

Therapeutic efficacy and safety of various botulinum toxin A doses and concentrations in spastic foot after stroke: a randomized controlled trial

Jiang Li¹, Ru Zhang¹, Bo-li Cui¹, Yong-xiang Zhang¹, Guang-tao Bai¹, Si-shan Gao², Wen-jian Li^{1,*}

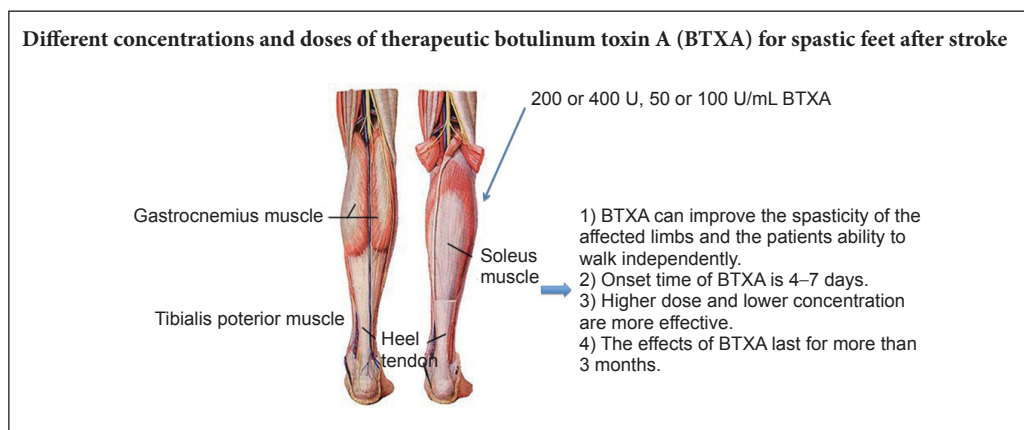
¹ Affiliated Hospital of Qingdao University, Qingdao, Shandong Province, China

² Department of Neurology, Traditional Medicine Hospital of Huangdao District, Qingdao, Shandong Province, China

How to cite this article: Li J, Zhang R, Cui BL, Zhang YX, Bai GT, Gao SS, Li WJ (2017) Therapeutic efficacy and safety of various botulinum toxin A doses and concentrations in spastic foot after stroke: a randomized controlled trial. *Neural Regen Res* 12(9):1451-1457.

Funding: This study was supported by a grant from the Shandong Science and Technology Development Plan Project in China, No. 2012YD18031.

Graphical Abstract



*Correspondence to:
Wen-jian Li, M.D.,
lwj917@sina.com.

orcid:
0000-0002-7731-4812
(Wen-jian Li)

doi: 10.4103/1673-5374.215257

Accepted: 2017-07-03

Abstract

No recommended guidelines currently exist for the therapeutic concentration or dose of botulinum toxin type A (BTXA) injected into the muscle to treat limb spasticity. Therefore, in this randomized controlled trial, we explored the safety and efficacy of two concentrations and two doses of BTXA in the treatment of spastic foot after stroke to optimize this treatment in these patients. Eligible patients ($n = 104$) were randomized into four groups. The triceps surae and tibialis posterior on the affected side were injected with BTXA at one of two doses (200 U or 400 U) and two concentrations (50 U/mL or 100 U/mL). The following assessments were conducted before as well as 4 days and 1, 2, 4, and 12 weeks after treatment: spasticity, assessed using the modified Ashworth scale; basic functional mobility, assessed using a timed up and go test; pace, assessed using a 10-meter timed walking test; and the ability to walk, assessed using Holden's graded scale and a visual analog scale. The reported results are based on the 89 patients that completed the study. We found significant differences for the two doses and concentrations of BTXA to improve the ability of patients to walk independently, with the high-dose/low-concentration combination providing the best effect. Onset and duration of the ameliorating effects of BTXA were 4-7 days and 12 weeks, respectively. Thus, BTXA effectively treated foot spasms after stroke at an optimal dose of 400 U and concentration of 50 U/mL.

Key Words: nerve regeneration; stroke; foot spasms; botulinum toxin type A; foot varus; foot drop; walking function; neural regeneration

Introduction

Stroke is currently one of the top three causes of death in China, with high morbidity, mortality, and disability, rates that markedly reduce the quality of life. Unfortunately, few effective treatments exist for stroke and its sequelae. Early thrombolysis is an evidence-based clinically effective therapy for ischemic stroke and rehabilitation, whereas rehabilitation can be more a "better late than never" approach. After experiencing a stroke, approximately 75% of patients will have a variety of dysfunctions, and spasticity is an important cause of some of these dysfunctions (Lundstrom et

al., 2010).

