Comparison of Monoclonal Antibody Delivery to Intracranial Glioma Xenografts by Intravenous and Intracarotid Administration

Yisheng Lee, Dennis E. Bullard, Carol J. Wikstrand, Michael R. Zalutsky, Lawrence H. Muhlbaier, and Darrel D. Bigner


ABSTRACT

Monoclonal antibody 81C6, which is directed against a human gliomasphere extracellular matrix antigen, was used to evaluate the potential advantage of intracarotid (i.c.) administration versus i.v. delivery to D-54 MG human glioma intracranial xenografts in immuno-suppressed rats. Two approaches were taken. In paired-label analysis, 125I-labeled 81C6 and 131I-labeled isotype control antibody were given to separate groups of animals by either the i.v. or i.c. route. Biodistribution measurements as a function of time were analyzed in terms of the percentage of injected dose/g of tissue and localization indices. No significant difference (P > 0.19 to P > 0.56) was demonstrated between the i.v. and i.c. routes. To control for the large localization variation inherent in the animal model used, an alternative experimental design, paired-injection analysis, was performed in which 125I- and 131I-labeled 81C6 were simultaneously administered by the i.c. and i.v. routes to the same animal. Significantly higher levels of percentage of dose/g of tissue and localization ratios (P < 0.05 to P < 0.005) were shown from Day 1 to Day 3 for 81C6 given i.c. Approximately 20% more antibody was delivered to the D-54 MG intracranial tumor by the i.c. route during the experimental period of 5 days. No difference in the levels of normal tissue exposure between the two routes of administration was seen. These data suggest an advantage exists for whole monoclonal antibody given i.c. and that, theoretically, a greater advantage may be present for smaller molecules such as Fab and F(ab')2 fragments.

INTRODUCTION

Proposed causes of treatment failure for malignant gliomas can be divided into two categories. The first includes those derived from tumor cell heterogeneity such as clonal variation in growth potential, differential drug resistance, and antigenic heterogeneity (1). The other category involves the effector arm of the therapeutic approach: poor drug delivery and lack of therapeutic agent specificity (2). These latter two problems are evaluated in this paper.

In order to increase drug delivery to specific tumor sites and to lower systemic toxicity, i.e., infusions have been used in both animal experiments and human trials (3–7). Up to 7-fold increased delivery to the brain of methotrexate, a water-soluble agent, has been achieved by the i.c. route in two animal models (3, 4). In the 9L rat gliosarcoma model, the lipid-soluble agent, a significantly lower dosage (50%) of BCNU can be given i.c. to achieve survival equivalent to that observed following full-dose i.v. administration (5). The relevance of these observations in animals and the results of human studies remain equivocal; increased delivery via the i.c. route has not been clearly quantitated for most therapeutic agents.

Pertinent to the problem of agent specificity, the development of Mabs to tumor-associated targets not only creates the possibility of designing tumor-specific agents for imaging and therapy of tumors, but also provides a better vehicle for selective drug delivery, an interaction lacking for other chemotherapeutic agents. The murine antiangioma Mab 81C6 defines a gliomasphere extracellular matrix antigen expressed in human glioma cell lines, glioma xenografts in nude mice, and primary human gliomas, but not in normal adult or fetal brain (8). Localization to subcutaneous and intracranial glioma xenografts (9) has been achieved and allows external imaging of intracranial xenografts as small as 20 mg (10). However, the amount of Mab localized in intracranial D-54 MG tumors has ranged from 1.11% to 2.90% of injected dose per g of tissue (10), whereas localized levels reached more than 10% in the subcutaneous D-54 MG tumor model system (9). This difference may reflect a different degree of antigen expression or accessibility of antibody from the vascular compartments. It is possible, therefore, that i.c. delivery could result in a significant increase in delivery to intracranial tumors and that a small percentage of increase in intracranial delivery may be significant for tumor therapy and diagnosis.

In order to evaluate the potential of increasing antibody delivery to intracranial D-54 MG tumors (by i.c. administration), two approaches were taken: a conventional paired-label analysis, in which each animal received labeled specific and nonspecific antibody by either the i.v. or i.c. route; and a paired-injection study, in which each animal served as its own control by receiving simultaneous injections of specific antibody by both routes. Subsequent analysis of the paired-label studies failed to demonstrate a significant difference between the two routes. In contrast, paired-injection studies showed significantly higher localization by i.c. administration at Days 1, 2, and 3 (P < 0.005) after injection. With 131I-labeled antibody, this difference represented approximately a 20% increase in radiation exposure of the tumor during the 5-day time period. A greater sensitivity of the paired-injection technique exists because of its compensation for the variability of tumor size in this model. Although the large molecular weight of molecules such as Mabs appears to preclude a true “first passage” advantage from intraarterial administration, other factors such as increased sequestration of Mab in an intracranial “third space” compartment, reduced uptake in visceral organs, and increased tumor blood flow after i.c. injection may explain the advantage seen in this model by i.c. delivery.

