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Original Article

Neurophysiological Double-Blind Trial of a Botulinum Neurotoxin Type A Free of Complexing Proteins

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Abstract

Objective:
Safety and efficacy of botulinum neurotoxin type A preparation NT 201 (Xeomin, Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany) were investigated over 52 weeks in a double-blind, randomized trial with 32 male volunteers.

Methods:
Electroneurographic assessments with surface electrodes were performed after single injections of NT 201 (2, 4, 16, or 32 units) into the extensor digitorum brevis (EDB) muscle and the same dose (Botox; Allergan Pharmaceuticals (Ireland) Ltd. Westport, Ireland) into the contralateral EDB.

Results:
All NT 201 and BTXCo doses achieved a statistically significant reduction of the compound muscle action potential M-wave amplitude in the EDB muscle. At week 4, the highest dose was statistically significantly more effective than the lowest dose (NT 201, $P = 0.019$; 95% confidence interval, 0.195–1.370; and BTXCo, $P = 0.002$; 95% confidence interval, 0.309–1.167). Duration of effect was dose dependent. The mean values of compound muscle action potential M-wave amplitudes in the adjacent muscles (abductor digiti quinti and abductor hallucis) were above the predefined threshold of effect, indicating that there was no relevant diffusion-induced reduction of muscle activity. NT 201 and BTXCo were well tolerated.

Conclusions:
NT 201 is effective and safe in inducing the desired paretic effect.

Key Words: botulinum neurotoxin type A, NT 201, extensor digitorum brevis (EDB) muscle, paretic effect, local diffusion

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Botulinum neurotoxin type A (BTX-A) is produced by anaerobic *Clostridium botulinum* bacteria as part of a high molecular weight complex containing hemagglutinins and other nontoxic nonhemagglutinin proteins. Botulinum toxin type A acts selectively on peripheral cholinergic nerve endings and inhibits the release of acetylcholine.[1] Thus, BTX-A decreases muscle activity hence serving as an effective treatment of a wide range of indications including strabismus, blepharospasm, hemifacial spasm, cervical dystonia, and spasticity.

A possible disadvantage of preparations containing biological components is their immunogenic potential, which may lead to formation of antibodies and secondary nonresponse to the treatment. In contrast to BTX-A preparations that have been used in recent years (Dysport, Ipsen Biopharm Ltd, Wrexham, UK, and BOTOX [BTXCo], Allergan Pharmaceuticals (Ireland) Ltd. Westport, Ireland), the new preparation NT 201 (Xeomin; Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany) is a BTX-A, which is highly purified and free of any accessorial complexing proteins. NT 201 contains approximately 600 pg of active clostridial protein (manufacturer's information). Because immunogenicity depends on protein load[2] and NT 201 contains only the protein required to achieve the desired pharmacological effect, the immunogenic potential of NT 201 can be expected to be minimal.

In this 52-week study, we assessed 4 doses of NT 201 with respect to the magnitude and duration of the paretic effect after injection into the extensor digitorum brevis (EDB) muscle of 1 foot as well as local diffusion of NT 201 into adjacent muscles. For intraindividual comparison, equal doses

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of BTXCo were injected into the EDB muscle of the contralateral foot.

METHODS

Subjects

Thirty-two healthy male volunteers (mean age, 26.4 years; range, 18 to 41 years) were enrolled at a single center (Department of Neurology, University of Leipzig, Leipzig, Germany). The demographic data were similar in the 4 dose groups.

To qualify for inclusion in the study, the subjects had to fulfill the following electroneurographic criteria at baseline: compound muscle action potential (CMAP) M-wave amplitude of EDB muscle of more than or equal to 4.0 mV, CMAP M-wave amplitude of abductor hallucis (AH) muscle of more than or equal to 5.0 mV, and CMAP M-wave amplitude of abductor digiti quinti (ADQ) muscle of more than or equal to 5.0 mV. In addition, volunteers had to have normal muscle power (score = 5) on the Medical Research Council (MRC) scale.[3]

Exclusion criteria were a history of childhood botulism, concurrent or previous treatment with BTXCo, and previous myotomy or denervation surgery in the muscles of interest (eg, peripheral denervation and/or spinal cord stimulation). Subjects were not allowed to take any medication affecting neuromuscular transmission for a minimum of 2 weeks before study start and during the study.

