Yttrium-90 and Iodine-131 Radioimmunoglobulin Therapy of an Experimental Human Hepatoma

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ABSTRACT

Therapeutic trials were performed on the HepG2 human hepatoblastoma implanted s.c. in the athymic nude mouse. Animals were treated with polyclonal and monoclonal antiferritin and control antibodies labeled with either iodine-131 (131I) or yttrium-90 (90Y). Administration of 400 μCi of 131I-labeled polyclonal antiferritin or 300 μCi of 90Y-labeled monoclonal antiferritin significantly increased survival (P < 0.001). There were no tumor cures with radiolabeled polyclonal antibody therapy. Animals treated with 200 or 300 μCi of 131I-labeled monoclonal antiferritin (QCI054) did not show increased survival compared to controls. Although 400 μCi of 131I-labeled QCI significantly prolonged survival, treatment resulted in no long-term survivors. Monoclonal antiferritin labeled with 90Y significantly prolonged survival of animals (P < 0.001) at doses of 100, 200, or 300 μCi compared with untreated controls. Fifty % of the animals treated with 200 μCi and 75% of the animals treated with 300 μCi showed no evidence of disease at 140 days following treatment. Four hundred μCi of 90Y-labeled QCI proved toxic to the animals. Increased survival was accompanied by a decrease in tumor mitotic rate and an increase in cellular polymorphism as determined by pathological examination. The radiation dose absorbed in the tumor correlated directly with tumor response following treatment. The absorbed dose in tumors for complete decay of the isotope ranged from 165 and 330 cGy at the periphery and center of small tumors for an administered activity of 200 μCi of 131I-labeled polyclonal antiferritin, to 7,573 and 12,400 cGy for 300 μCi of 90Y-labeled monoclonal antiferritin QCI.

INTRODUCTION

Radiolabeled antiferritin antibodies have been shown to selectively target hepatomas in both clinical and laboratory studies without depositing significant radiation to normal tissues, even those tissues having high ferritin concentrations (1-5). Specific localization depended upon ferritin concentration, tumor vasculature, tumor size, presence or absence of necrosis, and the amount of radiolabeled antiferritin administered (3, 4).

Two important features of radiolabeled antibodies which could amplify tumor dose deposition and tumor cytotoxicity are an increase in specific antibody and intensification of isotopic radiation. To study specific antibody titer, we compared polyclonal antiferritin, which is approximately 20% immunospecific and recognizes many epitopes on the antigen, with monoclonal antiferritin, which is approximately 85% immunospecific and recognizes a single antigenic epitope. In order to evaluate tumor cytotoxicity due to isotopic radiation, both antibodies were labeled with iodine-131 having a mean β energy of 0.18 MeV and an r95 range of 0.99 mm and yttrium-90 having a mean β energy of 0.94 MeV and an r95 range of approximately 5.9 mm (6). These studies support the conclusion that increased specific antibody titer and increased radiation energy from a more energetic isotope enhance tumor cytotoxicity from radiolabeled antibodies.

MATERIALS AND METHODS

HepG2 Cell Line. The HepG2 human hepatoblastoma cell line was maintained in Eagle’s minimal essential medium containing 10% fetal bovine serum. Fresh medium was added twice weekly and the cultures were transferred weekly. Eight-wk-old male athymic nude mice were given injections s.c. in the thigh with 0.1 ml of cell suspension containing 2 x 106 cells. Tumors were visible within 3 to 4 wk; experiments were initiated when tumors reached sizes of approximately 0.3 to 0.4 g.

Antiferritin Antibody Production. Ferritin was purified from the tumor infiltrates of spleens from Hodgkin’s disease patients as described previously (7). Polyclonal rabbit antiferritin IgG was produced, isolated, and tested for activity as described previously (8). Normal rabbit IgG was isolated from sera taken from the same animals prior to immunization. Chelated monoclonal antiferritin (QCI054) and control antibodies were supplied by Hybritech, Inc., San Diego, CA. QCI and control monoclonal antibody were both of the IgG1 subclass.

