Intravenous RMP-7 Selectively Increases Uptake of Carboplatin into Rat Brain Tumors

P. J. Elliott, N. J. Hayward, R. L. Dean, D. G. Blunt, and R. T. Bartus

Alkermes, Inc., Cambridge, Massachusetts 02139 [P. J. E., N. J. H., R. L. D., D. G. B., R. T. B.], and Department of Pharmacology and Experimental Therapeutics, Tufts University, Boston, Massachusetts 02111 [R. T. B.]

ABSTRACT

Rats implanted with RG-2 gliomas were administered i.v. RMP-7 and [14C]carboplatin. Changes in the permeability of the blood-brain barrier to carboplatin were determined using quantitative autoradiography. i.v. infusions of RMP-7 induced an increase in the permeability of the vascular barrier within the tumor to carboplatin. Additionally, permeability of brain tissue proximal to, but clearly outside the tumor mass, was also increased. Progressively less uptake of [14C]carboplatin was observed as distance from the tumor border increased. The increases in permeability induced by RMP-7 occurred in a dose-related fashion. No increase in carboplatin level was observed in several nonbrain tissues, including sciatic nerve, retina, heart, lung, liver, kidney, and spleen. Finally, the permeabilizing effects of RMP-7 were shown to occur independent of histamineergic or hypotensive mechanisms. These data provide additional insight into the permeabilizing effects and mechanism of RMP-7 and offer additional support for the therapeutic utility of this novel compound as an adjunctive treatment for human gliomas.

INTRODUCTION

Malignant gliomas represent a serious medical problem (1, 2). Despite the development of new and potent chemotherapeutic agents for use in other tumor types, most gliomas remain resistant to treatment, with the majority of patients dying within 1 year of initial diagnosis (3). Although several factors contribute to the poor prognosis, one of the most significant is that potentially effective chemotherapeutic agents are restricted from brain areas associated with the tumor due to the impermeable nature of the BBB (4–7). Although the vasculature supplying the tumor is characteristically more leaky than that of normal brain (8), it still presents a significant barrier to drug penetration.

A number of innovative methods have been developed to increase the concentration of chemotherapeutic drugs within brain tumors. The most direct approach involves infusion of maximum tolerated doses of chemotherapeutic drugs directly into the internal carotid artery, increasing local capillary drug concentrations in and around the brain tumor (9–11). However, because the efficacy of this technique remains dependent on diffusion across vascular barriers, the clinical effects of intracarotid drug treatment have been modest thus far (12, 13).

A more aggressive and nonspecific approach involves the physical disruption of the BBB by intracarotid infusion of high concentrations of hyperosmotic agents such as mannitol and arabinose (14–17). However, this approach has limited clinical utility due to the small increases in concentrations of chemotherapeutic drugs achieved in the tumor (18–21) relative to healthy brain tissue, as well as morbidity associated with this procedure (22–24).

Recent efforts have refined the intracarotid approach to open the vascular brain barriers by administering agonists which activate specific receptors on endothelial cells which regulate permeability (25). Perhaps the most promising preclinical results to date have been achieved with bradykinin agonists. Bradykinin is a member of the kinin family which is known to modulate vascular tissue interfaces throughout the body and can increase permeability of vascular beds, including the BBB (26, 27). RMP-7 is a peptidergic bradykinin B2 agonist that was developed to permit higher concentrations of therapeutic and diagnostic compounds to enter the brain. RMP-7 has a longer plasma half-life and greater selectivity for the B2 subclass of bradykinin receptors than does bradykinin (28–30). Recent electron microscopic studies have demonstrated that RMP-7 increases brain permeability by opening the tight junctions of brain endothelial cells (31). These tight junctions are believed to comprise the primary diffusion barrier to the brain for blood-borne hydrophilic compounds (32–34).

