Convection-enhanced delivery in clinical trials

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The poor prognosis associated with the current management of malignant gliomas has led investigators to develop alternative treatments such as targeted toxin therapy. The optimal method for administering these agents is under development but appears to be convection-enhanced delivery (CED).

The direct intratumoral infusion of targeted toxins was first performed in nude mouse flank tumor models of human malignant glioma. After the demonstration of in vivo efficacy, these potent cytotoxic compounds were tested in Phase I and Phase II clinical trials.

Using a high-flow microinfusion technique, volumes of up to 180 ml were infused by CED through catheters placed directly into brain tumors. Minor systemic toxicity was seen in the form of hepatic enzyme elevation. Neural toxicity manifested as seizure activity and hemiparesis resulted from peritumoral edema that followed the completion of the infusion. Peritumoral toxicity was believed to be more related to the concentration of the infused immunotoxin than to the infusion volume. In approximately half of patients treated with CED a stable disease course, a partial response, or a complete response was demonstrated in some clinical trials.

Targeted toxin therapy has clinical efficacy in patients with malignant gliomas. Convection-enhanced delivery appears to represent an effective method for administering these agents in patients with malignant brain tumors.

KEY WORDS • brain neoplasm • targeted toxin • glioma • convection-enhanced delivery

Although most tumor recurrence occurs within 2 cm of the tumor resection margin, tumor cell infiltration can extend well beyond the surgical site into normal brain parenchyma. The ability to target exclusively infiltrating tumor cells while sparing nontarget neural tissue could represent a superior treatment alternative for patients with malignant gliomas. This concept of targeted drug delivery to cancer cells is not new and was originally proposed in 1906 by Paul Ehrlich. Initially, a class of hybrid molecules was constructed (immunotoxins) that were composed of a protein toxin conjugated chemically to an mAb. In animal studies the poor penetration of these agents due to their large size led investigators to generate smaller recombinant forms known as fusion proteins or targeted toxins that use growth factors or single-chain antibodies as their carrier ligands.

As the development of these agents progressed toward clinical trials, the BBB was recognized rapidly as a potential problem that could interfere with the administration of targeted therapy in patients with brain tumor. To circumvent this issue, delivery techniques for the direct administration of these drugs into solid tumor tissue were created and tested. The presence of the BBB was also considered to be an advantage for treating patients harboring brain tumors with immunotoxins because it allowed the infused compound to become trapped within the CNS and allowed for the achievement of high local concentrations directly within the targeted tumor. The high-flow microinfusion delivery technique used within the CNS became known as CED.
CONVECTION-ENHANCED DELIVERY MICROINFUSION

Pharmacokinetic Considerations

The goal in delivering targeted toxins to brain tumors is to achieve diffuse homogenous distribution of the agent throughout the tumor and into the adjacent area of tumor cell—infiltrated brain parenchyma. Nonuniform distribution of the drug in tumors will ultimately influence the therapeutic efficacy. In animal studies in which radiolabeled compounds were administered into the lateral ventricle, there were several factors that influenced the volume of distribution of the drug in the adjacent caudate nucleus (unpublished data). The factors included the geometrical orientation of the tissue relative to the point of perfusion, the density of capillaries within a given area of perfusion, the diffusion properties of the compound across the BBB, and the rate of uptake of the compound by the surrounding cells (unpublished data).

To penetrate a tumor, a targeted toxin delivered into the brain tissue adjacent to the tumor must pass through the microvascular wall of the tumor, overcome the resistance to diffusion posed by the interstitial pressure within the tumor, and distribute uniformly throughout the lesion without being influenced significantly by the proximal antigen binding affinity (unpublished data). Because tumor vessels are tortuous, dilated, and saccular in their architecture and demonstrate arteriovenous shunting, they prevent uniform distribution of the targeted toxin throughout (unpublished data). The intercapillary distance between tumor vessels has been shown to be wider than that in normal blood vessels, thereby requiring a larger area of diffusion for immunotoxins to reach therapeutic levels. The surface area of capillaries decreases from the periphery of the tumor to the center resulting in central necrosis such that the vascular surface area decreases as the tumor enlarges. Tumor interstitial pressure is thought to be due to an accumulation of interstitial fluid, an increase in col-lagen and elastic fiber network, and a lack of effective drainage of interstitial fluid out of the tumor. Tumor interstitial pressure has been shown to increase with the size of the tumor and can reach a level of 30 mm Hg.

