Intravenous Cereport (RMP-7) Modifies Topographic Uptake Profile of Carboplatin within Rat Glioma and Brain surrounding Tumor, Elevates Platinum Levels, and Enhances Survival

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ABSTRACT
Several experiments studied the effects of i.v. infusions of the bradykinin agonist, Cereport (RMP-7), on permeability of the blood-brain tumor barrier in rat gliomas. First, the ability of Cereport to increase uptake of two poorly blood-brain barrier-penetrating drugs (lypophilic paclitaxel and hydrophilic carboplatin) was directly compared to provide new information regarding the scope of delivery effects achieved with Cereport. Next, the increased uptake of platinum into tumor and brain surrounding tumor was shown to closely parallel that of radio-labeled carboplatin, confirming that delivery of a biologically active moiety is increased with Cereport. This study also demonstrated that the elevated tumor levels of platinum persisted for at least 2 h. The enhanced carboplatin uptake was then examined using a novel, high spatial resolution analysis of autoradiography. This revealed that the effects of Cereport were not uniform throughout the tumor, because it especially modified those areas normally impermeable to carboplatin. Finally, a range of i.v. Cereport doses (3.0 and 9.0 μg/kg) was tested in combination with carboplatin to determine whether increased survival might be achieved and to define the relationship between Cereport dose, plasma levels, uptake of carboplatin, and enhanced survival. Survival was enhanced only by the high dose of Cereport; the high dose also produced robust increases in carboplatin uptake and plasma concentrations of Cereport estimated to achieve the $K_i$, whereas the low dose did not. These data offer fundamental information regarding the effects of Cereport on delivery of chemotherapeutic agents to brain tumors and provide new insight into receptor-mediated permeability of the blood-brain tumor barrier.

Gliomas are a tragic form of brain tumor that inevitably leads to death of the patient. Surgical debulking of the tumor and radiotherapy extend patient survival by only 6 to 12 months (Radhakrishnan et al., 1994), whereas chemotherapy has generally been even less successful. Despite the development of new chemotherapeutic agents, it is generally recognized that the blood-brain tumor barrier (BBTB) limits exposure of the tumor to many potentially efficacious chemotherapeutic drugs (Black, 1995).

Several different approaches have been explored for increasing permeability of the BBTB so that higher concentrations of chemotherapeutics might be achieved in the tumor. The majority of these approaches, including infusion of hypotonic mannitol (Neuwelt and Rapoport, 1984; Kroll and Neuwelt 1998), as well as infusion of leukotrienes (Black and Hoff, 1985; Black et al., 1990, 1994), histamine (Inamura et al., 1994a), and bradykinin (Inamura and Black, 1994; Black, 1995), require intra-arterial administration directly into the tumor vasculature. More recently, Cereport (RMP-7) was developed as a pharmaceutical analog of bradykinin, possessing greater selectivity for the constitutively expressed cerebrovascular bradykinin B2 receptor and a measurably longer half-life than bradykinin (Doctrow et al., 1994; Straub et al., 1994; Bartus et al., 1996b). Cereport increases permeability of the blood-brain barrier (BBB) by transiently disengaging the tight junctions comprising the barrier (Sanovich et al., 1995). Cereport is unique among BBTB-modulating agents in that it is effective after both intracarotid and i.v. administration, although as expected, dose-response comparisons demonstrate higher doses are required with i.v. administration to achieve comparable effects (Bartus et al., 1996b; Elliott et al., 1996b; Emerich et al., 1999). The opportunity to enhance delivery of chemotherapeutic agents to brain tumors via i.v. dosing protocols offers the clear advantages of greater simplicity, safety, and cost-effectiveness.