MATERIALS AND METHODS

Monoclonal Antibody. Monoclonal antibody 81C6, which recognizes a human glioma-associated extracellular matrix antigen (8), and a control monoclonal antibody of the same isotype IgG2b, 45.5TG1.7 (45.6), were purified from hybridoma culture supernatant using Protein A-Sepharose 4B (11). Immunoglobulin radiolabeling with either 131I or 125I (New England Nuclear) was done by the chloramine T method (12). Radiolabeled immunoglobulins were purified from unreacted radioiodine on a 10-ml Sephadex G-25 fine column. The trichloroacetic acid

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2To whom requests for reprints should be addressed.

3The abbreviations used are: i.c., intracarotid; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; Mab, monoclonal antibody; LI, localization index.
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Precipitability for the different preparations varied between 95% and 97%. Immunoreactive antibody was isolated after radiolabeling, by first absorbing it to an excess of D-54 MG tumor homogenate for an hour at room temperature. After washing, bound 81C6 antibody was eluted with 0.05 M glycine buffered at pH 3.0 and then neutralized immediately.

Human Tumor Xenografts. Human glioma-derived cell line D-54 MG was grown s.c. in nude mice as previously described (13). Tumor tissue homogenates were prepared from these nude mouse D-54 MG subcutaneous tumors by an equal volume of 1% methyl cellulose (Fluka, Switzerland) and minimal essential medium. F-344 rats were immune suppressed by first giving 50 μl of burro anti-T-lymphocyte antiserum i.p. twice weekly beginning 1 to 3 days after birth. In the fourth week, the antisera was increased to 100 μl with cyclosporin added at doses between 7.5 and 10 mg/kg/day (14). For nude rats, animals that weighed between 200 and 300 g were used. Intracranial tumor xenografts were prepared as described (10). Briefly, each animal received 5 μl of tumor homogenate in the right cerebral hemisphere at a depth of 4 to 5 mm delivered with a Hamilton syringe fitted with a No. 25 needle. Animal survival was recorded, and intracranial tumors were identified for each animal at the time of death.

Paired-Label Immunolocalization. Initial comparison of 81C6 immunolocalization in intracranial D-54 MG tumor-bearing rats following i.c. and i.v. (femoral veins) administration was first approached by paired-label analysis (15). This was used to define antibody localization kinetics in terms of localization index, percentage of dose localized per g of tissue, and tumor-to-tissue ratio.

Twelve to 14 days after tumor inoculation, animals were randomized into i.c. and i.v. groups. At this stage of tumor growth, animals were estimated to have 3- to 5-mm-diameter intracranial D-54 MG tumors. All rats underwent right carotid artery exposure with pterygopalatine and occipital artery ligation. Previous data (16) have shown such selective ligation to be necessary in the rat to approximate carotid injection in humans for first passage delivery of agents to the brain. Infusion of 0.2 to 0.225 ml of either normal saline or labeled immunoglobulin mixture (81C6 and 45.6) was given via a right carotid artery catheter. For i.v. injection, each animal received 0.2 to 0.225 ml of normal saline or labeled proteins, so that each animal received the same total volume and the same surgical manipulation.

Two experiments were performed. In Experiment 1, each animal received 0.806 μCi/2.5 μg of 81C6 and 1.76 μCi/2.5 μg of 45.6 (no. of animals: i.c., 22; i.v., 25); in Experiment 2, higher doses of radiation/μg of protein were given: 6.63 μCi/2.5 μg of 81C6 and 13.69 μCi/2.5 μg of 45.6 (no. of animals: i.c., 15; i.v., 12). Animals were then killed at different time points. Immediately prior to death, animals were given 0.2 ml of 3% Evans blue dye, i.v. in order to better delineate the intracranial tumors. Following blood collection by cardiac puncture, the animals were perfused with 5% dextrose in normal saline, and the heart, lung, liver, kidney, spleen, muscle, and brain were removed. Intracranial tumors were dissected free from normal brain. All tissues were weighed, and localized radiation was determined in a gamma counter. The levels of 125I and 131I in tissue samples were corrected for isotope decay and cross-over of 131I counts into the 125I channel. The localization index, percentage of dose/g, and tumor tissue ratios were calculated as described (17).

