Diffuse brainstem gliomas represent 10% to 15% of brain tumors in children and are universally fatal with a median survival of ~1 year (1–10). Clinical findings in patients with diffuse brainstem gliomas include ataxia, cranial nerve deficits, and long tract signs. Current therapy for brainstem gliomas, which includes radiation and chemotherapy, is palliative at best. Surgical excision is not possible due to the location and infiltrative nature of these tumors (11–21). Whereas a growing number of putative therapeutic compounds exist for treatment of diffuse brainstem gliomas, inadequate delivery using currently available techniques prevents their effective use.

Currently available potential techniques for brainstem drug delivery include systemic or intrathecal drug administration, which have a number of inherent limitations. Systemic delivery is restricted by systemic toxicity and the inability of many compounds to cross the blood-brain barrier. Penetration into the brainstem following intrathecal delivery relies on diffusion, which severely constrains tissue distribution and produces non-targeted, heterogeneous dispersion (22, 23). Due to the limitations of these delivery methods, potential therapeutic substances have remained ineffective in the treatment of brainstem gliomas.

Previous studies show that convection-enhanced delivery (CED) can be used to overcome many of the limitations of currently available delivery techniques (23–25). Because CED relies on the bulk flow of infusate that is driven by a small interstitial pressure gradient for distribution of compounds, it can be used to directly (bypassing the blood brain barrier) deliver small and large molecular weight substances and achieves homogenous distribution over clinically relevant volumes within the interstitial space of the central nervous system (CNS; refs. 26, 27).

Because of their large molecular size, poor diffusivity, and inability to cross the blood-brain barrier, promising antiangioma therapeutic proteins developed for the treatment of gliomas have not been successfully delivered to these tumors using conventional delivery techniques. Interleukin 13 bound to a Pseudomonas
Table 1. Rat toxicity infusion groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Animals</th>
<th>Infusion volume (µL)</th>
<th>IL13-PE (µg/mL)</th>
<th>Survival (postinfusion), d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>10</td>
<td>0.25</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>10</td>
<td>0.25</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>10</td>
<td>0.5</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>10</td>
<td>0.5</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>10</td>
<td>10</td>
<td>Vehicle</td>
</tr>
</tbody>
</table>

NOTE: Vehicle, 0.2% HSA in normal saline.

Toxin (IL13-PE), which is composed of a mutated *Pseudomonas* exotoxin fused to the human T-cell cytokine IL-13, has shown promising antitumor properties against gliomas (28–32). Although IL13-PE is a large (52 kDa) protein and does not penetrate the blood-brain barrier, it is selectively cytotoxic to glioma cells (31, 33) and is a potentially ideal agent for brainstem distribution and treatment of brainstem gliomas using CED.

To determine if the physical properties of CED permit effective drug distribution of IL13-PE in the brainstem, to examine the potential of monitoring the distribution of IL13-PE in the brainstem during co-infusion with gadolinium-bound albumin (Gd-albumin), and to examine the safety of perfusion of the brainstem via CED of a mixture of IL13-PE and Gd-albumin, we used CED to distribute IL13-PE and Gd-albumin in the brainstem of nonhuman primates during *in vivo* real-time magnetic resonance imaging (MRI).

Materials and Methods

Preparation of IL13-PE

Clinical grade IL13-PE (IL13-PE38QQR) was supplied by NeoPharm, Inc. (Lake Forest, IL) and stored at −80 °C before use. IL13-PE was diluted in normal saline with 0.2% human serum albumin (HSA; Sigma Chemical Co., St. Louis, MO) to the desired concentration before infusion.

Toxicity of IL13-PE in rat brainstem

All animal investigations were conducted in accordance with the NIH guidelines on the use of animals in research and were approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke.

