Radioimmunotherapy of Intracerebral Human Glioma Xenografts with 131I-labeled F(ab')2 Fragments of Monoclonal Antibody Mel-14

Edward V. Colapinto, Michael R. Zalutsky, Gerald E. Archer, Michael A. Noska, Henry S. Friedman, Stefan Carrel, and Darell D. Bigner


ABSTRACT

The administration of radiolabeled monoclonal antibodies to improve the treatment of malignant gliomas is dependent upon achieving effective tumor radiation dose while sparing normal tissues. We have evaluated the efficacy of 131I-labeled F(ab')2 fragment of monoclonal antibody Mel-14, an IgG2a reactive with the chondroitin sulfate proteoglycan antigen of gliomas, melanomas, and other neoplasms, in prolonging survival of athymic mice transplanted intracerebrally with D-54 MG human glioma xenografts. Studies indicated that in vitro immunoreactivity, affinity, and tumor localization in vivo of radiolabeled Mel-14 F(ab')2 were maintained at specific activities of 10–13 μCi/μg. Intravenous injection of 1500 μCi/115 μg or 2000 μCi/154 μg 131I-labeled Mel-14 F(ab')2 into mice 6–7 days after xenograft implantation resulted in significant survival prolongation over control animals (P = 0.009 using Wilcoxon rank sum analysis). In another experiment, 1500 μCi/126 μg 131I-labeled Mel-14 F(ab')2 improved survival significantly over controls (P = 0.006), while 1500 μCi/220 μg 131I-labeled nonspecific antibody did not (P = 0.2). Increasing the injected radiation dose to 3000 μCi 131I-labeled Mel-14 F(ab')2 did not significantly increase survival in tumor-bearing mice, because of supervening radiation toxicity. However, giving 3000 μCi 131I-labeled Mel-14 F(ab')2 in two doses of 1500 μCi 48 h apart, did significantly prolong animal survival over controls (P = 0.001). Estimated radiation dose to tumor was 915 rad after injection of 3000 μCi 131I-labeled Mel-14 F(ab')2, in two doses, a dose higher than that delivered to normal tissues. The results of this study suggest that radiolabeled Mel-14 F(ab')2 be evaluated as an agent for radioimmunotherapy trials.

INTRODUCTION

The capability of MAbs reactive with tumor-associated antigens to direct cytotoxic radionuclides selectively to tumors has been demonstrated in various animal tumor models (1–6). For example, we have shown that 131I-labeled antiangioma MAb 81C6 IgG2b has specific antitumor effects in s.c. and i.c. human glioma xenografts in athymic rodents (7, 8). However, in patients, radioimmunotherapy has had limited efficacy (9, 10). One explanation for this is the consistently low level of MAb uptake by tumor in human studies (11–13). The reasons for the low uptake in brain tumors include problems of access due to the complex nature of transcapillary transport in brain neoplasms (14), the inherent biological heterogeneity of malignant gliomas (15), and catabolism of radioisotope from MAbs in vivo, particularly dehalogenation of radiiodinated MAbs. Ultimately, the efficacy of radioimmunotherapy will depend upon developing strategies to increase the ratio of radiation dose between tumor and normal nontarget tissues. To this end, in this study we have evaluated one possible strategy, the use of F(ab')2 fragments to deliver therapeutic doses of 131I to an i.c. human glioma xenograft in athymic mice.

Frames of MAbs may be particularly effective localizing agents in brain tumors because of their smaller size. In human glioma xenografts in athymic mice, the high affinity F(ab')2 fragment of the MAb Mel-14, a murine IgG2a reactive with the high molecular weight glycoprotein antigen of glioma and melanoma (16, 17), has shown more ready access into tumor and earlier specific tumor localization than intact IgG (18). Moreover, radiation dosimetry calculations based on these studies predicted that 131I-labeled Mel-14 F(ab')2 would deliver a substantially higher radiation dose to tumor than to normal tissues and that, in most cases, tumor-to-normal-tissue dose ratios were markedly increased using the F(ab')2 fragment compared to intact Mel-14 IgG (18). These findings suggested a possible role for the F(ab')2 fragment of Mel-14 in brain tumor radioimmunotherapy.

