Antibody Guided Diagnosis and Therapy of Brain Gliomas using Radiolabeled Monoclonal Antibodies Against Epidermal Growth Factor Receptor and Placental Alkaline Phosphatase

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Twenty-seven patients with brain glioma were scanned using ^{123}I-labeled monoclonal antibodies against epidermal growth factor receptor (EGFR1) or placental alkaline phosphatase (H17E2). Successful localization was achieved in 18 out of 27 patients. Eleven out of 27 patients were also studied using a nonspecific control antibody (11.4.1) of the same immunoglobulin subclass and observable tumor localization was also achieved in five patients. The specificity of targeting was assessed by comparing images obtained with specific and nonspecific antibodies and by examining tumor and normal tissue biopsies after dual antibody administration. Ten patients with recurrent grade III or IV glioma who showed good localization of radiolabeled antibody were treated with 40–140 mCi of ^{131}I-labeled antibody delivered to the tumor area intravenously (n = 5) or by infusion into the internal carotid artery (n = 5). Six patients showed clinical improvement lasting from 6 mo to 3 yr. One patient continues in remission (3 yr after therapy), but the other five who responded initially relapsed 6–9 mo after therapy and died. No major toxicity was attributable to antibody-guided irradiation. Targeted irradiation by monoclonal antibody may be clinically useful and should be explored further in the treatment of brain gliomas resistant to conventional forms of treatment.

J Nucl Med 30:1636–1645, 1989

Brain gliomas comprise ~60% of all primary CNS tumors and are challenging areas both for clinical oncologists and laboratory scientists (*1*). The prognosis of these tumors has not changed significantly in the last decade despite advances in surgery (*2*), radiotherapy (*3–5*) and chemotherapy (*6*). Postoperative irradiation may improve the quality of life and extend survival in many cases (*3*). It has been suggested that higher doses of radiation may contribute to increased survival but this should be tempered by the side effects that may arise from high doses of radiation (*4,5*).

Targeting of radiation using monoclonal antibodies is an attractive concept and encouraging responses have been described in some instances (*7*) including a case report of brain glioma (*8*). Intravenous administration of radiolabeled antibodies results in very low uptake by the tumor (*9*). It has been suggested (*8*) and shown in clinical (*10*) and preclinical (*11*) studies that the intra-arterial administration of radiolabeled antibodies may be advantageous in terms of improved tumor targeting. Selection of appropriate targets for monoclonal antibody guided therapy is a challenging area. We have selected epidermal growth factor receptor (*12–14*) as one of the targets. Epidermal growth factor (EGF) (*15*)

Received Feb. 6, 1989; revision accepted May 18, 1989.

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promotes growth of a wide range of cell types, but in order to respond to EGF, a cell must express on its surface a receptor for EGF (*16*). It has been shown that a wide range of tumors (*17*) including some brain gliomas (*13,14*) express high levels of EGF receptors. Furthermore, in some cases, the tumor DNA shows rearrangement and amplification (*14*) compared to the EGF receptor gene normally present in human placental DNA. It is probable that a high concentration of EGF receptors facilitates growth of tumor cells in vivo (*18*) and it has been demonstrated that tumors with high levels of EGF receptors have a worse prognosis (*19*).

Our second target for antibody-guided localization and therapy was placental alkaline phosphatase (PLAP) (*20*). We previously found positive expression of PLAP on tumors (*21*) including some brain gliomas. The function of PLAP is not known at present, but it is possible that its presence on cells offers them a growth advantage. High levels of PLAP in some tumors have been associated with a bad prognosis (*22*).

Our study had three main objectives: (a) to investigate whether radiolabeled antibody can localize successfully in brain gliomas; (b) to test the specificity of localization by incorporating a nonspecific monoclonal antibody in imaging and biopsy studies; and (c) to assess toxicity and therapeutic efficacy of ^{131}I-labeled antibodies in the treatment of recurrent gliomas resistant to conventional therapy.

PATIENTS, MATERIALS, AND METHODS

Patients

Two groups of patients were studied. The first consisted of 27 patients, with known or suspected brain gliomas (Table 1 and 2) aged 14–71 yr (mean = 45 yr) were selected for the radioimmunolocalization study. Patients who later were found to have metastatic carcinoma were excluded from the study.

All patients were scanned by external scintigraphy immediately after the i.v. administration of the radiolabeled antibody, and then at 24 and 48 hr. Eleven patients were also imaged using a nonspecific antibody 2 wk after the study with the specific antibody. The studies with the specific and nonspecific antibody were performed in a similar fashion using the same isotope, the same route of administration and equal doses. The mean amount of injected protein per patient for external body scintigraphy was ~250 μg (200–300 μg).

