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Prolonged intracerebral convection-enhanced delivery of topotecan with a subcutaneously implantable infusion pump

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Intracerebral convection-enhanced delivery (CED) of chemotherapeutic agents currently requires an externalized catheter and infusion system, which limits its duration because of the need for hospitalization and the risk of infection. To evaluate the feasibility of prolonged topotecan administration by CED in a large animal brain with the use of a subcutaneous implantable pump. Medtronic Synchromed-II pumps were implanted subcutaneously for intracerebral CED in pigs. Gadodiamide (28.7 mg/mL), with or without topotecan (136 μM), was infused at 0.7 mL/24 h for 3 or 10 days. Pigs underwent magnetic resonance imaging before and at 6 times points after surgery. Enhancement and FLAIR+ volumes were calculated in a semi-automated fashion. Magnetic resonance spectroscopy-based topotecan signature was also investigated. Brain histology was analyzed by hematoxylin and eosin staining and with immunoperoxidase for a microglial antigen. CED of topotecan/gadolinium was well tolerated in all cases ($n = 6$). Maximum enhancement volume was reached at day 3 and remained stable if CED was continued for 10 days, but it decreased if CED was stopped at day 3. Magnetic resonance spectroscopy revealed a decrease in parenchymal metabolites in the presence of topotecan. Similarly, the combination of topotecan and gadolinium infusion led to a FLAIR+ volume that tended to be larger than that seen after the infusion of gadolinium alone. Histological analysis of the brains showed an area of macrophage infiltrate in the ipsilateral white matter upon infusion with topotecan/gadolinium. Intracerebral topotecan CED is well tolerated in a large animal brain for up to 10 days. Intracerebral long-term CED can be achieved with a subcutaneously implanted pump and provides a stable volume of distribution. This work constitutes a proof of principle for the safety and feasibility for prolonged CED, providing a means of continuous local drug delivery that is accessible to the practicing neuro-oncologist.

Keywords: brain tumor, convection-enhanced delivery, glioma, infusion, local delivery, topotecan.

Malignant gliomas present a difficult therapeutic challenge because the blood-brain barrier and systemic toxicity represent major limitations to the systemic administration of chemotherapeutic drugs.[1] Because malignant gliomas recur within centimeters of the original lesion,[2] convection-enhanced delivery (CED) is a treatment modality that directly addresses these challenges.[3,4] CED involves the surgical implantation of catheters into a tumor and/or the adjacent surrounding parenchyma. The catheters are coupled to a pump to provide continuous, positive-pressure microinfusion that maintains a pressure gradient between the infusion site and the surrounding parenchyma, distributing the infusate into the interstitium.[4,5] Mathematical and experimental models have demonstrated the advantages of bulk flow distribution provided by CED over simple diffusion methods, such as systemic chemotherapy or local passive delivery methods.[6–11]

Topotecan, a camptothecin analog and inhibitor of topoisomerase I, is cytotoxic to proliferating glioma cells but remains relatively innocuous to normal brain.[12] Although this agent demonstrated a potent anti-neoplastic effect in preclinical models of gliomas, it failed to show appreciable efficacy and was associated

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with high dose-limiting toxicity with systemic administration in patients with recurrent malignant gliomas.[8,10,11] Given the benefits of CED as a delivery strategy, our group performed a phase 1 trial of topotecan CED for patients with recurrent malignant gliomas in which it proved to be safe and efficacious.[13] In this trial, topotecan was administered through externalized catheters for a treatment period limited to 4 days to minimize the risk of infection. However, in a rat glioma model, our group demonstrated increased survival when the infusion duration of topotecan was prolonged (1 vs. 4 vs. 7 days), providing a rationale for prolonged CED of topotecan in future clinical trials.[14]

In the present study, we evaluated the safety and feasibility of 10-day intracerebral delivery of topotecan by CED in pigs, a large animal brain model that closely approximates the proportion of white matter seen in humans. Medtronic Synchromed II infusion pumps, which have been approved by the US Food and Drug Administration (FDA) for the treatment of spasticity and chronic pain, were implanted subcutaneously to facilitate chronic infusion. We hypothesized that a fully internalized infusion system would allow the extension of CED from days to weeks and ultimately provide the rationale and feasibility for prolonged CED in the outpatient setting.