In the present study, we have evaluated the use of 131I-labeled Mel-14 F(ab')2, to deliver radiotherapy to HGL D-54 MG i.c. xenografts in athymic mice. As part of these studies, we have evaluated the effects of administered radiation and protein dose, and the effect of dividing the administered dose, on delivering radiation to tumor. In these studies we demonstrated that the administration of 131I-labeled Mel-14 F(ab')2 had a specific and significant survival benefit over control antibody in animals bearing i.c. human glioma xenograft tumors and that Mel-14 F(ab')2 may be a potential agent for use in clinical trials of radioimmunotherapy of brain tumors.

MATERIALS AND METHODS

Human Tumor Xenograft Model. Six- to eight-week-old male athymic mice (nu/nu genotype, BALB/c background) weighing 22–30 g were used in all experiments and were maintained as previously described (19). HGL D-54 MG, the Duke University subline of A-172 established by Giard et al. (20), was used in all experiments. Xenografts were passaged s.c. in athymic mice, as previously described (21). For initiation of i.c. xenografts, tumor inoculum was prepared from athymic mouse s.c. D-54 MG tumors passed through a Cytosieve and mixed with an equal volume of 1% methyl cellulose (Fluka, Switzerland) in serum-free zinc option medium. Mice were anesthetized by the i.p. injection of sodium pentobarbitol (80 mg/kg). The head of the animal was then fixed in a small animal stereotactic frame (Kopf, Tujunga, CA) and an incision was made over the cranium in the midline. Using a Hamilton Syringe fitted with a No. 25 needle, the frame was used to direct the point of injection 4 mm anterior to the lambda, 1.5–2 mm to the right of the midline, and 2 mm deep; 10 μl of tumor homogenate were injected into the right cerebral hemisphere. The wound was closed with a single staple.

Monoclonal Antibodies. MAb Mel-14 was purified from athymic mouse ascites by Protein A-Sepharose affinity chromatography. Control IgG2a RPC 5 was purified in a similar manner from hybridoma cells (American Type Culture Collection, Rockville, MD). RPC 5 does not bind to any known antigen. F(ab')2 fragments were prepared by digestion with pepsin, as previously described (18). Fragments were...
stored in sterile aliquots in 115 mm phosphate buffer at 4°C.

Radioiodination and Assessment of Purity. MAb fragments were labeled with ¹²¹I and ¹³¹I (New England Nuclear, Boston, MA) using a variation of the iodogen method outlined previously (18). Trichloroacetic acid precipitability was greater than 92% for iodinated preparations. The purity of all fragments was assessed before and after radioiodination using size-exclusion HPLC, as described previously (18).

**In Vitro Binding Assays.** The binding characteristics of radioiodinated Mel-14 F(ab')₂ were examined in immunoreactivity and affinity assays. For the immunoreactivity assay, D-54 MG xenograft tissue was homogenized in phosphate buffer (pH 7.4, 115 mM) and washed extensively to remove all soluble material. One hundred ng of ¹²¹I-labeled Mel-14 F(ab')₂ were incubated for 1 h with 300 mg of washed D-54 MG tumor homogenate protein at room temperature. Incubation of the radioiodinated fragment with a similarly prepared pork liver homogenate was used to determine nonspecific binding, which was subtracted from the amount bound to tumor homogenate to arrive at the specific binding percentages of ¹³¹I-labeled Mel-14 F(ab')₂.

For the measurement of binding affinity, the high antigen-expressing HGL D-247 MG was used; an antigen-negative cell line U-373 MG was used to determine nonspecific binding. For each cell line, cells were plated in 48-well plates 3 days before assay. After glutaraldehyde fixation of both cell lines, serial dilutions of radioiodinated antibody were added to the lines in triplicate and incubated overnight. Scatchard analysis of the binding data was done using the software program EBDa, as modified by McPherson (22).