Ten patients, aged 14–65 yr (mean = 41 yr), with recurrent grade III or IV glioma who previously showed good antibody localization, were treated with iodine-131- (^{131}I) labeled antibody delivered to the tumor area by infusion into the internal carotid artery (N = 5) or by intravenous administration (N = 5) (Table 2).

The second group consisted of seven patients. They underwent tumor resection and radioactivity in tumor tissue specimens was estimated, 3 days after injection of the EGFR1 and the nonspecific antibody labeled with ^{131}I and ^{125}I, respectively.

No patient had received chemotherapy or radiotherapy within 6 wk of the study. Written and informed consent was obtained prior to entry into the study. Prior to injection patients were skin tested for allergy to mouse immunoglobulin and were given 120 mg potassium iodide daily for 7 days for diagnostic, and for 28 days for therapeutic studies, starting one day before antibody administration.

Monoclonal Antibodies

EGFR1. This mouse IgG1 antibody binds to the native folded external domain of the human and rat EGF receptors (*23*). It does not react with the v-erb B protein probably because it recognizes sequences not present in the truncated molecule.

H17E2. This is a mouse IgG1 that was raised against purified plasma membranes of normal term placenta (*24*). It precipitates placental alkaline phosphatase activity at a single band of 67,000 D consistent with the molecular weight of PLAP (*20*). It also reacts with the leucine-inhibitable form of alkaline phosphatase found at low levels in the normal testis and is cross reactive with the placental enzyme. It does not react with other nonplacental forms of alkaline phosphatase (*24*).

11.4.1. This is a mouse IgG1 that was raised against the mouse H-2KK antigen (equivalent to human HLA antigen) and does not cross react with any human tissues (*25*).

Radiolabeling

Iodine-123 (AERE Harwell), ^{125}I (Amersham International IMS 30.1) or ^{131}I (Amersham International IBS30) was added to immunoglobulin (10 mg/ml) and the iodination procedure carried out in iodogen coated tubes (*26*). Iodination proceeded for 5 min at room temperature, and the radiolabeled IgG was separated from free radioiodine by gel filtration on Sephadex G50 using phosphate buffered saline, pH 7.4, as elution buffer (*9*).

Immunoreactivity

This was tested in an enzyme linked immunosorbent assay with solid phase antigen (*27*). Furthermore, comparison of antibody immunoreactivity before and after radiolabeling was tested in a direct radioimmunoassay, including competition with unlabeled antibody as previously described (*9*). Purity of the antibody preparations was assessed by FPLC (Pharmacia, Sweden).

Immunohistology

After counting, fresh frozen tissue sections of the tumors were tested in an indirect immunoperoxidase reaction for antibody reactivity. The concentration of the antibody used in immunohistochemistry was 10 μg/ml.

Kinetics

Blood samples were taken at various intervals and urine was collected for 5 days following the administration of the antibody either for imaging or therapy. Aliquots of the blood and urine were counted in a gamma counter along with standards of the injectate for clearance studies. The content of the whole blood was calculated by estimating the expected blood volume from the patients body surface area (*28*). Renal ^{131}I excretion was calculated and expressed as cumulative excretion. Protein bound iodine in the serum was quantitated by chromatography on a column with Sephadex G50.

Imaging Studies

All the patients were scanned immediately after antibody administration labeled with 10 mCi of ^{123}I and then at 24 and 48 hr. A large field-of-view camera (General Electric 400T gamma camera) was used with a low-energy collimator. In general, 200k counts were used to produce the early scans and 100k counts for later scans. All the patients after antibody treatment, when ^{131}I was decayed enough, were scanned using a high-energy collimator.

Biodistribution Studies

Tumor and normal brain tissues were removed at operation, 3 days after antibody administration. Samples were washed with PBS with heparin, blotted dry, weighed immediately on an analytic balance and counted in a gamma counter, in order to establish the percentage of injected dose per gram of tissue. Specificity index was defined as the percent of the injected dose per gram of specific antibody divided by the percent of the injected dose of the nonspecific antibody in tumor tissue. In addition to the specificity index, the tumor to normal brain ratio was assessed and defined as the percent of the injected dose per gram of administered antibody in the tumor divided by the percent of the injected dose in normal tissue. Biopsies from necrotic areas were excluded from the calculation.

