PROTOCOL TITLE: The Movement of Botulinum Toxin Through the Lateral Gastrocnemius Muscle in Humans: An Expanded Examination

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BACKGROUND

Despite the wide-spread use of botulinum toxin (BT) to treat spasticity in central neurological disease, evidence-based guidance on dosing, dilution, and injection technique is limited. Current practice is based on expert opinion (1) or small clinical studies (2,3). There is a need for better evidence regarding injection technique, accuracy, individual muscle dosing, and number of injection sites per target muscle (1). This cannot be accomplished without knowing more about how BT is distributed within a muscle following injection (4,5). Hallet (5) and(6) have proposed terminology for BT. movement in muscle. Convection (spread) is the immediate distribution of BT and saline within the muscle and is related to the volume injected and the force of the injection. Diffusion (5,6) is movement of toxin beyond the immediate injection site by Brownian motion and is determined by the concentration gradient or dose of BT and the molecular size of BT. Few data are available on convection and diffusion in human muscle. Animal and human data are inconsistent and inconclusive in characterizing not only BT distribution within muscle after injection, but also factors associated with that distribution (7). The wide-spread use of spasticity management, expense of these agents, and detrimental impact of BT due to movement into non-injected muscles mandates a better understanding of BT movement within muscles (4-6). Our group at Weill Cornell Medicine recently published a proof-of-concept case series describing a novel MRI approach to better elucidate BT muscle effect (BTME) in spastic muscle (7). We reported on five subjects with stroke and clinically significant lower extreme spasticity who were naive to, but held the potential to benefit from, BT injection. The lateral gastrocnemius muscle (LGM) was selected as our index muscle of study. Subjects underwent a non-contrast MRI scan of the spastic leg at baseline before BT injection and then at 2 months (M) and 3M following injection. The LGM was injected at three sites whose approximate locations were determined through measurement using the baseline and subsequent scans. The proximal and distal injects contained a 25 unit dose of onabotulinumtoxinA (OBT-A, Botox) in .25 ml saline. The center injection contained .25 ml saline only. Other leg muscles were injected based on clinical need. Our analyses focused on the LGM, lateral soleus muscle (LSM), and tibialis anterior muscle (TAM). The LSM is the muscle adjacent to the LGM and was hypothesized to be most likely affected by diffusion of toxin through the muscle fascia. The TAM served as a control muscle, as it was not injected for any subject. For all

5 subjects, the muscles of interest were manually outlined on T2 maps at baseline, whereas 2M and 3M post-injections were mapped using software written in-house in IDL 8.1 (Exelis Inc, Boulder, CO). A histrogram of T2 relaxation times (ms) was constructed for the baseline scan for all voxels in the muscle. A Guassian function was used to calculate a mean and standard deviation for each muscle at baseline. We defined BTME a priori as only those voxels on subsequent scans with a T2 relaxation time ≥ 3 standard deviations (SD) above the baseline mean for that subject and muscle. Subject specific thresholds were required due to variation among both subjects and muscles. Knowing 8th/1e5/n19um4:0b6ePr M of abnormal voxels in a given muscle after BT inject for each MRI slice, a per muscle/per slice BTME volume (at .0015 voxel) could be calculated for each muscle and subject.

STUDY DESIGN

The primary objective of this series of experiments is to, in a research setting, better understand how spastic muscle and differing dilutions affect BT movement through muscle.

The secondary objective of this series of experiments is to, in a clinical setting, describe BT movement through a variety of lower extremity muscles with differing doses and dilutions of BT commonly used in clinical practice.