**Antibody Biodistribution.** The localization of Mel-14 F(ab')₂ radioiodinated at a high specific activity was evaluated by injecting an equal mixture of ¹²¹I-labeled Mel-14 F(ab')₂ (60 µCi/5 µg) and ¹²⁵I-labeled RPC 5 F(ab')₂ (45 µCi/5 µg) in the tail vein of athymic mice bearing 7-day-old s.c. D-54 MG tumors. Groups of three animals were sacrificed by halothane overdose at 12, 24, 48, and 72 h after antibody injection. Blood was obtained by transecting the inferior vena cava, and major tissues and tumor were dissected, weighed, and assayed for ¹³¹I and ¹²⁵I activity using a dual-channel gamma-counter. Data were corrected for crossover of ¹²¹I into the ¹²⁵I gate and for decay of the radioisotopes. Using injection dose standards, values of percentage of ID/g tissue were derived. Localization indices were calculated to determine specificity of antibody binding (23).

To assess the effect of antibody dose on uptake in tumor, athymic mice bearing 7-day-old i.c. tumor were used. Animals were given injections in the tail vein of ¹²¹I-labeled Mel-14 F(ab')₂ (11.2 µCi/5 µg); groups of 6 to 10 animals were given injections of a total of 5, 10, 150, and 500 µg, with the rest of the antibody dose made up with unlabeled Mel-14 F(ab')₂. Twenty-four h after antibody injection, the mice were sacrificed; blood, tumor, and tissues were taken and evaluated as above. Mice received i.v. injections of Evans’ blue dye prior to euthanasia to guide the dissection of the i.c. tumor. Percentage of ID/g was calculated as described above.

**Therapeutic Trials.** Lugol’s solution was introduced into the animals’ drinking water 48 h before antibody injection and was continued throughout the experiment to block thyroid uptake of free radiiodine. On the day prior to treatment, the animals were randomized according to body weight. Nine or 10 animals were used per treatment group. Treatment was begun 6 to 7 days after tumor implantation. Antibody was injected i.v. in a volume of 100 µl. The animals were individually housed in plastic cages, and bedding was changed 24 and 48 h after antibody injection, and thereafter twice weekly, to minimize nonspecific radiation exposure from radioactive excreta. Four therapeutic trials were performed. Animals in Experiment 1 were given injections by tail vein as follows: buffer (115 mM phosphate buffer) and 1000 µCi/77 µg, 1500 µCi/115 µg, and 2000 µCi/154 µg ¹²¹I-labeled Mel-14 F(ab')₂. In Experiment 2, the groups received 125 µg unlabeled Mel-14 F(ab')₂, 1500 µCi/126 µg ¹²¹I-labeled Mel-14 F(ab')₂, and 1500 µCi/220 µg ¹²¹I-labeled RPC 5 F(ab')₂. In Experiment 3, mice were treated with the following: buffer, 3000 µCi/239 µg ¹²¹I-labeled Mel-14 F(ab')₂, and 3000 µCi/221 µg ¹²¹I-labeled RPC 5 F(ab')₂. In Experiment 4, the mice received buffer, 150 µg unlabeled Mel-14 F(ab')₂, and 1500 µCi/160 µg ¹³¹I-labeled Mel-14 F(ab')₂. In 48 h later this group received a second i.v. injection of 1500 µCi/140 µg ¹³¹I-labeled Mel-14 F(ab')₂. All mice were examined twice daily for survival, and the duration of survival was counted from the time of tumor implantation. In Experiment 4, 16 additional animals were also given injections of 1500 µCi/160 µg ¹³¹I-labeled Mel-14 F(ab')₂; 48 h later, eight of the animals received a second injection of 1500 µCi/140 µg Mel-14 F(ab')₂. The animals were sacrificed 12, 24, and 48 h after the antibody injections and tissues were dissected, weighed, and stored in formalin. After being allowed to decay for 30 days, the tissues were assayed in a gamma-counter and compared to ¹³¹I standards of appropriate count rate to derive values of µCi/g tissue. Cumulative activity, in µCi/g, in each tissue was determined from the µCi/g data, using the trapezoid integration method (2). Radiation doses were calculated by multiplying the cumulative activities by the g-rad/µCi factor tabulated by the Medical Internal Radiation Dose Committee for the nonpenetrating radiation of ¹³¹I, 0.4313 (24).