Methods

Stability and Efficacy of Topotecan Maintained at 37°C

U87 human glioma cells (ATCC) were plated in quadruplicate at 1×10^5 cells per well in a 96-well plate at each time point assessed. Topotecan in an aqueous solution (LKT Laboratories) was placed in an incubator at 37°C for a total duration of 10 days. Cells were incubated in 200 μL of media containing 136 μM of topotecan at days 0 (baseline), 6, and 10. Control cells at matched time points were cultured in topotecan-free media. Culturing in these conditions occurred for 72 h before performing an MTT assay (ATCC) for proliferation status, in accordance with the manufacturer's protocol. The percentage of inhibition of proliferation was calculated using the formula, $1\text{-}(OD_{topotecan}/OD_{control})$.

Surgery

All procedures were approved by the Institutional Animal Care and Use Committee at Columbia University Medical Center and were compliant with the standard animal practices. The infusate consisted of 1:100 gadodiamide (28.7 mg/mL; Omniscan; GE Healthcare) plus/minus topotecan (136 μM; LKT Laboratories) was infused at a rate of 0.7 mL/24 h for 3 or 10 days. Twenty-kilogram male pigs underwent implantation of a catheter (0.7 mm I.D; Hermetic Lumbar Catheter; Integra Neurosciences), with the end of the catheter cut to have a single hole for infusion, into the anterior limb of the right internal capsule as follows. The catheter was primed with the infusate and connected to a Synchromed II pump (Model 8637-20; Medtronic); the pump was then fixed to the superficial fascia in the subcutaneous plane. A second incision was made at the scalp, and the catheter was tunneled to this site. A burr hole was made and the catheter was introduced with free-hand technique into the brain parenchyma to the predetermined depth. The coordinates for the target were obtained from a preoperative magnetic resonance image (MRI) using the bregma as surface reference. The catheter was fixed to the burr hole with Aquasil Ultra Monophase (Dentsply). Neurological status and evidence of systemic side effects or toxicity were routinely followed by the veterinary team with the porcine neurobehavioral score.[15]

Imaging

Serial MRIs were obtained on days -1, 0, 1, 2, 3, 6, and 10 relative to the operative day. T1 and FLAIR images were collected at each time point. T1 and FLAIR images were processed by subtracting the contralateral from the ipsilateral brain hemisphere to highlight regions of signal enhancement in a new "difference" image. Linear registration between the 2 hemispheres was performed with the FLIRT toolbox in the FMRIB software library[16] to minimize differences in image orientation. To further detect the boundaries of the volume of distribution (VOD) and to ensure consistency across scans and animals, the upper and lower 1 percentile of intensity values was calculated for each difference image and used as a threshold. The boundaries were manually defined by an imaging researcher (T.Y.) who had been blinded to the treatments and time points, and volumes were calculated in cubic millimeters. For volumetric comparisons between animals and time points, relative volume was calculated as follows: relative volume = X volume/maximal volume for that pig from all time points.

Magnetic Resonance Spectroscopy

A 1.5T MRI scanner (Intera/Achieva R2136; Philips Medical Systems) equipped with a transmit-receive head coil was used. A single voxel (SV) point-resolved 1H spectroscopy was performed on the site of injection in the brain as well as the contralateral side using the following parameters: TR/TE = 2000/35 ms; voxel size, ~1 cm^3; 1024 points; and 128 averages. To saturate the water signal and therefore preserve the metabolite signal, a chemical shift selective pulse with narrow bandwidth centered at the resonance frequency of water was used. The post-processing was performed via an in-house developed software (3DiCSI) that included residual water suppression, Gaussian filtering of 3Hz, zero-filling to 2048 points, and zero-order and first-order phase and baseline corrections.

