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Convection-enhanced delivery of botulinum toxin serotype A into the nonhuman primate cisterna magna and hippocampus

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Abstract

OBJECTIVE—Botulinum toxin serotype A (BoNT/A) was reported to raise the seizure threshold when injected into the seizure focus of a kindled rodent model. Delivering BoNT/A to the nonhuman primate (NHP) central nervous system via convection-enhanced delivery (CED) has not been performed. The objective of this study was to determine the toxicity and distribution characteristics of CED of BoNT/A into the NHP hippocampus and cisterna magna.

METHODS—Escalating BoNT/A doses were delivered by CED into the NHP hippocampus (n = 4) and cisterna magna (n = 5) for behavioral and histological assessment and to determine the highest nonlethal dose (LD0) and median lethal dose (LD50). Hippocampal BoNT/A was coinjected with Gd-albumin, a surrogate MRI tracer. Gd-albumin and radioiodinated BoNT/A (¹²⁵I-BoNT/A) were coinjected into the hippocampus of 3 additional NHPs to determine BoNT/A distribution by in vivo MRI and postmortem quantitative autoradiography. Scintillation counting of CSF assessed the flow of ¹²⁵I-BoNT/A from the hippocampus to CSF postinjection.

RESULTS—LD0 and LD50 were 4.2 and 18 ng/kg, and 5 and > 5 ng/kg for the NHP hippocampus and cisterna magna, respectively. Gd-albumin and ¹²⁵I-BoNT/A completely perfused the hippocampus (155–234 mm³) in 4 of 7 NHPs. Fifteen percent of BoNT/A entered CSF after hippocampal injection. The MRI distribution volume of coinjected Gd-albumin (Vd_{MRI}) was similar to the quantitative autoradiography distribution of ¹²⁵I-BoNT/A (Vd_{QAR}) (mean Vd_{MRI} =

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

139.5 mm³ [n = 7]; Vd_{QAR} = 134.8 mm³ [n = 3]; r = 1.00, p < 0.0001). No infusion-related toxicity was identified histologically except that directly attributable to needle placement.

CONCLUSIONS—Gd-albumin accurately tracked BoNT/A distribution on MRI. BoNT/A did not produce CNS toxicity. BoNT/A LD0 exceeded 10-fold the dose administered safely to humans for cosmesis and dystonia.

Keywords

botulinum toxin serotype A; convection-enhanced delivery; hippocampus; nonhuman primates

BOTULINUM toxin serotype A (BoNT/A) is a neurotoxin native to *Clostridium* bacteria that paralyzes synaptic release of the neurotransmitter acetylcholine at the neuromuscular junction.⁹ In a rodent kindling model, BoNT/A infused into the amygdala increased the afterdischarge threshold potential for 3–6 weeks.^{6,14,15} These findings identified focally applied BoNT/A as a potential treatment for drug-resistant epilepsy (DRE). While many patients with epilepsy are successfully treated with medication, up to 30% of patients will develop the drug-resistant form of the disease.³⁹ Although ablative surgery for epilepsy treatment is successful in eliminating seizures in 2 of 3 patients,⁴² recent experimental approaches for DRE have investigated less invasive, safer, more selective, and more effective treatment options.^{13,37} Convection-enhanced delivery (CED) is one such modality.

CED, a drug delivery method that uses stereotactic infusion cannula placement and hydrostatic pressure gradients to transport therapeutic compounds directly across the blood-brain and blood-nervous system barriers,^{5,20,30,32,34} has been used in earlier animal studies to examine the effects of neurotoxin infusion on epileptic activity.²¹ This technique has been studied extensively in rodents, nonhuman primates (NHPs), and humans using muscimol, a GABA-A receptor agonist.^{17,28,29} BoNT/A has not been studied to the same extent.

