apparent, while in the antigen-negative tumors indices remain flat over time. Our findings agree with the opinion that maximum tumor levels of antibody, specific or otherwise, have been attained by 24 h (36).

It has been presumed that an indicator of specificity is the continuing accrual of antibody in the tumor in the face of dropping peripheral levels (37). The fact that there is no significant difference between the levels of the nonspecific and the specific antibody at 24 h seems to indicate that the process for both antibodies was relatively similar over the initial distribution period; it is therefore unlikely that, at least over the first 24 h, increase in uptake is an indicator of specificity. Subsequently, the overall trend is a loss of radioactivity from the tumor site, in spite of higher levels of activity enduring in the circulation over the time period studied. This seems to be more rapid for both the nonspecific antibody 4D513 and for HMFG1 in negative tumors.

Often a tumor/normal tissue ratio, called RI, of >3 is arbitrarily assumed to denote specific uptake (38). This is not borne out by our data, because even the nonspecific antibody in our study often showed a RI of >3 when compared to adipose tissue, while the specific antibody rarely exceeded a 3:1 tumor/mucosa ratio (mean, 2.4 ± 0.7). From the results of our study we can only say that LI and SI seem to be a more reproducible marker of specific targeting as compared to an arbitrarily set RI. Nor is the absolute amount of antibody in a tumor a clear indicator of specific uptake for individual tumors. We can only argue that these tumors exhibit a longer antibody retention time. In our view, therefore, the inclusion of negative control antibody, especially if one is assessing the therapeutic potential of a radioimmunoconjugate, should be contemplated given the uncertainty in controlling the efficacy of antibody targeting. Various researchers have indicated that tumor

Table 3 Absolute uptake of antibody in the tissues represented in Fig. 5

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Antigen class</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>PEM positive</td>
<td>5.5 ± 1.8</td>
<td>5.0 ± 2.3</td>
<td>4.2 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>PEM negative</td>
<td>4.0 ± 1.1</td>
<td>2.3 ± 1.2</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>4D513</td>
<td>4.4 ± 2.3</td>
<td>1.8 ± 0.8</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>Mucoea</td>
<td>PEM (all)</td>
<td>3.1 ± 1.3</td>
<td>3.0 ± 1.2</td>
<td>1.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Blood at surgery</td>
<td>11.0 ± 3.2</td>
<td>7.7 ± 2.2</td>
<td>5.1 ± 1.6</td>
</tr>
</tbody>
</table>

* Mean ± SD.

*p < 0.05.

C-Mean ± 0.01.

Table 4 Mean absolute amounts of specific and control antibodies in each of the tissues studied

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Antigen class</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>PEM positive</td>
<td>5.0 ± 2.0</td>
<td>2.7 ± 1.4</td>
<td>2.8 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>PEM negative</td>
<td>9.1 ± 4.0</td>
<td>7.6 ± 3.0</td>
<td>8.3 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>4D513</td>
<td>2.3 ± 0.8</td>
<td>3.0 ± 1.6</td>
<td>2.7 ± 1.3</td>
</tr>
<tr>
<td>Mucoea</td>
<td>PEM (all)</td>
<td>0.9 ± 0.4</td>
<td>0.7 ± 0.3</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Blood at surgery</td>
<td>0.7 ± 0.3</td>
<td>0.7 ± 0.25</td>
<td>0.6 ± 0.2</td>
</tr>
</tbody>
</table>

* Mean ± SD.

Adipose tissue was available from only 2 patients in this category.

Table 5 Values obtained for the dosimetric calculations for the local tissues studied

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Antigen class</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>PEM positive</td>
<td>5.0 ± 2.0</td>
<td>2.7 ± 1.4</td>
<td>2.8 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>PEM negative</td>
<td>9.1 ± 4.0</td>
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<tr>
<td></td>
<td>4D513</td>
<td>2.3 ± 0.8</td>
<td>3.0 ± 1.6</td>
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<tr>
<td></td>
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<td>0.7 ± 0.3</td>
<td>0.7 ± 0.25</td>
<td>0.6 ± 0.2</td>
</tr>
</tbody>
</table>

* Mean ± SD.

Adipose tissue was available from only 2 patients in this category.
DOSEMETRY OF mAbs IN HEAD AND NECK CANCER

Table 6 Mean volume and mass of the studied tumors

<table>
<thead>
<tr>
<th>Larynx</th>
<th>PEM-positive tumors</th>
<th>PEM-negative tumors</th>
<th>Control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor volume</td>
<td>4.4 ± 3.1 (n = 8)</td>
<td>4.5 ± 3.8 (n = 8)</td>
<td>4.0 ± 2.9 (n = 12)</td>
<td>3.5 ± 2.9 (n = 6)</td>
</tr>
<tr>
<td>Tumor mass</td>
<td>4.4 ± 3.2 (n = 8)</td>
<td>4.6 ± 3.8 (n = 8)</td>
<td>4.1 ± 2.9 (n = 12)</td>
<td>3.6 ± 3.0 (n = 6)</td>
</tr>
</tbody>
</table>

*a Mean ± SD.

size plays a role in uptake, with larger tumors exhibiting lower relative antibody uptake per g of tissue than smaller tumors (39). Experimentally there seems to be a critical mass over which this becomes evident (40). In many of the published studies a wide variation of uptake, even in strongly positive tumors, has often been noticed and often the tumor size has been implicated as the causal factor. Table 6 presents the measurement of volume and mass for 20 of the tumors from our sample for which orthogonal diameter measurements were made. The fact that the uptake in the positive tumors we studied displayed a markedly narrow band of variations (2.4—8.02% i.d./kg) may reflect the narrow variation in mass that the tumors at this anatomic site manifested (4.3 ± 3.2 g; range 0.61—11.2 g). No difference in mass existed in our sample between the studied categories. Nor was a correlation of absolute uptake and tumor size within each category found.