Histology

The ex vivo brain was fixed in 4% paraformaldehyde immediately after necropsy. Histological analysis was done with hematoxylin and eosin staining. Immunoperoxidase analysis was performed on paraffin-embedded sections using an antibody to the ionized calcium-binding-adaptor molecule, Iba1 (rabbit anti-human; Wako), a macrophage-specific antigen. Cell counting of Iba1$^+$ cells was performed on 3 high-power fields per animal at 2 locations, peri-catheter and distant white matter (>2 high power fields away from catheter). The tissue preparation and histology were evaluated by a neuropathologist (P.C.) who was blinded with regards to treatment.

Statistical Analysis

Two-tailed *t* test was used for estimation of differences in volumes between different conditions (with or without topotecan), and repeated measures analysis of variance was used to test differences in T1 enhancing volume and FLAIR+ volume between groups over time. Differences in Iba1$^+$ staining in animals between the 2 conditions (with or without topotecan) were assessed using 2-tailed *t* test. All statistical analysis was done using SPSS software, version 18.0 (SPSS Inc), and Prism software, version 5 (Graphpad Software).

Results

Topotecan Maintains Bioactivity up to 10 Days Body Temperature

To assess the effect of prolonged physiologic temperature on the bioactivity of topotecan, defined as its ability to inhibit the proliferation of U87 human glioma cells in culture, proliferation assays were performed comparing topotecan maintained at 37°C for 6 or 10 days to freshly prepared drug. Inhibition of 52.8% ± 10.8% of U87 proliferation was seen at baseline using a concentration of 136 μM of topotecan. The percentage inhibition of proliferation at day 6 (53.3% ± 6.8%) and day 10 (52.6% ± 4.3%) showed no significant difference from day 0 (Fig. 1).

Prolonged Intracerebral CED of Topotecan is Well Tolerated

The subcutaneous implantation of the Medtronic Synchromed II pump allowed the infusion of the topotecan/gadolinium (t/g) combination for up to 10 days, compared with the 4-day period employed in the topotecan CED phase I clinical trial.[13] The infusion was well tolerated in 100% of the animals, with a complete absence of neurological signs according to the porcine neurobehavioral score, which assesses mental status, appetite, and motor function/gait ($n = 6$).[15]

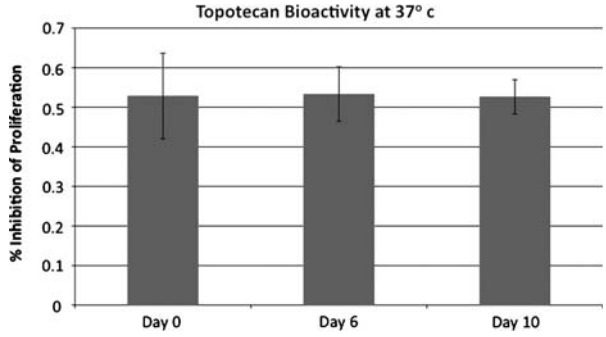

Fig. 1. Bioactivity of topotecan maintained at 37°C by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay on U87 cells on days 0, 6, and 10. No decrease in antiproliferative activity is seen with prolonged exposure to physiologic temperature.

Prolonged CED Leads to a Sustained Volume of Distribution

Imaging-based monitoring of gadolinium was used to evaluate the general effect of the length of infusion period on the VOD of a molecule in the brain. When infusion was maintained for 10 days, maximum enhancement volume was reached after 3 days, and this was followed by a relatively stable volume and intensity during days 6 and 10. The repeated measure ANOVA did not demonstrate a significant difference in gadolinium absolute and relative volumes over time between the 2 groups, although a trend in decreasing contrast enhancing volume was observed (Fig. 2). However, comparison of FLAIR+ relative volumes between 10-day and 3-day infusions demonstrated a significant difference upon cessation of infusion ($P = .03$) by analysis of variance. These findings suggest that in a t/g mix, a 10-day infusion maintains a constant VOD and concentration that is not sustained in a 3-day infusion, likely because of the rapid clearance of the infusate in the brain.[17,18]

