Convection-enhanced Delivery of Boronated Epidermal Growth Factor for Molecular Targeting of EGF Receptor-positive Gliomas

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ABSTRACT

Convection enhanced delivery (CED) is potentially a powerful method to improve the targeting of macromolecules to the central nervous system by applying a pressure gradient to establish bulk flow through the brain interstitium during infusion. The purpose of the present study was to evaluate CED as a means to improve the intracerebral and intratumoral (i.t.) uptake of a heavily boronated macromolecule (dendrimer; BD) linked to epidermal growth factor (EGF) for neutron capture therapy in rats bearing a syngeneic epidermal growth factor receptor (EGFR) + glioma. Boronated EGF was radiolabeled with 125I and administered by CED at a rate of 0.33 μl/min for 15, 30, and 60 min [infusion volumes (Vd) of 5, 10, and 20 μl, respectively], using a syringe pump connected to an indwelling cannula implanted into the right caudate nucleus of normal rats or i.t. in rats bearing either F98EGFR or F98 wild-type (F98 WT) gliomas. After infusion, rats were euthanized, and their brains were removed and serially sectioned. The uptake and biodistribution of 125I-boronated EGF in tumor or brain was studied by quantitative autoradiography and γ-scintillation counting. The volume of distribution (Vd) in brain was assessed using a computer interfaced image analysis system. After CED, the Vd increased from 34.4 to 123.5 μl with corresponding Vd ranging from 5 to 20 μl. The Vd of BD-EGF in the brain was 64.8 ± 13.4 μl with CED (V10 μl), and the Vd/Vi ratio was 6.5 compared with a Vd of 9.4 ± 1.6 μl and a Vd/Vi ratio of 0.9 after direct intracerebral injection. As determined by quantitative autoradiography and γ-scintillation counting at 24 h after CED, 47.4% of the injected dose per gram tissue (%ID/g) was localized in F98EGFR gliomas compared with 33.2%ID/g after direct i.t. injection and 12.3%ID/g in F98 WT gliomas. On the basis of these observations, we have concluded that CED is more effective than i.t. injection as a way to deliver boronated EGF to EGFR (+) gliomas for boron neutron capture therapy.

INTRODUCTION

BNCT is based on the selective delivery of a sufficient amount of nonradioactive 10B to tumor cells, followed by irradiation with low energy (0.025 ev) thermal neutrons, to produce high linear energy transfer α particles and recoiling 7Li nuclei. Each component of this binary system can be manipulated independently, so that the interval between administration of the 10B-containing agent and neutron irradiation can be adjusted to an optimal time at which the differential between 10B concentrations in normal tissues and tumor are maximized. In order for BNCT to be successful, there must be selective accumulation of 10B in the tumor (~20 μg/g); low levels in blood, endothelial cells, and normal brain; and a sufficient fluence of thermal neutron must be delivered to the tumor site. Interested readers are referred to several recent reviews and monographs that discuss these requirements in detail (1–4).

We have been interested in the possibility of using combinations of two LMW drugs, BPA and BSH (5, 6), together with HMW-targeting agents such as boronated monoclonal antibodies (7, 8) and EGF (9, 10) for BNCT of gliomas. One of the major challenges in treating high-grade brain tumors with BNCT is how to deliver the required amount (~100 atoms/cell) of the 10B-containing agents to individual tumor cells to sustain a lethal 10B(n, α)7Li capture reaction. There is a broad consensus of opinion that the blood-brain and blood-tumor barriers significantly limit the movement of a wide variety of therapeutic agents from the vascular compartment into the tumor and brain tissue around the tumor (11–14). The development of new classes of therapeutic agents, such as monoclonal antibodies and other receptor-targeting bioconjugates (15, 16), introduces a new set of problems for drug delivery to the brain. Because these agents are of HMW, this even more severely restricts their passage from the vascular compartment and their entry into brain tumors. Therefore, delivery methods that bypass the BBB and introduce agents directly into the extravascular space of the CNS have been used increasingly over the past decade (15–21). These approaches, all of which bypass the BBB, include interstitial (15–17), intrathecal (18), or direct i.t. injection (19, 20), the use of implantable, biodegradable, drug releasing polymers (21), and intracavitary instillation into the resection site of the tumor (15, 16).

