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Convection-enhanced Delivery of Free Gadolinium with the Experimental Chemotherapeutic Agent PRX321

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Abstract

Objectives—While convection-enhanced delivery (CED) is an effective delivery method that bypasses the blood-brain barrier (BBB), its utility is limited by infusate leakage spaces due to catheter misplacement. Therefore it is critical to evaluate drug distribution during CED infusion. Gadolinium conjugated to diethylenetriamine penta-acetic acid (Gd-DTPA) is a common, readily available MRI contrast agent which may be able to predict and actively monitor drug distribution. In this study, we assess the long-term safety and toxicity of intracerebrally infused Gd-DTPA along with an experimental targeted agent PRX321.

Methods—Fifty-four immunocompetent rats were implanted with intracerebral cannulas linked to subcutaneously placed osmotic pumps. After pump implantation, the rats were randomized into six groups of nine rats each in order to assess the toxicities of six different concentrations of human serum albumin (HSA) with and without Gd-DTPA and PRX321. The rats were monitored clinically for six weeks before they were autopsied and assessed for histological toxicity to their central nervous system (CNS).

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Results—There was one unexplained death in a group infusing low-concentration HSA, Gd-DTPA, and PRX321. Upon microscopic examination of the CNS in that animal, no unexpected histological toxicity was found. Additionally, there were no signs of clinical or histological toxicity in any of the remaining rats, which all survived until the end of the six-week observation period.

Discussion—Free Gd-DTPA can be safely infused via CED in a pre-clinical animal model. Future studies should include its use in predicting and actively monitoring CED drug infusions in early phase human clinical trials.

Keywords

Glioblastoma; brain; drug delivery systems; gadolinium; investigational therapies

Introduction

Effective treatment of CNS pathology is often hampered by the existence of the BBB, which either delays or impedes transport of systemically administered chemotherapeutics into neural tissue. The effect is even stronger with large biologic agents like antibodies. CED was first described by Bobo *et al.* in 1994 as a novel treatment modality[1] that would allow infused drugs to bypass the BBB, and the mechanism of delivery involves intracranial implantation of one or more catheters to allow direct drug infusion into the tumor bed. The transport phenomena and flow equations describing flow through a catheter annulus into porous brain tissue have been reviewed extensively by Morrison *et al.* in numerous publications[2, 3]. Since its description, CED has been used to deliver novel recombinant cytotoxins[4-7] into the brains of patients with recurrent malignant gliomas, for which the prognosis is poor [8] and standard treatment is not very effective. Additionally, CED research has expanded to include potential therapies for Parkinson's disease[9, 10], Gaucher disease[11-13], epilepsy[14, 15], and stroke[16].

While CED is a potentially powerful mechanism of achieving high intraparenchymal drug concentrations without the adverse effects experienced by systemic therapies, its actual efficacy is dependent on the ability to properly place the CED catheters and avoid leakage of infusate into subarachnoid or intraventricular spaces[17]. To do this, careful monitoring of drug distribution is required. Because CED delivers a high concentration of drug directly to the brain, the total amount of infused drug is generally small, so directly labeling the drug with an imaging tracer likely will not produce sufficient signal for imaging studies to be able to detect its distribution. As a result, various imaging methods and surrogate tracers and have been employed in several studies in order to predict drug distribution. Single photon emission computed tomography (SPECT) imaging[17], T2-weighted magnetic resonance imaging[18] (MRI), diffusion-weighted MRI[19, 20], and dynamic contrast-enhanced imaging[21] have been used to predict drug distribution. Gadolinium (Gd)-albumin conjugates[22-24], Gd-loaded liposomes[25, 26], and maghemite nanoparticles[27] have been attempted to be used as surrogate tracers, which means that by monitoring the distribution of these co-infused particles one can predict the distribution of the infused therapeutic.

