Intravenous Cereport (RMP-7) Enhances Delivery of Hydrophilic Chemotherapeutics and Increases Survival in Rats with Metastatic Tumors in the Brain

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Purpose. The following experiments determined whether intravenous infusions of Cereport enhance delivery of chemotherapeutics and prolong survival in rats with metastatic tumors in the brain.

Methods. Autoradiography and scintillation were used to examine uptake of the lipophilic (paclitaxel and carmustine) and the hydrophilic (carboplatin) chemotherapeutic agents, as well as the large hydrophilic marker, 70 kDa dextran. Cereport was also tested in combination with the chemotherapeutic drugs carboplatin, vinorelbine, gemcitabine and carmustine to determine if Cereport could enhance the survival benefit beyond that provided by chemotherapy alone.

Results. Cereport enhanced the uptake of carboplatin and dextran, but not paclitaxel or carmustine. The pattern of Cereport's uptake effect with carboplatin revealed that Cereport selectively increased the proportion of highly permeable regions. Survival was significantly enhanced when Cereport was combined with either carboplatin, vinorelbine, or gemcitabine, but not carmustine, compared to each chemotherapeutic agent alone.

Conclusions. These data provide the first evidence that Cereport, or any receptor-mediated approach intended to enhance the permeability of the blood-brain tumor barrier, can increase the delivery hydrophilic drugs to metastatic tumors in the brain, increasing survival in tumor-bearing rats.

KEY WORDS: blood-brain tumor barrier; bradykinin; brain metastases; carboplatin; carmustine; paclitaxel; vinorelbine; gemcitabine.

INTRODUCTION

Tumors that metastasize to the central nervous system represent one of the most devastating complications in patients with systemic cancer. They account for the majority of newly diagnosed brain tumors, with as many as 170,000 cancer patients in the United States developing brain metastases annually. Twenty-five to fifty percent of these patients die as a result of neurological complications (1). Single brain metastases can often be treated with stereotaxic radiosurgery or a combination of surgical resection and radiotherapy, in cases of large tumors. While aggressive treatment may add several months to a year to patients lives, recurrence of the tumor or the presence of new brain metastases, most often lead to death within three to six months following recurrence (2).

The contribution of chemotherapy to multi-modality treatment of brain metastatic disease remains controversial. Despite continual developments of new chemotherapeutic drugs, the treatment of brain metastatic tumors has not been impacted (3). At least one reason for the ineffectiveness of chemotherapy is the existence of the blood brain tumor barrier (BBTB) which inhibits diffusion of water-soluble compounds and large lipophilic compounds from the vessel lumen to the tumor interstitial space. Neuroradiology studies ((4)for a review), imaging studies (5) and electron microscopy (6,7) have all confirmed that the BBTB of brain metastatic tumors is generally leaky compared to the normal BBB. Nonetheless, many of the principle components of the barrier, including tight intracellular junctions, astrocytic processes and basal lamina (6,7), persist to form a barrier within the tumor vessels that produces a significant obstacle to the delivery of potentially valuable drugs to brain tumors.

In principle, increasing the permeability of the BBTB during administration of chemotherapeutic drugs should allow many existing hydrophilic drugs to gain access to the brain tumor, potentially providing significant patient benefit (8). Cereport® (RMP-7) is the first receptor agonist analog shown to increase permeability of the BBTB (9–12). Cereport is a bradykinin analog with a longer half-life and greater selectivity for the constituitively expressed bradykinin B2 receptor (compared to bradykinin itself) (12–14). Studies with animal models of glioma have demonstrated that intravenous (i.v.) Cereport selectively increases uptake of chemotherapeutics into gliomas (15–17) and recent imaging studies using PET and CT in human glioma patients have confirmed Cereport's selectivity in glioma tissue (18–20).

Despite the evidence for significant modulation of the glioma vascular barrier, no studies have examined the use of receptor-mediated modulation of the BBTB of metastatic tumors in the brain. Relative to glioma, the vasculature supplying metastatic tumors in the brain is generally considered more permeable (7,21–23), and its response to many vasoactive compounds also differs (24–26). It therefore remains an open question as to whether Cereport (or any other receptor-mediated method of increasing BBTB permeability) can increase uptake of chemotherapeutic agents into metastatic tumors in the brain. For this reason, a series of experiments were conducted in a rat metastatic brain tumor model to (1) determine the ability of Cereport to enhance uptake of multiple hydrophobic and hydrophilic compounds, (2) examine in greater detail the nature of the uptake effects achieved with carboplatin, and finally (3) determine whether Cereport extends the survival benefit of the chemotherapeutic agents carboplatin, carmustine, vinorelbine, and gemcitabine.

