Distribution kinetics of targeted cytotoxin in glioma by bolus or convection-enhanced delivery in a murine model

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Object. Interleukin-13 receptor (IL-13R)–targeted cytotoxin (IL-13–PE38) displays a potent antitumor activity against a variety of human tumors including glioblastoma multiforme (GBM) and, thus, this agent is being tested in the clinical trial for the treatment of recurrent GBM. In this study, the authors determined the safety and distribution kinetics of IL-13 cytotoxin when infused intracranially by a bolus injection and by convection-enhanced delivery (CED) in an athymic nude mouse model of GBM.

Methods. For the safety studies, athymic nude mice were given intracranial infusions of IL-13 cytotoxin into normal parenchyma by either a bolus injection or a 7-day-long CED. Toxicity was assessed by performing a histological examination of the mouse brains. For the drug distribution studies, nude mice with intracranially implanted U251 GBM tumors were given an intratumor bolus or a CED infusion of IL-13 cytotoxin. Brain tumor samples obtained between 0.25 and 72 hours after the infusion were assessed for drug distribution kinetics by performing immunohistochemical and Western blot analyses.

Based on the histological changes in the tumor and brain, the maximum tolerated dose of intracranial IL-13 cytotoxin infusion in nude mice was determined to be 4 μg when delivered by a bolus injection and 10 μg when CED was used. Drug distribution reached the maximum level 1 hour after the bolus injection and the volume of distribution was determined to be 19.3 ± 5.8 mm³. Interleukin-13 cytotoxin was barely detectable 6 hours after the injection. Interestingly, when delivered by bolus injections IL-13 cytotoxin exhibited superior distribution in larger rather than smaller tumors. Convection-enhanced delivery was superior for drug distribution in the U251 tumors because when CED was used the drug remained in the tumors 6 hours after the infusion.

Conclusions. These studies provide confirmation of a previous hypothesis that CED of IL-13 cytotoxin is superior to bolus injections not only for the safety of the normal brain but also for maintaining drug levels for a prolonged period in infused brain tumors. These findings are highly relevant and important for the optimal clinical development of IL-13 cytotoxin or any other targeted antitumor agent for GBM therapy, in which multiple routes of delivery of an agent are being contemplated.

Key Words: • brain tumor • cytotoxin • convection-enhanced delivery • continuous infusion • toxicity • maximum tolerated dose • mouse

Targeting cell-surface proteins or antigens on cancer cells by cytotoxins or immunotoxins is a fascinating approach to brain tumor therapy. Using this concept, we have identified plasma membrane receptors for the helper T cell type 2–derived cytokine IL-13 (IL-13R) on a variety of human solid-cancer cells including those found in brain tumors. The function of IL-13R on tumor cells is not completely known; however, IL-13 can mediate signaling and upregulate adhesion molecules in tumor cells. The IL-13Rα2 (also described as the IL-13Rα) chain, one of the prominent components of the IL-13R system, is overexpressed in glioma cell lines as well as in primary cell cultures derived from patients’ brain tumors but is not overexpressed in the normal brain or in astrocytes. Because this receptor chain plays an important role in ligand binding and internalization and is overexpressed in glioma samples in situ, IL-13R–directed cytotoxin therapy has been considered a promising approach. To target IL-13R on cancer cells we developed a recombinant chimeric protein called IL-13 cytotoxin (IL-13–PE38), which is composed of human IL-13 and a mutated PE. It has been proven that this recombinant protein has a potent antitumor activity against IL-13R–expressing tumors, including brain tumors in vitro and in vivo.

