



Characterization of diffusion and duration of action of a new botulinum toxin type A formulation

Hongran F. Stone, Zhao Zhu, Thai Q.D. Thach, Curtis L. Ruegg*

Revanche Therapeutics, Inc., 7555 Gateway Blvd., Newark, CA 94560, United States

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ABSTRACT

RT002, an injectable form of botulinum neurotoxin type A (BoNTA), comprised of a purified 150 kDa neurotoxin formulated in a novel formulation, is designed to limit the extent of diffusion and permit safe administration of longer acting doses. The aim of this study was to evaluate the degree of diffusion of RT002 in comparison to another commercially available BoNTA product, Botox® Cosmetic (OnabotulinumtoxinA, Allergan, Inc., Irvine, CA, USA), and establish the relative duration of effect for diffusion matched doses of the two BoNTA formulations using quantitative measurements in mice.

Measurement of muscle paralysis by muscle force generation (MFG) in mice at the injected gastrocnemius muscles indicated that RT002 and Botox are equipotent. Measurements of MFG inhibition in an adjacent muscle, the tibialis anterior muscle, indicated significantly less diffusion for RT002 as compared to Botox. When RT002 and Botox were dosed using the established diffusion matched doses, RT002 treatment resulted in an extended duration of drug effect as compared to Botox by 58–100% as assessed by either partial or complete recovery endpoints. Use of a daily voluntary running activity model provided confirmation that the two BoNTA formulations are equipotent by daily running distance and further confirmed the diffusion matched dose ratio assessed by degree of drug effect on body weight gain. Using these diffusion matched doses, RT002 treatment resulted in an extended duration of drug effect as compared to Botox (100–126% increase in duration) as assessed by either partial or complete recovery endpoints. Use of this model provides further evidence that the RT002 formulation limits diffusion with equipotency and thereby may permit safe administration of higher and more efficacious doses. In summary, data from two murine models suggest that RT002 may represent a next generation of BoNTA drug formulation offering superior degree and duration of effect at the intended target while controlling the unwanted diffusion and accompanying adverse effects of BoNTA at neighboring muscles and distal systemic targets.

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1. Introduction

Botulinum toxin type A (BoNTA) is a protein produced by the bacterium *Clostridium botulinum* which can block acetylcholine release at motor nerve terminals and induce

local muscular paralysis (Blasi et al., 1993; Turton et al., 2002). Since the first use of BoNTA on extraocular muscles in 1973 (Scott et al., 1973), it has been widely applied to relax muscles in treatment of muscle spasticity disorders (Jankovic, 1994; Brin, 1997; Koman et al., 2004; Cheng et al., 2006a,b). It has also been used clinically in aesthetics for cosmetic treatment of frown lines and wrinkles to improve facial appearance (Lowe, 2007; Flynn, 2007; Klein et al., 2008). However, the application of BoNTA in clinical use has been limited by side effects resulting

Abbreviations: RT002, a proprietary 150 kDa BoNTA formulation; BoNTA, botulinum toxin type A; MFG, muscle force generation.

* Corresponding author. Tel.: +510 742 3404; fax: +510 662 4817.

E-mail address: cruegg@revance.com (C.L. Ruegg).

from toxin diffusion and consequent adverse effects on unintended targets (FDA Gives Update on Botulinum Toxin Safety Warnings; Established Names of Drugs Changed, 2009). This in turn requires limiting the administered dose which leads to shorter duration and more frequent administration along with associated side effects due to multiple injections required to treat the target area. The diffusion to adjacent muscle revealed by animal studies (Aoki, 2001; Foster et al., 2006; Yaraskavitch et al., 2008) can cause side effects such as ptosis, heavy brow or a frozen face in facial aesthetics (de Almeida and De Boulle, 2007; Lowe, 2007). A new BoNTA formulation RT002 may address these issues by reducing diffusion and increasing duration so as to allow for less frequent retreatment and associated side effects of multiple needle injections into sensitive tissues.

2. Material and methods

2.1. Materials

2.1.1. Test articles

RT002, a proprietary 150 kDa BoTNA formulation, was produced by fermentation of the Hall strain of *Clostridium botulinum*. It was manufactured by Revance Therapeutics, Inc., Newark, CA, USA. RT002 (Lot #092-022-4) contained a nominal target of 170 U of BoNTA (tested at 158 U per vial) when using 0.9% saline for both reconstitution and serial dilution for its potency test to establish its unit equivalency with Botox. Botox was purchased from Allergan (Lot #C2310C3, Allergan, Inc., Irvine, CA, USA), and each vial contained a nominal target of 100 U BoNTA (as labeled on the vial, tested at 108 U per vial). The doses administered for these studies were based on the nominal values for RT002 and Botox potency.

2.1.2. Animals

The use of mice in this study was approved by the Institutional Animal Care and Use Committee (IACUC). All portions of the protocol were performed in accordance with the guidelines detailed in the *Guide for Care and Use of Laboratory Animals* published by the National Academy of Sciences. Mice were housed in a vivarium with a 12 h light/dark cycle and a controlled temperature. Food and water were provided *ad libitum*.

CD-1 female mice, ~20 g (Charles River Laboratories Inc, Hollister, CA), were used in muscle force generation (MFG) studies and BALB/cJ male mice at four weeks old (Charles River Laboratories, Hollister, CA) were used in the running wheel studies.