In a rat glioma model, intracarotid infusions of RMP-7 increase the permeability of the brain vascular barriers to a variety of hydrophilic compounds, ranging in molecular weight from the relatively small amino isobutyric acid (103), to moderately sized sucrose (343), carboplatin (373), and methotrexate (454), through much larger cytokines (18,000–20,000) and 70,000 dextran (27, 35–38). Interestingly, the increased permeability induced by bradykinin and RMP-7 is substantially greater in the tumor area, as compared to healthy brain tissue distal to the tumor, for all hydrophilic compounds tested. Thus, a bradykinin-mediated approach to chemotherapeutic drug delivery to the brain offers the advantage of achieving higher concentrations of drugs selectively within the tumor mass and proximal tissue, while having minimal effects on healthy brain tissue.

Recent observations with RMP-7, coupled with insight from the literature on the BBB and chemotherapeutic agents, permits one to define several essential characteristics that specific single-agent chemotherapeutics must have for them to benefit from combination with RMP-7 to treat gliomas. Collectively, these observations indicate that the appropriate chemotherapeutic agent should: (a) have adequate cytotoxicity against tumor cells and cells lines, in vitro, and in animal models of glioma; (b) have demonstrated significant clinical efficacy and an acceptable tolerability in the treatment of nonbrain tumors; (c) have generated some degree of clinical efficacy in the treatment of human gliomas and a rationale for dose intensification; (d) have limited brain tumor penetration due to its hydrophilicity; and (e) possess a pharmacokinetic profile suitable for use with RMP-7.

Although many anticancer drugs have shown activity against a wide variety of tumor cell types and in animal models and provided acceptable treatment for nonbrain tumors (39), few have demonstrated significant clinical efficacy against gliomas. Single-agent chemotherapeutics which produce acceptable effects in treating gliomas include
the nitrosoureas (carmustine or BCNU and lomustine or CCNU; Refs. 40 and 41) and the platinum (carboplatin and cisplatin; Refs. 12, 24, and 42–46). Because the nitrosoureas are lipophilic, they easily permeate the brain by diffusion through capillary walls (47). Since their brain uptake is not substantially affected by the BBB, brain tumor concentrations would not likely be increased by increasing the permeability of the BBB with RMP-7. This point was recently confirmed with BCNU in an animal model of glioma (38). Alternatively, the hydrophilic platinums diffuse very poorly through capillary walls, and most investigators believe they would provide even greater activity against gliomas if their permeation through the BBB could be increased (48).

Thus, carboplatin and cisplatin appear to satisfy the essential characteristics for use in combination with RMP-7 in the treatment of gliomas. Of the two agents, carboplatin has important advantages over cisplatin. First, carboplatin has an improved therapeutic index versus cisplatin, exhibiting less toxicity at therapeutic concentrations (49–51), with the apparent absence of the neurotoxicity which accompanies cisplatin (52, 53). Second, the pharmacokinetic profiles of the two drugs favor carboplatin. It is important that the platinums remain in unbound form both to cross the BBB (even with RMP-7) and to platinate DNA. Thus, the percentage of administered drug which remains unbound and its total time in the unbound form are important pharmacokinetic considerations. Recent studies have shown that the plasma clearance of cisplatin is several times greater than that of carboplatin, whereas the time for cisplatin to bind to plasma proteins is much faster (54). These characteristics combine to produce a 20-fold higher plasma concentration of unbound carboplatin compared to cisplatin. Measurements of the ability of systemically injected cisplatin and carboplatin to penetrate the brains of nonhuman primates demonstrated that the area under the curve in the cerebral spinal fluid was 160-fold higher for carboplatin over cisplatin; the cerebral spinal fluid:plasma area under the curve ratio was 8-fold higher as well (54). Thus, given comparable systemic doses of cisplatin and carboplatin, considerably higher concentrations of the therapeutically active species of carboplatin remain for longer durations, permitting increased opportunity for permeation of the BBB (54).

This article describes a series of experiments which are intended to build on recent research involving the development of RMP-7 as part of a combination therapy for treating human gliomas. Because the potential therapeutic advantage of bradykinin-mediated permeabilization could be exploited most easily in the clinical setting through conventional i.v. dosing protocols, a series of studies was conducted to investigate such an approach using a well-characterized rat glioma model. This article provides the first demonstration that i.v. RMP-7 selectively opens the BBB.