Table 2. Primate infusion groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Animals</th>
<th>Infusion volume (µL)</th>
<th>IL13-PE (µg/mL)</th>
<th>Survival (postinfusion), d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>100</td>
<td>0.25</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>100</td>
<td>0.50</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>90</td>
<td>10</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>60</td>
<td>10</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>100, 200</td>
<td>0.5</td>
<td>No</td>
</tr>
</tbody>
</table>

NOTE: 125I-IL13-PE, radiolabeled IL13-PE used for quantitative autoradiography.
cavities including the viscera. Tissues were then embedded in paraffin, cut into 5-μm-thick sections, and processed to slides stained with H&E. Representative tissue sections were obtained from all major organ systems, including (but not exclusive to) the central nervous system, peripheral nervous system, thyroid gland, parathyroid glands, adrenal glands, kidneys, skeletal muscle, bladder, respiratory tract, lungs, digestive system, heart, aorta, liver, spleen, ovaries, and bone marrow. The brainstem (including the region of infusion and cannula tract) was divided into five blocks and 5-μm tissue sections were taken from each block and processed to slides stained with H&E. Tissue sections were analyzed by a blinded pathologist. Any alterations in the tissues were graded based on severity of changes on a scale of 1 (minimal or barely detectable) to 4 (marked/very extensive).

**Accuracy of Gd-albumin as a surrogate MRI tracer for IL13-PE**

**Preparation of Gd-bound albumin.** 2-(p-Isothiocyanatobenzyl)-6-methyl diethylenetriamine pentaacetic acid (1B4M)-diethylenetriamine pentaacetic acid (DTPA; refs. 37, 38) was conjugated to HSA by modification of a previously described method (39). Briefly, 100 to 150 mg of HSA were dissolved in 20 mL of 50 mmol/L sodium bicarbonate, 0.15 mol/L NaCl at pH 8.5. To this solution, 45 μg of 1B4M-DTPA dissolved in 1 mL H2O (initial ratio of ligand to HSA of 30) were then added. The reaction mixture was rotated at room temperature overnight. The unreacted or free ligand was then separated from HSA conjugate by centrifugation. The final ligand to HSA ratio (CL/HSA)f was determined spectrophotometrically (40). The final volume of the purified HSA-1B4M-DTPA was adjusted to deliver a concentration of ~10 μg/mL HSA.

Gd (III) was then reacted with the HSA-1B4M-DTPA at an initial 2:1 molar ratio (Gd/1B4M) using a standard solution of Gd (III) [Gd(NO3)3] 6.42 × 10−3 mol/L. The pH of the Gd (III) solution was adjusted to 4.5 to 5.0 using 5 mol/L NH4OAc and was added to HSA-1B4M-DTPA dropwise while mixing the reaction. The mixture was allowed to proceed for 5 to 6 hours at room temperature with rotation. The unreacted Gd (III) was then removed by adding 0.5 mL of 0.1 mol/L EDTA solution and then centrifugation. The final concentration of albumin was determined spectrophotometrically by measuring the absorbance at 280 nm. The percent of Gd (III) incorporation was determined by repeating the measurement of the number of chelating agents on the protein and noting the decrease due to their occupation by Gd (III). Each HSA molecule was linked to 5 Gd molecules. A stock solution of the Gd-albumin (28 mg/mL) in PBS was then infused into the animals.

**CED and MRI.** Two animals were each co-infused with 60 μL of Gd-albumin and 125I-IL13-PE as described previously in text (Table 2). Infusions were done at 0.5 μL/min and T1-weighted MR images were obtained in all three planes (slice thickness, 1-1.5 mm; 0 mm spacing) every 20 to 40 minutes during infusion using a 3-T MR scanner (total infusion and imaging time of 120 or 240 minutes for the 100- and 200-μL infusions, respectively).