At low concentrations, BoNT/A selectively binds to the presynaptic terminals of cholinergic neurons and cleaves the synaptosomal nerve-associated protein 25 (SNAP-25) receptor.¹⁰ The blockade of cholinergic transmission peripherally at the neuromuscular junction is responsible for the flaccid paralysis and autonomic dysfunction characteristic of botulism. When injected into the CNS at higher doses than delivered to muscle for clinical purposes, BoNT/A blocks not only synaptic release of acetylcholine, but also other neurotransmitters, such as glutamate, nor-epinephrine, and serotonin.³ Glutamate is an excitatory neurotransmitter, and excessive release of glutamate has been shown in epileptic foci.¹² BoNT/A infusion into the epileptic brain may prevent the presynaptic release of glutamate, reduce the excitatory properties of epileptogenic neurons, and result in prolonged intervals of seizure suppression comparable to convective infusion of muscimol.

The objective of this study was to determine 1) preliminary toxicity and distribution data for BoNT/A delivered into the NHP hippocampus and cisterna magna by CED; 2) maximal BoNT/A concentration delivered safely; and 3) the infusion volume necessary to reach relevant volumes of epileptic tissue. This information can guide decisions regarding possible further testing of BoNT/A in clinical studies for the treatment of DRE.

Methods

Ethical Statement

This study was approved by our institutional Animal Care and Use Committee and conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*.³⁵

Animal Care

Twelve NHPs (*Macaca mulatta*), weighing between 5.7 and 9.8 kg (mean $\pm$ SD 7.23 ± 1.47 kg), were used in this study. All NHPs were housed in individual cages, on a 12-hour light/dark cycle, with free access to environmental enrichment. The minimum number of animals (12) were used to test our hypothesis.

Botulinum Toxin Serotype A

BoNT/A (Metabionics) has a molecular weight of 150 kD and a specific toxicity of 250 U/ng.

Surgical Procedure

The procedure for infusion cannula implantation in NHPs has been described earlier.^{19–21} NHPs were anesthetized with intramuscular ketamine (3 mg/kg) and medetomidine (0.1 mg/kg) in sterile saline. General anesthesia was maintained with isoflurane. Intramuscular atipamezole (0.1 mg/kg) was used to reverse medetomidine.

For BoNT/A infusion into the cisterna magna, the animal was placed prone, and sterile preparation of the skin over the posterior cervical and occipital areas was performed. A short (3 cm) posterior midline incision was made at the skull base, and the cisterna magna was exposed. The dura immediately below the foramen magnum was opened wide enough to visualize entry of the infusion catheter through the arachnoid into the cisterna magna. The infusion was then started and continued for 2 minutes (250 μ l/min), after which the catheter was removed. The dural and skin incisions were then closed with sutures.

For hippocampal infusions, after intramuscular injection of ketamine and medetomidine and induction of general anesthesia with isoflurane, the animal was placed prone in a stereotactic headframe (model 9-YSTI-35, Crist Instrument Co., Inc.). Preoperative T1- and T2-weighted sequences (slice thickness: 1–1.5 mm; spacing: 0 mm) were acquired using a 1.5T MRI scanner and analyzed to obtain the stereotactic coordinates of the infusion target in the head of the hippocampus.^{2,16} In the institutional NHP operating suite, an occipital burr hole was made, and the dura was opened to expose the occipital lobe cortex. The stereotactic frame electrode guide was aimed at the hippocampus through the parasagittal plane, with a superior-to-inferior angle of 20°–30°. An end-hole silicate catheter was inserted along this trajectory through the electrode guide, occipital lobe cortex and white matter, white matter inferior to the lateral ventricle atrium, and into the head of the hippocampus. The catheter depth from the occipital cortex to the head of the hippocampus was 40–43 mm.³⁸ Nylon screws were placed in the skull around the burr hole. The silicate catheter was secured to the skull with dental cement anchored to the Nylon screws. The scalp was closed with Nylon sutures.

The animals were transported back to the MRI machine for coinfusion of BoNT/A (1.3 nM) or ^{125}I -BoNT/A and gadolinium-bound albumin (Gd-albumin; 28 mg/ml), a stable chelate of Gd to albumin, synthesized as previously described in an earlier NHP study using a different macromolecule.³⁴ Intraoperative T1-weighted MR images were obtained in the axial, coronal, and sagittal planes (slice thickness: 1–1.5 mm; spacing: 0 mm) using a 4.7T MRI scanner to monitor infusion distribution. Hippocampal infusions (BoNT/A or ^{125}I -BoNT/A, coinfused with Gd-albumin) were administered at 0.5 $\mu\text{l}/\text{min}$ to a total volume of 60 μl . Infusion catheters were removed 10 minutes after infusion completion. Animals infused with ^{125}I -BoNT/A were euthanized with a lethal dose of phenobarbital immediately after infusion catheter removal.

