Intratumoral Administration of Recombinant Circularly Permuted Interleukin-4-*Pseudomonas* Exotoxin in Patients with High-Grade Glioma

Robert W. Rand, Robert J. Kreitman, Nicholas Patronas, Frederick Varricchio, Ira Pastan, and Raj K. Puri

Department of Neuro-Oncology, John Wayne Cancer Institute, Santa Monica, California 90404 [R. W. R.]; Laboratory of Molecular Biology, Division of Basic Sciences, National Cancer Institute, NIH, Bethesda, Maryland 20892 [R. J. K., I. P.]; Neuroradiology Department, NIH, Bethesda, Maryland 20892 [N. P.]; Division of Biostatistics and Epidemiology, Center for Biologies Evaluation and Research, United States Food and Drug Administration, Rockville, Maryland 20850 [F. V.]; and Laboratory of Molecular Tumor Biology, Division of Cellular and Gene Therapies, Center for Biologies Evaluation and Research, United States Food and Drug Administration, Bethesda, Maryland 20892 [R. K. P.]

Abstract

Human glioblastoma but not normal brain cells express numerous receptors for the cytokine interleukin (IL)-4. To target these receptors, we have investigated the safety and activity of directly infusing IL-4(38-37)-PE38KDEL, a chimeric protein composed of circularly permuted IL-4 and a truncated form of *Pseudomonas* exotoxin (PE), into recurrent malignant high-grade gliomas. IL-4(38-37)-PE38KDEL (IL-4-toxin) was infused over a 4–8-day period into gliomas of nine patients by one to three stereotactically placed catheters. No apparent systemic toxicity occurred in any patient. The infusion of IL-4-toxin in six of nine patients showed glioma necrosis as evidenced by diminished gadolinium enhancement on magnetic resonance imaging. Seven of nine patients underwent craniotomy because of increased intracranial pressure at 16–101 days after the beginning of infusion. In six of these seven patients, partial-to-extensive tumor necrosis with edema was confirmed pathologically. No histological evidence of neurotoxicity to normal brain was identified in any patient. Two patients were operated on; by magnetic resonance imaging, one showed mottled gadolinium enhancement, and the other showed extensive necrosis of tumor leading to complete remission; this patient remains disease-free >18 months after the procedure. We conclude that direct glioma injection of IL-4(38-37)-PE38KDEL is safe without systemic toxicity. Local toxicity seemed attributable mainly to tumor necrosis or occasionally to the volume of infusion. Histological evidence of toxicity to normal brain was not observed and in many patients, could be pathologically excluded. Additional patients are being treated to determine the maximal tolerated concentration and volume of IL-4(38-37)-PE38KDEL.

Introduction

Primary malignant astrocytomas and metastatic brain tumors are diagnosed in approximately 35,000 adult Americans each year (1, 2). Malignant gliomas, including the highly aggressive GBM, constitute at least 35% of all of the primary brain tumors and are a leading cause of death from cancer (3). Malignant astrocytomas represent the third leading cause of cancer-related deaths in the United States among adolescents and young adults between the ages of 15 and 34, and their frequency increases with age in adults over 55 years old (4). More than 17,500 Americans of all of the age groups are diagnosed with a malignant glioma (astrocytoma grade III and IV) each year. Despite recent advances in neuroimaging and neurosurgical techniques, the median survival of patients with malignant gliomas treated by surgical resection alone is only 4–6 months (5). Radiation therapy and stereotactic radiosurgery for local control eventually fail because they do not stop progression of the disease in the surrounding brain tissue into which glioma cells have migrated (6). Adjuvant chemotherapy is only minimally effective, with overall survival prolonged only a few weeks, and few patients survive more than 18–36 months (7, 8). Once patients have tumor progression, conventional chemotherapy has not been shown to prolong survival (4). The lack of specificity of both radiation therapy and chemotherapy for malignant cells has led to dose-related side effects and CNS toxicity. Therefore, other treatment modalities are needed that are specific and not toxic to the CNS.

We have shown that IL-4Rs are present on the surface of malignant glioma/astrocytoma cells (9). This surface protein is overexpressed on tumor cells but not on normal brain tissues (9, 10). To specifically target these receptors, we first produced a recombinant chimeric protein comprised of IL-4 and a mutated form of PE. This targeted toxin was highly cytoxic to glioma cells

---

Received 1/27/00; revised 3/9/00; accepted 3/9/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by the Kadoorie Foundation, the Lou Ehlers Foundation, the Frank G. Lyon foundation, and individual donations that were made in memory of patients.