Based on encouraging preclinical results, three Phase I/II clinical trials have been initiated in adults who have recurrent malignant glioma. The first clinical trial involves CED of IL-13 cytotoxin into recurrent unresectable malignant gliomas. Convection-enhanced delivery is a local delivery method in which a pressure gradient or bulk flow is used to control an infusate through the extracellular fluid.
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compartment. So far, this route of IL-13 cytotoxin administration appears to be very well tolerated with no neurotoxicity.\textsuperscript{11} The second clinical trial involves prerecession CED of IL-13 cytotoxin followed by postresection infusion of the cytotoxin into the brain adjacent to the resection cavity.\textsuperscript{23,24} This novel route of IL-13 cytotoxin delivery used in 27 patients has also been well tolerated. In the third clinical trial, IL-13 cytotoxin is infused directly into tumors by CED and tumor resection is performed later.\textsuperscript{29} Nineteen patients have been enrolled in this clinical trial and this particular route of IL-13 administration also appears to be safe. All three clinical trials have recently completed. Currently, a randomized open-label Phase III clinical trial is ongoing at numerous centers in the US, Canada, and Europe to test the efficacy of IL-13–PE in a large patient population with recurrent GBM.

Direct delivery of drugs to a brain tumor is one approach for efficient local distribution, partly because the blood–brain barrier prevents homing of systemically administered drugs to tumors.\textsuperscript{2} In addition to a bolus injection of the drug into tumors, CED has been demonstrated to achieve high concentrations of drug in animal studies and clinical trials.\textsuperscript{1,3,4,9,22,25–27} Although direct intratumoral or surrounding normal brain delivery of IL-13 cytotoxin is being performed in clinical trial settings, its drug distribution kinetics in brain tumors as well as in the normal brain have not been clearly delineated. In this study, we intratumorally injected IL-13–PE38 by bolus or CED into established U251 brain tumors in athymic nude mice. Brain tissue samples were analyzed by an immunohistochemical analysis with anti–IL-13 antibody or Western blot analyses with anti–IL-13 or anti-PE antibodies to assess drug distribution. In addition, the MTD of IL-13 cytotoxin when infused by a bolus injection or CED into the mouse brain was evaluated by a histological examination of infused brain parenchyma.

Materials and Methods

Cell Culture and Cytotoxin

The U251tg cell line was established from a single-cell suspension derived from the U251 GBM, which was aggressively grown subcutaneously in nude mice. The cells were cultured in RPMI-1640 with 10% fetal bovine serum, 1 mM HEPES, 1 mM L-glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin in a humidified incubator with an atmosphere of 5% CO\(_2\) and a temperature of 37°C. Recombinant IL-13–PE38 was generated by following a previously described procedure\textsuperscript{20} and was diluted in PBS containing 0.2% human serum albumin for all studies.

Intracranial Tumor Model

Six-week-old athymic nude mice (~22 g body weight) were anesthetized with ketamine and xylazine and separated placed in a stereotactic head frame. A sagittal skin incision (15 mm) was made from the skull bone to the neck region. A solution of U251tg cells, suspended in 10 μl of PBS, was stereotactically injected through an entry site located 1.5 mm posterior to the bregma and 1.5 mm to the right of the sagittal suture to a depth of 2.5 mm below the surface of the skull by using a 10-μl Hamilton syringe. The U251tg tumors developed 2 or 3 weeks after the injection and were used for the distribution kinetics studies. Animal care was in accordance with guidelines set by the National Institutes of Health Animal Research Advisory Committee.

Bolus and CED Treatment

For the drug distribution kinetics studies, we used 26 mice for the bolus injection and 16 mice for the CED infusion. Thirty other mice were assigned to a group to determine the dose of IL-13–PE38 that causes brain toxicity by bolus or CED infusion into the brain. The brains of an additional 16 mice were used to prepare samples for the Western blot analyses. Bolus infusions of IL-13–PE38 (0.02–10 μg in 10 μl of PBS containing 0.2% human serum albumin) were injected into the animals via a Hamilton syringe with a 26-gauge needle through a burr hole at a depth of 3 mm below the surface of the skull. To avoid backflow and leakage, we set the duration of the infusion at 20 minutes. Convection-enhanced delivery was performed by loading microsomatic Alzet pumps with 100 μl IL-13–PE38 (1–20 μg).\textsuperscript{24} The pumps were inserted subcutaneously into the intrascapular region of each mouse and connected to the polyvinyl chloride catheter tube (inside diameter 0.69 mm). A 28-gauge stainless steel tube with a cannula was inserted into a burr hole at the surface of the skull and fixed with an N-butyl cyanoacrylate adhesive. The entire length of the tubing and cannula was flushed with PBS before it was connected to the catheter tube to ensure that no air bubbles remained in the tube. After connecting the catheter tube to the cannula, the skin was closed with 6-0 silk sutures.

