NT401 (FasTox): a novel, rapid, and short-acting modified botulinum neurotoxin

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Summary

- Botulinum neurotoxin serotype A (BoNT/A) is a valuable treatment for numerous indications mainly in neurology, urology, and aesthetic medicine.
- All patients who have disorders that benefit from BoNT/A treatment share the need for life-long therapy, which fits well with the long duration of action of BoNT/A.
  - Its duration of action ranges from 3 months in skeletal muscle hyperinnervation, such as in cervical dystonia, to 9 months in autonomic dysfunctions, such as hyperhidrosis.
- The paralytic effect of BoNT/A develops over 3–7 days. Ideally, transient indications would be treated with a chemodenervating substance that has a rapid onset and a short duration of action; however, the pharmacokinetic (PK) profile of BoNT/A means that it is not a good match for such indications.
- In order to generate a neurotoxin that has the required rapid onset and short duration PK we have genetically engineered BoNT/E which, by nature, provides the shortest duration of action in humans (6 weeks) of the naturally occurring BoNT serotypes.[1]
- We have introduced an E3-ligase binding motif into the light chain of BoNT/E in order to increase the probability of cytosolic ubiquitination, thereby accelerating the proteasomal light chain degradation and shortening the duration of action.
- The modified neurotoxin (NT401, FasTox) is expressed in *Escherichia coli* and shows an onset of effect after intramuscular injection within 1 day in the mouse Digit Abduction Score (DAS) and the voluntary mouse running assay; the effect lasted 3–5 days.
- In non-human primates, the maximal effect developed within 1 day and lasted 2–4 weeks.

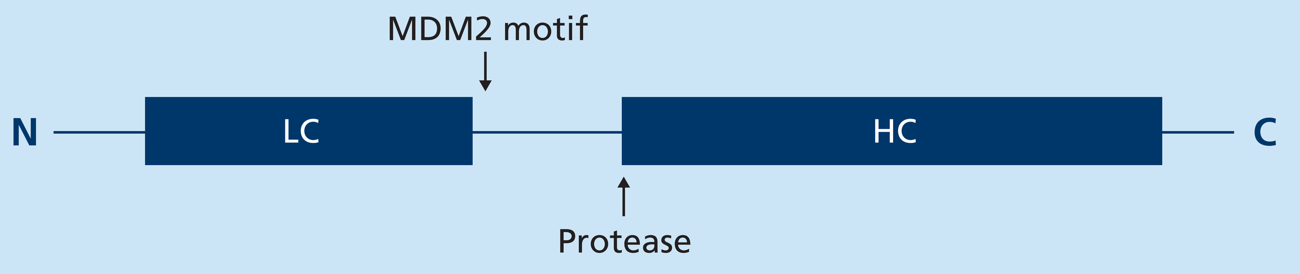
Background

- BoNT/A is a valuable cure for an increasing number of disorders.
- It is, however, limited to long-term treatment because of its extended duration of action, which ranges from 3 months (e.g. in cervical dystonia) to 9 months (e.g. in hyperhidrosis).
- For many physicians, a shorter-acting chemodenervating substance for the treatment of more acute disorders, predominantly in the surgical and orthopedic fields, would be welcomed.
- For this purpose, we have used genetic engineering to construct and express a modified BoNT/E-based neurotoxin using a well-controlled recombinant process.
- Clinical development could lead to the first recombinant neurotoxin on the market.

Methods

- We introduced an E3-ligase binding motif, mouse double minute 2 homolog (MDM2), into the linker of BoNT/E in order to increase the probability of cytosolic ubiquitination (**Figure 1**).

Figure 1: The rBoNT/E molecule


MDM2 motif
N
LC
HC
C
Protease

The molecule comprises a 50 kDa LC and a 100 kDa HC and was cloned into the proprietary expression vector pEVII.
The MDM2 motif (LTFEHNWAQLTS) and a protease cleavage site for posttranslational activation were introduced within the linker region connecting the LC and the HC.