Unfortunately many surrogate tracers, such as Gd-loaded liposomes, have very expensive and technically complex manufacturing processes, which can be major limiting factors when considering human clinical trials involving many patients. ^{123}I-labeled human serum albumin (HSA) with subsequent SPECT imaging proposed by Sampson *et al.,* is limited by the half-life of ^{123}I-HSA (13.2 hours) and the inherent limited imaging resolution of SPECT, although using SPECT or PET imaging to monitor delivery of a molecule by CED does have the advantage that it is highly quantitative[17]. Similarly, while diffusion-weighted MRI has

been able to show early cytotoxic tissue response[19, 20], like SPECT imaging it is also limited by its inherent resolution, which is less than that of conventional T1-weighted or T2-weighted MRI.

We propose that the direct intracerebral infusion of gadolinium conjugated to a chelating agent diethylenetriamine penta-acetic acid (Gd-DTPA) might be useful as a surrogate tracer despite its low molecular weight, since it is a readily available, commonly used contrast agent. Its uses are two-fold – Gd-DTPA can be co-infused along with the desired drug to (1) provide evidence of a frank leakage of infusate out of the target tissue and (2) to estimate, in near real-time, the infused agent's volume of distribution via conventional T1-weighted MRI. This active monitoring will ensure intraparenchymal delivery to the targeted area and allows early re-positioning of catheters if leakage or inadequate distribution of delivery is found. Since Gd-DTPA is a comparatively low molecular weight agent when compared to the high molecular weight therapeutic (*i.e.* chemotherapy, cytotoxin) with which it will be co-infused, Gd-DTPA will have higher diffusivity in porous neural tissue and be lost into cerebral capillaries at a higher rate. However, it may be possible to compensate for these differences in diffusivities and loss rates, thereby allowing Gd-DTPA distribution to closely predict the distribution of a co-infused higher molecular weight drug.

Although studies have shown pre-clinical and clinical safety of CED infusion of Gd-DTPA, the monitoring periods have been short[11, 19] (immediately following infusion) or the number of subjects have been very few[28, 29]. To date, there have not been any published systematic animal studies of the long-term safety and toxicity of CED Gd-DTPA infusion. In this study, an experimental anticancer drug, PRX321 (Protox Therpeutics, Inc., Vancouver, BC Canada), a recombinant toxin consisting of interleukin-4 fused to genetically modified *Pseudomonas* exotoxin previously known as NBI-3001[7, 30], was co-infused in order to evaluate the toxicity of Gd-DTPA delivered by CED. HSA was co-infused in this study as well, since albumin is routinely co-infused with drugs to prevent non-specific binding and sequestration of drugs in the catheter tubing. Prior work by Mardor *et al.* showed that infusates with high viscosities demonstrated improved convection[19], and therefore our study utilized infused solutions with both 0.2% and 3% HSA to test whether the higher concentration of HSA would affect long-term toxicity in neural tissue.

Materials and Methods

Intracerebral cannula implantation

Immunocompetent male Fisher 344 rats were maintained, according to institutional policy, in the Duke University Graduate Student Research Building II. All rats were approximately 3 months old at the time of surgery. The rats were anesthetized prior to surgery with our standard regimen of an intraperitoneal (i.p.) injection of a 50:50 mixture of ketamine (55 mg/mL stock solution) and xylazine (9 mg/mL stock solution) at a dose of 1 mg/kg. Following induction of anesthesia, they were placed into a stereotactic frame (Kopf Instruments, Tunjunga, CA). The cannula implantation procedure performed in our lab is identical to what we have previously described in Grossi *et al.* [31]. Briefly, a 25-gauge, 3-mm guide cannula (Plastics One, Inc., Roanoke, VA) was surgically implanted into the right caudate nucleus, at 1 mm anterior and 3 mm lateral to the bregma. The cannula was then permanently secured to the calvarium with cranioplastic cement (Plastics One, Inc., Roanoke, VA). A dummy cannula (Plastics One, Inc., Roanoke, VA) was placed into the guide cannula to occlude the lumen and prevent infection and the incision was closed with staples. The rats were given at least one week to recover from cannula implantation before undergoing the next series of operations for pump implantation.