Paired-Injection Radiolocalization. In later experiments, a second approach was utilized in which each animal acted as its own internal control. Specific antibody 81C6 labeled with either 125I or 131I was given simultaneously through either the i.c. or i.v. route (paired-injection). Animals were given injections as previously described. In experiment 3, 125I-labeled 81C6, 16 μCi/5 μg was given i.c., and 131I-81C6, 27 μCi/5 μg, i.v. In experiment 4, the route of isotope administered was reversed so that 131I-81C6, 11.5 μCi/5 μg, was given i.c., and 125I-81C6, 9.65 μCi/5 μg, was given i.v. Animals were killed at different time points, and tissues and tumors were analyzed as described previously.

Statistical Analysis. For paired-label experiments, both localization index and percentage of dose localized per g of tissue data obtained from i.c. and i.v. groups were analyzed by polynomial regression analysis. Best-fit models for localization kinetics were first determined for both routes. Further comparison between i.v. and i.c. route 81C6 delivery was then done in terms of kinetics (model parallelism) and dose delivery (line distance).

For paired-injection experiments, i.c. and i.v. data were considered matched pairs as each data set was derived from the same animal. The Wilcoxon signed-ranked test was carried out for each time point using a localization ratio (i.c./i.v.) and percentage of dose/g of difference (i.e. - i.v.) of each individual animal. Best-fit models for each route were generated through computer modeling using data from both experiments. Dosages delivered were calculated from the area under the curve for i.c. and i.v. injected 81C6 by the trapezoidal integration method (18). The radiation dose was then calculated for each route for 81C6 Mab labeled with isotopes of different half-lives using standard MIRD (Medical Internal Radiation Dose) formalism (19).

RESULTS

Intracranial D-54 MG Tumor Growth Characteristics. After intracranial injection of 10 μl of D-54 MG homogenate prepared from subcutaneous tumors, 100% tumor incidence was achieved. All animals died with a median survival time ranging from 16 to 18 days. Histological examination (Fig. 1) documented the presence of intracranial tumor. D-54 MG formed well-circumscribed tumors in the rat brain without prominent necrosis or hemorrhage. Hydrocephalus was also observed. The cause of death is thought to be brain stem compression from brain herniation directly related to tumor volume. The steepness of the survival curves reflected a homogeneous and rapid growth of these intracranial transplanted D-54 MG tumor cells.

Paired-Label Analysis. 125I-81C6 and 131I-45.6 were given together through either the i.v. or i.c. route, and the levels of each antibody in the intracranial tumors and normal tissues were determined at different time points after Mab injection. Two paired-label experiments were done with Mabs at different iodination ratios. As the degree of iodination may have an effect on Mab binding and in vivo clearance (18), serum Mab levels were evaluated. Fig. 2 demonstrated blood clearance of Mabs in these two experiments. No significant difference was found at any time point for i.c. and i.v. injected radiolabeled 81C6 in both experiments. The distribution and specificity of 81C6 binding in various tissues were further analyzed by the tumor:tissue ratio (Fig. 3). Both i.v. and i.c. administered 81C6 increased binding to tumors than normal tissues much faster.

Fig. 1. Intracranial D-54 MG tumors (7×) in the nude rats. D-54 MG formed well-circumscribed tumors without prominent necrosis and hemorrhage. Hydrocephalus was also seen occasionally.
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EXPERIMENT 1
PAIRED-LABEL

EXPERIMENT 2

EXPERIMENT 3
PAIRED-INJECTION

EXPERIMENT 4

Fig. 2. Blood clearance of i.v. and i.e. administered 81C6 Mab in both paired-label (Experiments 1 and 2) and paired-injection (Experiments 3 and 4) experiments. No significant difference was observed in either approach. Bars, SE.

than 45.6 in all tissues examined. There was no difference in the profiles of tumor: tissue ratio and thus normal tissue exposure between i.e. and i.v. administered 81C6.

