Article

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# 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

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**ABSTRACT**

The purpose of this study was to investigate if botulinum neurotoxin type-A (BoNT/A) had a preemptive antinociceptive effect in a formalin-induced orofacial pain model (FT). To test this hypothesis, male *Rattus norvegicus* were injected with isotonic saline solution 0.9% or BoNT/A administered as a 40 µl bolus, lateral to their nose, at 24 hours, 8, 15, 22, 29 or 36 days pre-FT. The procedures were repeated 42 days later. Influence on motor activity was assessed through the open-field test. Pain scores corresponded to the time spent rubbing and flicking the injected area. Animals pre-treated with BoNT/A at the first protocol (8 days subgroup) showed reduced inflammatory scores (p=0.011). For the other groups no significant results were observed at any phase. Motor activity was similar in both groups. BoNT/A showed to be effective preventing inflammatory pain up to eight days after the first treatment, an effect not reproduced on the second dose administration.

**Key words:** antinociceptive effect, orofacial pain, preemptive treatment, botulinum neurotoxin type-A.

**Toxina botulínica do tipo-A no tratamento preemptivo da dor trigeminal aguda: estudo pré-clínico duplo cego placebo controlado**

**RESUMO**

O objetivo deste estudo foi investigar o efeito preemptivo da neurotoxina botulínica do tipo/A (NTBo/A) através de um modelo de dor orofacial induzida pelo teste da formalina (TF). *Rattus norvegicus* machos foram injetados no lábio superior com solução salina isotônica 0,9% (SSI) ou NTBo/A (subgrupos 24 horas, 8, 15, 22, 29 ou 36 dias) antes do TF, em dois tratamentos farmacológicos e respectivas avaliações intercalados por 42 dias. Os escores da dor corresponderam ao tempo de fricção da região injetada. Após o primeiro pré-tratamento com NTBo/A no subgrupo 8 dias os escores da fase inflamatória foram menores do que no grupo SSI (p=0,011). Todas as outras comparações não foram significativas. Nos testes de atividade motora não ocorreram diferenças entre SSI e NTBo/A. A NTBo/A pode ser considerada como tratamento preemptivo das dores orofaciais quando utilizada até oito dias antes do estímulo álgico, não havendo consistência terapêutica após um segundo tratamento.

**Palavras-chave:** dor orofacial, efeito antinociceptivo, toxina botulínica do tipo-A, tratamento preemptivo.

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A noxious stimulus usually triggers a cascade of events in the peripheral and central pathways of the nervous system that ultimately produce nociception if the stimuli are of sufficient magnitude. Preemptive analgesia is an antinociceptive treatment that given before or during a noxious stimulus prevents its altered processing and the induction of central sensitization[1,2]. Local, regional, neuraxial, and systemic interventions alone or in combination, using single or multiple drug regimens have been examined in clinical protocols designed to test the efficacy of preemptive analgesia[3]. To be effective, preemptive treatment should be administered before or during the activation of the nociceptors by peripheral triggers and the treatment must be maintained until the end of inflammatory phase. A common problem found in preemptive analgesia drug trials is that the drug action is completed before the inflammatory phase is finished[1].

The rationale for using botulinum neurotoxin type-A (BoNT/A) for preemptive analgesia is twofold: [1] BoNT/A reduces the inflammatory phase of pain and inhibits the release of certain nociceptive mediators such as substance P (SP)[4], calcitonin gene-related peptide (CGRP)[5], as well as glutamate[6]; [2] Its analgesic effect lasts a long time, even from a single dose[7].

We conducted a double-blind placebo-controlled trial for three reasons: Firstly, to determine whether BoNT/A can be used as preemptive treatment; secondly, to determine the latency for the onset and duration of a presumed preemptive analgesic effect; and finally, to determine if repeated doses of BoNT/A could be effective. To test this hypothesis and to determine the patterns of a hypothetical preemptive response, a formalin-induced orofacial pain model in rats was used.

