SXN102342, a novel, recombinant botulinum neurotoxin type A1: *in vivo* characterization

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Introduction & objectives

- There is growing clinical evidence that botulinum neurotoxin (BoNT), a native protein product of *Clostridium botulinum* bacteria, can eliminate muscle and glandular hyperactivity in a wide range of medical conditions.
- Recently we have characterised a novel, recombinant BoNT A1 SXN102342 expressed in *Escherichia coli* and presented its synthesis and in vitro characterisation (Hooker et al. 2017).
- Here we describe in vivo characterisation of SXN102342, confirming its similarity to a native BoNT/A1.

Methods

All experimental procedures were approved by the Ethical Committee of Ipsen Innovation or 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 wheel running test in mice

- Adult male CD-1 mice (Charles River Laboratories, Saint-Germain-Nuelles, France) received intramuscular (i.m.) injection of SXN102342 (1.33-6.7 pg/animal), natural BoNT/A (1.33-6.7 pg/animal) or vehicle (Gelatine Phosphate Buffer; GPB) into the gastrocnemius muscle in the left hindlimb (n=8/group). They were placed in cages equipped with running wheels and were monitored continuously for 28 days.

The digit abduction score test in rats

- The assay is based on the digit spreading reflex evoked when the animal is lifted swiftly by the torso in the air. The reflex is inhibited in response to i.m. administration of BoNTs into the gastrocnemius-soleus muscles of the hind paw. The DAS response in each rat is scored on a five-point scale, from normal reflex (i.e. no inhibition; DAS 0) to full inhibition of the reflex (DAS 4). The abduction response was scored by the experimenter blind to the treatment.
- Adult, female Sprague-Dawley rats (Janvier Labs, Saint Berthevin, France) received i.m. injection of SXN102342 (1-20 pg/animal) or its vehicle (GPB) into the peroneus muscle of the *triceps surae* muscle group in the left hindlimb (Day 0) and were tested for DAS (n=6/group).

The muscle force test in rats

- The rat muscle force test was performed as described by Pickett et al. (2008). Anesthetised animals are placed on a specially-designed table, with the leg placed between blocks, so that only the tibiotarsal joint is mobile. A calibrated force transducer is secured to the foot so that the tibiotarsal angle is 90°. Transcutaneous electrodes are placed near the sciatic nerve and on the back. The sciatic nerve is electrically stimulated (40V, 50 µs at 0.5 Hz) and the muscle force generated by the *triceps surae* group is recorded with the aid of a computerised data acquisition system
- Adult, female Sprague-Dawley rats received i.m. injection of SXN102342 (1-50 pg/kg) or its vehicle (GPB)) in 0.1 mL/kg volume administered into the lateral head of the right gastrocnemius muscle (n=4/group). Animals were tested in the muscle force test twice before the injection (pre-tests) and once weekly over 4 weeks post-injection.

The muscle force test in rabbits

- Anesthetised animals are placed on a specially-designed table adapted to the animal's size. A calibrated force transducer is secured to the foot so that the tibiotarsal angle is 90°. The transcutaneous electrodes are placed near the tibial nerve and on the back. The tibial nerve is electrically stimulated (40V, 50 µs at 0.5 Hz) and the muscle force generated by the *triceps surae* group is recorded with the aid of a computerised data acquisition system.
- Adult, male, New Zealand White rabbits received SXN102342 (1-100 pg/kg) or its vehicle (GPB) in 0.1 mL/kg volume administered into the lateral and medial heads of the right gastrocnemius muscle (n=4/group). Animals were tested in the muscle force test twice before the injection (pre-tests) and once weekly over 4 weeks post-injection.

Results

The wheel running test in mice

- SXN102342, administered i.m., resulted in robust and dose-related reductions in the total distance travelled by mice in the running wheel, producing 80-100% suppression of the activity at 3 and 6.7 pg/animal ($p<0.001$) between 3 and 4 days post-injection (Figure 1A). Reduction in activity was followed by rapid recovery, with the activity returning to normal levels by post-injection days 13-22 (Figure 1A).
- BoNT/A also resulted in a robust and dose-related reduction in the total distance travelled by mice in the running wheel (Figure 1B). Specifically, 80% suppression of activity at 1.33 pg/animal ($p<0.01$) was followed by almost full suppression ($p<0.001$) of activity at higher doses between 3 and 4 days post-injection (Figure 1B). Reduction in activity was followed by a rapid recovery, with activity returning to normal levels by post-injection days 18-22.

The digit abduction score test in rats

- SXN102342 administered i.m., resulted in dose-related increases in DAS value, with full suppression of the digit abduction (DAS 4) at ≥25 pg/kg and an estimated ED_{50} value of 3.35 pg/kg (Figure 2A). After reaching their peak, DAS values showed time- and dose-dependent reduction, with all but 50 pg/kg returning to DAS 1 by day 8. There was no effect of the treatment on body weight gain at any of the doses tested.