Limb spasticity after stroke is part of upper motor neuron syndrome, and it detrimentally affects movement, leading to a deteriorated ability to care for oneself (Kaňovský et al., 2011; Intiso et al., 2014). Spastic foot is common after stroke, affecting the patients' ability to walk as well as their speed, limb position, and body stability, and is an active topic in stroke rehabilitation research (Esquenazi et al., 2009). Although a few treatments exist for spastic foot drop and varus, their outcomes differ across studies (Kaji et al., 2010; Pradon et al., 2011).

Therapeutic botulinum toxin A (BTXA) administration has met with success in treating spasticity after stroke. However, recommended doses and muscles selected for the BTXA injection still need to be standardized (Santamato et al., 2013b). Moreover, no guidelines offer a unified standard for the concentration or dose of BTXA, which vary widely across reports. We have treated limb spasticity in patients after stroke with BTXA for more than 10 years, using doses from 100 U to 600 U and concentrations of 40–100 U/mL with variable outcomes. Therefore, in the present study, we determined the therapeutic effect of different concentrations and doses of BTXA on spastic foot in patients after stroke.

Subjects and Methods

Subjects

This study was a randomized controlled trial. All enrolled patients were admitted to the Department of Rehabilitation Medicine at our hospital from August 2013 to August 2016 and randomized into four groups using a random number table based on the consecutive order of admission. The main inclusion criterion was a diagnosis of stroke according to the revised Diagnostic Criteria of the Fourth National Cerebrovascular Disease Conference (The Fourth National Cerebrovascular Disease Conference and Chinese Medical Association, 1996), and included individuals having cerebral infarction or cerebral hemorrhage with supratentorial lesions, and hemiplegia, verified by computed tomography scans or magnetic resonance imaging. Additional inclusion criteria included treatment for at least 1 month, the presence of spastic strephenopodia and foot drop on the affected side, the ability to walk with or without assistance, a score of $\geq 1^+$ on the modified Ashworth scale (Ashworth, 1964), ineffective or not very effective treatment with oral antispasmodics, no severe cardiovascular disease, and no history of allergy to BTXA. Importantly, the patients and their families also had to agree to the injection method and give informed consent. Patients meeting any of the following criteria were excluded: pregnancy, history of serious allergic reactions, the patient or their families declined to participate, severe cardiovascular disease, unstable hypertension, local infection at the planned injection site, or more than mild cognitive impairment, or mental disorder.

The population size required to conduct the study was calculated using the application “Sample size calculator for clinical research” (updated version of V1.1 <https://itunes.apple.com/app/id438080153>). The study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University of China (approval No. 20130617) and was performed according to the 1964 *Declaration of Helsinki*. All patients gave informed consent prior to taking part in any procedure in this study.

The patients were randomized into the following four groups based on the BTXA (trade name HengLi; Lanzhou Institute of Biological Products, Lanzhou, China) dose and concentration (Chinese Association of Rehabilitation Med-

icine, 2015) by using a random number table: low-dose/low-concentration group ($n = 21$), low-dose/high-concentration group ($n = 22$), high-dose/low-concentration group ($n = 24$), and high-dose/high-concentration group ($n = 22$). The low dose was 200 U, and the high dose was 400 U. The low concentration was 50 U/mL and the high concentration was 100 U/mL.

Intervention

For foot drop, the gastrocnemius and soleus were chosen as the injection sites, and for strephenopodia, the tibialis posterior.

The dosages and injection points for BTXA were as follows: (1) gastrocnemius, 100–150 U, 6–8 injection points; (2) soleus 50–150 U, 4–6 points; and (3) tibialis posterior, 50–100 U, 2–3 points. Each injection point was given the BTXA diluent of ≤ 0.5 mL, thus the BTXA dose was ≤ 50 U.

A low-frequency electric diagnostic instrument (Enraf-Nonius ENA-1550, Enraf-Nonius, Rotterdam, The Netherlands) was used to find the best injection site, that is, the site at which a low stimulation led to a maximum muscle contraction. The stimulation output was a single square wave (wave width, 100–300 nm; frequency, 300 ms; intensity, 0.2–0.8 mA). A plexus block needle (Stimuplex-A, Tianrui Company, Lianyungang, China) 50 mm in length was used.

To determine the best BTXA injection site, a surface electrode with a stimulation electric current intensity of 10–20 mA was used to select the position on skin that had the biggest response to the smallest stimulation and that site was marked. A Stimuplex A needle was used to penetrate the muscle through this marked site. The depth was changed to determine the best injection site, which was a site having visible muscle contraction (such as a range of motion that varied by at least 5°) when stimulated with minimal current intensity (such as approximately 0.1 mA), the so-called motor point (Van Campenhout and Molenaers, 2011). For deep muscles, the diagnostic instrument and block needle were used directly with a low simulating current to determine the point at which a minimal stimulus intensity caused the largest muscle contraction.

