

**TIME COURSE OF FUNCTIONAL RECOVERY OF SPASTIC SKELETAL MUSCLE AFTER APPLICATION OF BOTULINIUM TOXIN (DYSPORE)**

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*Botulinum toxin is successfully used in human and veterinary medicine. There are opposing opinions regarding the skeletal muscle recovery time after intramuscular administration of botulinum toxin and also disagreements regarding the frequency of application and the use of optimum doses which lead to improvement in patients with spastic cerebral palsy.*

*The objective of this research is to determine the time of skeletal muscle functional recovery, the frequency of application and the optimal dose of botulinum toxin in patients with cerebral palsy. The research was conducted in 30 patients with an average age of 16.1 years. The assessment of functional recovery time was made by monitoring the electromyoneurographic parameters and by determination of the muscle mass and strength. The assessment of frequency of application and optimal dose was based on the evaluation of degree and frequency of spasticity, range of motion, walking function, before and after botulinum toxin application in specified intervals.*

*We recommend the application of botulinum toxin in intervals of 3-6 months. The dose of 10 MU/kg of body mass proved to be optimal.*

*Key words: botulinum toxin, electromyoneurography, cerebral palsy, electromyoneurography, skeletal muscle*

## INTRODUCTION

Botulinum toxin (BT) is a protein produced by the anaerobic microorganism *Clostridium botulinum*. Once the spores reach an anaerobic environment they germinate and develop into microorganisms that excrete a protein substance, a toxin known to be the cause of poisoning (botulism), also used as a biological weapon. This is the substance with the highest toxic potential known in nature.

The main effect of this toxin is the blockage of cholinergic synapses, which causes the organs and tissues innervated by cholinergic synapses to stop

functioning. Thus, paralysis of smooth and striated muscles, as well as some exocrine glands occurs.

Botulinum toxin was first isolated in 1900. In 1919 it was discovered that there are two types of BT (A and B). In 1970 Scott performed the first experiment of implementation of BT on animals, and in 1980 it was implemented for the first time in human medicine, first in the treatment of strabismus, then blepharospasm, and very soon also in the treatment of muscle dystonia and spasm (Erbguth and Naumann, 2000; Lang, 2004).

Botulinum toxin is used extensively on global scale in the treatment of various disturbances in a growing number of medical and veterinary disciplines.

Structural contractures, fixed deformities of the joints and associated movement restriction are counterindications for the application of BT. One of the main reasons for the possible failure of therapy is the synthesis of antibodies to BT. BT antibodies have been proved by a bioassay on experimental animals and by ELISA test. The development of antibodies has been registered in 5 - 10% of patients with long-term BT application (Jankovic and Schwartz, 1995). Prevention of development of antibodies may be achieved by avoiding the administration large individual doses. The minimum time between two applications cannot be less than 3 months. Caution is required for females because a greater percentage of incidences of developing antibodies was registered compared to males. The possible causes of the appearance of antibodies can also be a poorly planned therapy and inadequate dosing (Dressler *et al.*, 2002).

It can be concluded from the available references that there are opposing opinions regarding the skeletal muscle recovery time after intramuscular administration of botulinum in patients with cerebral palsy. There are also disagreements regarding the frequency of application and the use of optimum doses which lead to improvement of subjective and objective symptoms (Koman *et al.*, 1994; Wissel *et al.*, 1999; Mancini *et al.*, 2005; Lowe *et al.*, 2006).

The objective of this research was to determine the skeletal muscle recovery time in patients with spastic cerebral palsy after application of BT, the optimum time period between applications, as well as the dose that will lead to the reduction of the degree and frequency of spasticity, and subsequent increase of the range of movement and improvement of walk functions.

#### MATERIAL AND METHODS

Intramuscular application of BT type A (Dysport) on certain muscle points was implemented in 30 patients handicapped by spastic cerebral palsy.

From the total number of analyzed patients 18 were male and 12 female. The average age of the patients was 16.1 years (11-20). Nine patients from each group had hemiplegic and paraplegic forms of cerebral palsy, and six patients diplegic and quadriplegic forms of cerebral palsy.

The botulinum application was conducted according to the following procedure: a bottle of Dysport was dissolved in 1 or 2.5 mL physiological solution yielding a solution containing 500 or 200 MU/mL. (MU -mouse unit, namely mean

lethal intraperitoneal dose for a mouse -LD50) was injected directly into the muscle, at the point of greatest spasticity localized by palpation (Figure 1).

A total of 39 ampoules of Dysport (19500 MU) was applied on 30 patients into 99 points (Table 1).

The average dose per point of application was 200 MU, i.e. 10 MU/kg of body weight. Immediately after injection the corrective splints (orthoses) were applied to the patients. Physical procedures were started after 5 - 7 days.