### MATERIALS AND METHODS

**Subjects and Housing Conditions.** Female Wistar rats (170–220 g; Charles River, Stoneridge, MA) were acclimatized to the vivarium for 5 to 10 days prior to tumor cell implantation. One week later, both femoral artery and jugular vein cannulations were undertaken. Throughout this period, animals had free access to food (Purina Rodent Chow; Purina, St. Louis, MO) and water and were housed in a 12-h dark:12-h light cycle. Room temperature and humidity were maintained at 22 ± 1°C and 50 ± 5%, respectively.

**Tumor Cell Implantation.** Rat glioma (RG2) cells were maintained as a monolayer culture in F10 medium (Life Technologies, Inc., Grand Island, NY) with iron-supplemented 10% calf serum until they were 70% confluent (3–5 days). On the day of implantation, they were resuspended in F10 medium with 1.2% methylcellulose (Sigma, St. Louis, MO) to provide a solution of 1 × 10^6 cells/5 μl.

Using a 4 ml/kg dose (i.m.) of a solution containing ketamine (25 mg/ml), xylazine (0.65 mg/ml), and acepromazine (0.33 mg/ml) to anesthetize the rats, RG2 cells (5 μl) were injected stereotaxically into the rat striatum over a 1-min period using a 22-gauge needle attached to a 10-μl Hamilton syringe. The coordinates used were: Bregma (+2.0 mm), midline (+3.0 mm), skull (−6.5 mm) with the incisor bar at +5.0 mm (55). After the injection of the cells, the needle remained in place for an additional 10-min period to allow cell dispersion from the needle tip. Subsequently, the rats were constantly monitored and allowed to recover from anesthesia (i.e., return of righting reflex) before being replaced in the vivarium.

**Blood Vessel Cannulation.** One week after tumor implantation and under urethane anesthesia (1.8 g/kg i.p.), cannulas (PE50) were placed in the jugular vein for drug administration and in both femoral arteries for the measurement of physiological parameters and the collection of blood used to calculate the uptake constant *Kc*.

**Drug Administration and Physiological Monitoring.** RMP-7 (Alkermes, Inc., Cambridge, MA) was dissolved in 0.9% saline and infused i.v. using an infusion pump (Razel Scientific Instruments Corp., Stanford, CT) at a rate of 0.05 ml/min over the entire 15-min administration period. 14C-carboplatin (specific activity, 144 μCi/mg; Amersham, Arlington Heights, IL) was given as a bolus (100 μCi/ml/kg) over a 3-s period into the jugular vein followed by an equal volume of heparinized saline. Body temperature was maintained at normothermic (37.0 ± 1.0°C) throughout the surgery and drug administration period by a regulated feedback-heating pad (Harvard Apparatus, South Natick, MA). Rats outside this range were not included in the uptake studies.

Arterial blood gases and pH were also measured using a blood gas analyzer (Ciba Corning, Norwood, MA) at the beginning and at the end of each infusion period. Animals with blood gas and pH values outside the normal ranges (pCO₂, 30–48 mm Hg; pO₂, 60–110 mm Hg; pH 7.4 ± 0.1) were removed from the study after the calculation of *Kc* values. Blood pressure was monitored continuously (MacLab Data Acquisition System) before and throughout the drug infusion; animals with basal mean arterial pressure of <65 mm Hg were not used in the study.

To help determine the uptake of carboplatin into brain tissue, arterial (femoral) blood was withdrawn into PE90 tubing at a constant rate (0.083 ml/min) throughout the 10-min period following administration of the radio-labeled bolus using a peristaltic pump. Once collected, the blood was removed from the tubing and prepared for scintillation counting (56).

At the end of the drug administration protocol, rats were decapitated, and their brains were rapidly removed. In some instances, other tissues (heart, kidney, liver, lung, retina, and sciatic nerve) were also removed. Tissues were then frozen on dry ice.

**Permeability of 14C-Carboplatin.** Brains were coronally sectioned (20 μm) on a cryostat (−15°C) throughout the length of the tumor and mounted on glass slides. Using standard autoradiographic techniques, the slides were exposed for 1 week to radiosensitive film (Kodak Biomax MR-1) with 14C calibration standards and then developed. The slides were then stained with H&E to verify tumor placement.