Antibody Radiolabeling. Polyclonal and monoclonal IgGs were labeled with 131I (DuPont, NEN, Boston, MA) by the lactoperoxidase glucose oxidase method as described by the manufacturer (Bio-Rad Laboratories, Richmond, CA) at a labeling ratio of 5 mCi per mg of IgG. The percentage of binding was determined by trichloroacetic acid precipitation. Polyclonal and monoclonal IgGs were labeled with 90Y by chelation following a procedure developed by Hybritech, Inc. The percentage of binding was determined by thin-layer chromatography. Samples were sterile filtered and pyrogen tested prior to injection.

Experimental Design. All experiments were initiated when the tumors were 0.3 to 0.4 g in size. Anesthetized animals were given intracardiac injections of radiolabeled antibody, control unlabeled antibody, or saline solution. Three animals from each group given injections of radiolabeled antibodies were sacrificed over the first 72 h following injection to ensure proper biodistribution as determined from previous experiments. Remaining animals of all groups were followed, with biweekly tumor measurements, for a period of 140 days, or until the tumors grew to a point where movement was significantly impeded; animals were then sacrificed. Results are reported in terms of tumor growth and the percentage of survival following treatment. Survival analysis of Kaplan-Meier curves was performed by log rank and generalized Wilcoxon tests.

Dose Measurements and Calculations. For each experiment, at least three animals were implanted with mini-TLDs3 prior to injection of radiolabeled antibody as described previously (9, 10). In brief, tumor-bearing animals were implanted with 0.2- x 0.4- x 5-mm3 TLDs, cut from CaSO4:TeFen matrix discs (Teledyne, Inc., Westwood, NJ), by insertion through a 20-gauge needle under metofane anesthesia. At various intervals following administration of radiolabeled antibody, animals were sacrificed and the tumor and normal organs were removed, weighed, and counted in an automatic γ-counting system. TLDs were removed, washed with decontaminating detergent, rinsed, and

3 The abbreviations used are: TLD, thermoluminescent dosimeter; RIT, radioimmunotherapy.
dried. The tumor was divided. One-half of the tumor was frozen, and the other half was fixed in phosphate-buffered formalin for pathological examination. The dose delivered to the TLD was measured as previously described (9).

From the tumor and normal organ biodistribution data, absorbed-dose calculations for 131I- and 90Y β particles were carried out as follows. Time-activity curves were generated using a nonlinear least-squares fitting program. Tumor thickness, the critical dimension that governed the fraction of the β-particle energy deposited within the tumor, was taken into account as detailed previously (11). The equilibrium dose for complete absorption of energy was also calculated for comparison.

All calculations were based on uniform distribution of activity.

Pathological Examination. Formalin-fixed tissues were embedded in paraffin, sectioned (7 µm), and stained with hematoxylin-eosin. All sections were evaluated without knowledge of treatment status, particular attention being given to the mitotic rate and the cellular morphology. The mitotic rate was estimated by counting the number of mitoses per field (x40 objective). The degree of cellular pleomorphism was assessed qualitatively according to the size and the shape of tumor cells, the number, shape, and tinctorial characteristics of the nuclei, and the nucleolar characteristics.

RESULTS

Tumor Growth Delay and Animal Survival. The results of the radiolabeled antibody treatment on the growth of the HepG2 tumor are shown in Figs. 1 to 3. Results are expressed as the log of the tumor volume divided by the volume at the start of therapy. Control tumor sizes are averaged measurements from 26 animals which were saline solution treated, unlabeled polyclonal and monoclonal antibody treated, and nonspecific radiolabeled antibody treated. There was no measurable difference in tumor growth between any of these groups. Polyclonal antiferritin labeled with 131I at any of the doses given failed to inhibit tumor growth. Two hundred and 300 µCi of 90Y-labeled polyclonal antiferritin delayed the growth of the tumor compared with control animals. Four hundred µCi of 131I-labeled QCI and 100, 200, and 300 µCi of 90Y-labeled QCI significantly inhibited the growth of the tumor. Two hundred and 300 µCi of 90Y-labeled QCI led to a dramatic reduction in tumor size. At autopsy (140 days), no evidence of viable tumor was observed; in two animals, patches of small numbers of tumor cells surrounded by fibrosis was found. No mitoses were detected in these areas.