Study Medication

NT 201 was produced in accordance with Good Manufacturing Practice and fulfilled the Good Manufacturing Practice safety and quality standards. BTXCo was obtained from commercial sources.

Both NT 201 and BTXCo were available in glass vials. Before intramuscular injection, the content of each vial was reconstituted in 4 mL of sterile 0.9% NaCl.

Study Design and Methods

This was a randomized (by foot and dose), double-blind, single-center study of NT 201 with intraindividual active control (BTXCo). Electroneurographic measurements were performed at 14 visits scheduled over a period of 52 weeks (Fig. 1). Surface electrodes of 11 mm diameter were used as recording electrodes (placed over the muscle belly) and reference electrodes (placed over the tendon of the corresponding muscle). The point of maximum response was marked with indelible ink to allow recording from the same site on subsequent occasions. Filter settings of 2 Hz to 10 kHz were attuned. Surface skin temperature of the left and right feet was ascertained to be 34.5°C before electroneurographic measurements. All recordings were performed after supramaximal stimulation and were carried out with the same device by 2 equally trained and experienced members of the site staff. Persons performing electroneurographic measurements

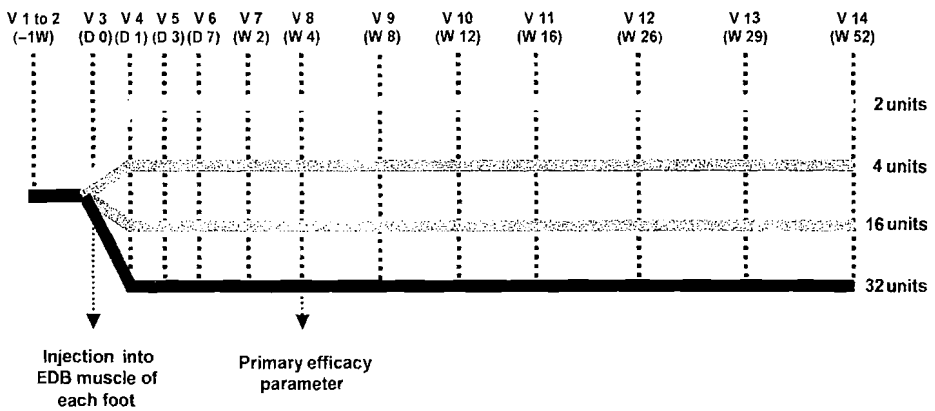

FIGURE 1. Outline of study design showing the 4 different dose groups of NT 201 and BTXCo, timing of study visits (visits 1 to 14), and time point of assessment of the primary efficacy parameter (week 4 and visit 8). D indicates day, V, visit, w, Week.

were blinded with respect to the dose and type of preparation (NT 201 or BTXCo).

The presence of subclinical peripheral neuropathy and/or an accessory peroneal nerve was excluded by electroneurographic measurements before treatment. The measurements at these visits (visits 1 to 3) included nerve conduction velocity, distal latency, and amplitude. These recordings were performed after proximal stimulation for sural nerve, tibial nerve (recordings from AH and ADQ muscles), and peroneal nerve (recording from EDB muscle).

At each visit, CMAP M-wave amplitudes were recorded from EDB muscle and adjacent AH and ADQ muscles after distal stimulation of the corresponding nerve. The largest 2 of the 3 values of CMAP M-wave amplitude recorded at any 1 visit were averaged to calculate the visit value. The largest 2 of the 3 preinjection visit values (visits 1 to 3) were averaged to calculate the baseline value.

At visit 3 (day 0), volunteers were randomly assigned to a dose of 2, 4, 16, or 32 units of NT 201 injected into the EDB muscle of 1 foot. For intraindividual comparison, the same dose of BTXCo was injected into the contralateral EDB muscle. The investigator responsible for injections was blinded with respect to the preparation, but it was not possible to blind the doses due to the different volumes. However, the investigator responsible for injection was not involved in performing the electroneurographic assessments.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki (18th World Medical Congress in Helsinki 1964 and amendments adopted in Hong Kong 1989 and Somerset West in 1996). In addition, the study was conducted in accordance with the German Drug Law (Arzneimittelgesetz September 1998), the Principles for the Proper Conduction of Clinical Trials of Pharmaceuticals (Bundesanzeiger, December 30, 1987), and the International Conference on Harmonisation Harmonized Tripartite "Guidelines for Good Clinical Practice (GCP) 17. January 1997." The study was approved by the local ethics committee (University Hospital of Leipzig, Leipzig, Germany), and all participants gave their written informed consent before the inclusion in the study.