Postoperative Care

Animals in the toxicity study were monitored daily for 6 weeks postinfusion for neurological dysfunction; alterations in appetite, food intake, and weight; wound healing, and evidence of toxicity. Face and limb weakness was assessed by monitoring spontaneous animal movement. BoNT/A infusion distribution animals were euthanized immediately after infusion completion.

Study Design and Groups

BoNT/A Infusion Toxicity Study Arm—To study the CSF toxicity of CED of BoNT/A and determine its highest nonlethal dose (LD0) and median lethal dose (LD50) in NHPs ($n = 9$), 5 NHPs received 500- μl infusions of BoNT/A at escalating doses (15, 22.5, and 30 ng; 2.2, 3.9, and 5 ng/kg) into the cisterna magna. Four other NHPs received unilateral 60- μl coinfusions of BoNT/A at escalating doses (15.3, 34.3, 117, and 129 ng; 1.7, 4.2, 18, and 21 ng/kg) and Gd-albumin into the hippocampus. Gd-albumin distribution volume on MRI (V_{dMRI}) was tracked by MRI during hippocampal infusion for comparison with the ^{125}I -BoNT/A distribution study described below. Animals in the toxicity study were observed for 6 weeks and then euthanized. Their brains were removed, sectioned, and stained with H & E for histology.

BoNT/A Infusion Distribution Study Arm—To study BoNT/A distribution in NHPs, 3 NHPs received unilateral 60- μl hippocampal coinfusions of Gd-albumin and ^{125}I -BoNT/A (115–126 ng; 16.9–18.4 ng/kg) (Fig. 1). V_{dMRI} was compared with the ^{125}I -BoNT/A distribution volume on quantitative autoradiography (QAR) (V_{dQAR}). Immediately after infusion completion, the animals were euthanized with a lethal dose of phenobarbital. Fifteen microliters of CSF was removed from the cisterna magna at one time point—the time of sacrifice—to assess ^{125}I -BoNT/A loss into the CSF from the hippocampus using scintillation counting. The brain was then removed, cut coronally into slabs, coated with O.C.T. (Tissue-Tek), and frozen in a bath of isopentane (methylbutane) in dry ice.

Image analysis software (MEDx, Sensory Systems, Inc.) was used to calculate V_{dMRI} from T1-weighted MR images, with an MR segmentation threshold set at 2 standard deviations above the surrounding noninfused region,³⁶ as described earlier.²⁰

For the QAR study, brains were sectioned into 20- μ m-thick coronal sections, and autoradiograms were prepared from sections spaced every 200 μ m. The tissue sections were exposed on imaging plates for 2 weeks (BAS-TR2025 plates, Fujifilm Medical Systems) and developed using a BAS-5000 Bio-Imaging Analyzer (Fujifilm Medical Systems). V_{dQAR} was determined by measuring the area of each autoradiographic section containing at least 10% of the ^{125}I -BoNT/A signal density at the infusion site, adding the areas together, and multiplying the sum of the areas by the distance between sections (200 μ m).^{29,36}

The designs for both the toxicity and distribution arms of the study are outlined in Fig. 1.

Statistical Methods

Since this was a small cohort study, descriptive statistics were reported, and the Spearman correlation coefficient was used to evaluate the correlation between Gd-albumin and ^{125}I -BoNT/A distribution volume. A p value < 0.05 was considered statistically significant.

Results

BoNT/A Infusion Toxicity Study: Behavioral Response

Cisterna magna infusions of 15 and 22.5 ng (2.2 and 3.9 ng/kg) BoNT/A doses did not produce toxicity in any of the 3 infused animals (Table 1). At a 30-ng (5 ng/kg) BoNT/A dose ($n = 2$), both cisterna magna-infused animals developed unilateral ptosis and decreased tone and activity within 3 days post-CM infusion completion. Poor appetite was observed in 1 animal. All signs of toxicity subsided within 10 days post-CM infusion. The LD0 was 5 ng/kg. LD50 was not determined but would presumably be > 5 ng/kg (Table 1).