After successful completion of cannula implantation, all healthy rats that showed normal weight, no neurological deficit, and no evidence of infection after one week of postoperative recovery time were then assigned a randomly generated, nonrepeating number between 1 and 54. The rats were then equally divided into six groups labeled 1-6, such that rats 1-9 were in group 1, 10-18 in group 2, 19-27 in group 3, 28-36 in group 4, 37-45 in group 5, and 46-54 in group 6. Group 1 received 0.2% HSA (Grifols USA, LLC, Los Angeles, CA) alone; Group 2 received 0.2% HSA and 7 μmol/mL Gd-DTPA (Bayer HealthCare Pharmaceuticals Inc., Wayne, NJ); Group 3 received 0.2% HSA and 7 μg/mL of the experimental drug PRX321 (Protox Therapeutics, Inc., Vancouver, BC Canada); Group 4 received 0.2% HSA, 1.5 μg/mL PRX321, and 7 μmol/mL Gd-DTPA; Group 5 received 3% HSA, 1.5 μg/mL PRX321, and 7 μmol/mL Gd-DTPA; Groups 6 received 3% HSA alone.. As discussed above, since previous studies have shown that more viscous solutions lead to more efficient fluid convection[19], we used 3% HSA in groups 5 and 6 to test the toxicity of higher concentrations of HSA.

Infusate preparation

The osmotic pumps infused at a rate of 10 μL/hr. In order to ensure an adequate supply of infusate for 5 days of continual infusion (1.2 mL of infusate over 120 hours), 25 mL of infusate was created for each group and numbered 1-6 corresponding to the group number for which it was used. For infusate 1, 20 μL of the 25% HSA stock solution was diluted with 24.98 mL of normal saline to create 25 mL of 0.02% solution. For infusate 2, 20 μL of the 25% HSA stock solution was mixed with 0.35 mL of Gd-DTPA stock solution and 24.63 mL of normal saline for a total volume of 25 mL. For infusate 3, 20 μL of the 25% HSA stock solution was mixed with 81.5 μL PRX321 and 24.899 mL of normal saline for a total volume of 25 mL. For infusate 4, 20 μL of the 25% HSA stock solution was mixed with 81.5 μL PRX321, 0.35 mL of Gd-DTPA stock solution and 24.549 mL of normal saline for a total volume of 25 mL. For infusate 5, 3 mL of the 25% HSA stock solution was mixed with 81.5 μL PRX321, 0.35 mL of Gd-DTPA stock solution and 21.569 mL of normal saline for a total volume of 25 mL. For infusate 6, 3 mL of the 25% HSA stock solution was diluted with 22 mL of normal saline for a total volume of 25 mL. Table 1 summarizes the final concentrations of each infusate component for all six groups.

Pump implantation and CED infusion

CED infusion was performed by an Alzet osmotic pump (Product 2MLI, ALZA Corp., Palo Alto, CA) connected by silicon tubing (Molded Rubber and Plastics, Butler, WI) to a 33-gauge, 7-mm infusion cannula (Plastics One, Inc.). The pumps were primed for 24 hours at 37°C before implantation. Prior to surgery, the rats were again anesthetized with an intraperitoneal (i.p.) injection of a ketamine (55 mg/mL) and xylazine (9 mg/mL) at a dose of 1 mg/kg. During pump implantation, the pump was implanted subcutaneously over the right shoulder and the 33-gauge infusion cannula was inserted into the previously implanted 25-gauge guide cannula. The pump was secured in place with surgical staples. The pumps were left in place for 5 days, after which the rats were anesthetized with an intraperitoneal (i.p.) injection of ketamine (55 mg/mL) and xylazine (9 mg/mL) at a dose of 1 mg/kg, the pumps were explanted, and the skin wounds were closed with surgical staples.