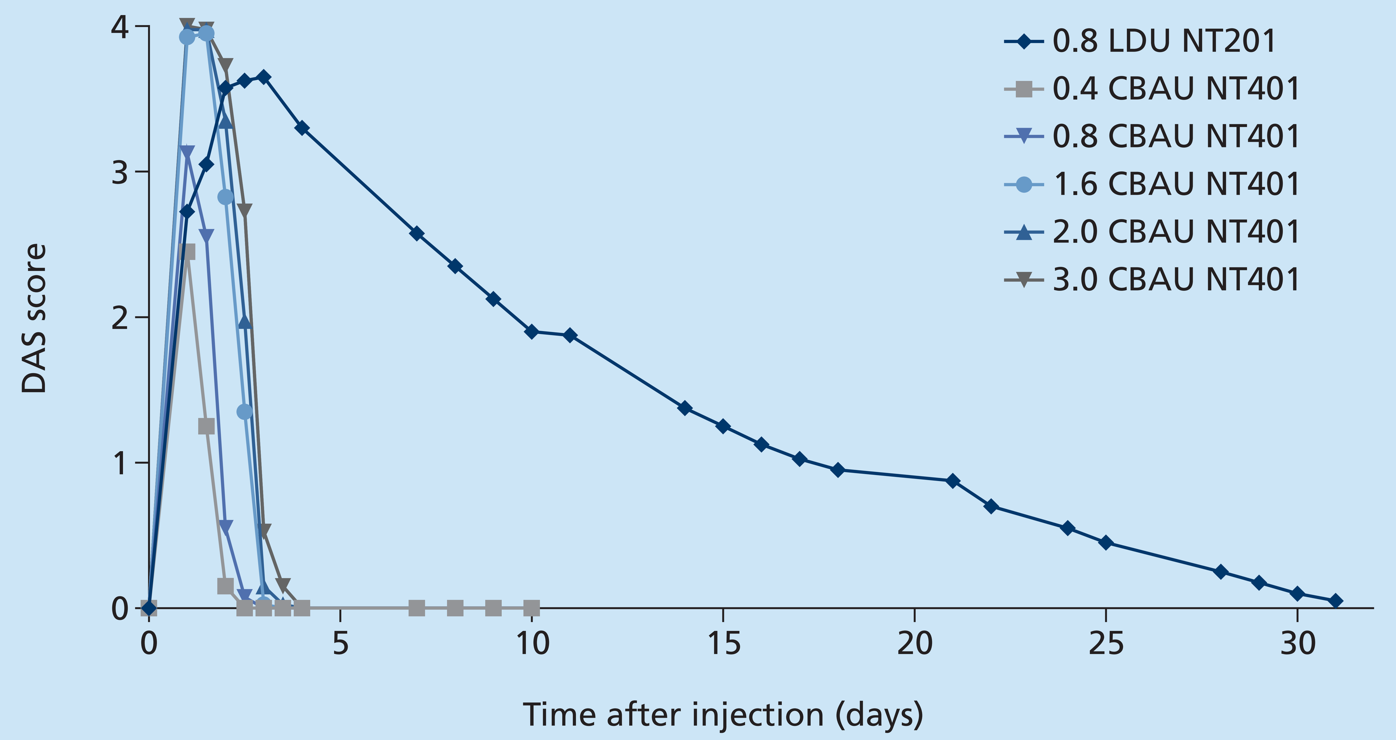
HC, heavy chain; LC, light chain; MDM2, mouse double minute 2; rBoNT, recombinant botulinum neurotoxin.

- This modification should result in an accelerated degradation of the light chain by the proteasome and, therefore, a shortened duration of neurotransmitter-release inhibition.
- NT401 is produced recombinantly using a state-of-the-art *E. coli* fermentation and purification process design. The potency of NT401 is measured in cell-based assay units (CBAU).
- *In vivo* efficacy was assessed in mice using the mouse DAS[2] and the mouse running assay.[3]
- In non-human primates, the effect of a single intramuscular injection on neuromuscular signaling was determined by electromyography using electrodes implanted in the gluteus (telemetry). For the evaluation of the paralytic effect, contraction of the hind limb after electrical stimulation of the tibial nerve was measured ([4]modified for monkeys).

Results

- *In vivo*, NT401 showed an onset of effect within 1 day on the mouse DAS (**Figure 2**) and mouse running assay (**Figure 3**). The effect lasted 3–5 days.
- The results were similar in a rat DAS.
- Preliminary experiments in non-human primates confirmed the fast onset of muscle paralysis within 1 day. Here, the paralytic effect lasted 2–4 weeks (at high doses, not shown).