## METHOD

### Subjects

Male rats (*Rattus-norvegicus*) (n=95) weighting from 240 to 340 grams were housed in standard plastic cages (4 per cage) with sawdust bedding in a temperature-controlled room (23±1°C) and maintained on a 12 hours light-dark cycle. Animals were allowed to have free access to food pellets and water. The trial was conducted at the Neurology Research Laboratory of the Universidade Federal do Paraná - Brazil. Animals were randomized in a double blind way to receive either isotonic saline solution 0.9% (ISS) as control group or neurotoxin botulinum type-A (Bo-NT/A) as an active drug. The ISS and BoNT/A groups were further divided into six subgroups of 8 animals each, named accordingly to the period before the formalin test (FT) at which the pre-treatment was administered: [1] 24-hours pre-FT subgroup; [2] 8-day pre-FT subgroup; [3] 15-days pre-FT subgroup; [4] 22-days pre FT subgroup; [5] 29-days pre-FT subgroup; and [6] 36-days pre-FT subgroup.

### Phases of the study

All animals were submitted two run periods, two treatments, two nociceptive assessment (formalin test) and two motor assessment (open field test) (Fig 1).

### Drugs and treatment

Two groups were divided: one group used ISS and other group used BoNT/A. For the experimental group, BoNT/A (Botox®, Allergan, Inc, Irvine, CA) was reconstituted in 2ml of ISS, and, for the Control Group, only ISS was used. All the doses of BoNT/A and ISS used were administered as a 40 µl bolus into the right upper lip, just lateral to the nose using a 0.5 ml syringe with a 29-gauge needle. The dose of BoNT/A was 12 units *per* Kilogram.

### Open field test

The open field test (OFT) assesses motor skills including the integrity and spontaneous exploratory behaviour of animals. As described, the TF is a behavioural test that depends upon other cortical functions, such as the integrity of motor circuitry of the animal. Since BoNT/A may diffuse and affect the motor activity of these animals, a way to exclude a BoNT/A influence on motricity that could hypothetically interfere with the FT results was to submit all animals to an OFT 30 minutes before each TF. The test area was formed by a circle of 97 centimeters (cm) diameter. The walls were circular, made of aluminium and measured 32.5 cm high. The arena was located on a wood floor painted in white. It was divided into three concentric circles. The smaller, the intermediate and the larger circles had diameters of 23 cm, 61 cm and 98 cm, respectively. Each circle was divided internally into areas of equal size. The numbers of areas within the inner, intermediate and outer circles were respectively: one, six and twelve units. A white 100 watts lamp was disposed 48 cm above the floor of the arena. The test consisted of observing the behavior of the animal for a period of five minutes after its placement in the inner circle. Before being admitted into the arena, the animals spent about 10 minutes adapting to the test room environment, in the same boxes and feeding conditions that they were kept at the bioterium. During the OFT the animals were deprived of food and water. The parameters evaluated were: [A] rearing frequencies (number of times the animals stood on their hind legs); [B] numbers of the squares (numbers of the time that the animal entered a new square with all four paws); [C] immobility time (number of seconds of lack of movement during testing). After five minutes the animals were removed from the OFT arena and transferred to a second room in which the FT was done. The OFT apparatus was washed with 5% ethanol before testing to eliminate possible bias due to odors left by previous mice.