2.2. Methods

Muscle paralysis at the injected target gastrocnemius muscle and muscle paralysis caused by toxin diffusion in the adjacent tibialis anterior muscle, were measured by muscle force generation (MFG), under both single twitch and tetanus conditions. The duration of toxin effect at the injected muscle was also monitored by MFG at different time points after toxin treatment. A second model consisting of monitoring daily voluntary running activity was used

to provide additional functional information on the degree and duration of muscle paralysis after toxin injection.

2.2.1. Muscle force generation (MFG)

Animals were anesthetized with isoflurane (1.25%, mixed with oxygen), and the test articles, RT002 or Botox (5 μ L in 0.9% saline) were unilaterally injected into the right gastrocnemius muscle based on body weight. Each test group included 8–12 mice. MFG was measured at the indicated time intervals post-injection under isoflurane anesthesia (2–3% for induction, 1–2% for maintenance; in oxygen), as described previously (Stone et al., 2007). Briefly, the hind limbs were fixed in position using pins. Gastrocnemius muscle, tibialis anterior muscle and the sciatic nerve were bilaterally isolated from surrounding tissues. The preparations were coated with mineral oil to keep tissues moist. The tendons of gastrocnemius muscle and tibialis anterior muscle were transected and attached to a force transducer (FORT250, WPI, Sarasota, FL) with thread. The force transducer was connected to an EMG/EP Measuring System (MEB-9400, Nihon Kohden, Japan) through a trans-bridge amplifier (SYS-TBM4M, WPI, Sarasota, FL). The position of the force transducer was adjusted until slight tension was placed upon the muscle. A platinum bipolar hook electrode (FHC, Bowdoinham, ME) was placed to electrically stimulate the sciatic nerve. The EMG/EP Measuring System was used to generate stimulation pulses and record the MFG. All measurements were generated in a blinded manner, where the sequence of measurement (such left or right leg), and the dose group was randomized and blinded to the technician who was conducting the measurement.

2.2.1.1. Single twitch. The stimulation intensity was gradually increased from 0.1 to 2 mA or until the maximum amplitude of MFG of single twitch was obtained. Ten pulses (0.5 ms, 1 Hz) at each intensity level were used to obtain an average of MFG for further analyses. The same procedure was performed on both limbs in a random order. The contralateral limb served as an internal reference.

2.2.1.2. Tetanus. A ten pulse train (0.5 ms, 1 mA) was used to evoke the tetanus MFG. The stimulation frequency was gradually increased from 10 to 200 Hz or until complete fusion was attained. Ten pulse trains at each frequency were used to obtain an average of MFG for further analyses.

2.2.1.3. Local diffusion. The mouse MFG model has been established as a useful tool to accurately measure the paralytic effect of BoNTA on the treated muscle (Stone et al., 2007). Based on this model, we have developed an *in vivo* mouse model to evaluate the local diffusion of toxin by measuring the MFG of tibialis anterior muscle (positioned adjacent to the gastrocnemius muscle). The single twitch and tetanus MFG of tibialis anterior muscle were measured bilaterally and the difference in MFG between the two tibialis anterior muscles was used to calculate the degree of paralytic effect on neighboring muscles as a result of diffusion.

2.2.1.4. Time course. To compare the time course of recovery from muscle paralysis (duration of action), seven time points ($n = 10$ per group) were examined at indicated

time points from Week 1 through Week 15 following injection, RT002 at Weeks 1, 2, 3, 6, 8, 10, and 15, and Botox at Weeks 1, 2, 3, 6, 8, 10, and 12. The final time point for RT002 was extended to 15 weeks based on higher MFG inhibition measured at 6, 8 and 10 weeks.

2.2.2. Voluntary running activity model

Mini size Super Pet Run-A Rounds Exercise Wheels with a diameter of 4.5" (PetSmart, part #2753362) were fitted with digital cycling computers (Model BC906, Sigma Sports) and placed into 47 × 26 × 14.5 cm cages. The digital cycling computer measures maximum running speed, total distance ran, and total time ran. These data were recorded at daily intervals from the computers and logged for each cage, and the computers were reset. To condition animals prior to treatment, groups of eight animals were housed with free access to exercise wheels. At approximate three-day increments animals were divided into groups of four then two per cage (Keller, 2006). Paired animals with consistent running distance per cage were then randomized to seven different treatment groups with 4 cages per test group. The seven treatment groups were saline control, RT002 at 5 U/kg, 10 U/kg, and 20 U/kg, and Botox at 4 U/kg, 8 U/kg and 16 U/kg, respectively. As shown in Table 1, there was no significant difference between randomized groups in the average of six-day group running distance prior to the beginning of the treatment ($p = 0.8370$, one way ANOVA). The ratio between RT002 and Botox doses was held constant at the diffusion matched dose ratio of 2.5:1 as established in the MFG model. Test articles were administered via intramuscular injections of 5 μ L to the right gastrocnemius muscle using a Hamilton syringe with 32 gauge needle. Running activity was recorded for a total duration of 42 days after dosing. Body weight was measured twice per week except for the first week when measured weekly.

