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Comparison of analgesic effects of single vs. repeated injection of botulinum toxin A in orofacial formalin test

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Abstract

Long-term effectiveness and repeated administration of botulinum toxin A are the basis for its use in both neuromuscular disorders and certain painful conditions. Botulinum toxin A has been recently approved for migraine treatment, and its off-label use extends to other craniofacial pain disorders. However, recently it was reported that, after repeated injection, botulinum toxin loses its antinociceptive efficacy in rats. In present study with a similar design we compared the effects of single and repeated injections of botulinum toxin in formalin-induced orofacial pain. No statistically significant differences were found between single or repeatedly treated animal groups. Our results are in line with clinical experience and suggest that botulinum toxin can be re-administered in orofacial pain treatment.

1. Introduction

Botulinum toxin A (BTX-A) is used in neuromuscular and autonomous disorders characterized by cholinergic hyperactivity, and in treatment of some forms of pain (Jankovic et al., 2004; Jabbari and Machado, 2011). Important for its clinical application is the long-term effectiveness and repeated use (Naumann et al., 2006). Apart from its off-label use in various painful conditions such as peripheral neuropathies, low back pain or various types of headache (Allam et al., 2005; Dodick et al., 2005; Foster et al., 2001; Jabbari and Machado, 2011). BTX-A has been recently approved for chronic migraine treatment (Dodick et al., 2010).

In experimental animals it has been shown that BTX-A exhibits long-term reduction of inflammatory acute pain, neuropathic pain, visceral and deep somatic pain (Cui et al, 2004; Bach-Rojecky and Lackovic, 2005; Bach-Rojecky et al., 2005; Chuang et al., 2004; Krug et al., 2009).

In a recent report its effectiveness was demonstrated in orofacial formalin pain 8 days after the BTX-A pretreatment. However, when toxin was re-administered 42 days after the first treatment, the effect of BTX-A was not reproduced (Piovesan et al., 2011). Mentioned report that BTX-A loses its analgesic effect upon repeated injection in experimental animals is potentially very important for its clinical use in migraine and in some other chronic pain conditions, where repeated use is of high importance (Naumann et al., 2006).

Aim of this study was to compare the effects of single or repeated injections of BTX-A in formalin-induced orofacial pain. In present study we did not find the difference between single and repeated injections of BTX-A.

2. Materials and methods

2.1 Animals

Adult male Wistar rats (University of Zagreb School of Medicine, Croatia) weighing 300 g at the beginning of experiment were used. Animals were kept in 12 h/ 12 h light and dark cycle, with unlimited access to food and water. Experiment was conducted according to the European Communities Council Directive (86/609/EEC) and recommendations of the International Association for the Study of Pain (Zimmerman, 1983). Animal procedures were approved by the Ethical Committee of University of Zagreb School of Medicine (permit No. 07-76/2005-43).

2.2 Drugs

Botulinum toxin type A (Botox®, Allergan Inc., Irvine, CA, USA) was dissolved in 0.9% NaCl. 5 U/kg dose was administered unilaterally as 20 µl bolus into the whisker pad tissue

using a 27 1/2 gauge needle. 20 µl 0.9% saline solution was injected to control rats. Facial formalin injection was carried out using 50 µl of saline-diluted 2.5% formalin (Kemika, Zagreb, Croatia).

2.3 Treatment and behavioral testing

Three months old adult rats were divided into three groups, each consisting of five to six animals. First group of six conscious rats was injected with BTX-A into the left whisker pad tissue, while other two groups were left untreated. After 42 days, first group of rats underwent their second treatment of BTX-A, while the second and third group were injected with BTX-A and saline, respectively. Period of 42 days between the two BTX-A injections was chosen based on study of Piovesan et al. (2011).

After a period of six days, orofacial formalin test was performed to assess the antinociceptive activity of BTX-A. Animals were placed inside transparent chambers for 10-minute acclimatization period. Following the acclimatization, conscious, restrained rats were injected with formalin into the whisker pad ipsilateral to BTX-A pretreatment and immediately returned to the transparent chamber for a 45-minute observation period. Observers were blind to the animal treatment. Observation period was divided into 15 blocks of 3 minutes, and the number of seconds the animal spent in ipsilateral face rubbing or grooming was measured during phase I (0-12 min.) and phase II (12-45 min.) of formalin-induced pain (Raboisson and Dallel, 2004)..

2.4 Statistical analysis

Data were represented as mean +- SEM, and analyzed by one-way ANOVA followed by Tukey's HSD test ($p<0.05$).

3. Results

Injection of BTX-A into whisker pad resulted in reduced movement of ipsilateral whiskers and their backward direction (Figure 1). In group receiving two injections of BTX-A no visible reduction of whisker movement was visible 42 days after the first treatment (prior to second BTX-A injection). Whisker paralysis after second BTX-A injection occurred again, as proof of efficiency of re-administered BTX-A (Figure 1C).

