The Properties and Longitudinal Experience of a Chinese Type A Botulinum Toxin for the Treatment of Focal Dystonia and Hemifacial Spasm

Wan Xinhua, Tang Xiaofu, Wang Yinchun
(Department of Neurology, PUMC Hospital, CAMS & PUMC, Beijing 100730, and Lanzhou Biological Products Institute, Lanzhou 730046, China)

Abstract

Objective: To introduce the properties of a Chinese type A botulinum toxin (LANTOX, made by Lanzhou Institute of Biological Products), and its long-term effect for focal dystonia and hemifacial spasm.

Methods: The purity and recovery of crude and crystalline toxin were tested. Long-term data from 305 patients with hemifacial spasm (HFS), blepharospasm (BS) and cervical dystonia (CD) were evaluated and subgroups of patients received LANTOX injections between 1994 and 2000 in at least six separate treatment sessions. They were followed up for 2 ~ 8 years. The therapeutic results of LANTOX injections in the last session were analyzed in comparison with those of the first session.

Result: LANTOX purity was high (2.55~2.60×10^7LD50/mgPr, A260/A280 ≤ 0.55, high molecular substance accounted for 99.2% of total proteins). Long-term treatment with LANTOX in patients with focal dystonia and HFS was not associated with any decline in benefit, and efficacy may improve slightly with repeat treatments. LANTOX is an excellent long-term treatment of HFS, BS and CD.

Conclusion: We conclude that LANTOX is of botulinum toxin therapy quality standard according to the results obtained from the basic study and the long-term clinical application. The re-injection of LANTOX significantly improves the quality of life of most patients and is a safe, effective and comparatively economical treatment for the patients with focal dystonia and HFS.

Key words: Botulnum Toxin Type A; Therapy; Focal Dystonia; Hemifacial Spasm

Introduction

Botox (Botulinum toxin type A, BTX-A, made in Allergan Inc.) has been successfully used in the treatment of abnormal muscle contractions and other clinical indications since it was approved by FDA in 1989 for strabismus, blepharospasm, and hemifacial spasm in patients 12 years and older[1-4]. A Chinese type A botulinum toxin (LANTOX), made by Lanzhou Biological Products Institute, had been approved by the Ministry of Health in China to be produced attemptably for the clinical use since October 1993.LANTOX, 25U/ng, 100~110U/ampoule, was prepared by acid precipitation of crude toxin, extraction with phosphate buffer, RNase treatment, DEAE-A50 ion exchange chromatograph (collect and mix the part of A260/A280 0.5~0.6 eluant) and dialyze against concentrated solution of ammonium sulfate to form crystal, then dilute and lophilize the crystalline toxin. Many reports of LANTOX also have provided evidence that the home-made preparation is a safe and effective therapy, but the long-term effects of repeated LANTOX have been seldom assessed [5]. We have treated numerous patients with Botox (old products, before 1999) and LANTOX in a wide variety of neuromuscular

disorders in the PUMC hospital since 1993, including hemifacial spasm, dystonia, tremor, tics, spasticity, Frey' syndrome, bruxism etc. [6-7] This report describes the basic properties of LANTOX and the longitudinal experience with LANTOX injections for the treatment of focal movement disorders. Although a few of these patients have had up to 15 treatment sessions, we have arbitrarily chosen six treatment sessions as the minimum number in order to derive an adequate sample size of patients. Since the most frequent application of LANTOX in our hospital included HFS, CD and BS, the analysis was limited to these disorders.

Methods

Toxicity test

The potency was expressed in units, 1 unit representing the estimated LD50 for mice. In accordance with Broff 's method, a volume of 0.1 ml original (or diluted) toxin is injected intravenously into each of 5 mice weight 14~16g. Calculated their mean time of death, checking the toxicity of original (or diluted) toxin from the standard curve of toxin dose (toxicity) vs time of death. Also, the toxicity of original or other toxin sample can be determined by routine serial dilution method, a volume of 0.5ml of each dilution is injected intraperitoneally into each of 4 mice weight 14~16g, the number of death is recorded over a period of 96h and the LD50 of original toxin or other sample were calculated by Read Muench method.