We performed a series of experiments using an RG2 rat glioma model to address several issues fundamental to the use of receptor-mediated modulation of the BBTB to increase delivery of chemotherapeutics for treating brain tumors and to the continued development of Cereport as the first drug of
this class. In the initial experiment, the ability of i.v. Cereport to enhance delivery of the chemotherapeutic agents, carboplatin and paclitaxel, was compared directly using a previously defined optimal dose (Bartus et al., 1996b; Elliott et al., 1996b). Paclitaxel was selected as an interesting, relatively new chemotherapeutic agent with promise for treating brain tumors. Despite its relative lipophilicity, paclitaxel does not normally achieve high concentrations in brain tumors (Heimans et al., 1994; Schinkel et al., 1996), and no information yet exists on the ability of Cereport to enhance its delivery across the BBTB. Carboplatin is a logical choice to be combined with Cereport for treating gliomas because it: 1) is hydrophilic (and therefore would benefit from the increased aqueous pathway created by Cereport across the BBTB), 2) has demonstrated cytotoxic effects against glioma both in vitro and in vivo, 3) has demonstrated dose-escalation effects (supporting the concept that increasing the concentration of carboplatin in the tumor will provide a greater chemotherapeutic effect), and 4) has an acceptable pharmacokinetic profile (i.e., a plasma half-life > 1 h with relatively little protein binding during that time). A separate experiment was performed to establish that Cereport actually increases levels of the cytotoxic platinum moiety in the tumor and brain surrounding tumor (BST) and to determine whether the increases achieved with Cereport persist beyond the time of BBTB modulation. Next, the enhanced uptake achieved with carboplatin after Cereport administration was studied in detail using a novel, high spatial resolution analysis of quantitative autoradiography within the tumor area and BST. This analysis provided new and unexpected information on the effects of Cereport within micro-subregions of the tumor and BST and revealed that the most marked effects of Cereport occur in areas of the glioma and BST that are normally relatively impermeable. Finally, the ability of i.v. Cereport to enhance the survival benefit of carboplatin was evaluated in this treatment-resistant rat glioma model. This experiment demonstrated significant effects when a Cereport dose was used that produced consistent and robust increases in carboplatin uptake and is estimated to increase plasma concentrations to the level of the $K_i$ of Cereport but not when a lower dose was used (which produces only modest increases in uptake and plasma levels that are below the $K_i$ of Cereport). Collectively, the data presented offer several new and important findings regarding the use of Cereport to increase delivery of chemotherapeutic agents to treat brain tumors and offer additional insight into the emerging field of receptor-mediated modulation of BBB permeability for treating brain tumors.

**Materials and Methods**

**Subjects.** Male Fischer rats (total $n = 183, 170$–$220$ g; Taconic Farms, Germantown, NY) were used in these studies. The rats were housed in pairs in polypropylene cages with free access to food and water. All procedures were reviewed and approved by Alkermes’ Animal Care and Use Committee and were conducted in a manner that met or exceeded National Institutes of Health standards.

**Tumor Cell Implantation.** Rat glioma (RG2) cells were maintained and implanted as described previously (Bartus et al., 1996a; Elliott et al., 1996b). Briefly, rats were anesthetized with a solution containing ketamine ($33.33$ mg/ml), xylazine ($1.0$ mg/ml), and acepromazine ($1.66$ mg/ml) and placed in a stereotaxic instrument. RG2 cells ($5 \times 10^5$ cells/$5 \mu$l) were injected unilaterally into the striatum using a stereotaxic-mounted $10$-µl Hamilton syringe with a $22$-gauge needle at the following coordinates: A-P ($+2.0$ mm), L ($+3.0$ mm), and V ($-6.5$ mm) (Pellegrino et al., 1986).

**Drug Administration and Physiological Monitoring during Uptake Studies.** Studies to quantify the uptake of radiolabeled carboplatin or paclitaxel into glioma were conducted 8 days after tumor implantation. Cannulas were placed in the jugular vein and into both femoral arteries under urethane anesthesia (1.8 g/kg; i.p.). The jugular cannula provided the means to infuse the drugs, whereas the femoral cannula provided a means for measuring physiological parameters and collecting the blood used to calculate the uptake constant, $K_i$.

