Do flexible inter-injection intervals improve the effects of botulinum toxin A treatment in reducing impairment and disability in patients with spasticity?

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Abstract

In patients treated with botulinum toxin-A (BoNT-A), toxin-directed antibody formation was related to the dosage and frequency of injections, leading to the empirical adoption of minimum time intervals between injections of 3 months or longer. However, recent data suggest that low immunogenicity of current BoNT-A preparations could allow more frequent injections. Our hypothesis is that a short time interval between injections may be safe and effective in reducing upper limb spasticity and related disability. IncobotulinumtoxinA was injected under ultrasound guidance in spastic muscles of 11 subjects, who were evaluated just before BoNT-A injection (T0), and 1 month (T1), 2 months (T2) and 4 months (T3) after injecting. At T1, in the case of persistent disability related to spasticity interfering with normal activities, patients received an additional toxin dose. Seven subjects received the additional dose at T1 because of persistent disability; 4 of them had a decrease of disability 1 month later (T2). Rethinking the injection scheme for BoNT-A treatment may have a major impact in the management of spasticity and related disability. Future studies with larger sample sizes are warranted to confirm that injection schedules with short time intervals should no longer be discouraged in clinical practice.

Key words: botulinum toxin; spasticity; disability assessment scale; global assessment scale; additional dose; Injection interval; antibody-induced treatment failure; booster injection

1. Introduction

The management of spasticity is a major challenge in neuro-rehabilitation. Primary goals are the improvement of patients' comfort and their functional level. Several effective procedures are now available, among which botulinum toxin-A (BoNT-A) treatment represents the first choice for focal spasticity [1].

While botulinum toxin is a highly effective treatment for spasticity, treatment effects are temporary and many patients experience partial to complete reemergence of symptoms towards the end of each injection cycle as the benefits of the previous dose begin to wear off. In the early days of BoNT-A treatment, if the first set of injections induced unsatisfactory effects patients were injected additional doses of toxin 2-4 weeks later [2]. Unfortunately, the early years of BoNT-A treatment were burdened by treatment failure due to the development of antibodies able to neutralize the toxin (neutralizing antibodies) [3]. The risk of BoNT-A antibody formation was directly related to the toxin dosage and the frequency of injections. Indeed, patients who experienced antibody-induced treatment failure had received injections with higher frequency (shorter time intervals) than patients who did not develop toxin resistance [4]. These observations marked the end of the use of additional doses of BoNT-A, and led to the empirical detection of injection intervals of 3 months or longer, still adopted in the nowadays clinical practice [5].

Clinical observations that led to the recommendation of ≥ 3 months injection interval were performed on patients treated with early formulations of onabotulinumtoxinA, known to be much more immunogenic than the current onabotulinumtoxinA [6] As a result, an obvious question is whether the availability of recent toxin formulations with reduced immunogenic potential [7] should prompt a reappraisal of the debate on whether the clinical benefits of adopting shorter and more flexible inter-injection intervals could be worth the risk of neutralizing antibody formation, at the moment recognized as significantly lowered [8].

We conjectured that this issue would be best analyzed having data on the immunogenic risk of neutralizing antibody formation on the one hand, but more meaningfully, by providing evidence on the clinical benefits deriving from the adoption of short inter-injection intervals on the other. Certainly, if adopting short injection intervals produced no increase in clinical benefit (an eventuality that cannot be excluded a priori), performing large clinical studies to investigate the risk of antibody formation would be inappropriate. As a matter of fact, recent data in children with lower limb spasticity show that increasing the inter-injection intervals from 12-monthly to 4-monthly confers no clinical advantage [9].

Therefore, we hypothesize that in patients with upper limb spasticity the clinical benefit induced by botulinum toxin treatment improves when adopting a short inter-injection interval. If this is the case, impairment and disability evaluated after administering an additional dose of incobotulinumtoxinA one month after a first set of injections, should be found reduced. Having this information would strengthen the rationale for designing and planning multicentre studies investigating the immunogenic risk of neutralizing antibody formation.

2. Methods

We designed this pilot study in patients with upper limb spasticity to test the hypothesis that adopting short inter-injection intervals improves the clinical benefit induced by botulinum toxin treatment on impairment and disability in patients with spasticity.