**Statistical Analysis.** The significance of treatment response was evaluated using the Wilcoxon rank sum test comparing animal survival times between buffer- or unlabeled antibody-treated control groups and treatment groups, as previously described (7).

**RESULTS**

**Intracerebral Tumor Model.** Following stereotaxic i.c. transplantation of D-54 MG tumor homogenate, 100% of control animals died between 9 and 21 days after tumor implantation. By day 6 after implantation, well established tumors (weight, 15 ± 4 mg; mean ± SD) were present in the right cerebral hemisphere of a group of mice killed in conjunction with the therapy experiments.

**Characterization of MAb.** MAb preparations were examined by HPLC before and after radioiodination, to assess purity. All preparations demonstrated greater than 95% purity by HPLC (data not shown).

**Radioiodinated Mel-14 F(ab')₂ preparations were tested for immunoreactivity by determining their specific binding to D-54 MG tumor homogenates in vitro. After correction for nonspecific binding, all preparations at specific activities of 9.4–13.0 µCi/µg showed specific binding ranging from 30 to 45%. This range is comparable to that observed previously for trace-labeled Mel-14 F(ab')₂ (18).

**Affinity constants were derived by Scatchard analysis of the binding data. Affinity constants of 2.0 × 10⁹ to 1.6 × 10¹⁰ M⁻¹ were found for high specific activity preparations, compared to 7.0 × 10⁸ M⁻¹ for radioiodinated Mel-14 F(ab')₂ published earlier in a trace-labeled study (18).**

**The in vivo biodistribution of co-administered ¹²¹I-labeled Mel-14 F(ab')₂; ¹²¹I-labeled RPC 5 F(ab')₂, labeled at specific activities of 12.0 and 9.0 µCi/µg, respectively, was evaluated in athymic mice bearing 7-day-old s.c. D-54 MG xenografts. ¹²¹I-labeled Mel-14 F(ab')₂ reached 9.7 ± 1.8% ID/g (mean ± SD) in tumor at 12 h after antibody injection. A tumor localization index of 27 was reached at 48 h; these values are similar to those obtained in trace label studies (18).**

**Effect of MAb Dose on Localization to Tumor.** The biodistribution of Mel-14 F(ab')₂ labeled with ¹³¹I, at an administered dose varying over a 10-fold range, was examined 24 h after MAb injection into mice bearing 7-day-old i.c. D-54 MG xenografts. Tumor size was 37 ± 17 mg (mean ± SD) at time of sacrifice. The mean percentage of ID/g of radioiodinated MAb reaching tumor varied inversely with the dose of injected protein (Fig. 1). At a dose of 5 µg, tumor uptake of Mel-14 F(ab')₂ (mean ± SD) was 10.2 ± 4.1% ID/g (in absolute amounts, 0.5 ± 0.2 µg/g); this fell to 6.0 ± 1.8% ID/g (3.0 ± 0.9 µg/g) at a dose of 50 µg, 2.3 ± 0.5% ID/g (3.5 ± 0.79 µg/g) at 150 µg, and 1.6 ± 0.4% ID/g (7.9 ± 2.2 µg/g) at 500 µg. Normal organ uptakes of MAb, in percentage of ID/g, did not change significantly.
significantly over this dose range (data not shown).

Therapy of Established Tumor. In light of the above findings, the lowest possible administered protein dose would have been desirable to increase tumor uptake of antibody in order to maximize the delivery of radiation to tumor. However, this consideration had to be counterbalanced by the limitation imposed by the increasing susceptibility of antibodies, particularly fragments, to immunoreactivity losses at higher specific activities. The working balance between these two factors resulted in the use of radiolabeled antibodies with specific activities of approximately 10–13 μCi/μg.