Sequential LI and percentage of dose per g of tissue for each experiment were examined to determine the localization kinetics of i.c. and i.v. administered 81C6 in intracranial D-54 MG tumors. Best-fit line models determined by polynomial regression analysis suggested a linear relationship between LI or data of percentage of dose per g of tissue and log time (Fig. 4). Further analysis of i.e. and i.v. best-fit lines established that there was no significant difference between the magnitude or rate of uptake of 81C6 in intracranial tumors and that the regression lines were basically parallel to each other (model parallelism). There was also no difference between the amount of antibody bound between the two different routes as analyzed by model difference (i.e. versus i.v.) of LI and percentage of dose per g of tissue (Table 1).

Paired-Injection Analysis. To further evaluate the question of potential advantage of administration routes while controlling for individual tumor size, a paired-injection experimental design was utilized in which each animal served as its own control (Experiments 3 and 4). The Wilcoxon signed-rank test was used to analyze the difference at each time point between i.v. and i.e. routes of the percentage of dose per g of tissue and localization ratio. Significant differences were found to exist in both parameters on Days 1, 2, and 3 (Table 2). Following Mab 81C6 given i.c., radioactivity consistently localized better than 81C6 given simultaneously via the i.v. route, regardless of which radioisotope ($^{131}$I or $^{125}$I) was administered by which route. To evaluate total administered dose by the two routes of administration, data from Experiments 3 and 4 were compared from day 1, a common time point. As the difference was not highly significant in either percentage of dose per g of tissue ($P > 0.016$) or localization ratio ($P > 0.056$), integration of data from both paired-injection experiments was performed to provide a computer-generated dose delivery curve for i.v. and i.e. routes (Fig. 5). Systemic exposure was also analyzed by the tumor: tissue ratio as in paired-label analysis. Again, no difference was found between 81C6 Mab administered i.e. or i.v. Such predicted values using the model curve indicated that 81C6 i.e. still localized better than i.v. 81C6 from Days 1 to 4 and there was no difference noted at 4 h and Day 5. Dosimetry calculations from the curves indicated that Mab 81C6 administered by the i.e. route delivered 19.3% more antibody than i.v. administered 81C6 Mab. However, for diagnostic and therapeutic purposes, Mab was usually labeled with isotopes of different half-lives. The effect of radiation decay on i.e. advantage was evaluated. When $^{131}$I is used for radiation dosage calculation, after correction for half-life, i.e. 81C6 still delivered 18.9% ($311.0 \mu$Ci/h/g versus $261.6 \mu$Ci/h/g, 1000 $\mu$Ci as initial dose injected) more radiation during the 5-day experimental period. This calculation is performed with the assumption that radiolabeling with

Fig. 3. Localization of $^{125}$I-81C6 Mab compared to $^{131}$I-45.6 Mab control. Tumortissue ratio as a function of time and route. Mab 81C6 localized much more specifically than 45.6 Mab, but there is no significant difference between i.v. and i.e. routes. a, blood, lung, and liver; b, kidney, cerebellum, and cerebrum.
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Fig. 4. Comparison of localization kinetics of i.v. (O) and i.e. (•) administered 81C6 using localization index and percentage of dose per g of tissue as parameters. The binding kinetics was parallel between the two routes. The lines represent the predicted values of i.v. and i.e. 81C6 Mab binding. Left, Experiment 1; right, Experiment 2.

Table 1. Statistical analysis of Mab 81C6 delivery to intracranial D-54 MG tumor by i.e. and i.v. routes, analyzed by localization index, and percentage of injected dose/g of tissue

Paired-label analysis (P value)

<table>
<thead>
<tr>
<th></th>
<th>i.v., i.c. model parallelism</th>
<th>i.v., i.c. difference</th>
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<tbody>
<tr>
<td>Experiment 1</td>
<td>Experiment 2</td>
<td>Experiment 1</td>
</tr>
<tr>
<td>Localization index (%)</td>
<td>0.19</td>
<td>0.35</td>
</tr>
<tr>
<td>% of dose/g</td>
<td>0.56</td>
<td>0.19</td>
</tr>
</tbody>
</table>

• Localization index for Experiments 1 and 2:

\[ LI = \frac{\text{tumor 81C6 % of dose/g/tumor 45.6 % of dose/g}}{\text{blood 81C6 % of dose/g/blood 45.6 % of dose/g}} \]

Table 2. Statistical analysis of Mab 81C6 delivery to intracranial D-54 MG tumor by i.e. and i.e. routes, analyzed by localization ratio and percentage of injected dose/g of tissue

In paired-injection analysis, 81C6, labeled with different isotopes, was given i.e. and i.v. to the same animal simultaneously. Data were analyzed at individual time points.