CED, by which agents are directly infused into the CNS, is an innovative method to increase drug uptake and distribution (22–30). Under normal physiological conditions, interstitial fluids move through the brain both by convection and diffusion. Diffusion of a drug depends upon its molecular weight, ionic charge, and concentration gradient within normal tissue and the tumor. The higher the molecular weight, the more positively charged the ionic species, and the lower the concentration, the slower the rate of diffusion (31). The slow diffusion of macromolecular agents within the brain, tumor, and brain around the tumor severely limits their distribution after direct i.c. administration (22). For example, after rapid i.t. injection of IgG into a tumor, distribution occurs primarily by diffusion and requires 3 days to diffuse 1 mm from the point of injection (32). However, unlike diffusion, convection or “bulk” flow results from a pressure gradient and is independent of the molecular weight of the substance. CED potentially can improve the targeting of both LMW and HMW agents to the CNS by applying a pressure gradient to establish bulk flow during interstitial infusion (22–24) in order to increase the volume of distribution (25). CED potentially can be used to efficiently deliver drugs (25, 26) and toxins (27) to large regions of the brain and spinal cord. 

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cord (28) without significant functional or structural damage, and can produce a more homogenous dispersion of the agent at higher concentrations than otherwise might be attainable.

We have reported previously that direct i.t. injection of a heavily boronated starburst dendrimer linked to EGF specifically targeted two genetically engineered, EGFR (+) rat brain tumors, the EGFR gene transfected subline of the rat C6 glioma (10, 33, 34) and F98EGFR gliomas (35), and that unreacted reagent was removed using a Bio-Spin P-6 column (Bio-Rad Laboratories, La Brea, CA), as described by us elsewhere (36). Fractinations containing peak concentrations of both protein and boron were pooled and used in the studies described in the following section.

Preparation and Purification of Boronated EGF Bioconjugate. A fourth-generation polyamido amino (“PAMAM”) dendrimer, called previously a “starburst” dendrimer (Dendritech, Midland, MI), was boronated with a methylviosocyanate substituted polyhedral boron anion, [Na(CH2)2,NBu3H2NCO]- to yield BD using a procedure described in detail by us elsewhere (9). The BD then was reacted with N-succinimidyl 3-(2-pyridyldithio) propionate, and the resulting product was cleaved with DTT to yield a sulphydryl containing BD. EGFR was derivatized with the heterobifunctional reagent m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester and linked to sulhydryl-containing BD to yield a BD-EGF bioconjugate (9). This was purified by column chromatography using a Sephadex G-50 column and eluted with 0.1 M TRIS and 0.2 M NaCl buffer (pH 8.5). One-ml fractions were collected, and protein concentrations were determined spectrophotometrically by measuring absorbance at 280 nm using a Beckman DU-6 spectrophotometer (Beckman Instruments, Inc., Irvine, CA). Boron was quantified by direct current plasma-atomic emission spectroscopy using a Spectrasonics VB spectrometer (Applied Research Laboratories, La Brea, CA), as described by us elsewhere (36). Fractions containing peak concentrations of both protein and boron were pooled and used in the studies described in the following section.

Radiodiodination of BD-EGF. The BD-EGF bioconjugates were reacted with Bolton-Hunter reagent (Pierce Chemical Co., Rockford, IL) to introduce a phenolic function into the bioconjugate. Briefly, a 10-fold molar excess of Bolton-Hunter reagent was added to BD-EGF and cooled on ice for 1 h after which unreacted reagent was removed using a Bio-Spin P-6 column (Bio-Rad Laboratories, Hercules, CA). BD-EGF then was radiiodiodinated with 125I-NaI by the procedure described by us (7) using 2 mg/ml of chloramine-T in 0.5 M phosphate buffer (pH 7.5; ICN Biomedicals, Inc., Costa Mesa, CA). 125I-labeled BD-EGF was shown to be stable and was not dehalogenated for at least 1 week when kept at 4°C.