2.2.3. Data analysis

The maximum MFG of single twitch and tetanus recorded from each individual muscle was used for comparison. The paralytic effect is expressed as the percentage of MFG inhibition measured by MFG:

$$\% \text{ inhibition} = (MFG_{\text{control side}} - MFG_{\text{treated side}}) / MFG_{\text{control side}} \times 100\%$$

The data were reported as the mean \pm standard error of the mean (mean \pm SEM). Student's *t*-test was used for statistical analyses. For statistical significance, a confidence level of $p < 0.05$ was used. In the MFG recovery time course study, logarithmic curve fits were formatted using Microsoft Office Excel 2003 (Microsoft, Redmond, Washington, USA) to compare duration of action of the two test articles (Fig. 3). In the voluntary running activity study, Prism[®] 4

(Graphpad, San Diego, California, USA) was used to perform non-linear regression analyses. The analysis equation was:

$$Y = \text{Span}^*(1 - \exp(-K*X)) + \text{Bottom}.$$

The onset of recovery was set as the minimal running distance, and the data points prior to the onset of recovery were excluded from the regression analyses (Keller, 2006).

3. Results

3.1. Paralytic effect on target muscle (gastrocnemius muscle)

Two groups of animals (1 U/kg RT002 and 1 U/kg Botox, $n = 12$ per group) were examined at Week 1 post-injection (approximating timing of peak effect, data not shown). The single twitch and tetanus MFG reductions were $65.3 \pm 3.4\%$ and $39.7 \pm 5.3\%$ of control in 1 U/kg RT002 group, whereas the reductions were $71.6 \pm 3.8\%$ and $47.1 \pm 6.4\%$ of control in 1 U/kg Botox group (Fig. 1a and b). There was no significant difference between RT002 and Botox groups in both single twitch ($p = 0.2283$) and tetanus ($p = 0.3782$) measurements.

3.2. Diffusion measured by MFG inhibition of the adjacent muscle (tibialis anterior muscle)

Local diffusion was assessed after treatment of the gastrocnemius muscle with BoNTA by measuring the degree of single twitch MFG inhibition of the tibialis anterior muscle. Different doses of RT002 and Botox were used to examine the dose level of local diffusion and to compare their potencies. The lowest doses resulting in significant MFG inhibition of tibialis anterior muscle as compared to the contralateral control muscle were 5 U/kg for RT002 ($12.7 \pm 3.4\%$ inhibition, $p = 0.0036$) and 2 U/kg for Botox ($17.4 \pm 5.4\%$ inhibition, $p = 0.0066$), respectively (Fig. 2a). These doses were defined as diffusion matched dose for RT002 (5 U/kg) and Botox (2 U/kg). The degree of inhibition of single twitch MFG for the tibialis anterior muscle measured for RT002 (12.7%) vs. Botox (17.4%), was not significant ($p = 0.4780$). At lower doses, RT002 and Botox did not significantly alter single twitch MFG of tibialis anterior muscle (data not shown). The matched diffusion dose ratio of RT002 to Botox was 2.5:1. When both preparations were dosed at 5 U/kg, the single twitch MFG reductions of tibialis anterior muscle were $12.7 \pm 3.4\%$ (RT002) and $31.5 \pm 4.9\%$ (Botox), respectively (Fig. 2b). Thus diffusion of Botox was significantly greater than RT002 at equivalent dose ($p = 0.0067$).

3.3. Time course of MFG recovery after toxin treatment

As shown previously in Section 3.2, the single twitch MFG inhibition of the tibialis anterior muscle was not

Table 1
Comparison of Average Running Distance Prior to Drug Treatment.

| | Saline Control | RT002 | | | Botox | | |
|---------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | | 5 U/kg | 10 U/kg | 20 U/kg | 4 U/kg | 8 U/kg | 16 U/kg |
| Average (km) +/- SE | 7.52 \pm 0.89 | 7.11 \pm 0.63 | 6.81 \pm 0.49 | 6.95 \pm 0.58 | 6.97 \pm 0.47 | 7.12 \pm 0.76 | 7.33 \pm 0.61 |