Paired-injection analysis

<table>
<thead>
<tr>
<th></th>
<th>4 h</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
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<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>20</td>
<td>7</td>
<td>14</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Localization ratio (%)</td>
<td>&gt;0.40</td>
<td>&lt;0.005</td>
<td>0.005</td>
<td></td>
<td></td>
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<tr>
<td>% of dose/g</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>0.005</td>
<td></td>
<td></td>
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<tr>
<td>Experiment 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>ND</td>
<td>17</td>
<td>ND</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Localization ratio (%)</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
<td>&gt;0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of dose/g</td>
<td>0.005</td>
<td>&lt;0.005</td>
<td>0.005</td>
<td></td>
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• ND, not done.

• Localization ratio for Experiments 3 and 4:

- tumor i.e. 81C6 % of dose/g/blood i.e. 81C6 % of dose/g
- tumor i.v. 81C6 % of dose/g/blood i.v. 81C6 % of dose/g

with a very short half-life such as 211At (astatine) and 212Bi (bismuth) (Table 3).

DISCUSSION

Paired-label analysis failed to show a significant i.e. advantage for Mab 81C6 delivery to intracranial D-54 MG tumor

different isotopes does not lead to significant changes in antibody binding and clearance. Similar degrees of advantage were obtained for 67Cu (copper) and 89Y (yttrium), but not in isotopes

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Table 3 Advantage of Mab 81C6 delivery to intracranial D-54 MG tumors by the i.e. route: effect of radiation half-life

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Decay half-life (h)</th>
<th>i.e. advantage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>131I (iodine)</td>
<td>193.9</td>
<td>19.3</td>
</tr>
<tr>
<td>90Y (yttrium)</td>
<td>64.1</td>
<td>17.5</td>
</tr>
<tr>
<td>64Cu (copper)</td>
<td>61.2</td>
<td>17.4</td>
</tr>
<tr>
<td>211At (astatine)</td>
<td>7.2</td>
<td>0.3</td>
</tr>
<tr>
<td>210Bi (bismuth)</td>
<td>1.01</td>
<td>-4.0</td>
</tr>
</tbody>
</table>

xenografts. This is consistent with previous paired-label comparison of i.c. versus i.v. delivery of Mab 81C6 to normal rat brains with or without blood-brain barrier disruption which also failed to demonstrate first passage advantage of i.c. infusion (20). However, examination of all the data available with this model (9, 20) demonstrated a broad range of brain and tumor uptake. This variability may have resulted not only from individual animal differences, but also from intratumoral heterogeneity of blood flow, blood-to-tissue transfer constant, and extraction fraction within different areas of individual tumors (21). It is assumed that this variability affected the data analysis and that the paired-label analysis commonly used in tumor localization studies may be inadequate to detect small differences when such variability is present, unless a very large number of animals are used.

To compensate for this variability, paired-injection analysis, which directly compared i.c. and i.v. delivery using each individual intracranial tumor as its own control, was performed. In these experiments, a significant difference in the percentage of dose per g of tissue delivery for i.v. and i.c. routes was found at all time points (P ≤ 0.005). However, after correction for plasma immunoglobulin levels (localization ratio), higher tumor dosages could be demonstrated only between Days 1 and 3 after i.c. injection of Mab 81C6. Our interpretation of the data is that the tapering off of the difference by Day 5 resulted from antibody recirculation at near saturation, of such immunoglobulin agents with long plasma half-lives. The 4-h time point, however, appears to contradict the commonly held hypothesis that i.c. advantage would only be a “first passage advantage” predicting largest differences at early time points. The large molecular weight of molecules such as Mab may preclude such a phenomenon. The high background concentration of circulating immunoglobulins which could not be totally washed out by tissue perfusion before sampling and the relatively low rate of specific tumor binding at early time points may also be responsible. Later (Days 1 to 3) advantage may have then resulted from sequestration of i.c. infused Mab in an intratumoral “third space” which could permit better delayed access to antigen. As such, i.c. infusion of Mab would act as a reservoir for later specific binding.