2 To whom requests for reprints should be addressed, at Laboratory of Molecular Tumor Biology, Division of Cellular and Gene Therapies, Center for Biologies Evaluation and Research, Food and Drug Administration, NIH, Building 29B, Room 2NN10, 29 Lincoln Drive, MSC 4555, Bethesda, Maryland 20892. E-mail: puri@cber.fda.gov.

---

3 The abbreviations used are: GBM, glioblastoma multiforme; MR, magnetic resonance; MRI, MR imaging; CNS, central nervous system; IL, interleukin; IL-4R, IL-4 receptor; PE, *Pseudomonas* exotoxin; PET, positron emission tomography; USFDA, United States Food and Drug Administration; MTD, maximum tolerated dose; ICP, intracranial pressure; V-P, ventriculoperitoneal (shunt); CSF, cerebrospinal fluid.
in a specific manner (10–13) but bound to IL-4R in glioma cells with 37-fold less affinity than native IL-4. To improve the binding affinity of the ligand, we altered the toxin ligand junction by producing a circularly permuted IL-4 and used it to make the recombinant toxin, IL-4-(38-37)-PE38KDEL (13–15). This single-chain recombinant molecule contains amino acids 38–129 of IL-4, fused via a peptide linker to amino acids 1–37, which in turn is fused to the toxin. PE38KDEL is composed of amino acids 253–364 and 381–608 of PE, with KDEL (an endoplasmic retaining sequence), at positions 609–612. This purified toxin was found to be highly cytotoxic to IL-4R-positive glioblastoma cells. IL-4-(38-37)-PE38KDEL bound to glioblastoma cells with 16-fold higher affinity than the native (non-circularly permuted) IL-4-toxins, IL-4-PE\textsuperscript{37}, or IL-4-PE38KDEL. More importantly, IL-4-(38-37)-PE38KDEL was 3- to 30-fold more cytotoxic to glioblastoma cell lines compared with the first generation IL-4-toxins (10). These \textit{in vitro} data indicated that glioblastoma cells are very sensitive to IL-4-toxins. In an \textit{in vivo} model of human tumor in mice, it was shown that the increased binding and cytotoxic activity of IL-4-(38-37)-PE38KDEL leads to increased antitumor activity (15, 16).

To assess \textit{in vivo} activity of the IL-4-toxin against GBM, immunodeficient nude mice were injected s.c. with human U251 glioma cells, which resulted in the development of tumors with a mean size of 13–60 mm\textsuperscript{3} by day 3–4. Intratumoral administration of IL-4-(38-37)-PE38KDEL into U251 glioblastoma flank tumors in nude mice on alternate days for three to four doses induced a complete regression of small (<13 mm\textsuperscript{3}) and large (<60 mm\textsuperscript{3}) tumors in all of the animals without any evidence of toxicity. Most of the IL-4-(38-37)-PE38KDEL remained localized on intratumoral administration. Significant antitumor activity was also observed when IL-4-toxin was administered via i.p. and i.v. routes. These results demonstrated that IL-4-(38-37)-PE38KDEL might have significant antitumor activity against human glioblastoma (16).

The blood-brain barrier, which is a major impediment to the entry of many “small molecule” drugs into the CNS, effectively precludes any method of administration of macromolecules except by direct injection. Direct infusions into brain tumors can be safe and effective. When directly infused into white matter/tumors, fluids containing the targeted toxins can distribute by bulk flow (convection) through the interstitial spaces and can spread to distant areas of the brain (17–19). High-flow microinfusion provides a high local drug concentration and enables a greater volume of tissue to be bathed with the drug than low-flow microinfusion or release of chemotherapeutic agents from tumor implants that rely on diffusion (20). In the current study, to evaluate the toxicity and antitumor activity, we infused IL-4-(38-37)-PE38KDEL in a dose-dependent schedule intratumorally by the high-flow microinfusion technique (17–20) in patients with recurrent malignant high-grade gliomas.

Materials and Methods

IL-4-(38-37)-PE38KDEL

Recombinant fusion protein IL-4-(38-37)-PE38KDEL was produced under current good manufacturing practices (cGMP) by Inland Labs (Dallas, TX) following our procedure (13). The recombinant toxin was purified by ion-exchange and size-exclusion chromatography as described previously (13–15). The drug was stored at −80°C. This drug was found stable for potency when stored at this temperature for at least 13 months and also stable when diluted material in carrier protein was stored at room temperature for 5 days (not shown).