2.1. Patients' selection

Patients were included in the study based on the following criteria: 1) upper limb spasticity resulting from a lesion in the brain or in the spinal cord; 2) evidence of difficulty, mainly caused by spasticity, in dressing or maintaining personal hygiene, pain or malposition of the upper limb, as demonstrated by a score ≥ 2 in at least 1 of the 4 domains of the Disability Assessment Scale (DAS); 3) a stable clinical picture in the 6 months preceding enrollment.
Exclusion criteria were: cognitive impairment interfering with the ability to provide informed consent; joint retractions and major muscle contractures in the affected upper limb; significant cutaneous or joint inflammation in the affected upper limb; ongoing neuromuscular diseases; a change in oral medication for spasticity in the previous three months; treatment with intrathecal baclofen.
The present study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; a written informed consent was obtained from all participants.

2.2. Study design

IncobotulinumtoxinA was injected in the upper limb muscles affected by spasticity under ultrasound guidance. Muscles to be injected and toxin doses were determined in each single subject, according to the clinical picture, in the aim to reduce spasticity and related disability. The subjects were evaluated just before BoNT-A injection (T0), and 1 month (T1), 2 months (T2) and 4 months

(T3) after injecting. At T1, in the case of persistence of selection criterion number 2, an additional BoNT-A dose was injected.

All subjects underwent physiotherapy during the two months following the first set of injections (from T0 to T2). Physiotherapy, which included muscle stretching and muscle training, was organized in three weekly sessions lasting 45 minutes each (24 sessions in total). Functional task-specific activities were incorporated.

2.3. Outcome measures

Muscle tone was evaluated using the *Modified Ashworth Scale (MAS)*, a 6-point scale ranging from 0 (no increase in tone) to 4 (limb rigid in flexion or extension) [10].

To evaluate the functional disability we used the *Disability Assessment Scale (DAS)* [11]. Four areas of disability were assessed (hygiene, dressing, limb position and pain) according to the following classification: 0 = no disability; 1 = mild disability not interfering significantly with normal activities; 2 = moderate disability (normal activities require increased effort and/or assistance); 3 = severe disability (normal activities limited). The scores of the 4 areas were summed.

The overall response to treatment was evaluated together by investigators, patients, and caregivers using the *Global Assessment Scale (GAS)* [12]. The GAS is a 9-point scale ranging from 0, unchanged, to +4, very marked improvement, or to –4, very marked worsening.

MAS and DAS were administered at each examination (T0, T1, T2 and T3), while GAS and adverse effects were evaluated only after the BoNT-A injection (T1, T2 and T3).

All measures of variability are reported as standard deviation.

2.4. Endpoints

Endpoint 1: number of enrolled subjects showing persistence of spasticity and related disability at T1 with DAS score ≥ 2 in at least 1 of the 4 domains.

Endpoint 2: number of subjects injected at T1 with DAS score at T2 < DAS score at T1.

3. Results

Table 1 shows the demographic and clinical features of the 11 enrolled subjects.

The mean dose of incobotulinumtoxinA injected at T0 was 220 ± 81 units (Table 2).

At T1, all the subjects showed a MAS score reduction of at least 1 point in at least 1 treated muscle (Table 3). Eight subjects (patients 1-8) underwent a reduction in DAS score, and all the 11 subjects

had a positive GAS score (Table 4).
In subjects 1-4 (mean dose of BoNT-A at T0 215 ± 39 units), DAS score at T1 was < 2 in all the 4 domains; therefore, they received no additional dose. In contrast, subjects 5-11 (mean dose of BoNT-A at T0 224 ± 101 units) showed persistence of spasticity and related disability at T1 with DAS score ≥ 2 in at least 1 of the 4 domains (***endpoint 1: 7 out of 11 subjects***) (Table 4). These 7 subjects received an additional dose of incobotulinumtoxinA (137 ± 73). In 3 of those subjects (6, 8 and 10), the additional dose was injected in the same muscles treated at T0. In other 3 subjects (5, 7 and 11), the early additional dose was injected both in muscles treated and not treated at T0. Subjects 9 received the additional dose only in a muscle not treated at T0 (Table 2).
At T2, subjects 1-4 (not receiving the additional dose) showed same DAS and GAS scores as those collected at T1. In contrast, among those who received the additional dose, 4 subjects (5-7 and 10) showed reduced DAS score (***endpoint 2: 4 out of 7 subjects***), and 5 subjects (5-7, 9 and 10) increased GAS score.
All subjects denied any adverse event.