CED of BSD-EGF. CD-Fischer rats (Charles River Laboratories, Wilmington, MA), weighing 200–220 g, were anesthetized with a 1.2:1 mixture of ketamine/xylazine at a dose of 120 mg of ketamine/20 mg xylazine per kg of body weight. After this, the animal was placed in a stereotactic headframe (David Kopf Instruments, Tujunga, CA), a skin incision was made in the midline of the head, and the underlying skull was exposed. A burr hole was drilled 0.5 mm anterior to and 2.5 mm to the right of bregma, and a small plastic screw (Arrow Machine Manufacturing, Inc., Richmond, VA) was embedded into the skull. For CED a plastic cannula was inserted stereotactically into the entry port of the plastic screw and was advanced 5 mm below the dura into the right caudate nucleus of nontumor-bearing animals or into the tumor of glioma-bearing rats. To distribute BD-EGF into the brain (or tumor) by CED, we developed a noncompliant delivery system that was gas-tight with no dead volume. A syringe pump (Harvard Apparatus Co., Cambridge, MA) was used to generate continuous pressure throughout the infusion, during which pressure was transmitted from the pump to a gas-tight, infusate-filled 25-μl Hamilton syringe by a hydraulic drive. This consisted of a water-filled polyetheretherketone (PEEK) tubing (inner diameter 0.020 inches and outer diameter 0.062 inches) attached at either end via a flangeless low-pressure union to a 250 μl gas-tight syringe. One syringe was placed in the pump and the other syringe transmitted the fluid pressure directly to the plunger of a second gas-tight Hamilton syringe that delivered the BD-EGF (24, 29).

125I-labeled BD-EGF was diluted with PBS to yield a concentration of 5 μCi/10 μg BD-EGF/10 μl. Three μl of Evans blue dye (4 mg/ml) were added to every 100 μl of 125I-BD-EGF solution so that the site of infusion subsequently could be visualized grossly within the brain parenchyma during animal preparation. Nontumor-bearing (i.e., normal) rats were divided into four groups of animals as follows: Group 1 received an i.c. injection of 5 μCi/10 μg BD-EGF/10 μl; groups 2, 3, and 4 received BD-EGF by CED at a rate of 0.33 μl/min for 15, 30, or 60 min with corresponding injection volumes of 5, 10, and 20 μl, respectively. The infusion rates and injection volumes in the brains of nontumor-bearing rats are summarized in Table 1.

Quantitative Autoradiographic Analysis of Distribution of 125I-BD-EGF in Normal Brain. Rats were euthanized immediately after or 12 h after infusion, and their brains were removed and frozen in isopentane (2-methylbutane), which had been cooled to approximately −150°C in liquid nitrogen, and then stored at −70°C until sectioning. Brains were cut coronally at 1-mm intervals rostral and caudal to the point of insertion of the cannula. Five serial sections from each coronal slice were cut on a cryostat (Miles Scientific, Naperville, IL) at a thickness of 20 μm each. One of the five sections was stained with H&E for histological examination. The percentage of uptake of radioactivity was quantified in two sections by γ-scintillation counting for 125I using a well counter (model 1185; Tm Analytic, Elk Grove Village, IL). The remaining two sections were processed for QAR by exposing them to either NTB-2 dipping emulsion or X-ray stripping film. After a 16-h exposure, the autoradiographs subsequently were developed.125I standards for QAR were prepared from homogenized brain at concentrations ranging from 0.01 to 0.8 relative to the 125I concentration of the infusate (22). The autoradiographs subsequently were analyzed by quantitative densitometry using a Macintosh-based computer image analysis system (Image 1.5, kindly provided by the NIH, Bethesda, MD via the Internet) using a Rodbard function to fit the optical densities of the radioactivity standards with known tissue equivalents (29). The Wilcoxon rank-sum test in SAS version 8-02 (SAS Institute, Cary, NC) was used to calculate exact P values for tumor uptake of 125I-BD-EGF.

