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SHORT COMMUNICATION

Lasting reduction of postsurgical hyperalgesia after single injection of botulinum toxin type A in rat

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ABSTRACT

A single injection of low doses of botulinum toxin type A (3.5 U/kg) completely abolished secondary mechanical hyperalgesia throughout its duration in a model of post surgical pain after gastrocnemius incision in rat.

INTRODUCTION

Nearly 50% of surgical patients suffer from intense post surgical pain [1]. Opioids and non-opioid drugs are used to alleviate this problem, but it persists to be an issue.

The most common experimental approach to the study of postsurgical hyperalgesia is the incisional model of pain [2]. Diverse drugs reduce incision-induced mechanical hyperalgesia in rats, but only morphine has been proven to be 100% effective, with results lasting only for a few hours [3].

There is a continually rising number of reports suggesting long-lasting antinociceptive effect of botulinum toxin type A (BTX-A) in both humans and animals [4,5]. It is generally assumed that the mechanism of BTX-A-induced antinociception might be the prevention of neurotransmitter release from the primary sensory neurons [6]. Here, we report that single injection of BTX-A completely abolished secondary hyperalgesia after gastrocnemius incision in rats.

MATERIALS AND METHODS

A total number of 50 male Wistar rats (300–350 g) were included in the study. The Principles of Laboratory Animal Care (NIH Publication 86-23, 1985) were followed, and approval was granted by the Ethical Committee, University of Zagreb School of Medicine.

Drugs

Botulinum toxin type A (BOTOX; Allergan, Irvine, CA, USA); chloral hydrate and ethanol (Sigma, St Louis, MO, USA).

Forty-five animals were anaesthetized by a single intraperitoneal injection of chloral hydrate (300 mg/kg). A 3-cm longitudinal incision was made through the skin of the midportion of the posterior hind limb starting 1–1.5 cm from the edge of the heel and extending to the popliteral region [2]. Longitudinal incision of gastrocnemius muscle (parallel with muscular fibers) was done. After hemostasis, the skin was sutured. Sutures were removed 3 days after the procedure.

Five unoperated rats served as control. Sensitivity to mechanical stimuli was measured in terms of paw withdrawal after painful pressure as described by Randall and Selitto [7]. The measurements were performed three times in 10-min intervals. The experimenter was unaware of the treatment groups. Animals which developed secondary hyperalgesia (27 out of 45)

after 24 h (paw-withdrawal threshold reduced for at least 35% compared to the unoperated controls) were injected subcutaneously either with saline or BTX-A (1, 3.5 and 7 U/kg) into the plantar surface of the hindpaw pad (in a volume 20 μL). In the time-course experiment, mechanical sensitivity was measured on day 1, 6, 10 and 14 following the BTX-A injection.

Results are presented as mean ± SE. Statistical analysis was performed using ANOVA and Newman–Keuls post hoc test. In the time-course experiment, two-way ANOVA for repeated measurements followed by Tukey's test was applied. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

BTX-A (3.5 and 7 U/kg) produced a complete reversal of mechanical hyperalgesia measured on day 6 after the toxin application (*Figure 1*). Since paw withdrawal was measured, results might be influenced by decreased muscular strength which, in turn, could be induced by BTX-A. However, it was previously shown by Cui et al. [8] (and in our laboratory: unpublished) that BTX-A in a dose of 7 U/kg did not affect motor performance in the rotarod test.

BTX-A (3.5 U/kg) did not influence mechanical pain threshold on day 1 after its application. However, on days 6 and 10, BTX-A completely abolished the secondary mechanical hyperalgesia (*Figure 2*). This is in line with our previous report on experimental neuropathy where BTX-A reduced hyperalgesia starting from day 5 and lasting for 10 more days [5]. On day 14 following the gastrocnemius incision, there was no difference between the tested groups (*Figure 2*), indicating that post surgical hyperalgesia ceased. Our results are in line with three open label clinical trials. Dohin et al. found that intramuscular BTX-A pretreatment decreases the duration of postoperative pain and improves the comfort in 9 children with cerebral palsy after limb surgery [9]. Wittekindt et al. [10] report reduced chronic and shooting pain lasting 4 weeks after subcutaneous injection of BTX-A in 16 patients after neck dissection. They also report similar results, pain reduction after a single subcutaneous injection of BTX-A in 13 patients after neck dissection, in their more recent dose-finding study [11].

