Cereport® (RMP-7) increases carboplatin levels in brain tumors after pretreatment with dexamethasone

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Accumulating evidence suggests that dexamethasone might decrease permeability of the blood-brain tumor barrier, further limiting the delivery of agents into brain tumors. The bradykinin B_2 receptor agonist, Cereport® (RMP-7), selectively increases permeability of the vasculature supplying brain tumors in both animal models and humans. The present study was conducted to characterize the effects of dexamethasone on the blood-brain tumor barrier and its potential interaction with Cereport's ability to enhance penetration of radiolabeled carboplatin. Dexamethasone (1.5 mg/kg/day, twice a day) was given to RG2 glioma-bearing rats via oral gavage for 3 consecutive days. After treatment, animals received a 15-min intracarotid infusion of Cereport (4.5 µg/kg) and a bolus of [^{14}C]carboplatin. The levels of [^{14}C]carboplatin (nCi/g) in the tumor and nontumor regions were determined at 1, 14, or 24 h after the last dose of dexamethasone. Dexamethasone, alone, significantly decreased the levels of radiolabeled carboplatin permeating the tumor (19%), although there were no significant differences between any of the time points examined. Cereport administration significantly increased levels of carboplatin in the tumor, independent of whether or not dexamethasone was given (46% with and 49% without). Although the relative effects of Cereport on tumor carboplatin levels were not affected by dexamethasone, the absolute levels achieved with Cereport were modestly reduced (44 nCi/g versus 55.5 nCi/g of [^{14}C]carboplatin, with and without dexamethasone, respectively). Thus, while the data support the use of Cereport as adjunctive therapy in the treatment of glioma patients, they also warn that the use of dexamethasone may reduce delivery of chemotherapeutic agents to brain tumors, even when special pharmacologic measures are employed to enhance delivery. *Neuro-Oncology 1, 268–274, 1999 (Posted to Neuro-Oncology [serial online], Doc. 99-12, September 9, 1999. URL <neuro-oncology.mc.duke.edu>)*

Despite substantial effort to develop new antineoplastic drugs, survival of malignant glioma patients has not been significantly improved (Nelson et al., 1993). One reason chemotherapeutic agents have not been more successful is the existence of the BBTB[2] (Groothius et al., 1982; Long, 1970; Neuwelt et al., 1982; Shibata, 1989). While the vasculature comprising the BBTB is known to be leaky compared with normal brain (Levin et al., 1975), it still offers significant resistance to water-soluble chemotherapeutic agents, thus impeding the entry of these drugs into the tumor.

Over the past several years, a number of different techniques have been explored to increase permeability of the BBTB, with hyperosmotic disruption of the barrier being among the earliest (Neuwelt et al., 1984, 1985; Neuwelt and Rapoport, 1984). More recently, bradykinin (Black, 1995; Inamura and Black, 1994; Matsukado et al., 1998; Nomura et al., 1994) and the bradykinin agonist Cereport® (RMP-7) (Bartus et al., 1996a, b; Doctrow et al., 1994; Straub et al., 1994) have been used to stimulate the receptors on the endothelial cells comprising the BBTB to increase barrier permeability. Cereport selectively increases permeability of the vasculature supplying brain tumors in rodent models of glioma (Bartus et al., 1996a, b; Elliott et al., 1996a, b; Inamura et al., 1994; Matsukado et al., 1996) as well as in glioma patients (Black et al., 1997; Cloughesy et al., 1999; Ford et al., 1996).