Assessment of toxicity

Toxicity was monitored by measuring the rat's weight three times per week and daily neurological function tests, consisting of stepping and placing reflex and incline ramp climbing ability. At the end of the 6 week study period, the rats were sacrificed by overdose with isoflurane. A full necropsy was performed and the neuroaxis was fixed in buffered neutral formalin for 7 days, and then placed in a decalcifying solution for 48 hours. Six coronal sections of the neuroaxis were subsequently taken for histological examination,

including (1) brain at the level of cannula implantation near the coronal suture and pituitary gland, (2) brain at the level of the cerebellum, (3) cervical spine, (4) thoracic spine, (5) lumbar spine, and (6) cauda equina. The sections were fixed in formalin and sent to pathology for analysis, where they were embedded in paraffin. 6 μm sections were stained with Luxol fast blue and hematoxylin and eosin (H&E) and examined by light microscopy.

Results

Survival

In 5 of the 6 groups, there were no deaths over the six-week observation period. In group 4, which was treated with 0.2% HSA, PRX321, and Gd-DTPA, one rat died two days after the start of infusion. Although we have no reason to believe the rat death in group 4 was caused by CED infusion of either PRX321 or Gd-DTPA, it has been included in our survival analysis for the sake of impartiality. In summary, there was 100% survival in groups 1 (0.2% HSA), 2 (0.2% HSA, Gd-DTPA), 3 (0.2% HSA, PRX321), 5 (3% HSA, PRX321, Gd-DTPA) and 6 (3% HSA) and 88.9% (8 out of 9) survival in group 4 (0.2% HSA, PRX321, Gd-DTPA). In the cohort of rats that received Gd-DTPA infusion, there was 96.3% survival (26 out of 27). In the cohort of rats that received PRX321 infusion, there was 96.3% survival (26 out of 27). It should be noted that two rats, after being randomized to group 1 (0.2% HSA), died due to adverse effects of anesthesia. One died during pump implantation (day 0) and the other died during pump removal (day 5). Both never awoke following i.p. injection of the ketamine/xylazine mixture and were therefore marked as anesthesia-related deaths, most likely due to overdose of anesthetic. These two rats were excluded from our survival analysis.

Clinical Toxicity

Neurological examination was normal for all of the rats at each daily check. The weights were recorded and the mean percent weight changes from baseline at the time of pump implantation for all groups are plotted in Figure 1. Overall there was a net increase in weight within each group over the six-week period. A repeated measures analysis showed that the patterns of percent weight change over time within the six groups were not significantly different ($p>0.999$ for test of interaction between group and time). By the end of the study, the final average weight changes with standard deviation for each group are as follows: Group 1 showed a 4.3±4.244%, Group 2 showed a 5.4±4.265% increase, Group 3 showed a 6.3±8.037% increase, Group 4 showed a 4.0±5.721% increase, Group 5 showed a 2.8±4.733% increase, and Group 6 showed a 2.7±1.689% increase. The rat from group 4 that died on day 2 post-infusion was excluded from the weight calculations and analysis. It should be noted that the deceased rat showed no neurological deficit or sudden weight change prior to death.

Histological assessment

Pathological examination of the rats' neuroaxes showed no toxicity attributable to HSA, Gd-DTPA or PRX321 at any level of the brain or spinal cord. The only abnormality seen was at the level of the caudate where the cannula was implanted. At that level, gliosis along the catheter entry tract was seen, which was expected to occur secondary to blunt tissue insult during initial implantation of the guide cannula (Figure 2). Gross inspection of the major internal organs at time of necropsy also revealed no significant abnormalities in any of the rats in the study. Additionally, microscopy showed no neuroaxial abnormalities in the rat from group 4 (0.2% HSA, PRX321, Gd-DTPA) that died shortly after the start of infusion (Figure 3).

