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ORIGINAL REPORT

EFFECT OF SHORT-TERM ELECTRICAL STIMULATION BEFORE AND AFTER BOTULINUM TOXIN INJECTION

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Objective: **To compare the effect of electrical stimulation applied before and after botulinum toxin injection.**
Subjects: **Eight healthy subjects.**
Methods: **Both extensor digitorum brevis muscles were injected with low fixed doses of botulinum toxin. Subjects received a 20-min session of electrical stimulation before botulinum toxin injection for the right foot and after the injection for the left foot. Percentage changes in compound muscle action potential amplitude were calculated at different intervals over a 60-day period.**
Results: **A reduction in compound muscle action potential percentage was measured at every time-point, both for the muscles stimulated before injection of botulinum toxin and for those stimulated after injection. The compound muscle action potential percentage was always lower on the side stimulated after injection of botulinum toxin. A reduction in compound muscle action potential percentage was measured on the 7th and 15th days in all extensor digitorum brevis muscles examined. On the 15th day the compound muscle action potential percentage was 38.8 (right foot) vs 24.1 (left foot) ($p = 0.0117$). A slow recovery was observed after this period.**
Conclusion: **Electrical nerve stimulation enhances the effect of botulinum toxin to a greater extent if applied after injection rather than before. The short stimulation time used in our study gave similar results to those seen in previous research using longer application times.**

Key words: botulinum toxin type A; electrical nerve stimulation; neurophysiology; spasticity.

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INTRODUCTION

Botulinum toxin (BT) is a neuromuscular blocker that can be used for several purposes. It is particularly useful in rehabilitation settings as it enables the focal treatment of spasticity (1–4). Various rehabilitative techniques have been used to favour the absorption of BT and thus enhance its effects on hyperactive muscles. Physicians combine BT injection with stretching, functional electrical stimulation, taping and therapeutic exercise (5). The basic concept is that the degree of motor activity may be an important factor in enhancing the effect of the neurolytic agent (6).

BT parasitizes the physiological retrieval process of synaptic vesicles (7). There are 2 main endocytosis pathways; a fast pathway called "kiss and run" (8–10) and a slow pathway called "clathrin-mediated endocytosis". The latter is the pathway mainly used by BT to enter nerve fibres (11, 12). It has been shown that nerve stimulation accelerates selectively clathrin-mediated endocytosis (13).

In all the published studies electrical stimulation has been applied after BT injection. There are no published studies of repetitive electrical stimulation applied before BT injection (14–17). Electrical stimulation may have a preparatory role, producing a favourable precondition for BT uptake through pre-activation of the clathrin pathway. The first aim of the present pilot study was to test whether electrical stimulation is more effective when applied before or after injection of BT.

No agreement has yet been reached concerning the frequency and duration times of electrical stimulation. Researchers have chosen different application times: from 24 h nonstop (18), to 30 min a day for 5 days, 6 times a day for 3 days, or twice a day for 1 month (6, 19, 20). It has been shown that the link phase between BT and receptors and the subsequent internalization lasts only a short time (21). Further immunological studies have shown that after 20 min anti-toxin antibodies have no inhibitory action on BT (16). Taking these data into consideration, the second goal of our study was to evaluate the effect of short (20 min) electrical stimulation on BT-injected extensor digitorum brevis (EDB) muscles.

METHODS

Eight healthy adults (7 women; mean age 45.4, age range 29–61 years) were enrolled in the study. Subjects had to be free from neurological and/or orthopaedic impairments. None of them had received BT before this study. All subjects entered the study voluntarily and gave their informed consent.

The study involved injecting 40 IU of BT type A Dysport® (Ipsen, Slough, UK) in fixed-dilution volumes (0.1 ml). A 30-gauge needle was used to make a single intramuscular injection of BT into the central part of the EDB muscle of both feet. The EDB muscle was chosen due to its isolated position in the foot, its easy accessibility for neurophysiological study, and the absence of clinical problems following its paralysis.