A potentially complicating factor with Cereport, or any pharmacologic approach intended to temporarily

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[2]Abbreviations used are as follows: BBB, blood-brain barrier; BBTB, blood-brain tumor barrier; SEM, standard error of the mean.

increase the permeability of the BBTB, is the possible interaction with other drugs commonly administered to glioma patients. For example, brain tumor patients typically receive corticosteroids, such as dexamethasone (Decadron®), to treat emesis and manage neurologic symptoms by decreasing intracranial pressure (reviewed in Glaser et al., 1997; Gutin, 1977; Koehler, 1995; Vecht and Verbiest, 1995). Accumulating evidence suggests that dexamethasone may decrease permeability of the BBB, although the picture is far from complete. Brain scans in human glioma patients have demonstrated that dexamethasone decreases contrast enhancement and uptake of radioisotopes into brain tumors (Hatam et al., 1983; Jarden et al., 1989; Yeung et al., 1993). In non–tumor-bearing rats, reductions in the concentration of markers have also been noted in several different brain regions (Neuwelt et al., 1990; Ziylan et al., 1988). Moreover, several studies using rodent models of glioma have shown that dexamethasone significantly decreases uptake of radiolabeled markers (Matsukado et al., 1997; Neuwelt et al., 1982; Shapiro et al., 1990) and the extravasation of proteins and dyes into tumors (Guerin et al., 1992; Neuwelt et al., 1993; Reichman et al., 1986). However, other studies have produced conflicting results. For example, some investigators failed to find a significant effect of dexamethasone in glioma models (Luthert et al., 1986; Nakagawa et al., 1987) while others (Molnar et al., 1995; Straathof et al., 1998) reported a decrease in permeability or levels of marker in the brain surrounding tumor, but none in the tumor itself.

It seems likely that at least some of these discrepancies are due to the widespread variation in dosing parameters and in other variables between studies. Certainly, the pharmacological effects of dexamethasone are complex, variable over time, and significantly affected by the dose and route of administration. Nonetheless, the data collectively raise concerns that a decrease in vascular permeability may be induced by dexamethasone, not only further limiting uptake of chemotherapeutics to the brain tumor but also possibly mitigating the effects of agents intended to deliver higher concentrations of chemotherapeutic drugs to the tumor by increasing the permeability of the barrier. Indeed, two experiments using osmotic disruption of the BBTB reported a loss of efficacy when preceded by dexamethasone treatment (Neuwelt et al., 1982, 1993).

To date, only a single study has reported on the interaction of dexamethasone and the bradykinin agonist Cereport (Matsukado et al., 1997). An overall decrease in tumor permeability was reported following dexamethasone treatment, as well as a reduction in the ability of Cereport to increase tumor permeability. Despite the effects of dexamethasone, uptake of carboplatin was still significantly greater under Cereport compared with vehicle. However, a number of factors may permit clinicians to discount the relevance of that particular study to the human situation. First, it employed bolus injections of a relatively high dose of dexamethasone. Second, it used an allogeneic model known to be complicated by immune responses to the tumor (Dean et al., 1996). The results were confounded by a significant decrease in the size of the tumor in the dexamethasone-treated rats. Third, only a single time point (post-dexamethasone administration) was tested. The present study, therefore, was conducted to further elucidate the effects of dexamethasone on the BBTB and the potential interaction of dexamethasone and Cereport, specifically selecting parameters that would complement those selected in the initial study (Matsukado et al., 1997). We employed an oral route of administration and a dosing schedule of dexamethasone more similar to that used clinically. Additionally, the effects on BBTB were evaluated at several different time points after dexamethasone administration. Finally, a syngeneic strain of rat for RG2 tumors was used to help reduce potential confounding effects involving the actions of dexamethasone on immune responses.

Materials and Methods

Animals

Male Fischer 344 rats (Taconic, Germantown, N.Y.) weighing 170–200 g (8–10 weeks of age, n = 71) were used in these studies. The rats were housed in pairs in a vivarium maintained on a 12-h light/dark schedule with a temperature of 22 ± 1°C and a relative humidity of 50 ± 5%. Food and water were available ad libitum to all study animals. All procedures were reviewed and approved by Alkermes Animal Care and Use Committee and were conducted in a manner that met or exceeded standards of the National Institutes of Health.