Determination of Volume of Distribution. The volume of distribution was defined as the tissue volume in which the local concentration of the infused 125I-BD-EGF relative to concentration of the infusate uniformly equaled or exceeded an arbitrary fraction (>1%) of the concentration of the infusate (29). To define the boundaries of infusion, a threshold equal to ~15% of the maximum tissue equivalent was used. The Vd was estimated by multiplying the area of perfusion, as measured by computer analysis, by the distance between sections and summing across all of the slices (29). The percent

<table>
<thead>
<tr>
<th>Group number and route</th>
<th>Time (min)</th>
<th>Volume (Vd) mm³</th>
<th>Rate (ml/min)</th>
<th>Distribution volume (Vd) mm³</th>
<th>Vd/Vi ratio</th>
<th>% Recovery of infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 i.c.</td>
<td>2</td>
<td>10.0</td>
<td>5.0</td>
<td>9.4 ± 1.6</td>
<td>0.9 ± 1.6</td>
<td>86.6 ± 5.2</td>
</tr>
<tr>
<td>2 CED</td>
<td>15</td>
<td>5.0</td>
<td>5.0</td>
<td>34.4 ± 0.3</td>
<td>6.9 ± 1.2</td>
<td>81.5 ± 8.6</td>
</tr>
<tr>
<td>3 CED</td>
<td>30</td>
<td>10.0</td>
<td>0.33</td>
<td>64.8 ± 13.4</td>
<td>6.5 ± 1.4</td>
<td>82.6 ± 2.3</td>
</tr>
<tr>
<td>4 CED</td>
<td>60</td>
<td>20.0</td>
<td>0.33</td>
<td>123.5 ± 19.2</td>
<td>6.1 ± 1.0</td>
<td>88.6 ± 7.5</td>
</tr>
</tbody>
</table>

* Volume of distribution was determined by QAR, and % recovery was determined by γ-scintillation counting in groups of 4 animals each, and the means and SDs of Vd, the Vd/Vi ratio, and % recovery were calculated.

Table 1 Vd of 125I-BD-EGF after i.c. injection or CED into normal rat brain
recovery was determined by obtaining the cpm of $^{125}$I in the tissue sample using a Tru Analytic $\gamma$ scintillation counter. To determine the total amount of radioactivity in the five coronal sections, three of which had been used for other purposes, the amount of radioactivity (cpm) in the remaining two sections were multiplied by 2.5 to determine the total amount of radioactivity within the tissue volume. To calculate the total amount of radioactivity delivered, a 1 $\mu$l calibration sample was collected at the end of each infusion and cpm were determined and multiplied by the infusion volume. The percentage of recovery was determined by dividing the total radioactivity of the recovered infusate within the tissue by the calculated total amount of radioactivity delivered. Homogeneity of delivery was determined from autoradiographs made from coronal sections of the brains of animals that had received $^{125}$I-BD-EGF, and cross-sectional concentration profiles were generated. The tissue concentration of $^{125}$I-BD-EGF, in sequential 0.1-mm increments, was determined by converting the absorbance of the infused regions of the autoradiographs to tissue equivalents ($\mu$Ci/g tissue) by using appropriate $^{125}$I standards and the NIH Image analysis software. Tissue uptake of $^{125}$I-BD-EGF was determined by gamma counting of weighed samples of blood, liver, kidney, muscle, and skin from each animal.