Patients

Patients were eligible who showed MRI-documented recurrence or progression of a malignant brain tumor (GBM) after standard therapy and received no other treatment within 3 weeks of inclusion in this protocol. All of the patients were previously treated with external beam radiation but failed to show improvement. The tumors that had no satellite lesions stereotactically accessible and no significant mass effect were treated. Adult patients over 18 years of age with Karnofsky performance score of ≥60 were enrolled. Men and women of childbearing potential were instructed to practice birth control. Women of childbearing age had a negative serum or urine pregnancy test within 7 days of study entry. Patients were able to give informed consent, were willing and medically capable of undergoing the surgical operation, and were not receiving other investigational agents for the treatment of malignant astrocytoma. All of the patients had adequate baseline organ function as assessed by the following laboratory parameters on their preoperative visit (e.g., within 21 days of commencement of study drug infusion): serum creatinine, <2 mg/dl; platelet count, >100,000/mm\textsuperscript{3}; absolute neutrophil count, >1,500/mm\textsuperscript{3}, hemoglobin, >9.5 mg/dl; prothrombin time/partial thromboplastin time, at or below the upper limit of normal; bilirubin, <2 mg/dl; and aspartate aminotransferase and alanine aminotransferase, less than twice the upper limit of normal.

Patients with diffuse subependymal or CSF disease, with anaplastic oligodendroglioma, with tumors involving the brainstem, cerebellum, or both hemispheres, unable or unwilling to give informed consent, with an active infection requiring treatment or having an unexplained febrile illness were excluded from the study. Institutional Review Boards of the United States Food and Drug Administration and of St. John’s Hospital (Santa Monica, CA) approved the protocol. The investigational new drug exception (IND 7004) was authorized by the USFDA in February 1997.

Treatment

All of the patients underwent a standard stereotactic biopsy under magnetic resonance/computed tomography guidance under appropriate anesthesia before placement of catheters as described by Laske et al. (20). Tumor biopsy of the enhancing tissue was performed for histology. After a positive biopsy for recurrent tumor, up to a maximum of three silastic-infusion catheters (2.1-mm outer diameter, Pudenz catheter(s), Medtronics, PS Medical, Galeta, CA) were placed with the tip at a selected site in the tumor using stereotactic guidance through small-twist drill holes. The shortest possible routes were chosen to place catheters into tumors. The number of catheters was determined based on the tumor size and the volume of drug to be administered to ensure maximal saturation of tumor bed and margins in the designated period of time. Patients who received the largest volumes were implanted with three catheters. Whenever three catheters were used, one catheter tip was advanced to
escalating the concentration of drug from 6 determined in this trial. In ongoing multicenter trials, we are grade-2 CNS or systemic toxicity. However, no MTD was dose at which two or more patients experienced higher than drug application, the MTD was defined as a dose lower than a view to estimate the tumor volume. In the investigational new volume of normal brain tissue. The volume of each infusion was deter-

the center of the tumor, and the second and third catheter tips were placed at opposing poles of the tumor adjacent to the largest volume of white matter. The proximal ends of the catheters were sewn to the scalp. To maintain patency of the catheter and attached tubing, the intratumoral catheter and tubing were filled with IL-4-toxin after insertion. After surgery, the externalized catheters were connected to Medex 2010 micropumps (Medtronic, Inc., Minneapolis, MN) and filled with IL-4 (38-37)-PE38KDEL. These procedures were performed under local and general anesthesia as necessary.

Infusion within each catheter began immediately, or within 24 h after catheter insertion, at a very slow pace over a 4–8 day period (0.3–0.6 ml/h). The pump was filled once only. Patients were treated with steroids and antibiotics at standard doses. The catheters were removed after completion of therapy at the bed site and antibiotics were discontinued; steroid dosage (up to 20 mg/day in divided doses) was slowly tapered to maintenance level. Some patients were given diuretics to relieve edema. A MR scan was performed, and patients were discharged after they were stable and had undergone an interim physical and neurological examination and had blood drawn for chemistry and hematology.