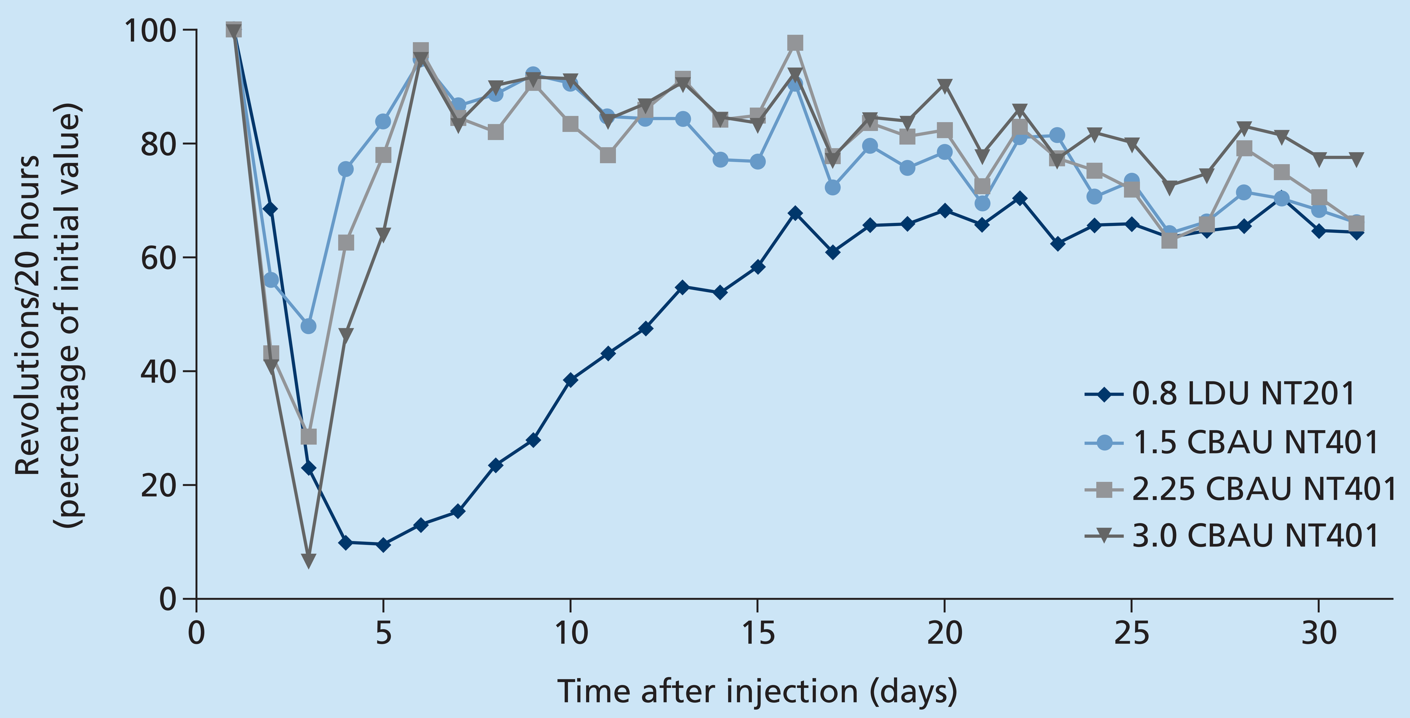
Figure 2. Digit abduction scoring after NT 401 (FasTox) or NT 201 (BoNT/A) injection into the right gastrocnemius muscle in mice


DAS score
0
1
2
3
4
0
5
10
15
20
25
30
Time after injection (days)
0.8 LDU NT201
0.4 CBAU NT401
0.8 CBAU NT401
1.6 CBAU NT401
2.0 CBAU NT401
3.0 CBAU NT401

The maximum score of 4 on the y-axis reflects the maximum paralytic effect of NT 401 or BoNT/A resulting in an abolished toe-spread reflex. Mean of 10 mice per group.

BoNT, botulinum neurotoxin; CBAU, cell-based assay units; DAS, digit abduction score; LDU, LD_{50} units.

Figure 3. Mouse voluntary running assay after NT 401 (FasTox) or NT 201 (BoNT/A) injection into the right gastrocnemius muscle in mice