In orofacial formalin test, phase I of rubbing/ grooming behavior was not affected by BTX-A treatment, while phase II in both BTX-A treated groups revealed significant reduction in number of seconds spent rubbing/grooming, in comparison with control (Figure 2). There was no statistically significant difference in time of facial grooming between the animals administered once or twice with BTX-A (Figure 2).

4. Discussion

According to experimental results recently published by Piovesan et al. (2011), BTX-A injections 8 days before formalin test inhibit orofacial pain. However, repeated treatment after 42 days had no effect. This observation could have far reaching clinical consequences because it questions the usefulness of repeated injections of BTX-A in pain conditions. Basically, it is also intriguing because it might suggest that, after single injection, BTX-A makes permanent functional changes. Such putative changes might be connected either with mechanism of its uptake by peripheral sensory nerves mediated by SV2 proteins (Mahrhold et al., 2006), or with the mechanism of antinociceptive action, which is connected with axonal transport from periphery to central sensory nuclei, and likely with central SNAP-25 cleavage (Bach-Rojecky et al., 2009, Matak et al., 2011, Filipović et al., 2012). It might also suggest immunoresistence to BTX-A or possibly yet unknown long-term effect on synaptic plasticity caused by BTX-A.

However, in contrary to results from Piovesan et al. (2011), both single and repeated injection of BTX-A were equally effective in our study, which is in line with clinical experience in pain treatment. It also suggests that BTX-A does not induce permanent functional changes which could lead to ineffectiveness of repeated injection. Presence of ipsilateral whisker paralysis after second injection of BTX-A verifies that no immune resistance towards BTX-A occurred and the second bolus showed equal effectiveness on neuromuscular junction as in animals that were injected only once, which is in agreement with repeated clinical use in muscular disorders (Naumann et al., 2006).

There are few basic differences between the two studies performed. Adult rats of the same age were used in our experiment, whereas the animals used in study from Piovesan et al. (2011) were juvenile when treated with BTX-A and tested with formalin for the first time. In the aforementioned study, formalin was applied twice to the same experimental animals. Cellular protein precipitation and possible permanent peripheral nerve ending damage in rats treated with formalin upon the first nociceptive testing might have interfered with second nociceptive testing or BTX-A uptake into the sensory neurons after second injection. Therefore, in our study the formalin test was conducted only once in all experimental groups. As described in previous literature phase I of the formalin test, caused by direct stimulation of nerve endings with formalin, is measured for the first 12 minutes of the test, while phase II represents central sensitization induced by peripheral nerve activity and is measured for the next 33 minutes (12-45 min) (Raboisson and Dallel, 2004). In our experiments, formalin-induced pain was measured during the whole period of 45 min while Piovesan et al. (2011) measured pain for 30 minutes only.

Our results did not confirm recent preclinical study from Piovesan et al. (2011), and suggest that BTX-A can be re-administered in orofacial pain and probably in BTX-A treatment of other forms of pain.

REFERENCES

Allam N, Brasil-Neto JP, Brown G, Tomaz C (2005) Injections of botulinum toxin type A produce pain alleviation in intractable trigeminal neuralgia. Clin J Pain 2: 182-184

Bach-Rojecky L, Lacković Z (2005) Antinociceptive effect of botulinum toxin type A in rat model of the carrageenan and capsaicin induced pain. Croat Med J 46: 201-208

Bach-Rojecky L, Relja M, Lacković Z (2005) Botulinum toxin type A in experimental neuropathic pain. J Neural Transm 112: 215–219

Bach-Rojecky L, Lacković Z (2009) Central origin of the antinociceptive action of botulinum toxin type A. Pharmacol Biochem Behav 94: 234-238

Chuang YC, Yoshimura N, Huang CC, Wu M, Chiang PH, Chancellor MB (2008) Intraprostatic botulinum toxin a injection inhibits cyclooxygenase-2 expression and suppresses prostatic pain on capsaicin induced prostatitis model in rat. J Urol 180: 742-748

Cui M, Khanijou S, Rubino J, Aoki KR (2004) Subcutaneous administration of botulinum toxin A reduces formalin-induced pain. Pain 107: 125-133

Dodick DW, Mauskop A, Elkind AH, DeGryse R, Brin MF, Silberstein SD; BOTOX CDH Study Group (2005) Botulinum toxin type A for the prophylaxis of chronic daily headache: subgroup analysis of patients not receiving other prophylactic medications: a randomized double-blind, placebo-controlled study. Headache 45: 315-324

Dodick DW, Turkel CC, DeGryse RE, Aurora SK, Silberstein SD, et. al.on behalf of the PREEMPT Chronic Migraine Study Group (2010) OnabotulinumtoxinA for treatment of chronic migraine: Pooled results from the double-blind, randomized, placebo-controlled phases of the PREEMPT Clinical Program. Headache. 50: 921-936

Filipović B, Matak I, Bach-Rojecky L, Lacković Z (2012) Central action of peripherally applied botulinum toxin type a on pain and dural protein extravasation in rat model of trigeminal neuropathy. PLoS One 7: e29803

Foster L, Clapp L, Erickson M, Jabbari B (2001) Botulinum toxin A and chronic low back pain: a randomized double blind study. Neurology 56: 1290–1293