For all uptake studies, either [14C]carboplatin (mol. wt. = $371$, specific activity = $144 \mu$Ci/mg, Amersham, Arlington Heights, IL) or [3H]paclitaxel (mol. wt. = $854$, specific activity = $6.5$ Ci/mmol; Moravek Biochemicals, Brea, CA) were given as a 15-min infusion (100 $\mu$Ci/kg) into the jugular vein. Ten minutes after the initiation of this infusion, Cereport (9.0 $\mu$g/kg; Alkermes, Inc., Cambridge, MA) or the saline vehicle was continuously infused i.v. for 10 min (at a rate of $0.05$ ml/min) into the same jugular vein by using a Y adapter. This dose was selected on the basis of several prior studies demonstrating that it fell well within the active dose range for i.v. Cereport (see Bartus, 1996b; Elliott et al., 1996b; Emerich et al., 1999). Throughout the uptake experiments, body temperature was maintained normothermic ($37.0 \pm 1.0^\circ$C), and arterial blood gases, pH, and blood pressure were monitored; animals with physiological values outside the normal ranges (10–15% of all animals) were not used.

**Scintillation Studies.** To compare the ability of Cereport to enhance uptake of carboplatin and paclitaxel into tumor and BST, animals received infusions of [14C]carboplatin or [3H]paclitaxel as described above. At the end of drug administration, rats were decapitated, brains were removed, and the tumor was dissected free. Equal amounts of tissue were taken from the ipsilateral and contralateral cortices and the contralateral striatum. Tissue samples were weighed and incubated overnight at $40^\circ$C in $1.0$ ml of Solune (Packard Instrument Co., Inc., Meriden, CT). The following day, $10$ ml of Hionic Fluor (Packard Instrument Co., Inc.) was added, and radioactivity (nanocuries per gram) was computed using scintillation counts. For determination of carboplatin and paclitaxel uptake into tumor tissue, arterial blood was withdrawn into PE90 tubing at a constant rate ($0.004$ ml/min) throughout the period after administration of the radiolabel by using a peristaltic pump. Once collected, the blood was removed from the tubing and prepared for scintillation counting. Four groups of animals were used: 1) carboplatin and saline, $n = 8$; 2) carboplatin and Cereport, $n = 8$; 3) paclitaxel and saline, $n = 18$; and 4) paclitaxel and Cereport, $n = 14$.

**Histology and Autoradiography.** For autoradiographic determinations of carboplatin uptake into tumor tissue, animals received carboplatin infusions combined with either saline ($n = 7$) or Cereport ($n = 9$) as discussed above. At the end of each drug administration, the rats were decapitated, and their brains were rapidly removed and stored at $-20^\circ$C until they were sectioned. The brains were cut at $20$-µm intervals on a cryostat ($-16^\circ$C) throughout the length of the tumor, and the sections were thaw-mounted onto glass microscope slides. Using standard autoradiographic techniques, the slides were exposed to radioisensitve film (Kodak Biomax MR-1) with $^{14}$C calibration standards (0.002 to $3.58 \mu$Ci/g; American Radiolabeled Chemicals, St. Louis, MO) for 1 week and then developed. The slides were then removed and stained with hematoxylin and eosin (H&E) to verify tumor placement.

$K_i$ Calculations. The unidirectional transfer constant, $K_i$, was calculated as described earlier (Ohno et al., 1978; Zilfan et al., 1988; Inamura and Black, 1994; Inamura et al., 1994a). The $K_i$ value represents the rate of [14C]carboplatin or [3H]paclitaxel taken up into tissue. The values from the autoradiographic film were quantified as nanocuries per gram of tissue; radioactivity within tumor and brain tissue were defined as Ct and Chr, respectively. Blood volumes
of both tumor and brain were derived from previously published work and were calculated to be 9.4 and 3.7 μl/g, respectively (Inamura et al., 1994b). Cbl was defined as the whole blood radioactivity (nanocuries per milliliter) at the time of sacrifice. Cbt was defined as the total blood radioactivity (nanocuries per milliliter per minute), collected throughout the infusion period by continuously sampling blood (T) via a cannula implanted into the femoral artery. The formula for calculating the $K_t$ is as follows:

$$K_t = \frac{Ct \text{ or } Cbr - (Tissue \text{ blood volume } \times Cbl)}{\int_0^T Cbt \cdot dt}$$