HPLC of crystalline toxin was done according convention.

Clinical procedure

1. Preparations. The vials of LANTOX were stored frozen (-20) until the freeze- dried white powder toxin were diluted with sterile normal saline just before injections and never used after 4 hours of preparation to ensure its efficacy. The concentration of the diluted solution was 25u in 1ml.

2. Patients. Among the patients with focal movement disorders, who were treated with LANTOX, 305 cases fulfilled our inclusion criteria for analysis of treatment results (Table 1). We enrolled 187 patients with HFS, 36 patients with BS and 82 patients with CD who received LANTOX injection between 1994 and 2000 in at least six separate treatment sessions.

3. LANTOX treatment. The methods of LANTOX administration and response assessment were previous described in our reports [6-7]. Selection of muscles and determination of the LANTOX dose injected were based on the parts involved, the extent and degree of hyperactivity and reaction to the injection. Because the abnormal movement and posture vary considerably from patient to patient, we had to have some flexibility in the injection schedule for maximizing benefit and minimizing adverse effects just as most studies had done. EMG was used if there was uncertainty as to which muscles were most involved or if patients failed to respond to injections placed by clinical judgment alone and booster injections were given. The objective improvement was evaluated according to Cohen's (HFS and BS), Tsui's (CD) scale, emphasizing examination at rest and during various activities that worsened the dystonic clinical picture. Considering the improvement of motor function, the amount of pain relief and the other changes in the quality of living, the patients reported their subjective assessment arbitrarily on a scale from 1 to 10 after injection. All the enrolled patients were followed up by regular clinic visits for 2~8years.

Latency was defined as the interval (in days) between the injection and the first sign of improvement following the injection. The benefit lasted at least two weeks was considered valid. The therapeutic efficacies were evaluated as no effect, moderate improvement, marked improvement and excellent according to the changes of the Cohen's and Tsui's scores obtained from the injections and the patients' subjective assessment.

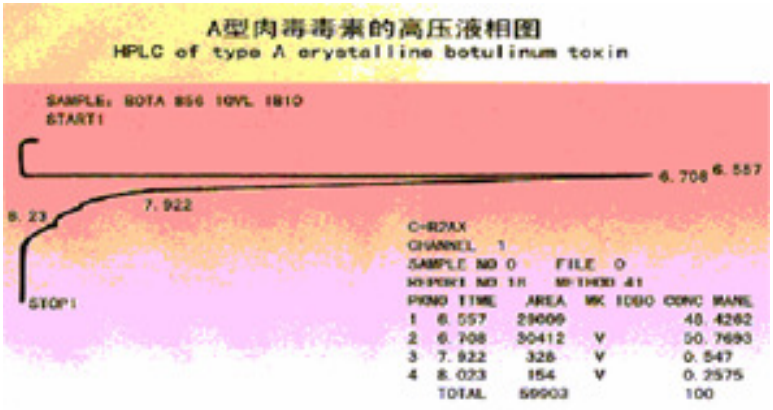
Two-tailed t-test and Ridit test were used to compare ordinal and variables respectively.

Results

Toxicity test

The purity and recovery of crude and crystalline toxin (Table 2).

HPLC of crystalline toxin. The experimental conditions were shown in Fig.1. The chromatograph results of Lot.85-6 was presented. A high and sharp peak occurred and its area in total exceeded 99.2%, it proved the high purity of crystalline toxin.



Treatment results

Our 305 patients received a total 1997 treatment sessions over a 6-year period, mean 6.55 sessions. Most patients ameliorated within the first week after injections, the improvement was delayed for up to 4 weeks in some. The relief patients felt from a single treatment of LANTOX can normally be sustained for approximately three to six months, the benefit in some patients had lasted up to 1~2 years. Most of them noticed a gradual fading of its effects. At this point they had to return to us for their next treatments.