4. Discussion

This study confirmed the hypothesis tested, that adopting short inter-injection intervals improves the clinical benefit induced by botulinum toxin treatment on impairment and disability in patients with spasticity.
We found that a single set of BoNT-A injections into the spastic muscles of the upper limb resulted, 1 month after the treatment, in an improvement in disability in 8 of the 11 enrolled subjects. BoNT-A reduced hypertonia as measured as MAS score and improved the GAS scores in all subjects. These findings are in line with previous results obtained in a large cohort of subjects [11].
For this study on additional toxin doses administered at short inter-injection intervals, we used incobotulinumtoxinA because it induced no formation of neutralizing antibodies in a large cohort of patients with post-stroke spasticity [13], nor in any patients with spasticity or dystonia enrolled in a safety study and treated with very high doses up to 1200 U/session [14].

4.1. Endpoint 1

The first endpoint of the present study was to find out how many subjects still had a disability related to spasticity with a DAS score of 2 or higher in at least 1 domain at T1, i.e. 1 month after the first set of injections. This endpoint is essential because not only it represents as a marker of

efficacy, but also as an indication for treatment. Since the aim of the treatment of spasticity is the reduction of disability and improvement in quality of life [15], in the case of a DAS score of 1 (moderate disability due to muscle over-activity not interfering significantly with normal activities) or 0 (no disability due to muscle over-activity), the use of BoNT-A may be unnecessary.
We found that, at T1, 7 subjects scored 2 or higher in at least 1 domain of DAS. Therefore, this pilot study suggests that one month after injection, despite an overall improvement, more than half of the subjects still manifest incomplete benefit on selective domains of the upper limb residual function (hand hygiene, dressing, limb position and pain).
Since we enrolled subjects having a stable chronic clinical picture and showing no progression of spasticity in the short-period, reasonably disability at T1 derives from an insufficient effect of the first set of injections, which can be explained by several factors. The first and simplest might be that the dosage injected at T0 was insufficient. Although the absolute doses were in the range recommended by current guidelines for the target muscle, it is likely that some patients would have needed a higher amount of toxin, an observation that can obviously be made only a few weeks after injection, at a time when toxin action becomes evident. The second factor might be due to scarce sensitivity of the clinical examination in evaluating the activity of a specific muscle among synergists. It may happen that the functional weight attributed to the overactivity of single muscles targeted by injection at T0 is inadequate, especially when synergists are activated with different orders of magnitude. In subjects injected at different time points in both agonist and antagonist muscles (e.g. subject 11, first injected in the triceps and then in forearm flexors), it is also possible that the muscle overactivity causing high DAS score at T1 increased its functional weight in producing disability after BoNT-A exerted its action in antagonist muscles injected at T0. Finally, we cannot exclude that residual disability at T1 derives from features of the upper motor neuron syndrome other than spasticity (i.e. muscle contracture, weakness, loss of dexterity, fatigue [16]). In addition, we must also mention that those patients who were more disabled at T0 were less likely to obtain full benefit from a single treatment. Indeed, all patients scoring DAS higher than 10 at T0 deserved and received the early additional dose at T1.

4.2. Endpoint 2

The second endpoint of the study was to find out the number of subjects in whom disability decreased 1 month after the early additional dose (T2).
While none of the 4 subjects receiving no early additional dose showed a reduction in disability from T1 to T2, 4 subjects who received the additional dose (5-7, 10) had a further reduction of

disability. In these subjects, the tone of the injected muscles was reduced, and the GAS scores increased at T2.

This further functional improvement deserves some comments. A first comment is that at T1 the re-injected muscle exhibited residual overactivity allowing further toxin uptake. The degree of muscle activation is known to influence both the speed and rate of toxin binding and internalization [17]. Second, not only did the newly internalized toxin prove effective, but it also acted with a time course overlapping that of the toxin injected at T0. Finally, whatever the balance between factors producing disability at T1 (see discussion on endpoint 1), they proved sensitive to additional toxin action.