**F98 EGFR Glioma Model and in Vivo Studies.** The F98 EGFR glioma model has been described recently by us in detail (35). This was produced by transfecting the parental F98 glioma cells (37) with an expression vector containing EGFR cDNA (35). F98EGFR cells expressed $5 \times 10^5$ EGFR sites per cell compared with an undetectable number of EGFR on F98 WT cells and the affinity constant ($K_a$) for EGF was $8 \times 10^7$ M$^{-1}$. As described in detail elsewhere (5), $10^7$ F98WT or F98EGFR glioma cells in a 10 $\mu$l volume were stereotactically implanted into the right hemisphere of CD-Fischer rats. The rats were weighed three times per week to monitor their clinical status after tumor implantation. Twelve to 14 days later, when clinical signs of a progressively growing i.c. tumor were evident (weight loss, lethargy, hunching, and ataxia), the rats were divided into four experimental groups consisting of 8–10 animals each. Animals in groups 1 and 3 had F98EGFR gliomas and those in groups 2 and 4 had F98WT tumors. Rats in groups 3 and 4 received an i.t. injection of $^{125}$I-labeled BD-EGF (5 $\mu$Ci/10 $\mu$l) and, those in groups 1 and 2 received an equal volume of $^{125}$I-BD-EGF, delivered by CED over 30 min at a rate of 0.33 $\mu$l/min. Both i.t. injection and CED were carried out via the central hole of the plastic screw, which had been embedded into the calvarium. Ten $\mu$l volumes of all test agents were injected by a 25 $\mu$l Hamilton syringe with a 28-gauge needle equipped with a plastic collar to limit the depth of the needle point to the same as that used for tumor implantation. The biodistribution of $^{125}$I-BD-EGF was studied at 6 and 24 h after administration. Animals were euthanized by an overdose of halothane. Tumors and normal tissues, consisting of brain, blood, liver, kidney, and muscle were removed and weighed, and tissue and organ uptake of $^{125}$I was determined by $\gamma$-scintillation counting for $^{125}$I-BD-EGF. Each tissue sample was counted together with triplicate samples of the injectate to correct for decay of the isotope before gamma counting. Some brains were sectioned for autoradiographic analysis. Boron concentrations in tumor, brain, and other normal tissues were determined by direct current plasma-atomic emission spectroscopy (36).

**RESULTS**

**Distribution of $^{125}$I-BD-EGF in Normal Brain.** The $V_d$ of $^{125}$I-labeled BD-EGF after i.c. injection or CED in normal (i.e., nontumor-bearing) rat brains is summarized in Table 1 and shown graphically in Fig. 1. The $V_d$ of the infusate, which contained >1% of the amount of $^{125}$I-BD-EGF that was infused, doubled with doubling increments of $V_i$ (Fig. 1). The $V_d$ were 34.4, 64.8, and 123.5 $\mu$l after CED, and the corresponding $V_i$ were 5, 10, and 20 $\mu$l, respectively (Table 1). The $V_d$ after i.c. injection of 10 $\mu$l was 9.4 $\mu$l, and the $V_d$/$V_i$ ratio was 0.9 compared with 6.9, 6.5, and 6.1 for CED. CED resulted in a 6.9-fold increase in $V_d$ compared with that attained after i.c. injection (Table 1). These differences were clearly evident in a series of autoradiographs that were made from coronal section of brains from nontumor-bearing rats (Fig. 2). As determined by quantitative densitometry, immediately after completion of CED of 20 $\mu$l of $^{125}$I-BD-EGF for 60 min ~80% of the rat gray matter (~25% of the infused hemisphere) had received >1% of the infusion concentration (Fig. 2). The mean percentage of radioactivity recovered from the brain was >80% (81.5, 82.6, and 88.6%) for $V_i$ of 5, 10, and 20 $\mu$l, respectively (Table 1). The $V_d$ in animals euthanized 12 h after CED or i.c. injection was slightly increased over those determined immediately after CED or i.c. injection (Fig. 3). The concentrations of the biocompound in normal brain, as determined by QAR, were homogeneous (Fig. 4). Microscopic examination of the brains of nontumor-bearing animals that had undergone CED did not reveal any acute neuropathological changes along the path of the infusion cannula or in the remainder of the infused cerebral hemisphere.

**Distribution of $^{125}$I-BD-EGF in F98EGFR Glioma-bearing Rats.** Radiolocalization of $^{125}$I-labeled BD-EGF after i.c. injection or CED into either F98EGFR or F98WT glioma-bearing rats is summarized in Table 2. As determined by $\gamma$-scintillation counting of individual animals, between 1 and 6 h after i.t. injection or CED, 60–80% ID/g of BD-EGF was nonspecifically localized in F98EGFR gliomas, and the differences between the two groups were not statistically significant (Fig. 5). However, by 24 h after CED 47.4% ID/g was localized in F98EGFR gliomas compared with 33.2% ID/g after i.t. injection and...
The CED and i.t. groups were highly significant (P < 0.008), as were differences between the F98 EGFR and F98 WT groups. The amount of radioactivity in the ipsilateral (tumor-bearing) and contralateral (nontumor-bearing) cerebral hemispheres were similar (6.5–10.1% ID/g) after CED and i.t. injection, irrespective of whether animals had F98 EGFR or F98 WT tumors, although the highest tumor to brain ratio was observed after CED in F98 EGFR glioma-bearing rats. Similar results were observed by us (38) after CED of a monoclonal antibody, L8A4, directed against EGFRvIII (39), which has a more restricted pattern expression on high grade gliomas (40). As reported