Immunohistochemical Analysis

Brain samples were collected at indicated time points after intracranial IL-13–PE38 infusion, fixed in 10% formalin, and embedded in paraffin. Sagittal brain sections (0.1-mm thickness) were cut, deparaffinized using xylene treatment, and washed with decreasing grades of alcohol (100–50%) and with PBS. Slides containing tissue were incubated with anti–human IL-13 mAb (1 μg/ml) or an isotype control for 18 hours at 4°C. The immunohistochemical analysis was performed using the avidin–biotin complex peroxidase method. The tissue was subsequently developed using diaminobenzidine substrate–biotinylated peroxidase reagent and counterstained with hematoxylin.

Analysis of the IL-13–PE38 Distribution

The stained brain tissues or tumor sections were subjected to image analysis by using an inverted microscope connected to a digital camera and a computer loaded with image analysis software. Sections of tissue were digitally photographed and the area stained with anti–IL-13 mAb in each section was quantitated by applying a semiautomated process. The area of each section (distance on x axis [from ear to ear] × distance on z axis [vertical]) was multiplied by the interval to the next section (y axis, representing the distance from front to back [0.1 mm]); the values for each section were summed to determine the V\(_d\). The point marked zero is considered to represent the site of the infusion needle tip.

Western Blot Analysis

Protein extracts from whole-brain cerebral cortex were mixed with SDS sample buffer and heated for 5 minutes at 70°C. Proteins (20 μg/lane) were separated using 4 to 12% SDS-PAGE and transferred onto a polyvinylidene difluoride membrane. Membranes were blocked for 1 hour in TBST (10 mM Tris–HCl [pH 7.5], 150 mM NaCl, and 0.5% Tween 20) and blocking buffer. After blocking, the membranes were incubated for 1 hour with rat anti–IL-13 antibody or rabbit anti-PE antibody in TBST and then washed. Membranes were incubated with horseradish peroxidase–conjugated secondary antibody in TBST for 30 minutes and were developed by chemiluminescence for the detection of PE. For IL-13 detection, membranes were incubated with biotin-conjugated secondary antibody followed by alkaline phosphatase–avidin D in TBST and developed using the substrate.

Sources of Supplies and Equipment

The U251 human GBM cell line was originally obtained from the National Cancer Institute, Frederick Cancer Research Facility, Division of Cancer Treatment Tumor Repository Program (Frederick, MD). The cell culture reagents were purchased from Biosource International, Inc. (Camarillo, CA), and the athymic nude mice were obtained from the National Cancer Institute Frederick Cancer Center Animal Facilities (Frederick, MD).

The stereotactic frame was manufactured by David Kopf In-
and edema were observed in brain tissue samples collected from mice treated with an 8-μg bolus injection of IL-13–PE38. All of these animals remained healthy and maintained normal posture, movement, and feeding patterns. On the other hand, administration of 10 μg of IL-13–PE38 proved to be lethal to all mice that received a bolus injection.

In contrast to the bolus injection, when nude mice received an intracranial infusion of IL-13–PE38 by CED brain necrosis was not detectable following doses up to 10 μg (Table 1). Brain necrosis was, however, observed at the doses of 12.5 μg and 15 μg. All animals injected with 20 μg IL-13–PE38 by CED died of drug toxicity. These results indicate that higher doses of IL-13–PE38 can be safely administered intracranially by CED compared with bolus infusion.