Dosimetry

Macroscopic dosimetry calculations were performed on the evidence from biopsy and scans, as well as from body clearance data. No attempt at microdosimetry was made although it could be assumed that local variations in radioactive concentration would give rise to undefined errors in dose calculation. The basic formula for tissue dose from radiation distributed within that tissue is

$$K \times T\frac{1}{2} \times C = \text{absorbed dose (cGy)},$$

where K is a constant representing the absorbed fraction for a particular radioactive emmission and incorporates a time integration constant. The effective half-life in this tissue is $T\frac{1}{2}$ and C is the measured concentration in the tissue (*29*). The effective half-life is related to the physical and biologic half-lives by the following expression:

$$1/Tef = 1/Tb + 1/Tp.$$

Gamma camera images compared with images of known concentration of isotopes in suitable phantoms in the water bath provided the data to estimate the tumor uptake after both the scan and therapy injections (*30*). By using a similar geometric arrangement between patient and phantom studies direct comparison may be made and few corrections are needed to account for scatter and attenuation.

The bone marrow dose following the treatment was estimated by integrating the ^{131}I radioactivity in the blood over 200 hr after treatment, at which time most of the ^{131}I (>95%) had been excreted or decayed. Bone marrow is extremely vascular and macromolecules would rapidly reach equilibrium with the blood. Active marrow constitutes 2.2% of the body weight, that is 25 to 31% of the blood weight. It was assumed in our calculations that 25% of the integrated blood activity was in the bone marrow.

Therapy

Monoclonal antibodies radiolabeled with ^{131}I were delivered to the tumor area by a 5-min infusion into the internal carotid artery (N = 5) or by an i.v. administration (N = 5). Patients received i.v. antibody if their internal carotid artery could not

TABLE 1
Imaging Study with EGFR1 Monoclonal Antibody

Patient no.	Age/sex (yr)	Histology	Previous treatment	Specific AB	Nonspecific AB
1	14/F	Glioma (brain stem)	RT	(+)	
2	55/M	Glioma (brain stem)	RT	(+)	(+/−)
3	41/M	Glioma (grade III)	Surgery, RT	(+)	(−)
4	37/M	Glioma (grade III)	Surgery, RT	(+)	(−)
5	41/F	Glioma (grade IV)	Surgery, RT	(+)	
6	60/M	Glioma (grade IV)	RT	(+)	(+)
7	50/M	Glioma (grade IV)	Surgery, RT	(+)	
8	71/M	Astrocytoma (grade III)	Surgery, RT	(−)	
9	43/M	Glioma (grade III)	RT, chemotherapy	(+)	(−)
10	42/F	Astrocytoma (grade III)	Surgery, chemotherapy	(−)	
11	69/M	Astrocytoma (grade IV)	Surgery	(+)	(+)
12	66/M	Glioma (grade IV)	Surgery	(−)	

be satisfactorily or safely catheterized or if the blood supply of the tumor was not primarily derived from one internal carotid artery (e.g., the patient with brain stem glioma). We have not made a quantitative comparison between the arterial and i.v. routes of antibody administration in this small group of patients.

Response Evaluation

All patients underwent a pre-study evaluation consisting of history and physical examination as well as full blood count, biochemical profile, x-ray, and computed tomographic (CT) scanning. The response in each patient after antibody treatment was assessed clinically and radiologically. Clinical evaluation consisted of symptoms and signs resulting from increased intracranial pressure, seizure attacks, neurologic deficits with disturbances of motor, speech, sensory, visual, or intellectual function or personality changes. The patients who demonstrated an improvement in their clinical state (improvement in neurologic signs and Karnofski performance score) after antibody treatment were classified as having "clinical response". After antibody therapy there was no concomitant treatment added which could have improved the clinical state.

Computed tomograms were performed 4 wk after the treatment and then at various intervals, to compare the size of the tumor and the persistence or not of cerebral edema. Tumors that had decreased by 25% in two perpendicular diameters were classified as having a "radiographic response". In many cases it was not possible to obtain an accurate measure of tumor volume from a series of CT scans. This was largely because of the presence of edema, necrosis, and the varied shape of the tumor. The use of two perpendicular diameters in our measurements only provides a semiquantitative estimate of tumor volume change. Response categories were: (a) patients who clinically and radiologically were in response; (b) patients who clinically but not radiologically were in response; and (c) patients with no response.

Human Antimouse Immunoglobulin Response

Patients were seen weekly following treatment. Serum samples were obtained from all patients before and after antibody administration (10 days, 2 and 4 mo). The same murine monoclonal antibodies were used as antigen in the enzyme linked immunosorbent assay, as previously described (*31*).