MATERIALS AND METHODS

Subjects

Male Fischer rats (N = 229, 170–220g; Taconic Farms, Germantown, NY) 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

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Committee and were conducted in a manner which met or exceeded NIH standards.

Cell Maintenance and Implantation

MATB-III cells (ATCC# CRL-1666) were grown and maintained as a suspension culture in McCoy's 5A Medium supplemented with 10% fetal bovine serum, 20mM HEPES 50 units/ml penicillin, 50 μg/ml streptomycin and 0.125 μg/ml amphotericin B. Cells were harvested and centrifuged at 1500g for 4 minutes at 10 °C prior to suspending at a density of 2 × 106 cells/ml in HEPES-buffered serum-free media containing 1.2% methyl cellulose. Rats were anesthetized with a solution containing ketamine (24 mg/ml), xylazine (1.3 mg/ml) and acepromazine (0.33 mg/ml) and placed in a stereotaxic instrument. MATB-III cells (1×104cells/5μl) were injected unilaterally into the striatum using a stereotaxic-mounted 10 μl Hamilton syringe with a 22 gauge needle using previously described methods (17).

Drug Administration

Uptake studies were conducted eight days after tumor implantation. Under urethane anesthesia (1.8 g/kg; i.p.), cannulae were placed in the jugular vein for drug administration and into both femoral arteries for the measurement of physiological parameters and the collection of blood used to calculate the uptake constant, Ki (11,15). Either [4C] carboplatin (MW = 371, S.A. = 144 μCi/mg, Amersham, Arlington Heights, IL) [14C] dextran (MW = 70 kDa; S.A. = 1.14 nCi/g, Amersham, Arlington Heights, IL), [14C] carmustine (MW = 214, S.A. = 73 μCi/mg, Amersham, Arlington Heights, IL) or [3H] paclitaxel (MW = 854, S.A. = 6.5 Ci/mmol; Moravek Biochemicals, Brea, CA) were given together with a Cereport (6.0 μg/kg; Alkermes, Inc., Cambridge, MA) or saline vehicle infusion.

Scintillation Studies

To compare the ability of Cereport to enhance uptake of dextran, carmustine and paclitaxel into tumor and brain surrounding tumor (BST), animals received infusions of the radiolabel from 0–15 minutes with an overlapping Cereport (6.0μg/kg) or saline infusion from 10–20 minutes. At the end of drug administration, the tumor, ipsilateral cortical tissue and contralateral striatal tissue was processed as previously described (21). Six groups of animals were used: (1) dextran and saline, N = 10, (2) dextran and Cereport, N = 10, (3) carmustine and saline, N = 9, (4) carmustine and Cereport, N = 10 (5) paclitaxel and saline, N = 10, and (6) paclitaxel and Cereport, N = 8.

Autoradiography

For autoradiographic determinations of carboplatin uptake into tumor tissue, animals received a 10 minute infusion of either saline (N = 7) or Cereport (N = 9), together with a 2–3 second bolus injection of radiolabled carboplatin two minutes into the infusion. At the end of drug administration, the brains were removed and stored at –20 °C until they were sectioned. The brains were cut at 20 μm intervals on a cryostat (–16 °C) throughout the length of the tumor and the sections were thaw mounted onto glass microscope slides. Using standard autoradiographical techniques, the slides were apposed to radiosensitive film (Kodak Biomax MR-1) with 14C-calibration standards (0.002 to 3.58 μCi/g, American Radiolabeled Chemicals, St. Louis, MO) for 1 week and then developed. The sections were then stained with hematoxylin and eosin (H&E) to verify tumor placement.

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 H&E-stained sections were digitized to define the exact tumor location and boundary. This coronal section was then overlaid upon 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 were measured. Uptake was also quantified within the ipsilateral and contralateral cortices and the contralateral striatum as previously described (19).

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 used to quantify uptake of radiolabeled carboplatin with extremely sensitive spatial resolution (4.68 μm2 area) (27). 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 nCi/gm, 11–20 nCi/gm, 21–30 nCi/gm, etc).

