Using the muscle force test in the monkey to measure biological activity of botulinum neurotoxins

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Introduction & objectives

- Assays in rodents have been used for understanding the potency and duration of action of botulinum neurotoxins (BoNTs) *in vivo*. However, studies in larger species, that are closer to humans in their muscle size and anatomy are needed to better predict effects of new, modified recombinant BoNTs, developed to address unmet medical needs of patients.
- Here, we characterised the biological activity of abobotulinumtoxinA (aboBoNT-A; Dysport) in the monkey after a single intramuscular (i.m.) injection in the gastrocnemius muscle of the *triceps surae* group of the hindlimb. We used the muscle force test as it mimics functional activity of the muscle, impaired in patients with spasticity. The gastrocnemius muscle was chosen for the injection as it is among those lower limb muscles that are treated with BoNTs in the clinic. Cynomolgus monkeys were chosen as, due to similarity in the central/peripheral nervous systems as well as the relative size and organization of the muscles, their sensitivity to BoNTs better estimates the response in humans.
- The first objective of the study was to assess functional activity of aboBoNT-A by measuring the force developed by the *triceps surae* group of the hindlimb ipsilateral to the toxin injection.
- The second objective of the study was to assess potential distal effects of the toxin, using the muscle force of the *triceps surae* group contralateral to the injection.
- The weight of diaphragm and muscles on the ipsilateral and contralateral sides was measured at the end of the study as an additional measure of the proximal and distant effects of aboBoNT-A.

Methods

Subjects

- Adult, purpose-bred, Cynomolgus monkeys (*Macaca fascicularis*) were supplied by Noveprim (Mauritius). The weight range of animals at initiation of treatment was 2.0 to 3.5 kg. Animals were group-housed (up to 3 animals) and maintained on a 12-h light/dark cycle (lights on from 06:00 to 18:00) under constant temperature (22 ± 3°C) and relative humidity (> 35 %).
- All experimental procedures were approved by the Ethical Committee Charles River Laboratories, France and are in full compliance with the European Communities Council Directive 2010/63/EU and the French National Committee Decree No 2013-118.

The muscle force test

- Anesthetised animals were placed on a specially-designed restrain chair so that only the tibio-tarsal joints were mobile. An isometric force transducer (model K1000; HSE-HARVARD Apparatus) was secured to the forefoot so that the tibio-tarsal angle was 90°. A pair of stimulating needle electrodes were then placed near the tibial nerve. The nerve was electrically stimulated (Simulator C type 224, HSE-Harvard Apparatus), using square shocks (40V, 50 µs at 0.5 Hz) and the muscle force generated by the *triceps surae* group was recorded from a force transducer with the aid of a computerised data acquisition system (Notocord-hem software; Notocord Systems, Croissy-Sur-Seine, France).
- The muscle force was recorded from each animal twice before the injection (pre-test; 2 to 4 days apart), on post-injection Days 2, 3, 5 and once weekly from post-injection Week 2. The absolute muscle force generated by each hindlimb for at least 10 consecutive stimulations was normalised to the mean pre-test muscle force value of each hindlimb.

Respiratory function evaluation and clinical signs

- For each animal, respiratory function was assessed immediately after the muscle force test. The pneumotachographic and diaphragmatic EMG signals (using telemetry devices implanted into the body cavity several weeks in advance) were recorded simultaneously (Clement et al 1991). On each study day, the animals were observed for clinical signs (with particular attention to breathing, ptosis of the abdomen and palpebras) and food consumption. Body weight was measured once weekly.

Organ collection

- At the end of the study animals were sacrificed and the following organs were collected for weight assessment: gastrocnemius (left and right), soleus (left and right), plantaris (left and right), triceps brachialis (left and right), diaphragm.

Toxin

- AbobotulinumtoxinA (aboBoNT-A; Dysport®; Ipsen Limited, Wrexham, UK) was reconstituted in saline and administered i.m. into the right gastrocnemius at 1, 5, 10 and 12.5 U/kg (n=6/dose). Each dose was administered as two volumes of 0.05 mL/kg aimed at the right and left heads of the muscle. The left gastrocnemius was injected with saline. An additional group of control animals (n=3) received i.m. injection of vehicle (saline) into the right and left gastrocnemius muscles (two volumes of 0.05 mL/kg)

Results

The muscle force test

- Intramuscular administration of aboBoNT-A (1-12.5 U/kg) resulted in rapid, robust, and dose-related reductions in muscle force of the ipsilateral *triceps surae* group (Figure 1). Specifically, a trend of activity at 1 U/kg was followed by 75% reductions in 5 and 10 U/kg-treated animals and more than 80% reductions in 12.5 U/kg-treated animals (Figure 1). Maximal reductions in muscle force were reached on post-injection Week 2.
- Reductions in the muscle force were followed by dose-related recovery, as 5 U/kg returned to approximately 45% suppression levels by Week 12, while 10 and 12.5 U/kg –treated groups showed slower rates of recovery (Figure 1).
- Following i.m. administration of aboBoNT-A (1-12.5 U/kg), there were no signs of distal effects of the toxin, as changes in the muscle force seen in vehicle-treated *triceps surae* group contralateral to the aboBoNT-A injection were within the range of variability linked to the assay (Figure 2).
- Post-mortem analysis of the muscle weight confirmed significant and dose-related reduction in the weight of aboBoNT-A-injected gastrocnemius, as well as reductions in the weight of ipsilateral (right) plantaris in animals treated with 12.5 U/kg aboBoNT-A (Table 1)
- There were no changes in muscle weight of any of the muscles on the contralateral side or in muscle weight of the diaphragm (Table 1).