Intracerebral RG2 Cell Implantation

Rat glioma (RG2) cells were maintained and prepared as previously described (Elliott et al., 1996b). Prior to implantation, animals were anesthetized with an intramuscular injection of a solution consisting of 25 mg/ml ketamine, 1.3 mg/ml xylazine, and 0.25 mg/ml acepromazine. Five microliters (5×10^4 cells) of RG2 cells were unilaterally injected into the rat striatum (coordinates, A-P [+2.0 mm], L [+3.0 mm], D-V [–6.5 mm] with the incisor bar at +5.0 mm) (Pellegrino et al., 1986) over a 1-min period using a 22-gauge needle attached to a stereotaxic-mounted 10-µl Hamilton syringe. The incision was closed with wound clips, and the rats were allowed to recover from anesthesia before being returned to the vivarium.

Dexamethasone Treatment

Beginning on the sixth day following tumor implantation, animals were randomly assigned to two treatment groups: vehicle (water; n = 15) or dexamethasone (n = 56). Dexamethasone sodium phosphate (4 mg/ml, Steris Laboratories, Phoenix, Ariz.) was diluted with distilled water to a final concentration of 0.75 mg/ml dexamethasone (free base). Vehicle or dexamethasone (1.5 mg/kg/day, given twice daily at 8 a.m. and 8 p.m.) was given via oral gavage for 3 consecutive days. In an effort to attenuate the loss of body weight due to dexamethasone, all groups were given 12–15 pieces of a mixture of

highly palatable, high caloric breakfast cereals in their home cage after each oral dosing.

Dose Administration and Physiological Monitoring

One, 14, or 24 h following the last dose of dexamethasone (9–10 days following tumor implantation) and under urethane anesthesia (1.56 g/kg, i.p.), a PE10 cannula was placed in the left external carotid artery for infusion of Cereport. Additional cannulae (PE50) were placed in the jugular vein for [^{14}C]carboplatin administration and one femoral artery for measurement of mean arterial blood pressure/heart rate.

Cereport (4.5 μg/kg) or vehicle (0.9% normal saline) was infused intra-arterially into the internal carotid using an infusion pump at a rate of 0.05 ml/min over a 15-min period. [^{14}C]carboplatin (specific activity = 142 μCi/mg, Amersham, Arlington Heights, Ill.) was given as a bolus (100 μCi/ml/kg) over a 3-s period into the jugular vein 5 min after the start of the Cereport or vehicle infusion. All animals were killed immediately after the end of the 15-min Cereport infusion. To further control between-group variability, equal amounts and concentrations of the radiolabeled tracer were carefully given (on a μCi/kg basis) over a standardized 3-s bolus infusion to help assure a consistent plasma area under the curve between rats. Accordingly, the increase in tumor permeability is comparably affected by Cereport whether using nCi/g or the uptake constant (K_i) as the dependent measure (e.g., see Elliott et al., 1996a). Body temperature, blood pressure, blood gases, and blood pH were maintained within normal ranges throughout the drug administration period. Rats outside these ranges (approximately 10% of the animals) were not included in the carboplatin tumor permeation study.

Quantitation of ^{14}C-labeled Carboplatin Levels

At the end of the drug administration protocol, rats were decapitated and the brain was rapidly removed. The brain was cut longitudinally along the midline, and the tumor was carefully dissected free. An equal amount of tissue was removed from the contralateral striatum. Cortical tissue was taken from both ipsilateral and contralateral hemispheres. Each tissue sample was then weighed and placed into a scintillation vial containing 1 ml of Solulene-350 (Packard Instrument Co., Downers Grove, Ill.) and 10 ml of Hionic-Flor (Packard Instrument Co.). The levels of [^{14}C]carboplatin radioactivity were then computed (as nCi/g of tissue) for each region using liquid scintillation counting.