Assessments

The parameters measured included classification on the modified Ashworth scale (MAS) to assess spasticity (Blackburn et al., 2002; Nakhostin Ansari et al., 2012), the 6-meter timed up and go (TUG) test of basic functional mobility (Podsiadlo and Richardson, 1991), a 10-meter timed walking test, and the ability to walk as scored on Holden’s graded scale (Holden et al., 1984). The scores on the MAS were as follows: grade 0, no increase in muscle tone; grade I: able to perform a passive flexion or extension, a slight increase in muscle tone, and a sense of attachment at the end of the movement; grade I^+ : a slight increase in muscle tone, after 50% of range of motion, a slight sense of congestion, and accompanied by a certain resistance; grade II: muscle tension

increases significantly during most of the range of motion, but the affected part is still easy to move; grade III: severely increased muscle tone, passive activity is difficult; grade IV: the flexion and extension of the joint are limited and stiff. Scoring method: grade 0, score 0; grade I, score 1; grade I⁺, score 1.5; grade II, score 2; grade III, score 3; grade IV, score 4. For 6-minute TUG task, the overall time was recorded for the patient to stand from a seated position, move forward in a straight line for 3 meters, return to the same seat, and sit down. Data were assessed twice, with a 1-minute interval, and the average value was calculated.

For the 10-meter timed walking assessment, a 14-meter long, flat surface on the ground was selected and marks were placed at 2 and 12 meters. The patient walked across the floor, and the time for the patient to traverse the middle 10 meters was recorded. The average value of two measurements obtained with an interval between the trials of 1 minute was recorded.

Holden's scale was scored using the following 5 levels: Level 0 (no function), cannot walk or needs assistance from more than two persons; level 1 (requires continuous assistance), needs one person's continuous assistance to walk; level 2 (requires a little assistance), able to walk but has poor balance, is unsafe, and needs 1 person in the vicinity to apply either continuous or intermittent contact with the body to help, or needs to use assistive devices to ensure safety; level 3 (some care or verbal guidance): able to walk, but not normally or not safely and needs one person to monitor or guide by verbal instruction but not by contact with the body. Level 4 (independence on the ground), can walk on flat ground, but walking on a slope, uneven ground, or stairs is still difficult and thus needs someone to assist or offer some care. Level 5 (complete independence), able to walk anywhere safely.

These parameters were evaluated before (baseline) and 1, 2, 4, and 12 weeks after BTXA administration by the same clinician and therapist, who were well trained and blinded to patient information. All patients were evaluated under similar conditions.

Statistical analysis

The SPSS 16.0 software (SPSS, Chicago, IL, USA) was used for statistical analysis. Normally distributed data were presented as the mean \pm SD. All measurements were analyzed with one-way analysis of variance followed by the least significant difference test, or chi-square test followed by Kruskal-Wallis test. Count data are shown as frequencies or percentages and were compared using the chi-square test. The effect of time was examined using linear-by-linear associations. For all tests, a value of $P < 0.05$ was considered statistically significant.

Results

Participants

Eligible patients ($n = 104$) diagnosed as having spastic foot and prolapsed foot were enrolled in this

study. In total, 89 patients completed the study and were recorded. Of these, 65 were men and 24 were women; and the overall age range was 35 to 79 years (mean \pm SD, 58.75 \pm 17.87 years). Among them, 63 patients had cerebral infarction (46 men, 17 women; mean age \pm SD, 57.66 \pm 15.83 years) and 26 had cerebral hemorrhage (19 men and 7 women, mean age, 59.53 \pm 18.31 years). There were no significant differences in patient characteristics across the four groups at the start of the study ($P > 0.05$; **Table 1**). A flow diagram of patient progress throughout the trial is shown in **Figure 1**.

Effect of BTXA injection on lower-extremity motor function in patients with spastic foot after stroke