Gd-albumin and BoNT/A coinfusion into the hippocampus produced toxicity in 3 of 4 animals. No toxicity signs were observed in the animal receiving 15.3 ng (1.7 ng/kg) of hippocampal BoNT/A. A 34.3-ng (4.2 ng/kg) dose produced mild bilateral ptosis and dull facial expression within 2 days post-hippocampal infusion, subsiding 15 days after hippocampal infusion completion. Bilateral ptosis, labored breathing, and impaired swallowing were observed in animals receiving hippocampal BoNT/A doses of 117 ng and 129 ng (18 ng/kg and 21 ng/kg) within 1 day after hippocampal infusion, which resulted in death 5 and 9 days posthippocampal infusion completion, respectively. Histological analysis of the brains did not show any neural toxicity evidence following hippocampal BoNT/A infusion. LD0 and LD50 were 4.2 and 18 ng/kg, respectively (Table 1).

BoNT/A Infusion Distribution Study: Autoradiograms, MRI, and Scintillation Counting

Gd-albumin distribution into the hippocampus, CSF, and around the catheter was seen on T1-weighted MRI (Fig. 2A–C). The distribution of Gd-albumin and ^{125}I BoNT/A were similar, as demonstrated by V_{dMRI} and V_{dQAR} (Table 2), with a mean relative difference of 1.2% ($n = 3$) (range 7.9% to -30.1%). The Spearman correlation between V_{dMRI} and V_{dQAR} was statistically significant ($r = 1.00$, $p < 0.0001$; $n = 3$). In the 3 NHPs from which CSF was removed to assess ^{125}I BoNT/A distribution using scintillation counting, 14.7%–16.4% of hippocampal-infused ^{125}I BoNT/A reached the CSF space (Table 2).

Across all infused animals, mean $V_{dMRI} = 139.5 \text{ mm}^3$ ($n = 7$) and $V_{dQAR} = 134.8 \text{ mm}^3$ ($n = 3$) (median $V_{dMRI} = 155.2 \text{ mm}^3$, $V_{dQAR} = 60.7 \text{ mm}^3$; range $V_{dMRI} = 44.6\text{--}233.8 \text{ mm}^3$, $V_{dQAR} = 51.4\text{--}292.3 \text{ mm}^3$). The mean ratio of V_{dMRI} to volume of infusate with respect to Gd-albumin distribution was 2.26 ± 1.26 ($n = 7$).

Discussion

In this study, CED of BoNT/A into the NHP CNS was tolerated at or below doses of 3.9 ng/kg. At higher doses ($> 4.2 \text{ ng/kg}$), enough BoNT/A escaped into the systemic circulation and reached neuromuscular junctions to cause dose-dependent muscular weakness (Table 1).

The use of NHPs in this study was essential because the NHP brain is much larger than the rodent brain, providing a hippocampus that is large enough to test if BoNT/A delivered by single catheter CED would achieve a volume of distribution relevant to the volume of the human hippocampus. The data obtained from rodents alone in earlier studies were inadequate to predict the doses of BoNT/A that would be toxic to the human hippocampus, the primary treatment target for surgically remediable DRE.^{6,14,15}

In the toxicity study arm, the intracisternal BoNT/A dose that produced nonfatal toxicity (LD0) in NHPs was 30 ng (5 ng/kg), presumably a safe dose of BoNT/A to infuse into the human hippocampus because it would be tolerated even if BoNT/A infused into the hippocampus escaped entirely into the surrounding CSF spaces. Lower doses of BoNT/A infusion into the hippocampus either did not produce toxicity (1.7 ng/kg) or resulted in short-term toxicity (4.2 ng/kg) because only about one-sixth of hippocampal BoNT/A escaped into the CSF. However, higher doses (18 ng/kg and 21 ng/kg) resulted in death, which would represent a dose of about 3 ng/kg into the CSF. Further study with NHPs could determine with more certainty the LD50 of intracisternal BoNT/A infusion ($> 5 \text{ ng/kg}$ in this study). Intraparenchymally infused BoNT/A leaking into the CSF of the temporal horn of the lateral ventricle or subarachnoid cisterns would reach the CM, which communicates with both compartments. Intracisternal infusion in animals is a model for studying the distribution, effects, and toxicity of drugs delivered via the CSF that does not require penetrating the skull and brain as required by intraventricular delivery.⁴⁵