Efficacy and Safety Parameters

Paretic effect was prospectively defined as a reduction to less than or equal to 80% of the baseline value of CMAP M-wave amplitude. The primary efficacy parameter was the percentage reduction of the EDB CMAP M-wave amplitude (CAmR [%]) at week 4 (visit 8) compared with the baseline value.

Secondary efficacy parameters were analyzed during the course of the 52-week observation period. These included EDB CAmR (%) at visits 4 to 14 and time to maximum EDB CAmR (%). In addition, duration of effect, defined as the time between the injection and the time point when the CMAP M-wave amplitude returned to more than 80% of the baseline value, was assessed.

Local diffusion was determined by the CAmR (%) in the ADQ and AH muscles at week 4 (visit 8) relative to the baseline value. An additional analysis of diffusion, defined as the mean of the CAmRs (%) at weeks 1, 2, and 4, was performed for ADQ and AH muscles. This additional analysis allowed to assess diffusion of the preparations at different time points.

To assess the quality of electroneurographic measurements, a coefficient of variation of CMAP M-wave amplitudes was calculated from all 3 recordings obtained at 3 different time points (9 recordings in total) before the injection.

Muscle power of the toes was measured 1 week before the injection (visit 1), on the day of injection (day 0), and after 4, 16, 26, 39, and 52 weeks, using the MRC scale, ranging from 0 (= no contraction) to 5 (= normal power).[3,4] Vital signs, laboratory parameters, physical and neurological examinations, and adverse events (AEs) were recorded for safety reasons at various time points during the 52-week observation period.

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Statistical Analyses

All efficacy data were analyzed for the intent-to-treat (ITT) population. The primary efficacy parameter was tested sequentially to derive the smallest dose that was more effective than 2 units on the logarithmic (log) transformed CAmR (%) values. This procedure was performed by 1-sided *t* tests at the level of α equal to 0.025 with Satterthwaite-Welch approximation controlling the variance in homogeneity between the compared dose groups. Secondary efficacy parameters were analyzed for treatment effects within and differences between dose groups using 2-sided statistical tests. The significance level was α equal to 0.05 and for evaluation of diffusion α equal to 0.1. No adjustments were made for multiple comparisons. In addition, the primary efficacy parameter was analyzed in an analysis of covariance model with the following covariables: age, body mass index (BMI), baseline EDB CMAP M-wave amplitude, and left-right footedness to investigate the influence of these covariables on the treatment effect.

Safety parameters were analyzed for the evaluable-for-safety population using exploratory statistics or lists.

RESULTS

Efficacy

Efficacy was evaluated for the ITT population (N = 26). Of the 32 volunteers randomized, 6 were excluded from the efficacy evaluation because of randomization failures. The decision to exclude these volunteers was made before unblinding. Data from at least 6 volunteers were available in each dose group (2 units, n = 7; 4 units, n = 7; 16 units, n = 6; 32 units, n = 6).

Percentage Reduction in EDB CMAP M-wave Amplitude at Week 4 (Primary Efficacy Parameter)

At week 4, the paretic effect, measured as EDB CAmR (%);was statistically significant for all dose groups of NT 201 and BTXCo (ITT population, N = 26; Fig. 2). Confirmatory testing using a *t* test with Satterthwaite-Welch approximation controlling the variance in homogeneity between the dose groups revealed a statistically significantly larger log transformed EDB CAmR (%) in the group receiving 32 units than the group receiving 2 units for both NT 201 (P = 0.019; 95% confidence interval [CI], 0.195–1.370) and BTXCo (P = 0.002; 95% CI, 0.309–1.167) at week 4. The comparisons between the 2-unit and 16-unit dose groups were not statistically significant at week 4 for either NT 201 or BTXCo.