Four days after BTXA administration, only the high-dose/high-concentration group showed a difference in the assessed parameters as compared with baseline values (**Table 2**). Although the MAS scores showed some improvement in this group, the difference was not statistically significant compared with either its baseline assessment or with scores of other groups ($P > 0.05$). One week after BTXA administration, however, the MAS scores in the high-dose/low-concentration and high-dose/high-concentration groups were significantly lower than their respective values both 4 days after treatment and at baseline ($P < 0.05$), whereas the scores in the other two (low-dose) groups showed no significant changes for the same comparisons ($P > 0.05$). No significant change was detected in walking speed or Holden or TUG scores for any group between baseline and 4 days or 1 week after the BTXA administration. At 2 weeks, the MAS scores in all four groups were significantly lower than those recorded at both previous time points ($P < 0.05$). At 4 weeks, few changes were observed in the measured parameters in both low-dose groups compared with the previous assessments in these groups. However, significant differences in MAS scores were observed in both the high-dose/low-concentration and high-dose/high-concentration groups. Walking speed and TUG scores had significantly improved in both the high-dose groups compared with those before and at 2 weeks after treatment ($P < 0.05$). Holden scores were significantly improved in the low-dose/high-concentration and high-dose/low-concentration groups at 4 weeks compared with those before treatment ($P < 0.05$). At 12 weeks, MAS scores had almost returned to their original values in both low-dose groups, but remained improved in both high-dose groups. By contrast, compared with baseline values, walking speed, Holden scores, and TUG values were significantly improved 12 weeks after treatment in all groups.

Onset and duration of therapeutic effects following BTXA administration to patients with spastic foot after stroke

We determined the time of onset and duration of therapeutic effects of BTXA and found statistically significant differences both between and within groups ($P < 0.05$; **Table 3**). Among the four groups, the high-dose/low-concentration group

Table 1 Characteristics by group of participating patients with spastic strephenopodia and foot drop after stroke

Group	n	Gender		Age (year)	Cerebral infarction	Cerebral hemorrhage
		Male	Female			
Low-dose/low-concentration BTXA	21	15	6	57.38±17.35	14	7
Low-dose/high-concentration BTXA	22	14	8	59.33±15.62	17	5
High-dose/low-concentration BTXA	24	19	5	58.27±14.75	16	8
High-dose/high-concentration BTXA	22	17	5	57.94±18.05	16	6
Total	89	65	24	58.75±17.87	63	26

Low dose: 200 U; high dose: 400 U; low concentration: 50 U/mL; high concentration: 100 U/mL. Target muscles were the triceps surae and tibialis posterior on the affected side, and injection sites on each muscle were as similar as possible across patients. Data are expressed as number of participants, except age, which is expressed as the mean ± SD. BTXA: Botulinum toxin A.

Table 2 Effects of BTXA treatment on assessed measures in patients with spastic strephenopodia and foot drop after stroke

	MAS (score)	TW-10 (second)	Holden Grading (score)	VAS-WF (score)	TUG (second)
Low-dose/low-concentration BTXA group (n = 21)					
Before injection	3.75±0.71	18.77±5.04	3.52±0.31	3.12±0.43	26.45±6.03
4 days	3.72±0.69	18.92±4.76	3.56±0.63	3.18±0.50	25.97±6.10
1 week	3.67±0.51	17.45±5.31	3.43±0.59	3.11±0.39	25.21±5.98
2 weeks	3.21±0.43*	17.36±5.52	3.51±0.64	3.19±0.48	24.37±6.23
4 weeks	3.18±0.54*	16.85±4.87	3.57±0.57	3.21±0.51	22.16±6.07
12 weeks	3.13±0.51*	13.78±5.19*	3.73±0.56*	5.42±0.55*	20.37±5.35*
Low-dose/high-concentration BTXA group (n = 22)					
Before injection	3.78±0.41	17.87±5.10	3.33±0.43	3.22±0.47	25.86±7.01
4 days	3.76±0.39	17.48±5.45	3.37±0.54	3.13±0.49	25.59±7.43
1 week	3.63±0.46	17.14±6.03	3.44±0.55	3.12±0.44	25.32±6.23
2 weeks	3.33±0.31*	16.40±5.36	3.51±0.46	3.13±0.47	24.58±6.79
4 weeks	3.25±0.48*	13.84±3.86*	3.69±0.53*	3.21±0.53	21.12±7.21*
12 weeks	3.26±0.46*	13.03±3.28*	3.75±0.51*	5.53±0.58*	20.78±6.96*
High-dose/low-concentration BTXA group (n = 24)					
Before injection	3.74±0.47	17.35±4.27	3.37±0.58	3.10±0.46	26.02±7.32
4 days	3.73±0.53	17.42±5.12	3.31±0.53	3.18±0.50	25.75±6.86
1 week	3.55±0.61*	16.34±5.24	3.36±0.55	3.11±0.41	24.51±6.83
2 weeks	3.23±0.58*	16.32±5.57	3.51±0.61	3.16±0.40	23.88±6.79
4 weeks	2.61±0.57*#	13.11±5.15*	3.68±0.59*	3.21±0.45	20.38±6.17*#
12 weeks	2.84±0.61*	11.76±4.67*#	3.85±0.50*	5.71±0.61*	19.72±7.09*#
High-dose/high-concentration BTXA group (n = 22)					
Before injection	3.69±0.63	17.47±4.53	3.35±0.52	3.25±0.48	26.17±8.15
4 days	3.67±0.58	16.86±5.19	3.48±0.59	3.19±0.46	25.76±7.26
1 week	3.26±0.60*	16.67±5.71	3.51±0.51	3.12±0.42	24.33±7.66
2 weeks	2.96±0.54*#	16.31±6.23	3.56±0.53	3.18±0.50	23.32±6.83
4 weeks	2.85±0.61*#	13.26±3.01*#	3.43±0.49	3.24±0.53	18.49±6.97*#
12 weeks	2.73±0.57*†	12.32±2.67*†	3.89±0.54*#†	5.66±0.65*	17.83±6.81*#†