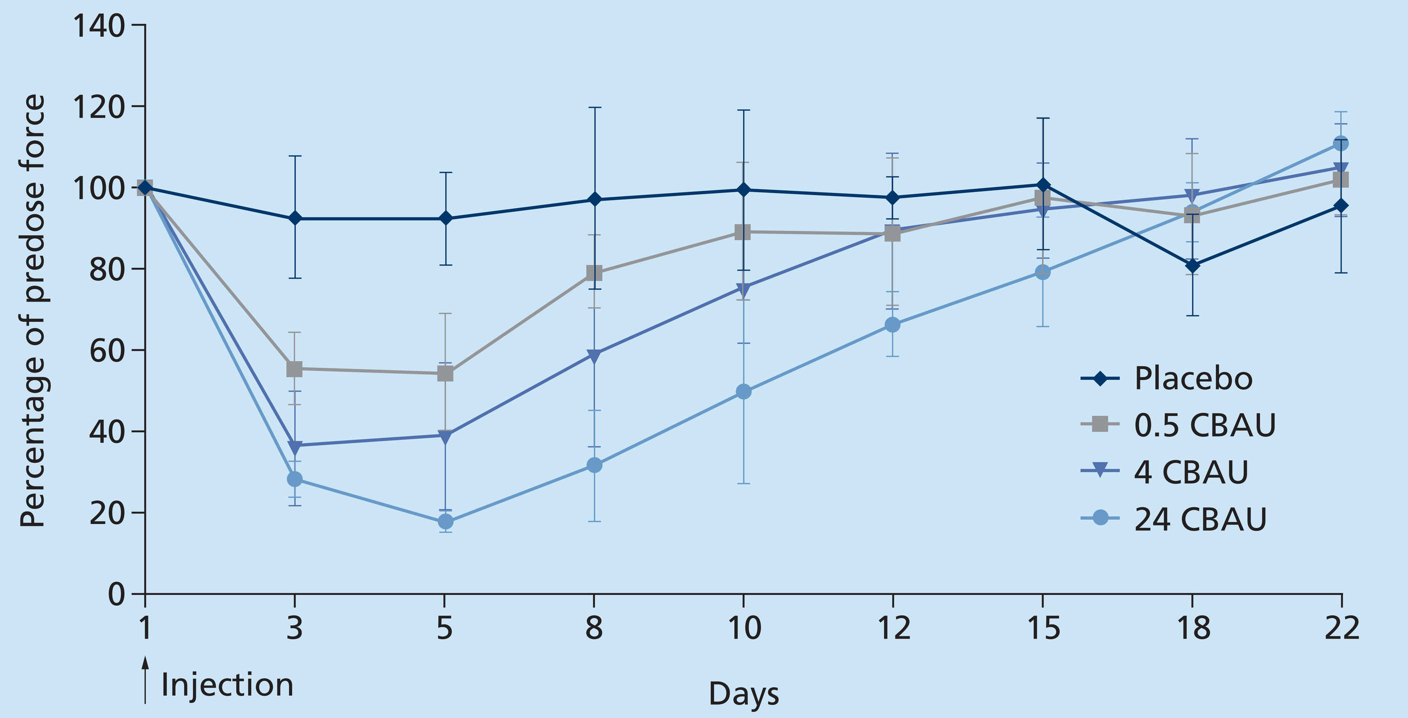

Revolutions/20 hours
(percentage of initial value)
0
20
40
60
80
100
0
5
10
15
20
25
30
Time after injection (days)
0.8 LDU NT201
1.5 CBAU NT401
2.25 CBAU NT401
3.0 CBAU NT401

The daily sum of wheel revolutions is given as the percentage of the initial run sums on the y-axis.
Zero % reflects the maximum paralytic effect of NT 401 or BoNT/A resulting in an immobile mouse.
Mean of 8 mice per group.

BoNT, botulinum neurotoxin; CBAU, cell-based assay units; LDU, LD_{50} units.

- No clear dose–response relationship could be seen with electromyography (data not shown); however, in the muscle force study the extent and duration of the paralytic effect was dose-dependent in the selected dose range (**Figure 4**).

Figure 4. Muscle force in the gastrocnemius muscle of the Cynomolgus monkey


Percentage of predose force
0
20
40
60
80
100
120
140
1
3
5
8
10
12
15
18
22
Injection
Days
Placebo
0.5 CBAU
4 CBAU
24 CBAU

Developed force is expressed as a percentage of predose force determined on two occasions prior to treatment. NT401 induced a dose-dependent reduction in muscle force. Maximal effects were observed between days 3 and 5. The duration of effect was dose-dependent. After peak effect, muscle force returned to pre-test levels between 8 and 18 days after dosing.
Under anesthesia, hind limb muscle contraction was induced by electrical stimulation of the tibial nerve. Developed force, by the corresponding foot pressing a pedal, was measured before and after injection of doses of NT401 or placebo into the right gastrocnemius muscle.
Mean (standard deviation) results of 6 Cynomolgus monkeys per group.

Conclusions

- We have generated a modified, recombinantly produced botulinum neurotoxin that has a rapid onset of effect and a shorter duration of action than that previously demonstrated for BoNT/A or expected for BoNT/E.
- This novel, first-in-class chemodenervating agent opens entirely new therapeutic avenues that cannot be accessed using the neurotoxins currently available.

References

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