RESULTS

Patients

Details of patients investigated are shown in Tables 1, 2, and 3. Details of patients treated are shown in Table 4. No reactions were recorded after the skin test with mouse immunoglobulin.

Radiolabeling—Immunoreactivity

Monoclonal antibodies were satisfactorily radiolabeled with labeling efficiency of ~90% and specific

TABLE 2
Imaging Study with H17E2 Monoclonal Antibodies

Patient no.	Age/sex (yr)	Histology	Previous treatment	Specific AB	Nonspecific AB
1	32/M	Astrocytoma (grade III)	Surgery, RT	(+)	(−)
2	63/F	Glioma (grade III–IV)	Surgery, RT	(+)	
3	41/M	Glioma (grade IV)	Surgery, RT, and chemotherapy	(+)	
4	26/M	Glioma (grade IV)	Surgery, RT	(+)	
5	65/F	Astrocytoma (grade IV)	Surgery, RT	(+)	
6	60/F	Astrocytoma (grade III)	Surgery	(+)	
7	19/M	Glioma (grade IV)	Surgery, RT, and chemotherapy	(−)	(−)
8	42/M	Astrocytoma (grade II–III)	Surgery, RT, and chemotherapy	(+)	(+)
9	34/M	Glioma (grade III–IV)	Surgery, RT, and chemotherapy	(−)	(−)
10	53/M	Astrocytoma (grade III)	Surgery, RT, and chemotherapy	(+)	
11	35/F	Glioma (grade II)	RT	(−)	
12	38/F	Glioma (grade II)	Surgery, RT	(−)	
13	52/M	Glioma (grade IV)	Surgery, RT	(+)	(+)
14	40/M	Glioma (grade IV)	RT	(−)	
15	36/F	Glioma (grade III)	Surgery, RT	(−)	

activity between 5–8 mCi/mg. No significant loss of immunoreactivity, or aggregate formation was found. In the presence of antigen excess more than 70% and 60% of iodinated EGFR1 and H17E2, respectively, was capable of binding to antigen.

Immunohistology

EGFR1 monoclonal antibody showed positive staining in an indirect immunoperoxidase reaction against the majority of glioma tissue sections. This was less clear with H17E2 antibody because only a small number of glioma tissues have been tested thus far (work in progress). We scored as positive when >30% of malignant cells within each glioma tissue reacted with antibody as visualized by low power microscopy.

Kinetics

Kinetic studies were performed with all antibodies used in this study. Blood clearance was not significantly different between ^{123}I- and ^{131}I-labeled antibodies or any one of the three antibodies used. Iodine clearance was biphasic with a mean half-life of the first component T½a = 20 ± 5.5 hr and of the second component T½b = 34 ± 8 hr. The cumulative urinary excretion of the ^{123}I and ^{131}I over 5 days was ~60% and 70% of the administered dose, respectively. The protein bound radioactivity in the serum using ^{123}I-labeled antibodies was 90% to 95% (mean 93%), and with ^{131}I-labeled antibodies for treatment was 85% to 96% (mean 91%).

Imaging Studies

Successful antibody guided localization of brain gliomas was shown in nine out of 12 patients who received EGFR1 radiolabeled monoclonal antibody and in nine out of 15 patients who received H17E2 radiolabeled monoclonal antibody. The specificity of targeting was studied in 11 patients by comparing imaging after administration of specific and nonspecific antibodies. Observable tumor localization was also achieved in five out of 11 patients who received the nonspecific monoclonal antibody. Figure 1 shows an antibody scan using EGFR1-^{123}I in a patient with

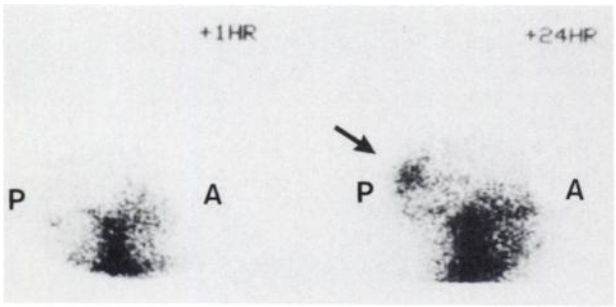

FIGURE 1
Antibody scan of the head with ^{123}I-labeled EGFR1 monoclonal antibody immediately after antibody administration and at 24 hr. The region of glioma is clearly seen (arrow). "A" and "P", anterior and posterior, respectively.