The effects of radiolabeled antibody therapy on animal sur-

![Fig. 1. Effects of 131I- and 90Y-labeled polyclonal antiferritin antibody treatment on tumor growth in vivo. Results are expressed as the log of the ratio of tumor size compared with the tumor size at the start of therapy. Therapy was initiated when tumors reached approximately 0.3 to 0.4 g. O, control saline solution injected; +, 200 µCi of 131I-polyclonal antiferritin; x, 400 µCi of 131I-polyclonal antiferritin.

![Fig. 2. Effects of 131I-labeled monoclonal antiferritin antibody treatment on tumor growth in vivo. Results are expressed as the log of the ratio of tumor size compared with the tumor size at the start of therapy. Therapy was initiated when tumors reached approximately 0.3 to 0.4 g. O, control saline solution injected; +, 200 µCi of 131I-QCI054; x, 300 µCi of 131I-QCI054; o, 400 µCi of 131I-QCI054.

![Fig. 3. Effects of 90Y-labeled monoclonal antiferritin antibody treatment on tumor growth in vivo. Results are expressed as the log of the ratio of tumor size compared with the tumor size at the start of therapy. Therapy was initiated when tumors reached approximately 0.3 to 0.4 g. O, control saline solution injected; +, 100 µCi of 90Y-QCI054; x, 200 µCi of 90Y-QCI054; o, 300 µCi of 90Y-QCI054.

![Fig. 4. The percentage of survival of tumor-bearing animals following therapy with 131I- and 90Y-labeled polyclonal antiferritin. Therapy was initiated when tumors reached approximately 0.3 to 0.4 g. O, control saline solution injected; +, 200 µCi of 131I-polyclonal antiferritin; x, 400 µCi of 131I-polyclonal antiferritin; o, 200 µCi of 90Y-polyclonal antiferritin; □, 300 µCi of 90Y-polyclonal antiferritin.
Treatment with 200 or 300 µCi of 131I-labeled monoclonal antiferritin QCI did not significantly increase survival compared with controls. Treatment of tumor-bearing animals with 400 µCi of 131I-labeled QCI significantly increased survival (P < 0.001) without permanent remission. Treatment with 100, 200, or 300 µCi of 90Y-labeled QCI significantly prolonged survival (P < 0.001). At the conclusion of the experiment at 140 days, 75% (6 of 8) of the animals treated with 300 µCi and 50% (4 of 8) of the animals treated with 200 µCi had no evidence of viable tumor upon pathological examination. As a result of an animal maintenance problem, two additional animals in the group treated with 200 µCi died at 105 days due to an inadvertent accident. These animals also had no evidence of tumor by pathological examination, although they were scored as fatalities for experimental purposes. Four hundred µCi of 90Y-labeled QCI proved toxic and resulted in hematologic death in 9 of 10 animals by Day 17.

Radiation-absorbed Dose. The doses absorbed in the tumors by radiolabeled polyclonal and monoclonal antiferritin as measured by implanted TLDs and by calculation from data derived from sacrificed animals are reported in Tables 2 and 3. Good correlation was seen between the measured and calculated results. Those animals showing a significant increase in survival received a minimum of 1,200 cGy (300 µCi of 90Y-polyclonal antiferritin), and up to 12,400 cGy (300 µCi of 90Y-QCI) to the center of the tumor, assuming uniform distribution and complete decay of the isotope. The dose delivered with 400 µCi of 131I-labeled polyclonal antiferritin, which significantly increased survival, was not calculated because there were no animal data from early time points. Based on previous saturation studies, the 400 µCi of radiolabeled polyclonal antiferritin would have delivered approximately 800 cGy to the tumor. Radiation doses greater than 12,000 cGy could be achieved with higher injected activities, but the toxicity associated with the higher doses was unacceptable.

The calculated equilibrium doses for complete absorption of energy and the calculated dose for complete decay of the isotope in these small tumors are also shown in Tables 2 and 3. These values were similar for 131I but varied by a factor of approximately 2 to 2.5 for 90Y because of the longer 90Y β particle range and higher energy.