Sample Size Justification The proposed sample sizes (6 patients for experiment #1, 15 patients for a within-group design in experiments #2-#4, or 25 patients for a between-groups design in experiments #2-#4) are primarily based on logistic considerations and conform to the exploratory (i.e., hypothesis-generating) nature of this pilot study. Because this is a pilot study, a formal sample size/power calculation is not required. Statistical Analyses For Experiment #1, descriptive analysis will be conducted to help inform the study design for the remaining experiments. The mean, standard deviation and morphology (either 3D reconstruction or number of MRI slices detecting a toxin effect) of BTME volume following injection in the LGM and MGM will be calculated for all 3 groups. If injections in the two muscles are similar (BTME volume +/- 20%) and there is no evidence of toxin movement outside of the injected muscle, then a within subject design will be implemented as described above. If not, we will use a between-subject design. Given the resources required for this study and the time commitment of the subjects, we propose a preliminary analysis for Experiments #2 and #3 after completion of MRI2 in 12 subjects if using a between subjects design (8 in each in standardized and 4 in the experimental injection groups using a 3:2 ratio) or 12 subjects if using the within-subjects design (12 comparison). For experiment #2 with a within-subjects design, we will examine the difference in BTME volume from baseline to 2 months following injection between the muscle sites injected with standard and experimental BT dilution in each patient using a paired t-test or Wilcoxon signed-rank test, as appropriate. For a between-subjects design, experiment #2 will be analyzed using a two-sample t-test or Wilcoxon rank sum test, as appropriate, to compare the mean 2-month difference in BTME volume in patients who received a standard dilution injection vs. those who received an experimental dilutio8n/15i/n1j9e4c:t0io6nPM in the spastic LGM. For either a within- or between-subjects design in experiment #3, we will use the paired t-test or Wilcoxon signed-rank test, as appropriate, to examine the difference in BTME volume from baseline to 2 months following injection between the spastic and non-spastic muscle sites injected in each patient. Experiment #4 will be analyzed descriptively, showing mean, standard deviation, median, and range of 2-month change in BTME volume for each combination of clinically injected site, dose, and dilution. This information will be used to generate hypotheses for future studies. Observational Group: N=6 observational subject MRI data will be analyzed the same as data from Experiment #4. All p-values will be two-sided with statistical significance evaluated at the 0.05 alpha level. Ninety-five percent confidence intervals for means will be calculated to assess the precision of the obtained estimates. Lack of any observed statistically significant differences between groups will not constitute evidence of equivalence or non-inferiority between dilution or spasticity groups; rather, estimates in each group will serve as preliminary data for further investigation (i.e., hypothesis-generating). All analyses will be performed in SAS Version 9.4 (SAS Institute, Inc., Cary, NC). Note: The statistical analysis section was written in conjunction with Debra D'Angelo, MS, in the Division of Biostatistics and Epidemiology, Department of Healthcare Policy & Research.

The research questions for the present study series are as followed:

1. How does the movement and morphology of BT muscle effect (BTME) differ between standardized, research injections into spastic and non-spastic LGM?

2. How does the movement and morphology of BTME differ between a standardized, research injection into spastic LGM versus an injection using the same dose in a 100% greater dilution?

3. Is there a predictable BTME within a given muscle following lower extremity BT clinical injections based on clinical need?

We have two hypotheses.

First, we predict that BT muscle effect (BTME) will be greater in normal muscle than in spastic muscle.

Second, we hypothesize that BTME will increase with increasing dilution.

The standard BT injection will be an injection of 25 units of onobotulinumtoxinA (Botox®) diluted in 0.25cc of saline.

The experimental BT injection will be an injection of 25 units of onobutilinumtoxinA (Botox®) diluted in 0.50cc of saline.

Four experiments will be conducted to explore the research questions. At baseline, subjects will receive research injections that are decided based on the research protocol. Given the very small dosage of the research BT injections, we do not anticipate seeing any symptomatic effects in subjects. At 3 months following research injections, subjects will receive clinical injections that are decided based on their clinical need and are anticipated to result in clinical benefit for subjects. For all experiments subjects will have an MRI at baseline (MRIB), at 2M following research injections (MRI2), and at 2M following clinical injections (MRI3). If, for enrolled subjects who have previously had botulinum toxin injections below the knee, evidence of present botulinum toxin effect is seen on MRI, the subject will be ineligible for the research injections, MRI2, and MRI3 and will instead be offered to receive the clinical injections and then be exited from the study.

Experiment #1:

After a baseline MRI, 6 subjects will be randomized into three groups (N=2 in each group): standard injection in LGM, experimental injection in LGM, or experimental injection in MGM. All 6 subjects will receive a standard injection to the non-spastic LGM. Subjects will undergo a second MRI 2 months after the research injections, which will be used to confirm the design of the subsequent experiments.

If, for Experiment #1, we find on MRI2 a similar appearance and volume of BTME (BTME volume +/- 20%) for both the LGM and MGM receiving the experimental injection and that the BTME is contained within each muscle, we will have the option of proceeding with the within- subject design where each subject serves as his or her own control, receiving either the research or experimental injection in the spastic LGM and the alternative injection in the spastic MGM. If the within-subject design is used, 15 subjects will be recruited. If the BTME volumes for the LGM and MGM receiving the experimental injection are not within +/- 20% of each other, or we see on the MRI that the BTME is not contained

within each injected muscle, then we will use a between subject design where subjects will be randomized to receive either the experimental or standard injection to the spastic LGM and 25 subjects will be recruited.