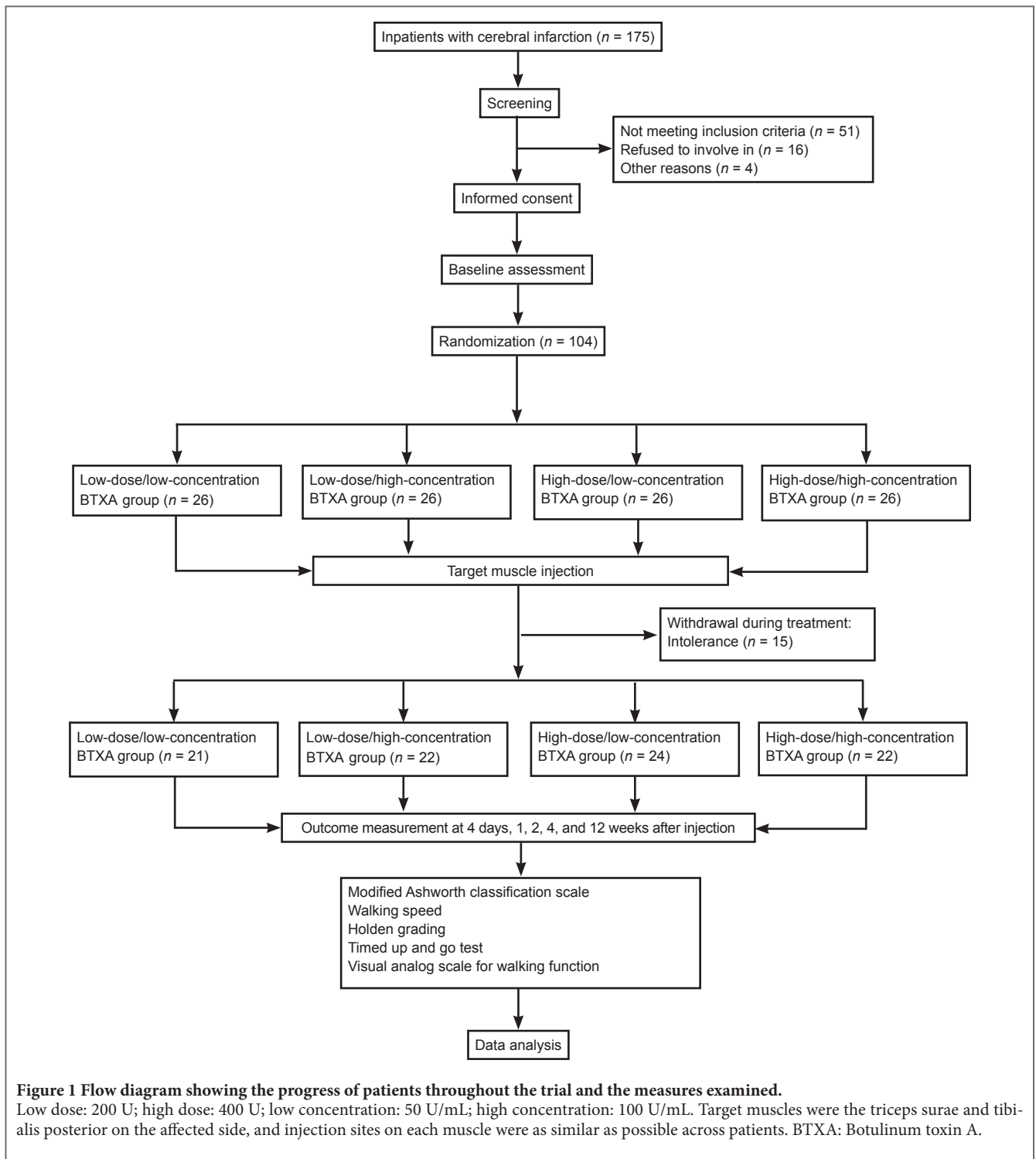
Low dose: 200 U; high dose: 400 U; low concentration: 50 U/mL; high concentration: 100 U/mL. Target muscles were the triceps surae and tibialis posterior on the affected side, and injection sites on each muscle were as similar as possible across patients. Data are expressed as the mean ± SD and were analyzed with one-way analysis of variance followed by the least significant difference test. * $P < 0.05$, vs. before treatment and 4 days after treatment; # $P < 0.05$, vs. previous assessment; † $P < 0.05$, vs. other groups assessed at the same time point. VAS-WF: Visual analog scale for walking function; BTXA: Botulinum toxin A.