A Fisiocomputer ET2/BF® (J&S, Roma, Italy) device was used for electrical stimulation. The surface-stimulating electrode was positioned directly on the belly of the muscle. Continuous trains of rectangular current pulses were applied at a set frequency of 5 Hz. The electrical current was supplied intermittently every 4 seconds. The delivery was maintained 3 seconds and the single stimulus duration was 0,2 ms. The stimulus intensity was sufficient to elicit a visible muscle contraction (28–31 mA) and was adjusted according to the patient's tolerance. The electrical stimulation was always well tolerated. Subjects received 20 min of electrical stimulation to the right EDB muscle immediately before BT injection and 20 min to the left EDB muscle immediately after BT injection.

The compound muscle action potential (CMAP) was used as an electrophysiological measure to investigate the action of BT. The CMAP was recorded in every EDB, using a recording surface electrode placed over the EDB belly, with the neutral reference electrode placed over the tendon of the EDB, and the ground electrode at ankle level.

A bipolar supramaximal electrical stimulation was applied to the peroneal nerve in the ankle. Stimulus duration ranged from 0.1 to 1 ms, depending on the different skin impedance and voltage intensity (millivolts). An electromyography (EMG) device was used to generate the stimuli and record all responses. Every CMAP was recorded 3 times following supramaximal stimulation of the peroneal nerve in the ankle, moving the registering electrode a few mm along the EBD each time. In order to quantify the CMAP in each EDB muscle, we measured the peak to peak amplitude of the CMAP at time 0, before injection of BT, and subsequently on the 7th, 14th, 30th and 60th days for each subject. The progressive percentage variation in CMAP amplitude over time was quantified and compared with baseline for each individual. In particular, the ratio between the CMAP amplitude recorded at different times (CMAP-t), and the CMAP amplitude at baseline (CMAP-b), was determined using the following ratio formula:

$$\frac{CMAP\text{-}t}{CMAP\text{-}b} \times 100 = CMAP\%$$

The trend variation in CMAP% for EDB stimulated before or after BT injection were compared.

Statistical analysis was performed using a non-parametric test for paired data, because the sample number was small and values had a non-Gaussian distribution. The Wilcoxon test for paired data was used to compare the CMAP% value recorded for EDB muscles stimulated before or after BT injection. It was tested at 4 time-points after botulinum toxin administration. A p-value <0.05 was used to reject the null hypothesis.

RESULTS

A significant change in CMAP% was observed in all of the 8 subjects treated with BT. Table I shows the course of CMAP% registered in EBD muscles stimulated before (right foot) or after (left foot) BT injection and measured on different days over a period of 60 days. Values for each subject and the mean data are reported. Fig. 1 illustrates the time course of the mean CMAP% for 8 subjects for both sides.

All subjects showed a reduction in CMAP%, as a blocking effect, both in the left and the right foot 7 days after BT injection. A further reduction in CMAP% was observed in all EDB muscles on the 15th day after the first evaluation. On the 15th day the mean value of CMAP% was 38.8 for the pre-stimulated side, and 24.1 for the post-stimulated side. This difference was significant at every time-point (see Table I).

DISCUSSION

It is difficult to quantify the effect of BT in a clinical setting using objective and reproducible methods (22, 23). Several approaches have been used to evaluate the reduction in muscular tone and improvement in range of motion after BT injection, such as visual observation or application of a clinical assessment scale (the Modified Ashworth Scale). The disadvantage of these methods is that they are subjective and therefore not reproducible.

An objective method is to use electromyographic and neurographic techniques. Some authors have recorded CMAP amplitude before and after BT injection in order to measure the paralysing effect of BT on the muscle (19, 21, 23–27). On the basis of these previous studies, we chose to measure the decrease in CMAP in EDB muscles after BT injection.

Our results show that, apart from the use of electrical stimulation, there is a significant homogeneous trend in CMAP% over time among the subjects examined after BT injection, of a reduction on the 7th and the 15th day and subsequent stabilization.

The EDB test can therefore be considered a valid and reproducible method, despite the presence of several factors that might influence the reliability of the method (electrode position, skin resistance, electrical stimulation device position, skin temperature of examined subject's foot, etc.).