12.3% ID/g in F98 WT gliomas (Fig. 5). Using the Wilcoxon paired rank-sum test to calculate exact P values, the differences between the CED and i.t. groups were highly significant (P = 0.008), as were differences between the F98 EGFR and F98 WT groups. The amount of radioactivity in muscle, liver, skin, and kidneys after CED were <1% of the injected dose for all of the infusion volumes and times after infusion. Autoradiographs clearly demonstrated that CED was far superior to i.t. injection as a means to distribute 125I-BD-EGF within the tumor (Fig. 6). Although the brains of glioma-bearing rats showed significant tumor-related neuropathologic changes, none appeared to be related specifically to insertion of the cannula. Tumor weights ranged from 160–300 mg and, as determined from H&E-stained coronal sections, tumor sizes ranged from ~3 to 5 mm in diameter.

### Discussion

In the present study, we have shown that CED can significantly increase the V_d of BD-EGF in both normal brain and in F98 EGFR glioma-bearing rats compared with those attainable by either i.c. or i.t. injection. The V_d after CED of 10 μl of 125I-BD-EGF in normal rat brain was increased 7.2-fold over i.c. injection. As determined by QAR, CED of 20 μl of 125I-BD-EGF for 60 min resulted in a sharply delimitated spherical region within the gray matter that contained a high concentration of BD-EGF. As determined by γ-scintillation counting 24 h after CED, the uptake of 125I-BD-EGF in tumor was 1.5 times greater than that observed after i.t. injection. There was a 3.9-fold increase in the amount of radioactivity localized in F98 EGFR compared with F98 WT gliomas after CED and a 3.4-fold increase in F98 EGFR compared with F98 WT after i.t. injection, thereby establishing that there was specific molecular targeting of the bioconjugate.

The amount of radioactivity in the ipsilateral (tumor-bearing) and contralateral (nontumor-bearing) cerebral hemispheres were similar (6.5–10.1% ID/g) after CED and i.t. injection, irrespective of whether animals had F98 EGFR or F98 WT tumors, although the highest tumor to brain ratio was observed after CED in F98 EGFR glioma-bearing rats. Similar results were observed by us (38) after CED of a monoclonal antibody, L8A4, directed against EGFRvIII (39), which has a more restricted pattern expression on high grade gliomas (40). As reported

### Table 2: Radiolocalization of 125I-BD-EGF in rats bearing F98EGFR or F98WT gliomas at 24 h after either i.t. injection or CED

<table>
<thead>
<tr>
<th>Group number</th>
<th>Route/tumor</th>
<th>Tumor</th>
<th>Brain (ipsilateral)</th>
<th>Brain (contralateral)</th>
<th>Liver</th>
<th>Blood</th>
<th>Brain IPAR</th>
<th>Brain CPAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CED/F98EGFR</td>
<td>47.4 ± 8.2</td>
<td>10.1 ± 7.9</td>
<td>3.9 ± 4.2</td>
<td>0.8 ± 0.8</td>
<td>0.8 ± 0.7</td>
<td>7.8 ± 5.5</td>
<td>19.1 ± 12.3</td>
</tr>
<tr>
<td>2</td>
<td>CED/F98WT</td>
<td>12.3 ± 3.6</td>
<td>6.5 ± 1.8</td>
<td>2.8 ± 1.1</td>
<td>0.8 ± 0.4</td>
<td>1.1 ± 0.3</td>
<td>2.1 ± 0.5</td>
<td>8.4 ± 5.7</td>
</tr>
<tr>
<td>3</td>
<td>i.t./F98EGFR</td>
<td>32.2 ± 6.6</td>
<td>9.4 ± 4.9</td>
<td>2.1 ± 1.4</td>
<td>0.9 ± 1.1</td>
<td>0.7 ± 0.1</td>
<td>3.5 ± 1.7</td>
<td>10.9 ± 4.6</td>
</tr>
<tr>
<td>4</td>
<td>i.t./F98WT</td>
<td>9.4 ± 1.0</td>
<td>7.1 ± 2.0</td>
<td>1.4 ± 0.8</td>
<td>1.0 ± 0.8</td>
<td>0.9 ± 0.3</td>
<td>1.7 ± 0.7</td>
<td>9.7 ± 7.5</td>
</tr>
</tbody>
</table>