Image-Based and Histological Signature of Topotecan CED

To investigate imaging characteristics of topotecan CED, infusion with t/g was compared with infusion with gadolinium alone ($n = 5$). There was no difference in the enhancement volume on T1 between the pigs infused with gadolinium (3820 ± 545 μL) versus g/t (3438 ± 342 μL). In contrast, t/g infusion led to a FLAIR+ signal volume (5879 ± 660 μL) that was larger than that seen by infusion of gadolinium (3635 ± 701 μL). This difference in FLAIR volumes between these 2 conditions was due to an increase of FLAIR volume with respect to T1 for t/g (1.7 ± 0.18 FLAIR/T1), as opposed to the infusion of gadolinium (0.95 ± 0.03 FLAIR/T1) (Fig. 3). The difference in FLAIR signal between t/g and gadolinium did not reach statistical significance; thus, a larger number of subjects might be required to evaluate the role of

FLAIR volume as a surrogate of topotecan CED in the brain.

To further investigate the image-based signature of the biological effects of topotecan infusion into brain parenchyma, magnetic resonance spectroscopy was performed in the presence of t/g and compared to that of gadolinium infusion alone. Gadolinium infusion for 2 days had no detectable effect on the spectra of porcine brains (Fig. 4A and B). On the other hand, the mixture of t/g seemed to have an effect on parenchymal metabolite spectroscopy. The N-acetylaspartate (NAA), total creatine (Cr), and choline (Cho) peaks declined ipsilateral to the infusion catheter, but not in the contralateral voxel (Fig. 4C and D). NAA is an amino acid and a neural integrity marker in the brain, and its decline is mostly correlated with neural damage. Cho consists of contributions from phosphocholine groups and free choline and is involved in membrane synthesis and degradation. Cho increase is mostly observed in tumors, whereas its reduction is seen in the case of necrosis. Cr also consists of both creatine and phosphocreatine compounds and is involved in energy metabolism via the creatine kinase reaction to generate ATP. Total Cr is usually reported to be of constant concentration, although a few studies suggest that it may change in acute and chronic infarction.[19,20] Overall, the characteristic FLAIR and magnetic resonance spectroscopy features of topotecan infusion were suggestive of parenchymal damage.

Histological analysis of the brains showed a confined area of necrosis along the catheter track for both conditions using hematoxylin and eosin staining. Immunohistochemistry for Iba1 revealed peri-catheter macrophage/microglia infiltration in animals that received t/g and those that received gadolinium only ($71.0\% \pm 1.8\%$ vs. $67.3\% \pm 7.5\%$; $P = .68$). However, macrophage/microglia infiltration in the ipsilateral distant white matter was higher upon infusion with t/g ($74.7\% \pm 4.9\%$ vs. $28.2\% \pm 6.7\%$; $P = .06$), although this finding did not reach statistical significance, likely because of the limited sample size (Fig. 5).