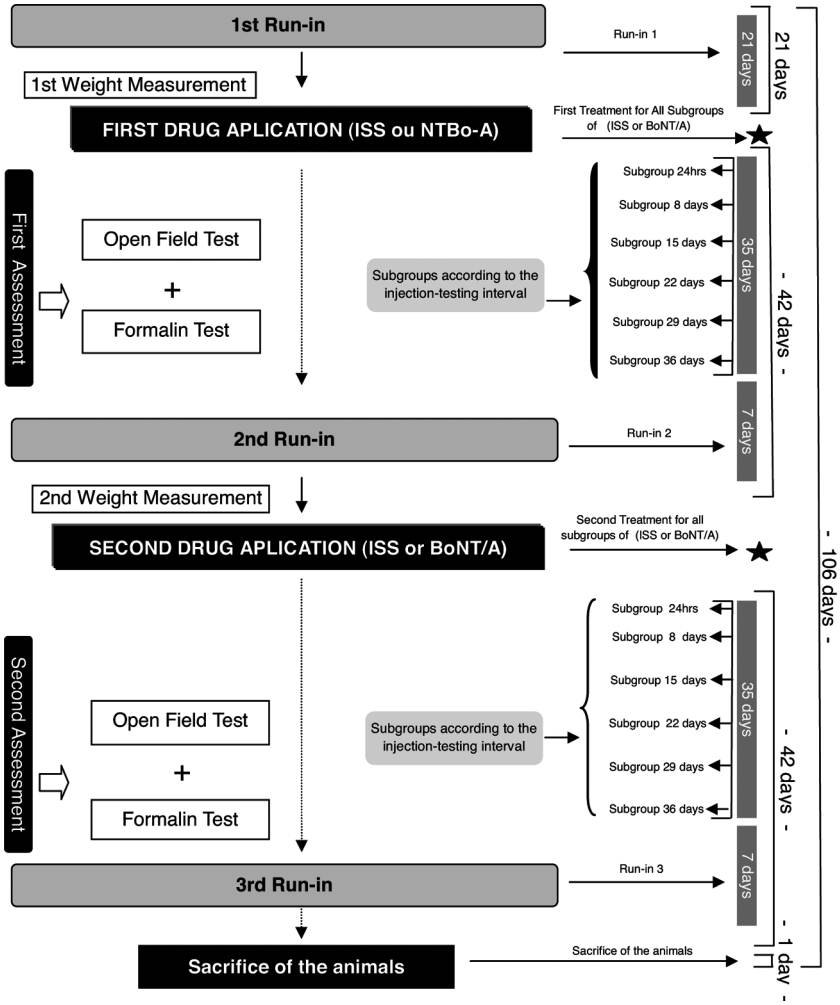


**Fig 1.** Timeline and steps of the study.

### Formalin test

A formalin solution was diluted in ISS to a concentration of 2.5%. Formalin test (FT) took place between 11:00 a.m. and 07:00 p.m. in a quiet room maintained at 23-24ºC. After the corresponding period for each subgroup the animals were weighted and placed inside a Plexiglas® observation chamber for an acclimatization period of 30 minutes. After this period the animals were placed inside of a test box measuring 30×30×30 centimeters with three mirrored sides. Rats were not allowed to eat or drink during the test. A 40 µl bolus of 2.5% of formalin was then injected into the right upper lip, just lateral to the nose, using a 0.5 ml syringe with a 29-gauge needle. The recording time was divided into 10 blocks of three minutes each and the pain score was determined for each block by measuring the numbers of seconds (amplitude of response) that the animal spent rubbing and flicking (R/F) the injected area with the ipsilateral fore paw or hind paw. The data collected between 0-3 minutes post-formalin injection represented phase 1 (neurogenic or phasic phase) and data collected between 12-30 minutes post-formalin injection represent phase 2 (inflammatory or tonic phase). The formalin test was repeated after 42 days (Fig 1).

### Regulatory aspects

All the experiments adhered to the guidelines of Committee for Research and Ethical Issues of IASP (Pain 1983). The experimental procedures were reviewed and approved by the regulatory committee of the Universidade Federal do Paraná, Health and animal Sciences Sector.

### Statistical analysis

For statistical analysis, each rat was considered as a single unit, and the subgroups were considered as independent samples. The Student t-test was used for comparisons between paired subgroups. For comparisons between unpaired subgroups the Mann Whitney test was used. For all tests, only "p" values smaller than 0.05 were considered significant.