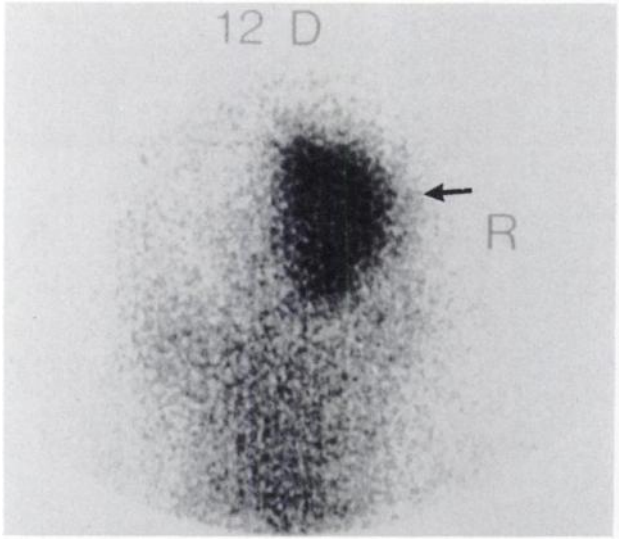

FIGURE 2
Antibody scan of the head 12 days after the treatment with ^{131}I-labeled H17E2 monoclonal antibody. The region of glioma is clearly seen (arrow).

glioma. Figure 2 shows an antibody scan of the head, 12 days after treatment.

Biodistribution Studies

An analysis of the percentage of injected dose per gram of tissue in the tumor specimens is shown in Table 3. The mean percentage of injected dose per gram of tumor and normal brain tissue with the specific antibody was ~0.004 and 0.0015, respectively. As shown in Table 3 more specific antibody accumulated in the tumor than nonspecific but the difference was small. The tumor to normal brain ratio with specific and nonspecific antibodies is shown in Figure 3.

Therapy Results

Ten relapsed patients, who had previously shown good localization of radiolabeled monoclonal antibodies were treated with 40–140 mCi of ^{131}I. The dose of ^{131}I administered was 40 mCi in two patients, 75 mCi in two patients, 100 mCi in four patients, 140 mCi in one patient and one patient was treated twice with 100 mCi each time. Six out of ten patients showed clinical improvement lasting from 6 mo to 3 yr. In these patients there was improvement in neurologic parameters, i.e., one patient showed movement in a hemiplegic arm and another patient partially regained her sense of balance without having further focal motor events. Neurologic improvement in these patients allowed for further reduction in their steroid dosages. In two of these six patients there was also radiologic improvement and one patient, who was treated twice continues in remission 3 yr after therapy. Four out of the six patients who responded initially, relapsed and died 6–9 mo after antibody therapy (Table 4).

TABLE 3
Biodistribution Studies

Patient no.	Age/sex (yr)	Histology (grade)	% ID/g of tumor Specific	% ID/g of tumor Nonspecific	Specificity index (*)
1	28/M	Glioma (II)	0.0008 center 0.0009 edge	0.0033 center 0.0030 edge	0.24 0.30
2	42/F	Glioma (II)	0.0043 center	0.00018 center	23.89
3	40/F	Glioma (III)	0.0044 center 0.0020 edge	0.00326 center 0.0018 edge	1.35 1.11
4	56/M	Glioma (III)	0.0060 center 0.0030 edge	0.0032 center 0.0017 edge	1.87 1.76
5	48/F	Glioma (IV)	0.0050 center 0.0049 edge	0.00334 center 0.00196 edge	1.50 2.50
6	64/M	Glioma (IV)	0.0057 center	0.0030 center	1.90
7	32/M	Glioma (IV)	0.00566 center	0.00465 center	1.22

* Specificity index is defined as tumor ratio of specific versus nonspecific antibody.

Dosimetry

Static gamma camera images obtained at between 5 and 15 days after therapy showed a mean effective half-life in the tumor region of 40 hr. The maximum uptake by a 25-cc tumor was ~1 mCi from a 100-mCi injectate giving an upper limit for tumor dose of 1,250 cGy. The integrated dose is sharply dependent on the shape of the uptake/clearance curve representing the time before the first measurement at 4 days. These doses are only rough calculations because biopsies of treated areas were not obtained. Accurate dosimetry and microdosimetry in particular are very difficult to be performed clinically. Other studies of microdosimetry in experimental models showed a very wide range of tumor doses even within the same tumor mass (*32*).

The mean absorbed dose by bone marrow in the patients who received 100 mCi and 140 mCi was estimated to be ~140 cGy and 260 cGy, respectively.