Table I. *Variation in compound muscle action potential percentage (CMAP%) over time in extensor digitorum brevis (EDB) muscles stimulated before botulinum toxin (BT) injection (right side) or after BT injection (left side) for 8 subjects*

		Days after BT injection (CMAP%)									
		CMAP-b (mV)		7		15		30		60	
Subject	Sex/age, years	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
1	F/38	2.0	2.1	29.4	19.9	35.3	9.26	40.2	10.2	66.6	16.2
2	F/32	8.5	9.1	52.5	33.3	47.0	25.0	51.7	40.6	52.9	38.5
3	F/55	5.7	9.9	36.5	19.4	15.2	11.3	30.2	22.2	20.0	14.3
4	F/57	6.6	5.5	48.5	27.3	46.6	15.3	35.7	33.4	32.7	24.7
5	F/29	10.9	10.3	45.8	50.5	49.5	44.6	49.5	46.6	44	46.6
6	M/32	7.2	11.9	62.5	39.5	47.2	33.6	73.6	33.6	100	36.1
7	F/59	19	13.9	38.7	23.0	29.5	24.5	31.6	33.1	–	–
8	F/61	11.4	13.8	46.0	16.6	39.9	29.7	41.2	34	42.1	30.4
Mean	45.4	8.9	9.5	45.0	28.7	38.8	24.1	44.2	31.7	51.2	29.6
p[a]				0.00173		0.0117		0.0173		0.0280	

[a]Difference in CMAP% for pre-stimulated vs post-stimulated (EDB) muscle.
CMAP-b: CMAP amplitude at baseline.

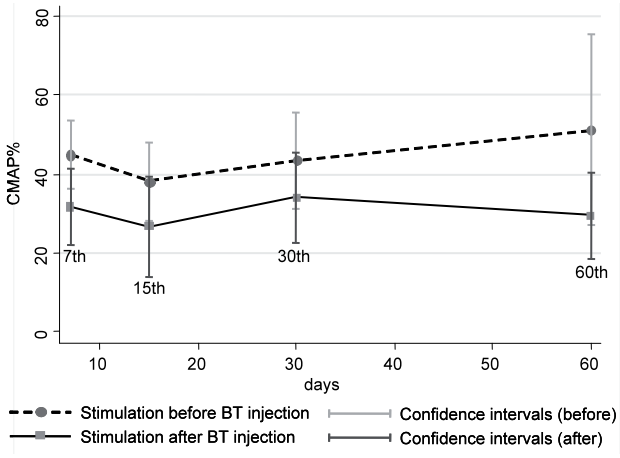

Fig. 1. Variation in mean compound muscle action potential percentage (CMAP%) at different recording times for the right side (stimulated before botulinum toxin (BT) injection) and the left side (stimulated after BT injection). Ordinate: mean CMAP% after botulinum toxin type A (BT-A) injection. The solid line shows the course of the post-stimulated extensor digitorum brevis (EDB) muscle. The dotted line shows the course of the pre-stimulated EDB. The abscissa shows the time. The vertical bars show the 95% confidence intervals of each mean value. CMAP was measured before BT injection on day 0 and afterwards on the 7th, 15th, 30th and 60th day following the BT injection.

The reproducibility of the test confirms that the factors that might affect the results did not have significant relevance. Furthermore, as also pointed out by other authors, the EDB muscle is easily accessible because of its isolated position in the foot and its paralysis does not lead to subsequent clinical problems.

Some studies support the use of repetitive nervous stimulations (18, 19, 24, 27), while others conclude that there is no significant increase in the efficacy of BT after their application (28, 29).

The level of motor activity is a relevant factor in strengthening the effect of BT toxin.

It has been shown that when electrical stimulations are applied at a frequency of 5 Hz, many synaptic vesicles merge with neuronal membrane to release acetylcholine (13).

Ninety-five percent of synaptic vesicles are retrieved through 2 endocytosis pathways; a fast pathway termed "kiss and run" (8–10), and a slow pathway termed "clathrin-mediated endocytosis". The first pathway is characterized by an incomplete fusion of vesicles, while in the second pathway the clathrin provides a gem-shape to the synaptic membrane invaginations. Clathrin-mediated endocytosis is the main pathway used by BT to enter nerve fibres, and it is accelerated selectively by electrical nerve stimulation (11–13).