Results

Levels of ^{14}C-labeled Carboplatin in Tumor and Brain

The effects of dexamethasone treatment and Cereport infusion on carboplatin levels were compared with the effects of saline in the tumor region using two-way analysis of variance. No interaction between treatment (Cereport/vehicle) and time after last dose of dexamethasone (1, 14, or 24 h) on changes in levels of carboplatin in tumor was seen ($P > 0.6$, data not shown). The data for each time point were, therefore, pooled. Pretreatment with dexamethasone for 3 days resulted in a statistically significant decrease in carboplatin levels in tumor (19%) compared with vehicle-treated animals ($P < 0.05$, one-tailed, based on the directional hypothesis and abundant empirical support that dexamethasone decreases permeability). The modest decrease in the ability of carboplatin to enter the tumor caused by dexamethasone did not impact the ability of Cereport to increase levels of radiolabeled carboplatin in tumor (Fig. 1; Table 1). Statistical analysis revealed that Cereport increased carboplatin levels in both the dexamethasone-treated (46%) and non–dexamethasone-treated animals (49%) ($P < 0.05$, two-tailed t test) and that the difference in the effect of Cereport in the two conditions was comparable ($P > 0.10$).

There were no effects of either dexamethasone or Cereport on carboplatin permeation of any other brain regions examined ($P > 0.10$; Table 1), demonstrating that under these dosing conditions, the effect of these drugs on enhancing carboplatin levels is specific to the tumor.

Tumor Weight

Dexamethasone treatment decreased the weight of the previously implanted striatal tumor by 28% compared with tumors from vehicle-treated rats (8.927 mg ± 0.001 SEM versus 6.452 mg ± 0.004 SEM; one-way analysis of variance, $P < 0.015$).

Body Weight

An analysis of variance with repeated measures revealed that after 3 days of oral dexamethasone administration (1.5 mg/kg/day, given twice daily), the tumor-implanted rats lost an average of approximately 10% of their body weight (195.8 g ± 2 SEM to 176.5 g ± 2.0 SEM; $P < 0.007$, two-tailed t test). In contrast, the vehicle-treated

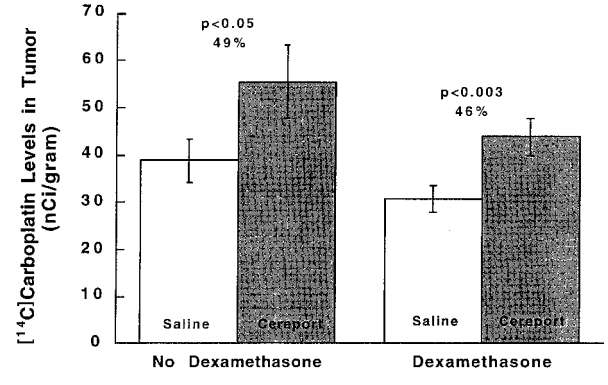

Fig. 1. Lack of interaction between dexamethasone and Cereport. Three days of oral dexamethasone pretreatment (1.5 mg/kg/day) decreased levels of radiolabeled carboplatin in brain tumor by 19%. Intracarotid infusion of Cereport (4.5 μg/kg) enhanced carboplatin levels in the tumor by nearly the same percentage regardless of whether the animals were pretreated with dexamethasone (46%) or not (49%). Data are shown as mean ± SEM.

Table 1. Effect of Cereport and dexamethasone on regional levels of [^{14}C]carboplatin

Treatment	*n*	Tumor (nCi/g ± SEM)	Contralateral striatum (nCi/g ± SEM)	Ipsilateral cortex (nCi/g ± SEM)	Contralateral cortex (nCi/g ± SEM)
Vehicle					
Saline	6	37.2 ± 5.1	12.1 ± 0.9	12.4 ± 1.7	12.3 ± 1.1
Cereport	9	55.5 ± 6.1*	13.1 ± 1.9	14.3 ± 2.4	12.9 ± 2.1
Dexamethasone					
Saline	28	30.1 ± 2.8	14.0 ± 1.5	14.4 ± 1.6	14.2 ± 1.6
Cereport	28	44.0 ± 3.7*	13.5 ± 1.0	15.2 ± 1.5	14.0 ± 0.7

Abbreviations: SEM, standard error of the mean.