Survival Studies

Animals received i.v. infusions of carboplatin, vinorelbine, gemcitabine, or carmustine alone or in combination with Cereport on day 7 and 9 post-cell implantation (i.e., one day prior to and one day following the days that uptake studies were performed). Using previously published procedures (31), animals received 10 mg/kg of carboplatin, 5.0 mg/kg of vinorelbine, 15.0 mg/kg of gemcitabine, or 2.5 mg/kg of carmustine, in combination with saline or Cereport (6 μg/kg). In separate survival studies, animals were divided into one of three treatment groups: (1) saline infused from 0–20 minutes, (3) chemotherapeutic drug (carboplatin, vinorelbine, gemcitabine, or carmustine) infused from 0–15 minutes with an overlapping saline infusion from 10–20 minutes, and (3) chemotherapeutic drug infused from 0–15 minutes with an overlapping Cereport (6.0μg/kg) infusion from 10–20 minutes. Separate animals received saline infusions from 0–15 minutes with an overlapping Cereport (6.0μg/kg) infusion from 10–20 minutes. On day 9, all animals received a second treatment, identical to the first, under awake, lightly restrained conditions. Animals were monitored daily and any animal showing signs of morbidity was euthanized via CO2 asphyxiation and that date recorded for calculating survival data. Necropsies

were performed on each animal to determine the cause of death.

Statistics

The effects of Cereport on uptake into tumor, BST and normal brain were compared in rats using a one-way analysis of variance. (JMP, SAS Institute Inc., Cary, N.C.). Data from the high spatial resolution analysis of carboplatin uptake (i.e., pixel analysis) were analyzed using X2. Survival data were analyzed using Kaplan-Meier methods and the Log-Rank test. Minimal statistical significance in all cases was defined as $p < 0.05$.

RESULTS

Cereport Enhances Uptake in Tumor and Brain Surrounding Tumor

All MATB-III tumors were located within the striatum. Light microscopy of H&E sections revealed that the brain metastatic tumors were similar in size, location, and appearance to striatal glioma in previous rodent models using Cereport (9–12,15–17). Quantitation of the H&E sections from the present autoradiography studies determined that the maximal cross-sectional tumor area was 3.99 ± 0.46 mm2. The tumors were generally localized and well-defined, although cells could occasionally be seen migrating short distances from the tumor mass along larger striatal blood vessels. Quantitative autoradiography revealed that i.v. Cereport enhanced uptake into the brain tumor and BST. As shown in Figure 1, i.v. Cereport (6.0 μg/kg) increased carboplatin levels in the tumor by 87% ($p<0.015$) and in the BST by 98% ($p<0.02$). Calculations of Ki revealed similar increases in tumor (122%) and BST (123%). Consistent with previous reports (8,9,11,15,16,19,20), the effect of Cereport on permeability of normal, non-neoplastic brain regions was less robust and not as reliable as that seen in tumor and BST (contralateral cortex and striatum $p>0.10$; ipsilateral cortex $p<0.03$).

Scintillation studies were used to examine the generality of uptake into metastatic tumors in the brain following Cereport. Cereport significantly increased uptake ($p<0.01$) of the high molecular weight marker, 70 kDa dextran into metastatic tumors in the brain by 74% (Figure 2). No effects were seen in any other brain region examined ($p>0.10$). In contrast to the enhanced delivery of dextran into tumor, no effect of Cereport on uptake of the hydrophobic agents carmustine or paclitaxel was seen in tumor or any brain region examined ($p>0.10$).

High Spatial Resolution Analysis of Carboplatin Uptake into Tumor and BST

The uptake profile for the vehicle-treated rats generated by the high spatial resolution analysis revealed that the permeability of the BBTB is normally very heterogeneous in this model. While 50% of the pixels within the tumor displayed less than 80 nCi/gram of radioactive carboplatin (indicating a relatively low level of permeability), a small, but measurable number of areas displayed levels in excess of 200 nCi/gram (indicating relatively leaky areas of the BBTB).

Under Cereport, the pattern of uptake was clearly different ($p<0.0001$ vs. vehicle), for Cereport increased exposure of virtually every portion of the tumor to carboplatin, with the most marked effect being an increase in the proportion of highly permeable regions (Figure 3). For example, under vehicle, only 2% of the tumor area had carboplatin levels of 200 nCi/gram or greater, whereas under Cereport, this was increased to 40%. Indeed, 10% of the tumor area under Cereport had carboplatin levels of 300 nCi/gram or higher. In addition to the robust effect of Cereport on the highly permeable regions of the tumor, other changes in the uptake profile included shifting the proportion of tumor area with less than 80 nCi/gram (i.e. highly impermeable regions) from