Quantitative Determination of Carboplatin Uptake in Tumor, BST, and Normal Brain. Quantitative analysis of the regional radioactivity in the brain sections was performed using an image analysis system (ImagePro Plus; Media Cybernetics, Silver Spring, MD). Individual coronal brain sections stained with H&E were digitized to define the exact tumor location and boundary. This coronal section was then overlaid on the identical autoradiographic film image, and the image was digitized. Using the H&E-stained section to define the tumor boundary, the total radioactivity within it and in a 1-mm ribbon of the BST immediately surrounding but clearly outside the tumor were measured. Ipsilateral brain tissue was defined as cortical tissue at least 2 mm from the tumor border but within the ipsilateral hemisphere. Contralateral tissue was defined as striatal or cortical tissue on the opposite hemisphere of the brain. To convert the film images into units of radioactivity, the optical densities of images produced by the $^{14}$C standards were determined, and a standard curve relating optical density to tissue radioactivity was generated for each film. Based on this curve, the amount of radioactivity (nanocuries per gram) within the various brain regions was computed. For each animal, the mean total radioactivity was calculated from three separate coronal sections containing the largest cross-sectional area of the tumor.

High Spatial Resolution Analysis of Uptake in Tumor and BST. To provide a more detailed analysis of the effects of Cereport within the tumor and BST, a method was developed to quantify uptake of radiolabeled carboplatin with extremely sensitive spatial resolution (4.68-μm² area). This was accomplished by separately evaluating each individual pixel comprising the digitized autoradiographic images. The level of radiolabeled carboplatin from each individual pixel within the tumor and BST was computed and placed within bins representing varying levels of radioactivity (e.g., 0–10, 11–20, 21–30 nCi/g, etc.). This permitted a high spatial resolution profile to be constructed, reflecting both the extent of permeability in the tumor and BST as well as its spatial variability. By comparing the shape of these profiles under vehicle and Cereport conditions, it should be possible to gain greater insight into the uptake effects achieved with Cereport, and in particular, where within the tumor and BST Cereport may be exerting its greatest effects.

Platinum Determinations and Measurement of Residence Time in Tumor. To confirm that the increases in carboplatin levels achieved by Cereport reflect increases in platinum in the tumor and to determine whether the increases remain elevated over time, a separate study was performed using unlabeled (i.e., cold) carboplatin. Two groups were given i.v. carboplatin (10 mg/kg) only, and two received i.v. carboplatin plus Cereport (9.0 μg/kg) as described earlier for the uptake studies. One carboplatin group (n = 12) and one carboplatin plus Cereport group (n = 12) were sacrificed immediately after the drug infusion (corresponding to the timing of the uptake studies), whereas the two other groups were sacrificed 2 h later (n = 12/group).

All rats were decapitated, their brains were rapidly removed, and the tumors were carefully dissected. Tissue samples were weighed and incubated overnight at 40°C in 1 ml of Soluene. The following day, the samples were frozen on dry ice and processed for quantitative determinations of platinum levels using atomic absorption spectrophotometry. Briefly, all samples were digested in 10 ml of nitric acid for 24 h before analysis. Using graphite furnace atomic adsorption spectrophotometry equipped with Zeeman background correction, the amount of platinum was determined by comparing the signal of the sample against known platinum calibration standards at a wavelength of 265.9 nm.

Survival Studies. To determine whether the enhanced uptake of carboplatin into glioma produced by Cereport prolongs survival, animals received i.v. infusions of carboplatin and/or Cereport/vehicle on days 7 and 9 postglioma implantation (i.e., 1 day before and 1 day after the days that the uptake studies were performed).

Seven days post-tumor implantation, separate groups of animals were anesthetized using the ketamine, xylazine, and acepromazine solution and received chronic indwelling intraintrajugular cannulas for...
After infusion, the cannula was flushed with 100 µL of a solution containing heparin (20 U/ml) and vancomycin (1 mg/ml). All cannulas were flushed between treatments to maintain patency. On day 9, all animals received a second treatment, identical with the first, under awake, lightly restrained conditions. Animals were monitored daily for signs of ill health, and any animal showing signs of morbidity was euthanized via CO₂ asphyxiation, and that date was recorded for calculating survival data.