There are three sources of potential bias for the subsequent macrodosimetric calculation to be addressed. The ideal case of acquiring time dependent information in the same tumor is impossible with the biopsy-based strategy used. However, there is currently no other method available with which one could obtain biodistribution data at the anatomical level needed for the purposes of our study. Necessarily the time-dependent information is an amalgamation of information from samples from a number of tumors and normal tissues that we studied dispersed at three time points. Second, no information on what happens to the antibody in the tumor after day 3, or the manner in which it is acquired in the tumor over the first 24 h, can be derived accurately from our data. On the one hand, Begent et al. (41) have shown, for an anti-CEA antibody, that manipulation of antibody levels in blood by using anti-antibody sera leads to, respectively, lower antibody levels in the tumor, leading to the tentative conclusion that isotope may be lost from the tumor at a faster rate when circulating levels fall. On the other hand it is maintained that antibody may be bound to antigen irreversibly (42). Furthermore detailed studies using extracorporeal immunoadsorption to reduce circulating levels of radioactivity, both in animal models and in patients (43), have demonstrated very little perturbation of the absolute amount of radioactivity in tumor, in contrast to other parenchymal organs where radioactivity levels tail off sharply.

The final identifiable source of error from the data is that they have been derived using radiodines and would be relatively accurate for radioimmunotherapy using 131I but will not accurately reflect the uptake and retention of chelated radiometals (e.g., 90Y) (44—46) or other isotopes susceptible to enzymatic degradation other than dehalogenation e.g., 32P-kemptide (47). Nevertheless a relative idea of the amount of isotope needed to be injected into the patient to achieve a sufficient dose for the purposes of this study can be arrived at, without too much error, if these points are borne in mind.

From our data we have calculated that a single injection of around 150 mCi of 131I-labeled HMFG1 would carry a radiation dose to the marrow within the 1.6-Gy upper limit consistent with an admissible dose to this tissue (48, 49). We therefore anticipate that a fractionated RIT treatment consisting of two administrations of 131I-labeled HMFG1 (300 mCi in total) given within the 6-week period of a routine external beam radiotherapy fractionated course should theoretically achieve this ≥10% dose increase (around 9—10 Gy) to an antigen-positive tumor during this time. Dosimetry calculations for an 131I-HMFG1 radioimmunoconjugate presented in Table 5 led to the conclusion that an overall compensatory decrease in the external beam dose of around 3.4 Gy may be needed to safeguard against radiation toxicity to mucosa, which is the critical acute local tissue toxicity. More detailed dosimetry to local normal tissues is presented elsewhere (50). The dosimetry for 90Y is not favorable. One can probably safely administer only 10—12 mCi/m² with the currently best chelating agent isothiocyanato-pentamethylenetetraminepentaaetic acid which would lead to a maximum dose of ~1.6 Gy/administration to a tumor prior to severe bone marrow toxicity. This amount would be inadequate in delivering the dose increment essential for the predetermined goal of the study.

Experimental data indicate that the concurrent use of these two radiation modalities may be synergistic at both a physiological and a radiobiological level. It has been shown that external radiotherapy may induce an increase in the uptake of antibody through altered physiology of the irradiated milieu (51—53), this would suggest that the best time to administer the first injection would be the appearance of “tumoritis” which may manifest itself as early as 4—5 days into the treatment (54). Conversely, radiobiological synergy of protracted RIT type radiation treatment, combined with external beam radiotherapy, has been identified as an altered radiosensitivity of these exposed cell lines due to (a) cell cycle synchrony (G2 block) or (b) protracted exposure sensitization (55). The first mechanism may be operative at dose rates of about 10 cGy/h or more and the latter during protracted exposure (72 h) at dose rates of 1 cGy/h or more, with the overall effect being an up 4-fold increase in efficacy depending on cell line sensitivity. Because G2 block is achievable for only short durations it was proposed that the optimum time to insert a RIT dose is after the first week of external beam radiotherapy. The exploitation of the second mechanism means that a dose rate of 0.5—3 cGy/h should be maintained for as long as possible during the prescribed period of external beam radiotherapy.

We conclude the discussion on a final cautionary note. Choosing patients, as we have advocated, by immunohistochemistry criteria may improve targeting but may also insert selection bias into the treated population. Cortesina et al. (16) demonstrated that intense staining with anti-mucin antibodies is a marker of increased survival, possibly due to the well-differentiated nature of the tumors, on the other hand, the existence of high levels of EGFr (EGFr in these cancers have been proposed as possible immunotherapy targets) is a grave prognostic indicator (56). Results therefore must be interpreted individually for each antibody, and comparisons between treated populations with different antibodies must be made with care.
REFERENCES


Pharmacokinetics, Biodistribution, and Dosimetry of Specific and Control Radiolabeled Monoclonal Antibodies in Patients with Primary Head and Neck Squamous Cell Carcinoma

Anthony Maraveyas, Nick Stafford, Gail Rowlinson-Busza, et al.


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