Subjects will receive clinical injections 3 months following the research injections and undergo a final MRI 2 months following clinical injections. Recruitment for the subsequent experiments will begin after data analysis from the second MRI in experiment #1 is complete.

Experiment #2 (Effect of dilution, answer Research Question #1):

If using a within-subject design, subjects will be randomized to receive a standard injection to either the LGM or MGM. The experimental injection will be delivered to the muscle not receiving the standard injection. If using a between-subjects design, subjects will be randomized to receive either a standardized injection in the LGM or an experimental injection in the LGM. On the same day of, but before the injection, the MRIB will be acquired. Using the localization schema proposed in our proof-of-concept study, the baseline scan will be used to determine the coordinates and depth of the injection into a given muscle. Two months (+/- 1 week) after the injection, subject will report for MRI2 and will be considered finished with Experiment #2.

He/she will be scheduled for the "clinical" injection 5 weeks (+/- 1 week) from that time, which will be evaluated in Experiment #4.

Experiment #3 (spastic vs. non spastic muscle, answer Research Question #2):

Experiment #3 will take place simultaneously and within the same subject population as Experiment #2. Regardless of whether a within- or between subject design is adopted in Experiment #2, all subjects will also receive a standard injection to the non-spastic LGM. The same technique using MRIB for muscle localization and the same protocol for obtaining MRI2 employed in Experiment #2 will be used for Experiment #3, at the same time points. Subjects will be scheduled for the "clinical" injection 5 weeks (+/- 1 week) from the time of MRI2, as mentioned under the description for Experiment #2.

Experiment #4:

As described previously, all subjects that participated in Experiments #2 and #3 will undergo a cycle of clinically-based BT injections to the spastic lower extremity no sooner than 3 months after the research injections and about 1 month after MRI2. Potentially, any lower extremity muscle or combination of muscles may be injected based on clinical evaluation and need. We reserve the right to limit the total dose of toxin injected to no more than 200 units of onobotulinumtoxinA. This would be a reasonable dose in clinical practice for the first cycle of lower extremity injections in a toxin-naive patient. All subjects will receive a third and final leg MRI3 2 months following the clinical injection, marking the end of this study approximately 5 months after the initial randomization in Experiment #2.

Observational Descriptive Study

An additional N=6 subjects who are receiving onobotulinumtoxinA injections in their lower leg on their spastic side for clinical purposes as part of standard clinical care will be recruited from the PI's clinical population. Qualifying subjects will be patients who are receiving onobotulinumtoxinA injections for any lower extremity muscle or combination of muscles and are naïve to previous onobotulinumtoxinA injections in the lower extremity. Subjects participating in this observational portion of the study will be

asked to undergo to MRI exams of their legs, one prior to their clinical onobotulinumtoxinA injections and one two months post injection. The research team will document the muscle groups injected and the dosage and dilution of the injections for data analysis purposes.

The reasoning behind adding this additional group of subjects is threefold:

1. We plan to use these additional data to practice and refine our interpretation of MRI images in preparation for analysis of the MRI images of subjects participating in Experiments 1-4

2. We plan to use these additional data to inform Research Question 3 (Is there a predictable BTME within a given muscle following lower extremity BT clinical injections then expected based on clinical need?)

3. We have had greater difficulty prospectively recruiting subjects for Experiments 1-4 and believe we will be more successful in recruiting subjects and obtaining data for this observational subject group while we continue to recruit for Experiments 1-4.

INCLUSION AND EXCLUSION CRITERIA

Experimental Group: Subjects will include males and females aged 30-75 with the diagnosis of any stroke (ischemic or hemorrhagic, first occurrence or recurrent) with clinically significant lower extremity spasticity as assessed by the principal investigator who would benefit from treatment with BT. Subjects must be ambulatory with or without a device and without assistance at a household or greater level. Any combination of spastic lower extremity muscle injections are acceptable so long as there is an indication to inject the gastrocnemius muscle. The goals of treatment may include improvement of gait, ankle range of motion, ankle foot orthosis fit, heel strike, and ankle position in stance phase. Also, decreased clonus or relief from painful muscle spasms may be goals. Subjects will be naïve to BT of any serotype, as well as, phenol or alcohol treatment in any lower extremity muscle for any reason. Prior upper extremity treatment is allowed, however.

Observational Descriptive Group: Subjects will include males and females aged 30-75 receiving clinical injections for any lower extremity muscle or muscle group with the diagnosis of spasticity of any etiology. Subjects must be ambulatory with or without a device and without assistance level at a household or greater. Subjects must be naive to botulinum toxin in the lower extremity only.