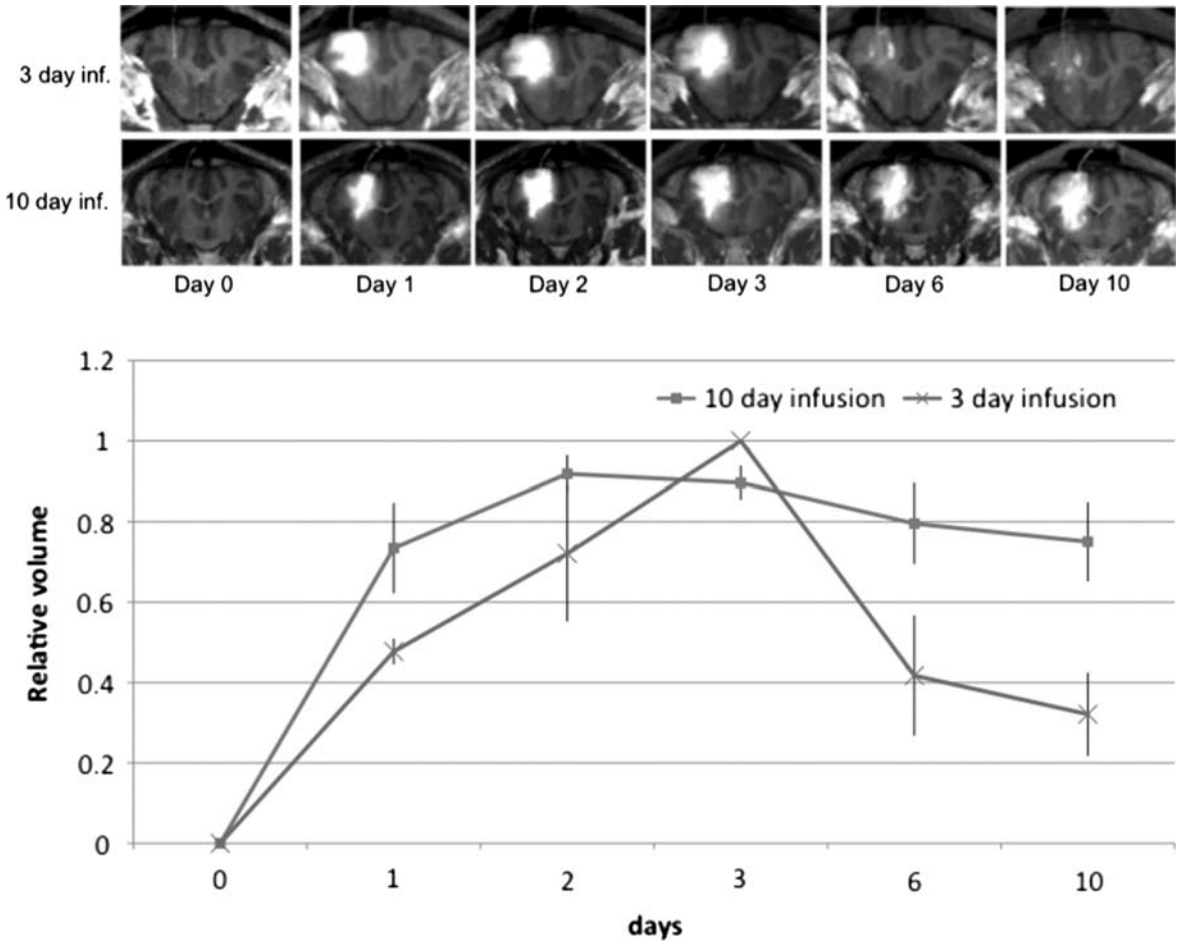

Fig. 2. Relative volume changes in 10-day versus 3-day infusions of topotecan-gadolinium. In both time periods of infusion, the maximum relative volume was reached 2–3 days after infusion. A trend toward a decrease in enhancing volume is seen when the infusion is discontinued at day 3, although statistical significance was not achieved.