Over all the injections, 32.0% were rated as excellent, 60.6% as marked improvement, 7.1% as moderate improvement. Secondary nonresponse was seen in approximately 2% of patients (6 cases, 4 with CD and 2 with BS), partial and complete therapy failure occurred respectively in 3 cases (Table 3). Among them, 4 patients with CD received a little higher dose per session (mean 287.5U) without shorter re-injection intervals (mean 25.5 weeks) than LANTOX-responsive patients of CD.

Symptom relapse can be effectively treated by repeated LANTOX injection. The results of LANTOX treatment for HFS, BS and CD are tabulated in Table 4. The clinical effects of the last session with LANTOX injection were analyzed in comparison with those of the first session. There were no differences in the dose per treatment cycle, the latency of response and the average relapse interval between the first and last session, in most cases, subsequent subjective assessments were similar too, but the pretreatment scores of the

last injection were slightly lower in all 3 diagnostic groups. Moreover, a progressive reduction in posttreatment scores was observed in HFS and CD. Our calculations showed neither a significant increase in the dosage of LANTOX nor a significant reduction in the relapse intervals after at least 6 treatment sessions.

Complications of LANTOX injection were usually mild and transient, including localized pain, tenderness and/or bruising which may be associated with the injection. Local weakness of the injected muscle(s) represents the expected pharmacological action of botulinum toxin. However, weakness of adjacent muscles may also occur due to spread of toxin. The most concerned complications after injections were ptosis and dysphagia, which were often minor and disappeared automatically within a few weeks, none required special medical treatment in our experience.

There were skin rashes appeared within a few days after injections in 3 cases (8 sessions), which disappeared or decreased in 2 weeks following the common used oral anti-allergic chemicals. There were 5 patients (4 with CD and 1 with BS,) who reported their pronounced weight loss (5~11 kilogram) after repeated injections(6~9 sessions) without dysphagia and malignant conditions or other clear reasons.

DISCUSSION

BTX-A affects the neuromuscular junction through binding, internalization, and inhibition of acetylcholine release. It must enter the nerve endings to exert its chemodenervating effect. Once inside the cholinergic nerve terminal cell, BTX-A inhibits the docking and fusion of acetylcholine vesicles at the pre-synaptic membrane. Duration of effect is usually 3 to 4 months. Gradually, muscle function returns by the regeneration or sprouting of blocked nerves forming new neuromuscular junctions. The ability of BTX to block acetylcholine release in a long-lasting but reversible fashion with few side effects has made it an important tool for the treatment of focal movement disorders.

Purified crystalline LANTOX, which retained hemagglutinin showed single, homogeneous needle form and had high purity ($2.55\sim2.60\times10^7$LD50/mgPr, A260/A280 $\leq$ 0.55, high molecular substance accounted for 99.2% of total proteins). As a result of LANTOX therapy in our series from PUMC Hospital, the functioning of patients with involuntary muscle spasms of various origins has been improved remarkably. Over all the injections in this study, 92.6% sessions were evaluated as excellent and marked improvement. The patients have less discomfort from spasms and their life quality has become better. The proper choice of dose and administration site are the most important determinants of a favorable response to BTX-A treatment. The dose and localization of LANTOX injections should be determined individually based on clinical examination as well as EMG in some patients with cervical dystonia. In our experience, electromyographic assistance seems to be useful both for selection and targeting of dystonic muscles. Changes in response also may require dose adjustment. The improvement seems to depend particularly on the number of involved muscles. Increased complexity of the clinical picture provides less possibility for successful treatment.

Since the specific purpose of this study was to determine the long –term effects of LANTOX, the analysis focused on the patients having received six or more injections. On the basis of the present data, therapy of focal dystonia and HFS with LANTOX seems to be safe and yields good stable results even after 8 years of treatment. Changes occurring

with subsequent LANTOX injections may be less dramatic than the first injection. Long-term treatment led to progressive improvement of subsequent pretreatment scores. Moreover, a progressive reduction in subsequent posttreatment scores was observed in CD and HFS with the latency of response, duration of improvement and doses unchanged.