To gain information regarding the relationship between the dose of Cereport, efficacy, and plasma levels, standard pharmacokinetic modeling techniques (PCNONLIN software, version 4.0, 1992; SCI Software, Apex, NC) were used to estimate the Cereport plasma concentrations during and immediately after the 10-min Cereport infusion. This estimate was calculated for rats of 250 g with a blood volume of 54.3 mg/kg. The half-life for Cereport was estimated to be approximately 2 min (based on unpublished results, R. T. Bartus, Alkermes). Finally, for all calculations, it was assumed that within the tight temporal confines of the current dosing schedule, Cereport is evenly distributed in blood but not within organ/tissue extravascular space.

**Statistics.** The effects of Cereport on uptake of carboplatin into tumor, BST, and nontumor brain regions (including contralateral striatum, ipsilateral cortex, and contralateral cortex) is depicted. Note the significant and selective effects of Cereport in the tumor and BST with much less robust and consistent effects in the nontumor brain regions.

### Results

#### Experiment 1: Effect of i.v. Cereport on Uptake of Carboplatin and Paclitaxel
Using scintillation counting, it was determined that Cereport produced a significant and robust increase in uptake of carboplatin into tumor (P < .0001; Fig. 1). In nontumor brain regions, only nonsignificant trends were seen (data not shown; P > .05). No effect of Cereport on uptake of paclitaxel was seen in tumor or any brain region (P > .10; Fig. 1).

#### Experiment 2: Retention of Elevated Platinum Levels in Tumor with Cereport
Atomic absorption spectrophotometry confirmed that the increase in carboplatin uptake achieved with Cereport reflects an elevation in platinum levels within the tumor. The platinum levels from the rats sacrificed at the end of the Cereport infusion (i.e., 5 min after the end of the carboplatin infusion) were over 2-fold higher than those from the vehicle controls (Fig. 2; P < .001). The 2-fold difference achieved by Cereport persisted at the 2 h time point, although apparent clearance of carboplatin from the interstitial brain fluid produced a significant decrease in signal in both groups.

An ANOVA confirmed the significant effect of Cereport and time (P < .001), with no interaction of the two, indicating the persistence of the effects of Cereport over time. A direct comparison of saline versus Cereport at the 2-h time point
confirmed that the platinum levels achieved with Cereport at the 2-h time point remained significantly greater than those with vehicle ($P < .01$; Fig. 2).

**Experiment 3: High Spatial Resolution Analysis of Carboplatin Uptake into Tumor and BST.** Using quantitative autoradiography of the entire tumor and BST, it was determined that Cereport increased uptake of carboplatin by over 2-fold in each (130%, $P < .001$; Fig. 3). In brain regions not associated with the glioma, nonsignificant trends were observed ($P > .05$; Fig. 3). Collectively, these data are consistent with previous reports of the effects of Cereport on uptake of carboplatin in rat glioma models (Inamura et al.,

![Graphs showing relative frequency of carboplatin uptake in tumor](image)

**Fig. 4.** High spatial resolution profile of carboplatin uptake within tumor. Top, graphs depict the proportion of tumor displaying varying degrees of radiolabeled carboplatin. Each vertical bar represents progressively greater degrees of permeability (i.e., progressively greater amounts of radiolabeled carboplatin). Note the heterogeneity in permeability displayed under both saline vehicle (left) and Cereport (right) conditions. Note also that Cereport both significantly reduced the proportion of tumor that was relatively impermeable (i.e., 0–20 nCi/g) and also increased the proportion of tumor where carboplatin levels were relatively high (i.e., >50 nCi/g). Bottom, graph presents the same data in the form of cumulative frequency (i.e., percentage of pixels within the tumor containing increasingly greater amounts of radiolabeled carboplatin) plotted as a function of the level of radioactive carboplatin. Note the significant drop in the frequency of low nanocurie-level pixels with Cereport together with a substantially higher proportion of higher nanocurie-level pixels. See Materials and Methods for methodological details. This figure depicts the same raw data that are averaged in Fig. 3, Tumor. Error bars represent ±S.E.M.
1994b; Elliott et al., 1996a,b; Matsukado et al., 1996, 1998) and in human glioma patients (Warkne et al., 1995; Ford et al., 1996; Black et al., 1997).