(dose, 400 U; concentration, 50 U/mL) had the shortest on-set and longest duration of action ($P < 0.05$).

Adverse effects of BTXA administration in patients with spastic foot after stroke

Eight patients experienced adverse effects, including local bleeding at the injection site, transient dysphagia, allergic

reaction, and fainting during treatment; however, none of these adverse effects led to severe consequences. Three of the patients reported feeling intolerable pain, and received local anesthesia before the injection. Swelling was seen at the injection site in five patients on the day of the injection, but this swelling was ameliorated with the application of cold compresses.



Discussion

BTXA has been used clinically for more than half a century. In recent years, patients with upper motor neuron syndrome have been administered BTXA, which has shown good effects, but the optimal dose and concentration as well as standardized recommendations have not been established (Baricich et al., 2008; Wang and Gao, 2008). Currently, only a few countries, including Great Britain, China, and the United

States, produce BTXA preparations (Béseler et al., 2012). In China, only the Lanzhou Institute of Biological Products is licensed to produce BTXA. The name of this product is HengLi, and each bottle contains 100 U. BTXA was officially registered for commercial use in the United States in 1989 under the trade name Botox (Wang and Gao, 2008). Although BTXA is a toxic substance, it has been clinically used safely for 50 years in humans (Cigna et al., 2014; Schramm et

Table 3 Onset and duration of therapeutic effects following BTXA administration to patients with spastic strephenopodia and foot drop after stroke

Group	Onset time (day)				Action time of BTXA (month)		
	3–6	7–10	11–14	> 14	2–3	4–5	> 6
Low-dose/low-concentration BTXA (n = 21)	1(5)	8(38)	10(48)	2(10)	6(29)	15(71)	0(0)
Low-dose/high-concentration BTXA (n = 22)	2(9)	10(45)	9(41)	1(5)	8(36)	14(64)	0(0)
High-dose/low-concentration BTXA (n = 24)	7(29)	12(50)	5(21)	0(0)	1(4)	19(79)	4(17)
High-dose/high-concentration BTXA (n = 22)	5(23)	9(41)	8(36)	0(0)	2(9)	19(86)	1(5)

Low dose: 200 U; high dose: 400 U; low concentration: 50 U/mL; high concentration: 100 U/mL. Target muscles were the triceps surae and tibialis posterior on the affected side, and injection sites on each muscle were as similar as possible across patients. Data are expressed as number (percentage). BTXA: Botulinum toxin A.

al., 2014).

In China, the current consensus of domestic experts is that the maximum safe dose of the product called Botox is 600 U per patient (Rousseaux et al., 2014). Long-term clinical observations and reports have confirmed that the domestic product, HengLi, is similar to Botox in dose and efficacy, but no large-scale clinical observation trials with HengLi have been reported until now.

HengLi was used in this controlled clinical trial and our results showed that this product was effective for treating spastic foot. Although both high and low doses were effective, the higher dose led to faster therapeutic onset. A high dose at a low concentration showed the most rapid onset of effects, whereas the therapeutic onset was slowest at a low dose with a low concentration. The duration of action (averaging 5–7 months) in both high-dose groups was longer than that in the two low-dose groups, in which the effects lasted 3–5 months on average. This result is consistent with those of previous reports, and no severe adverse effects were observed (Fung et al., 2012; Santamato et al., 2013a). Regarding the visual analog scale for walking function assessments, no significant improvement was found in any group during the first 12 weeks, but significant differences were observed after 12 weeks. This may indicate that restoring the ability to walk in patients with hemiplegia is quite challenging, and satisfactory recovery is generally achieved after participation in a comprehensive rehabilitation program.

In this clinical trial, we examined patients with spastic foot after stroke who were randomized to four groups based on the treatment dose and concentration of BTXA. This was the first time we had conducted this study with HengLi, and no other similar study was found in our search of published articles in the PubMed database. However, a recent study used a higher dose of incobotulinumtoxin A than usual (up to 840 U), and found it was safe (Santamato et al., 2017). In another clinical trial, the authors compared the effects of Botox to another similar compound, letibotulinumtoxin A (Botulax), and found that the compounds had similar effects (Do et al., 2017).