Figure 1. Monkey muscle force (% of baseline) generated by the right (ipsilateral) *triceps surae* group following i.m. administration of abobotulinumtoxinA (1-12.5 U/kg) or vehicle (saline) into the gastrocnemius muscle at 2 x 0.05 mL/kg volume.

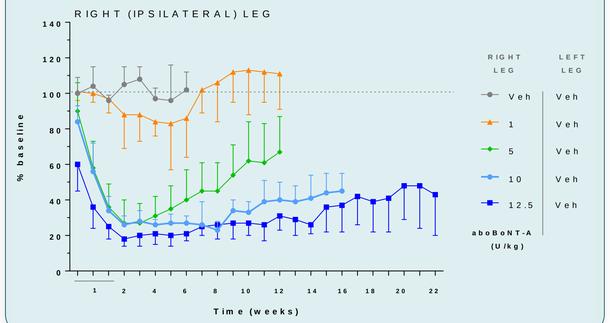

Figure 2. Monkey muscle force (% of baseline) generated by the left (contralateral) *triceps surae* group following i.m. administration of vehicle (saline) into the gastrocnemius muscle at 2 x 0.05 mL/kg volume.

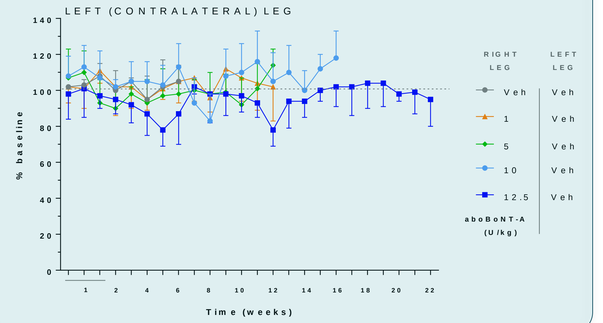

Table 1. Organ weights (mean values) from animals with i.m. injection of vehicle (saline) or abobotulinumtoxinA (1-12.5 U/kg) at the end of the testing period. The results are expressed as absolute weights (g) of the left (contralateral) muscles and weights of right (ipsilateral) muscles as percentage of the corresponding left muscles.

Treatment	Vehicle	1 U/kg	5 U/kg	10 U/kg	12.5 U/kg
Week of tissue collection	W7	W14	W14	W17	W23
n	3	6	6	6	6
MUSCLE					
Gastrocnemius					
Left weight (g)	14.8	16.4	17.1	16.5	17.5
Right weight (%)	+7	-1	-16	-31	-36
Soleus					
Left weight (g)	5.7	7.3	7.0	6.2	6.4
Right weight (%)	+10	-6	-11	-3	-4
Plantaris					
Left weight (g)	4.7	5.4	5.2	5.3	5.5
Right weight (%)	+6	0	-5	-3	-16
Triceps brachialis					
Left weight (g)	28.7	32.8	35.3	30.8	29.2
Right weight (%)	+5	-1	-6	-8	-6
Diaphragm					
Weight (g)	10.5	11.3	10.4	10.6	13.5
Diaphragm/body weight (%)	0.32	0.34	0.31	0.32	0.38

Conclusions

- *In vivo*, a single i.m. administration of aboBoNT-A (1-12.5 U/kg) resulted in robust, dose-, and time-related reductions in the muscle force of the *triceps surae* group ipsilateral to the injection.
- There were no signs of distal effects of aboBoNT-A, as the treatment did not change the muscle force of the contralateral (vehicle-injected) *triceps surae* group.
- AboBoNT-A (1-12.5 U/kg) was well-tolerated, as animals showed no changes in respiratory function, no clinical signs, no changes in food consumption or body weight. In accord, weights of muscles on the contralateral side to the injection as well as the diaphragm were similar to those of vehicle-treated animals.
- The monkey muscle force test is a robust pharmacological tool for measuring potency and duration of action of BoNTs and thus can be used to characterise the biological activity of new, modified recombinant BoNTs developed as novel therapies for unmet medical needs.

References

1. Clement Ma G, Albertini M, Aguggini G (1991) Effects of PGF2α on the EMG of costal and crural parts of the diaphragm of the newborn pig. *Prostaglandins Leukotrienes and Essential Fatty Acids* 43: 167-173.

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