The effects of tumor size on calculated dose deposition in small tumors were determined as shown in Tables 2 and 3 and compared directly in Table 4. Calculated doses were evaluated for a tumor thickness of 0.1 cm, 0.15 cm, and 0.2 cm with the assumption of the same deposited activity per g of tumor and the same effective half-life. The doses varied by a factor of 2 as the tumor size increased from 0.1 to 0.15 to 0.2 cm. The doses, as measured by implanted TLDs, were in good agreement with,

### Table 1 Radiolabeled antibody therapy of the HepG2 tumor grown s.c. in the athymic nude mouse

<table>
<thead>
<tr>
<th>Antibody (dose/isotope)</th>
<th>Median survival (days)</th>
<th>No. that survived &gt;140 days</th>
<th>Antibody (dose/isotope)</th>
<th>Median survival (days)</th>
<th>No. that survived &gt;140 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline solution</td>
<td></td>
<td></td>
<td>QCI antifer (200 µCi/131I)</td>
<td>35</td>
<td>0/9</td>
</tr>
<tr>
<td>Cold poly* (50 µg)</td>
<td>26</td>
<td>0/10</td>
<td>QCI antifer (300 µCi/131I)</td>
<td>45</td>
<td>0/5</td>
</tr>
<tr>
<td>Cold QCI (50 µg)</td>
<td>30</td>
<td>0/5</td>
<td>QCI antifer (400 µCi/131I)</td>
<td>80</td>
<td>0/10</td>
</tr>
<tr>
<td>Nonspec mono (200 µCi/90Y)</td>
<td>50</td>
<td>0/6</td>
<td>QCI antifer (100 µCi/90Y)</td>
<td>80</td>
<td>0/9</td>
</tr>
<tr>
<td>Poly antifer (200 µCi/131I)</td>
<td>45</td>
<td>0/8</td>
<td>QCI antifer (200 µCi/90Y)</td>
<td>105*</td>
<td>4/8</td>
</tr>
<tr>
<td>Poly antifer (400 µCi/131I)</td>
<td>60</td>
<td>0/8</td>
<td>QCI antifer (300 µCi/90Y)</td>
<td>140*</td>
<td>6/8</td>
</tr>
<tr>
<td>Poly antifer (200 µCi/90Y)</td>
<td>45</td>
<td>0/8</td>
<td>QCI antifer (400 µCi/90Y)</td>
<td>17</td>
<td>0/8</td>
</tr>
<tr>
<td>Poly antifer (300 µCi/90Y)</td>
<td>75</td>
<td>0/7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Cold poly, unlabeled polyclonal antiferritin; nonspec mono, nonspecific radiolabeled monoclonal antiferritin; poly antifer, radiolabeled polyclonal antiferritin; QCI antifer, radiolabeled QCI antiferritin.

* Two animals died of an inadvertent accident. These animals also had no evidence of tumor by pathological examination, although they were scored as fatalities for experimental purposes. Four hundred µCi of 90Y-labeled QCI proved toxic and resulted in hematologic death in 9 of 10 animals by Day 17.
and fall within, the ranges of doses calculated for the three tumor sizes.

Pathological Examination. Histological examination of tumors was performed 3 and 14 days following saline solution injection or therapy with 200 µCi of 90Y-labeled control nonspecific monoclonal antibody; or 200, 300, or 400 µCi of 131I-labeled QCI; or 100, 200, or 300 µCi of 90Y-labeled QCI. The results are summarized in Table 5, and histological characteristics of untreated and treated tumors are illustrated, respectively, in Fig. 7, a, b, and c.

Tumors taken from control animals and animals treated with nonspecific antibody were similar and were characterized at 14 days following treatment by a high mitotic rate and by the presence of a monomorphic population of cells. Tumors taken from animals treated with 200 µCi of 131I-labeled QCI at 14 days following treatment showed a slightly reduced mitotic rate and a slightly increased cellular pleomorphism. Higher doses of 131I-labeled QCI induced a larger increase in mitotic rate and a greater cellular size and pleomorphism in the tumors.