In the distribution study arm, Gd-albumin (MW [molecular weight] 75 kD) accurately tracked therapeutic distribution of the macromolecule, BoNT/A (MW 150 kD), within NHP CNS structures, consistent with earlier work in which the smaller molecule, Gd-DTPA (MW 0.39 kD), accurately tracked the smaller molecule, muscimol (MW 0.11 kD).^{19,20} Across all infused animals, the mean $V_{dMRI} = 139.5 \text{ mm}^3$ ($n = 7$) and $V_{dQAR} = 134.8 \text{ mm}^3$ ($n = 3$), and the mean V_{dMRI} /infusate volume ratio was 2.26 ± 1.26 . The average volume of the adult human hippocampus is about 3.5 cm^3 , so the distribution volumes achieved in the NHP model would only cover about 4% of the human hippocampal volume.^{24,44} The infusion volume would need to be increased significantly in humans to perfuse the entire hippocampal volume. Lower infusion volumes covering only the area in the hippocampus from which seizures originated may be enough to control seizures.⁴¹

The accuracy of Gd-albumin as a surrogate tracer in our study is consistent with the findings of earlier studies of macromolecule infusion into the NHP brain via CED, which have also reported Gd-albumin as an accurate surrogate tracer of therapeutic infusion distribution.^{23,30,34} However, on T1-weighted imaging, Gd-albumin tracked only the parenchymal distribution of ¹²⁵I BoNT/A infused into the hippocampus and not its distribution into the CSF space. As in an earlier study,²³ future studies should utilize FLAIR sequences in addition to T1-weighted imaging to optimize both the sensitive and accurate detection of Gd-albumin distribution in the brain. Additionally, although Gd-albumin was an accurate surrogate tracer in this NHP study and others,^{23,30,34} further study of Gd-bound compounds for larger macromolecules, such as BoNT/A, is warranted to develop an appropriate surrogate tracer for use in humans analogous to the use of Gd-albumin in our study. It is plausible that standard Gd-bound compounds, however, could be useful for infusing larger therapeutic agents into the human brain via CED, as evident in a recent clinical trial of Gd-DTPA and IL-13 *Pseudomonas* exotoxin,¹⁸ and that the development of an entirely new tracing agent is unnecessary.

Histological assessment of brain sections did not reveal any direct toxic effect of botulinum toxin on the brain. Binding of BoNT/A to brain prevented much of the infused BoNT/A from entering the systemic circulation, because the LD50 of BoNT/A was above 4.2 ng/kg when administered into the NHP hippocampus, which far exceeded the LD50 of 1 ng/kg reported for most laboratory animal species when the toxin is administered intravenously, subcutaneously, or intraperitoneally.³¹ However, as shown in the distribution arm of our study, small amounts of BoNT/A infused into the hippocampus reached the CSF space (Table 2) and presumably escaped into systemic circulation. A mechanism of toxicity via systemic circulation, and not via CNS effects, is thus plausible and also supported by our histological findings that BoNT/A did not cause neuronal injury.

The highest BoNT/A dose used in this study (> 20 ng/kg) was similar to that previously reported as effective in raising kindling threshold for 3–6 weeks in rodent seizure experiments.¹⁴ However, such a high concentration of BoNT/A is much greater than one would expect to be necessary for the action of a receptor-specific agent like BoNT/A. Such high concentrations would increase the amount of BoNT/A that could be distributed by diffusion versus convection. A lower concentration of BoNT/A by CED would have more predictable distribution and might be enough to suppress epileptic activity in humans. Furthermore, an earlier study reported that infusion of 1.5 µl of a 50-nM (7.5 ng/µl) BoNT/E solution into the rodent hippocampus dramatically reduced kainic acid-induced seizures for more than 3 weeks.¹¹ Considering that BoNT/E has an LD50 similar to that of BoNT/A,¹¹ and because the antiepileptic effect of botulinum toxin is mediated by binding to the synaptic vesicle protein on hippocampal neurons, our toxicity and distribution findings for BoNT/A may be applicable to hippocampal infusions of BoNT/E.