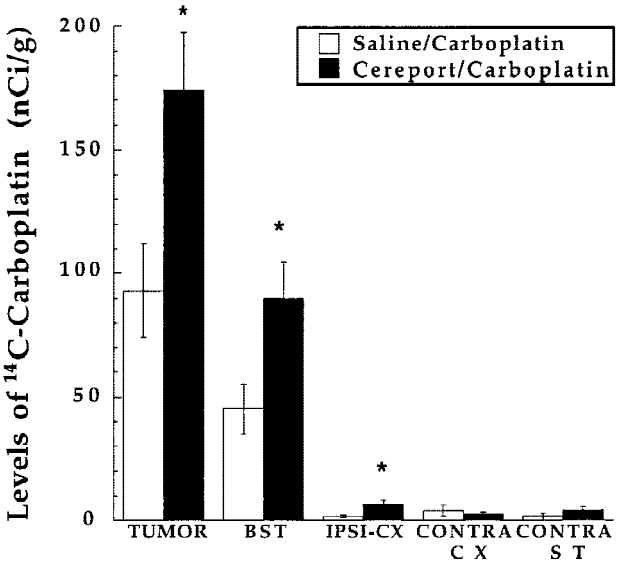

Fig. 1. Effect of i.v. Cereport (6.0 μg/kg) on uptake of 14C-carboplatin, as measured by quantitative autoradiography. The levels of 14C-carboplatin for the entire area of tumor, brain surrounding tumor (BST), and non-tumor brain regions (e.g., ipsilateral cortex, contralateral cortex and contralateral striatum) are depicted. Cereport significantly enhanced carboplatin uptake in the tumor (87%) and BST (98%). Carboplatin levels are shown as nCi/g to facilitate comparisons with the more detailed pixel analyses shown in Figures 3 and 4. Determinations of Ki revealed similar increases in tumor (122%) and BST (123%). Error bars represent ±SEM. *$p<0.01$ vs control.

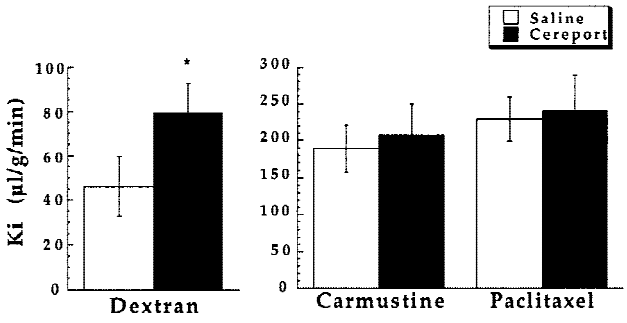

Fig. 2. Comparison of differences in uptake of 14C-dextran 14C-carmustine and 3H-paclitaxel, achieved with i.v. Cereport (6.0 μg/kg) versus saline vehicle, as determined by scintillation counts. Note that while a 74% increase in uptake of the hydrophilic, dextran, was achieved in tumor, no increase in uptake of lipophilic carmustine or paclitaxel was observed. Error bars represent ± SEM. *$p<0.01$ vs control.