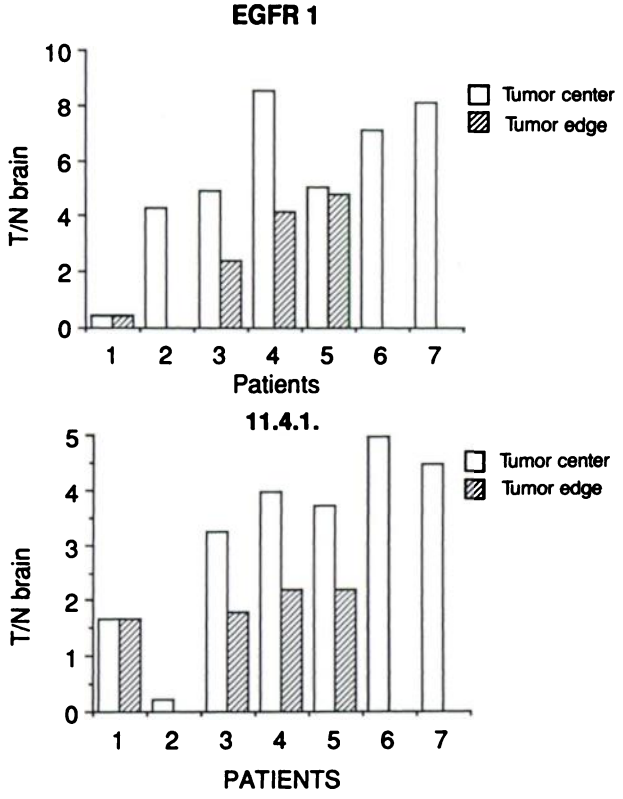

FIGURE 3
Tumor to normal brain ratio of antibody uptake 3 days after i.v. administration of EGFR1 monoclonal antibody (upper) and 11.4.1 monoclonal antibody (lower).

Toxicity

No acute toxicity was encountered in any of the patients. Furthermore, we did not observe any impairment in liver or renal function tests. The patients treated with 100 mCi developed mild neutropenia and thrombocytopenia (leucocytes 2–2.9 × 10^6 cells/l and platelets 50–74 × 10^9 cells/l) 3 to 5 wk after therapy. The patient who received 140 mCi developed moderate (leukocytes 1.0–1.9 × 10^6 cells/l and platelets 25–49 cells/l) but reversible thrombocytopenia (nadir at 30 days) and neutropenia (nadir 40 days) recovering 10–14 days after the nadir. The degree of marrow suppression was related to marrow doses: 140 cGy for mild toxicity; 260 cGy for moderate toxicity. Transient hemiparesis was observed in one patient (H17E2 3) due to the insertion of the intraarterial catheter into the internal carotid artery. This lasted for 5 min and was self limiting.

Human Anti-Mouse Response

One out of ten patients who had therapy developed human anti-mouse immunoglobulin response after treatment with radiolabeled monoclonal antibody. Nine patients did not develop an immune response above that demonstrated by healthy controls. Our ob-

TABLE 4
Therapy

Patient no.	Age/sex (yr)	Histology	Previous therapy	Antibody	Route	Dose	Clinical improvement	Radiologic improvement	Survival rate
EGFR1 #1	14/F	Glioma (brain stem)	RT	EGFR1	i.v.	40 mCi	Walking better Headache relieved	(−)	6 mo
EGFR1 #2	55/M	Glioma (brain stem)	RT	EGFR1	i.v.	40 mCi	No	(−)	42 days
H17E2 #3	41/M	Glioma (grade IV)	Surgery, RT, and chemotherapy	H17E2	i.a.	75 mCi	Headache relieved, no focal motor events	(−)	6 mo
H17E2 #5	65/F	Astrocytoma (grade IV)	Surgery, RT	H17E2	i.v.	75 mCi	Could walk unaided able to grasp objects with left hand	(−)	6 mo
H17E2 #4	26/M	Glioma (grade IV)	Surgery, RT	H17E2	i.a.	100 mCi	Became fully mobile	(+)	9 mo
H17E2 #2	63/F	Glioma (grade III–IV)	Surgery, RT	H17E2	i.a.	100 mCi	No	(−)	3 mo
EGFR1 #4	37/M	Glioma (grade III)	Surgery, RT	EGFR1	i.v.	100 mCi	Headache relieved Speech improved	(−)	6 mo alive
EGFR1 #5	41/F	Glioma (grade IV)	Surgery, RT	EGFR1	i.v.	100 mCi	No	(−)	4 mo
H17E2 #1	32/M	Astrocytoma (grade III)	Surgery, RT	H17E2	i.a.	140 mCi	No	(−)	4 mo
EGFR1 #11	41/M	Glioma (grade III)	Surgery, RT	EGFR1	i.a.	100 mCi (× 2)	Headache relieved, no seizures, walking improved	(+)	3 yr alive and in remission

servations on the phenomenon of human anti-mouse globulin response in patients has been described in detail elsewhere (*31*). In the patients studied by radioimmunoscintigraphy we could not detect human antimouse IgG antibody (other than preexisting response) during 4 mo of follow-up, even in those patients who received two administrations (specific and nonspecific antibody).