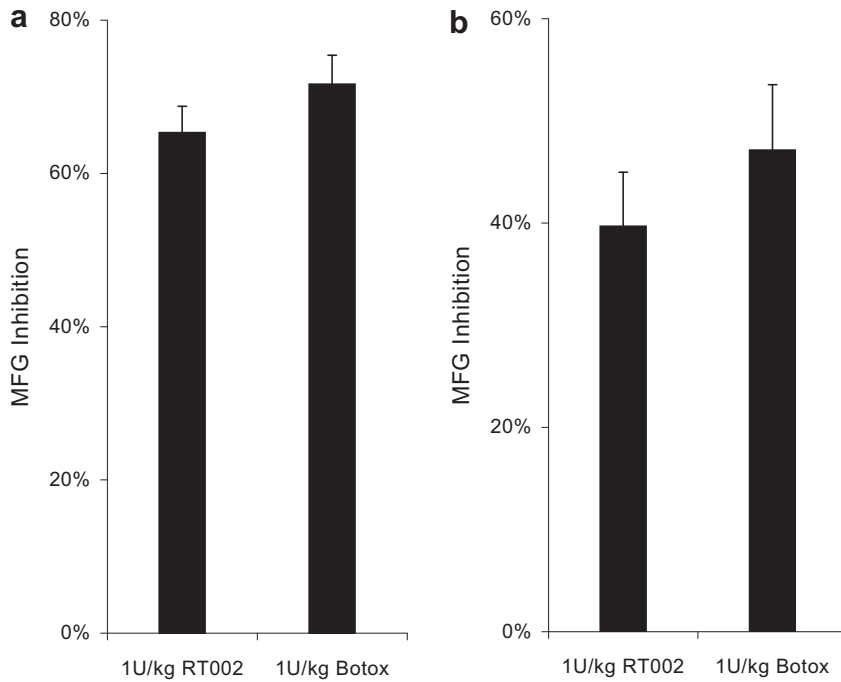


Fig. 1. Paralytic effect of BoNTA on gastrocnemius muscle. (a) Single twitch. (b) Tetanus. The y axis shows the degree of MFG inhibition at one week post dose for gastrocnemius muscle following treatment of 1 U/kg RT002 or Botox®. Error bars display SEM.

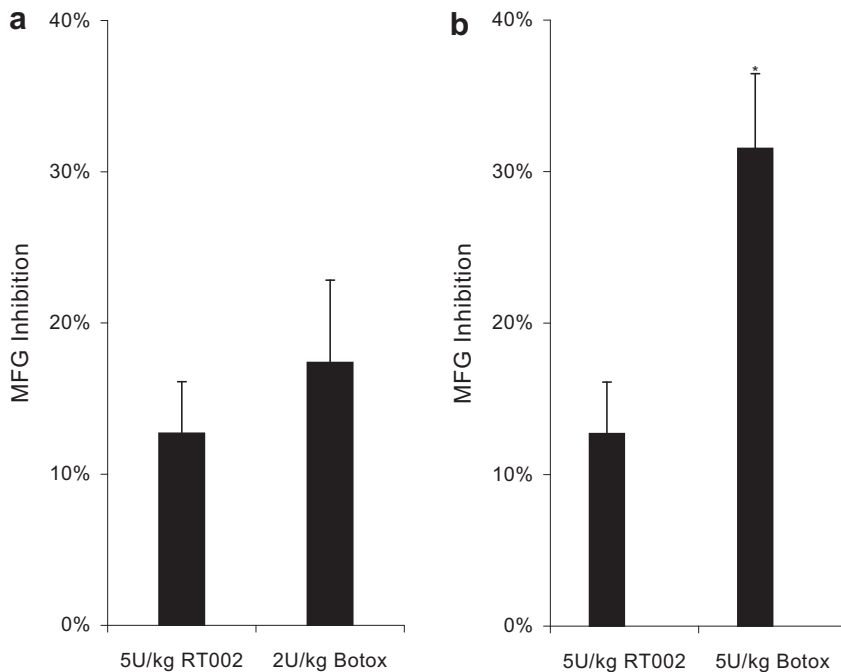


Fig. 2. BoNTA diffusion measured by MFG inhibition at tibialis anterior muscle (a): The threshold doses of local diffusion for single twitch were 5 U/kg and 2 U/kg in RT002 and Botox groups, respectively. (b): At the same dose of 5 U/kg, the paralytic effect diffusing to tibialis was significantly higher in the Botox treated group than in RT002 treated group. *: $p < 0.05$, significant difference when compared to RT002 treatment; error bars display SEM.

significantly different in mice treated with 5 U/kg RT002 and 2 U/kg Botox. This result suggested that the extent of local diffusion and the risk of paralysis for non-injected adjacent muscles were equivalent for these two treatments. These two treatments (5 U/kg RT002 and 2 U/kg Botox) were used to compare the time course of MFG inhibition and duration in the gastrocnemius muscle. A total of seven time points spanning 15 weeks post dose were collected from each group in this study. Logarithmic curve fits were made to the data to evaluate duration

of action. The MFG inhibition in gastrocnemius muscle was greater in the RT002 group than in the Botox group at each time point, and the duration to any fixed level of partial or complete recovery was longer for the RT002 group than for the Botox group. The durations to full recovery (inhibition returns to 0% level) of single twitch MFG for the RT002 group and the Botox group were 31.6 and 15.8 weeks, respectively. The duration of RT002 effect was two-fold longer in RT002 group than in Botox group (Fig. 3a).