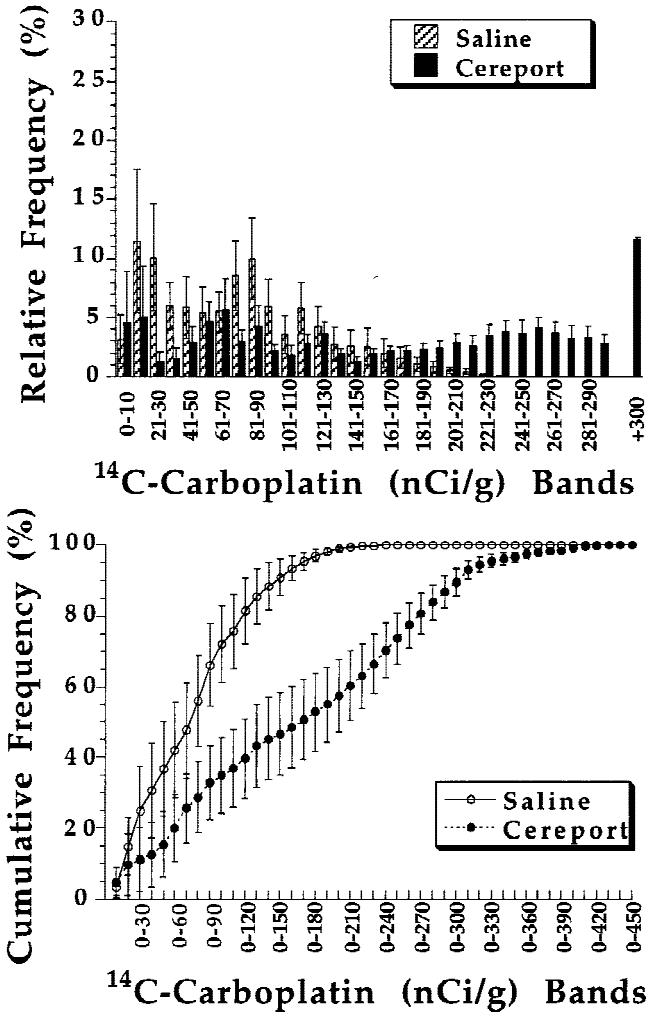

Fig. 3. High spatial resolution profile of 14C-carboplatin uptake within tumor. Top graph depicts the proportion of tumor displaying varying degrees of radiolabeled carboplatin grouped in 10 nCi bands ranging from 0 nCi/gram to greater than 300 nCi/gram for both saline and Cereport animals. Each vertical bar represents progressively greater degrees of permeability (i.e., progressively greater amounts of radiolabeled carboplatin). Bottom graph presents same data in the form of cumulative frequency (i.e., % of pixels within tumor containing increasingly greater amounts of radiolabeled carboplatin), plotted as a function of level of radioactive carboplatin. This figure depicts that same raw data illustrated as mean increase within the entire tumor in Figure 1. Error bars represent ± SEM.

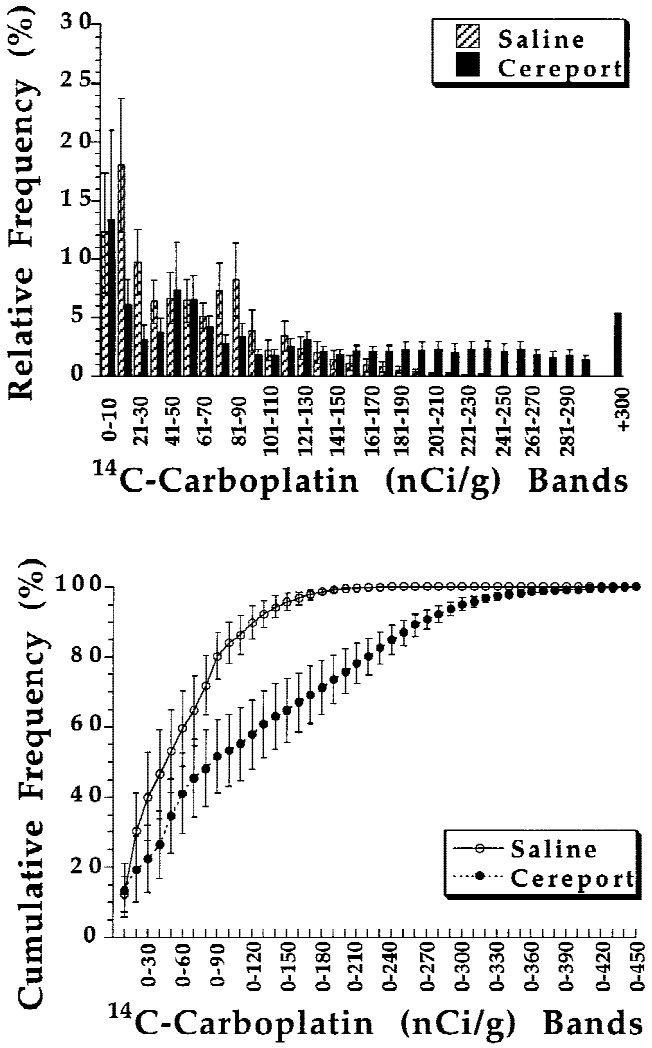

Fig. 4. Analysis of individual pixels of 14C-carboplatin uptake within brain surrounding tumor (BST) as described for tumor in Figure 3. The top figure shows the relative frequency of pixels grouped in 10 nCi bands ranging from 0 nCi/gram to greater than 300 nCi/gram for both saline- and Cereport-treated animals. The bottom panel shows the same data presented as the cumulative frequency of groups of 10 nCi bands of carboplatin uptake. Each bar/point represents the mean ± SEM.

nearly 50% under vehicle to approximately 25% under Cereport (Figure 3).

A similar phenomenon was observed in the BST in that the most apparent effect was a dramatic increase in the proportion of tissue that was highly permeable to carboplatin (Figure 4). Under vehicle conditions, less than 1% of the BST contained carboplatin that exceeded 200 nCi/gram, whereas following Cereport, 25% of the BST contained over 200 nCi/gram, and 5% of these pixels contained over 300 nCi/gram of carboplatin.