**Distribution Kinetics of IL-13–PE38 by Bolus Intratumoral Infusion**

To evaluate the distribution kinetics when infused into intracranial U251tg tumors by a bolus injection, 1 μg of IL-13–PE38 was infused into the tumor mass, and tumor and brain samples were collected at 0.25, 0.5, 1, 3, 6, 24, 48, and 72 hours. Tumor development was confirmed 3 weeks (in Experiment 1) or 2 weeks (in Experiment 2) after cell injection. Sequential sagittal brain sections (0.1 mm each; y axis) were subjected to immunohistochemical analysis by using anti–IL-13 antibody. The clearly IL-13–positive area of each section was measured in both x and z axes. The point of infusion is given the position of 0. As shown in Fig. 1A (Experiment 1) and B (Experiment 2), when measurements were made toward the x axis compartment (from ear to ear), IL-13–positive staining was detectable at 0.25 hours (Fig. 1A, x axis 17 μm and y axis [diameter] 13 μm; Fig. 1B, x axis 7.4 μm and y axis [diameter] 19 μm), reaching maximum levels at 1 hour (Fig. 1A, x axis 29.6 μm and y axis [diameter] 27 μm; Fig. 1B, x axis 13.5 μm and y axis [diameter] 31 μm). The distribution area decreased 3 hours after bolus administration. No staining with anti–IL-13 antibody was observed in samples collected at 24, 48, and 72 hours (data not shown). The IL-13–positive area also reached the maximum level toward the z axis compartment (vertical) at 1 hour (Fig. 1A, 21.6 μm; Fig. 1B, 19.8 μm), and the positive-staining region was visible in 6 hours. From these values at each time point, we estimated the V_t of IL-13–PE38 when given by bolus infusion. As shown in Fig. 1C, the V_t increased linearly after the injection, reaching 19.3 ± 5.8 mm^3/1 hour after the infusion, and then decreased to a nondetectable distribution by 24 hours. Because the V_t of IL-13–PE38 was 10 μl/injection, the V_t/V_n ratio at 1 hour after bolus drug infusion was 1.93 ± 0.58.

As shown in Fig. 1D, strong staining of IL-13 was detected in brain tissue around the region near the site of the injection when the samples were analyzed 0.25 hours after the bolus infusion. Interestingly, denucleated dead cells were detected as a sign of tumor necrosis in the IL-13–positive region. At 1 hour after the bolus drug infusion, an expanded IL-13–positive region was visible (at a 40× low-power field) and dying tumor cells were also detected (Fig. 1E). The IL-13–PE38 infusion, represented by staining with anti–IL-13 antibody, became undetectable by 24 hours after bolus infusion (Fig. 1F). These results indicate that brain tu-

**TABLE 1**

<table>
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<tr>
<th>Total Dose (mg)</th>
<th>Toxicity†</th>
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<tr>
<td>0.02</td>
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<tr>
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</tr>
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<td>severe necrosis in brain</td>
</tr>
<tr>
<td>10.00</td>
<td>100% lethal</td>
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*C Thirty athymic nude mice that received intracranial administration of IL-13–PE38 into the normal parenchyma by bolus injection (10 μl volume) or CED (100 μl volume, 7 days of infusion) were killed 4 days after treatment and their harvested brain samples were analyzed by histological changes.

† Toxicity is defined as clear evidence of necrosis and hemorrhage observed after H & E staining.