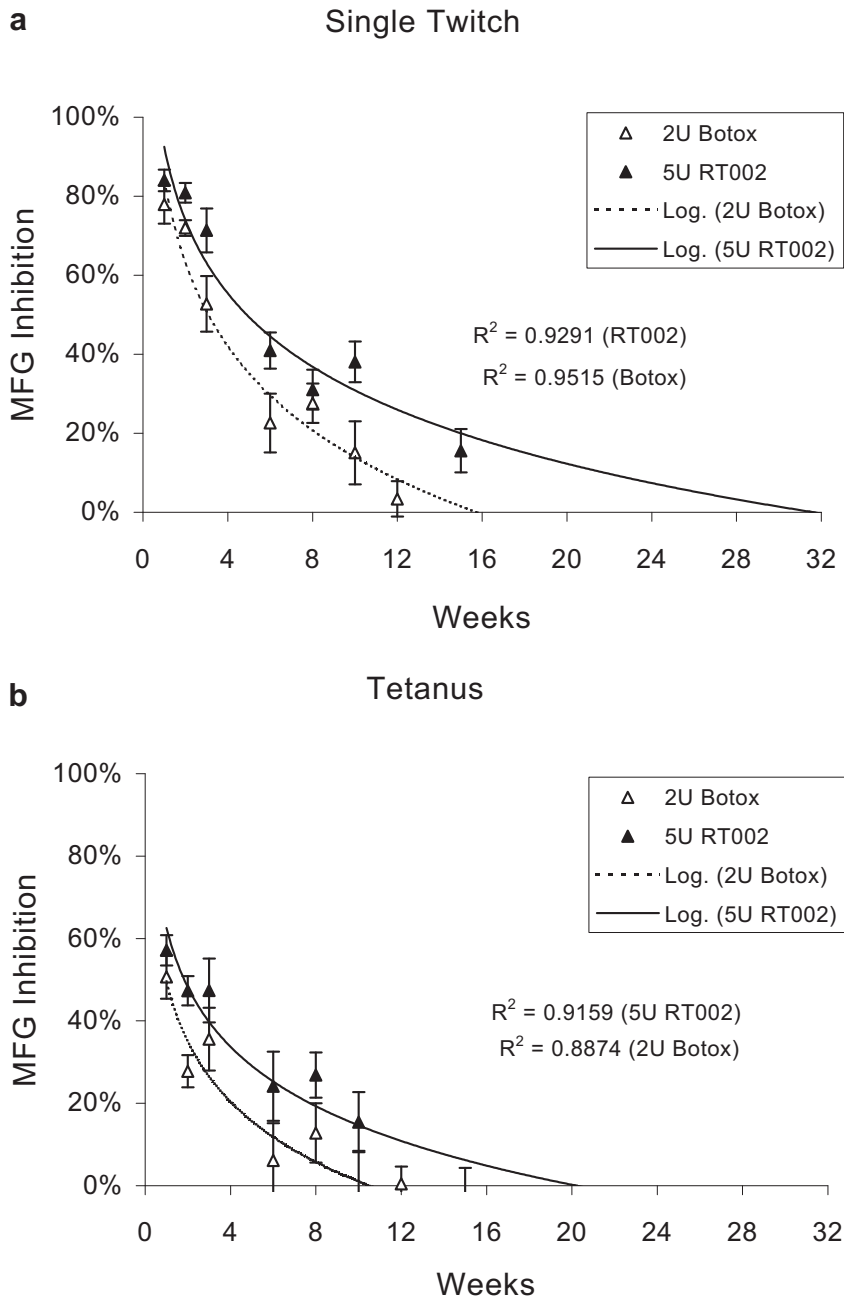


Fig. 3. Time course of MFG recovery after BoNTA treatment. Both single twitch (a) and tetanus (b) MFG of gastrocnemius muscle were tested from week 1 through week 15. Logarithmic curve fits were performed to evaluate the duration of action. R-squared values of curve fit are shown for the corresponding curves.

Table 2
MFG recovery time (Weeks) - ^aSingle Twitch/^bTetanus.

| % Recovery | RT002 | Botox | % Increase duration for RT002 |
|------------|-----------|-----------|-------------------------------|
| 50% | 4.9/1.8 | 3.1/1.0 | 58%/80% |
| 75% | 12.4/6.1 | 7.0/3.2 | 77%/91% |
| 100% | 31.6/20.2 | 15.8/10.5 | 100%/92% |

^a Regression equations used for RT002 ($y = -0.2679\ln(x) + 0.9255$) and Botox ($y = -0.3065\ln(x) + 0.845$).

^b Regression equations used for RT002 ($y = -0.2081\ln(x) + 0.6258$) and Botox ($y = -0.2107\ln(x) + 0.4957$).

Consistent with data shown in Section 3.1, the MFG inhibition was less pronounced for tetanus (Fig. 3b) as compared to single twitch (Fig. 3a) in both RT002 and Botox groups. The duration of effect was shorter in tetanus than in single twitch as well however the relative degree of longer duration of effect of RT002 was also observed for tetanus as for single twitch. The durations of full recovery of tetanus MFG in the RT002 group and the Botox group were 20.2 and 10.5 weeks, respectively (Fig. 3b). The duration of RT002 effect was also approximately two-fold longer as compared to the Botox group for tetanus MFG.

The durations for 50%, 75% and 100% recovery based on the non-linear analyses regression in Fig. 3 are listed in Table 2.

Table 3
Maximum reduction in running distance (%).

| | RT002 (10 U/kg) | Botox (8 U/kg) | RT002 (20 U/kg) | Botox (16 U/kg) |
|-------------|-----------------|-----------------|-----------------|-----------------|
| % Reduction | 52.2% ± 5.8% | 52.9% ± 6.1% | 91.7% ± 2.0% | 95.3% ± 1.0% |

3.4. Voluntary running activity

Voluntary running activity (Allen et al., 2001 and Turner et al., 2005) was used as an additional functional endpoint to study the degree and duration of muscle paralysis after toxin injection (Keller, 2006). In this study, RT002 and Botox were injected at 2.5:1 diffusion matched ratio as established in the MFG model and the data for running activity and body weight are presented in paired dose groups. As reported previously (Bambrick and Gordon, 1989; Dodd et al., 2005; and Luvisetto et al., 2003), when animals are treated with toxin, the drug effect can range from local muscle paralysis, muscle mass loss, to more severe systemic toxicity such as body weight loss in a dose-dependent manner. In this study, in addition to the daily running distance, the body weight was also monitored as an indication of the degree of systemic toxicity caused by