The starting dose was selected based on the preclinical animal studies. The first dose level of 0.2 µg/ml was 0.002 times lower than the dose that produced histological damage to normal brain when administered intra-brain parenchyma in rats and 0.0333 times of MTD injected intrathecally in monkeys. The dose was escalated one log in the next cohort of three patients and then one-half-logs to the highest dose (6 µg/ml). The volume of fluid infused was determined based on the tumor volume determined by MR scan, including 1- to 2-cm margins of normal brain tissue. The volume of each infusion was determined by the MRI computer including the space between each view to estimate the tumor volume. In the investigational new drug application, the MTD was defined as a dose lower than a dose at which two or more patients experienced higher than grade-2 CNS or systemic toxicity. However, no MTD was determined in this trial. In ongoing multicenter trials, we are escalating the concentration of drug from 6 µg/ml to 15 µg/ml.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Craniotomy</th>
<th>Radiation</th>
<th>Chemotherapy</th>
<th>Tumor site</th>
<th>Tumor, volume, (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64/M</td>
<td>10/15/95; 11/20/96; 11/12/97</td>
<td>5000 cGy</td>
<td>BCNU</td>
<td>Right temporal lobe</td>
<td>27.1</td>
</tr>
<tr>
<td>2</td>
<td>59/F</td>
<td>04/27/97</td>
<td>3000 cGy</td>
<td>None</td>
<td>Right temporal lobe</td>
<td>21.5</td>
</tr>
<tr>
<td>3</td>
<td>49/F</td>
<td>05/08/97</td>
<td>5940 cGy</td>
<td>Carboplatin</td>
<td>Right temporal lobe</td>
<td>38.9</td>
</tr>
<tr>
<td>4</td>
<td>54/M</td>
<td>03/19/97</td>
<td>6000 cGy and γ knife</td>
<td>Vincristine</td>
<td>Left fronto-temporal lobe</td>
<td>184.0</td>
</tr>
<tr>
<td>5</td>
<td>26/F</td>
<td>12/30/96; 11/10/97; 02/17/98</td>
<td>6000 cGy</td>
<td>BCNU</td>
<td>Right temporal lobe</td>
<td>121.0</td>
</tr>
<tr>
<td>6</td>
<td>56/M</td>
<td>06/07; 10/02/97</td>
<td>6000 cGy; iodine seeds implant</td>
<td>None</td>
<td>Right parietal lobe</td>
<td>208.0</td>
</tr>
<tr>
<td>7</td>
<td>71/M</td>
<td>06/24/97; 12/04/97</td>
<td>2000 cGy</td>
<td>None</td>
<td>Right parietal lobe</td>
<td>22.0</td>
</tr>
<tr>
<td>8</td>
<td>52/F</td>
<td>07/29/97</td>
<td>5500 cGy</td>
<td>None</td>
<td>Left temporal lobe</td>
<td>48.6</td>
</tr>
<tr>
<td>9</td>
<td>49/F</td>
<td>07/03/97</td>
<td>5000 cGy</td>
<td>CCNU</td>
<td>Left parietal lobe</td>
<td>23.0</td>
</tr>
</tbody>
</table>

a All of the patients were diagnosed with GBM.
b Pretreatment tumor volume was determined by measuring the volume of each view of the glioma (slice) and adding the 2.5-mm space volume between each view of the gadolinium-enhanced T1-weighted axial MRI images.
c BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; CCNU, (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea.

deformed catheters were connected to Medex 2010 micropumps (Medtronic, Inc., Minneapolis, MN) and filled with IL-4 (38-37)-PE38KDEL. These procedures were performed under local and general anesthesia as necessary.

Infusion within each catheter began immediately, or within 24 h after catheter insertion, at a very slow pace over a 4–8 day period (0.3–0.6 ml/h). The pump was filled once only. Patients were treated with steroids and antibiotics at standard doses. The catheters were removed after completion of therapy at the bed site and antibiotics were discontinued; steroid dosage (up to 20 mg/day in divided doses) was slowly tapered to maintenance level. Some patients were given diuretics to relieve edema. A MR scan was performed, and patients were discharged after they were stable and had undergone an interim physical and neurological examination and had blood drawn for chemistry and hematology.

The starting dose was selected based on the preclinical animal studies. The first dose level of 0.2 µg/ml was 0.002 times lower than the dose that produced histological damage to normal brain when administered intra-brain parenchyma in rats and 0.0333 times of MTD injected intrathecally in monkeys. The dose was escalated one log in the next cohort of three patients and then one-half-logs to the highest dose (6 µg/ml). The volume of fluid infused was determined based on the tumor volume determined by MR scan, including 1- to 2-cm margins of normal brain tissue. The volume of each infusion was determined by the MRI computer including the space between each view to estimate the tumor volume. In the investigational new drug application, the MTD was defined as a dose lower than a dose at which two or more patients experienced higher than grade-2 CNS or systemic toxicity. However, no MTD was determined in this trial. In ongoing multicenter trials, we are escalating the concentration of drug from 6 µg/ml to 15 µg/ml.

<table>
<thead>
<tr>
<th>Patient</th>
<th>IL-4 (38-37)-PE38KDEL concentration (µg/ml)</th>
<th>Volume infused (ml)b</th>
<th>Total dose (µg)</th>
<th>Number of catheters</th>
<th>Length of infusion (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>30</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>2a</td>
<td>0.2</td>
<td>30</td>
<td>6</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>68</td>
<td>13.6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>119</td>
<td>238</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>5a</td>
<td>2.0</td>
<td>120</td>
<td>240</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>6.0</td>
<td>84</td>
<td>504</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>6.0</td>
<td>120</td>
<td>720</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>6.0</td>
<td>60</td>
<td>360</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>6.0</td>
<td>54</td>
<td>324</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

a The volume of infusion was determined based on tumor volume plus enough volume for 1–2-cm margins to saturate the brain surrounding the tumor. In some cases, the total drug volume could not be infused because these patents experienced volume-related symptoms. The concentration of the drug was kept constant and escalated first one-log in two patients and then one-half-log in four patients.
b The number of catheters was determined based on tumor volume and shape and were placed at the margins (n = 2 or more catheters) or in some cases in the center of the tumor (n = 1 catheter).