The survival curves of mice treated with 1000 μCi/77 μg, 1500 μCi/115 μg, and 2000 μCi/154 μg 125I-labeled Mel-14 F(ab')2 are shown in Fig. 2A. Injections of 1000 μCi increased median survival 25% over control animals that had received buffer, and the statistical significance of this survival prolongation was P = 0.06. Increasing the dose of 125I-labeled Mel-14 F(ab')2 to 1500 and 2000 μCi produced enhancement of survival of greater statistical significance (P = 0.009) (Table 1).

The issue of specificity of the 125I-labeled Mel-14 F(ab')2 therapeutic effect was examined in another experiment where the therapy of i.c. tumor-bearing mice was evaluated after the i.v. injection of 1500 μCi/220 μg 125I-labeled Mel-14 F(ab')2, fragment of RPC 5, a nonspecific control IgG2a (Fig. 2B). The injection of 1500 μCi 125I-labeled Mel-14 F(ab')2 improved median survival 23% over animals that received unlabeled Mel-14 F(ab')2 (P = 0.006), while 125I-labeled RPC 5 F(ab')2 did not have a significant therapeutic effect (P = 0.22), increasing median survival only 8%.

With a view toward improving the therapeutic effect of 125I-labeled Mel-14 F(ab')2 by increasing the administered radiation dose, the maximum nonlethal dose of i.v. injected 125I-labeled Mel-14 F(ab')2 tolerated by athymic mice was determined. Nontumor-bearing mice were given 2000, 3000, and 4000 μCi 125I-labeled Mel-14 F(ab')2. All mice survived over 6 weeks and gained body weight over this time period. Therefore, in Experiment 3 the radiation dose was increased to 3000 μCi/239 μg 125I-labeled Mel-14 F(ab')2 and 3000 μCi/221 μg 125I-labeled RPC 5 F(ab')2 in i.c. D-54 MG tumor-bearing athymic mice.

Table 1. Radioimmunotherapy of D-54 MG i.c. xenografts

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Treatment</th>
<th>Dose (mCi/μg)</th>
<th>Median survival (days)</th>
<th>IMS (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Buffer (control)</td>
<td>10.0/77</td>
<td>17.5</td>
<td>25</td>
<td>0.06</td>
</tr>
<tr>
<td>1</td>
<td>Mel-14 F(ab')2</td>
<td>1.5/115</td>
<td>17.5</td>
<td>25</td>
<td>0.009</td>
</tr>
<tr>
<td>1</td>
<td>Mel-14 F(ab')2</td>
<td>2.0/154</td>
<td>18.0</td>
<td>29</td>
<td>0.009</td>
</tr>
<tr>
<td>2</td>
<td>Mel-14 F(ab')2 (control)</td>
<td>3.0/239</td>
<td>16.5</td>
<td>14</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>Mel-14 F(ab')2</td>
<td>3.0/220</td>
<td>17.0</td>
<td>17</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>Buffer (control)</td>
<td>15.0</td>
<td>13.0</td>
<td>0.5</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>Mel-14 F(ab')2</td>
<td>1.5/160</td>
<td>15.0</td>
<td>13</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>Mel-14 F(ab')2</td>
<td>1.5/220</td>
<td>17.3</td>
<td>31</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Percentage increase in median survival of treatment group over control group.

B. 125I-Mel-14 F(ab')2-Buffer —— 125I-Mel-14 F(ab')2

Fig. 2. Treatment of i.c. D-54 MG xenograft tumors in athymic mice with 125I-labeled Mel-14 F(ab')2. Survival from time of tumor implantation for 9 or 10 animals/treatment group is shown. A, Experiment 1; B, Experiment 2; C, Experiment 3; D, Experiment 4.