Jabbari B, Machado D (2011) Treatment of refractory pain with botulinum toxins- an evidence-based review. Pain Med 12: 1594-1606

Jankovic J (2004) Botulinum toxin in clinical practice. J Neurol Neurosurg Psychiatry 75: 951-957

Krug HE, Frizelle S, McGarraugh P, Mahowald ML (2009) Pain behavior measures to quantitate joint pain and response to neurotoxin treatment in murine models of arthritis. Pain Med 10: 1218-1228

Mahrhold S, Rummel A, Bigalke H, Davletov B, Binz T (2006) The synaptic vesicle protein 2C mediates the uptake of botulinum neurotoxin A into phrenic nerves. FEBS. Lett 580: 2011-2014

Matak I, Bach-Rojecky L, Filipović B, Lacković Z (2011) Behavioral and immunohistochemical evidence for central antinociceptive activity of botulinum toxin A. Neuroscience 186: 201–207

Naumann M, Albanese A, Heinen F, Molenaers G, Relja M (2006) Safety and efficacy of botulinum toxin type A following long-term use. Eur J Neurol Suppl 4:35-40

Piovesan EJ, Leite L da Silva, Teive HG, Kowacs PA, Mulinari RA, Radunz V, Utiumi M, Campos HG, Werneck LC (2011) Botulinum toxin type-A effect as a preemptive treatment in a model of acute trigeminal pain -A pre-clinical double-blind and placebo-controlled study. Arq Neuropsiquiatr 69: 56-63

Raboisson P, Dallel R. The orofacial formalin test (2004) Neurosci Biobehav Rev 28: 219–226

FIGURE LEGENDS

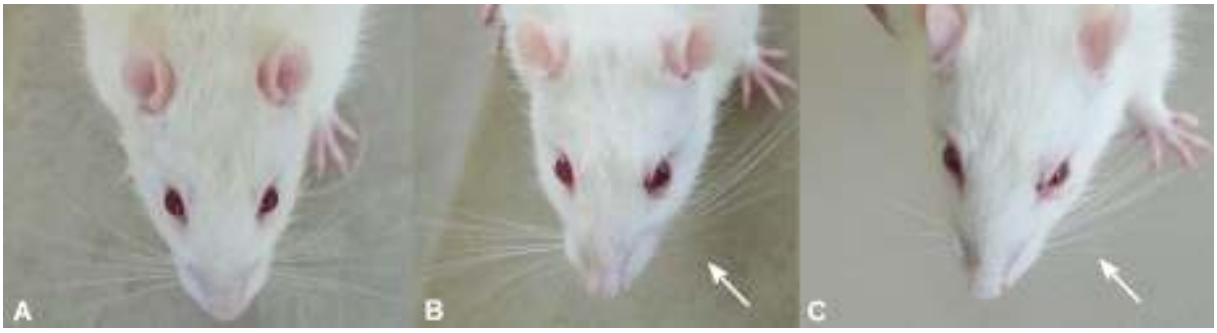

Figure 1 Whisker appearance 1 day after administration of A) physiological saline, B) BTX-A first injection, C) BTX-A second injection. Saline and BTX-A were administered to the left whisker pad.

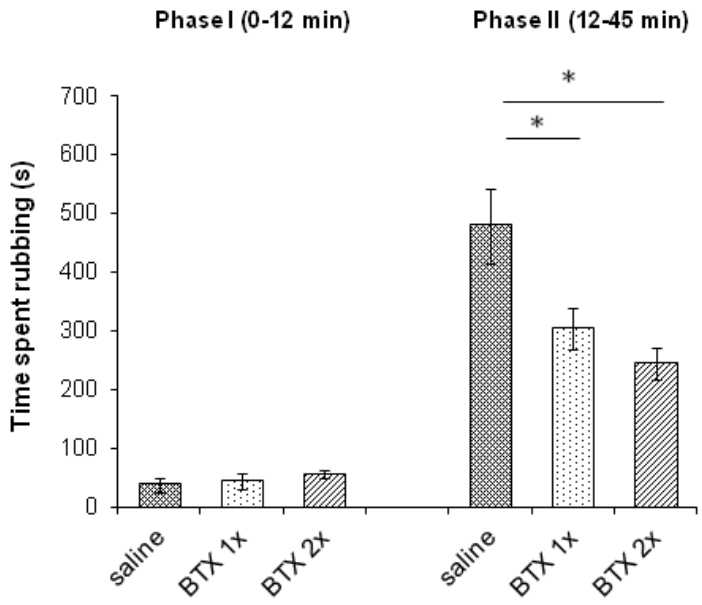

Figure 2 Repeated BTX- A reduces orofacial pain induced by 2.5% formalin injection into the whisker pad. Animals were pretreated into ipsilateral whisker pad one time or two times with 5 U/kg BTX-A (42 days period between the two injections). Nociceptive testing was performed 6 days following the single or second, repeated injection of BTX-A. Data are represented as mean+-SEM, * - $p<0.05$ (Tukey's HSD test).