Nonbrain tissues were sectioned (20 μm) on a cryostat (−20°C), and slices were processed as above. Sections were exposed to film for 9 days, except kidney which was exposed to the radiosensitive film for 2 days.

Quantitative analysis of the regional radioactivity was performed using an image analysis system (MCID: Imaging Research Corp., St. Catherines, Ontario, Canada). Individual coronal brain sections, stained with H&E, were digitized, and the same coronal section on the autoradiogram was then exactly overlaid and digitized. Using the stained section to locate the tumor boundary, the total radioactivity within it and at various distances from it (i.e., other regions of interest such as BST, ipsilateral, and contralateral tissue) were measured. Areas of necrosis within the tumor (as defined by H&E staining) were occasionally noted (1–2% of tumors) and were omitted from the tumor uptake calculations. Ipsilateral brain tissue was defined as cortical tissue at least 2.0 mm away from the tumor border but within the ipsilateral hemisphere. Contralateral tissue was defined as cortical tissue on the opposite side of the brain. Radioactivity within nonbrain tissues was quantitated in a manner similar to that of brain tissue to obtain nCi/g values, except that the rats in these studies were perfused for 5.0 min at the end of the RMP-7 infusion intracarically with 50 ml of 0.9% saline.
To convert the film images into units of radioactivity, the absorbancies of images produced by the ¹⁴C standards were determined, and a standard curve relating absorbance to tissue radioactivity was generated for each film. Based on this curve, the amount of radioactivity (nCi/g) was computed for each region. For each brain, the mean radioactivity from three adjacent coronal sections containing striatal tumor was calculated. Sections used in this analysis were taken from a region representing the largest cross-sectional area of the tumor.

Calculations. The unidirectional transfer constant, $K_r$, was calculated as described earlier (57, 58) and expressed in $\mu$g/min. As such, the $K_r$ value represents the amount of $[¹⁴C]$carboplatin taken up into tissue. The values from the autoradiographic film were quantitated as nCi/g tissue; radioactivity within tumor and brain tissue at time of sacrifice were defined as $C_T$ and $C_B$, respectively. Blood volumes of tumor and brain were derived from previously published work (35) and calculated to be 9.4 and 3.7 $\mu$l/g, respectively. $C_B$ was defined as the whole-blood radioactivity at the time of sacrifice (nCi/ml) or as the total blood radioactivity (nCi/ml/min) collected throughout the radiolabel infusion period ($T$), respectively.

$$K_r = \frac{C_T (T) or C_B (T)}{\int_0^T C_B (T) \cdot dt} (\text{tissue blood volume} \times C_B (T))$$

Mechanistic Studies. In an attempt to determine the mechanisms through which RMP-7 may induce its permeabilizing effects, additional studies were undertaken using the histamine antagonists mepyramine (Spectrum, Gardena, CA) and cimetidine (Sigma) and with the adenosine agonist NECA (Research Biochemicals International, Natick, MA). All drugs were given as a 20 min i.v. infusion starting 5 min prior to the RMP-7 infusion.

Statistics. The effect of RMP-7 on uptake of $[¹⁴C]$carboplatin into tumor or brain tissue was compared with vehicle-treated rats using an ANOVA and a post hoc Dunnett’s $t$ test.

RESULTS

Permeability of $[¹⁴C]$Carboplatin into Brain Tissue

Using quantitative autoradiography in conjunction with histological techniques, it is possible to demonstrate differences in the uptake of carboplatin into histologically defined brain tumor and surrounding areas (Fig. 1). In an initial study, i.v. infusion of RMP-7 (4.5 $\mu$g/kg) produced a significant increase in $[¹⁴C]$carboplatin uptake in the RG2 tumor mass and the brain tissue surrounding the tumor, with smaller but significant effects observed in ipsilateral and contralateral brain tissue (Fig. 2). Additional studies demonstrated that this permeabiliz-
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Fig. 2. Initial study demonstrating the effect of i.v. RMP-7 (4.5 μg/kg) on carboplatin uptake in various brain regions from BST (1.0 mm from tumor boundary), ipsilateral cortical tissue (Ipsi), and contralateral cortical tissue (Contra). Data are presented as mean uptake (K₁) values. *, P < 0.005; **, P < 0.0001. □, vehicle-treated groups (n = 34); and ◇, RMP-7-treated groups (n = 36). Bars, SE.