**Clinical and histologic analysis.** Animals were observed daily for medical or neurologic difficulties following infusion (28-day observation). Animals were sacrificed at the completion of the observation period (Table 2) and perfused with buffered saline containing heparin followed by 10% formalin. A necropsy was done, which included an examination of the external surfaces, all orifices, and the cranial, thoracic, and pelvic cavities including the viscera. Tissues were then embedded in paraffin, cut into 5-μm-thick sections, and processed to slides stained with H&E. Representative tissue sections were obtained from all major organ systems, including (but not exclusive to) the central nervous system, peripheral nervous system, thyroid gland, parathyroid glands, adrenal glands, kidneys, skeletal muscle, bladder, respiratory tract, lungs, digestive system, heart, aorta, liver, spleen, ovaries, and bone marrow. The brainstem (including the regions of infusion and cannula tract) was divided into five blocks and 5-μm tissue sections were taken from each block and processed to slides stained with H&E. Tissue sections were analyzed by a blinded pathologist. Any alterations in the tissues were graded based on severity of changes on a scale of 1 (minimal or barely detectable) to 4 (marked/very extensive).

**Results**

**Toxicity of IL13-PE in rat brainstem.** None of the rats (IL13-PE concentrations of 0.25, 0.5, and 10 μg/mL) exhibited clinical deficits during short-term (3 days) or long-term (28 days) evaluation (Table 1). Histologic analysis of the brainstems revealed only minimal gliosis in the region immediately (a maximum radius of 25 μm) surrounding the infusion cannula track. There was no evidence of systemic organ toxicity.

**Toxicity of IL13-PE in primate brainstem.** Animals infused with IL13-PE at concentrations of 0.25 and 0.5 μg/mL had no...
Clinical or histologic evidence of toxicity (Fig. 1) over the observation period (Table 2). Brainstem histology revealed only minimal gliosis in the region immediately (a maximum radius of 50 μm) surrounding the infusion cannula track. There was no evidence of systemic toxicity.

Three of four animals receiving the highest concentration of IL13-PE (10 μg/mL; Table 2) developed clinical deficits, beginning 10 to 12 days after infusion. Clinical findings included left gaze preference, difficulty turning to left, and lethargy. These deficits necessitated early sacrifice of two animals (14 days postinfusion; Table 2). Histologic analysis of all four animals revealed necrosis and inflammation in the infused region. There was no evidence of systemic toxicity.

**Accuracy of Gd-albumin as a surrogate MRI tracer for IL13-PE.**

MRI of the imaging surrogate tracer, Gd-albumin, accurately tracked the distribution of 125I-IL13-PE (Fig. 2). Quantitative autoradiography of 125I-IL13-PE revealed that the MRI of the co-infused Gd-albumin overlapped precisely with the actual anatomic distribution and Vd of the 125I-IL13-PE. The mean difference between the Vd of IL13-PE predicted by MRI of Gd-albumin and actual 125I-IL13-PE distribution was 4.8% (Table 3), translating to a difference of <0.2 mm between the diameter of imaged infusate and the actual diameter of drug delivered (Fig. 2).

**Real-time, in vivo MRI of Gd-albumin and IL13-PE in primates.** Co-infusion of Gd-albumin and nonradiolabeled IL13-PE (concentration, 0.5 μg/mL) permitted safe and effective monitoring of IL13-PE distribution in the primate brainstem. Real-time imaging done during delivery showed that the anatomic region infused with Gd-albumin was clearly distinguishable from the surrounding noninfused tissue (Fig. 3). The pontine region surrounding the tip of the cannula steadily filled with infusate until the anatomic region was nearly filled with infusate (Fig. 4).

Neither animal co-infused with Gd-albumin and IL13-PE showed evidence of systemic or neurologic toxicity over a 28-day observation period (Table 2). Histologic analysis of the brainstem revealed normal tissue architecture and minimal gliosis that was limited to the region immediately (a maximum radius of 50 μm) surrounding the infusion cannula track. Complete necropsies revealed no evidence of systemic toxicity.