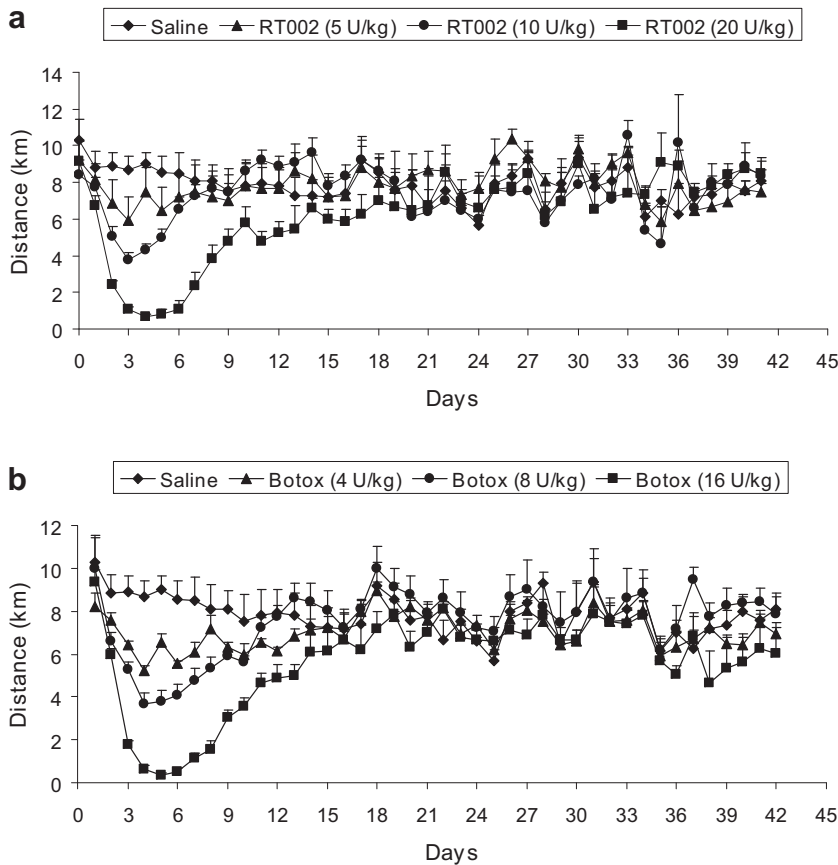


Fig. 4. Dose-Response of BoNTA formulations by voluntary running distance, (a) RT002: 5 U/kg (▲), 10 U/kg (●), and 20 U/kg (■), together with saline control (◆). Each symbol represents the mean of four cages. (b) Botox: 4 U/kg (▲), 8 U/kg (●), and 16 U/kg (■), together with saline control (◆). Each symbol represents the mean of four cages with SEM shown by error bars.

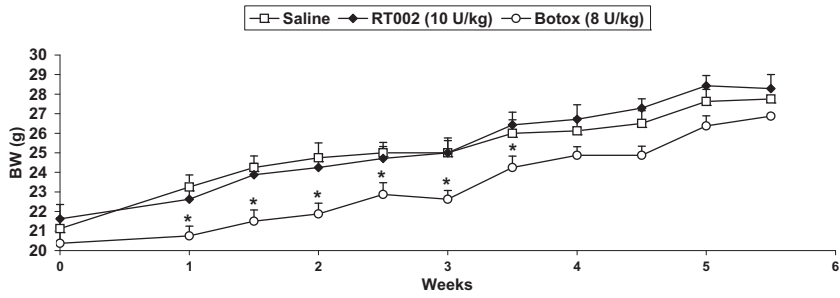


Fig. 5. Effect of BoNTA treatment on mean body weight change. Animals were injected with doses of: RT002, 10 U/kg (◆) vs. Botox, 8 U/kg (○), or saline control (□). Each symbol represents the average of eight animals with SEM shown by error bars. *: $p < 0.05$, significant difference when compared to control or RT002 treatment group.

toxin diffusion. As shown in Fig. 4, the running distances remained generally constant and were not affected by injection of saline. When mice were injected with RT002 (Fig. 4a) or Botox (Fig. 4b), the reduction in running distance was proportional to the amount of BoNTA injected; the peak reduction in running distance occurred at

approximately 3 days after BoNTA treatment and the subsequent rate of recovery over the ensuing days or weeks was a function of BoNTA doses both for RT002 and Botox. As summarized in Table 3, there is no significant difference in the maximum percentage reduction in running distance when BoNTAs, RT002 and Botox, were injected at similar