Fig. 1. Effect of antibody dose on localization in i.c. D-54 MG xenografts in athymic mice. Mean values of percentage of ID/g present in xenografts 24 h after a single injection of 5–500 μg 125I-labeled Mel-14 F(ab')2 are shown for 8–10 animals/group. Bars, SD.
F(ab')2 at this dose level showed marked splenic atrophy, with mean spleen weights of 29 ± 7 mg at 48 h after injection. Control tumor-bearing animals showed spleen weights of 78 ± 17 mg. In comparison, spleen weights of non-tumor-bearing athymic mice are generally 100 mg.

In Experiment 4, a group of i.c. D-54 MG xenograft-bearing animals were given injections of a total dose of 3000 μCi 131I-Mel-14 F(ab')2 in two doses. On day 6 after tumor implantation, the mice were given injections of 1500 μCi/160 μg and then again of 1500 μCi/140 μg 48 h later. Animals treated in this manner had their median survival prolonged 31% over the control mice, which had received only buffer (P = 0.001) (Fig. 2D; Table 1). Animals given injections of unlabeled Mel-14 F(ab')2 showed no improvement in median survival over control animals (P = 0.5). In this experiment, 1500 μCi Mel-14 F(ab')2 increased median survival only 13% over controls, an increase of marginal statistical significance (P = 0.1). The levels of radioactivity assayed in tumor and normal tissues, in μCi/g, after the two MAb injections are shown in Fig. 3 and illustrate that the second injection of 131I-labeled Mel-14 F(ab')2 increased the delivery of radioactivity to the xenografts. At 12 h after a single injection, tumor activity reached a peak of 13.7 μCi/g and declined to 7.4 μCi/g at 48 h; 12 h after the second injection (and 60 h after the first), tumor radioactivity reached a peak of 27.8 μCi/g (Fig. 3) and was maintained at 13.8 μCi/g at 48 h. Tumor:blood ratios rose from 1.0 at 12 h to 6.3 at 48 h after a single injection; after the second injection, tumor: blood ratios went from 2.6 at 12 h to 9.4 at 48 h. 

Radiation dosimetry estimates showed that 3000 μCi 131I-Mel-14 F(ab')2, given in two doses delivered approximately 915 rad to the tumor, a dose higher than that to normal tissues, for example, 225 rad to the blood and 16 rad to normal brain. Liver and spleen received 316 and 454 rad, respectively. Only kidney received a radiation dose approaching that of tumor, at 749 rad (Table 2). In comparison, a single dose of 1500 μCi/160 μg would deliver 390 rad to tumor and 122 rad to blood (Table 2).

DISCUSSION

Previously, we have reported that Mel-14 F(ab')2 can localize selectively in i.c. glioma xenografts, suggesting that its use in radioimmunotherapy might be feasible (18). Only one animal study has employed radiolabeled fragments in therapy; Buchegger et al. (26) used a mixture of 131I-labeled F(ab')2 fragments and IgG to treat s.c. colon carcinoma xenografts in mice. MAb fragments have been typically considered less suitable for radioimmunotherapy than intact IgG because of their lesser uptake and retention in tumor (25). Nevertheless, in the present study, we have used 131I-labeled F(ab')2 fragments to treat established i.c. human glioma xenografts in athymic mice, and we have shown that a single injection of 1500 μCi 131I-labeled Mel-14 F(ab')2 can produce statistically significant prolongation of animal survival and that this effect is specific, when compared with similar doses of 131I-labeled control F(ab')2 fragment.

The dose of radioactivity delivered to tumor by an i.v. administered radiolabeled MAB depends on both the amount of MAB uptake in the tumor and the specific activity of the radiolabeled MAB. MAB uptake in tumor is clearly dependent upon the immunoreactivity and affinity of the radiolabeled antibody for antigen, which, in turn, can be influenced by the specific activity of the MAB. In the present study, we have shown that the F(ab')2 fragment of Mel-14 labeled with 131I up to a specific activity of 13 μCi/μg using the iodogen method retained an immunoreactivity of 30–45%. This level of immunoreactivity may appear, on the surface, to indicate a substantial loss; however, the assay used was only meant as a quick quality control check and was not meant to indicate the immunoreactive fractions as would be determined by performing a multipoint assay and extrapolating to infinite antigen excess. Further, the immunoreactivity and affinity of the high specific activity labeled F(ab')2 fragment was comparable to that previously described for trace-labeled antibody. The 131I-labeled Mel-14 F(ab')2 labeled at higher specific activity also retained its ability to localize specifically in D-54 MG tumor in vivo.