Several recent studies have investigated the use of other neurotoxins in animal models for selective pharmacological neuronal inactivation. The intrathecal infusion of diphtheria toxin-substance P fusion protein in a chronic pain rat model was well tolerated.⁴ The transient receptor potential vanilloid 1 (TRPV1) agonist resiniferatoxin (RTX) proved effective in relieving animal distress and increasing activity when delivered intrathecally to client-owned

dogs with lower-extremity bone cancer.⁷ In a recent human clinical study, 100 U of BoNT/A was used ex vivo to improve vessel graft patency in cerebral revascularization surgery.⁴³ All of these studies employed the strategy of using a neurotoxin to target a unique tissue receptor, block harmful physiological processes, and treat CNS disease.

The same site of delivery but a shorter duration of infusion was used for BoNT/A in this study compared with earlier studies using muscimol, a GABA-A receptor agonist, in NHPs and in a phase I trial on DRE.^{17,21} In the phase I trial, muscimol infusion was safe but not conclusively effective.¹⁷ The neuroinhibitory effect of BoNT/A is more sustained than muscimol, providing persistent neural suppression after infusion,^{1,8} which could chronically affect epilepsy networks like electrical stimulation.²⁷ However, there are factors limiting further development of BoNT/A DRE treatment: 1) the expense of Good Laboratory Practice (GLP) preclinical investigations in an epilepsy model, 2) the risk and expense of a clinical study involving BoNT/A infusion in human patients every few months, and 3) the uncertainty regarding whether a competing, permanent, and minimally invasive treatment for DRE would be preferred to BoNT/A even if BoNT/A showed efficacy and safety and received FDA approval as a new therapeutic.

When considering potential human studies, one clinically approved BoNT/A formulation is Botox (Allergan), which has a specific toxicity of 20 U/ng.¹⁰ The maximal administered dose is 500 U. However, in this study, the BoNT/A formulation (Metabionics) used had a specific toxicity of 250 U/ng. Thus, each dose of BoNT/A administered in our study was more than 10 times more potent, based on U/ng BoNT/A, than the clinical formulation, Botox (Allergan).

The starting dose of botulinum toxin for hippocampal administration would be predicated on doses found safe in animal models and humans. The lethal dose of intravenously or intramuscularly administered BoNT/A is 90–150 ng for a 70-kg human.⁴⁰ The doses of Botox (Allergan) used to treat spasticity and chronic lower-back pain are about 230 U and 200–500 U²² per session, respectively. The maximal Botox dose currently administered to humans (500 U), equivalent to 25 ng or about 0.3 ng/kg, is still far less than the 34-ng hippocampal dose (4.2 ng/kg) of a more potent formulation of BoNT/A that was tolerated by NHPs in our study. For intramuscular injection to treat dystonia, Botox is delivered as a 100-U (5 ng) powder diluted in 1–8 ml of 0.9% NaCl solution.¹ The concentrations of the BoNT/A solutions (2 ng/μl and 500 pg/μl) used in our NHP hippocampal infusion experiments were far greater than those used for intramuscular injection to treat dystonia in humans (0.005 ng/μl or 5 pg/μl).¹ BoNT/A doses in our study were administered based on NHP mass because the BoNT/A LD₅₀ is related to respiratory neuromuscular blockade, which is related to toxin dose per body mass (U/kg).^{26,40} On the other hand, because BoNT/A could produce local toxicity unrelated to body mass, studies delivering neurotoxins as therapeutic agents to the brain should also report toxicity in relation to the concentration and volume of the infused neurotoxin solution.