The high spatial resolution analysis in the vehicle-treated rats (computed by quantifying the radioactivity in individual 4.68-μm² pixels within each area of interest) confirmed that the permeability of the BBTB is normally very heterogeneous in this model. Although approximately 40% of the pixels within the tumor displayed less than 20 nCi/g radioactive carboplatin (indicating a very low level of permeability), a small but measurable percentage displayed radioactivity levels in excess of 70 nCi/g (indicating relatively leakier areas of the BBTB). Additionally, the mode (i.e., bin with the greatest number of pixels) for the vehicle condition was only 11 to 20 nCi/g, suggesting an overall low level of permeability for this tumor (Fig. 4).

With Cereport, the pattern of uptake was clearly different ($P < .0001$, relative to vehicle), reflecting in part the greater than 2-fold increase in carboplatin uptake into the tumor and BST. The mode was increased significantly with Cereport (31–40 nCi/g) compared with vehicle (11–20 nCi/g). Importantly, the proportion of pixels displaying less than 20 nCi/g radiolabeled carboplatin with Cereport decreased to approximately 3% with Cereport (compared with 40% with vehicle). This demonstrated that with Cereport almost no area of very low permeability remained in the tumor. Finally, a marked increase in the more highly permeable areas was also noted with Cereport. For example, nearly 20% displayed greater than 70 nCi/g versus less than 4% with vehicle. Importantly, the change with Cereport was greater than what would be expected if the radioactivity associated with each pixel in the vehicle group was simply increased 2-fold in a uniform fashion across the tumor. This is shown in Fig. 5, which depicts the pattern of uptake achieved with Cereport versus a simulation generated by uniformly increasing each pixel score by 130% for the vehicle-treated animals ($P < .001$).

In the BST, a profile very similar to that seen in the tumor was observed in the vehicle group. Additionally, the change in the pattern of uptake in BST with Cereport ($P < .0001$ versus vehicle; Fig. 6) was similar to that of glioma. Again, the most apparent Cereport effect was reflected in a dramatic reduction in proportion of impermeable micro-regions.

**Experiment 4: Increased Survival after Cereport and Carboplatin.** Animals treated with saline exhibited a median survival of 19 days and a maximum survival of 24 days. Rats given Cereport only (9.0 μg/kg) displayed a nearly identical survival curve to vehicle-treated rats (Fig. 7). Carboplatin (10 mg/kg, given on days 7 and 9) significantly enhanced both median and maximum survival (Fig. 7), increasing each by approximately 10 days ($P < .05$). The low dose of Cereport (i.e., 3.0 μg/kg) in combination with carboplatin failed to further increase survival beyond that achieved with carboplatin alone. However, the higher dose of Cereport (9.0 μg/kg) produced a marked increase in survival, increasing median survival to 36 days and maximum survival to 60 days (in each instance, nearly a 2-fold increase over carboplatin alone; $P < .01$).

The plasma concentrations achieved with 3.0 μg/kg were estimated to range from 8 nM (2 min from the initiation of the infusion) to 16 nM (at the end of the 10-min infusion, at which time the $C_{\text{max}}$ was reached). In contrast, the 9.0-μg/kg dose was estimated to produce plasma levels ranging from 25 to 50 nM (using the same temporal points of reference). These latter estimates are consistent with the $K_i$ values established for Cereport (10 to 50 nM), which are associated with binding to $B_2$ bradykinin receptors and induction of bradykinin second messenger responses in vitro (Doctrow et al., 1994; Bartus et al., 1996b).

**Discussion**

The experiments reported here establish several novel and important points, demonstrating that: 1) the increased uptake of radiolabeled carboplatin reported previously reflects a genuine increase in platinum levels in the tumor and BST; 2) the residence time for the elevated platinum lasts at least 2 h; 3) Cereport is particularly effective in eliminating those portions of the BBTB (in both tumor and BST) that are normally impermeable to carboplatin; 4) contrary to the effects achieved with hydrophilic carboplatin, lipophilic paclitaxel does not benefit from Cereport despite the fact that it also does not easily penetrate the BBB; and 5) i.v. doses of Cereport that increase carboplatin uptake and were estimated to achieve plasma levels approximating the $K_i$ of Cereport significantly enhance survival when combined with carboplatin; a lower dose of Cereport, which produces marginal uptake effects and Cereport plasma levels, had no effect on survival when combined with carboplatin.