Once a maximum tolerated concentration is achieved, we intend to escalate the volume of infusion to determine maximum tolerated volume.

**Table 1** Patient demographics

**Table 2** Treatment schedule

**Patient Evaluation**

**Pretreatment.** All of the patients underwent a complete history and physical examination and blood and serum chemistry tests to assess the functioning of all of the major organs. These patients also underwent baseline MRI scans, with and without gadolinium enhancement. MRIs were reviewed by neuroradiologist (N.P.), and tumor histology slides were reviewed by a neuropathologist (F.V.).
**Table 3** IL-4 (38-37)-PE38KDEL treatment and outcome

<table>
<thead>
<tr>
<th>Patient</th>
<th>Adverse events&lt;sup&gt;a&lt;/sup&gt; (NCI&lt;sup&gt;b&lt;/sup&gt; toxicity grade&lt;sup&gt;c&lt;/sup&gt; 1–5)</th>
<th>Posttreatment after initial infusion</th>
<th>Radiographic&lt;sup&gt;d&lt;/sup&gt; MRI (days after infusion)</th>
<th>Intraoperative&lt;sup&gt;e&lt;/sup&gt; (days after infusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>Craniotomy; chemotherapy</td>
<td>IE (66)</td>
<td>PN (97)</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>Two additional cycles of IL-4-toxin infusion; craniotomy with gliadel</td>
<td>IE (26)—1st infusion</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Headache (1)</td>
<td>Craniotomy; CPT-11; SU5461</td>
<td>IE (34)—2nd infusion</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Weakness and nausea (2/3)</td>
<td>Craniotomy with gliadel</td>
<td>DE (5)—3rd infusion</td>
<td>PN (27)—3rd infusion</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>Craniotomy; one additional cycle of IL-4-toxin infusion</td>
<td>DE (7)</td>
<td>EN (14)</td>
</tr>
<tr>
<td>6</td>
<td>Weakness and seizures (2/3)</td>
<td>Craniotomy; BCNU</td>
<td>DE (5)—1st infusion</td>
<td>PN (66)—1st infusion</td>
</tr>
<tr>
<td>7</td>
<td>Anomia (1 transient)</td>
<td>None</td>
<td>IE (5)—2nd infusion</td>
<td>NN (18)—2nd infusion</td>
</tr>
<tr>
<td>8</td>
<td>Dysphasia (1/2) communicating hydrocephalus</td>
<td>CCNU; V-P shunt</td>
<td>DE (9)</td>
<td>EN (10)</td>
</tr>
<tr>
<td>9</td>
<td>Seizures — 2/3; communicating hydrocephalus</td>
<td>V-P shunt; craniotomy</td>
<td>NC (55)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> During and within 1 week of IL-4-toxin infusion.
<sup>b</sup> NCI, National Cancer Institute; BCNU, 1,3-bis (2-chloroethyl)-1-nitrosourea; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; IE, increased enhancement; DE, decreased enhancement; NC, no change; PN, partial necrosis with infiltrating tumor; EN, extensive necrosis with some viable tumor; NN, no necrosis.
<sup>c</sup> Severity of NCI toxicity grade: 1, mild, or grade 1; 2, moderate, or grade 2; 3, severe, or grade 3; 4, life threatening/debilitating, or grade 4.
<sup>d</sup> Radiographic tumor response was assessed by MRI scans on days indicated in parentheses. Anterior-posterior and transverse diameters of contrast-enhancing lesions were measured and compared pre- and postinfusion.
<sup>e</sup> Tumor response was assessed intraoperatively on days posttreatment (indicated in parentheses). Tumor necrosis was confirmed by pathological examination of necrotic tissue.

**During and After Treatment.** During infusion, the patient’s vital signs and neurological status were monitored closely each day of the infusion. No symptoms of increased ICP were observed during infusion. Blood samples from every day of treatment and on the third day posttreatment were obtained for complete blood count and serum chemistry and demonstrated no appreciable abnormalities.