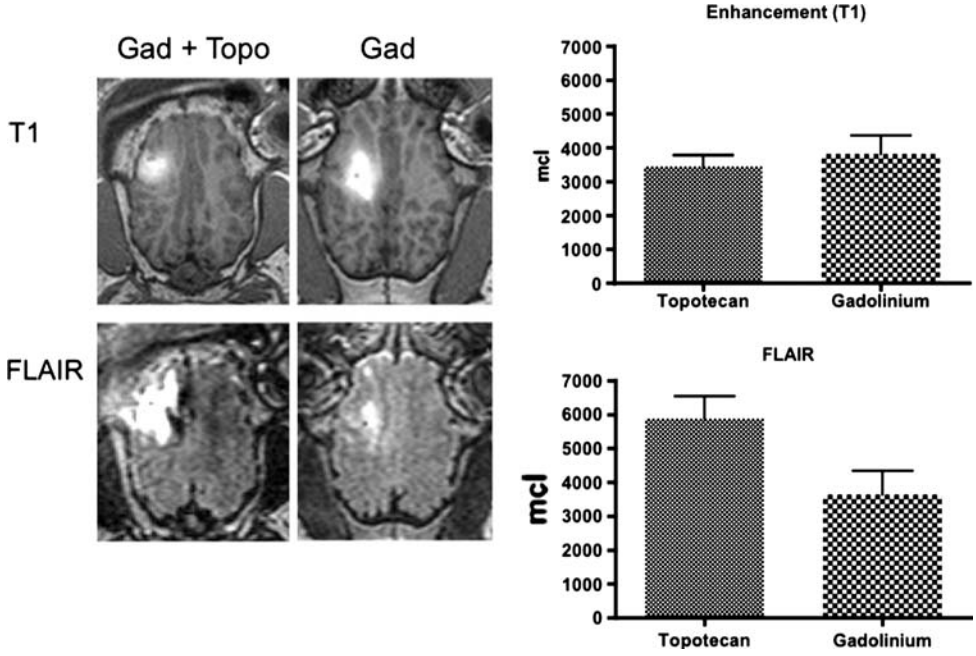

Fig. 3. FLAIR+ volume differences between topotecan-gadolinium versus gadolinium-infused animals for 10 days. A trend toward larger FLAIR+ volume is seen in animals with topotecan present in the infusate.