## RESULTS

### Open field test

The results showed that there was no difference be-

**Table 1.** Descriptive and comparative assessment for Open Field Test in different subgroups for BoNT/A and ISS groups. Numbers of squares, rearing frequency and immobility time after the first treatment.

| | Group | M | Media | ±SD | Median | Min | Max | p†,‡ 1 versus 2 |
|---|---|---|---|---|---|---|---|---|
| Numbers of squares | | | | | | | | |
| • 24 hours | BoNT/A | 8 | 72.75 | 36.80 | 73 | 13 | 121 | p=0.901† |
| | ISS | 8 | 74.75 | 25.38 | 77 | 45 | 112 | |
| • 8 days | BoNT/A | 8 | 75.88 | 35.77 | 77.5 | 14 | 124 | p=0.607† |
| | ISS | 8 | 66.38 | 36.50 | 59 | 22 | 128 | |
| • 15 days | BoNT/A | 8 | 40.38 | 46.38 | 19 | 0 | 106 | p=0.096† |
| | ISS | 8 | 79.88 | 41.89 | 65.5 | 35 | 145 | |
| • 22 days | BoNT/A | 8 | 76.25 | 37.93 | 76.5 | 19 | 139 | p=0.176† |
| | ISS | 8 | 69.63 | 23.69 | 64.5 | 39 | 101 | |
| • 29 days | BoNT/A | 7 | 54.43 | 19.12 | 54 | 24 | 81 | p=0.779‡ |
| | ISS | 7 | 44.29 | 21.83 | 39 | 11 | 75 | |
| • 36 days | BoNT/A | 8 | 42 | 28.71 | 44 | 0 | 93 | p=0.293† |
| | ISS | 8 | 65.88 | 54.72 | 50.50 | 2 | 147 | |
| Rearing frequency | | | | | | | | |
| • 24 hours | BoNT/A | 8 | 15.13 | 7.33 | 15 | 7 | 31 | p=0.099† |
| | ISS | 8 | 24.63 | 13.32 | 18.5 | 13 | 47 | |
| • 8 days | BoNT/A | 8 | 22.38 | 10.46 | 24.5 | 6 | 34 | p=0.578† |
| | ISS | 8 | 27 | 20.44 | 22 | 7 | 68 | |
| • 15 days | BoNT/A | 8 | 14 | 16.51 | 6 | 0 | 45 | p=0.428† |
| | ISS | 8 | 20 | 12.63 | 18 | 3 | 39 | |
| • 22 days | BoNT/A | 8 | 46.50 | 78.98 | 22.5 | 4 | 239 | p=0.954† |
| | ISS | 8 | 19.00 | 10.11 | 20 | 4 | 31 | |
| • 29 days | BoNT/A | 8 | 13.43 | 6.87 | 13 | 5 | 25 | p=0.779‡ |
| | ISS | 7 | 10.71 | 6.89 | 10 | 2 | 20 | |
| • 36 days | BoNT/A | 8 | 15 | 9.62 | 13.50 | 0 | 29 | p=0.329† |
| | ISS | 8 | 20.88 | 13.30 | 19 | 0 | 41 | |
| Immobility time | | | | | | | | |
| • 24 hours | BoNT/A | 8 | 116.50 | 56.69 | 108.5 | 42 | 204 | p=0.195† |
| | ISS | 8 | 79.38 | 52.21 | 65 | 11 | 170 | |
| • 8 days | BoNT/A | 8 | 182.63 | 57.49 | 184.5 | 87 | 253 | p=0.680† |
| | ISS | 8 | 172.13 | 40.60 | 174.5 | 115 | 232 | |
| • 15 days | BoNT/A | 8 | 171.88 | 102.81 | 197 | 12 | 266 | p=0.134† |
| | ISS | 8 | 101.38 | 71.65 | 104.5 | 9 | 176 | |
| • 22 days | BoNT/A | 8 | 125.63 | 72.32 | 136.5 | 9 | 210 | p=0.148† |
| | ISS | 8 | 171.50 | 44.25 | 200 | 111 | 208 | |
| • 29 days | BoNT/A | 8 | 109.29 | 51.53 | 117 | 37 | 183 | p=0.955‡ |
| | ISS | 7 | 130 | 74.07 | 94 | 45 | 257 | |
| • 36 days | BoNT/A | 8 | 210.75 | 80.03 | 235.5 | 56 | 300 | p=0.244 † |
| | ISS | 8 | 149.88 | 116.80 | 156 | 15 | 291 | |

Group 1 (botulinum neurotoxin type-A); Group 2 (isotonic saline solution 0.9%); (Min) minimum; (Max) maximum; • Subgroups; †t-Student test; ‡Mann-Whitney test. Significance p<0.05. Values showed in seconds or units.

tween all the parameters studied between the subgroups after the first (Table 1) and second treatments (Table 2) for BoNT/A and ISS.