**Discussion**

**CED**

CED relies on bulk flow that is driven by a small pressure gradient to distribute substances within the interstitial spaces of the CNS. Unlike intraventricular or intrathecal delivery, which relies on diffusion, convection is not limited by the molecular weight, concentration, or diffusivity of the infusate (22, 44). Because CED distributes molecules directly into the CNS parenchyma, it permits targeting of selected regions of the CNS in a manner that bypasses the blood-brain barrier (22, 45). Convective delivery has been shown to safely and reproducibly distribute small and large molecules homogeneously over clinically relevant volumes throughout the CNS (23, 35, 37).

**IL13-PE**

IL13-PE is a recombinant tumor-targeted toxin that exploits the overexpression of IL13 receptors on malignant glioma cells and the lack of expression of IL13 receptors on normal neural tissues to preferentially destroy tumor cells. To use receptors for IL13 as a therapeutic target, the protein toxin IL13-PE was developed by linking the human T-cell cytokine IL-13 with a mutated form of *Pseudomonas* exotoxin (46). *Pseudomonas* exotoxin is produced by *Pseudomonas aeruginosa* bacteria and is a single-chain protein made up of three major domains. The NH2-terminal domain Ia (IL-13 ligand) binds to the glioma cell, and the ligand-receptor complex undergoes receptor-mediated internalization to allow processing of the toxin. Domain II is a site of proteolytic cleavage and is responsible for catalyzing translocation of the toxin into cytosol. Domain III, located at the COOH terminus, possesses an ADP ribosylation activity that inactivates elongation factor 2, halting protein synthesis, leading to glioma cell death (47).

**Current study**

**Safety.** Clinical and histologic data confirmed that CED of concentrations up to and including 10 μg/mL of IL13-PE were well tolerated in the rat brainstem over short-term (3 days postinfusion) and long-term (28 days postinfusion) evaluations. These findings are consistent with previous short-term (<14 days) rat toxicity studies that have shown lack of toxicity with perfusion of the brainstem (48) or the striatum with IL13-PE.

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Quantitative autoradiography Vd (mm3)</th>
<th>MRI Vd (mm3)</th>
<th>%Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>262</td>
<td>280</td>
<td>6.8</td>
</tr>
<tr>
<td>2</td>
<td>358</td>
<td>370</td>
<td>3.4</td>
</tr>
<tr>
<td>Mean</td>
<td>310</td>
<td>325</td>
<td>4.8</td>
</tr>
</tbody>
</table>

*Vd of 125I-IL13-PE as compared with Vd of co-infused Gd-albumin.*

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**Fig. 2.** Coronal T1-weighted MRI of primate brains after CED of 60 μL of radiolabeled 125I-IL13-PE co-infused with Gd-albumin as a surrogate imaging tracer. Inset, corresponding autoradiogram showing the anatomic and spatial accuracy of Gd-albumin as a surrogate tracer for IL13-PE.
concentrations of up to 100 μg/mL (49, 50). The delayed evidence of toxicity at the highest concentration infused of IL13-PE (10 μg/mL) in the primate indicates that IL13-PE toxicity is likely related to nonspecific, concentration-dependent immunotoxin effects on perfused tissue and is not related to CED per se or to the total infused volume of IL13-PE. The lack of toxicity at 10 μg/mL in the rat likely represents an interspecies difference in response to IL13-PE.

Despite the evidence of toxicity at an IL13-PE concentration of 10 μg/mL in primate, the ability to homogeneously perfuse the brainstem using convective delivery with an IL13-PE concentration of 0.5 μg/mL safely represents more than a magnitude of order increase over the in vitro concentration (glioma toxic concentration of <1 ng/mL) determined to be cytotoxic to malignant glioma cell lines (33). Moreover, convective intraparenchymal perfusion of supratentorial malignant gliomas has been done safely in phase I studies using CED and IL13-PE at concentrations of 0.5 μg/mL and volumes larger than needed for brainstem perfusion (51).