* a 125I-BD-EGF (5 μCi/10 μg of EGF) was administered i.t. or by CED, and animals were euthanized 24 h later. Radioactivity was determined by γ-scintillation counting. Tissue uptake is expressed as % ID/g tissue, and each value represents the arithmetic mean ± SD of 4–5 rats. As determined by the Wilcoxon paired rank-sum test, the differences between the CED and i.t. groups were highly significant (P = 0.008), as were differences between the F98 EGFR and F98 WT groups.

* b The tumor:brain ratios were calculated for each individual animal after which then means ± SD were computed for both the ipsilateral (tumor-bearing) and contralateral (nontumor bearing) cerebral hemispheres.
previously by others (22–30) and confirmed by us, interstitial brain infusions were well tolerated and were not associated with hemodynamic instability during infusion. In a variety of experimental models (22–30), cerebral edema did not result in neurological dysfunction, as long as intracranial pressure was not appreciably elevated. During our study, microscopic examination of the brains of normal and F98EGFR glioma-bearing rats after CED showed the expected cerebral edema, and the only other change was disruption of tissue along the insertion track of the infusion cannula.

Although it was possible to determine $V_d$ after CED of $^{125}$I-BD-EGF to normal brain, which is a histologically homogeneous tissue, this could not be determined in F98EGFR gliomas. In contrast to normal brain, tumors showed considerable histological heterogeneity (41) with areas of actively growing or infiltrating tumor cells, necrosis, and frequently a cystic center, corresponding to the site where the glioma cells, suspended in gelatin containing medium, originally had been implanted. A single histological section would not have been representative of the tumor $V_d$, which was calculated by multiplying the area perfused by the distance (in $\mu m$) between sections and summing across all of the coronal sections. This would not have provided a true value for $V_d$ within the tumor and additional refinement of the image analysis system will be required to determine $V_d$ within the tumor. Nevertheless, our study has convincingly demonstrated that CED improved the uptake and distribution of a HMW receptor targeting agent in an experimental brain tumor model. There have been a number of reports on the use of CED, which also has been given a variety of other names (42–44), to improve the uptake of therapeutic agents in brain tumors. Kaiser et al. (45) have reported that CED (or as they have called it, “intracerebral clysis”) of topotecan to C6 glioma-bearing rats produced long-term survival in $>90\%$ of animals compared with death in $<4$ wks in animals that received i.p. administration of the drug. Similar results with topotecan have been reported by Pollina et al. (19) in nude rats bearing U87 gliomas and with temozolomide in nude rats bearing i.c. implants of the D54-MG human glioma (46).