In 3 patients (8, 9 and 11), DAS scores remained unchanged at T2 indicating insufficient impact of the additional dose on hygiene, dressing, limb position and pain. In subject 8, flexor digitorum superficialis (FDS) muscle was injected at T0 with no effect on muscle tone. The early additional dose was re-injected in FDS, once again with no change in MAS score at T2 (see table 3). As all other muscles injected at T0 underwent a significant tone reduction, plausibly soft tissue changes or a very low degree of muscle activity, may have prevented the beneficial effects of toxin action. A similar mechanism presumably operated also in subject 11, who manifested reduced hypertonia in all muscles injected at T0 and T1, but not in the *pectoralis* muscles whose MAS score remained unchanged at all times. Although hypertonia due to muscle contracture does not vary with the velocity of muscle stretch, in a clinical setting it may be difficult to distinguish muscle contracture from muscle over activity, especially in the proximal arm district. In subject 11, however, over activity of shoulder adduction synergists not injected at T0 nor at T1 may have also played a role in disability. In the absence of neurophysiological testing, the sole clinical evaluation may have overestimated the role of the *pectoralis* muscles as prime movers in the patient's abnormal posture. In subject 9, whereas hypertonia disappeared at T2, disability remained unchanged. Discrepancy between MAS and DAS scores suggests that in this subject disability was determined by features of the upper motor neuron syndrome other than spasticity. Interestingly, GAS score increased at T2. We consider this finding important and encouraging, because treatment of focal spasticity with BoNT-A is required not only to increase patients' functional level, but also to improve their comfort (reflected by GAS score).

4.3. Limitations of the study

The first limitation of this study is the small sample size. This limitation is relevant for both endpoint 1 and endpoint 2. In particular, as far as endpoint 1 is concerned, investigating more

subjects could have allowed further knowledge about the proportion of patients requiring short time intervals between injections according to their level of disability at baseline.
The second limitation is the lack of placebo controls, which is more relevant for endpoint 2.

5. Conclusions

This pilot study designed to test the hypothesis that adopting short inter-injection intervals would improve the clinical benefit induced by botulinum toxin treatment on impairment and disability in patients with upper limb spasticity, shows that:

- one month after the first set of toxin injections, despite an overall improvement, the majority of the enrolled subjects (7 subjects out of 11) still manifest disability related to spasticity interfering with normal activities;
- residual disability one month after the first set of toxin injections proves sensitive to an additional dose of BoNT-A delivered at short time interval from the previous set of injections, as shown by positive GAS scores in 5 and decreased DAS scores in 4 at T2;
- reasonably, in subjects unresponsive to the additional dose, factors other than muscle over activity concurred to produce disability.

Our results suggest that rethinking the injection scheme for BoNT-A treatment may have a major impact on managing patients with spasticity. Investigating the risk of neutralizing antibody formation was not in the scope of this pilot study, but evidence that all the currently available BoNT-A formulations exhibit low clinically detectable levels of antibodies should no longer discourage clinicians to test injection schedules with short time intervals in clinical practice. Nevertheless, present results warrant large long-term follow-up trials aimed at verifying that short time inter-injection intervals can be adopted safely in clinical practice.

Disclosure statement

The authors report no conflicts of interest related to the present research.

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TABLE 1: demographic and clinic features of the 11 enrolled subjects

Subject	**Age** (years)	**Gender**	**Affected side**	**Spasticity duration** (years)	**Lesion type**	**Previous injections** (number)	**Last injection** (months before T0)
1	43	M	R limbs	43.0	cerebral palsy	>20	4
2	61	M	R limbs	1.5	haemorrhagic stroke	0	=
3	69	M	R limbs	3.5	haemorrhagic stroke	4	5
4	42	F	R limbs	15.0	Ischemic stroke	4	5
5	33	M	R upper limb	2.0	neuro-SLE	2	4
6	44	F	R limbs	1.5	ischemic stroke	1	4
7	20	F	L upper limb	15.0	epileptic encephalopathy	9	5
8	68	M	R upper limb	2.0	cervical myelopathy	0	=
9	47	M	L upper limb	5.0	multiple sclerosis	0	=
10	78	M	L limbs	27.0	haemorrhagic stroke	5	4
11	64	M	L limbs	2.5	haemorrhagic stroke	4	4

M: male; F: female; SLE: systemic lupus erythematosus; R: right; L: left

TABLE 2: injected muscles and BoNT-A doses (units)