An analysis of covariance with factors age, BMI, footness, and baseline CMAP M-wave amplitude supported the results of the primary efficacy analysis (P = 0.07) and indicated a tendency toward higher efficacy of NT 201 in younger subjects and those with a lower BMI.

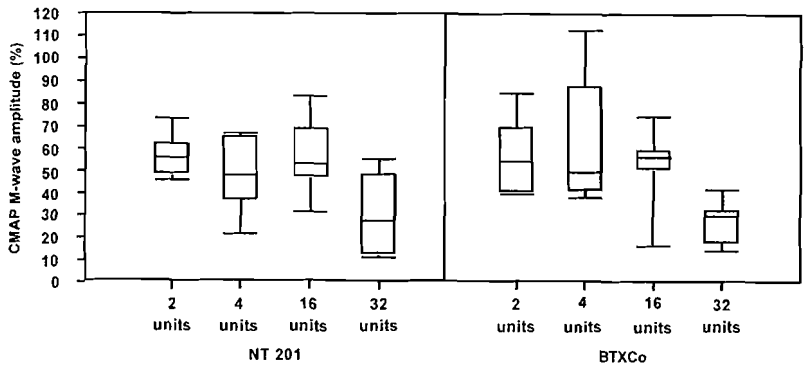

FIGURE 2. Primary efficacy parameter, box plot of EDB CMAP M-wave amplitude in percentage relative to baseline (baseline = 100%) at week 4 for NT 201 and BTXCo (ITT population, n = 26). The plots show the median, minimum, and maximum, and 25% and 75% percentiles.

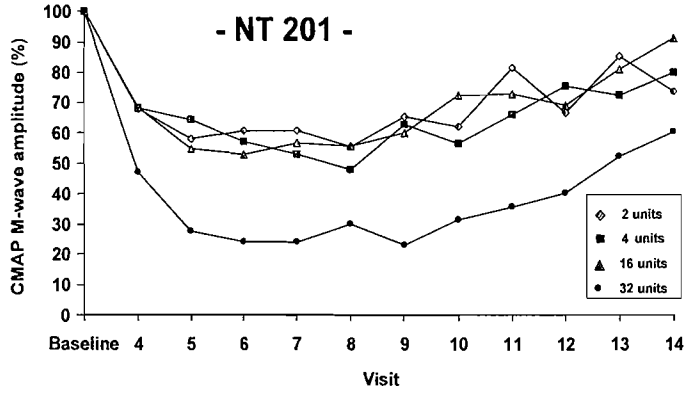

FIGURE 3. Mean values of EDB CMAP M-wave amplitude in percentage relative to baseline (baseline = 100%) during the study (visits 4 to 14) for NT 201 (ITT population, n = 26).

Secondary Efficacy Parameters

Overall, EDB CAmR (%) at visits 4 to 14 was considerably higher in the 32-unit dose group than the dose groups receiving 2, 4, and 16 units of either preparation (Figs. 3 and 4). Onset of effect was observed on day 1 (visit 4) in all dose groups and for both preparations. Mean time to maximum EDB CAmR (%) was 3.91 weeks for BTXCo and 4.97 weeks for NT 201.

Mean duration of effect was dose dependent and was shortest in the group receiving 2 units (Fig. 5). The effect lasted slightly longer with NT 201 than BTXCo in the groups receiving 2 units (NT 201: 22.6 ± 17.85 weeks vs BTXCo: 16.3 ± 17.84 weeks), 4 units (NT 201: 37.6 ± 21.13 weeks vs BTXCo: 21.7 ± 19.62 weeks), and 16 units (NT 201: 31.0 ± 23.45 weeks vs BTXCo: 25.8 ± 21.77 weeks). In each volunteer receiving the highest dose (32 units), the effect was still present at week 52 (Fig. 5).

Diffusion Into Adjacent Muscles

For all dose groups of NT 201 and BTXCo, the mean CMAP M-wave amplitude was more than 80% of the baseline value in ADQ and AH muscles at week 4 as well as at all postinjection visits (Figs. 6 and 7) up to the end of the study (week 52). Intraindividual comparisons between mean CMAP M-wave amplitudes obtained for NT 201 and BTXCo in each dose group did not show any statistically significant differences. These results were supported by the additional analysis that considered the mean value of ADQ and AH CAmR (%) of weeks 1 to 4. Thus, there was no noteworthy diffusion-induced reduction of muscle activity.