Poor drug delivery has been one of the major causes for the disappointing therapeutic responses that have been observed after chemotherapy of brain tumors (47, 48). There is a paucity of data quantifying brain tumor uptake of cytoreductive chemotherapeutic agents (49, 50) and virtually none on their cellular distribution within brain tumors. In contrast, there is a large body of data in the BNCT literature on the uptake of BPA (41, 51–54) and BSH (55–59) in both human (51–53, 58, 59) and rat (41, 54–57) brain tumors. In large part this is because of the critical importance that tumor boron content has for calculating the radiation dose delivered to the tumor by the $^{10}$B(n, $\alpha$)$^7$Li capture reaction (2, 3). Data that we (53, 60, 61) and others (59) have obtained show that there is considerable variation in boron concentration within various regions of the same tumor or from subject to subject after i.v. or intra-arterial administration of BPA and BSH. This is the most likely explanation for the broad range in survival times from a modest increase to cure that has been observed in both the F98 glioma (5, 6, 41, 53, 54) and the MRA 27 melanoma models after BNCT (61). Intracarotid injection with or without BBB disruption significantly improved tumor boron concentration and cellular microdistribution (5, 6). CED might additionally improve both tumor uptake and microdistribution of LMW drugs, as well as HMW receptor targeting agents, such as BD-EGF, as shown in the present study, and boronated monoclonal antibodies (7) and liposomes (62, 63). Our ultimate goal is to use CED to improve tumor uptake and microdistribution of HMW, EGF targeting, and boron containing bioconjugates. Because BNCT is a binary system in which the interval between administration of the boron-containing agent and neutron irradiation can be optimized, this is especially advantageous when combined with CED.

Clinically, CED has been and is being used to deliver a variety of agents to patients after surgical resection of their brain tumors to eradicate residual infiltrative tumor cells (27). Between one and three catheters have been inserted into the resection cavity, and infusion volumes as high as 420 ml have been administered over a 3-week interval without any significant adverse effects.4,5 If CED were to be used clinically for the administration of boronated EGF or monoclonal antibodies directed against EGF (65) or a mutant isoform of the receptor, EGFRvIII (66), it probably would be carried out over a much shorter period of time. After this, there would be a break to allow for clearance of the bioconjugate from normal brain, and then BNCT would be initiated. A similar approach has been used by us for BNCT of F98EGFR glioma-bearing rats (35). We have reported recently that i.t. injection of BD-EGF, either alone or in combination with i.v. administration of BPA, to F98EGFR glioma bearing rats, followed by BNCT, resulted in a significant prolongation in survival times compared with those observed in animals bearing EGF (–) F98 WT tumors (35). This study provided proof-of-principle for targeting an EGF (+) tumor with a boronated bioconjugate and is paradigmatic for future studies using receptor targeting agents either alone or in combination with LMW drugs for BNCT. It also demonstrated that direct i.t. injection could not deliver the critical amounts of $^{10}$B to the tumor to achieve a cure and that more effective methods of delivery are needed.

CED may be especially useful for administration of receptor targeting macromolecules such as monoclonal antibodies and EGF. After localization of a drug in the brain interstitium, additional movement within the brain or tumor occurs by diffusion, and this significantly limits $V_d$. For HMW agents, there is even less diffusion within the brain or tumor relative to tissue clearance, which additionally reduces $V_d$. The small $V_d$ and steep concentration gradients associated with diffusion severely limit the effectiveness of diffusive drug delivery for the regional therapy of brain tumors. Studies by Boucher et al. (67) and Jain et al. (68) have demonstrated that the interstitial pressure and diffusion coefficients vary from one experimental tumor model to another, as well as within the tumor itself. This can produce signifi-

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4 E. H. Oldfield, personal communication.
5 C. J. Wikstrand, personal communication.
cent variations in diffusion-driven drug concentrations within the tumor (69). Although there still may be variations in drug concentrations within the tumor after CED, these should be much less than those that would occur after either systemic administration or direct i.t. injection. CED, which produces high-flow microinfusion with volumetric inflow rates of 0.33 μl/min for 30 min (23), for example, can deliver the same amount of agent to much larger volumes of brain and brain tumor than would otherwise be possible by direct interstitial injection, which has low-flow (diffusion) but a smaller Vd (70). The present study has shown that CED can improve the delivery of BD-EGF to much larger volumes of brain and tumor than could be achieved by i.t. injection with a significant pharmacodynamic advantage over systemic administration, where concentrations of HMW agents attain CNS concentrations that are only 0.01–0.0001% of the plasma concentration (34, 71). Our future studies on molecular targeting of gliomas expressing amplified EGFR using boronated bioconjugates for BNCT will use CED either alone or in combination with systemic administration of the LMW drugs BPA and BSH.

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Convection-enhanced Delivery of Boronated Epidermal Growth Factor for Molecular Targeting of EGF Receptor-positive Gliomas

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