Experimental Group: Subject will have no history of concomitant neurological disease (central or peripheral) other than stroke. Subjects will be medically stable (as determined by the PI) and have no contraindications to intramuscular injection of BT. Subjects with intrathecal baclofen pumps will be excluded. Subjects must have no contraindication for MRI. Subjects with MRI-compatible hip replacements may participate, but not those with total knee replacements (due to artifact.)

Observational Descriptive Group: Subjects with intrathecal baclofen pumps will be excluded. Subjects must have no contraindication for MRI. Subjects with MRI compatible hip replacements may participate, but not those with total knee replacements (due to artifact).

DATA AND SAFETY MONITORING PLAN

Subjects will be evaluated continuously by the PI and the research team for any abnormal reactions to the botulinum toxin injections or the MRI scans throughout the course of the study, including at the time of the baseline study visit, one week post-injection phone calls, and at 2 month and 5 month follow-up study visits. Efficacy of the botulinum toxin injections will not be recorded for research purposes, as the purpose of this study is to develop better imaging techniques to evaluate and measure the movement of the toxin itself through the muscle.

Subjects have the right to stop participating in the study at any time, for any reason, without recourse. If at any point in the study a subject no longer meets the inclusion criteria of the study or meets the exclusion criteria, they will be removed from the study. If at any point in the study the PI believes that it is not in the best interest of the subject to continue participating in the study, they will be removed from the study.

Subjects will be removed from the study if they have a change in medical status or location which prevents them from completing the post-injection MRIs at follow up. Subjects will also be removed if they are unable to withstand the MRIs or experience a complication as a result of the MRI or botulinum toxin injection.

Botulinum Toxin Injection: The side effects of botulinum toxin are minimal and affect a small portion (less than 3%) of patients within 24 hours. The symptoms include temporary pain at the injection site, joint and muscle pain, cold or flu-like symptoms, and in very rare instances, possible allergic reaction such as itching, rash, red itchy welts, wheezing, asthma symptoms, or dizziness or feeling faint. When receiving any injection, there is always the small risk of bleeding and infection if proper sterilization procedures, skin preparation, and local pressure subsequent to each injection are not followed. In addition, subjects may experience decreased muscle strength and function due to hypotonia (low tone). The effects of botulinum toxin are temporary and last for approximately 12 weeks. Dr. Michael O'Dell, who will be responsible for performing botulinum toxin injections, has had extensive experience injecting botulinum toxin over the past decade with very few side effects. Magnetic Resonance Imaging: The effects of magnetic fields in an MRI scanner have been extensively studied and there are no known significant risks with an MRI exam. The subject may be bothered by feelings of confinement, or claustrophobia, as well as the noise made by the scanner during the procedure. Subjects will be asked to wear earplugs or earphones while in the magnet.

Monitoring will occur continuously throughout the duration of the study protocol. Subjects will have access to a research team member throughout the course of the study to describe any abnormalities or possible complications. In addition, researchers will make sure to check in with the subject, including to evaluate the hea8li/n1g5/1o9 f4:0th6ePM injection site, as well as the subject's overall well-being during each study visit. Subjects will be called within one week post injections for follow-up to identify any adverse events. Safety monitoring and evaluation of adverse events will be formally assessed at 3 time points throughout the duration of the study by a panel of WCMC Physiatrists, described in Questions 19, 20, and 21 below.

Because the first cycle of injections given to subjects are for research purposes and contain a very small amount of toxin, we do not anticipate subjects to gain any functional benefit from the first cycle of injections. The second cycle of injections provided for subjects are based on clinical need and therefore will not differ from treatment for spasticity that may occur in a clinical setting, which subjects will still have access to at the discretion of their physician and insurance. The MRI scans are for research purposes only and thus would not cause any negative impact on subjects or loss of benefit if the study was closed or the subject was terminated from the study.

Adverse events will be graded based on their severity, which will be independently scored apart from the attribution of the event. Adverse events will be graded based on their attribution, as either not related, possibly related, or related to the study procedures. All possible risks related to the study procedure are evaluated based on their unexpectedness, as either possible or rare. All adverse events that are unexpected, possibly related or related to the protocol, and place subjects at a harm greater than was previously known will be reported to the IRB following the Immediate Reporting Policy for Weill Cornell Medicine research involving human subjects. All adverse events will be reported to the PI and the panel of WCMC physiatrists for formal review at 3 time points throughout the study's duration.

All unexpected adverse events will be reported immediately to the PI Dr. O'Dell. Additionally, the appropriate incident report will be filed and reported to the IRB as per the WCMC AE reporting policy.