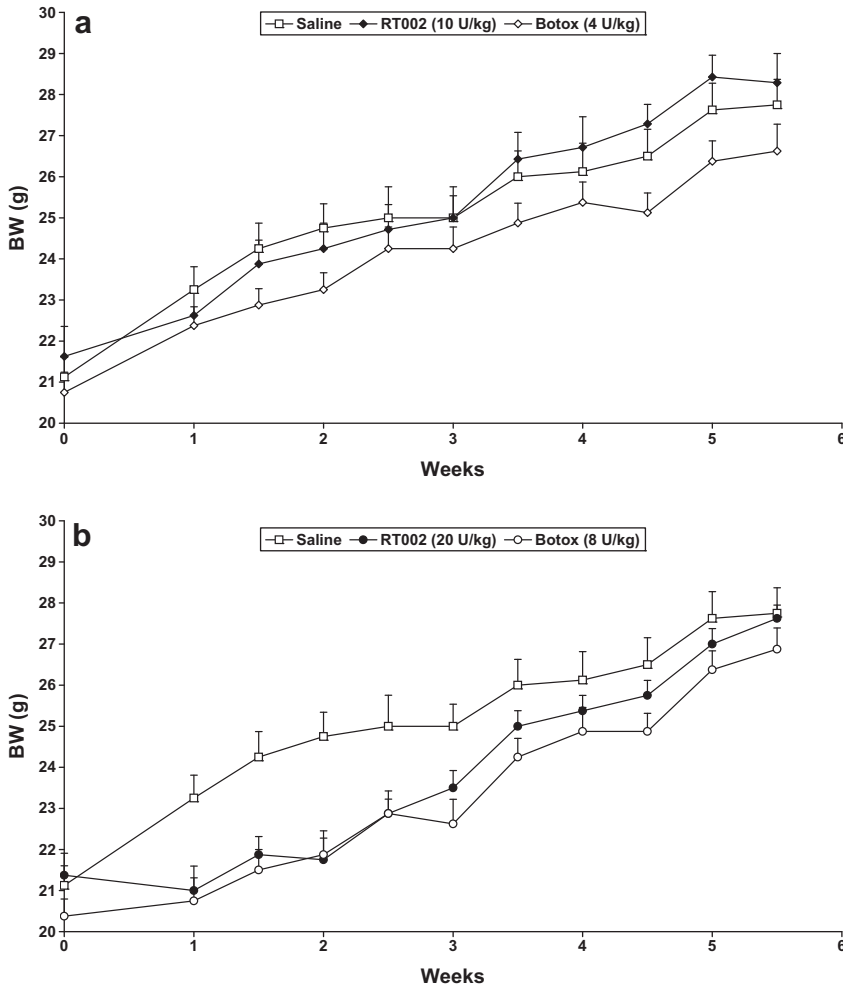


Fig. 6. Dose-response of BoNTA formulations by body weight. (a) Animals were injected with doses of: RT002, 10 U/kg (◆) vs. Botox, 4 U/kg (◇), or saline control (□). Each symbol represents the average of eight animals with SEM shown by error bars. (b) Animals were injected with doses of: RT002, 20 U/kg (●) vs. Botox, 8 U/kg (○), or saline control (□). Each symbol represents the average of eight animals with SEM shown by error bars.

concentrations (10 U/kg RT002 vs. 8 U/kg Botox, $p = 0.9368$, and 20 U/kg RT002 vs. 16 U/kg Botox, $p = 0.4086$), indicating the two BoNTA formulations were dosed equivalently. As shown in Fig. 5, at the beginning of the study, there was no significant difference in body weight among the three dose groups (one way ANOVA, $p = 0.7562$). The group treated with BoNTA complex exhibited significantly reduced body weight compared to control animals from week 1–3.5 weeks. RT002 treated animals continued to gain weight during the course of the study and at no time demonstrated a significant difference in body weight compared to the control group (Fig. 5). These results indicate that at a similar dose level, RT002 has less diffusion leading to less systemic toxicity than BoNTA complex measured by body weight gain.

During the period of this study, control animals continued to gain body weight throughout the study. For the treatment groups, body weight decrease or reduction in body weight gain occurred in a dose-dependant manner in the RT002 and Botox treated groups. A similar degree of body weight loss/reduction in body weight gain was observed when animals were treated with 10 U/kg RT002 and 4 U/kg Botox (Fig. 6a, $p > 0.05$ for Botox versus saline control or RT002 versus saline control during the course of the study), as well as 20 U/kg RT002 and 8 U/kg Botox (Fig. 6b, $p < 0.05$ for Botox versus saline control or RT002 versus saline control), indicating that these doses were matched in terms of degree of diffusion and resulted in equivalent systemic toxicity. These data provide further evidence that the RT002 and Botox formulation are equipotent but the RT002 formulation limits diffusion and thereby may permit safe administration of higher and more efficacious doses. When these diffusion matched doses were used to compare the degree and duration of therapeutic effect as measured by running distance, RT002 exhibited a greater peak effect and longer duration of effect

with the mice taking approximately twice as long to return to baseline running distance as compared to Botox (Fig. 7). The durations for 50%, 75% and 100% recovery to baseline running distance based on the non-linear regression analyses in Fig. 6 are listed in Table 4.

4. Discussion

The quantitative effects of two BoNTA formulations on MFG and voluntary running activity were evaluated from three perspectives: 1) potency, 2) degree of side effects measured by diffusion to neighboring muscle or effects on body weight, and 3) duration of action. The results demonstrate that the two BoNTA formulations, RT002 and Botox are equipotent at the target muscle. The differentiating factor between these two toxin formulations is that RT002 exhibited less diffusion and could be dosed to a higher level and with consequently greater degree and duration of effect compared to another BoNTA preparation, Botox.