The relatively small sample size is a limitation of the present study that should be taken into consideration when interpreting our results, and we plan to increase the sample

size and include additional subgroups, such as different types of botulinum toxin injected into various sites, in a future study.

In conclusion, despite individual differences in patient responsiveness, the therapeutic use of BTXA in lower limb spasticity shows a positive dose-effect relationship (Picelli et al., 2014). The results of our study indicate that the higher dose and lower concentration of BTXA used in this study can be safely administered in the clinic to treat patients with spastic foot after stroke. Reducing spasticity is not the ultimate aim of BTXA administration (Ding et al., 2015). Instead, the goal of this therapy is to provide sufficient time for other rehabilitation measures, such as correcting deformities (Chinese Association of Rehabilitation Medicine, 2015), that will allow patients to achieve a maximum degree of selfcare and to eventually restore their abilities to perform their normal daily activities and fully function in society.

Author contributions: JL participated in study conception and design. RZ and BLC collected and analyzed data. YXZ and GTB participated in case collection and treatment. SSG and WJL were in charge of case collection and data interpretation. JL and WJL wrote the paper or provided critical revision of the paper for intellectual content. All authors approved the final version of the paper.

Conflicts of interest: None declared.

Research ethics: This clinical trial was approved by the Ethics Committee of Affiliated Hospital of Qingdao University of China (approval No. 20130617). This trial was conducted in accordance with the Declaration of Helsinki, formulated by the World Medical Association and Related Ethical Requirements of Human Research in Affiliated Hospital of Qingdao University of China. Written informed consent was provided by each patient or their family members after they indicated that they fully understood the treatment plan.

Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Data sharing statement: The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Plagiarism check: Checked twice by iThenticate.

Peer review: Externally peer reviewed.