The tumors taken from animals treated with 100 µCi of 90Y-labeled QCI showed a slight reduction of the mitotic rate and a moderately increased size and cellular pleomorphism. Two hundred and 300 µCi of 90Y-labeled QCI induced a dramatic decrease in mitotic rate as early as 3 days posttreatment and a high degree of cellular pleomorphism by 14 days.

Histological examination was also performed on long-term survivors bearing either regressed or regrowing tumor treated 2 to 7 mo earlier. Regressed tumors often consisted of fibrous tissue containing only a few pigment-laden macrophages. A tumor cell focus was observed in only one case. Regrowing tumors were comprised of densely packed, slightly pleomorphic cells with high nucleocytoplasmic ratios and hyperchromatic
Table 4  Effect of tumor size on the calculation of dose deposited with \(^{90}\text{Y}\)-labeled antibody in very small tumors

<table>
<thead>
<tr>
<th>Time postinjection (h)</th>
<th>Tumor (0.1 cm) with calculated dose at surface/center (cGy)</th>
<th>Tumor (0.15 cm) with calculated dose at surface/center (cGy)</th>
<th>Tumor (0.2 cm) with calculated dose at surface/center (cGy)</th>
<th>TLD (cGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>488/551</td>
<td>664/777</td>
<td>808/976</td>
<td>734</td>
</tr>
<tr>
<td>72</td>
<td>635/717</td>
<td>864/1010</td>
<td>1051/1269</td>
<td>739</td>
</tr>
<tr>
<td>144</td>
<td>870/983</td>
<td>1186/1386</td>
<td>1442/1741</td>
<td>1088</td>
</tr>
</tbody>
</table>

Activity per gram of tumor and effective half-life are constant in all examples.

Table 5  Effects of radiolabeled antibody therapy on tumor pathology

<table>
<thead>
<tr>
<th>Antibody treatment</th>
<th>Mitotic rate Day 3</th>
<th>Mitotic rate Day 14</th>
<th>Cellular pleomorphism Day 3</th>
<th>Cellular pleomorphism Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, no treatment</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(^{90}\text{Y})-control antibody (200 µCi)</td>
<td>ND</td>
<td>++++</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>(^{131}\text{I})-QCI054 (200 µCi)</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(^{131}\text{I})-QCI054 (300 µCi)</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(^{131}\text{I})-QCI054 (400 µCi)</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(^{90}\text{Y})-QCI054 (100 µCi)</td>
<td>ND</td>
<td>++++</td>
<td>ND</td>
<td>++++</td>
</tr>
<tr>
<td>(^{90}\text{Y})-QCI054 (200 µCi)</td>
<td>+</td>
<td>++++</td>
<td>+</td>
<td>++++</td>
</tr>
</tbody>
</table>

* ND, not determined.

nuclei containing large nucleoli. The mitotic rate was high (8 mitotic figures per high-power field). Areas of necrosis were present in such tumors, and in two cases pulmonary metastases were seen.

**DISCUSSION**

This paper presents a detailed, quantitative comparison of tumor response in a model system between monoclonal and polyclonal antibodies and between \(^{131}\text{I}\)- and \(^{90}\text{Y}\)-labeled antibodies. The data demonstrate that, in our model system, monoclonal antibodies can deliver doses 8.4 times that of polyclonal antibodies, and \(^{90}\text{Y}\)-labeled antibodies deliver up to 7.3 times the dose delivered by the same antibodies labeled with \(^{131}\text{I}\). Tumor response as measured by growth kinetics, animal survival, and histology indicates that such response is probably dose dependent and is, thus, independent of type of antibody or radionuclide utilized, except to the extent that they deliver different doses to the tumor.