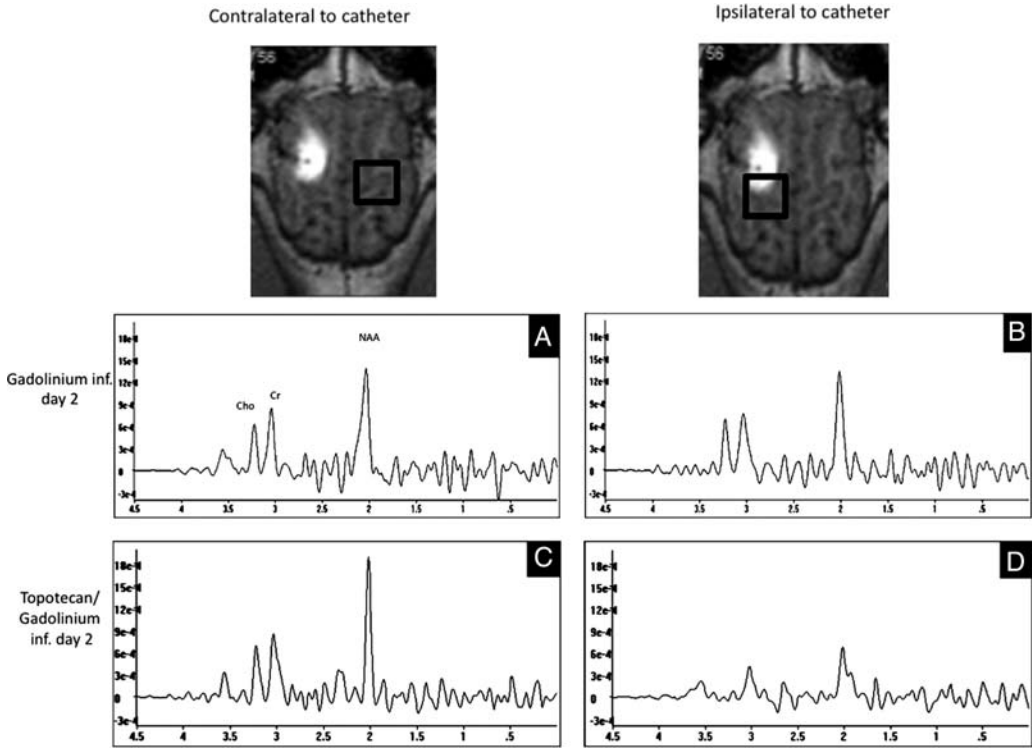

Fig. 4. Magnetic resonance spectroscopy in the presence of topotecan. SV 1H magnetic resonance spectroscopy of ~1 cm^3 on the side ipsilateral to the infusion catheter (right) or contralateral hemisphere (left). During 2-day period of gadolinium infusion, there was no difference in the metabolite spectra between the contralateral (A) and ipsilateral sides of infusion (B). In contrast, during a 2-day period of infusion of topotecan-gadolinium (t/g) mixture, N-acetylaspartate (NAA), creatine (Cr), and choline (Cho) declined ipsilateral (D) but remained unaffected contralateral to the infusion catheter (C).

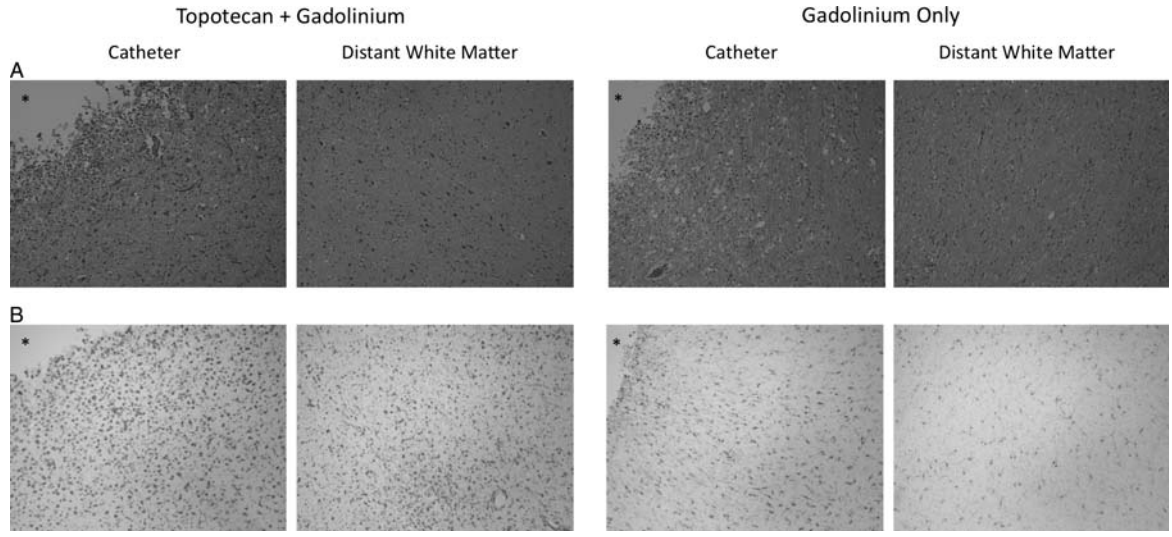

Fig. 5. Histological sections after 10 days of topotecan-gadolinium or gadolinium alone at catheter site (*) and distant white matter. (A) Hematoxylin and eosin staining and (B) immunohistochemistry for Iba1.

Discussion

This study demonstrates the safety and feasibility of intracerebral topotecan CED for prolonged periods in a large animal brain using a subcutaneously implantable pump. We employed gadolinium co-infusion to demonstrate volume dynamics, which is an established and widely used method in the literature to assess imaging findings associated with convection-enhanced delivery.[21–25] By following the gadolinium VOD and enhancement intensity by MRI in a 3-day versus 10-day infusion comparison of t/g, we demonstrated that a longer infusion period may lead to a sustained volume and concentration of an agent that is otherwise cleared by the brain during shorter infusions. Because of the methodological and anatomical variability in this experimental design and the stringent criteria of our analyses, the gadolinium volume changes did not reach statistical significance. However, the significant decrease in FLAIR+ volume that emerges once infusion is stopped indicates loss of infusate. In this study, the method of free-hand catheter insertion into the brain caused variability in the location of the catheter tip, especially in proximity to CSF spaces, which would affect achievable volume. In clinical applications, the complex geometries and variable locations of lesions in patients with glioma would likely affect volume in a similar fashion.