**Results**

**Induction of Brain Toxicity by IL-13–PE38**

To determine the dose of IL-13 cytotoxin that causes brain toxicity when infused into the normal parenchyma intracranially, nude mice receiving either a bolus injection (10-μl volume) or CED (100-μl volume, 7 days of infusion) were killed 4 days after treatment. To determine any histological changes, paraffin-embedded tissue samples were subjected to immunohistochemical analysis by using anti–IL-13 antibody. The clearly IL-13–positive area of each section was measured in both x and z axes. The point of infusion is given the position of 0. As shown in Fig. 1A (Experiment 1) and B (Experiment 2), when measurements were made toward the x axis compartment (from ear to ear), IL-13–positive staining was detectable at 0.25 hours (Fig. 1A, x axis 17 μm and y axis [diameter] 13 μm; Fig. 1B, x axis 7.4 μm and y axis [diameter] 19 μm), reaching maximum levels at 1 hour (Fig. 1A, x axis 29.6 μm and y axis [diameter] 27 μm; Fig. 1B, x axis 13.5 μm and y axis [diameter] 31 μm). The distribution area decreased 3 hours after bolus administration. No staining with anti–IL-13 antibody was observed in samples collected at 24, 48, and 72 hours (data not shown). The IL-13–positive area also reached the maximum level toward the z axis compartment (vertical) at 1 hour (Fig. 1A, 21.6 μm; Fig. 1B, 19.8 μm), and the positive-staining region was visible until 6 hours. From these values at each time point, we estimated the V_t of IL-13–PE38 when given by bolus infusion. As shown in Fig. 1C, the V_t increased linearly after the injection, reaching 19.3 ± 5.8 mm^3/1 hour after the infusion, and then decreased to a nondetectable distribution by 24 hours. Because the V_t of IL-13–PE38 was 10 μl/injection, the V_t/V_n ratio at 1 hour after bolus drug infusion was 1.93 ± 0.58.

As shown in Fig. 1D, strong staining of IL-13 was detected in brain tissue around the region near the site of the injection when the samples were analyzed 0.25 hours after the bolus infusion. Interestingly, denucleated dead cells were detected as a sign of tumor necrosis in the IL-13–positive region. At 1 hour after the bolus drug infusion, an expanded IL-13–positive region was visible (at a 40× low-power field) and dying tumor cells were also detected (Fig. 1E). The IL-13–PE38 infusion, represented by staining with anti–IL-13 antibody, became undetectable by 24 hours after bolus infusion (Fig. 1F). These results indicate that brain tu-

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FIG. 1. Distribution kinetics of IL-13 cytotoxin infused into the mouse brain by a bolus intratumoral injection. Nude mice harboring a xenografted U251tg intracranial tumor (two animals/group) each received a bolus injection of IL-13–PE38 (1 μg in a 10-μl volume of PBS containing 0.2% human serum albumin). Brain samples were collected at 0.25 to 72 hours after drug infusion, fixed with 10% formalin, and embedded in paraffin. Sequential sections were subjected to immunohistochemical analysis using anti–IL-13 antibody. A–C: Graphs demonstrating drug distribution from position 0 (needle tip). The distribution is represented by the y axis (sequential 0.1-mm sagittal front–back section) compartment compared with the x axis (ear–ear) compartment or the y axis compartment compared with the z axis (vertical) compartment. Experiment 1 (A). Experiment 2 (B). The IL-13 cytotoxin was detectable in samples up to 6 hours postinjection (C). The Vd value was determined from each time point. Data are presented as means ± standard deviations of two separate experiments. D–F: Representative photomicrographs of brain tumor sections obtained 0.25 (D), 1 (E), or 24 hours (F) after a bolus injection. Arrow in E indicates the needle track and brown staining indicates the IL-13–positive area. Tu = tumor. Anti–IL-13 antibody with hematoxylin counterstaining.
when infused into U251tg tumor–bearing mice by CED (5
hand, the protein levels of the actin control remained un-
distributed within the tumor bed without dissociation be-
cate that the IL-13 and PE components in IL-13–PE38 were
declined over the course of 3 to 6 hours and, beyond that
time no IL-13–PE38 was detected. The PE staining provid-
eed identical results to the IL-13 staining. These results indi-

Fig. 2. Distribution of bolus IL-13 cytotoxin in whole-brain extracts determined by Western blot analysis. Whole-brain extracts were prepared from brain samples collected after bolus intratumor-
al infusion of IL-13–PE38 at indicated time points (0–48 hours) af-
fter injection. Samples (20 μg/lane) were separated by SDS–PAGE, 
transferred to a polyvinylidine difluoride membrane, and hybrid-
ized with the IL-13 and PE anti–antibody. Actin served as an 
internal control. Brain extracts were pooled from two individual 
mice to recover enough protein to minimize technical variations. 
Western blot experiments were repeated several times.

cor cells were susceptible to the cytotoxic effect of IL-13– 
PE38 as early as 0.25 hours after a bolus intratumoral infusion 
and that the drug distribution reached maximum levels at 
1 hour and decreased to undetectable levels by 24 hours 
postinjection.