Figure 1. Distance travelled (cm) by mice in automated running wheels following a single i.m. injection of SXN102342 (1.33-6.7 pg/animal) and its vehicle (GPB) or natural BoNT/A (1.33-6.7 pg/animal) when the injections were made into the right gastrocnemius muscle (n=6/dose)

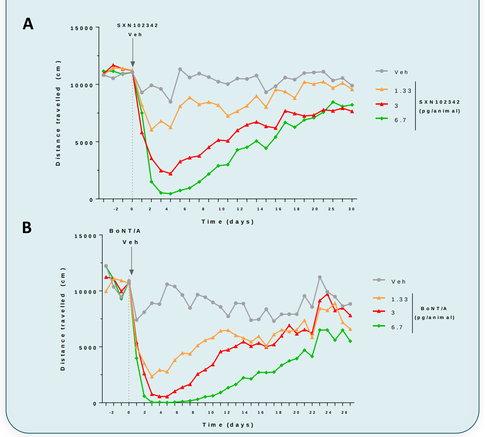


Figure 2. DAS responses in the rat following a single i.m. injection of SXN012342 (2.5-50 pg/kg), and its vehicle (GPB; panel A) or natural BoNT/A (2.5-50 pg/kg) and its vehicle (GPB; panel B) into the left peroneus muscle (n=6/dose)

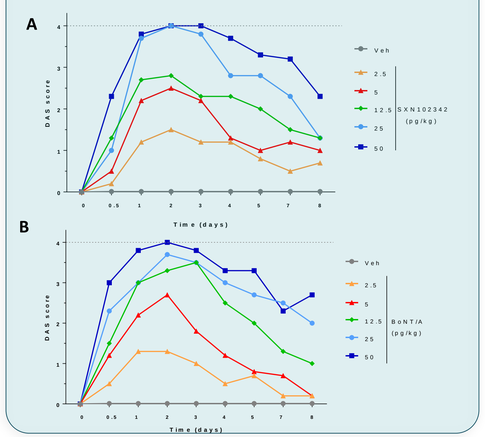


Figure 3. Muscle force generation in the right and left *triceps surae* group of muscles in the rat following a single i.m. treatment with SXN102342 (1-50 pg/kg) into the right gastrocnemius muscle and no injection into the left one (n=4/group).

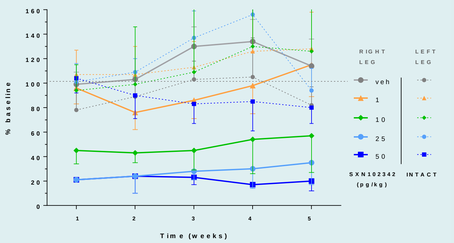
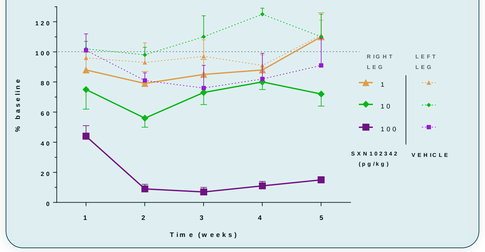


Figure 4. Muscle force generation in the right and left *triceps surae* group of muscles in the rabbit following a single i.m. treatment with SXN102342 (1-100 pg/kg) and its vehicle (GPB), into the right and left gastrocnemius muscles, respectively (n=4/dose)



- BoNT/A also resulted in dose-related increases in the DAS score, with full suppression of the digit abduction (DAS 4) at ≥25 pg/kg and an estimated ED_{50} value of 3.35 pg/kg (Figure 2B). The DAS scores showed time- and dose-dependent reduction as groups treated at 2.5 – 12.5 pg/kg showed DAS scores of ≤1 by day 8 (Figure 2B). There was no effect of the treatment on body weight gain at any of the doses tested.

The muscle force test in rats

- SXN102342 resulted in dose-related reductions of the muscle force in the toxin-injected (right) muscle, resulting in 20 and 60% reductions at 1 and 10 pg/kg, and 80% reductions at 25 and 50 pg/kg (Figure 3). The muscle force activity of 1 pg/kg-injected animals returned to baseline by Week 4, while activity of other groups remained stable. The muscle force activity of the intact (left) muscle remained variable showing no clear toxin-mediated effects. There was no effect of the treatment on body weight gain.

The Muscle Force test rabbits

- SXN102342 resulted in dose-related in dose-related reductions in the muscle force in the toxin-injected (right) muscle, resulting in up to 20% reductions at 1 pg/kg treatment, and 40 and 90% reductions at 10 and 100 pg/kg (Figure 4). The muscle force activity of 1 pg/kg-injected animals returned to baseline by Week 4, while other groups showed only trends of recovery over time. The muscle force activity of the vehicle-injected (left) muscle remained variable showing no clear toxin-related effects. There was no effect of treatment on body weight gain.

Conclusions

- *In vivo*, a single i.m. administration of SXN102342 resulted in robust, dose-, and time-related effects in a range of assays performed in multiple species.
- SXN102342 reduced the running wheel activity in mice, increased the digit abduction scores in rats and reduced the muscle force in rats and rabbits, indicative of its muscle-relaxant activity.
- Activity of SXN102342 was time-dependent, with higher doses typically associated with longer duration of action across assays.
- As seen in the mouse running wheel and the rat DAS assays, activity of SXN102342 is similar to that of natural BoNT/A1
- SXN102342 represent a useful pharmacological tool to be used as a reference compound in characterisation of novel, modified, recombinant botulinum neurotoxins.

References

1. Hooker A, Palan S, Beard M (2017) Protein engineering and process development of recombinant botulinum neurotoxin serotype A1. TOXINS, 18-21 Jan 2017, Madrid, Spain.

2. Pickett A, O'Keeffe R, Judge A, Dodd S (2008) The in vivo rat muscle force model is a reliable and clinically relevant test of consistency among botulinum toxin preparation. *Toxicon* 52: 455-464.

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