Botulinum toxin type A in experimental neuropathic pain

Short Communication

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Summary. A peripheral application of botulinum toxin type A (7 U/kg) has significantly reduced thermal and mechanical hypersensitivity in rats with the partial sciatic nerve transection as a classical model of surgical neuropathy.

Keywords: Botulinum toxin, neuropathic pain, rat, antinociception.

Introduction

Botulinum toxin A (BTX-A) paralyses skeletal muscles by proteolytic cleavage of one of the proteins termed SNAP-25 which is essential for vesicle post-docking and fusion prior to acetylcholine release (Aoki, 2001). Accordingly, over the last 20 years, BTX-A has been used as a treatment for a variety of disorders characterized by increased muscle contraction.

Recent clinical observations suggested that BTX-A alleviates pain caused or associated with increased muscle tonus. Hence, according to open-label and some double-blind studies, BTX-A treatment was found to be effective in chronic tension-type headaches (Relja and Telarovic, 2004; Schulte-Mattler and Krack, 2004), cervical dystonia (Lu et al., 1995), chronic low back pain (Foster et al., 2001), pain of the musculoskeletal origin (Sheen, 2002) and piriformis syndrome (Childers et al., 2002; Fishman et al., 2002). Contrary to these studies, there are only a limited number of case reports suggesting that BTX-A might be useful in pain which is not associated with a muscle spasm, particularly in neuropathic pain (Klein, 2004). In preclinical experiments, BTX-A was heretofore investigated only in the formalin (Aoki, 2003; Cui et al., 2004) carrageenan and capsaicin models of pain (Bach-Rojecky and Lackovic, 2005).
The mechanism of putative antinociceptive action of BTX-A is poorly understood (Bach-Rojecky et al., 2004; Silberstein, 2004). So far, it has been suggested that the toxin inhibits the release of several neurotransmitters involved in neurogenic inflammation, i.e. glutamate and substance P, thus reducing peripheral sensitization (Aoki, 2003). Since nerve injury is able to increase the release of peripheral inflammatory mediators, peripheral BTX-A treatment might theoretically alleviate some of the pain symptoms of neuropathy. In the present paper, we report on the efficacy of BTX-A on the peripheral neuropathy induced by partial nerve transection in rats.

**Materials and methods**

**Animals**

Male Wistar rats (Medical School, Zagreb, Croatia) weighing about 200 g were used in all experiments. The experiments were carried out in accordance with the Croatian law on animal welfare. The “Principles of Laboratory Animal Care” (NIH Publication No. 86-23, 1985) were followed.

**Drugs**

Botulinum toxin type A (BOTOX, Allergan, USA) was dissolved in 0.9% NaCl. BTX-A 7 U/kg in a volume of 20 μl was injected into the dorsal surface of the rats’ hind paw.

**Methods**

**Surgical neuropathy**

Under general anesthesia (chloral hydrate 300 mg/kg), rats were subjected to the partial right sciatic nerve transection as described by Lindenlaub and Sommer (2000). On the left, contralateral side of these animals, as well as in the control animals, a sham operation was performed. Two weeks following the peripheral nerve injury, only animals which developed neuropathy, i.e. became hypersensitive to the thermal and mechanical stimuli (at least 20% change from the mean of the sham-operated group), were included in further experiments (Coudore-Civiale et al., 1998). Each group consisted of 5–7 animals, which were peripherally injected with either botulinum toxin type A 7 U/kg or saline. Nociceptive measurements began 24 h following the drug treatment. The dose of BTX-A was based on our preliminary experiments. In the first experiment we found that 3 U/kg of BTX-A failed to reduce enhanced sensitivity to mechanical stimuli in neuropathic animals on day 5 after the toxin application while higher dose of the toxin (4 U/kg) increased the pain thresholds for 44.6 ± 7.6% compared to untreated neuropathic rats while in second experiment we observed that 4 and 7 U/kg of BTX-A are equally potent (31.3 ± 6.1% vs. 31.8 ± 7.9%). Cui et al. (2004) also found that 7 U/kg of BTX-A is effective in the formalin model of experimental pain. Accordingly a dose of 7 U/kg was used in present study.

**Modified unilateral hot plate test**

The latency time for paw withdrawal from the hot-plate surface (52 ± 0.5°C) was recorded (Baamonde et al., 2002).

**Paw pressure test**

The rats underwent the modified paw-pressure test (Randall and Selitto, 1957). Nociceptive thresholds were measured following the application of gradually increasing pressure (expressed in grams) to the hind-paw until paw-withdrawal or overt struggling was elicited.