To determine the impact of tumor volume on drug dis-
stribution, smaller and larger tumors were injected with IL-
13–PE38. As shown in Fig. 1, the distribution measured in 
Experiment 1 was superior to that measured in Experiment 
1 compared with Experiment 2. In Experiment 1, the max-
imum drug distribution along the x axis was 30 μm com-
pared with 13.5 μm in Experiment 2 (p < 0.05). This dis-
tribution correlated well with tumor size because the tumors 
in Experiment 1 (tumors developed for 3 weeks) were larger 
than those in Experiment 2 (tumors developed for 2 
weeks).

Discussion

In this study, we determined the MTD of IL-13–PE38 
when infused into the normal brain by a bolus injection 
or by CED. In addition, we determined the distribution ki-
netics of IL-13–PE38 in a human glioma in the immuno-
deficient mouse brain following either a bolus injection or 
CED infusion, as assessed using immunohistochemical and 
Western blot analyses.

In the toxicity studies, the MTD of intracranial IL-13– 
PE38 infusion in nude mice was determined to be 400 μg/ 
ml or 200 μg/kg total when the delivery method was bo-
lus infusion and more than 700 μg/ml (100 μg/ml daily) or 
500 μg/kg total when it was CED. In our previous studies, 
Sprague–Dawley rats that received an intracranial bolus in-
jection of IL-13 cytotoxin demonstrated no toxicity at a 
dose level of 100 μg/ml, but did display severe brain necro-
sis at the 500-μg/ml dose. The 400-μg/ml dose was not 
evaluated; however, our current results following a bolus 
IL-13–PE38 injection into the normal brain parenchyma 
in mice (400 μg/ml) were generally consistent with results
Distribution kinetics of IL-13–PE in human brain tumor

from studies of rats, even though the size of the brain differs between mouse and rat.

It is important to highlight that the MTD obtained using CED when administered into the normal brain parenchyma was higher than that obtained using a bolus administration. The mechanisms behind the differences in MTD between bolus injection and CED is unknown. It is possible that the peak distribution area under the curve will be higher for CED than for the bolus administration. In addition, the rate of drug clearance would differ between CED and bolus administration. As interstitial IL-13–PE38 would be cleared by proteolytic cleavage, a larger volume of distribution achieved by CED would expose normal cells to a lower concentration of drug, leading to a lower nonspecific toxicity compared with a high local concentration delivered by bolus administration.

When intracranial GBMs in mouse brains received bolus injections of IL-13–PE38, the distance of drug distribution was higher in larger tumors than in smaller tumors. Because GBM cells express type 1 IL-13R, which is composed of the IL-13Rα2 chain as well as the IL-13Rα1 and IL-4Rα chains, and IL-13 binds to the IL-13Rα2 chain with the highest binding affinity, it is possible that the reason IL-13 cytotoxin may be better distributed in larger tumors is because of the higher number of IL-13R–expressing GBM cells than that found in smaller tumors. Alternatively, the higher interstitial pressure in larger tumors may have created a larger convection effect compared with smaller tumors.

We determined the distribution kinetics of IL-13–PE38 by performing immunohistochemical (Fig. 1) and Western blot (Fig. 2) analyses and obtained identical results. We previously demonstrated that IL-13 cytotoxin was degraded 2 to 4 hours after an intratumoral bolus injection in subcutaneously xenografted U251 tumors in nude mice. Our current findings indicate that IL-13 cytotoxin remained present longer in intracranial brain tumors than in subcutaneous tu-