Infusion of targeted toxins in patients with brain tumors must overcome the resistance of the brain to reach infiltrating tumor cells. By administering saline into the brains of animals, it was found that the mean initial resistance to flow was 6.42 mm Hg/ml/min and decreased to 0.81 × 10^3 mm Hg/ml/min with an increase in the total volume infused. The increased interstitial pressure initially encountered was believed to be due to the inherent resistance of the brain; however, distention of the extracel-lular space allowed for accommodation to infused fluid volume. Opening 0.01% of the interstitial channels in the brain to 30 to 70 times their normal size will increase the hydraulic conductivity 100- to 2500-fold (unpublished data).

The distribution of immunotoxins in the interstitial fluid of the brain is also based on a concentration gradient termed diffusion. Diffusion of immunotoxins is dependent on their concentration, molecular weight, polarity, and the avidity of the immunotoxin for the target antigen (unpublished data). The time required for an immunotoxin to diffuse a distance L can be approximated by the formula \( L^2 / 4D \) where D is the diffusion coefficient of the immunotoxin. The distribution of immunotoxins within the brain improves with CED. The CED of \(^{131}\)I-radiolabeled transferrin in the cat brain achieved a diffusion distance of 1.5 cm in 2 hours compared with \(^{131}\)I-radiolabeled sucrose (359 D) which diffused 0.5 cm further in the same period. This difference represents a significant reduction in time compared with a similar weight-based immunoglobulin G molecule that required 8 months to diffuse 1 cm (unpublished data).

Preclinical Studies

Intratumoral Administration. In a nude mouse flank tumor model of human GBM in which the immunotoxin composed of transferrin conjugated to the mutant diphtheria toxin CRM 107 was directly infused into the tumor, greater than 95% tumor regression was demonstrated by Day 14. No tumor recurrence developed by Day 30. The tumors measured 0.5 to 1 cm in diameter and were treated every other day with 10 \( \mu \)g of either transferrin CRM107 or antitransferrin receptor mAb ricin A chain immunotoxin (454A12-RA) for a total of four doses. In animals receiving 454A12-RA tumor volume decreased 30% by Day 14. A dose-response relationship was seen with transferrin-CRM107 when using the doses 0.1, 1, or 10 \( \mu \)g.

Two other targeted toxins have been tested in nude mouse human GBM flank tumor models, DAB EGF (the diphtheria toxin mutant DAB adj attached to EGF) and IL-4(38-37)-PE38KDEL (IL-4 bound to a circularly permuted \textit{Pseudomonas} exotoxin mutant). When either 1 or 10 \( \mu \)g of DAB adj EGF was injected intratumorally twice a day for three doses into U87 human GBM flank tumors in nude mice, significant tumor growth was inhibited. The intratumoral administration of 250 \( \mu \)g/kg of IL-4(38-37)-PE38KDEL on alternate days for three or four doses caused the complete regression of both small and large U251 human GBM nude mouse flank tumors. Intravenous and intraperitoneal injections of IL-4(38-37)-PE38KDEL also caused significant antitumor activity in this same animal model.

Distribution Studies. The spatial distribution of immunotoxins in solid tumor has been examined using quantitative autoradiography. In an animal model of subcutaneous human rhabdomyosarcoma, radiolabeled immunotoxins directed against the transferrin receptor were administered intravenously and their spatial distribution in the tumor was determined at 2, 6, and 24 hours. When autoradiography was performed, diphtheria toxin had a uniform distribution throughout the tumor. Punctate patterns of distribution were seen throughout the tumor for immunotoxins composed of either a monoclonal immunoglobulin G1 antibody or an mAb Fab' fragment, both of which were conjugated to CRM107. A more homogenous pattern of spatial distribution was seen when a nonbinding immunotoxin was administered intravenously compared with either targeted toxin, although the pattern was not as uniform as that which was demonstrated with diphtheria toxin. The heterogeneous distribution of the mAb-direct-ed immunotoxins was believed to be due to their binding to tumor cells.
Clinical Trials