Patients returned as outpatients for follow-up evaluation every 4 weeks (± 5 days), unless otherwise indicated, for 16 weeks and every 8 weeks (± 5 days) thereafter. At those visits, patients underwent an interim physical and physical/neurological examination and had blood drawn for chemistry and hematology and a brain MR scan, MR spectroscopy, or PET scan to determine antitumor effect. MR spectroscopy was performed in the last four patients who showed elevation of choline constant preoperatively with active malignant glioma.

**Results.** In this open-label dose escalation trial, nine patients (4 male and 5 female; 26–71 years old; median age, 54 years) who had histologically confirmed recurrent GBM and who showed MRI-confirmed progression of disease were enrolled (Table 1). All of these patients had undergone craniotomy and radiation therapy before entry into the trial. The Karnofsky performance score of these patients was at least 60. These nine patients with malignant gliomas were infused with IL(38-37)-PE38KDEL via one to three small diameter catheters. Because the goal of this trial was to saturate the entire glioma bed and surrounding brain tissue, the IL-4-toxin was infused at a very slow pace over a 4–8 day period (0.3–0.6 ml/h) using micropumps. Pudenz catheter(s) of 2.1-mm outer diameter were placed into and around the malignant glioma and positioned accurately after stereotactic tumor biopsy, and IL-4(38-37)-PE38KDEL was infused using one or more Medex 2010 micropumps. None of these patients showed any additional discomfort with IL-4-toxin infusion. Patient 2 received two additional infusions of IL-4-toxin in month 2 and 3 and patient 5 received one additional infusion 1 month after the initial infusion. Four infusions were done at the 0.2-µg/ml dose, three at the 2.0-µg/ml, and five at the 6.0-µg/ml dose.

**Toxicity and Response to Therapy.** Patients were infused with 30–185-ml drug volume of IL-4(38-37)-PE38KDEL (Table 2). There were no treatment-related deaths. Cerebral edema causing corresponding signs and symptoms of increased ICP developed without evidence of previous cerebral edema on days 27, 14, and 10 in patients 2, 4, and 6, respectively. Four other patients developed increased ICP between 43 and 97 days post-IL-4-toxin infusion (Table 3). Craniotomy was performed with tumor resection in seven patients because decadron (up to 20 mg/day) and antidiuretic medications could not control the increased cerebral edema. The symptoms and signs of the increased ICP improved as the pressure diminished.

The first cohort of three patients received the lowest dose of IL-4(38-37)-PE38KDEL (0.2 µg/ml) except for patient 2, who was retreated with two additional courses (Table 2). All of these patients showed a slight but significant decrease in the thickness of gadolinium-enhancing rim 4–9 days after IL-4-toxin therapy. However, repeat scans about 1–4 months after infusion showed an increase in necrotic cavity but also an increase in gadolinium enhancement with new brain involve-
ment or progression of tumor that grew at a site distant from the infusion. Patient 3 developed two new cavities attributable probably to tumor necrosis with ring enhancement as assessed 33 days postinfusion. These cavities may have been generated because of catheter-related tissue necrosis. All of these patients died after progression of their disease.

Patients 4 and 5 received the 2-μg/ml dose, whereas patient 5 also received an additional cycle at 6 μg/ml. Patient 4 showed a mottled pattern of gadolinium uptake, which decreased 7 days after IL-4-toxin infusion. In patient 5, MRI at the end of the first infusion (day 5) showed a decrease in gadolinium enhancement. However, 64 days after the first infusion, an increase in tumor size was observed. Craniotomy was performed because of uncontrolled ICP on day 66. The second infusion was begun on day 104 of first infusion. About 1 month after the second infusion, gadolinium enhancement was further increased. A repeat craniotomy was performed 18 days after the second infusion that showed no IL-4-toxin induced further necrosis of rapidly growing glioma. Both of the patients expired because of tumor progression.

The next four patients received the 6-μg/ml dose level. In patient 6, MRI showed a marked decrease in gadolinium enhancement 9 days after the infusion (Fig. 1). However, the patient underwent craniotomy to relieve increased ICP with a subsequent improvement of contralateral extremity weakness. The tumor at craniotomy appeared to be extensively necrosed with residual areas of GBM and focal oligodendrogial differentiation. Sixteen weeks after IL-4-toxin infusion and surgery, the patient went into coma shortly before his death. A premorbid PET scan showed no evidence of increased metabolic activity in the region of glioma (not shown). The cause of death was attributed to uncontrollable mass effect caused by ongoing edema, but because an autopsy was refused, it could not be histologically confirmed whether viable tumor remained.