An important and often dose-limiting side effect of BoNTA treatment is paralytic effect outside of the target muscle due to local diffusion, which could cause ptosis, heavy brow or a frozen face in facial aesthetics (de Almeida and De Boule, 2007; Lowe, 2007), as well as other serious adverse effects for other therapeutic indications. To quantitatively assess and compare diffusion of BoNTA formulations, Revance developed two in vivo mouse models, wherein BoNTA is injected directly into the gastrocnemius muscle and the degree of diffusion is examined by the MFG change in a non-injected adjacent muscle, such as the tibialis anterior muscle, or by the overall system toxicity measured by changes in body weight. Results from these models indicate that RT002 exhibits less diffusion than Botox thus enabling administration of higher therapeutic doses while maintaining equivalent safety. Interestingly, a recent study published by Carli (Carli et al., 2009)

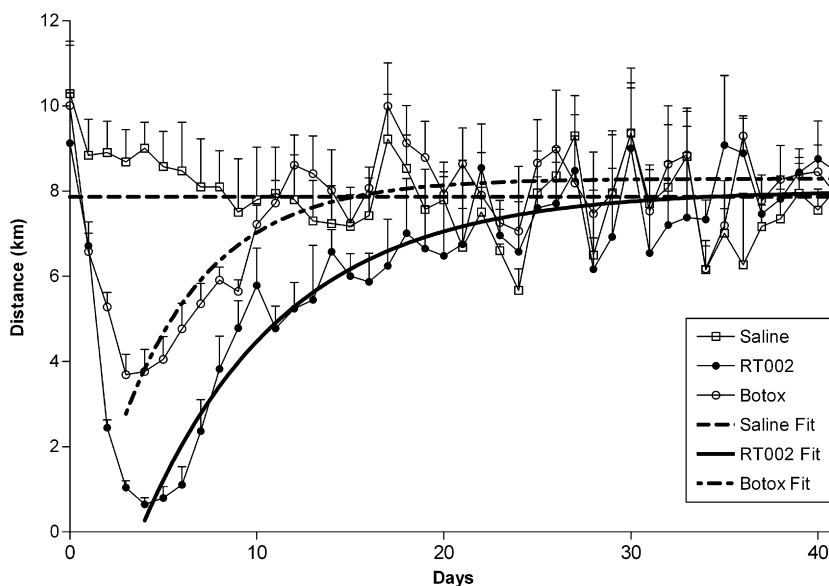


Fig. 7. Effect of BoNTA on voluntary running distance. RT002, 20 U/kg (●) vs. Botox, 8 U/kg (○), or saline control (□). The dotted line represents the average daily running distance of saline injected animals. The solid line and broken line are non-linear regression fits for RT002 and Botox injected animals, respectively. The equation used for regression was $Y = \text{Span} \cdot (1 - \exp(-K \cdot X)) + \text{Bottom}$. Each symbol represents the mean of four cages, 2 animals per cage.

Table 4
Voluntary running distance recovery time (days).

| % Recovery | RT002 | Botox | % Increase duration for RT002 |
|------------------|-------|-------|-------------------------------|
| 50% of baseline | 8.9 | 4.1 | 117% |
| 75% of baseline | 14.0 | 7.0 | 100% |
| 100% of baseline | 34.3 | 15.2 | 126% |

indicated that there was no significant difference between Botox (Allergan), Dysport (Ipsen), and Xeomin (Merz Pharmaceuticals) with respect to diffusion into adjacent muscles in the mouse leg using a novel and highly sensitive test based on neural cell adhesion molecule (N-CAM) expression in muscle. These three BoNTA products differ in molecular weight where Botox and Dysport are 900 kDa BoNTA complex, and Xeomin is 150 kDa BoNTA. However, it has been documented that the complex is rapidly disrupted at physiologic pH thereby liberating the 150 kDa BoNTA quickly once injected. The fact that there is no difference observed for diffusion for these products is consistent with that effect. We hypothesize that in RT002 formulation, BoNTA and the novel polycationic excipient interact with one another and possibly with local tissue polyanions which in turn limits the diffusion of the BoNTA component of RT002 to adjacent tissues.

When dose titration to a diffusion threshold was performed and these diffusion matched doses were used to compare RT002 to Botox, it was shown that inhibition of MFG for target muscle lasted approximately 58–100% longer for RT002 as compared to Botox. Using voluntary running activity as a second model and comparing doses of RT002 and Botox in proportion to their relative diffusion matched ratio, it was found that the duration of therapeutic effect for RT002 lasted 100–126% longer than Botox while causing an equivalent or lesser degree of systemic adverse effects as measured by body weight changes. The reduced diffusion observed for RT002 would lead one to expect that it should display a safer profile as compared to Botox and therefore may permit the administration of higher relative doses of RT002 resulting in increased efficacy and improved duration of effect while maintaining desired safety.

5. Conclusion

RT002 exhibited equipotency with less diffusion to non target muscle per given dose as compared to Botox in two murine models. When doses of RT002 and Botox matched for equivalent diffusion as measured by MFG and comparable systemic exposure as measured by body weight were administered, RT002 produced a greater maximal degree of effect and prolonged drug effect as assessed by MFG and voluntary running distance models.

Ethical statement

The use of mice in this study was approved by the Institutional Animal Care and Use Committee (IACUC). All portions of the protocol were performed in accordance with the guidelines detailed in the *Guide for Care and Use of Laboratory Animals* published by the National Academy of Sciences.

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Conflict of interest

All authors are employees of Revance Therapeutics, Inc. and may receive stock as part of their compensation.

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