Open access statement: This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

References

- Ashworth B (1964) Preliminary trial of carisoprodol in multiple sclerosis. *Practitioner* 192:540-542.
- Béseler MR, Grao CM, Gil A, Martinez Lozano MD (2012) Walking assessment with instrumented insoles in patients with lower limb spasticity after botulinum toxin infiltration. *Neurologia* 27:519-530.
- Baricich A, Carda S, Bertoni M, Maderna L, Cisari C (2008) A single-blinded, randomized pilot study of botulinum toxin type A combined with non-pharmacological treatment for spastic foot. *J Rehabil Med* 40:870-872.
- Blackburn M, van Vliet P, Mockett SP (2002) Reliability of measurements obtained with the modified Ashworth scale in the lower extremities of people with stroke. *Phys Ther* 82:25-34.
- Chinese Association of Rehabilitation Medicine (2015) Botulinum toxin for treatment of limb spasticity in adults. *Zhongguo Kangfu Yixue Zazhi* 30:81-110.
- Cigna E, Maruccia M, Fanelli B, Scuderi N (2014) Botulinum toxin type A in the healing of chronic lesion following bilateral spasticity of gluteus muscle. *Int Wound J* 11:412-415.
- de Niet M, de Bot ST, van de Warrenburg BP, Weerdesteyn V, Geurts AC (2015) Functional effects of botulinum toxin type-A treatment and subsequent stretching of spastic calf muscles: a study in patients with hereditary spastic paraplegia. *J Rehabil Med* 47:147-153.
- Ding XD, Zhang GB, Chen HX, Wang W, Song JH, Fu DG (2015) Color Doppler ultrasound-guided botulinum toxin type A injection combined with an ankle foot brace for treating lower limb spasticity after a stroke. *Eur Rev Med Pharmacol Sci* 19:406-411.
- Do KH, Chun MH, Paik NJ, Park YG, Lee SU, Kim MW, Kim DK (2017) Safety and efficacy of letibotulinumtoxinA (BOTU-LAX®) in treatment of post stroke upper limb spasticity: a ran-domized, double blind, multi-center, phase III clinical trial. *Clin Rehabil* 1:269215516689331.
- Esquenazi A, Mayer NH, Elia AE, Albanese A (2009) Botulinum toxin for the management of adult patients with upper motor neuron syndrome. *Toxicol* 54:634-638.
- Fung S, Phadke CP, Kam A, Ismail F, Boulias C (2012) Effect of topical anesthetics on needle insertion pain during botulinum toxin type A injections for limb spasticity. *Arch Phys Med Rehabil* 93:1643-1647.
- Holden MK, Gill KM, Magliozzi MR, Nathan J, Piehl-Baker L (1984) Clinical gait assessment in the neurologically impaired. Reliability and meaningfulness. *Phys Ther* 64:35-40.
- Intiso D, Simone V, Di Rienzo F, Iarossi A, Paziienza L, Santamato A, Maruzzi G, Basciani M (2014) High doses of a new botulinum toxin type A (NT-201) in adult patients with severe spasticity following brain injury and cerebral palsy. *NeuroRehabilitation* 34:515-522.
- Kaji R, Osako Y, Suyama K, Maeda T, Uechi Y, Iwasaki M, GSK1358820 Spasticity Study Group (2010) Botulinum toxin type A in post-stroke lower limb spasticity: a multicenter, double-blind, placebo-controlled trial. *J Neurol* 257:1330-1337.
- Kaňovský P, Slawek J, Denes Z, Platz T, Comes G, Grafe S, Pulte I (2011) Efficacy and safety of treatment with incobotulinum toxin A (botulinum neurotoxin type A free from complexing proteins; NT 201) in post-stroke upper limb spasticity. *J Rehabil Med* 43:486-492.
- Lundstrom E, Smits A, Borg J, Terent A (2010) Four-fold increase in direct costs of stroke survivors with spasticity compared with stroke survivors without spasticity: the first year after the event. *Stroke* 41:319-324.
- Nakhostin Ansari N, Naghdi S, Forogh B, Hasson S, Atashband M, Lashgari E (2012) Development of the Persian version of the Modified Modified Ashworth Scale: translation, adaptation, and examination of interrater and intrarater reliability in patients with poststroke elbow flexor spasticity. *Disabil Rehabil* 34:1843-1847.
- Picelli A, Dambruoso F, Bronzato M, Barausse M, Gandolfi M, Smania N (2014) Efficacy of therapeutic ultrasound and transcutaneous electrical nerve stimulation compared with botulinum toxin type A in the treatment of spastic equinus in adults with chronic stroke: a pilot randomized controlled trial. *Top Stroke Rehabil* 21 Suppl 1:S8-16.
- Podsiadlo D, Richardson S (1991) The timed "Up & Go": a test of basic functional mobility for frail elderly persons. *J Am Geriatr Soc* 39:142-148.
- Pradon D, Hutin E, Khadir S, Taiar R, Genet F, Roche N (2011) A pilot study to investigate the combined use of Botulinum toxin type-a and ankle foot orthosis for the treatment of spastic foot in chronic hemiplegic patients. *Clin Biomech (Bristol, Avon)* 26:867-872.
- Rousseaux M, Daveluy W, Kozłowski O, Allart E (2014) Onabotulinumtoxin-A injection for disabling lower limb flexion in hemiplegic patients. *NeuroRehabilitation* 35:25-30.
- Santamato A, Micello MF, Panza F, Fortunato F, Pilotto A, Giustini A, Testa A, Fiore P, Ranieri M, Spidalieri R (2013a) Safety and efficacy of incobotulinum toxin type A (NT 201-Xeomin) for the treatment of post-stroke lower limb spasticity: a prospective open-label study. *Eur J Phys Rehabil Med* 49:483-489.
- Santamato A, Panza F, Ranieri M, Frisardi V, Micello MF, Filoni S, Fortunato F, Intiso D, Basciani M, Logroscino G, Fiore P (2013b) Efficacy and safety of higher doses of botulinum toxin type A NT 201 free from complexing proteins in the upper and lower limb spasticity after stroke. *J Neural Transm (Vienna)* 120:469-476.
- Santamato A, Panza F, Intiso D, Baricich A, Picelli A, Smania N, Fortunato F, Seripa D, Fiore P, Ranieri M. (2017) Long-term safety of repeated high doses of incobotulinumtoxinA injections for the treatment of upper and lower limb spasticity after stroke. *J Neurol Sci* 378:182-186
- Schramm A, Ndayisaba JP, Auf dem Brinke M, Hecht M, Herrmann C, Huber M, Lobsien E, Mehnert S, Reuter I, Stenner A, van der Ven C, Winterholler M, Kupsch A, Wissel J (2014) Spasticity treatment with onabotulinumtoxin A: data from a prospective German real-life patient registry. *J Neural Transm (Vienna)* 121:521-530.
- The fourth national cerebrovascular disease conference, Chinese medical association (1996) All kinds of cerebrovascular disease diagnosis points. *Zhonghua Shenjingke Zazhi* 29:379-381.
- Van Campenhout A, Molenaers G (2011) Localization of the motor endplate zone in human skeletal muscles of the lower limb: anatomical guidelines for injection with botulinum toxin. *Dev Med Child Neurol* 53:108-119.
- Wang Y, Gao B (2008) A dose-response relationship research on botulinum toxin type A local intramuscular injections of lower extremity spasticity in children with cerebral palsy. *Childs Nerv Syst* 24:545-547.

Copyedited by Smith T, de Souza M, Yu J, Li CH, Qiu Y, Song LP, Zhao M