The seventh patient had complete regression of tumor. This 71-year-old patient was treated with surgical resection and radiation and relapsed twice before treatment with IL-4-toxin. The recurrent tumor was approximately 2.2 × 2.2 cm in size surrounding the prior craniotomy resected area as demonstrated by PET scan of patient 7, 16 months posttreatment. The normal activity of the cortex is interrupted by a hypometabolic area at the tumor site (arrows) indicating tumor suppression.
enhanced MRI scans. The patient tumor was infused at 6 μg/ml with a total of 360 μg of IL-4-toxin. After the infusion, MRI coronal sections showed two lesions. This patient showed a decrease in size of the tumor cavity and gadolinium enhancement on weeks 3, 16, and 24. PET scans 4, 10, and 16 months after IL-4-toxin infusion showed hypometabolism in the area of the malignant glioma (Fig. 2). An enhanced MRI 9 months (Fig. 3) and 15 months postinfusion demonstrated no evidence of neoplasm recurrence (data not shown). The right lateral ventricle had expanded into the area of the original tumor site. The patient is without neurological deficits and, 18 months postinfusion, is well, doing daily gardening and holding a part-time job. This patient is currently not taking any anticonvulsive medications.

In patient 8, MRI showed an increase in necrotic cavity. The enhancing rim thickness, however, was also increased. Nine weeks after the infusion, the lesion had mottled enhancement consistent with necrosis. In patient 9, MR scans 20 days after infusion showed a decrease in gadolinium enhancement. A subsequent scan (14 days after the last MRI) showed no change in enhancement. However, another two scans performed on day 53 and day 66 showed an increase in ring enhancement. An increase in edema, which resulted in midline shift, was also noted in each scan. Craniotomy was performed on day 50 because of increased ICP, at which time necrosis of most of tumor was confirmed pathologically, which suggested that tumor necrosis caused the edema. Patients 8 and 9 both developed communicating hydrocephalus possibly because of necrotic glioma cells in the arachnoid villae. Both of the patients were treated successfully by V-P shunt placements.

Of the nine patients treated, six patients (patients 2, 4, 5, 6, 7, and 9) showed necrosis of their treated tumor as determined by MRI obtained 7 days to 15 months after IL-4-toxin infusion. Among these, three patients (patients 3, 4, and 6) were operated on 2–12 days after MRI scanning to reduce ICP that was not controlled by decadron and antidiuretic medications. Tumor necrosis was documented histologically in all three of these patients, as well as in a third patient (patient 2) who did not have evidence of tumor necrosis by MRI. Fig. 4 shows sections from three of these patients (patients 2, 3, and 6) in which normal brain was included in the sample of necrotic tumor. The surgical procedure in patients 3 and 6 was performed after the first cycle of the 0.2-μg/ml and 6-μg/ml dose levels, respectively, whereas patient 3 had a third cycle at the 2-μg/ml dose level. Thus, whereas pathological confirmation of selective targeting of glioma was not possible in all of the patients, Fig. 4 represents evidence at all of the 3 dose levels that IL-4(38-37)-PE38KDEL treatment was associated with necrosis of tumor but not of normal brain.

Discussion

IL-4(38-37)-PE38KDEL is a targeted fusion protein that specifically attaches to cells expressing IL-4Rs reported to be present on many different solid tumor cells of varying pathology (21). After binding to the IL-4R, the fusion protein is internal-
ized, and then the toxin portion of the molecule causes the ADP ribosylation of elongation factor 2 and arrests protein synthesis (22). The cell dies by apoptosis attributable to the lack of new protein synthesis (23). Human brain tumor cells are particularly enriched in the expression of IL-4Rs on their cell surface (9, 10). Although numerous preclinical safety and efficacy experiments have been performed with IL-4(38-37)-PE38KDEL, this targeted drug has not been administered to humans before. Here we demonstrate for the first time that intraglioma administration of IL-4(38-37)-PE38KDEL to patients is safe. No treatment-related deaths or life-threatening toxicities were observed in any patient. In six of nine patients, administration of IL-4(38-37)-PE38KDEL induced pronounced necrosis of their treated tumor parenchyma in a dose-dependent manner as demonstrated by decrease in gadolinium enhancement and increase in cavitation on MRI scans. In seven patients who underwent surgery after this treatment, necrosis of tumor but not normal brain tissue was confirmed by gross and microscopic examination. Increase in neurological signs and symptoms in four patients were attributable to increased ICP, which was relieved by craniotomy and resection of the necrotic tumor. This resulted in improvement of these signs and symptoms. Delayed intracranial edema appeared 10–97 days after drug infusion, which indicated that this event was not directly related to drug administration. In fact, edema seemed to be a result of massive tumor necrosis caused by IL-4(38-37)-PE38KDEL infusion. Two patients did not need reoperation. Patient 7 did not require any additional IL-4-toxin treatment and continues to be a complete responder 18 months after the initial drug infusion. His recent MRI scan showed minimal residual enhancement and hypometabolic tissue by PET scan.