Discussion

CED is an effective means of transporting drugs into the brain without having to contend with the blood brain barrier[1]. Still, achieving adequate intraparenchymal delivery while preventing subarachnoid or intraventricular leakage is crucial to the success of CED therapy[4, 17]. Since CED is hampered by somewhat unreliable distribution of delivered drug, there needs to be an effective but simple way to monitor the volume of drug distribution using current imaging modalities. We believe that direct intracerebral Gd-DTPA, a widely used radiographic enhancement agent is the ideal agent to overcome these difficulties. It can be used in conjunction with conventional T1-weighted MRI to provide high-resolution, accurate predictions of leak and estimations of distribution of a co-infused agent with a high molecular weight, such as conventional chemotherapeutics or recombinant cytotoxins.

As we have shown in our systematic pre-clinical study, intracerebral gadolinium is neither acutely toxic nor does it produce long term sequelae in neural tissue. Groups 5 and 6 in our study co-infused an experimental toxin along with Gd-DTPA and demonstrate how this experimental setup is safe, which will permit future studies where this co-infusion can be used in conjunction with normal MRI for near real-time monitoring of CED infusion shortly after catheter implantation. This will allow for immediate adjustment of the catheter trajectory if it is leaking into the subarachnoid space or if it is not sufficiently infusing the tumor bed. While the cause of death of the rat in group 4 (0.2% HSA, PRX321, Gd-DTPA) that died on day 2 post-infusion is unclear, we hypothesize that it may be a delayed anesthesia-related reaction. Otherwise it could be related to acute intracerebral edema from infusion, although we believe this to be less likely. We do not believe it was related to toxicity of either Gd-DTPA or PRX321 infusion, since the remainder of group 4 survived with no evidence of toxicity. Furthermore, all rats in group 5 (3% HSA, PRX321, Gd-DTPA) received the same doses of PRX321 and Gd-DTPA, and they demonstrated 100% survival and no signs of toxicity. It is important to note that no histological toxicity was seen in any of the rats (Figure 2) including the rat that died in group 4 (Figure 3).

Gd-DTPA is a small molecule which will diffuse through tissue and be lost into circulation faster than a co-infused high molecular weight drug. Therefore, certain calculations need to be made in order for Gd-DTPA infusion to be able to accurately predict the distribution of a higher molecular weight drug. For the short time scale of a few hours, which would correspond to the time immediately following infusion during which monitoring for catheter misplacement and frank leakage is most imperative, the difference in diffusion between these molecules of different sizes is negligible, so the primary physical factor that will cause disparity in the distributions of a small and large molecule is the difference in loss rates. If the loss rates of these different molecules are calculated, the ratio of small to large molecule loss rate can be determined. This ratio can be used to compensate for molecular size difference and the higher loss rate of the small molecule by infusing the smaller molecule at a concentration that is equal to the concentration of the large molecule multiplied by the ratio of small to large molecule loss rate. As a simplified hypothetical situation, if the small molecule is lost 100 times faster than the large molecule, infusion of the small molecule at 100 times the concentration of the large molecule will compensate for the difference in loss rates, allowing the small molecule to be able to predict the distribution of the large molecule. On a longer time scale of infusion of several hours to a day, the difference in diffusion rates between these different molecules is more important, since the increased diffusion of the small molecule will make its concentration profile wider than that of the large molecule. While the calculations involving diffusivity are complex and beyond the scope of this paper, the difference in diffusivities, much like the difference in loss rates, may be able to be measured and appropriate correction factors can be determined. Thus, although the distribution of Gd-DTPA on imaging will not exactly correspond to the distribution of a

larger molecule, the convection profile of the larger agent can be calculated from the distribution of Gd-DTPA.