Keeping in mind the number of patients suffering from postsurgical pain and inadequate treatments, our finding that single BTX-A injection completely abolished hyperalgesia for at least 10 days in the experimental model of postsurgical pain seems to be of major importance. Together with three before-mentioned clinical trials, our findings emphasize the need for further controlled clinical trials.

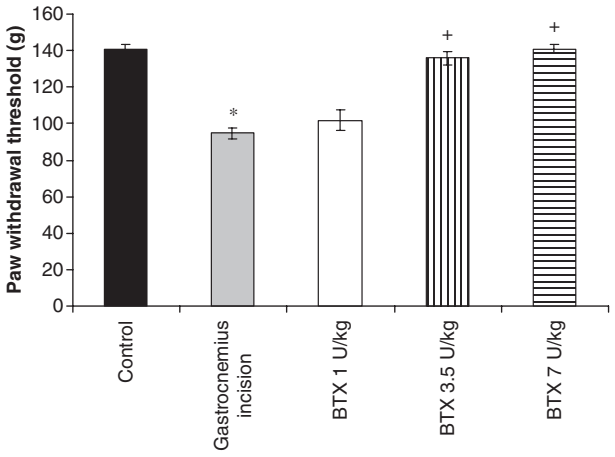

Figure 1 Dose-dependent antinociceptive effect of BTX-A on mechanical hyperalgesia in experimental model of postsurgical pain. Measurements were done on day 6 after BTX-A subcutaneous application into the rat hindpaw. Control, saline-treated unoperated animals ($n = 5$); gastrocnemius incision, saline-treated group after gastrocnemius incision ($n = 6$); BTX 1 U/kg ($n = 7$), BTX 3.5 U/kg ($n = 8$) and BTX 7 U/kg ($n = 6$)—BTX-A-treated groups after gastrocnemius incision. Mean ± SE, $^{*}P < 0.001$ compared to control; $^{+}P < 0.001$ compared to gastrocnemius incision (ANOVA and Newman–Keuls post hoc test).

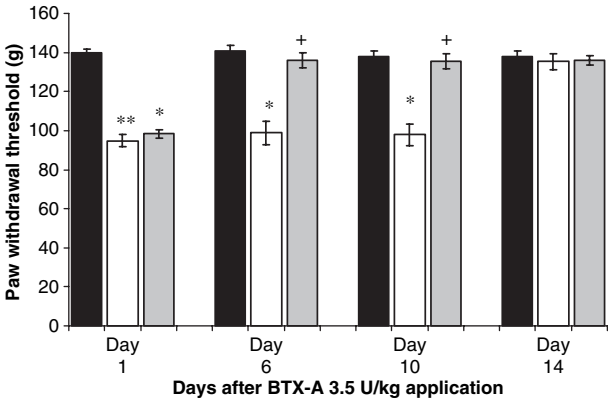

Figure 2 Time-course of the antinociceptive effect of BTX-A (3.5 U/kg) on mechanical hypersensitivity in experimental model of post surgical pain. Black bars, saline-treated unoperated controls ($n = 5$); White bars, saline-treated group after gastrocnemius incision ($n = 6$); Gray bars, BTX-A treated group after gastrocnemius incision ($n = 8$). Mean ± SE, $^{*}P < 0.01$, $^{**}P < 0.001$ compared to saline-treated unoperated controls; $^{+}P < 0.01$ compared to saline-treated group after gastrocnemius incision (ANOVA for repeated measurements followed by Tukey HSD test).

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