Permeability of human brain tumor to $^{99m}$Tc-glucoheptonate and $^{99m}$Tc-albumin

Implications for monoclonal antibody therapy

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The variable penetration of chemotherapeutic drugs into brain and tumor is more dependent upon lipid solubility than upon size. In contrast, the molecular weight of virus- and tumor-specific monoclonal antibodies appears to limit uptake. The authors have studied eight patients with malignant brain tumors in order to compare tumor uptake of an iodinated contrast agent evaluated by computerized tomography scanning with uptake of the low and high molecular weight imaging agents technetium-$^{99m}$ ($^{99m}$Tc)-glucoheptonate and $^{99m}$Tc-albumin, respectively, measured by radionuclide brain scanning. The agent $^{99m}$Tc-labeled albumin was chosen for evaluation because its molecular weight (68,000) is similar to that of the most clinically promising monoclonal antibody fragment, the immunoglobulin (Ig) G Fab monomeric fragment. The radionuclide brain scans in the eight patients showed highly variable permeability of brain tumor to these markers, with uptake of the high molecular weight marker in the tumor being much less than that of the low molecular weight radionuclide. A clinical implication of these studies is that the success of monoclonal antibody therapy in the treatment of malignant brain tumors may require techniques to increase permeability of the blood-brain barrier and blood-tumor barrier to protein.

KEY WORDS · brain tumor · blood-brain barrier · radionuclide brain scanning · monoclonal antibody

For most small molecular weight substances, including most chemotherapeutic agents or antibiotics, lipid solubility rather than molecular weight is the major limiting factor in penetration of either the blood-brain barrier or the blood-tumor barrier. Perhaps for this reason, molecular weight as a limiting factor in delivery of these agents remains less intensively studied despite the development of monoclonal antibodies directed against tumor-associated antigens.

However limited the data may be, they clearly suggest that delivery of high molecular weight proteins, such as monoclonal antibodies, to brain tumors is significantly more restricted than delivery of conventional lower molecular weight water-soluble chemotherapeutic agents. This phenomenon has been studied in the nude rat, an animal genetically deficient in T lymphocytes which permits growth of human tumor xenografts. In this rat model, human small-cell carcinoma of the lung has been grown both intracerebrally and subcutaneously in the same animal. In the intracerebral tumor the blood-tumor barrier almost totally prevents the uptake of Evans blue albumin, a protein marker with a molecular weight (MW) of 68,000, in contrast to permitting variable uptake of small molecular weight substances such as methotrexate (MW 454) and fluorescein (MW 376). Evidence that this is not a specific characteristic of the tumor cells is shown by the greater uptake of Evans blue albumin or the cytotoxic agent methotrexate in tumor grown subcutaneously in the same animal.

Anecdotal evidence suggests that the same phenomenon occurs in human brain tumors. In a Phase I trial, Houghton, et al., evaluated mouse monoclonal antibodies administered to patients with malignant melanoma. One of their patients, in whom there was a greater than 50% regression of all measurable lesions systemically, concomitantly developed progres-
Human brain-tumor permeability to radiolabeled agents

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Histological Diagnosis</th>
<th>Enhancement by CT</th>
<th>Radionuclide Uptake Ratio</th>
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* CT = computerized tomography; 99mTc = technetium-99m; GBM = glioblastoma multiforme; -- = tumor not visualized.
† For a description of the method of determining the ratio of radionuclide uptake in tumor versus normal brain see text.

Results of CT and radionuclide brain scanning in eight patients with primary and metastatic malignant brain tumors

Enhanced CT scans were obtained 30 minutes after the administration of 90 gm (200 cc) of meglumine iothalamate (Conray 43) by intravenous infusion. Radionuclide brain scans were obtained 3 hours after injection of 18 to 22 mCi of either 99mTc-glucoheptonate (MW 226, specific activity 0.5 mCi/mg) or 99mTc-albumin (MW 69,000, specific activity 1 to 2 mCi/mg). These two radionuclide scans were obtained 1 to 30 days apart and within 1 to 30 days after the enhanced CT scan.