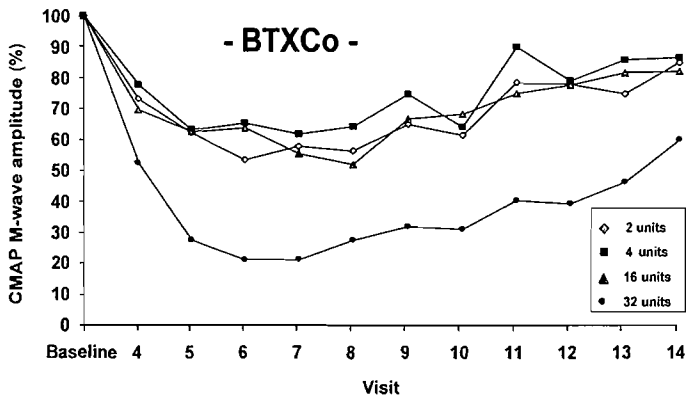

FIGURE 4. Mean values of EDB CMAP M-wave amplitude in percentage relative to baseline (baseline = 100%) during the study (visits 4 to 14) for BTXCo (ITT population, n = 26).

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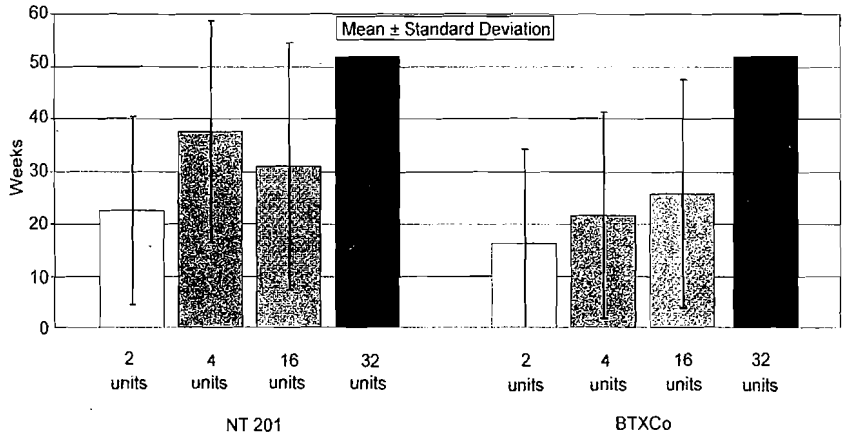

FIGURE 5. Duration of paretic effect of NT 201 and BTXCo in EDB muscle (ITT population, n = 26). The figure shows the duration (weeks) from injection of NT 201 or BTXCo to first return to more than 80% of baseline EDB CMAP M-wave amplitude. Data are mean ± SD.

Quality of Electroneurographic Measurements

The coefficients of variation of the CMAP M-wave amplitudes calculated from the baseline values were low in all muscles investigated (EDB, 17.32% ± 8.77%; AH, 12.39% ± 5.46%; ADQ, 13.48% ± 5.36%; means ± SD), indicating that the intraindividual variability was less than 20% on average and the electroneurographic measurements were highly reliable. This confirms the appropriateness of the definition of the paretic effect as a reduction of more than or equal to 20% from the baseline CMAP M-wave amplitude (reduction to ≤80%).

Clinical Assessment of Muscle Power (MRC Scale)

Clinical assessment of muscle power in the toes of both feet revealed normal muscle power (score = 5) in all subjects and at all assessment points.