In addition, we established the safety and the imaging and histological signature of topotecan CED for 10 days by comparing the infusion of t/g with that of gadolinium alone. These findings establish an approach for prolonged CED suitable for the outpatient setting in patients with malignant gliomas.

These findings are relevant because CED of topotecan was well tolerated in patients with brain tumors, and prolongation of topotecan delivery was shown to enhance its efficacy against rat gliomas.[14] One major pharmacologic concern of utilizing an internal pump system for the chronic delivery of chemotherapeutics is the stability of the drug with prolonged exposure to physiologic temperature. Our study demonstrates no loss of the biologic activity of topotecan maintained at 37°C in inhibiting the proliferation of U87 glioma cells for up to 10 days.

One of the main determinants of CED efficacy relies on optimizing the VOD, and targeting the drug delivery to the area of interest. Noninvasive means of assessing the VOD and detecting the effects of chemotherapeutic agents on the brain are essential for this goal. In this study, we demonstrated that magnetic resonance spectroscopy provides a signature that is specific to the presence of topotecan. In the presence of topotecan, decreases in the NAA, Cr, and Cho peaks are observed by magnetic resonance spectroscopy. Moreover, preliminary imaging data show that infusion of t/g leads to a FLAIR+ signal volume greater than gadolinium alone. These findings would have to be reexamined in the context of underlying pathology, such as malignant tumors, which are associated with significant edema and peri-tumoral FLAIR+ volume. The biological effects of topotecan leading to these imaging findings are not clear but are suggestive of a reactive process. Furthermore, the greater macrophage/microglia infiltrate observed during histological analysis of the t/g-infused subjects supports the observation that topotecan has an effect on the brain independent from the mechanical effects of CED. Despite histological observations, long-term (10-day) topotecan CED was well tolerated in all animals, with proof of the safety of this treatment. Moreover, it is important to note that the topotecan concentration used is within the range of that proven safe in the phase I topotecan CED trial.[13]

The gadolinium-related enhancement suggests that prolonged infusion does not lead to a constant expansion of VOD, but rather a constant volume. The

equilibrium between an agent's delivery and clearance, local anatomical factors, and pressure dynamics might play a role in this phenomenon. A sustained drug concentration is desirable because topotecan targets cycling cells and malignant gliomas have cell populations that remain relatively quiescent, limiting the efficacy of a transient drug delivery.[26,27] Thus, this study provides a rationale of prolonged CED therapy to maintain a stable concentration of an agent in a local area, especially for an agent that is otherwise rapidly cleared by the brain. An important limiting factor in VOD is loss of infusate through backflow around the catheter and into CSF spaces, which has been encountered in previous trials.[28] Although these factors may limit the VOD observed in our study to a certain degree, we hope to demonstrate the establishment of stable volumes in spite of these factors.

This study demonstrates the feasibility of intracerebral topotecan CED for up to 10 days, and we provide a proof of principle of the use of a subcutaneously implanted FDA-approved infusion pump as a means to achieve long-term CED. This approach allows constant local/regional infusion of agents into the brain parenchyma for prolonged periods of time, while the use of subcutaneous pump allows the possibility for refilling the pump in the outpatient setting. Long-term CED has immediate potential therapeutic implications for patients with brain tumors who would benefit from local delivery of high drug concentrations in a focal area of brain parenchyma. In addition, the clinical potential of long-term CED could include other CNS diseases, such as epilepsy or Parkinson disease.[29–32]

Supplementary Material

Supplementary material is available online at *Neuro-Oncology* (http://neuro-oncology.oxfordjournals.org/).

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