The time of skeletal muscle functional recovery after application was assessed by analysis of electromyoneurographic (EMNG) parameters and measurements of muscle mass and strength.

A detection EMNG was performed prior to application in all patients, and then at intervals of 3 days, 7 days, 14 days, 6 weeks, 3 months, 6 months and one year after the BT application. The insertion activity and terminal latency were observed and the amplitude and duration of action potential were studied in the EMNG analysis. We used "Meditronic Keypoint 4" apparatus.

The muscle mass was measured in centimeters (cm) on respective parts of the extremities. The muscle strength was measured by a dynamometer prior to botulinum application, 7 days, 14 days, 6 weeks, 3 months, 6 months and a year after botulinum application.

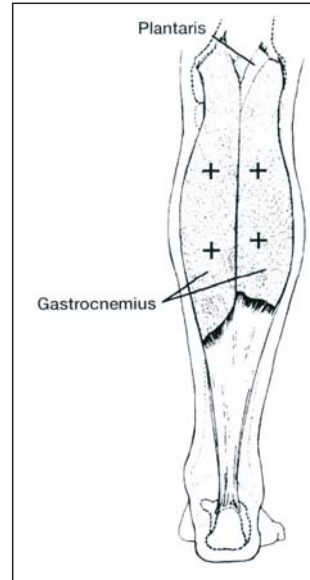


Figure 1. Points of application of Botulinum toxin into the Gastrocnemius muscle

Table 1. Muscles and number of applications

Muscles		Number of applications (points)
Hip adductors		12
Medial hamstrings		36
M. biceps femoris		24
M. gastrocnemius	pars medialis	12
	pars lateralis	12
M. tibialis posterior		3

Determination of the optimum time between two applications, as well as the effects of the applied dose of 10 MU/kg was estimated on the basis of analysis of clinical indicators: degree and frequency of spasticity, range of passive and active motion and walking function of the patients. The degree of spasticity was

determined according to the modified Ashwort scale, before application, and in intervals of 7, 14 days, 6 weeks, 3 and 6 months and a year after application (Bergfeldt *et al.*, 2006). The scale of spasticity was used to measure the average daily spasm before and within the intervals after BT application: 0 (no spasticity), 1 (one spasm a day), 2 (between 1 and 5 spasms a day), 3 (between 5 and 9 spasms a day), 4 (10 and more spasms a day).

Range of passive and active motions in the joints was determined.

The evaluation of walk was made on the basis of functional mobility scale (FMS) (Kerr *et al.*, 2004). The average follow-up of the patients was 24 months (15–38).

## RESULTS

The existence of spontaneous discharge of polyphase action potentials during the insertion of the needle electrode was determined in all patients (insertion activity) by EMNG tests prior to application of BT. On follow-up examination 3 days later, there was a significant change in EMNG findings in all tested patients, with a significant reduction of spontaneous discharge during the insertion activity, extension of terminal latency and reduction of amplitude and duration of action potential. The measurement showed that the maximum extended terminal latency has been achieved on the third day after application, when it was about 43% longer than the average measured before application. Afterwards, it became gradually shorter and returned to the usual value 6 months to one year after application. Contrary to this, the changes in the amplitude and duration of the action potential were the greatest on the seventh day after application, and returned gradually to the initial values within a period of 6 months to one year (Figure 2).

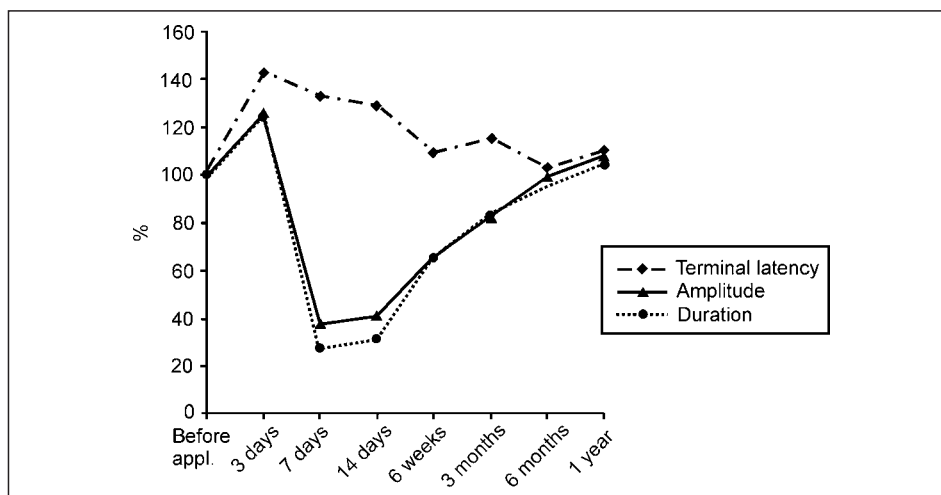


Figure 2. Terminal latency, amplitude and duration of action potential

The muscle mass reached the minimum about the 7<sup>th</sup> day after application and remained at the achieved level up to 3 months after the application, then it gradually increased and reached the values before the application within one year. The muscle strength reached its minimum also on day 7, then grew rapidly until about 3 months after application, only to be followed by a very slight increase within one year after application (Figure 3).