*Significantly different from saline-treated animals, $P < 0.05$.

tumor-bearing animals remained at the same weight (197.2 g ± 2.6 SEM to 198.8 g ± 3.0 SEM; $P > 0.1$).

Discussion

Gliomas are the most common form of primary brain tumor and are nearly always fatal (Preston-Martin, 1999). Chemotherapy currently does little to improve the prognosis of glioma patients because, in part, the BBTB restricts diffusion of blood-borne substances into the tumor. The B_2 receptor bradykinin agonist Cereport was developed to increase levels of chemotherapeutic agents into brain tumor and surrounding tissue by enhancing permeability of the BBTB (Bartus et al., 1996a, b; Bartus, 1999). Cereport selectively enhances permeability in the tumor and proximal tissue in rodent models of glioma (Bartus et al., 1996a, b; Elliott et al., 1996a, b; Inamura et al., 1994) and enhances survival as compared with carboplatin alone (Matsukado et al., 1996). Results in the clinic confirm these findings, indicating enhanced, selective uptake of agents into tumor with Cereport (Black et al., 1997; Ford et al., 1966) and preliminary evidence of improved patient outcome when combined with carboplatin (Black et al., 1997; Cloughesy et al., 1999; Gregor et al., 1997).

In addition to the cytotoxic agents intended to kill tumor cells, neuro-oncology patients typically receive other drugs to help manage their symptoms. Corticosteroids, in particular dexamethasone (Decadron), are routinely administered to improve the neurological symptoms that result from the elevated intracranial pressure produced by the brain tumor (reviewed in Glaser et al., 1997; Gutin, 1977; Koehler, 1995; Vecht and Verbiest, 1995). Ironically, while improving the symptoms, dexamethasone may also lower delivery of chemotherapeutic agents into brain tumor tissue by decreasing BBTB permeability (Koehler, 1995; Yamada et al., 1983). The present study was conducted in a well-established syngeneic rat glioma model to determine whether dexamethasone significantly reduces the ability of carboplatin to enter the tumor, as well as whether it impairs the ability of Cereport to further increase levels of carboplatin in the tumor.

The most efficacious clinical dose of dexamethasone, as measured by improved neurological symptoms and mental status, varies between patients (Renaudin et al., 1973). Most neuro-oncology patients are treated with the empirically determined dosage of 4 mg given 4 times a day (French and Galicich, 1964), and the dosage is then titrated up or down depending on the patient's response to dexamethasone. However, some patients require a daily dosage as high as 100 mg to achieve clinical improvement (Lieberman et al., 1977; Renaudin et al., 1973). In the present study, the animal dosage of dexamethasone selected (1.5 mg/kg/day) approximated the 100 mg human dosage on a mg/kg/day basis. In an effort to test a clinically meaningful dosing schedule, the dose of dexamethasone was distributed across two oral administrations per day (12 h apart). Based on the prolonged plasma and biological half life of dexamethasone (Posner, 1992; Vecht and Verbiest, 1995), this dosing regime should have produced sustained effects of dexamethasone throughout the experiment.

In the present study, three days of dexamethasone treatment resulted in a modest but significant decrease in the ability of carboplatin to permeate the BBTB, reflected by a 19% reduction in the level of radiolabeled carboplatin in the tumor. Cereport significantly increased the ability of carboplatin to permeate the BBTB in both the non–dexamethasone- and dexamethasone-treated animals. Although the absolute effect of Cereport was reduced by dexamethasone, the relative effect on carboplatin levels (compared with the appropriate vehicle-treated rats) was equivalent. Because it has consistently been shown that Cereport increases delivery of agents to tumors by enhancing permeability of the BBTB and not by concentration-dependent diffusion (Bartus et al., 1996a, b; Elliott et al., 1996a, b; Inamura et al., 1994; Matsukado et al., 1996), it seems reasonable to conclude that Cereport maintained its effects on the BBTB in the presence of dexamethasone. This outcome is particularly noteworthy because the effects of Cereport in this study (even without dexamethasone) were lower than the changes typically observed. Most studies using intracarotid infusion of Cereport achieve approximately a twofold increase in uptake (e.g., Bartus et al., 1996a; Elliott et al., 1996b; Inamura et al., 1994; Matsukado et al., 1996). Even though, by chance, Cereport only increased carboplatin levels by 50% in the present study, it was still able to overcome the effects of dexamethasone on the BBTB.