The permeabilizing effect of RMP-7 was dose related, producing 30–80% increases in uptake in brain tumor tissue (P < 0.002; Fig. 3). The amount of [¹⁴C]carboplatin uptake observed in tissue distal from the tumor boundary was less pronounced, and diminished with increasing distance from the tumor, in both vehicle and RMP-7 treated groups (Fig. 3). The permeabilizing effect of RMP-7 was higher than that of the vehicle in all of the nontumor tissues examined: BST tissue, ipsilateral cortex, and contralateral striatum (P < 0.01; Fig. 3). Dose-related hypotension was also noted in these studies with very mild (<15%) and transient (<3 min) changes seen up to 4.5 μg/kg, and the most profound effect was observed at 18 μg/kg (Fig. 5A). Similar decreases in blood pressure have also been reported previously with bradykinin (59).

In a separate study, the amount of radioactivity present within concentric bands (constant width, 0.25 mm) from the tumor edge was examined in vehicle- and RMP-7-treated groups (Fig. 4). Again, RMP-7 elicited an increase in the uptake of [¹⁴C]carboplatin, with greater effects observed in the tumor mass and progressively less effects seen more distal to the tumor boundary (P < 0.01). In this particular study, 4.5 and 18 μg/kg RMP-7 increased the tumor uptake of [¹⁴C]carboplatin 34 and 77% above control levels, respectively.

Permeability of Nonbrain Tissues

Effects of i.v. infusion of RMP-7 (4.5 μg/kg) on uptake of [¹⁴C]carboplatin in peripheral tissue are presented in Table 1. To enable a direct comparison across tissue types, the data are presented as radioactivity within tissue (nCi/g). RMP-7 was found to increase uptake of

Fig. 3. Follow-up study demonstrating that i.v. administration of RMP-7 produces a significant dose-related increase in the uptake of [¹⁴C]carboplatin in rat brain over a range of doses (1.5–18 μg/kg). Data are presented as mean uptake (K₁). Statistical significance was determined using ANOVA. □, vehicle, n = 10; ◇, 1.5 μg/kg RMP-7, n = 12; ■, 4.5 μg/kg RMP-7, n = 14; □, 9.0 μg/kg RMP-7, n = 15; ◇, 18 μg/kg RMP-7, n = 13. Bars, SE.
Table I Effect of iv. RMP-7 (4.5 pg/kg) on \[^{14}\text{C}\]carboplatin uptake into central and peripheral tissues

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Treatment group</th>
<th>Tumor</th>
<th>BST</th>
<th>Contralateral striatum</th>
<th>Retina</th>
<th>Sciatic nerve</th>
<th>Heart</th>
<th>Liver</th>
<th>Lung</th>
<th>Kidney</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle (n = 8)</td>
<td>97 ± 13</td>
<td>50 ± 5</td>
<td>6 ± 1</td>
<td>7 ± 2</td>
<td>134 ± 6</td>
<td>12 ± 3</td>
<td>127 ± 6</td>
<td>39 ± 12</td>
<td>2234 ± 486</td>
<td>86 ± 8</td>
</tr>
<tr>
<td></td>
<td>RMP-7 (n = 8)</td>
<td>139 ± 10(^a)</td>
<td>75 ± 6(^a)</td>
<td>8 ± 1</td>
<td>5 ± 1</td>
<td>134 ± 5</td>
<td>11 ± 2</td>
<td>128 ± 3</td>
<td>30 ± 11</td>
<td>2603 ± 409</td>
<td>86 ± 4</td>
</tr>
</tbody>
</table>

\(^a\) P < 0.05.