## Formalin test

The responses of the animals to the formalin 2.5% injection into the right upper lip resulted in a biphasic pattern: two periods of intensive rubbing/flinching (R/F) activities were observed between 0-3 minutes (neurogenic phase) and 12-30 minutes (inflammatory phase) with almost no nociceptive response between 3-12 minutes. After the first pre-treatment the BoNT/A 8-days pre-FT subgroup disclosed an attenuated response to formalin in the inflammatory phase (p=0.011; Table 3). No significant response was recorded for the other subgroups during the inflammatory or neurogenic phases (Table 3, Fig 2). At the second experiment, conducted 42 days later, no significant response was recorded at any phase for

**Table 2.** Descriptive and comparative assessment for Open Field Test in different subgroups for BoNT/A and ISS Groups. Numbers of squares, rearing frequency and immobility time after the second treatment.

| | Group | N | Media | ±SD | Median | Min | Max | p †,‡ 1 versus 2 |
|---|---|---|---|---|---|---|---|---|
| Numbers of squares | | | | | | | | |
| • 24 hours | BoNT/A | 8 | 15.75 | 11.73 | 10 | 5 | 39 | p=0.885 † |
| | ISS | 8 | 17 | 20.88 | 9 | 5 | 67 | |
| • 8 days | BoNT/A | 8 | 18 | 12.82 | 16 | 2 | 42 | p=0.668 † |
| | ISS | 8 | 23.25 | 31.32 | 12.50 | 4 | 98 | |
| • 15 days | BoNT/A | 8 | 15.5 | 20.18 | 5.50 | 0 | 48 | p=0.504 † |
| | ISS | 8 | 10.13 | 9.15 | 6.50 | 2 | 26 | |
| • 22 days | BoNT/A | 8 | 23.63 | 15.46 | 26 | 8 | 49 | p=0.229 † |
| | ISS | 8 | 16.88 | 12.15 | 16.50 | 4 | 40 | |
| • 29 days | BoNT/A | 8 | 8 | 8 | 5 | 0 | 26 | p=0.163 ‡ |
| | ISS | 7 | 16.71 | 14.37 | 13 | 3 | 46 | |
| • 36 days | BoNT/A | 8 | 28.63 | 31.80 | 20.50 | 0 | 97 | p=0.807 † |
| | ISS | 8 | 25 | 26 | 18 | 0 | 78 | |
| Rearing frequency | | | | | | | | |
| • 24 hours | BoNT/A | 8 | 2.81 | 2.47 | 2.50 | 0 | 7 | p=0.919 † |
| | ISS | 8 | 2.75 | 2.37 | 2 | 0 | 7 | |
| • 8 days | BoNT/A | 8 | 6 | 6.39 | 4.50 | 1 | 19 | p=0.754 † |
| | ISS | 8 | 7.13 | 7.64 | 4.50 | 0 | 23 | |
| • 15 days | BoNT/A | 8 | 4.25 | 8.63 | 4 | 0 | 25 | p=0.884 † |
| | ISS | 8 | 3.75 | 4.09 | 3 | 0 | 13 | |
| • 22 days | BoNT/A | 8 | 6.88 | 4.05 | 8 | 0 | 11 | p=0.814 † |
| | ISS | 8 | 6.25 | 6.15 | 5 | 0 | 18 | |
| • 29 days | BoNT/A | 8 | 2.25 | 2.37 | 1 | 0 | 6 | p=0.397 ‡ |
| | ISS | 7 | 4 | 5.06 | 2 | 0 | 15 | |
| • 36 days | BoNT/A | 8 | 5 | 5.78 | 3.50 | 0 | 14 | p=0.835 † |
| | ISS | 8 | 7.13 | 9.77 | 5.50 | 0 | 30 | |
| Immobility time | | | | | | | | |
| • 24 horas | BoNT/A | 8 | 227.63 | 43.90 | 242 | 131 | 269 | p=0.628 † |
| | ISS | 8 | 236.38 | 46.93 | 232.50 | 141 | 276 | |
| • 8 dias | BoNT/A | 8 | 154.38 | 112.47 | 199.50 | 8 | 279 | p=0.735 † |
| | ISS | 8 | 172.25 | 94.10 | 177 | 39 | 280 | |
| • 15 dias | BoNT/A | 8 | 241.75 | 67.78 | 269.50 | 86 | 291 | p=0.255 † |
| | ISS | 8 | 202.38 | 64.99 | 228.50 | 116 | 271 | |
| • 22 dias | BoNT/A | 8 | 251.75 | 43.25 | 267 | 151 | 286 | p=0.846 † |
| | ISS | 8 | 277.63 | 9.69 | 278.50 | 258 | 290 | |
| • 29 dias | BoNT/A | 8 | 267.50 | 31.30 | 277 | 204 | 300 | p=0.820 ‡ |
| | ISS | 7 | 210.14 | 78.59 | 227 | 50 | 277 | |
| • 36 dias | BoNT/A | 8 | 238.88 | 78.83 | 269 | 62 | 300 | p=1 † |
| | ISS | 8 | 238.25 | 49.62 | 249 | 148 | 284 | |