Conclusions

It has been shown that efficacy of an infused toxin is dependent on its ability to achieve sufficient delivery into parenchymal tissue[17]. It is hoped that ultimately, theoretical models of CED infusion will be accurate enough that drug distribution can be predicted based simply on the physical properties of the catheter and surrounding neural tissue anatomy, thereby rendering the need for surrogate tracers as obsolete[2, 3]. However, the prediction software is currently not yet near this level of sophistication and surrogate tracers are still very necessary to ensure adequate drug delivery. Gd-DTPA, a clinically available contrast agent, can fill this role and can quickly become an integral component of infusion monitoring. Direct delivery of intracerebral Gd-DTPA should be applied to the clinical setting, where it can be used with a variety of infused toxins. This systematic study demonstrates that intracerebral delivery of Gd-DTPA via CED is safe in the acute and chronic setting, and Gd-DTPA co-infusion should be a great asset to investigators involved in CED clinical trials for years to come.

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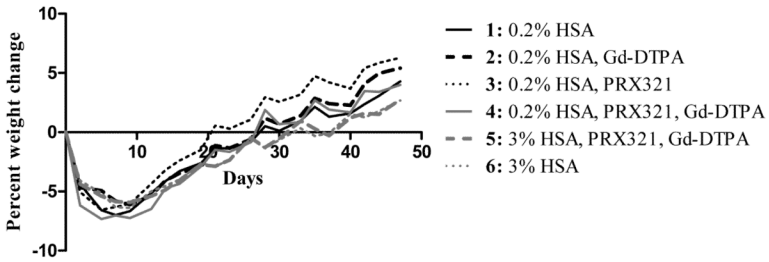

Figure 1.

Average percent change in weight over time for all 6 groups. A repeated measured analysis showed no statistically significant difference in weight change between groups, $p > 0.999$. All groups had gained weight relative to baseline by the end of the six-week study period. In the 0.2% HSA, PRX321, Gd-DTPA group, one rat that died two days after initiation of infusion was excluded from percent weight change analysis.

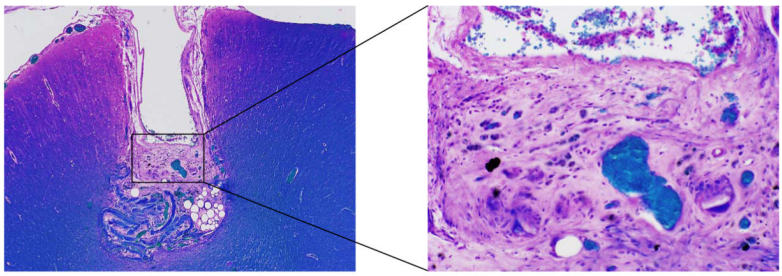

Figure 2.

H&E stain of a coronal cross-section of a representative study rat brain, shown at 4x (left) and 20x magnification (right). There were no pathological findings except for gliosis along the catheter tract.

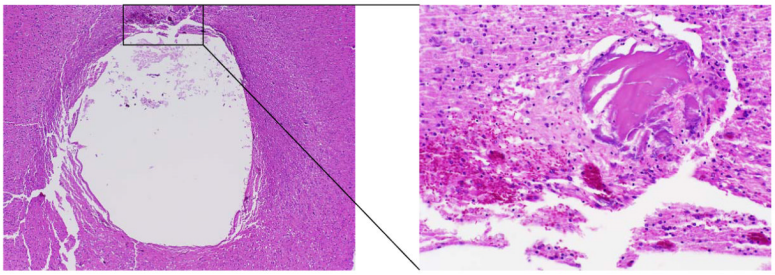

Figure 3.
H&E stain of a coronal section of brain from the rat in group 4 (0.2% HSA, PRX321, Gd-DTPA) that died 2 days following the start of infusion, shown at 4x (left) and 20x (right) magnification. Histological findings were consistent with other rats from the same group and from other groups and noted the only abnormality to be gliosis along the catheter tract.

Table 1

Study group infusate concentrations

Final concentrations of infusate components			
Group	**HSA**	**PRX321**	**Gd-DTPA**
1	0.20%	0	0
2	0.20%	0	7 μmol/mL
3	0.20%	1.5 μg/mL	0
4	0.20%	1.5 μg/mL	7 μmol/mL
5	3%	1.5 μg/mL	7 μmol/mL
6	3%	0	0