Brain tumor capillaries have a blood-tumor barrier that limits delivery of antitumor agents to tumor tissue and the immediately adjacent brain tissue. We sought, therefore, to develop a safe and effective method to increase drug delivery to brain tumors. Ideally, this approach should be selective and increase permeability in the primary tumor mass and distant microscopic tumor foci, but it should not increase drug delivery to normal brain tissue. Because many antitumor compounds are also toxic to normal brain, increasing delivery to normal tissue could result in increased toxicity. The approach should also be reversible and not result in brain edema and increased mass effect.

In a recent series of experiments, the use of low-dose intracarotid infusion of bradykinin as a method to deliver antitumor agents selectively to brain tumors was examined. These studies in experimental models of brain tumors in rats demonstrated that intracarotid bradykinin infusion selectively increased the permeability for compounds ranging in molecular weight (M) from 100 to 70,000 D. The permeability of high-molecular-weight dextran (M, 70,000) was increased 12-fold in tumors after bradykinin infusion, whereas the permeability of cytokines (M, 20,000) and sucrose (M, 342.3) were increased six- and twofold, respectively. The duration of the effect of intracarotid bradykinin was approximately 20 minutes and permeability in normal tissue was not increased.

The synthetic bradykinin analog receptor-mediated permeabilizer (RMP)-7 is a nonapeptide containing unnatural amino acids at positions 3, 5, and 8 and a reduced peptide bond between positions 8 and 9, and it is composed of amino acids with the L-configuration. The biological half-life of RMP-7 is longer than that of bradykinin. Bradykinin increases tumor permeability via B2 receptors, and RMP-7 exhibits more selectivity for B2 relative to B1 receptors compared to bradykinin. Increased brain tumor permeability after intracarotid RMP-7 infusion occurs at
1/100th the dose needed for bradykinin. The bradykinin analog RMP-7 has been shown to increase delivery of the antitumor drugs methotrexate and carboplatin to experimental brain tumors. The survival of rats with gliomas is also significantly increased with intracarotid infusion of RMP-7 and carboplatin compared to carboplatin without RMP-7. Based on these findings, Phase I and II clinical trials were initiated using intravenous and intracarotid carboplatin and RMP-7 infusions in patients with malignant brain tumors.

Positron emission tomography (PET) scanning using $^{68}$Ga ethylenediamine tetraacetic acid (EDTA) is a quantitative technique to determine the passive and noncarrier-mediated transport constant ($K_t$) of compounds from plasma to brain tissue in humans. We report the results of $^{68}$Ga EDTA PET studies in patients with recurrent malignant gliomas with and without intracarotid infusion of RMP-7.

Clinical Material and Methods

Patient Population

Nine adult patients with recurrent malignant gliomas were entered into this study. The protocol was approved by the University of California at Los Angeles human subject protection committee. Entry criteria included anaplastic astrocytoma or glioblastoma multiforme proven by biopsy. All patients had received prior regional radiation therapy with at least 5500 rad, which had failed. In some patients prior treatment with adjuvant chemotherapy had also failed. Recurrent tumor was determined by an increase in tumor size on imaging studies. Patients were admitted on the day prior to RMP-7 infusions. No food or fluid intake was allowed after midnight, at which time intravenous hydration was started with saline at 100 ml/hour.