With respect to the CNS, targeted toxin trials have been conducted in which these agents were administered intravenously, intrathecally, and intratumorally. The intravenous injection of anti-EGFR mAb in doses ranging from 760 to 2400 mg was performed in 16 patients with recurrent GBMs.12 No patient experienced a partial or complete response, although in seven of the 13 patients evaluated the disease course was stable for 1 to 4 months. A median survival time of 15.6 months was reported in 25 patients with malignant astrocytomas who underwent resection and external-beam radiotherapy and who received either intravenous or intrathecal radiolabeled anti-EGFR mAb.3

Although the first immunotoxin clinical trial was conducted in patients harboring leptomeningeal neoplasms with the 454A12-RA immunotoxin, advances in genetic engineering have led to the development of recombinant fusion proteins in which DNA sequences for the carrier growth factor and various bacterial toxins are inserted into bacteria to generate a homogeneous, pure compound.13 In the trial in which 454A12-RA was administered intrathecally as a single dose in eight patients, 1.2 to 1200 μg was delivered, and in four patients there was a greater than 95% transient reduction in their CSF tumor cell counts.12

The intratumoral administration of a murine anti-EGFR mAb in eight patients with primary or recurrent GBM resulted in histologically detected necrosis in three patients and CT scanning–documented necrosis in three patients.11 Human anti–mouse antibody responses were observed in six of eight patients. In 62 patients with malignant gliomas (58 GBM and four anaplastic astrocytoma) who underwent intratumoral administration of an anti-tenascin mAb conjugated to 1–131, 23 patients experienced complete responses in a median period of 12 months before tumor progression.14 In this series, 31 patients harbored newly diagnosed tumors and 31 patients had recurrent tumors. The overall median survival for the entire group was 23 months. The mean radiation dose delivered to the tumor was calculated at 526 Gy per cycle. Dose-dependent human anti–mouse antibody responses were demonstrated.

Using a high-flow interstitial microinfusion that induced fluid convection within the brain, 15 patients harboring malignant gliomas (nine GBM, five anaplastic astrocytoma, and one anaplastic oligodendroglioma) were treated with intratumoral injections of transferrin-CRM107.15 There were two other patients with lung carcinomas also treated in the trial. In this Phase I/II trial, tumor volumes were reduced by more than 50% in nine patients. Tumor volume reduction was not observed for 1 month posttreatment, and in four patients was not maximized for 6 to 14 months. The median survival for the nine patients in whom the lesions responded to treatment was 75 weeks, with three of the survivors still alive at the time of the publication of the results (range 102–142 weeks). There were two patients in whom complete responses were demonstrated. In the first of these, disease progression did not occur for 23 months and in the second patient, who harbored an anaplastic astrocytoma, the lesion recurred 5 months after treatment. Of the 16 patients who underwent MR imaging within 6 weeks of treatment, reduction in tumor volume or a zone of necrosis within the tumor occurred in 14 (88%).

The therapeutic response in this trial appeared to be dose dependent. In two of eight patients who received total doses of 0.5 to 12.8 μg at a concentration of either 0.1 or 0.32 μg/ml, partial responses were observed. When a concentration of 1 μg/ml or greater was administered, two of four tumors receiving total doses of 20 to 128 μg had complete responses and the other two had partial responses. At intermediate dose levels, the therapeutic response correlated more with the total dose administered than with the concentration of the drug that was infused. There was no correlation between the pretreatment tumor volume and the therapeutic response. In the patients treated with a total dose of 60 μg or higher, responses were all partial; however, peritumoral toxicity was also noted in two of three patients who received a total dose of 60 μg. When a concentration of 0.66 μg/ml or less was infused in 40 ml, no peritumoral toxicity occurred.

The intratumoral infusion volumes ranged from 5 to 180 ml.15 The infusions were well tolerated without causing any treatment-related deaths or life-threatening toxicity. A total of 44 infusions were performed, and the transient worsening of a preexisting neurological deficit was demonstrated in only three patients. All three patients improved clinically after hyperosmolar therapy and corticosteroid agent treatment. Four patients, known to suffer seizures, experienced seizures during the trial and peritumoral edema developed in three. When the infused concentration of the targeted toxin was greater than or equal to 1 μg/ml, focal peritumoral brain injury developed 2 to 4 weeks posttreatment. In three patients in whom hemiparesis occurred and which resolved in two, serpentine strips of increased signal were revealed on T1-weighted noncontrast-enhanced MR imaging. Thrombosed cortical venules and capillaries were observed in biopsy samples obtained from these abnormal areas depicted on the MR images.