Safety

Safety was assessed in the evaluable-for-safety population (N = 32). Laboratory

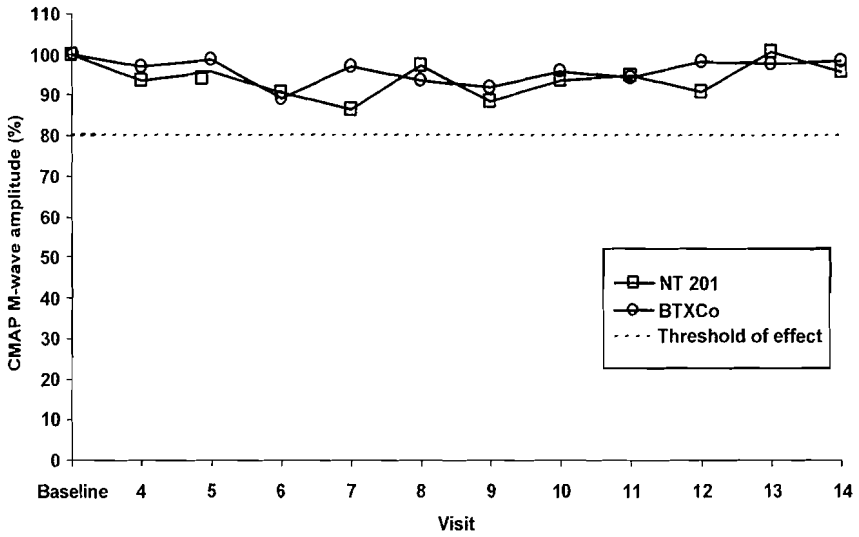

FIGURE 6. Mean values of ADQ muscle CMAP M-wave amplitude in percentage relative to baseline (baseline = 100%) during the study for NT 201 and BTXCo (ITT population, n = 26). The mean values of CMAP M-wave amplitudes in the ADQ muscles are above the predefined threshold of effect, indicating that there is no relevant diffusion-induced reduction of muscle activity in the adjacent muscle.

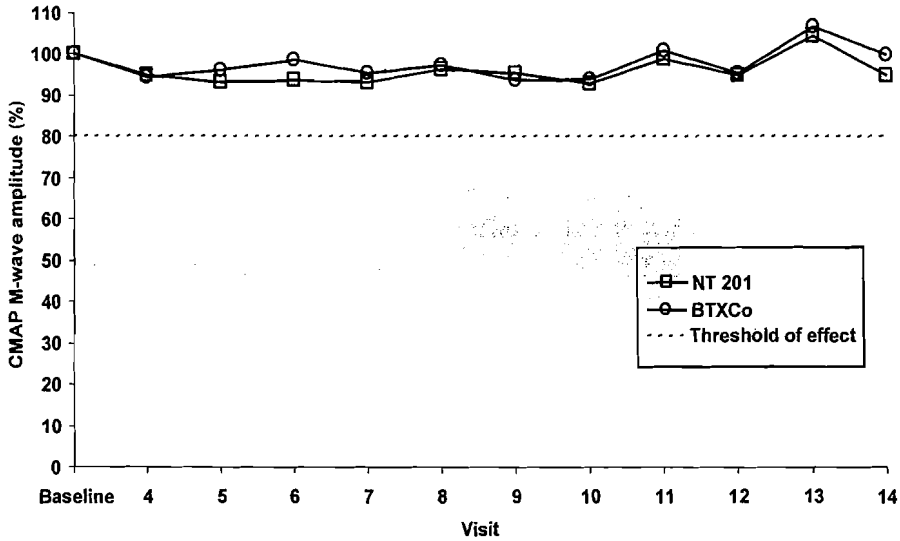

FIGURE 7. Mean values of AH muscle CMAP M-wave amplitude in percentage relative to baseline (baseline = 100%) during the study for NT 201 and BTXCo (ITT population, n = 26). The mean values of CMAP M-wave amplitudes in the AH muscles are above the predefined threshold of effect, indicating that there is no relevant diffusion-induced reduction of muscle activity in the adjacent muscle.

tests, vital signs, and physical/neurological examinations did not reveal any abnormal findings. Of 13 AEs reported by 8 volunteers, 2 events (paresthesia in a foot injected with NT 201 and weakness in a foot injected with BTXCo) were considered drug related. One serious AE (nucleus pulposus prolapse L5/S1, radiculopathy S1 left) occurred, but there was no relationship to the study treatment as judged by the investigator and medical safety officer of Merz Pharmaceuticals GmbH.

DISCUSSION

In this study, we assessed the safety and efficacy of NT 201 after injection into the EDB muscle of 1 foot, in comparison with BTXCo injected into the EDB muscle of the contralateral foot in healthy male volunteers. The use of equal doses of NT 201 and BTXCo was based on the findings in previous studies, where comparable results were achieved with similar doses of NT 201 and BTXCo.[5–7] In our study, the doses of NT 201 and BTXCo could not be blinded because of the varying injection volumes. However, this was not thought to influence the study results because the persons performing the electroneurographic measurements were blinded with respect to both the dose and study preparation.