Intracarotid Infusions

While the patient was on the PET scanner table, procedures were performed using a portable digital subtraction x-ray imaging unit (Angioplus; OEC-Diasonics, Salt Lake City, UT). A foley or condom catheter had been placed prior to the patient being transported to the PET scanner suite and 30 mg ondansetron was administered intravenously as an antiemetic. Conscious sedation was induced in the patient using either intravenous propofol (250–900 mg) or intravenous Versed (0.5–4 mg) and fentanyl (50–900 mg). Vital signs including blood gas levels, blood pressure, heart rate, respiratory rate, and body temperature were monitored throughout the procedure; electroencephalographic changes were also monitored. In each patient, one groin was shaved, prepared with povidone–iodine, and covered with a sterile drape. After induction of local anesthesia in the patients, their femoral artery was punctured and catheterized with a No. 6 French sheath. This sheath also allowed arterial blood samples to be obtained. To induce anticoagulation, 5000 U of heparin was administered as an intravenous bolus followed by 1000 U intravenously per hour. A No. 5 French guiding catheter (Medtronic–MIS, Sunnyvale, CA) was placed in the proximal internal carotid artery (ICA) and an angiogram was obtained. Using standard methods of microcatheterization, a microcatheter (Jet Stream 18; Medtronic–MIS) was placed in the C2 segment of the ICA beyond the ophthalmic artery. Several injection tests were performed in a pulsatile fashion using contrast material to evaluate any inhomogeneity in drug delivery and to determine the stability of the microcatheter. Manual pulsatile delivery was always used to infuse the drugs to avoid streamlining and to obtain a homogeneous distribution of the drugs in the ICA and the cerebral circulation. Although the infusion rate was 1 ml/minute, the injection was delivered by vigorous boluses of approximately 0.2 ml, thus eliminating the streaming associated with slow injections. When the position of the microcatheter was adequate, the mobile fluoroscopy equipment was removed and the patient was moved into the PET scanner. The RMP-7 was obtained from the pharmacy and diluted in 10 ml saline to one of the following four concentrations: 0.1, 0.3, 1, or 3 mg/ml; a total RMP-7 dose of 10, 30, 100, or 300 ng/kg, respectively, in the four different groups. The RMP-7 was injected into the ICA at a volume rate of 1 ml/minute during a 15-minute period (10 minutes of the RMP-7 delivery alternating with 5 minutes of carboplatin infusion; the total volume of RMP-7 solution delivered was therefore 10 ml). Five minutes after the initiation of the RMP-7 infusion, carboplatin was infused into the ICA at a concentration of 4 mg/ml and at a rate of 3 ml/minute. The total dose of carboplatin infused was 100 mg. When RMP-7 and carboplatin were infused simultaneously, each compound was infused alternately via the microcatheter for approximately 30 seconds. When the RMP-7 infusion was terminated, carboplatin alone was infused for 3 minutes. After the infusion, the guiding catheter and the microcatheter were removed. The femoral artery sheath was left in place until the end of the PET study. At the end of the PET study, the heparin was reversed with protamine sulfate, the arterial sheath was removed, and manual compression was exerted over the puncture site for 15 minutes.

Positron Emission Tomography

Two $^{68}$Ga EDTA PET scans were obtained in each patient using a PET scanner (Siemens Corp., Erlangen, Germany) on 2 different days (Day 0 for baseline study; Day 1 following RMP-7 administration). The patient’s head was positioned within the axial field of view of the scanner (14.4 cm covered by 47 imaging planes with 0.3125-cm separation between planes). The “septa-in” option was selected for the data collection mode of the scanner. A transmission scan (using gallium-68 rotating rod source) was obtained to provide attenuation information for later attenuation correction of the emission scans.

For baseline studies, a line was placed to obtain arterial blood samples. A single bolus of $^{68}$Ga EDTA (5–10 mCi) was injected intravenously. For RMP-7 studies, $^{68}$Ga EDTA was injected intravenously 5 minutes after initiating the intracarotid RMP-7 infusion. A sequence of PET scans (5 × 1, 8 × 3, 7 × 5 minutes; total of 64 minutes, 20 frames) was performed simultaneously with injection of gallium-68 to obtain dynamic images. Approximately 20 to 25 arterial blood samples were taken in each PET scan.
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study. Blood samples were taken every 30 seconds for the first 5 minutes and then at 6, 8, 10, 15, 20, 30, 45, and 60 minutes after injection of tracer. The radioactivity in arterial plasma samples was counted in a well counter to give the input function.

To correct for patient movement during the dynamic PET scans, frame–frame image registration was used. Usually, Frame 16 was chosen as the reference frame. After frame–frame alignment, all image frames were summed to give a sum image. The two sum images (with and without RMP-7) were coregistered to MR images.