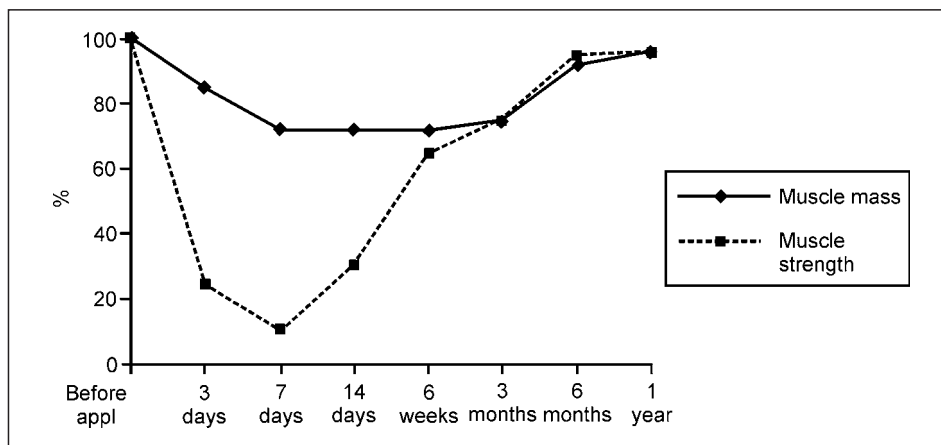


Figure 3. Muscle mass and strength

The degree of spasticity before BT application was graded 2 in 78 muscles according to the modified Ashwort scale and it was graded 3 in 21 muscles. Six weeks after application the spasticity degree in 18 muscles was graded 1, in 42 it was graded 1+, in 24 it was graded 2, and in 15 it was graded 3 according to the modified Ashwort scale.

The spasticity frequency before application had been between 2 and 3, and a week after application from 0 to 1. It remained at this level up to three months after application, when it gradually increased up to 6 months from application. After this period a sudden increase of spasticity frequency was marked in almost all tested patients.

The passive range of motion was improved in all patients seven days after application. The active range of motion was improved two weeks after application in 21 patient. The unchanged range of motion remained in 9 patients. Three months after the application there was a reduction of the active range of motion of 10% in 18 out of 21 patients. After six months reduction was 25%, while the passive range of motion remained unchanged in all patients up to six months after application.

Six weeks after application walking improved in 18, and remained unchanged as before the application in 9 patients (3 patients did not walk either before or after the application). The effect of walk improvement is gradually waning and a significant aggravation is observed after three months. After six

months the walk pattern in almost all patients was as it had been prior to application.

#### DISCUSSION

In the assessment of time of functional recovery the following electromyoneurographic parameters were monitored: terminal latency, amplitude and duration of action potentials). Muscle mass and muscle strength were evaluated.

A significant extension of the terminal latency in our study begins as early as the third day after application and is maintained up to six months (Figure 2).

The amplitude and duration of the action potential reached the lowest value on the 7<sup>th</sup> day after application, thereof the values increased gradually and normalized between six months and a year after application (Figure 2). This normalizing of values coincides with the establishment of initial neurotransmission and cessation of impulse transfer through the newly created nerve sprout terminals.

The maximum degree of muscle atrophy is achieved 14 days after application, when the muscle mass is reduced to about 70% of the initial mass. Some authors state that a greater muscle mass loss, as far as 42% of the initial value, is experienced within the period of 14 days (Adler *et al.*, 1996; Adler *et al.*, 2001; Ma *et al.*, 2004). In the researches published by these authors the experiments were made on animal models, with the use of different doses, as well as a different pharmacological form of BT. However, in most of the studies, as well as in ours, it is confirmed that the maximum muscle atrophy occurs approximately on the 14<sup>th</sup> day after application. There is still not a valid explanation for the appearance of this phenomenon two weeks after application. It is obvious that up to the third month after application there is a plateau, namely, no significant increase in muscle mass, and then it begins to grow and this growth is most pronounced in the period between the third and sixth month. In the following six months it slowly approaches the initial value which is achieved up to one year after application (Figure 3). The slow recovery of the muscle mass in the period between the 2<sup>nd</sup> week and the third month can be explained by the changes in neurotransmission. Earlier studies show that the terminal branching of the nerves towards the neuromuscular plates is responsible for neurotransmission during the first month after application. The original innervation is then gradually established and the newly crated terminal sprouts disappear. The proliferation, and then elimination of terminal nerve sprouts could be the explanation of the slow recovery of muscle mass in the period until 3 months after the application of BT (De Paiva *et al.*, 1999).