No serious nor life threatening conditions occurred during the past 8 years of uninterrupted experience at our hospital. There was one case of anaphylatic shock reported about LANTOX injection recently, but it was very mild and rapidly recovered (30minutes) while given adrenaline. Among the patients who had skin rashs in previous injection, none gave up the subsequent treatment due to the allergic issue, and skin rashs didn't appear following each injection. Rash also occurred in the patients with Botox. We noticed that there were 5 patients lost their weight after repeated injections without other clear reasons; the relationship between them is not very sure. No other systemic indication of previously unknown adverse event was observed.

The most concerned issue about the long-term therapeutic efficacies of BTX-A is the development of serum antibody against toxin. Formation of neutralizing antibodies to BTX-A may reduce the effectiveness of BTX-A re-injection. Actually, the final products of BTX-A all are a mixture of toxin and toxoid. The results from some studies suggest that BTX-A injections at more frequent intervals or at higher doses may lead to greater incidence of antibody formation. The potential for antibody formation may be minimized by injecting with the lowest effective dose given at the longest feasible intervals between injections [8-10]. The formation rate of neutralizing antibodies in patients receiving LANTOX has not been well studied. It was reported that plasma exchange or immunoadsorption on a protein A column performed before BTX-A readministration may provide an alternative strategy in treating selected secondary non-responders who are severely disabled [11].

There was no significant difference in the clinical effects and SFEMG changes between the Botox and LANTOX in our previous studies [6-7, 12], and favorable results with both preparations have been published, but they still have been distinctive on some aspects. First, they are different in production procedure. The addition of human serum albumin in Botox for protecting the toxin from rapid detoxification would make patients treated in the potential danger of hematogenous infection. Second, for same reason, the U/ng of toxin in final product was 4 for Botox (old products, before 1999) and 20~25 for LANTOX. There was 50~90% toxicity loss in Botox during dilution and lyophilization while only 10~20% in LANTOX [5, 13]. It is said that the current Botox preparation has lower antigenic potency. Third, theoretically there should be equipotency for two preparations of BTX-A when strength is expressed as mouse units, but actually the dose of Botox and LANTOX cannot be directly compared. In our experience, the requested dose of Chinese preparation which produced the similar effects was statistically little higher than that of Botox. The technique for measurement of biological activity might be different between the manufacturers. Furth, the price of Botox is 8~10 times higher than that of LANTOX in the same units.

In conclusion, Chinese LANTOX preparation has met the quality standard for the BTX-A therapy according to the results both from the basic study and the long-term clinical application. The re-injection of LANTOX significantly improves the quality of life of most patients, is a safe, effective and comparatively economical treatment for the patients with focal dystonia and HFS in a long run.

References

1. American Academy of Neurology Assessment: The clinical usefulness of botulinum toxin-A in treating neurologic disordors. *Neurology*, 1990;40: 1332-1336
2. Denislic M, Pirtosek Z, Vodusek DB, *et al*. Botulinum toxin in the treatment of neurological disorders. *Annals New York Academy of Sciences*, 1994; 710:76-87
3. Hermanowicz N, Truong DD. Development of botulinum toxin therapy. *West J Med*, 1995;163:472-473
4. Schantz EJ, Johnson EA. Botulinum toxin: the story of its development for the treatment of human disease. *Persp Biol Med*, 1997; 40(4): 317-327.
5. Wang YC, Tang XF. Botulinum toxin and the clinical use. *Chinese Journal of Neuroimmunology and Neurology*, 1996; 3: 124-127.
6. Tang XF, Wan XH, Huang G, *et al*. The treatment of focal muscle spasm with Botox and CBTX-A. *Chinese Journal of Neurology*, 1999; 32(3): 135-138.
7. Wan XH, Tang XF. Comparison of Botox and a Chinese type A botulinum toxin in cervical dystonia. *National Medical Journal of China*, 1998; 78: 131-134.
8. Kessler KR, Skutta M, Benecke R Long-term treatment of cervical dystonia with botulinum toxin A: efficacy, safety, and antibody frequency. J Neurol. 1999 Apr; 246(4):265-74.
9. Dressler D. Clinical features of antibody-induced complete secondary failure of botulinum toxin therapy. *Eur Neurol*, 2002 Jul; 48(1): 26-9.
10. Schnider P, Birner P, Moraru E, et al. Long-term treatment with botulinum toxin: dosage, treatment schedules and costs. *Wien Klin Wochensch,* 1999 Jan 29; 111(2): 59-65.
11. Naumann M, Toyka KV, Mansouri Taleghani B, Depletion of neutralising antibodies resensitises a secondary non-responder to botulinum A neurotoxin. *J Neurol Neurosurg Psychiatry*, 1998 Dec; 65 (6): 924-7.
12. Wan XH, Tang XF, Cui LY. Remote effects of local injection of botulinum toxin type A: Single-Fiber EMG studies. *Acta Academiae Medicinae Sinicae*, 1999; 21(5): 362-367
13. Schantz EJ. Historical perspective. In: Jankovic J,Hallett M,eds. Therapy with botulinum toxin. New York, Marcel Dekker, 1994, XXV.