Fig. 8 presents a summary of the data from Tables 2 and 3. These data demonstrate that the monoclonal antibody QCI054 chelated to \(^{90}\text{Y}\) deposits approximately 8.4 times (ratio of slopes) the dose to tumors than does rabbit polyclonal antiferritin labeled in an identical manner. Since the polyclonal antibody preparation contains 12 to 17% of ferritin-specific immunoglobulin, as determined by affinity chromatography, this targeting ratio is in the general range that would be predicted, if the ferritin-specific fraction only was effective in delivering dose to the tumor. Further, the data in this figure indicate that QCI, when labeled with \(^{90}\text{Y}\), deposits approximately 7.3 times the dose deposited (ratio of slopes) when the same monoclonal antibody is labeled with \(^{131}\text{I}\). This value is higher than that expected based on the relative energy deposited by each radio-
At the highest doses, 75 to 124 Gy, obtained with 200 and 300 growth delay as well as more pronounced survival extension.

Tumor doses of 10 to 15 Gy produce a delay in tumor growth response in Figs. 1 to 6, the following general description of doses. From the data shown in Fig. 8 compared with tumor different antibody-radionuclide conjugates deliver different dependent on antibody and radionuclide, to the extent that 90Y must be paired with an isotope such as 'In for scanning which greatly reduces patient costs. As a ß emitter, however, a pure ß emitter and does not require a shielded hospital radiation dose to the tumor for the same deposited activity, as isotope deposition, (c) 90Y has a mean ß energy of 0.94 MeV and an r95 range of approximately 0.99 mm for 131I, resulting in a greater dose rate and higher total radiation dose to the tumor for the same deposited activity, as well as a more homogeneous dose across the tumor, even assuming microscopic inhomogeneities (6). (d) Unlike 131I, 90Y is a pure ß emitter and does not require a shielded hospital environment for administered activities greater than 30 mCi, which greatly reduces patient costs. As a ß emitter, however, 90Y must be paired with an isotope such as 11In for scanning and in vivo dosimetry (21).

Response of the tumors appears related primarily to dose, dependent on antibody and radionuclide, to the extent that different antibody-radionuclide conjugates deliver different doses. From the data shown in Fig. 8 compared with tumor response in Figs. 1 to 6, the following general description of dose response of the HepG2 tumor xenograft can be proposed. Tumor doses of 10 to 15 Gy produce a delay in tumor growth and an extension in survival, but they show no detectable regression. Similarly, doses of 20 to 50 Gy produce greater growth delay as well as more pronounced survival extension. At the highest doses, 75 to 124 Gy, obtained with 200 and 300 µCi of 90Y-labeled monoclonal, there are considerable tumor regression and 50 to 75% survival at 140 days. These data demonstrate that radiation doses delivered at rates achieved by RIT are capable of regressing tumors and, for higher doses, ablating them.

The histological effects seen in the treated tumors are consistent with radiation damage and radiation-induced tumor death. Immediate radiation effects were observed for dividing cells, characterized by a rapid decrease in mitotic rate. This was followed by the formation of enlarged atypical cells and abnormal mitoses in the tumor. The long-term effects included coagulative necrosis and fibrosis in the region of the tumor heterograft. At higher doses toxic to the animal (400 µCi for 90Y-QCI), marked bone marrow atrophy, petechia, and, eventually, hematological death were observed.

To evaluate the implications of the present work for clinical application, three areas must be considered: (a) the kinetics of monoclonal and polyclonal antibodies in patients, as distinct from kinetics in laboratory animals; (b) the toxic effects of radiolabeled antibodies in patients, compared with their effects in laboratory animals; and (c) the dose response, including the effects of heterogeneous dose distribution, of human tumors in situ, compared with xenografts treated in the nude mouse. Vriesendorp et al. and Order et al. have begun to compare QC1054 and polyclonal antiferritin in selected patients. Initial studies performed with these same antibodies and labeling procedures show important and critical differences between this model system and clinical application. (a) Monoclonal rodent antibodies, as do mouse antibodies, have a shorter effective half-life when injected into patients compared with other species. (b) QC1054 in a small group of patients has not proved more effective in delivering a radiation dose to human tumors than polyclonal antibodies in these preliminary observations.