The EDB model has been used in earlier studies to assess efficacy of botulinum neurotoxins, and it is therefore well established.[6,8–18] The paretic effect of the preparations was measured electroneurographically using surface electrodes. This method was preferred over needle electromyography because it causes less discomfort. The low coefficients of variation confirmed the validity of the electroneurographic measurements in our study.

The number of subjects per dose group was at least 6, which was considered sufficient for statistical evaluation.

Statistically significant paretic effects were observed in all dose groups of NT 201 and BTXCo. The paretic effect observed in the 4-unit dose group in the present study was similar to that described in a previous phase I trial involving 4 units of NT 201 and BTXCo.[6] A dose-response relationship was apparent when comparing the highest dose (32 units) and the lowest dose (2 units) but not when comparing lower doses (≤16 units) with each other. This finding was consistent for both NT 201 and BTXCo and was thought to be the result of the considerable intervolunteer variability of response.[15]

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Although a dose-dependent effect of BTX-A in the EDB model was described in some studies,[15,16] the dose-response relationship was less conclusive in other trials.[12,19]

In accordance with our results obtained at week 4, the reduction in the EDB CMAP amplitude was similar with 2, 4, and 16 units of both NT 201 and BTXCo at all visits.

BTXCo had a slightly shorter mean time to maximum EDB CAmR (%) than did NT 201. A similar time to peak decline in CMAP M-wave amplitude (3 weeks) was reported for BTXCo,[11] but other research groups reported a shorter time to maximum effect (approximately 1 week).[8–10,14,15] These discrepant data may be explained by the observation that the reduction in EDB CMAP M-wave amplitude does not vary to any extent between weeks 1 and 4 after an injection. Because only small changes in the CMAP M-wave amplitude occur during this period, the time point when the reduction reaches its maximum is of less clinical relevance than the time of onset. The effect observed in this trial occurred already 1 day after the injection, in accordance with findings from previous trials with BTX-A.[6,18]

Duration of the paretic effect of both NT 201 and BTXCo was dose dependent. The effect tended to be somewhat longer with NT 201 than BTXCo in lower-dose groups (≤16 units). As the effect was still present at the end of the study in all volunteers receiving the highest dose (32 units), the 52-week observation period was too short to accurately determine the duration of paretic effect in this dose group. Therefore, a comparison of duration of effect between NT 201 and BTXCo was not possible for the 32-unit dose group.

In a similar study involving a longer observation period, 22% of the original muscle paralysis was still present 57 weeks after BTXCo injection, with the degree of paralysis depending on the dose of neurotoxin.[16] In most other trials, the observation period ranged from 3 months to 250 days, and an effect was consistently reported to still be present at the end of the study.[6,8,10,11,14,19]

In our study, there were no substantial reductions of CMAP M-wave amplitudes in the ADQ or AH muscles indicative of a relevant diffusion-induced reduction of muscle activity in the adjacent muscles. Similar results regarding the diffusion of BTX-A into ADQ and AH muscles after injection into the EDB muscle were previously reported.[18]

Overall, NT 201 and BTXCo were well tolerated during the 52-week observation period, and no subject discontinued the study prematurely because of an AE. Among the 13 AEs reported, only 2 were considered treatment related. Local paresthesia after NT 201 injection was not unexpected because this is a known side effect of botulinum toxin.[20] The other treatment-related AE was weakness and early fatigue in the foot 11 days after BTXCo injection. This event was not unexpected because weakness is a known side effect of botulinum toxin that has also occurred in other studies using the EDB model.[9,15]

Although NT 201 and BTXCo had a marked paretic effect on the EDB muscle, there was no obvious loss of muscle power of the toes in clinical assessments (MRC scale). This may be due to the activity of the extensor digitorum longus muscle which seems to compensate for the loss of activity in the EDB muscle.

CONCLUSIONS

NT 201 was effective and well tolerated in healthy male subjects. As a highly purified BTX-A preparation free of complexing proteins, NT 201 is expected to be of therapeutic value in the treatment of involuntary muscle contraction.

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