The influence of antibody dose on MAB uptake by tumor is important in this tumor model. The percentage of the injected dose localized in tumor 24 h after injection fell over the dose range of 5–500 μg; considering the small size of the i.c. tumors used in this study, this observation likely reflected saturation of tumor antigen sites by antibody. Alternatively, Rogers et al. (27) postulated that dehalogenation of the fragment may increase with higher antibody doses, resulting in less circulating radioantibody available for uptake by tumor. Practically, a protein dose that would give maximum localization to tumor (in terms of percentage of ID/g 24 h after injection) was not achievable in this study because of the constraints imposed by limiting the specific activity to 10–12 μCi/μg, a level at which antibody immunoreactivity was maintained at acceptable levels. The effect of antibody dose on localization to tumor xenografts had some important implications in the therapy experiments. In Experiment 1, doubling the injected radiation dose from 1000 to 2000 μCi and, consequently, the protein dose from 77 to 154 μg, would result in an increase of only approximately 10% in radiation delivery to tumor, in μCi/g, extrapolating from the dose-uptake relationship depicted in Fig. 1. Similarly, in Experiment 4, the marginally significant increase in median animal survival after the injection of 1500 μCi 131I-labeled Mel-14 F(ab')2 is explainable by the larger protein dose employed (160 μg), which would decrease the proportion of the 1500 μCi localized to the xenografts by at least 20%, compared to that
localized in Experiments 1 and 2 when 77 and 126 μg were used, respectively.

An attempt to increase tumor radiation dose by an infusion of 3000 μCi 131I-labeled Mel-14 F(ab')2; in tumor-bearing mice did not prolong survival significantly over controls which received buffer alone or similarly labeled nonspecific MAb. This observation was likely the result of supervening radiation toxicity to the hematopoietic system, as evidenced by the marked splenic atrophy in the treated animals. The reduction in spleen weight in mice bearing i.c. tumors changed the absorbed radiation dose to such organs and rendered the animals more susceptible to radiation toxicity, compared to the non-tumor-bearing mice used in the toxicity experiment. Badger et al. (1), in AKR/J SL2 lymphoma-bearing AKR Cu mice, showed that, while 500 μCi 131I-labeled anti-Thy 1.1 intact MAb significantly prolonged animal survival, 1000 μCi did not and that the mice treated with 1000 μCi died as a result of tumor growth and toxicity to the bone marrow.

Dividing the 3000 μCi of administered radiation into two doses of 1500 μCi 48 h apart resulted in significantly increased survival of the tumor-bearing animals over the controls. The second dose of MAb doubled the amount of radiation localized in tumor, in μCi/g, while still maintaining tumor:blood ratios of greater than 2, a consequence of the rapid clearance of Mel-14 F(ab')2 from the blood pool after the first injection. Other factors may be affecting MAb access to tumor when multiple injected doses are used. For example, tumor growth between injections may influence the permeability of tumors to drugs, including MAbs. In two transplantable tumor models in rats, RT-9 and Walker-256 carcinoma cells, there was found to be a direct relationship between tumor size and tumor permeability (14); yet only a “weak correlation” was described between tumor permeability and tumor size in D-54 MG xenografts in rat (28). An additional consideration is that the radiation delivered to tumor by the first injection of radiolabeled MAb may have altered tumor capillary permeability and thereby improved the access of subsequently injected MAbs to tumor; the radiation delivered by implanted 125I seeds has been shown to disrupt the blood-brain barrier in normal canine brain for up to 1 year (29).