The degree of blood-tumor barrier permeability on the radionuclide scan was assessed from Polaroid color photographs of the rainbow color-coded output on the Medical Data Systems computer in which the highest average counts/pixel. A 3 x 3-pixel unit tumor ROI using a 64 • 64 matrix was then applied to tumor areas in each of the equalized images. An array of colors was then obtained, and mean ratios for the three views from each study were calculated.

In one patient the different blood-clearance characteristics of glucoheptonate and albumin were evaluated with sequential blood samples. The counts were corrected for decay and then found to be nearly linear on a log-log plot of counts per minute (cpm) versus time.

**Results**

We made two main observations in reviewing the data from the eight patients studied. The first observation was that highly variable tumor permeability occurred in lesions identified in seven patients by the more sensitive imaging technique of enhanced CT scanning. In comparison, clear uptake of either the low or high molecular weight radiopharmaceutical was seen in only three patients (Cases 1 to 3) with radionuclide scanning (Table 1). The more important observation, however, was that different tumor permeability was shown in those three patients in whom the radionuclide brain scans were clearly positive. In these cases, uptake of the low molecular weight 99mTc-glucoheptonate was markedly better than that of the high molecular weight 99mTc-albumin. This result is dramatically revealed from the radionuclide studies in Case 1 (Fig. 1) and was confirmed by semiquantitative analysis of ratios of color-coded count levels in normal brain and tumor read directly from the images (Table 1).

Since the clearance of albumin in plasma is slower than that of glucoheptonate (Fig. 2), the potential effect of the two different radionuclides on the blood pool of the brain was assessed. In the special ROI analysis, ROI values in normal brain on the various scans were equalized before tumor:brain ratios were obtained for comparison. The results of the special ROI technique are outlined in Table 2 and show that the difference between tumor uptake of glucoheptonate and albumin ranging from white to dark blue, with the "hottest" (white) pixel being 100%. The color-coded count-percentage photographs were then semiquantitated by the observational assignment of an area-weighted mean percentage uptake to each brain hemisphere and a maximum percentage uptake to the brain tumor. The tumor and contralateral brain hemisphere percentages were then divided to obtain ratios of tumor to hemisphere for each of the three views; anterior, vertex, and posterior. The mean ratio for each radiopharmaceutical per patient study was then calculated.

In addition, the variation in background (blood pool) was controlled by a special region of interest (ROI) analysis in which the non-tumor-bearing hemisphere ROI counts in all images from both studies in each patient were normalized to the single image with the highest average counts/pixel. A 3 x 3-pixel unit tumor ROI using a 64 x 64 matrix was then applied to tumor areas in each of the equalized images. An array of tumors was then obtained, and mean ratios for the three views from each study were calculated.
FIG. 1. Color-coded radionuclide brain scans, posterior view, in Case 1 demonstrating a right parieto-occipital tumor (a primary central nervous system lymphoma). As indicated on the color scale, white is the “hottest” pixel and blue the “coldest.” For a further description see the Materials and Methods section. Left: Scan obtained 3 hours after intravenous infusion of technetium-99m (99mTc)-glucoheptonate. Right: Scan obtained 3 hours after intravenous infusion of 99mTc-albumin. Note the decreased uptake of radionuclide in the tumor on this image as compared with left.

has been maintained. We therefore attribute the difference in radionuclide uptake in tumor to permeability characteristics of the blood-tumor barrier.

Discussion

In this clinical trial we used radionuclide brain scans to further evaluate the variable tumor permeability that has been shown in recent animal studies of human tumors grown intracerebrally and subcutaneously in the nude rat. As in the animal studies, tumor permeability in our patients was variable. When high versus low molecular weight compounds were administered to the rats, the high molecular weight substances penetrated the tumors grown intracerebrally to a significantly lesser extent than they penetrated the same tumors grown subcutaneously. The findings of our current study support these animal studies by demonstrating that malignant human brain tumors are more permeable to the low molecular weight glucoheptonate than to the high molecular-labeled albumin.