The PET-to-MR image registration method developed by Lin, et al., was used in this study. Gadolinium-enhanced T2-weighted MR images were segmented into four main tissue types (muscle, tumor, cerebrospinal fluid, and brain tissue). Simulated PET images were generated (with relative concentration levels in the ratio of 1:1:0:0 for muscle/tumor/cerebrospinal fluid/normal brain tissue) to imitate the PET-measured 68Ga EDTA brain images. The simulated PET images were then used as the reference to register against the measured PET images.

The region of interest (ROI) analysis method was used to calculate tracer uptake into brain tissue. Tumor and normal tissue ROIs were defined on the contrast-enhanced T2-weighted MR image. Defined ROIs were copied to registered PET dynamic images of Day 0 and Day 1 studies for calculating tissue–time activity curves. The T2-weighted contrast enhancing area was used as the “tumor” ROI. Normal tissue ROIs included regions of comparable size in the ipsilateral hemisphere and regions of comparable size in the mirror location in the contralateral hemisphere with no abnormality on T1- or T2-weighted images.

The first-order linear compartment model (plasma and brain tissue) was used to describe tracer transport across the blood-brain barrier (BBB). The rate of uptake of 68Ga EDTA into brain after intravenous administration is given as follows:

\[
\frac{dX}{dt} = K_e \cdot b(t) - k_e \cdot X(t)
\]

\[
\frac{dC_{i,n}(t)}{dt} = X(t) + CBV \cdot b(t).
\]

Where \(K_e\) is the unidirectional transfer constant from plasma to tissue (milliliter/minute/gram), \(k_e\) is the rate constant of efflux (liter/minute), \(X(t)\) is the extravascular tracer concentration at time \(t\) (microCurie/gram tissue), \(b(t)\) is the tracer activity in plasma, and \(C_{i, n}(t)\) is the total tracer concentration (microCurie/gram tissue) in brain tissue. The cerebral blood volume (CBV) is the plasma volume in tissue (milliliter/gram). The \(C_{i, n}(t)\) calculated from the above equation was used to fit the PET-measured tissue–time activity curves. Nonlinear regression (using a Levenberg–Marquardt algorithm) was used for the curve fitting to estimate the parameters of \(K_e\) and CBV simultaneously. To reduce the variability of the estimates, \(k_e\) was fixed to a small value (0.0002 L/minute) to assume negligible efflux of the tracer from tissue during the study time (60 minutes). The software package BLD was used to perform the model fitting and parameter estimation.

Protocol for MR Imaging

The MR images were obtained with a 1.5-tesla scanner (Signa; General Electric Medical Systems, Milwau-kee, WI) to provide the anatomical information. The T1-weighted fast spin–echo images (TE 90 msec, TR 2000 msec, 3-mm slice) were acquired to define abnormal tissue. The T1-weighted spin-echo images (TE 20 msec, TR 500 msec, 3-mm slice) were acquired before and 2 hours after intravenous administration of 0.2 mmol/kg MR contrast agent (ProHance; Bracco Diagnostics, Inc., Princeton, NJ) to delineate areas of BBB disruption. Baseline MR images were obtained within 7 days of the Day 0 PET scans. A second T1-weighted MR image was obtained 2 hours after the RMP-7 PET study (Day 1 PET). For both MR studies, contrast material was injected intravenously 2 hours prior to MR imaging. For the RMP-7 study, contrast material was injected 5 minutes after the start of the intracarotid RMP-7 infusion. The volumes of the T2-weighted lesion and contrast-enhanced lesions on MR images obtained with and without RMP-7 infusion were determined using a graphic workstation to perform three-dimensional graphic reconstruction of the lesion based on segmentation of the abnormal area on each image. Student’s t-test was used to determine statistical significance.