![Fig. 3. Distribution kinetics of IL-13 cytotoxin infused by CED into the mouse brain. Nude mice harboring a xenografted U251tg intracranial tumor (two animals/group) each received a CED infusion of IL-13–PE38 (5 μg in 100-μl volume over a 7-day period). Brain samples were collected at 6 and 24 hours after the end of the drug infusion, fixed with 10% formalin, and embedded in paraffin. Sequential sections were prepared and subjected to immunohistochemical analysis using anti–IL-13 antibody. A and B: Representative photomicrographs of brain sections obtained 6 and 24 hours after completion of the CED. Anti–IL-13 antibody with hematoxylin counterstaining. Original magnifications ×100 (A) and ×600 (B). Arrow in A indicates the needle track. C: Graphs depicting drug distribution from position 0 (needle tip), represented as x axis (ear–ear), y axis (sagittal), and z axis (height) compartments. D: Bar graph showing the Vₜ determined from the values shown in C for each time period. Ne = necrosis.]
tumors that were injected intratumorally. This may be due to differences in absorption, clearance, and interstitial pressure. This property of a longer half-life in intracranial glioma will be exploited for cancer therapy.

Although various investigators have used mixed-target cytotoxins or immunotoxins with fluorescent or radiolabeled markers to evaluate drug distribution in the brain,9,30,32 we used immunohistochemical staining of tissues with a specific antibody to IL-13 to assess the IL-13–PE38 distribution in the tumor bed and beyond the tumor in the normal brain. When compared with previous studies of CED in which 14C-sucrose, fluorescein isothiocyanate–dextran, or boronated epidermal growth factor had been used, the V₆₃ using IL-13–PE38 was lower.9,30,32 Although the precise reason for this variation is unknown, it is possible that the amount of the drug and the detection method (study of fluorescent or radiolabeled markers as opposed to an immunohistochemical analysis) might have been responsible for this difference. One drawback with immunohistochemical analysis in the study of drug distribution is the semiquantitative nature of this technique. On the other hand, the clearance and distribution of fluorescent or radiolabeled markers may differ from those of immunotoxins or cytotoxins, or the labeled material may be cleaved during or after the drug infusion, resulting in a nonspecific assessment of the drug distribution. We did not have that latter problem in our study, however, because the status of intact IL-13–PE38 was assessed and confirmed using a specific antibody to IL-13, which detected the complete IL-13–PE38 molecule. In addition, our Western blot analyses confirmed our detection of intact IL-13–PE38 because two antibodies specific to both IL-13 and PE demonstrated drug distributions similar to those observed using immunohistochemical analysis. Because the Western blot analyses included two different assays that detected their targeted proteins and showed a similar trend in band intensity, we believe that the dissociation of IL-13–PE did not occur, at least to a significant degree. Minor dissociation may not be appreciated by these semiquantitative techniques and may require much more sensitive assays.

Conclusions

We have demonstrated the following. 1) The MTD of IL-13–PE38, when infused into the normal brain, is higher after CED than after bolus administration. 2) The distribution kinetics of IL-13–PE38 in a human glioma in the mouse brain after a bolus injection of drug provided identical results when assessed using immunohistochemical and Western blot analyses. 3) The distribution kinetics of IL-13–PE38 in a human brain tumor in the mouse after a CED drug infusion is quite different from those in a similar tumor after a bolus injection of drug. Our results indicate that higher concentrations of IL-13–PE38 can be infused into animal brains by CED than by a bolus injection. In addition, IL-13–PE38 remains in the brain tumor longer when infused by CED. Although we found that the distribution of IL-13–PE38 reached maximum after 1 hour of bolus injection, complete clearance of the drug from the brain occurred 24 hours after the drug injection. When the delivery method was CED, however, IL-13–PE38 levels were still detectable 24 hours after the completion of drug infusion. These findings are critically important for the development of this drug and for the clinical use of IL-13–PE38 in the treatment of GBM.

References

Distribution kinetics of IL-13–PE in human brain tumor


Manuscript received March 5, 2004.
Accepted in final form August 11, 2004.

These studies were conducted as part of a collaboration between the Food and Drug Administration and NeoPharm, Inc., under Cooperative Research and Development Agreement (CRADA). The views presented in this article do not necessarily reflect those of the Food and Drug Administration.

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