DISCUSSION

In a previous study (*8*), we showed that a radiolabeled tumor-associated monoclonal antibody, when given by an internal carotid artery infusion, could result in tumor regression and was of clinical benefit to a patient with recurrent grade IV glioma resistant to conventional therapy. This prompted us to perform this larger study to examine the reproducibility of that report, and to include controls such as the nonspecific antibody and different routes of administration (*33*) in order to assess if sufficient discrimination between antibody uptake in normal tissue and tumor could be achieved. This was primarily a study to determine toxicity and the limits of tolerance for normal organs. At the same time it provided limited information on efficacy of this approach. We carried out biopsies after administration of paired antibodies and we compared the uptake of both specific and nonspecific antibodies. As shown in Table 3 the absolute amount of specific antibody accumulated in the tumor was relatively small. However, in six out of seven cases, specific antibody (EGFR1) localized in higher amounts than nonspecific antibody (11.4.1). There are different factors which could account for the low accessibility of monoclonal antibodies in the brain tumors including the blood-brain barrier, lack of vascularity, tumor necrosis, etc. We have not made any measurements to assess the relation between the disruption of the blood-brain "barrier" and the antibody uptake in these patients. However, it is likely that breakdown of the blood-brain "barrier" in the tumors is, at least, in part responsible for both the nonspecific and the specific antibody uptake. Our findings from both the biopsy as well as imaging data demonstrate an element of nonspecific uptake. In five out of nine patients we showed positive radioimmunolocalization

of tumor with the nonspecific antibody. The relation between specific and nonspecific tumor immunolocalization is a complex phenomenon which may be different for every antibody-tumor system. This problem has not been adequately highlighted in the past by performing dual antibody radioimmunoscintigraphy studies. Therefore, for meaningful antibody-guided imaging studies of brain glioma we recommend the use of specific and nonspecific antibodies so that the issue of specificity can always be resolved.

There is a wide choice of antigens for targeted radiotherapy using monoclonal antibodies. There have been several reports of monoclonal antibodies reacting against human gliomas (*34–38*). We selected two molecules which, in addition to their increased expression on brain gliomas, may play a fundamental role in carcinogenesis and tumor promotion. Previous in vitro studies have shown a relationship between EGF receptor concentration and tumor growth (*18*). Gliomas express epidermal growth factor receptors (*13,14*) and this expression may play a role in carcinogenesis (*39, 40*). Comparison of the complete sequence of the EGF receptor gene with that of a transforming protein (V-erb B) present in avian erythroblastosis virus, showed that the latter was homologous with the transmembrane and cytoplasmic domains of the EGF receptor, but lacked the majority of extracellular sequences (*41*). One of the monoclonal antibodies used in this study (EGFR1) (*23*) binds to the external domain of the human EGF receptor but does not react with the V-erb-B protein, presumably because it does not recognize sequences present in the truncated molecule. Prior immunohistochemical testing of brain gliomas produced positive staining with EGFR1 antibody, indicating that most of these tumors synthesize the complete molecule of EGF receptors. Placental alkaline phosphatase (PLAP) may be another suitable target (*20*). It is normally found on term placenta, but its function is not known at present. Its ectopic expression on rapidly dividing tumors, such as germ cell neoplasms of the testes, may indicate that this enzyme is associated with rapidly progressing tumors. In ovarian cancer, high levels of serum PLAP are associated with a poor prognosis (*22*). The fact that PLAP is expressed at low levels or not at all in normal tissues, allows a further advantage with regard to tumor targeting, in contrast to EGF receptors that are present at high levels in some normal tissues such as the small bowel. However, it is not clear, what proportion of human gliomas express PLAP (work in progress).