Effect of iv. RMP-7 (4.5 pg/kg) on [\(^{14}\text{C}\)carboplatin uptake into central and peripheral tissues

iv. administration of RMP-7 (4.5 pg/kg) produces a significant increase in the uptake of [\(^{14}\text{C}\)carboplatin in rat brain tumor compared to vehicle-treated rats. This effect diminishes as distance increases from the tumor edge as determined using concentric bands of constant 0.25-mm width. CONTRA-ST, contralateral striatal tissue. Data are presented as mean uptake (nCi/g) values ± SE.

Distance from tumor edge (mm)

![Graph showing the effect of RMP-7 on carboplatin uptake into tumor at various distances from the tumor edge](image)

Fig. 4. Effect of RMP-7 on carboplatin uptake into tumor and at various distances from the tumor edge. iv. administration of RMP-7 (4.5 and 18 μg/kg) produces a significant increase in the uptake of [\(^{14}\text{C}\)carboplatin in rat brain tumor compared to vehicle-treated rats. This effect diminishes as distance increases from the tumor edge as determined using concentric bands of constant 0.25-mm width. CONTRA-ST, contralateral striatal tissue. Data are presented as mean uptake (K). □, vehicle, n = 10; □, 4.5 μg/kg RMP-7, n = 14; □, 18 μg/kg RMP-7, n = 13. Bars, SE.

\[^{14}\text{C}\]carboplatin into the brain tumor and surrounding tissue (P < 0.05) but not into contralateral brain. Moreover, they also show that RMP-7 did not affect [\(^{14}\text{C}\)carboplatin uptake into the lungs, kidney, heart, spleen, liver, retina, or sciatic nerve.

Mechanistic Studies

To further define the mechanism(s) through which RMP-7 induces its enhanced permeabilizing effects, two additional studies were conducted.

Role of Histamine. In one study, the potential role of histamine was investigated. Under conditions where high doses (10 mg/kg) of selective H1 and H2 histamine antagonists (mepyramine and cimetidine, respectively) were coinfused, the permeabilizing effects of 4.5 μg/kg RMP-7 were unaffected (P = 0.72), whereas RMP-7 significantly increased uptake of carboplatin into tumor (P = 0.04). K (values (mean ± SE) for [\(^{14}\text{C}\)carboplatin uptake into tumor (n = 9–11) were: vehicle, 23.61 ± 2.21; histamine antagonists alone, 25.23 ± 2.28; RMP-7 alone, 30.45 ± 2.39; and

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RMP-7 in combination with both histamine antagonists, 28.62 ± 2.63.

Role of Hypotension. Because higher uptake effects were observed with the highest doses of RMP-7 (i.e., 18 µg/kg; Fig. 3) and this dose produced substantial hypotension, only RMP-7 increased the uptake of [14C]carboplatin into rat brain. Although both agents produced substantial hypotension, only RMP-7 increased the uptake of [14C]carboplatin into rat brain. 

DISCUSSION

i.v. infusion of RMP-7 selectively increased the uptake of carboplatin into the brain tumors and surrounding brain tissue of rats, corroborating other reports involving intracarotid administration of RMP-7 (35–38). Moreover, these effects exhibited a clear dose relationship, with higher doses of RMP-7 producing greater effects on the permeability of the vascular barriers. Additionally, RMP-7 significantly increased uptake of carboplatin into brain areas proximal to, but clearly outside the tumor mass. As such, a distinct topographic gradient was observed, with decreasing uptake of carboplatin occurring at progressively further distances from the tumor. This observation has potential therapeutic relevance, since the vascular barriers within brain regions outside the tumor mass are relatively impermeable to carboplatin and other hydrophilic drugs (5, 60). Tumor recurrence after surgery and chemotherapy occurs most often at or near the site of the original tumor (61–65), presumably because small numbers of glioma cells infiltrate brain tissue outside the main tumor mass and survive, in part, due to the impermeable nature of the vascular barriers. Since RMP-7 selectively elevates drug levels in brain tissue closely associated with the tumor, when used as an adjunctive treatment with chemotherapeutic agents, RMP-7 should provide greatest cytotoxic effects in and near the tumor, with minimal effects occurring in tissue distal from the tumor. Combined with data from recent clinical trials indicating that carboplatin alone can produce modest benefits for glioma patients (12, 64, 65), the present data suggest that the combination of RMP-7 and carboplatin may provide even greater therapeutic effects. This point is further supported by a recent rat glioma study in which intracarotid RMP-7 combined with carboplatin was shown to significantly improve survival of glioma-bearing rats over carboplatin alone (37). Thus, RMP-7 represents a novel approach to the more traditional polypharmacy strategy for treatment of fatal brain cancer conditions.