Tumor Response

The objective of this study was to determine whether intracarotid infusion of RMP-7 could increase transport of 68Ga EDTA into human gliomas. However, patients in this study with no evidence of toxicity or tumor progression could continue treatment with intracarotid RMP-7 and 100 mg of carboplatin monthly for up to 6 months. Response was determined at 8-week intervals and the following criteria were used: 1) complete response, disappearance of all demonstrable tumor on MR imaging; 2) partial response, decrease in size of tumor by 50% or more by volume; 3) minor response, decrease in tumor size by between 25% and 50% by volume; 4) stable disease, no definite change in tumor size or decrease in size of less than 25% tumor volume; and 5) progressive disease, increase in tumor size as measured by MR image.

Results

There were seven men and two women included in this study, with a median age of 39 years (range 35–68 years). Four of the tumors studied were anaplastic astrocytomas and five were glioblastomas multiforme. All patients had received at least 5500 rad of regional radiation therapy previously. The mean dexamethasone dose was 8 mg/day. The dexamethasone dose was stable in all patients for 2 weeks prior to PET studies, but ranged from 0 to 32 mg/day between patients.

Physiological parameters including blood pressure and arterial blood gas levels did not change significantly during RMP-7 infusions. The heart rate was slightly increased during RMP-7 infusions, from a mean of 62 ± 8 bpm to 76 ± 8 bpm. Burst suppression seen on encephalography occurred in Case 7 due to sedation with propofol. This patient also had the lowest percentage increase in \(K_e\), at 2%. A typical PET–MR registration result for Case 3 is shown in Fig. 1.
The $K_i$ for the “defined” tumor region was determined by PET in the nine patients and is summarized in Table 1. The $K_i$ of the defined tumor increased an average of $46 \pm 42\%$ with RMP-7. The $K_i$ in ipsilateral and contralateral normal brain increased an average of $13 \pm 31\%$ and $17 \pm 37\%$, respectively. The change in $K_i$ in ipsilateral or contralateral normal brain was not significant. The $K_i$ in ipsilateral and contralateral normal brain before RMP-7 infusion was $0.000235 \pm 0.000097$ and $0.000243 \pm 0.000179$ ml/minute/g, respectively.

The total abnormal volume in milliliters on $T_2$-weighted images was increased $14\%$ and the total volume of gadolinium enhancement was increased $20\%$ after RMP-7 infusion. However, the increases in $T_2$-weighted volume and $T_1$-weighted enhancement were not statistically significant.

No correlation was seen between dexamethasone dose and the increase in $K_i$ after RMP-7 infusion in this study using linear regression analysis.

Three of three patients treated with 10, 30, or 100 ng/kg of RMP-7 and carboplatin had progressive disease. Three of six patients treated with 300 ng/kg RMP-7 and carboplatin had progressive disease. Three of six patients treated with 300 ng/kg RMP-7 had stable disease at 2 months and one of the six treated with 300 ng/kg RMP-7 had progressive disease.

### Discussion

This study shows that intracarotid infusion of RMP-7 will selectively increase transport of $^{68}$Ga EDTA into brain tumors without increasing its transport into normal brain tissue in patients with recurrent malignant gliomas. This indicates that intracarotid infusion of RMP-7 will also increase the transport of carboplatin selectively into brain tumor tissue, because the molecular size, charge, weight, and water solubility of $^{68}$Ga EDTA (M, 460) and carboplatin (M, 371.3) are similar. Some authors have suggested that a twofold increase in chemotherapy delivery to tumors results in a 10-fold increase in tumor cell kill. Intracarotid infusion of RMP-7 has been shown to increase transport of carboplatin to experimental brain tumors 2.7-fold. Intracarotid infusion of RMP-7 together with carboplatin has also been shown to increase survival significantly in rats with gliomas. Although the number of patients is too small to draw conclusions, three of six patients treated with the 300-ng/kg dose of RMP-7 and carboplatin had at least a 50% reduction in tumor size (Fig. 2).

The volume of enhancement on MR imaging was not significantly increased by RMP-7 infusions. Changes in MR imaging enhancement, unlike those in PET studies, are not sensitive markers to determine changes in drug delivery. Of importance, MR imaging demonstrated that...
Intracarotid infusion of RMP-7

RMP-7 infusions did not increase mass effect in patients with brain tumors.