These findings raise several important implications. The 2-fold increase in platinum levels observed (using atomic absorption spectrophotometry) near the end of the Cereport infusion closely mirrored the increase in [14C]carboplatin obtained via scintillation and autoradiography. This establishes for the first time that the increased uptake of radiolabel seen in these and other studies indeed reflects increased uptake of carboplatin.
levels of active chemotherapeutic moiety. It also establishes an effect using therapeutic (as opposed to trace) levels of carboplatin. Importantly, the 2-fold advantage in platinum levels achieved with Cereport persisted for at least 2 h, providing the first demonstration that the increased levels achieved with Cereport persist beyond the transient opening of the BBTB, perhaps providing enough residence time to invoke a cytotoxic effect on the tumor cells.

As in previous studies using this and similar glioma models (Inamura et al., 1994b; Bartus et al., 1996a,b; Elliott et al. 1996b,c; Matsukado et al., 1996, Black et al., 1997; Emerich et al., 1999) as well as human glioma patients (Ford et al., 1996; Black et al., 1997; Cloughesy et al., 1999), the increased uptake achieved with Cereport was found to be much greater and more consistent in tumor and BST than in brain tissue distal to tumor. In this study, the greater than 2-fold increases in carboplatin achieved with Cereport in the tumor and BST are contrasted with only marginal increases in other nontumor brain regions.

We used a more detailed and novel, high spatial resolution analysis of the uptake within tumor and BST to further characterize the Cereport-induced changes. This analysis revealed that the BBTB in this model is normally highly heterogeneous (similar to that reported for human gliomas). Interestingly, Cereport did not simply uniformly enhance the uptake of carboplatin to produce the >2-fold change, because with Cereport, almost no portion of any region remained relatively impermeable to carboplatin. Additionally, a

Fig. 6. High spatial resolution profile of carboplatin uptake within BST. The analysis is the same as that used for tumor shown in Fig. 4. Note that the BST under vehicle conditions tends to be somewhat less permeable than the tumor (i.e., greater proportion of area represented by low nanocurie levels). Note also that the effects of Cereport on BST appear to be similar to those in tumor with a dramatic reduction in the proportion of areas that are relatively impermeable to radiolabeled carboplatin. This figure depicts that same raw data that are averaged in Fig. 3, BST. Error bars represent ±S.E.M.
greater proportion of highly permeable areas was generated. Thus, the effect of Cereport is not manifested as an indiscriminate doubling of carboplatin levels because the shape of the uptake profile was clearly modified (Figs. 4 and 6). This is most obvious when the pattern of uptake with Cereport in tumor is directly compared with that of a simulated profile achieved by increasing all the raw scores from the vehicle group by 130% (i.e., the difference in overall uptake between Cereport and vehicle; Fig. 5). Of particular interest is the change in the pattern of carboplatin delivery to normally impermeable areas of the tumor and BST, which is significantly different with Cereport from what would be expected from a simple 130% increase in carboplatin uptake. In certain subareas of the tumor, the increased uptake achieved with Cereport is severalfold greater than that achieved with vehicle. This change in the topographic uptake profile with Cereport, involving dramatic differences in certain aspects of carboplatin uptake, may help explain the marked increase in survival associated with the 2-fold increase in overall carboplatin levels with Cereport. Further research will be required to confirm the therapeutic importance of this modified uptake profile as well as to determine the means by which Cereport changes the distribution of carboplatin within tumor and BST. It is surprising that although Cereport is much less effective in nonpermeable normal brain, it is able to substantially reduce areas of impermeability within the tumor and BST. This finding suggests that the relative selectivity Cereport displays for tumor and tumor-associated brain tissue is not simply a function of the tumor tissue being more leaky (see Bartus, 1999 for more detailed discussion of alternative explanations).