The method of analyzing blood-tumor barrier permeability employed in this study is analogous to that used in correlating the uptake of 99mTc-glucoheptonate and the degree and extent of barrier modification achieved in our clinical study of patients with malignant brain tumors who underwent osmotic opening of the blood-brain barrier. Based on 219 clinical blood-brain barrier disruption procedures evaluated by radionuclide scans, uptake ratios of tumor to background ranging from 1.0 to greater than 2.17 were obtained. This range was subdivided into four categories corresponding to nil, moderate, good, or excellent degrees of disruption clinically. Extrapolating from extensive animal and more limited human cerebrospinal fluid (CSF) studies, it appears that an excellent disruption is associated with up to a 100-fold increase in drug delivery to normal brain or CSF. With this as a basis

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*99mTc = technetium-99m. For a description of the method of determining the ratio of radionuclide uptake in tumor versus normal brain using the special region of interest analysis see text.
for interpreting the ratios, a small numerical increment reflects a significant change in the continuum from a nil to an excellent blood-brain or blood-tumor barrier disruption.

The primary clinical implication of these observations relates to the administration of antibodies directed against tumor-associated or virus antigens. The molecular weights of the two most commonly used antibody classes are 160,000 for immunoglobulin (IgG) and 1,000,000 for IgM. Since IgG seems to be the most promising antibody and since Fab fragments of IgG have less nonspecific binding, the Fab fragment of IgG holds the most promise for clinical studies. The molecular weight of the monomeric IgG fragment (60,000) is similar to that of albumin, and this is the reason we chose albumin for evaluation in our present study.

The few animal and clinical reports that have been published, as well as anecdotal reports of administration of these antibodies to patients with brain tumors, suggest that penetration into the tumor has been less than ideal. The question that arises from our current findings is whether the problem of protein delivery can be overcome by using techniques such as osmotic blood-brain barrier disruption to increase delivery. Initial animal studies evaluating the delivery of antibodies and enzymes to normal brain and labeled albumin and antibody to tumor grown in brain suggest that barrier modification can increase delivery to both tumor and surrounding brain. Whether this increase in delivery by osmotic barrier modification can result in improved uptake ratios of tumor to background remains to be determined.

Addendum

In support of the need for osmotic blood-brain barrier modification, we evaluated delivery of melanoma-specific iodine-131 (131I) monoclonal antibody (Mab) in a pilot study of three patients with melanoma metastatic to the central nervous system (CNS) (Neuwelt EA, Specht HD, Barnett PA, et al: Increased delivery of tumor-specific monoclonal antibodies to brain after osmotic blood-brain barrier modification in patients with melanoma metastatic to the CNS. Neurology 36 (Suppl 1):291, 1986 (Abstract)).

Each patient's tumor demonstrated excellent reactivity by immunohistochemistry with our Federal Drug Administration-approved Mab 96.5 specific for a 97,000-molecular weight melanoma antigen (P97) and/or Mab 48.7 specific for a lower-molecular weight proteoglycan melanoma antigen. All three patients received 131I tumor-specific Mab (5 to 7 mg, 1 mCi/mg) intravenously and, on a separate occasion, two received 131I nonspecific ("nonsense") Mab (5 to 7 mg, 1 mCi/mg). There was no uptake of either antibody into the region of the tumor (as documented by brain scan). However, there was increased uptake in the tumor-bearing hemisphere in all three patients, only when radiolabeled tumor-specific Mab was administered intravenously in conjunction with osmotic blood-brain barrier opening. Serial brain scans showed that virtually all radiolabeled antibody had cleared from the brain by 72 hours. There did appear to be increased uptake in the tumor region in one patient, but antibody clearance from that region occurred at the same rate as from surrounding, apparently tumor-free, brain. Studies are continuing to improve the degree and persistence of Mab localization to such CNS tumors.

References


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