Intracarotid infusion of bradykinin or RMP-7 in animal models is reported to increase transport of high- and low-molecular-weight compounds selectively into tumor tissue but not into normal brain tissue.²⁻⁵,¹²⁻¹₆,²₄⁻²⁶ In most animal studies, quantitative autoradiography was used to determine a unidirectional Kᵢ in tumor and normal brain tissue. The calculations of Kᵢ with quantitative autoradiography in animal studies are similar to calculations of the Kᵢ in human PET studies. In animal studies using quantitative autoradiography, intracarotid infusion of RMP-7 selectively increased transport into tumors of [¹⁴C]α-aminoisobutyric acid (Mᵩ 103), [¹⁴C]carboplatin (Mᵩ 371.3), [¹²⁵I]tumor necrosis factor (Mᵩ 20,000), and [¹⁴C]dextran (Mᵩ 70,000) by 2.8-, 2.7-, 6.6-, and 10.3-fold, respectively. The larger the molecule (up to an Mᵩ of 70,000 D), the larger the percentage increase in transport of antitumor agents into lesions via intracarotid RMP-7 infusion. This indicates that intracarotid infusion of RMP-7 is even more ideal for delivery of higher-molecular-weight antitumor compounds such as cytokines.¹⁸

In animal models of glioma, intracarotid infusion of RMP-7 resulted in a 2.7-fold increase in transport of carboplatin into tumors. In this study in humans, RMP-7 resulted in an average increase of 46% in transport of ⁶⁸Ga EDTA into tumors. At least two possibilities could explain the lower transport in this study. The first is that, in animal models, tissue radioactivity in smaller ROIs within the tumor was measured and areas of necrosis were avoided. Autoradiograms obtained in studies of rats were correlated directly to areas of tumor defined on stained histological sections. In the PET study in humans, the entire tumor area defined on MR images was measured as the ROI. Thus, in the PET study, a large increase in permeability at the growing tumor margin would be averaged with regions of tumor necrosis (which lack capillaries and should show no increase in permeability), reducing the overall measured increase in Kᵢ. The second possibility is that the use of dexamethasone in these patients reduced the increase in permeability after RMP-7 infusion. Recent animal studies have demonstrated that pretreatment with dexamethasone for 3 days prior to RMP-7 infusion reduced the increase in Kᵢ for transport of carboplatin from 2.7- to 1.9-fold.¹⁷ The averaging of large ROIs and the use of dexamethasone most likely contributed to the smaller increase in Kᵢ in this study compared to prior animal studies. Increased transport of antitumor agents into lesions by means of RMP-7 might be optimized by limiting dexamethasone use in patients. A direct correlation between dexamethasone dose and increase in Kᵢ could not be shown in this study.

The increase in permeability of tumor capillaries after bradykinin or RMP-7 infusion can be prevented by pretreating animals with β₂ receptor antagonists.³ The increase in permeability with bradykinin can also be blocked by inhibiting nitric oxide synthase (NOS) and reversed by infusion of L-arginine.¹⁹ This would indicate that the mechanism of increased permeability in tumors with RMP-7 infusion is mediated through β₂ receptors and is dependent on NOS. The NOS levels are increased in tumor compared to nontumor tissue. The higher NOS levels in tumor may account for the selective effect of increased permeability by RMP-7 in tumors in contrast to nontumor tissue.