The dose comparison of Cereport in the survival studies provides new and important information. It provides the first evidence for a therapeutic benefit in brain tumors with any i.v. modification of the BBTB. The higher dose of Cereport (9.0 $\mu$g/kg) was selected because it falls well within the optimal portion of the i.v. dose-response function established previously (Bartus et al., 1996b; Elliott et al., 1996b; Emerich et al., 1999), and it was estimated to produce Cereport plasma levels that range from 25 to 50 nM during the 10-min infusion (i.e., within the range of the $K_i$ of Cereport for stimulating bradykinin B$_2$ receptors) (Doctrow et al., 1994; Bartus et al., 1996b). Importantly, this dose enhanced the survival benefit of carboplatin by greater than 2-fold. In contrast, 3.0 $\mu$g/kg falls within the Cereport dose range where the carboplatin uptake effects are marginal (Bartus et al., 1996b; Elliott et al., 1996b) and the Cereport plasma levels are estimated to range from 8 to 16 nM during the 10-min infusion (i.e., on the extreme low end of the $K_i$ of Cereport) (Doctrow et al., 1994; Bartus et al., 1996b). Interestingly, this dose did not affect survival when combined with carboplatin. The dose-related survival effects reported here, therefore, provide further insight into the plasma concentrations of Cereport required to achieve significant biological efficacy. Using the identical pharmacokinetic methods to model Cereport plasma levels in human patients, it is estimated that doses in the range of 1200 to 1500 ng/kg are likely required to achieve similarly effective plasma levels of Cereport in humans. However, initial phase II studies of Cereport combined with carboplatin used a Cereport dose of only 300 ng/kg (Prados et al., 1997; Gregor et al., 1999), which would produce Cereport plasma levels substantially below those that these data argue are necessary to achieve efficacy in gliomas. For these reasons, higher Cereport doses are now being studied in combination with carboplatin in brain tumor patients.

The lack of significant uptake effects with the lipophilic drug, paclitaxel, are perhaps as interesting as the positive effects achieved with carboplatin. The lack of any increase in paclitaxel uptake with Cereport is reminiscent of previous data with BCNU (another lipophilic chemotherapeutic drug) (Bartus et al., 1996a). Together, these data are consistent with the action of Cereport on the tight junctions of the BBB (Sanovich et al., 1995), whereby Cereport creates an aqueous pathway for water-soluble agents to diffuse from the blood, between the endothelial cells of the BBB, and into the brain. Because lipophilic agents diffuse into the brain through the lipid phase of the membranes of endothelial cells, they nei-
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ther need nor are aided by the transient aqueous corridor created by Cereport. Interestingly, despite its lipophilic nature, paclitaxel does not reach high concentrations within brain or brain tumors, in part because it is rapidly transported back to the luminal side of the cerebral vessels by the P-glycoprotein system (Schnikel et al., 1996). The inability of Cereport to increase paclitaxel concentrations in the tumor provides circumstantial evidence that Cereport does not inhibit the P-glycoprotein transport system at least within the confines of these dosing parameters.

Cereport was developed to enhance the effects of concomitantly administered chemotherapeutic agents that cannot gain access to brain tumors because of the tight junctions of the BBTB. As a treatment option for brain tumors, it is unique in that it has no cytotoxic activity by itself. To be optimally effective, a delivery agent like Cereport must satisfy several important goals. First, the amount of chemotherapeutic delivered to the tumor and BST should be significantly increased. Secondly, the distribution of the chemotherapeutic within the tumor and BST should be improved so that no area of the tumor or BST can escape the chemotherapeutic agent. Third, the time that the chemotherapeutic is elevated should be increased. Ideally, all of these effects should occur simultaneously and selectively in tumor-associated tissue (i.e., tumor and BST) with little or no effect in normal brain distal to tumor. These data demonstrate that i.v. Cereport achieves all of these goals with carboplatin in the RG2 rat gloma model, making it the only BBTB drug delivery method to date to do so. Moreover, when the identical dosing paradigm is used (substituting therapeutic concentrations of cold carboplatin for radiolabeled carboplatin), a significant 2-fold increase in survival is achieved. These data, therefore, offer clear evidence that modulating cerebrovascular bradykinin activity with selective B2 agonists such as Cereport represents a novel and potentially valuable means of enhancing the chemotherapeutic effects of carboplatin in gliomas and perhaps other types of brain tumors.