Two patients developed communicating hydrocephalus corrected by V-P shunt procedures. The exact mechanism of this phenomenon is not known. However, a reasonable explanation is that necrotic malignant glioma cells that were adjacent to the subarachnoid spaces gained access to the cerebrospinal fluid, which damaged the arachnoid villae similar to what often happens after a serious subarachnoid hemorrhage from a ruptured cerebral aneurysm. Recent studies have hypothesized that inflammatory reactions triggered by the inflammation or blood-clotting products could result in obstruction of CSF flow through arachnoid villi into the venous sinus (24). Thus, if
necrosed malignant glioma tissue is present adjacent to the subarachnoid spaces, postinfusion resection of the tissue in the second week may prevent this complication.

Targeted toxins can be enormously tumor-specific, and they have limited systemic toxicity when administered directly into the tumor site (25–29). Hypoxia renders neoplastic cells more resistant to radiation therapy and/or chemotherapy but may have less effect on the benefit of targeted toxins. Gliomas, which are radiation- or chemotherapy-resistant, may be sensitive to targeted toxins because of a difference in mechanism of action (30). Moreover, the cytotoxic potency of targeted toxins is five to ten times greater than that of chemotherapeutic agents (26). Thus targeted toxins provide a unique approach to treating cancers that express unique tumor-associated antigen or receptors. Many targeted toxins have been tested in the clinic. LMB-1 and LMB-2 were administered to numerous patients with solid cancer (31) and refractory hairy cell leukemia (32, 33), respectively. LMB-1 was found to be active in 5 of 38 patients with solid tumors. IL-2-diphtheria toxin has been tested against cutaneous T-cell lymphoma and has been found to be effective in this disease (34). A mouse CD22 monoclonal antibody, chemically coupled to deglycosylated ricin A chain, has been tested in the clinic and found effective in 20% of patients with B-cell lymphomas (35, 36). Recently, transferrin-receptor-targeted transferrin-diphtheria toxin has been administered to 18 patients with brain tumors using high-flow microinfusion technique similar to that used in our present study (20). Transferrin-CRM107 was found to cause brain tumor regression in 60% of the patients, including complete responses in two patients. This study demonstrated that direct interstitial infusion could be used successfully to distribute a large protein \( M \sim 140,000 \) in the tumor bed and surrounding brain tissue. Despite these successes, transferrin-CRM107 was found to show peripheral brain injury at doses \( \geq 1 \mu g/ml \) This brain injury occurred up to 4 cm from the infusion point (20). However, in our studies, no normal-brain-tissue injury was identified at drug concentration up to 6 \( \mu g/ml \). These data indicate that IL-4(38-37)-PE38KDEL is highly specific for cells that express large numbers of IL-4Rs. The lack of toxicity to normal brain is consistent with our previous observation of the lack of IL-4Rs in normal brain tissues from six individuals (10).

The results of our study demonstrate that regional administration of targeted toxin that is directed at IL-4Rs on GBM can elicit antitumor response while limiting systemic drug exposure, inasmuch as no systemic toxicity was observed in any patient. This observation is consistent with our previous study in monkeys in which, on intrathecal administration of IL-4(38-37)-P338KDEL, high concentration of drug in the CSF was detected, although no detectable systemic drug levels or systemic toxicities were observed (10).

Although most patients showed significant necrosis of their treated tumor as a result of IL-4(38-37)-PE38KDEL infusion, all but one patient exhibited tumor recurrence and death attributable to progressive disease. Most patients recurred at the same sites or at different sites that were not treated with our drug. One patient who remains a complete responder had a smaller tumor recurrence, which suggests that we succeeded in saturating the entire tumor bed with IL-4-toxin. Additional patients with similar tumor volume need to be treated at this and higher dose levels to confirm our initial observation. On the basis of these observations, our extended clinical trial is ongoing at fourteen additional centers in the United States and Germany.

We conclude that direct glioma injection of IL-4(38-37)-PE38KDEL toxin can be accomplished without systemic toxicity and is associated with a high incidence of cerebral edema that appears to be related to necrosis of the treated high-grade glioma. Additional clinical trials are under way to fully explore efficacy of IL-4(38-37)-PE38KDEL for malignant high-grade glioma, a particularly deadly form of human cancer.

Acknowledgments

We thank Pamela Dover and Drs. Bharat Joshi and S. Rafat Husain of Laboratory of Molecular Tumor Biology, United States Food and Drug Administration, for providing considerable technical and scientific support to these studies; and Drs. Richard Youle and Edward Oldfield for helpful discussions regarding the method of administration of intratumoral toxin.

References