Our animal study, and previous rodent studies showing that BoNT/A suppressed epileptic activity, supports the feasibility of delivering BoNT/A to the human hippocampus for the treatment of DRE. There would be little expected morbidity using doses of BoNT/A that are

presently used for dystonia treatment. If we used the maximal Botox dose currently used for dystonia treatment (500 U, 25 ng), the infusate concentration would be 1.25–2.5 U/ μ l (0.062–0.125 ng/ μ l), much lower than the concentration delivered to and tolerated by NHPs in our study. CED provides more uniform and broader tissue distribution than diffusion and would be preferred to bolus infusion as a method to deliver BoNT/A to the hippocampus as shown in earlier studies with other therapeutic agents.^{25,33}

Conclusions

In this NHP study, we found that BoNT/A infusions into the hippocampus and cisterna magna CSF were tolerated by CNS tissues and that toxicity arose related to BoNT/A producing diffuse muscle weakness at high doses. BoNT/A distribution was accurately tracked using MRI to detect coinjected Gd-albumin. Evaluation of CED of hippocampal BoNT/A in patients with DRE would require a clinical trial as well as identification of an appropriate surrogate tracer to monitor infusion distribution. The LD₀ of BoNT/A infused into the NHP hippocampus and cisterna magna exceeded the doses administered safely to humans for cosmesis and dystonia, suggesting that BoNT/A could be administered without toxicity to the human brain. BoNT/A effects last only a few months. Thus, treatment of DRE would require repeated dosing of BoNT/A at intervals providing ongoing suppression of epileptic activity.

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ABBREVIATIONS

BoNT/A	botulinum neurotoxin serotype A
CED	convection-enhanced delivery
DRE	drug-resistant epilepsy
LD₀	nonlethal dose
LD₅₀	median lethal dose
NHP	nonhuman primate
QAR	quantitative autoradiography
V_{dMRI}	distribution volume on MRI
V_{dQAR}	distribution volume on QAR

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FIG. 1.

Flowchart illustrating the NHP study design. Twelve NHPs (*Macaca mulatta*) were divided into a toxicity group and a distribution group. Gd-albumin distribution following infusion into the hippocampus was compared with ¹²⁵I-BoNT/A distribution measured by QAR in the 3 NHPs from the distribution study.

FIG. 2.

MR images obtained in an NHP that underwent coinjection of Gd-albumin and ^{125}I -BoNT/A to the right hippocampus. The images are thresholded to demonstrate signal intensity 10% above background intensity. There is gadolinium signal backtracking (*white*) along the catheter trajectory within the hippocampus with a volume of distribution of 208 mm^3 , shown in the axial (A), coronal (B), and sagittal (C) planes.

TABLE 1.

Toxicity of BoNT/A infused into the NHP cisterna magna and hippocampus

Infusate(s)	Location	Dose (ng)	Dose (ng/kg body weight)	Toxicity Units (U/kg)	No. of Animals Infused	Signs of Toxicity	No. Dead
BoNT/A	CM	15	2.2	440	1	No	0
BoNT/A	CM	22.5	3.9	780	2	No	0
BoNT/A	CM	30.0	5	860	2	Ptosis, decreased tone & activity (2); poor appetite (1)	0
Gd-albumin + BoNT/A	HPC	15.3	1.7	340	1	No	0
Gd-albumin + BoNT/A	HPC	34.3	4.2	840	1	Mild bilat ptosis & dull facial expression	0
Gd-albumin + BoNT/A	HPC	117	18	3600	1	Ptosis, labored breathing, impaired swallowing	1
Gd-albumin + BoNT/A	HPC	129	21	4200	1	Ptosis, labored breathing, impaired swallowing	1

CM = cisterna magna; HPC = hippocampus.

Toxicity units (U/kg) are based on the specific toxicity of BoNT/A (200 U/ng).

TABLE 2.

Distribution study using MRI, QAR, and scintillation counting

Vd (mm ³)		Relative Difference (%)	125I-BoNT/A Infused (ng)	125I-BoNT/A in CSF (pM)*	125I-BoNT/A in CSF (ng)**†	125I-BoNT/A Reaching CSF (%)*
QAR	MRI					
61.0	65.8	7.9	115	9.4	16.9	14.7
51.4	64.4	25.3	118	10.7	19.3	16.4
292.3	204.2	-30.1	126	19.6	19.6	15.6

Gd-albumin and 125I-BoNT/A were coinfused into the hippocampus of 3 NHPs.

*125I-BoNT/A distribution in CSF after hippocampal infusion.

† Calculated based on CSF volume of 15 µl.