Group 1 (botulinum neurotoxin type-A); Group 2 (isotonic saline solution 0.9%); (Min) minimum; (Max) maximum; • Subgroups; †t-Student test; ‡Mann-Whitney test. Significance p<0.05. Values showed in seconds or units.

any of the subgroups, neither for those pertaining to the BoNT/A group nor the ISS group (Table 4; Fig 2).

## DISCUSSION

In spite of the lack on motor activity testing in the previous literature, we considered it to be essential since BoNT/A may diffuse and have a potential effect on motricity[8], so the OFT was performed. The OFT demonstrated that rearing frequencies, the numbers of squares and the immobility times were similar for all subgroups of both groups.

The results also showed that BoNT-A was able to inhibit the behaviour of pain during the inflammatory phase only in the BoNT/A 8-day pre-FT subgroup. Con-

**Table 3.** Descriptive and comparative assessment for formalin test 2.5% in different subgroups for BoNT/A and ISS Groups. Neurogenic and inflammatory phases after the first treatment.

| | Group | N | Média | ±SD | Mediana | Min | Max | p†,‡ BoNT/A versus ISS |
|---|---|---|---|---|---|---|---|---|
| Neurogenic phase | | | | | | | | |
| • 24 hours | BoNT/A | 8 | 70.25 | 77.50 | 31.01 | 37 | 108 | p=0.195† |
| | ISS | 8 | 73.63 | 71 | 31.90 | 9 | 110 | |
| • 8 days | BoNT/A | 8 | 49.63 | 59.50 | 25.20 | 0 | 70 | p=0.680† |
| | ISS | 8 | 55.50 | 64.50 | 34.13 | 4 | 96 | |
| • 15 days | BoNT/A | 8 | 58 | 65.5 | 26.28 | 14 | 94 | p=0.134† |
| | ISS | 8 | 59.13 | 60.5 | 26.87 | 24 | 105 | |
| • 22 days | BoNT/A | 8 | 28.50 | 14.50 | 34.31 | 0 | 107 | p=0.148† |
| | ISS | 8 | 41 | 41 | 16.06 | 21 | 65 | |
| • 29 days | BoNT/A | 8 | 58.25 | 62 | 12.65 | 41 | 78 | p=0.955‡ |
| | ISS | 7 | 72.14 | 84 | 24.45 | 36 | 104 | |
| • 36 days | BoNT/A | 8 | 35.63 | 23 | 25.03 | 9 | 73 | p=0.244† |
| | ISS | 8 | 32.88 | 36.5 | 18.1 | 2 | 58 | |
| Inflammatory phase | | | | | | | | |
| • 24 hours | BoNT/A | 8 | 210.38 | 192 | 139.56 | 84 | 514 | p=0.705† |
| | ISS | 8 | 230.63 | 244 | 156.34 | 0 | 430 | |
| • 8 days | BoNT/A | 8 | 106.13 | 66 | 108.22 | 12 | 309 | p=0.011† |
| | ISS | 8 | 327.13 | 342 | 178.35 | 14 | 552 | |
| • 15 days | BoNT/A | 8 | 331.38 | 299.50 | 191.31 | 78 | 712 | p=0.798† |
| | ISS | 8 | 310 | 317.50 | 184.08 | 0 | 528 | |
| • 22 days | BoNT/A | 8 | 166.13 | 123.50 | 117.80 | 54 | 355 | p=0.607† |
| | ISS | 8 | 203.38 | 236 | 121.90 | 9 | 323 | |
| • 29 days | BoNT/A | 8 | 331.50 | 106.90 | 106.90 | 163 | 489 | p=0.203‡ |
| | ISS | 7 | 265.43 | 97.28 | 97.28 | 165 | 465 | |
| • 36 days | BoNT/A | 8 | 206.88 | 192.50 | 98.64 | 30 | 346 | p=0.084† |
| | ISS | 8 | 296.88 | 301 | 140.14 | 122 | 506 | |