Transient elevation of serum alanine and serum aspartate aminotransferases was demonstrated in 14 patients.13 Hypoalbuminemia occurred in 12 patients. Antidiphtheria antibodies were present in all patients before treatment and a twofold increase was seen in six of 14 patients more than 4 weeks after treatment. No correlation was noted between antibody titer and therapeutic response. In five deceased patients in whom autopsies were performed after treatment there was no evidence of systemic toxicity in any organ system. In six deceased patients in whom the brain was examined at autopsy, changes believed to be due to prior radiotherapy were seen, and chronic vasogenic edema not related to the infusion was present.

The results of the Phase II trial involving transferrin-CRM107 at nine centers in the US were recently presented in poster format at the Society for Neuro-Oncology meeting (unpublished data). Patients received two direct intratumoral continuous infusions of the targeted toxin via two catheters between 4 and 10 weeks apart. Each infusion was performed over 5 to 7 days and delivered 0.67 μg/ml in a volume of greater than 32 ml to 40 ml. Each treatment was intended to deliver 21.4 to 26.8 μg of transferrin-CRM107. Thirty-one of 44 patients ranging in age from 18 to 76 years underwent both treatments. There were five patients (11%) with complete responses, seven (16%) with partial responses, nine (20%) with stable disease, 13 (30%) with tumor progression, and 10 (23%)
could not be evaluated. Stable disease, a partial response, or complete response was demonstrated in nearly half of the patients. The median survival time was 37 weeks for all patients and 13 (30%) were still alive 12 months after the first infusion. Systemic toxicity included the transient elevation of hepatic enzymes, which was not clinically significant. There were eight episodes of transient cerebral edema, four focal seizures, two generalized seizures, and three hemipareses. The findings obtained in the expanded Phase II trial were believed to be comparable with the results of the Phase I/II study.

In a recent Phase I/II study, 12 patients with GBM underwent intratumoral CED of the targeted toxin IL-13 conjugated to a mutated *Pseudomonas* exotoxin (IL13-PE) (unpublished data). Following examination of a brain biopsy sample to confirm the presence of recurrent tumor, patients underwent a 48-hour infusion of IL13-PE. All patients underwent tumor resection on Day 8 and in five of nine patients tumor necrosis, which was not present preoperatively, was found. At the time of the report, the time until tumor recurrence ranged from 1 to 42 months (unpublished data).

Results of a Phase I study in which investigators studied intratumoral therapy of recurrent malignant gliomas with NBI-3001, a fusion targeted toxin composed of circularly permuted IL-4 and a mutated form of *Pseudomonas* exotoxin have been presented at the 2001 Congress of Neurosurgeons meeting (unpublished data). Thirty-one patients with malignant gliomas were treated at eight centers in the US and Germany. After confirmation of tumor was obtained by evaluating samples, one to three catheters were stereotactically placed in and around the tumor and varying concentrations and volumes of NBI-3001 were administered. No systemic toxicity was noted in any patient. Treatment-related significant adverse events were primarily involved the CNS and were generally transient. Drug-related Grade 3 to 4 toxicity was observed in 39% of patients in all dose groups and 22% of patients at the maximum tolerated dose of 6 μg/ml × 40 ml. The overall median survival was 8.2 months and median survival was 5.8 months for patients with GBM. The investigators concluded that their results indicated an acceptable safety profile for NBI-3001 at the maximum tolerated dose of 6 μg/ml × 40 ml and that further trials were necessary to assess its safety and efficacy. In a subsequent Phase I/II study, however, investigators demonstrated that the 6-μg dose was not consistently tolerated in the larger cohorts of patients. This recently completed study has suggested that 1.5 μg/ml administered in volumes up to 60 ml is well tolerated by patients (personal communication with sponsor, 2002), but a final analysis has not yet been released.

**CONCLUSIONS**

Targeted toxin therapy appears to have therapeutic efficacy in patients with malignant gliomas. Because of the size of these compounds and the presence of a partially intact BBB, direct intratumoral delivery of these compounds appears to be the best route for their administration. Convection-enhanced delivery of targeted toxins has demonstrated efficacy in animal studies and more recently has been shown to have therapeutic benefit in Phase I and II clinical trials. The optimal dosing regimens that include the flow rate, infusion volume, and concentration of the infused agent have not yet been firmly established and will vary based on which targeted toxin is chosen for treatment.

**References**