At a stimulation frequency of 30 Hz only 78% of completely exocytosed vesicles are retrieved via the same mechanism; thus, if the stimulation frequency is increased the retrieval of synaptic vesicles is slower (13). At present there is no clear agreement concerning the optimum frequency of electrical stimulation (high or low) and duration times of application. Some studies have used high frequencies (27), some low (18), while others have compared high and low frequencies (19, 29). Frasson et al. (19) showed that low-frequency stimulations lead to a faster and longer lasting reduction of CMAP compared with high-frequency stimulations. The lower efficacy of high-frequency stimulations can be explained by the hypothesis that prolonged high-frequency stimulation may reduce excitability at the site of the nerve fibre stimulation.

Eleopra et al. (18) injected low doses of BT-type A (Botox®, 3 IU) in EDB muscle in adult subjects with normal EDB muscles. They applied electrical stimulation with a frequency of 4 Hz for 24 h immediately after BT injection in the treated muscle. After 7 days they found an mean CMAP% of 17 for the stimulated muscle, and 33 for the non-stimulated muscle. Fifteen days after BT injection the values were 22 and 40, respectively; and on the 30th day the values were 30 and 42, respectively.

The results of our study show that the reducing trend in CMAP% amplitude for the side stimulated at low-frequency following BT injection is comparable to that shown by both Eleopra et al. (18) and Frasson et al.(19) in low-frequency stimulation muscle.

Previous studies of botulinum toxin treatment combined with electrical stimulation have involved spastic and larger muscles and different application times of electrical stimulation.

The threshold intensity of electrical stimulation for each subject was based on visible muscle contraction (6, 19). Thus, larger and smaller muscles can both be treated in the same way using the same application times. The necessary condition is that the whole muscle has to be stimulated.

Esquenazi & Mayer (20) examined elbow flexor and ankle plantarflexor muscles in patients with spasticity. Using clinical methods they found an increased duration effect of of BT in those patients who had received active electrical stimulation.

Different application times of nerve stimulation were used in previous studies, for example, 24 h continuous stimulation (18), 30 min per day for 5 days (19), 30 min 6 times per day for 3 days (6), 30 min twice daily for a month (20), or other combinations. Our study used a shorter stimulation time.

Immunological studies (11, 16) have shown that after 20 min antitoxin-antibodies exert no further inhibitory action on BT. Therefore, by the end of this period BT has already been completely internalized.

Ravichandran et al. (16) injected a specific antiserum in rats treated with BT, and found that when neutralizing antibodies were mixed with BT prior to BT injection, BT neurotoxicity was abolished. When antibodies are used after BT injection, their ability to neutralize BT decreases progressively over time. After 20 min there is no further antibody action.

In comparison with the results of Eleopra et al. (18), we conclude that a short-lasting electrical stimulation applied after BT injection does not result in any substantial difference from that of a longer lasting stimulation.

Considering our objective, if we compare the results obtained using electrical stimulation of the right EDB muscle for 20 min before BT injection, and of the left EDB muscle for 20 min immediately after BT injection, in both cases a significant reduction in CMAP% amplitude was obtained by the 7th day. However,

the reduction was more evident on the left, post-stimulated side, being almost double that of the opposite side.

Considering that motor units with a higher activity are able to internalize a larger quantity of toxin, 20 min of electrical stimulation, applied either before or after BT injection, may increase the efficacy of BT.

This method could be used in clinical settings to reduce the amount of BT required and thus improve the relationship between therapeutic outcome and side-effects.

We conclude that BT injection followed by short-term electrical stimulation could have the same effect in both healthy and spastic muscles. We do not yet have sufficient data on the effect of electrical stimulation before BT injection to be able to generalize our results from healthy subjects to spastic and weak patients. Electrical stimulation before BT injection may have different effects in spastic and healthy muscles due to the different muscular activity.

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