The methods calculating radiation doses to tissues after the injection of a radioactively labeled compound are well documented (*29*). By using the Medical Internal Radiation Dosimetry Committee formula a dose can be ascribed to each individual organ and to the whole body provided a knowledge of the physiologic pathways and kinetics has been obtained. The specific information required is the amount of radioactivity, the residence time in any organ, and the size of that organ. In the case of the patients in this study, these three sets of data were obtained from separate groups of patients since those who were selected for treatment were not those who underwent the biopsy procedure. The size of the tumor was assessed where possible by CT scan. The radioactive concentration in the tumor was measured by either biopsy at 72 hr or by gamma camera images following the scanning injection, or between 4–15 days after the treatment when the activity had fallen to a manageable level, and by comparing these to images of calibrated water phantoms. The residence time in the tumor was also calculated from successive gamma camera images over a period of days. Each measurement has its own peculiar limitation and associated error. The most pessimistic dose estimates use uptake data from the biopsy and residence time from the diagnostic scans and assume a uniform infusion of radioactivity throughout the diseased tissue. Using this straightforward method the resulting tumor doses are in the region of only 100 or 200 cGys. This value could scarcely have had any beneficial effect. There is, however, some encouraging evidence to suggest that an effective adjuvant dose had been delivered to the tumor. First, the treatments were delivered, where possible, by way of the carotid artery, offering an immediate advantage of perhaps a factor of two (*10,11,33*). Second, the percentage of injected radioactivity in the tumor region at 4–15 days after treatment appeared to be more than that at the same time after the diagnostic scans indicating a value of 0.02% of the injected dose per gram of tumor. Third, the time of residence in the tumor following the treatment dose again would indicate a higher initial uptake although early local concentrations could not be measured because of the high background activity. The calculated doses are not sufficient on their own to sterilize brain gliomas. On the other hand, such doses could make an important contribution if used in conjunction with radical external beam radiotherapy in patients with poor prognosis grade III or IV gliomas.

Bone marrow toxicity was noted at doses of 100 mCi of ^{131}I-labeled antibody which was correlated with the estimated bone marrow dose. The hemopoietic bone marrow is extremely vascular and is irradiated by its circulating blood. We have estimated the radiation dose to marrow to be 140 cGy in patients receiving 100 mCi and 260 cGy in patients receiving 140 mCi of ^{131}I. These findings are in agreement with our previous studies where ^{131}I-labeled monoclonal antibodies were administered for the treatment of advanced ovarian cancer (*7,42*).

We were encouraged that six out of the ten patients treated showed clinical responses even after only one therapeutic administration. One patient, who was

treated on two occasions, continues in remission 3 yr post-therapy. We are not certain that the theoretic advantages of intraarterial infusion of drugs in terms of increased drug delivery to the tumor and decreased systemic toxicity (*33*), are applicable to macromolecules such as antibodies. The so-called first-pass advantage should be minimal in the case of macromolecules such as immunoglobulins, if the vascular and tumor areas were considered as a two-compartment model. Previous clinical (*10*) and recent preclinical studies (*11*) have shown an advantage for the intracarotid administration of antibodies. A possible explanation for this may be that intraarterial administered antibody sequestrates into a tumor "third space" which may act as a reservoir allowing for slow release and access to tumor antigen (*11*). We recognize that there is a case for the study of mathematic modeling in relation to arterial and venous methods of administration. We did not measure first-pass uptake at the time of treatment because of the difficulties of using a gamma camera in the angiography/catheterization room.

On the basis of this data further studies should be conducted to determine the exact role of the monoclonal antibodies for the treatment of brain gliomas. These results may be improved if one could use smaller molecules such as antibody fragments or genetically engineered fragments (*43*), radionuclides such as yttrium-90 (*44*) that may have more favorable radiobiologic characteristics than ^{131}I as well as drugs such as mannitol which utilize osmotic blood-brain barrier disruption and could increase antibody uptake (*45*).

In conclusion, we have shown that monoclonal antibodies against EGF receptors and placental alkaline phosphatase can be used to target brain gliomas but the difference between specific and nonspecific antibodies was small. In fact 50% of our patients with positive specific antibody scan showed successful immunolocalization with nonspecific antibody. In six out of ten patients with recurrent glioma who underwent antibody-guided therapy we demonstrated a clinical benefit, with no significant toxicity. Monoclonal antibody guided irradiation may be clinically useful and should be investigated further in the treatment of brain gliomas resistant to conventional forms of treatment.

ACKNOWLEDGMENTS

This work was supported by the Imperial Cancer Research Fund. The authors thank Sir Walter F. Bodmer, M. Waterfield, and P. Goodfellow for the provision of the antibodies and their constructive comments.

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