Based on the obtained results it can also be observed that there is a significant reduction of muscle strength up to 3 months after application (Figure 3). The terminal nerve sprouts created by branching are thin and not myelinated, have a higher threshold of action potential and conduct the impulses more slowly. For this reason it is necessary to apply a stronger (supermaximum) stimulus to the

muscle in order to achieve the trigger threshold. After establishing the original innervation the muscle strength also returns to its original value.

When evaluating the interval between applications and the optimal dose we have measured the degree and frequency of spasticity, range of passive and active motions and analyzed walk.

Based on the obtained results of the degree and frequency of muscle spasticity it can be concluded that a significant improvement is achieved very soon after application, and that the achieved effect is maintained for a period of up to six months.

Better results are achieved in the passive range of motion which occurs somewhat earlier compared to the active range of motion. The effect after BT application is maintained longer in the passive range of motion than in the active range of motion, where a certain aggravation can be observed already three months after application, and after six months there is a 25% reduction of the active range of motion. The passive range of motion is maintained at the achieved level without significant changes six months after application of BT.

This study shows that the effect of BT on the walking function remained at a satisfactory level between three and six months after application.

Generally, the effects of BT on the observed clinical parameters are maintained for a quite a long time. The effects on most of the parameters last between three and six months. There are two possible explanations for the duration of BT A effects. First, the toxin persists in the end nerve sprouts, which enables it to remain proteolytically active over a longer time period (Bartels *et al.*, 1994; De Paiva *et al.*, 1999; Adler *et al.*, 2001). Secondly, the sections of SNAP – 25 fragment (synaptosome associated protein of 25 kDa) that are split by the effect of the toxin are unable to be degraded from the terminal nerve sprouts, and thus prevent binding of new SNAP-25 molecules (Eleopra *et al.*, 1998; De Paiva, 1999; Raciborska and Charlton, 1999).

The BT dose in the treatment of cerebral palsy in clinical practice is based on the body weight of the patient. Although some authors suggest that the dose of 6 MU/kg of body mass is optimal for the treatment of spasticity in children with cerebral palsy, the average doses applied in our study are about 10 MU/kg of body mass (Graham *et al.*, 2000). Some authors state that greater doses can result in a long-term effect on the neuromuscular transmission (Borodic *et al.*, 1994; Billante *et al.*, 2002).

Clinical and electromyoneurographical examinations after BT application show that the true effects appear between the 3<sup>rd</sup> and 7<sup>th</sup> day after application. After the BT application a skeletal muscle in patients with cerebral palsy recovers during the period between three and six months (exceptionally up to 9 months) after application. After that the transmission of impulses to the neuromuscular synapses is reestablished, which can be monitored by following up the normalizing of EMNG parameters and muscle mass and strength parameters which return to the state before BT application.

In order to maintain the achieved effects, the application has to be renewed within a period between 3-6 months from the previous application. The dose of 10 MU/kg body mass seems to be optimal in patients with spastic cerebral palsy



because it led to improvement of all monitored clinical parameters. With this dose, the period between applications was between three and six months. Therefore, frequent applications which could lead to creation of antibodies to BT were avoided.

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## **VREME FUNKCIONALNOG OPORAVKA SPASTIČNOG SKELETNOG MIŠIĆA NAKON APLIKACIJE BOTULINUM TOKSINA (DYSPORT)**

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### SADRŽAJ

Botulinum toksin se danas uspešno primenjuje u humanoj i veterinarskoj medicini. Postoje oprečna mišljenja u pogledu vremena oporavka skeletnog mišića nakon intramuskularnog davanja botulinum toksina kao i neslaganja u pogledu učestanosti aplikacija i primene optimalnih doza u cilju postizanja poboljšanja stanja pacijenata sa spastičkom cerebralnom paralizom.

Cilj ovog istraživanja je bio određivanje vremena funkcionalnog oporavka skeletnog mišića nakon aplikacije botulinum toksina kod pacijenata sa cerebralnom paralizom, kao i određivanje učestanosti aplikacija i optimalne doze. Istraživanje je sprovedeno na 30 pacijenata prosečne starosti od 16,1 godine. Procena vremena funkcionalnog oporavka vršena je praćenjem elektromioneurografskih parametara i određivanjem mišićne mase i snage. Učestanost aplikacija i optimalna doza procenjavani su na osnovu evaluacije kliničkih parametara: stepena i frekvencije spazma, obima pokreta i funkcije hoda, u određenim intervalima pre i nakon aplikacije botulinum toksina.

Kao optimalno rešenje preporučuje se aplikacija botulinum toksina u intervalima od 3-4 meseca u dozi od 10 MU/kg telesne mase.