The radiation dosimetry calculations for this study were done using the trapezoid rule and medical internal radiation dose. Based on Berger’s calculation for the 95th percentile distance of the β-particles of 125I, and assuming a tumor of spherical dimension with the radius and unit density for the tissue, the radiation dosimetry of tumors as small as 4.1 mg can be reliably determined using this method (30). Calculated radiation dosimetry showed that 3000 μCi 131I-labeled Mel-14 F(ab')2; given in two doses would result in higher radiation doses to tumor (915 rad) than to normal tissues. The radiation dose to tumor is less than that theoretically derived in trace-labeled studies (1208 rad after a 1000-μCi injection) (18) and represents the influence of the higher administered MAb protein dose on uptake in tumor. Lee et al. (7) reported tumor dosimetry of 1585 rad in i.c. D-54 MG xenografts in athymic rats using 131I-labeled MAb 81C6. The only normal tissue to receive a significant dose of radiation was the kidney. This is most likely due to the fact that kidney is the organ most involved with the excretion of the rapidly cleared F(ab')2 fragments. The possible toxicity associated with this dose of radiation may be minimized by increased frequency of voiding.

Although no “cures” were demonstrated in any of the experiments, as Lee et al. (8) reported using 131I-labeled intact MAb 81C6 IgG2b in an i.c. D-54 MG tumor xenograft in athymic rats, most investigators have been unable to ablate well established xenograft tumors with 131I-labeled MAbs (1, 3, 5, 26, 31, 32). Cheung et al. (4) have reported complete tumor ablation with 131I-labeled MAb 3F8 in well established neuroblastoma xenografts; however, neuroblastoma is a highly radiosensitive tumor.

Other methods to improve the efficacy of tumor radioimmunotherapy are promising. Iodine-131 is probably not the optimum radionuclide for use in radioimmunotherapy, particularly for use with faster clearing F(ab')2 fragments. The choice of 131I for this study was to facilitate comparison with radioimmunotherapeutic studies in the literature. The other β-emitting nuclide which has been used in animals for therapy is 90Y. However, 90Y has a larger 95th percentile distance than 131I, making it an even poorer choice for use in small tumors. Shorter-lived α-emitting isotopes, such as astatine-211 (33) and bismuth-212 (34), are more toxic to tumor cells and avoid the γ-radiation of 131I and they may be particularly appropriate for use with F(ab')2 fragments. Regional administration of antibody, such as intrathecal injection (35), improved radiolabeling techniques, producing a labeled MAb with higher retention of label in vivo (36); and combinations of intact MAbs and fragments (26) may all increase tumor uptake.

In summary, we have shown that a single injection of a radiolabeled F(ab')2 fragment of a MAb can specifically deliver a therapeutic dose of radiation to an i.c. human glioma xenograft and that a divided dose may reduce toxicity. A number of avenues need to be explored in order to improve this therapeutic effect and result in better treatment of patients with malignant brain tumors. The efficacy demonstrated in this study supports continuing evaluation of Mel-14 F(ab')2; in clinical trials, particularly using regional routes of administration aimed at further improving tumor uptake relative to normal tissues. The efficacy data reported herein was a factor in the recent approval by the Food and Drug Administration of a Phase I trial of 131I-labeled Mel-14 F(ab')2 for intrathecal administration to evaluate toxicity and efficacy in patients with leptomeningeal tumor dissemination with Mel-14 F(ab')2-immunoreactive tumor cells.

ACKNOWLEDGMENTS

The authors thank Ann Tamariz for her assistance in the preparation of the manuscript.

REFERENCES

RADIOIMMUNOTHERAPY WITH 131I-LABELED F(\text{ab}')\_2 FRAGMENT


Radioimmunotherapy of Intracerebral Human Glioma Xenografts with \(^{131}\text{I}\)-labeled F(ab\(^\prime\))\(_2\) Fragments of Monoclonal Antibody Mel-14

Edward V. Colapinto, Michael R. Zalutsky, Gerald E. Archer, et al.


Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/50/6/1822](http://cancerres.aacrjournals.org/content/50/6/1822)

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.