Because malignant brainstem gliomas are often associated with surrounding edema, a concern related to CED of IL13-PE to the brainstem is the potential for exacerbation of preexisting neurologic dysfunction or initiation of neurologic difficulties during and/or after infusion. However, previous studies have shown that CNS CED of therapeutic agents in the setting of tumor and edema can be done safely and can be achieved without significant elevation of interstitial pressure in normal or tumor tissues at the delivery rates used in this study (23, 51–54). Subsequently, the addition of infusate to the tumor or surrounding region rarely leads to exacerbation of neurologic symptoms and if neurologic difficulties did arise as a result of infusion, they are temporary and resolved with infusion cessation or adding/increasing corticosteroids.

**Imaging of IL13-PE distribution.** To determine the adequacy of treatment and potential therapeutic efficacy of a drug using CED, it is critical to accurately track and determine the distribution during treatment. The recent development of MRI (37, 43) and computed tomography (41, 43) surrogate tracers now permits noninvasive tracking of drug delivery during CED. Critical to the accuracy of tracking a drug during CED with a surrogate tracer is the expression of similar properties of drug and surrogate tracer, including molecular weight, metabolic degradation in the interstitial space, diffusivity, and receptor binding. Gd-albumin was chosen as a surrogate imaging tracer for IL13-PE because of its similar physical properties. Gd-albumin is a large molecular weight protein (72 kDa) similar in size to IL13-PE (52 kDa), and neither compound crosses the blood-brain barrier. Both compounds have similar interstitial
metabolic profiles and because there are little or no IL13 receptors on normal nervous system tissue, interstitial binding should not impede IL13-PE distribution during convective delivery (32, 55). These physical properties permit IL13-PE to not only be tracked accurately by Gd-albumin but to remain in perfused regions of tissue for efficacious periods of time (51).

The accuracy of in vivo MR image tracking of IL13-PE by Gd-albumin was confirmed by quantitative autoradiography of $^{125}$T-IL13-PE. $V_d$'s computed from autoradiographic analysis and MRI differed by only 4.8% (Table 3; Fig. 2) and this is within the measurement error of either technique. This $V_d$ difference corresponds to a difference of <0.2 mm in diameter on cross-sectional imaging in any plane. Thus, Gd-albumin is an accurate and effective imaging tracer for noninvasive, real-time monitoring of IL13-PE distribution on MRI. Moreover, co-infusion of IL13-PE at a concentration of 0.5 μg/ml and Gd-albumin showed no evidence of clinical or histologic toxicity, showing a lack of any synergistic toxicity between the two agents.

**Potential applications**

The potential of CED to distribute IL13-PE or other therapeutic agents effectively to brainstem tumors and the ability to monitor the distribution of infusate noninvasively in real time with a surrogate tracer should prove indispensable in the treatment of these lesions. Because Gd-albumin accurately tracks the distribution of IL13-PE, this delivery paradigm will permit noninvasive monitoring of drug delivery and ensure therapeutic distribution in individual patients with brainstem gliomas, whose tissue properties to differ from naive parenchyma, given the dynamic nature of infiltrative tumor cells and surrounding edema. Although the majority of brainstem gliomas are pontine and the goal of this study was to perfuse the pontine region, extension of tumor into the midbrain and medulla in patients should also be amenable to treatment using this technique by increasing the volume of infusion or targeting these regions separately.

**Conclusions**

CED can be used safely and effectively to infuse glioma-toxic concentrations of IL13-PE to the primate brainstem. Moreover, co-infusion of Gd-albumin can be used to accurately track distribution of IL13-PE in real-time using MRI. This should allow direct therapeutic application of IL13-PE to brainstem gliomas while monitoring its distribution to ensure effective perfusion of tumor.

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**References**

35. Lonser RR, Gogate N, Morrison PF, Wood JD, Oldfield EH. Direct convective delivery of macromo-
Real-time, Image-Guided, Convection-Enhanced Delivery of Interleukin 13 Bound to Pseudomonas Exotoxin

Gregory J.A. Murad, Stuart Walbridge, Paul F. Morrison, et al.