Increased Survival Following Cereport and Chemotherapy

The survival curves obtained from Kaplan-Meier methods are shown in Figure 5. The intrastriatal tumor was uniformly fatal to saline-treated animals with a median survival of 22–27 days and maximum survival of 27–32 days post-tumor implantation (Figure 5, Table I). Cereport alone did not impact survival. Intravenous infusions of all chemotherapeutics tested (carboplatin, vinorelbine, gemcitabine, and carmustine) modestly increased survival (Figure 5, Table I). In contrast, combining i.v. Cereport with the hydrophilic drugs carboplatin, vinorelbine, and gemcitabine produced substantially robust increases in survival relative to chemotherapy alone. Consistent with the lack of uptake effects with carmustine, i.v. Cereport did not prolong survival relative to carmustine alone. In this model, carboplatin benefited the most from being combined with Cereport. Median survival was approximately 4 times longer with Cereport, compared to carboplatin alone, while maximum survival was approximately 9 times longer when carboplatin was combined with Cereport. Two animals in the Cereport plus carboplatin group were long-

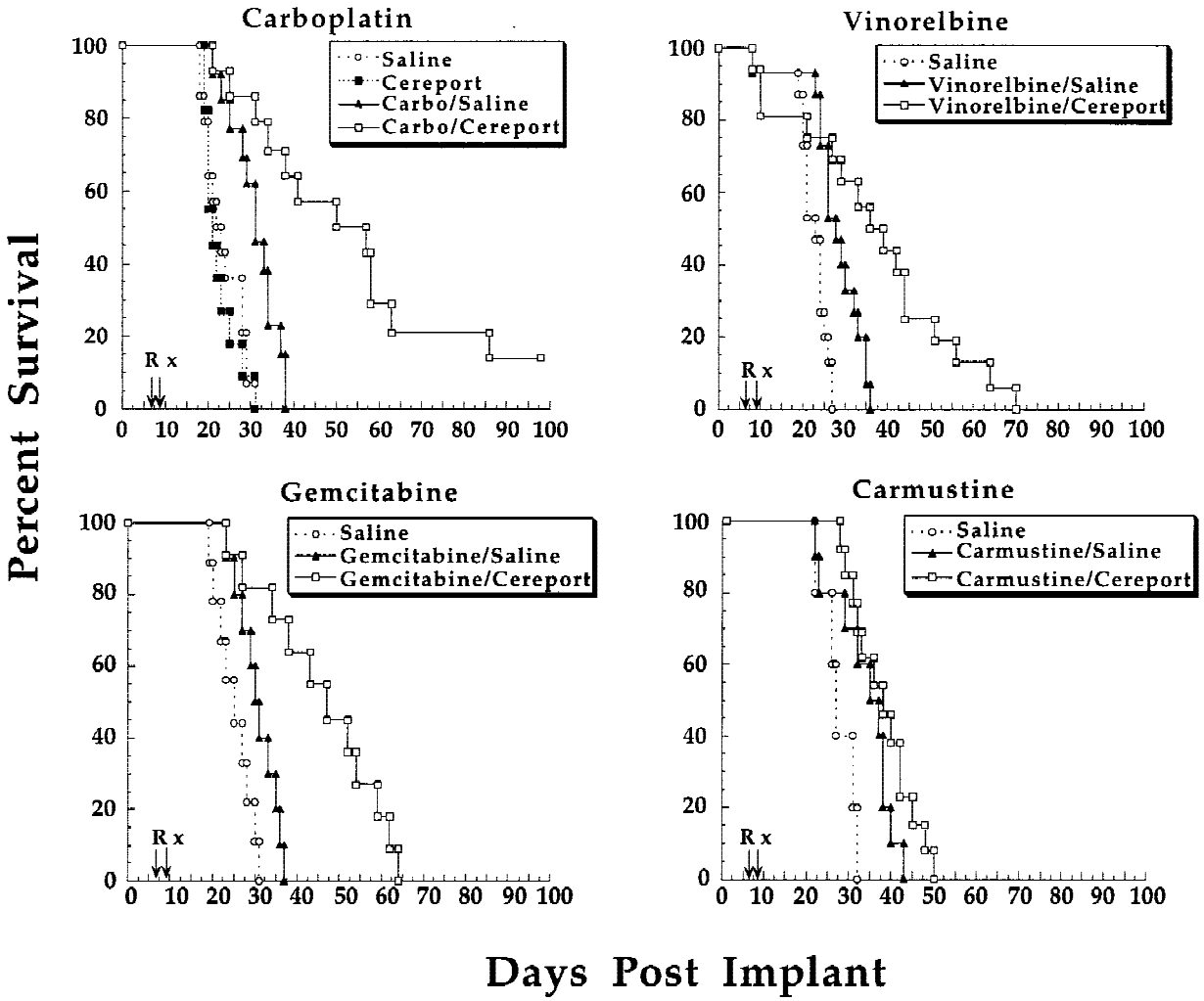

Fig. 5. Kaplan-Meier plots for rats implanted with MATB-III cells and treated 7 and 9 days later with either carboplatin, vinorelbine, gemcitabine, or carmustine in combination with Cereport and/or saline vehicle (see text for details).