Subjects	T0/T1	PM	PMr	TB	BB	BRA	BR	PT	PQ	FCR	FCU	EDC	FDS	FDP	FPL	FPB	AP	Total
1	T0							40	30	45	45							160
2	T0					50		30	20				60	40	20			220
3	T0					40		40	20				50	60	40			250
4	T0					30	50	30	20				40	25	20		15	230
5	T0				40			70	50									160
5	**T1**					**40**		**30**	**30**									**100**
6	T0							30	20	70	60		100	100				380
6	**T1**							**10**		**60**	**60**		**80**	**50**				**260**
7	T0									50	50	25	40				20	185
7	**T1**												**30**		**10**	**5**	**5**	**50**
8	T0	50								50			50					150
8	**T1**												**90**					**90**
9	T0				100													100
9	**T1**					**100**												**100**
10	T0							40	30				100	100	20			290
10	**T1**												**90**	**70**				**160**
11	T0	150		150														300
11	**T1**	**50**	**50**		**50**	**50**												**200**

Injected muscles at T0 and T1 and the BoNT-A doses (units). Subjects 1-4 were injected only at T0; subjects 5-11 were injected both at T0 and T1 (bold).

PM: *pectoralis major*; PMr: *pectoralis minor*; TB: *triceps brachii*; BB: *biceps brachii*; BRA: *brachialis*; BR: *brachioradialis*; PT: *pronator teres*; PQ: *pronator quadratus*; FCR: *flexor carpi radialis*; FCU: *flexor carpi ulnaris*; ED: *extensor digitorum*; FDS: *flexor digitorum superficialis*; FDP: *flexor digitorum profundus*; FPL: *flexor pollicis longus*; FPB: *flexor pollicis brevis*; AP: *abductor pollicis*.

TABLE 3: Modified Ashworth Scale (MAS) scores

Subjects	SA	FE	FF	FP	WF	FDS	FDP
1				4 0 0 4	3 1+ 1+ 3		
2			1+ 1 1 1+	1+ 1 1 1+		3 1 1 3	3 0 0 3
3			3 2 2 3	2 1 1 2		3 0 0 3	3 0 0 2
4			2 0 0 2	2 0 0 2		3 2 2 3	3 1 1 3
5			2 1+ 1+ 1+	4 3 1 3			
6				4 2 1 3	4 3 1 3	4 4 1 1	4 4 1 1
7					4 1 1 4	4 4 2 3	
8	3 0 1 3				4 1+ 1+ 4	4 4 4 4	
9			3 2 0 2				
10				2 1 1 2		4 4 4 4	3 3 1 2
11	4 4 4 4	4 2 3 4	3 3 1 3				

Modified Ashworth Scale (MAS) scores at T0, T1, T2 and T3 (for instance, "3 0 1 3" means MAS 3 at T0, MAS 0 at T1; MAS 1 at T2 and MAS 3 at T3).

SA: shoulder adductors; FE: forearm extensors; FF: forearm flexors; FP: forearm pronators; WF: wrist flexors; FDS: flexor digitorum superficialis; FDP: flexor digitorum profundus.

TABLE 4: DAS and GAS scores

	Disability Assessment Scale				Global Assessment Scale		
Subjects	**T0**	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**
1	2 2 1 3	1 1 1 1	1 1 1 1	2 2 1 3	3	3	0
2	2 1 2 2	1 1 1 1	1 1 1 1	2 1 2 2	3	3	0
3	2 3 2 3	1 1 1 1	1 1 1 1	2 3 2 3	3	3	0
4	2 2 2 2	1 1 1 1	1 1 1 1	2 2 2 2	3	3	0
5	1 2 2 2	1 2 1 2	1 1 1 1	1 2 2 2	2	3	1
6	3 3 3 3	3 3 3 2	1 3 3 1	2 3 3 2	2	3	2
7	3 2 3 3	3 2 3 2	2 2 2 2	3 2 2 3	2	3	1
8	3 3 2 3	2 2 1 2	2 2 1 2	3 3 2 3	3	3	0
9	1 1 2 2	1 1 2 2	1 1 2 2	1 1 2 2	1	2	0
10	3 3 1 3	3 3 1 3	2 3 1 3	3 3 1 3	1	2	0
11	3 3 3 3	3 3 3 3	3 3 3 3	3 3 3 3	1	1	0

MAS scores and DAS scores were collected at each examination (T0, T1, T2 and T3), while GAS scores were collected only after the BoNT-A injection (T1, T2 and T3). GAS scores at each examination (T1, T2 and T3) define the variation with respect to T0. The scores of the 4 domains of DAS are reported for each examination (for instance, “3 3 1 3” means hygiene score 3, dressing score 3; pain score 1 and posturing score 3).