Higher doses of RMP-7 induce a transient hypotensive effect similar to that observed with i.v. administration of its parent peptide bradykinin (59). To address the possibility that the increased permeability observed with RMP-7 may simply be related to a reduction in blood pressure, we administered similarly hypotensive doses of the adenosine agonist NECA and determined the changes in permeability. Results demonstrate that although both NECA and a high dose of RMP-7 were able to induce a profound and long-lasting hypotension, only RMP-7 increased carboplatin uptake into the tumor and surrounding tissue. This result, in conjunction with those from other recent studies, indicates that hypotension is neither necessary nor sufficient for RMP-7 to increase permeability of the BBB (37, 67).

Although the mechanisms responsible for the permeabilizing effects of RMP-7 are still being elucidated, the important role of B2 receptor activation has been established. Using both in vitro and in vivo systems, the selective B2 receptor antagonist HOE 140, but not the B1 receptor antagonist (des-Arg^10)HOE 140, completely blocked the effects of RMP-7 (30, 66). The role played by other systems has not yet been thoroughly investigated. In particular, histamine and bradykinin are known to often function in tandem, with common factors inducing the activity of both (68). Moreover, like bradykinin, histamine has been reported to permeabilize the BBB (69–71). Finally, in certain circumstances, bradykinin increases the release of histamine (72, 73). Thus, the possibility that some histamine-related mechanism might mediate the effects of RMP-7 was investigated. Results showed that coinfusion of histamine receptor antagonists (mepyramine and cimetidine) did not affect the permeabilizing activity of RMP-7. These data suggest that RMP-7 induces its effects on blood-brain permeability independent of histamine mechanisms.

The ability of RMP-7 to increase carboplatin delivery to various peripheral (i.e., nonbrain) tissues was also investigated. These data demonstrated that with the dosing paradigm used here, increases in
uptake of carboplatin were only seen in the brain, whereas the retina, the sciatic nerve, and a number of other peripheral organs were unaffected. Thus, an extra margin of safety seems to be afforded by i.v. RMP-7, with the greatest uptake occurring in tumor-associated tissue, relatively smaller increases in healthy brain distal to tumor, and no apparent effects on peripheral tissue. It might therefore be anticipated that treatment complications with the toxicity of conventional chemotherapeutic agents will not be exacerbated (and might even be reduced) when RMP-7 is used as an adjunctive therapy for neuro-oncological indications. The anticipated safety of RMP-7 is further supported by recent pathological examination of brains from RMP-7-treated swine, which showed no evidence of vasogenic brain edema or neuropathology. This observation is consistent with other RMP-7 permeability studies using electron microscopy (31), and light microscopy (30, 37). Thus far, no study has reported any sign of neuropathology or other adverse effects on central nervous system tissue following administration of RMP-7, a fact most likely related to the transient nature of the receptor-mediated permeability induced by RMP-7 (30).

The results demonstrate that i.v. infusion of RMP-7 significantly and selectively enhances the uptake of carboplatin into brain tumor and into adjacent brain tissue. Moreover, a greater margin of safety likely exists compared to other methods intended to increase delivery of drugs or diagnostic agents to the brain. The particular characteristics of RMP-7 and carboplatin make it an attractive drug combination for treating gliomas. The present data further support the use of RMP-7 as an adjunct to chemotherapy and greatly expand this opportunity by providing the first demonstration of significant increases in carboplatin uptake into tumor following i.v. infusion of RMP-7. Phase II clinical trials are currently underway in recurrent glioma patients to evaluate the therapeutic utility of i.v. RMP-7 in combination with carboplatin.

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