term survivors and were sacrificed 98 days post tumor-implantation. The tumor implantation areas within the brains of these two remaining treated animals revealed only a small necrotic area remaining within the previously implanted striatum. In contrast, H&E analysis revealed that at sacrifice, all other animals had large intracranial tumors that encompassed much of the implanted hemisphere.

DISCUSSION

These experiments are the first to evaluate the potential of pharmacological modulation of the BBTB to enhance the delivery of chemotherapeutic agents to metastatic tumors in the brain. Using the bradykinin agonist, Cereport, several new and important points were established. First, exposure of a brain metastatic tumor, and its surrounding brain tissue, to carboplatin can be significantly increased without increasing systemic doses of the chemotherapeutic agent or increasing exposure of normal brain far removed from the tumor. Secondly, Cereport increases the permeability of metastatic tumors in the brain to the larger hydrophilic compound, 70 kDa dextran, but not to the lipophilic compounds carmustine and paclitaxel. Finally, and most importantly, i.v. Cereport significantly prolonged survival over that achieved with several different chemotherapeutic agents alone.

The uptake effects achieved with i.v. Cereport in the current metastatic brain tumor model are reminiscent of those previously reported in glioma rat models using RG2 cells (8,11,12,17). With both types of brain tumors, a similar, approximate two-fold overall increase was seen in the tumor and BST. However, the modification of the uptake profile achieved with Cereport (as determined by pixel analysis) differed markedly between glioma and metastatic tumors in the brain. A major part of Cereport's effects in gliomas involved a decrease in the proportion of the tumor that was poorly permeabilized (27), leaving virtually no portion of the tumor and BST without significant exposure to carboplatin. In contrast, the major effect of Cereport in brain metastatic tumors was to markedly increase the proportion of highly permeable micro-regions. For example, Cereport increased the proportion of metastatic tumor with >200 nCi/gram of 14C-carboplatin (i.e. highly permeable areas) from only 2% to 40% (relative to vehicle) with approximately 10% of the tumor area exhibiting carboplatin levels in excess of 300 nCi/g, compared to none for vehicle (Figures 3 and 4). In gliomas, no micro-region of the tumor even approached 200 nCi/gram,

Table 1. Survival Following Cereport and Chemotherapy in Brain Metastatic Tumors

Treatment	N	Median survival (days)	% Change (vs saline)	Maximum survival (days)	% Change (vs Saline)	Statistics
Saline	14	22		31		
Cereport	11	21	–5	31	0	p>0.10 vs saline
Carboplatin + Saline	13	31	+41	38	+23	p<0.001 vs saline
Carboplatin + Cereport	14	57	+159	>98*	+217	p<0.001 vs carbo.
Saline	15	23		27		
Vinorelbine + Saline	15	28	+22	36	+34	p<0.001 vs saline
Vinorelbine + Cereport	16	36	+57	70	+160	p<0.01 vs vinorel.
Saline	9	25		31		
Gemcitabine + Saline	10	31	+24	37	+20	p<0.01 vs saline
Gemcitabine + Cereport	11	47	+57	70	+106	p<0.001 vs gemcit.
Saline	15	27		32		
Carmustine + Saline	10	35	+30	43	+35	p<0.01 vs saline
Carmustine + Cereport	13	38	+41	50	+57	p>0.10 vs carmustine

* 2/14 animals were long-term survivors and were sacrificed 98 days after tumor implantation for histological analysis (see text for details).

despite robust effects of Cereport within the poorly permeable micro-regions of the glioma and BST (27). Thus, exposure of certain areas within the metastatic brain tumor and BST to carboplatin was very high and substantially greater than suggested by the mean two-fold change reflected in the traditional autoradiographic analysis (Figure 3). It should also be noted that the range of permeable regions under normal conditions differed between the present metastatic tumors and the rodent glioma in previous Cereport studies. Under saline conditions, approximately 40% of the pixels within the glioma displayed less than 20nCi/gm radioactive carboplatin (indicating a very low level of permeability). A small but measurable percentage displayed radioactivity levels in excess of 70nCi/gm (indicating areas of relatively leaky BBTB). In the metastatic tumors, the pattern was different with 50% of the pixels within the tumor displaying less than 80 nCi/gram of radioactive carboplatin and a small, but measurable number of areas displaying levels in excess of 200 nCi/gram. Further research will be required to: (a) identify the means by which Cereport changes the distribution of carboplatin uptake within tumor and BST; (b) determine whether the pattern observed with carboplatin also occurs with other hydrophilic compounds; (c) define the reasons for the apparent differences in pattern of uptake between metastatic tumors in the brain and gliomas; and (d) establish the therapeutic significance and possible relative advantages of these differential patterns.