Another extremely important caveat in interpreting the present data in terms of possible clinical application of RIT lies in the differences in levels of toxic effects induced in rodents and human beings. If injected activity is expressed in mCi of radiolabeled immunoconjugate per kilogram of idealized body weight, the human being is probably over 5 times more sensitive to hematopoietic toxicity than is the nude mouse. Vriesendorp et al. have predicted a human LD-50, the administered dose sufficient for 50% mortality, for bone marrow-related death for 90Y-labeled polyclonal antiferritin in the range of 2.5 mCi/kg. Studies demonstrate that nude mice die from 400 µCi (16 mCi/kg) of 90Y-labeled QCI at times consistent with the hematopoietic syndrome. Generally, clinical trials have been limited to injected levels of 0.14 to 0.6 mCi/kg of 90Y-labeled antiferritin antibodies. Autologous bone marrow transplant is needed, however, to mitigate toxicity above injected levels of 0.35 mCi/kg.

Counterbalancing this human sensitivity is the observation that human tumors are targeted better in situ than in xenografts for some immunoconjugates. Injected levels of 0.3 to 0.6 mCi/kg of polyclonal 90Y-labeled polyclonal antiferritin antibodies can produce up to 26 Gy in Hodgkin’s disease tumor masses and up to 15 Gy in hepatomas (6, 21). Thus, for reasons not clear at this point, tumor masses in patients treated with antiferritin antibodies acquire much higher radiation doses, perhaps up to 10-fold, than for the human xenograft model grown in the nude mouse given injection of the same activity per body weight in the present studies. Although the percentage of injected dose deposited in tumors may be 1000-fold less in patients than in tumor-bearing nude mice, the absorbed dose based...
on activity administered per kg/body weight may be 10-fold higher. These comparisons between xenografts in mice and tumors in patients are also only valid if the relative biodistributions of the immunoconjugates in normal tissues are similar, and if the half-life in the two hosts is similar. Most murine monoclonal antibodies when injected into human patients tend to accumulate at high rates in the liver and to be cleared rapidly from the blood. Thus, the extrapolation of the dose achievable in xenografts to tumors in humans is not valid for murine monoclonal antibodies. An ancillary consideration in comparing targeting in xenografts in nude mice with targeting in human tumors in situ is that of physical dimensions. The size of the tumors in these experiments was 2 to 3 mm, which is nearly 2 to 3 times the range of the β particle emitted by 131I (0.99 mm), but is only a fraction of the range of the β particle emitted by 90Y (5.9 mm) (6). Thus the fraction of maximum possible dose delivered approaches 1.0 for 131I, but is considerably less for 90Y, since a greater amount of the radiation is deposited beyond the 2 to 3-mm dimension of the tumors. This would imply that the ratio of dose delivered by 90Y to 131I would be greater for larger tumors.

Our studies demonstrate the necessity for accurate dosimetry in the comparison of different immunoconjugates. In general, we found good agreement between the two methods used to estimate dose: calculation based on time-activity patterns; and measured by micro-TLD insertion. Observed differences may be due to placement of the TLDs, nonuniform distribution of the isotope, or error in the tumor size measurements. (a) Partial TLD insertion or placement other than in the center of the tumor could account for low readings, since the measured TLD dose reflects an average dose for the entire TLD. (b) Areas of necrosis or nonuniform distribution due to intratumor pressure or tissue damage caused by insertion of the TLD could result in differences between the calculated and measured doses due to the short range of the 131I β particle. (c) As shown in Table 5, errors in tumor size measurements as small as 0.05 to 0.1 cm could result in a 2-fold difference in tumor dose calculations. This is especially true for isotopes with energetic β particles such as 90Y.

In summary, radiolabeled antibodies can deliver large, presumably curable, doses of ionizing radiation to human tumor xenografts growing in the nude mouse. Radiation dose appears to be the critical parameter. In the system studied here, monoclonal antibodies against ferritin can deliver up to 8.4 times more radiation to the tumor than can polyclonal antibodies raised against the same antigen, when both antibody populations are labeled identically. When labeled with 90Y, ferritin is monoclonal antibodies can deliver up to 7.3 times more radiation to the tumor than when labeled with equivalent levels of 131I. This efficiency of murine monoclonal as carriers of therapeutic levels of radioisotopes is, unfortunately, probably not possible in human patients, and new human or chimeric antibodies must be developed. These studies suggest that antibodies labeled with 90Y may offer a powerful tool in the treatment of human cancer.

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