Table 1. Demograghic characteristics

	n	F/M (sex)	Age (yr), X ± SD, range	Symptom duration (mo), X ± SD, range
HFS	187	113/74	46.3 ± 12.6 (16~77)	62.2 ± 52.4 (2~432)
BS	36	26/10	55. 1 ± 12.2 (23~73)	53.0 ± 58.3 (2~312)
CD	82	51/31	36. 4 ± 13.5 (12~69)	46.6 ± 51.3 (3~348)

Table 2. The purity and recovery of crude and crystalline toxin

Lot No.	Original Toxin		Crystalline Toxin				Recovery
	Volume (ml)	Toxicity (LD_{50}/ml)	Volume (ml)	Toxicity (LD_{50}/ml)	Protein Con. (mg/ml)	Purity (LD_{50}/mgpr)	(%)
85-4	34300	$1.25X10^6$	55.0	$1.40 X 10^8$	5.49	$2.55X10^7$	18.06
85-5	36300	$0.90X10^6$	80.5	$1.48X10^8$	5.69	$2.60X10^7$	36.5
85-6	34000	$1.02X10^6$	54.0	$1.28X10^8$	5.02	$2.55X10^7$	19.90

Table 3. The therapeutic efficacies of CBTX-A injections

No. of patients		No. of sessions	Excellent		Marked improvement		Moderate improvement		No improvement	
			n	%	n	%	n	%	n	%
HFS	187	1226	417	34.0	779	63.5	30	2.4	0	0
BS	36	261	68	26.1	164	62.8	27	10.3	2	0.8
CD	82	510	154	30.2	268	52.5	84	16.5	4	0.8
Total	305	1997	639	32.0	1211	60.6	141	7.1	6	0.3

Table 4. Long-term result of CBTX-A treatment

	Dose per treatment cycle (U)	Latency of response (days)	Relapse interval (weeks)	Score	
				Pre-CBTX-A	Post-CBTX-A
HFS n=187					
First	40.4 ±8.5	3.7 ± 2.1	15.6 ± 4.2	3.6 ± 0.5	0.8 ± 0.7
Last	38.2 ± 9.2	4.0 ± 2.6	16.3 ± 4.8	3.0 ± 0.2	0.6 ± 0.7
P	>0.05	>0.05	>0.05	< 0.01	<0.05
BS n=36					
First	64.2 ± 18.1	3.6 ± 3.0	16.1 ± 4.8	3.7 ± 0.6	1.3 ± 0.9
Last	68.5 ± 19.5	4.1 ± 1.9	17.6 ± 5.2	3.4 ± 0.8	0.9 ± 0.8
P	>0.05	>0.05	>0.05	< 0.05	>0.05
CD n=82					
First	246.6 ± 83.7	5.3 ± 3.9	23.5 ± 16.6	12.7 ± 3.1	3.8 ± 2.4
Last	210.0 ± 68.1	5.7 ± 3.5	25.2 ± 10.9	8.6 ± 3.8	3.0 ± 2.1
P	>0.05	>0.05	>0.05	< 0.01	<0.05