Dexamethasone is known to exert a number of other independent biological effects. Preclinical studies using multiple administrations of dexamethasone in acute rodent models of brain tumors are often complicated by loss of body weight (Luthert et al., 1986; Tonolo et al.,

1988) and a decrease in tumor size (Luthert et al., 1986; Matsukado et al., 1997; Reichman et al., 1986; Straathof et al., 1998; Tjuvajev et al., 1996; Tonolo et al., 1988; Wolff et al., 1993). In the present study, despite using a dose and dosing schedule designed to mimic the clinical situation, similar confounding effects were observed, with dexamethasone decreasing body weight by 10% and tumor mass by 28% (although the reduction in body mass did not affect plasma concentrations of the radiolabel and, therefore, did not confound the permeation of carboplatin into the tumor). The results reported here are consistent with and build upon the observations of Matsukado, et al. (1997), the only other group to study the interaction of dexamethasone and Cereport on tumor permeability. Using an allogeneic rat glioma model with a daily dosing schedule consisting of 3 bolus injections of a relatively high dose of dexamethasone (3 mg/kg), a 39% decrease in tumor size compared with vehicle-treated animals was reported (apparently greater than the changes seen in the present study; changes in body weight were not presented in the prior study). Following intracarotid Cereport infusion, a 92% increase in tumor uptake was reported despite a decrease (26%) in the endogenous permeability of the tumor after pretreatment with dexamethasone. This effect of Cereport was nonetheless 78% lower than in animals not pretreated with dexamethasone, an outcome that differed from the present study.

The present study differed methodologically from the initial study of Matsukado et al. (1997) in several potentially important ways. First, a syngeneic rat model was used to reduce the likelihood that a potential immune response to the tumor would confound the effects of the steroid at the tumor site. Second, recognizing the complexity of the pharmacological effects of dexamethasone, which vary by dose and route, we used dexamethasone orally in conjunction with a dose and treatment schedule to reflect more closely the dexamethasone dosing schedule used in the clinic to treat cancer patients (Glaser et al., 1997; Gutin, 1977; Koehler, 1995; Vecht and Verbiest, 1995). We also determined the effects of dexamethasone on the BBTB over several time points. Despite these methodological differences, similar, although not identical, effects of both dexamethasone and Cereport on the BBTB were seen in these studies. Taken together, they demonstrate that Cereport increases the ability of carboplatin to permeate the BBTB after pretreating with dexamethasone. However, the absolute effect of Cereport on the BBTB is dampened compared with non–dexamethasone-treated animals.

In summary, the results of this study demonstrate a significant decrease in tumor carboplatin levels after an oral dexamethasone protocol designed to closely reflect clinical practices (twice daily for 3 days); no apparent changes to normal (nontumor) brain tissue; a reduction in the absolute effects of Cereport (compared with non–dexamethasone-treated rats), but no difference in the relative increase in carboplatin achieved with Cereport (i.e., when compared with rats given dexamethasone and vehicle). Thus, while Cereport increased the ability of carboplatin to permeate the BBTB following dexamethasone pretreatment, the total amount of carboplatin delivered to the tumor was reduced (compared with non–dexamethasone-treated Cereport animals). This was simply due to a decrease in baseline permeation of carboplatin induced by dexamethasone pretreatment. Together these data provide additional support for the use of Cereport as an adjunct therapy to increase delivery of chemotherapeutic agents to brain tumors. Furthermore, they suggest that future clinical protocols attempt to carefully minimize patient exposure to dexamethasone prior to delivering chemotherapeutic agents to brain tumors.

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