Twenty minutes after termination of RMP-7 infusion, the increase in permeability is reversed.³ Interestingly, if the infusion of bradykinin or RMP-7 is continued beyond

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Tumor Type</th>
<th>RMP-7 Dose (ng/kg)</th>
<th>Pre-RMP-7 Kᵢ (µg/min/g)*</th>
<th>Post-RMP-7 Kᵢ (µg/min/g)*</th>
<th>Percentage Increase†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GBM</td>
<td>10</td>
<td>2.2 ± 0.13</td>
<td>3.6 ± 0.23</td>
<td>64</td>
</tr>
<tr>
<td>2</td>
<td>AA</td>
<td>30</td>
<td>1.9 ± 0.20</td>
<td>2.3 ± 0.19</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>GBM</td>
<td>100</td>
<td>0.8 ± 0.14</td>
<td>1.9 ± 0.13</td>
<td>138</td>
</tr>
<tr>
<td>4</td>
<td>AA</td>
<td>300</td>
<td>1.5 ± 0.20</td>
<td>1.8 ± 0.09</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>GBM</td>
<td>300</td>
<td>0.7 ± 0.10</td>
<td>0.9 ± 0.07</td>
<td>29</td>
</tr>
<tr>
<td>6</td>
<td>GBM</td>
<td>300</td>
<td>1.3 ± 0.13</td>
<td>2.2 ± 0.13</td>
<td>69</td>
</tr>
<tr>
<td>7</td>
<td>GBM</td>
<td>300</td>
<td>6.8 ± 0.26</td>
<td>6.9 ± 0.24</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>AA</td>
<td>300</td>
<td>2.1 ± 0.19</td>
<td>2.4 ± 0.14</td>
<td>14</td>
</tr>
<tr>
<td>9</td>
<td>AA</td>
<td>300</td>
<td>0.9 ± 0.13</td>
<td>1.5 ± 0.12</td>
<td>58</td>
</tr>
</tbody>
</table>

* Standard error of the estimate of Kᵢ, as estimated by model fitting.
Abbreviations: AA = anaplastic astrocytoma; GBM = glioblastoma multiforme.
† Mean ± standard deviation = 46 ± 42%, p < 0.05.
20 minutes, the increased permeability in tumor capillaries begins to return to baseline anyway. This phenomenon suggests exhaustion of a second messenger. Therefore, we believe that the optimum increase in tumor permeability with RMP-7 is achieved with a 15-minute intracarotid infusion.

Although delivery of drugs into the carotid artery is more complex than intravenous delivery, preclinical results suggest that intracarotid infusion of RMP-7, compared to intravenous infusion, is a superior method to enhance drug delivery into tumors. In animal studies, 0.1 μg/kg/minute of intracarotid RMP-7 infusion for 15 minutes significantly increased transport of [14C]carboplatin to tumors. In contrast, 0.1 μg/kg/minute of intravenous RMP-7 infusion did not change transport in tumors.

Other approaches have been used to deliver drugs across the BBB. Intracarotid infusions of mannitol have been used to open the BBB in animals and humans. Hyperosmotic infusions will increase drug delivery across the barrier. However, this therapy has certain limitations: the increase in delivery to normal brain may increase 40- to 50-fold more than the increase in delivery to tumor tissue, and some authors report that the increase in K, within actual tumor may only be as high as 25%. Thus, the dose of neurotoxic drugs injected may need to be reduced to avoid injury to normal brain when using osmotic BBB disruption. Another approach to drug delivery in the brain is the coupling of drugs that are normally not transported across the BBB to drug delivery transport vectors in the brain, that is, modified proteins or monoclonal antibodies that undergo receptor-mediated transcytosis via the BBB. However, these receptor systems may be lost in tumor capillaries, again resulting in higher transport to normal brain tissue.

In contrast to these strategies for drug delivery to the brain, biochemical opening of the BBB uses the observation that brain tumor capillaries are biochemically different from normal brain capillaries. As a result, intracarotid infusion of bradykinin or RMP-7 can selectively increase drug delivery within brain tumors without increasing capillary permeability in normal brain. Whether this approach will improve survival in patients with malignant brain tumors will be determined in ongoing clinical investigations.

Acknowledgments

We thank Drs. William Graney for his assistance with this study and William Pardridge for his comments regarding this paper. We also thank Felicia Boyd and Rashidah Shakir for their editorial assistance.

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Manuscript received June 20, 1996.
Accepted in final form November 25, 1996.
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