When the integrated exposures (concentration × time integral) were compared, 81C6 given by the i.c. route delivered approximately 20% more antibody dose to intracranial D-54 MG tumors. The biological significance of this is difficult to predict. The direct translation of a drug level to its pharmacological activity is usually not warranted because most drug dose-response curves are not linear but sigmoidal. If drug levels are on the asymptotic portion of the response curve, even a large increase in drug level may result in only a very small increase in response rate (22). This relationship is suggested by our previous experiments using the 9L rat intracranial tumor model where i.c. administered BCNU provided no further benefit in animal survival when the threshold of tumor cytotoxicity for BCNU-sensitive cells was reached (5). In contrast, when on the exponential portion of the curve, small increases may provide a steep dose-response pattern. It is clear, however, that before generalizations can be made, more data must be accrued for both drugs and Mab. The knowledge of the tissue level achieved and the dose-response relationship for cytotoxic agents, chemotherapeutic drugs, or radiation will be helpful in determining the risk:benefit ratio of i.c. administration. Our data and calculation thus preclude the use of isotopes with short half-life (211At and 212Bi) for i.c. delivery using Mab as carriers.

As for systemic toxicity, the most important determinant is the loss of agents by extraction or metabolism during their first passage through the tissue perfused by the infused artery (23, 24). In this study, the specific binding to intracranial tumors at early times (10 min) was less than 1% of the injected dose, and no significant difference could be seen in Mab 81C6 clearance or recirculation concentration integrals. When normal tissue levels were expressed as tumor:tissue ratio, i.c. administered Mab 81C6 reached the same level as the i.v. at all time points. Thus in terms of systemic exposure, no difference was seen with i.c. injection of anti-glioma Mab 81C6.

The theoretical advantages of intraarterial infusion of drugs include increased drug delivery to target tissues and decreased systemic toxicity. In practice, however, multiple factors are involved in the determination of the so-called “first passage” advantage. It is possible to theoretically establish their relative values from mathematical analyses and computer modeling (23-25), but true benefit will have to be determined by direct experimentation. An analysis of the pharmacokinetics and physiological parameters involved in 81C6 Mab localization in D-54 MG intracranial and subcutaneous xenografts (21) demonstrated significantly lower mean tumor blood flow (53.5 ± 18.4 ml/100 g min) than that of tumor-free cortex (198.2 ± 58.4 ml/100 g min). This provided a favorable condition for higher i.c. delivery of immunoglobulins (23, 24). However, when immunoglobulin clearance from blood was examined, long half-life of approximately 3 days was observed. This was considered to be counterindicated to a i.c. route advantage. BCNU, a drug which has shown beneficial effects from i.c. delivery (5), has a half-life of 21 min (26). Quantitative data derived from experiments using methotrexate (triphasic clearance, half-lives: 0.75, 2.0 to 3.5, and 10.5 h) demonstrated 7-fold increase after i.c. administration during the first 10 min after injection (3). Thus, if only two factors are considered, such observations would suggest that the i.c. benefit obtained for large water-soluble molecules such as immunoglobulins with very long plasma half-life would be small. Agents with short plasma half-life and large extraction fractions would have the greatest i.c. delivery advantage.

However, several differences exist between immunoglobulins and other chemotherapeutic agents: the slow clearance of immunoglobulins from extracellular space in tumors and the specificity of tumor-specific antibody for antigens. The binding of Mab 81C6 to intracranial D-54 MG tumors reached peak levels at 24 to 48 h and persisted for at least 9 days. This is especially important as a drug delivery advantage should not be
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evaluated at only a single time point, but rather by total drug exposure over time. Considerable discrepancies between drug delivery and clinical results may result from predicting responses from transient high drug levels at a single time point which may have little benefit in tumor killing (22, 23). Total exposure can be determined by (concentration) \times (time) integrals (22). With the use of a tumor-specific agent such as a Mab, additional factors that may be involved include: epitope expression in other tissues and specific binding to shed antigens. With antigens such as carcinoembryonic antigen, which are present in the plasma, more direct delivery via the i.c. route may provide an additional advantage not seen with 81C6 (27).

In summary, studies using the paired injection technique and intact tumor-specific Mab demonstrated i.c. injection to deliver approximately 20% more Mab and 131I radiation dosage to rat intracranial D-54 glioma xenografts than did i.v. administration. With i.c. Mab injection, systemic exposure was not decreased from that achieved by the i.v. route. The results supported the i.c. advantage as predicted by theory and computer modeling (25). This approach would also suggest that F(ab')2 and Fab fragments of 81C6, which have a relatively faster plasma clearance, may provide an even greater advantage via the i.c. approach. This hypothesis and the determination of the eventual clinical utility of these observations need to be determined in future experiments.

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