The survival benefit produced by combining Cereport and carboplatin also appears to be greater in the present metastatic tumor model than in glioma-bearing rats. In the glioma-bearing rats, both median and maximum survival increased by about two-fold, relative to carboplatin, alone (27). However in the current metastatic brain tumor model, median survival was approximately 4 times longer with Cereport, compared to carboplatin alone, while maximum survival was approximately 9 times longer (Figure 5). Since carboplatin, alone, produced similarly modest increases in survival in the two models, these data suggest that Cereport may be even more beneficial in metastatic tumors in the brains, relative to gliomas. One possible explanation for this difference could be that the unusually high proportion of the metastatic tumor that is exposed to very high concentrations of carboplatin under Cereport produced even greater tumor cell death. These data therefore raise the possibility that survival benefits may reflect the pattern of increased uptake within the tumor and BST more than the overall mean increase in these same regions.

The enhanced survival in the present study with metastatic tumors in the brain was achieved using an i.v. Cereport dose rate comparable to that which significantly enhanced both uptake (17) and survival (27) in a glioma model (i.e., 0.6 μg/kg/min). By comparison, in the prior glioma survival study, a lower dose (involving 0.2 μg/kg/min) did not enhance survival over that achieved with carboplatin alone (27). The lower dose was estimated to produce Cereport plasma levels ranging from approximately 8 to 16 nM during the infusion period, while the higher, effective dose was estimated to achieve levels of approximately 25 to 50 nM during the infusion (27). Given that Cereport's Ki for binding to the bradykinin B2 receptor and for initiating second messenger responses is about 10 to 50 nM (13,16), the differential dose effects in survival seen when Cereport is combined with carboplatin are not likely coincidental. Importantly, only recently have clinical studies with Cereport begun to test doses (e.g., >1200 ng/kg) in brain tumor patients that are estimated to achieve similar plasma levels of Cereport. All prior studies in glioma patients used a dose nearly a half order of magnitude lower; i.e., 300 ng/kg (18–20).

The results presented in this paper offer the initial empirical evidence that Cereport might be beneficial in the treatment of metastatic tumors in the brain, by increasing exposure of the tumor and BST to higher concentrations of chemotherapeutic agent. While the bulk of work in this paper focused on carboplatin, other hydrophilic chemotherapeutic agents may similarly benefit from Cereport, since uptake of the large hydrophilic, 70 kDa dextran, was also significantly increased, whereas uptake of two lipophilic compounds (paclitaxel and carmustine) was not. The range of hydrophilic chemotherapeutics (carboplatin, vinorelbine, and gemcitabine) that increased survival when combined with Cereport greatly expands the potential opportunities to use Cereport to

improve treatment of metastatic brain tumors. As an agonist for a natural receptor-mediated system, Cereport was anticipated to offer certain safety advantages as a drug delivery agent to brain tumors. Indeed, all preclinical and clinical studies, to date, have confirmed this expectation (18–20,28), including direct infusion of Cereport into the arterial cerebrovasculature, followed by semiquantitative analysis of the brain and its vasculature. The safety of Cereport is further supported by its transient effects, for not only is the BBTB restored soon after the Cereport infusion is terminated (12,16), but the barrier also self-regulates, in that spontaneous restoration occurs after several minutes of continuous Cereport infusion (12,16).

In summary, the data presented in this manuscript are the first to demonstrate that pharmacological modulation of the BBTB can significantly increase the uptake of hydrophilic chemotherapeutic agents into metastatic tumors in the brain. Moreover, these effects achieved with Cereport were selective for tumor associated tissue in that comparatively small changes were seen in brain tissue far removed from the tumor. Finally, the increased delivery of hydrophilic chemotherapeutics from i.v. Cereport resulted in significantly prolonged survival, relative to chemotherapy alone. Together, these data provide important support for the concept that Cereport and perhaps other receptor-mediated approaches to BBB modulation might be developed to enhance delivery of chemotherapeutic agents to treat metastatic tumors in the brain.

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