BoNT/A (botulinum neurotoxin type-A); ISS (isotonic saline solution 0.9%); (Min) minimum; (Max) maximum; • Subgroups; †t-Student test; ‡Mann-Whitney test. Significance p<0.05. Values showed in seconds.

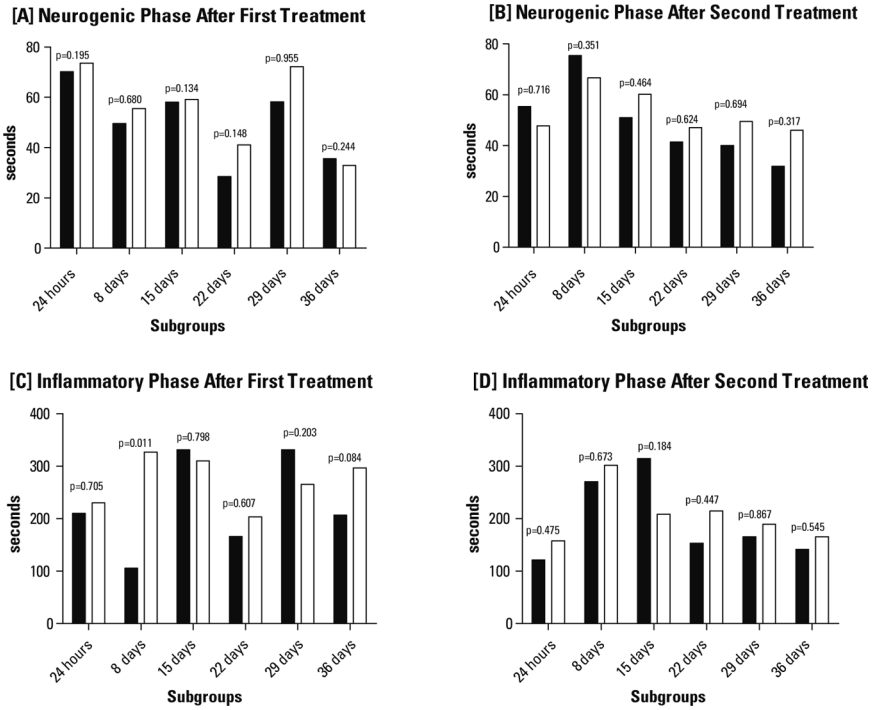


**Fig 2.** [A] Results from neurogenic phase after the first treatment; [B] Results from neurogenic phase after the second treatment; [C] Results from inflammatory phase after the first treatment; [D] Results from inflammatory phase after the second treatment.

**Table 4.** Descriptive and comparative assessment for formalin test 2.5% in different subgroups for BoNT/A and ISS groups. Neurogenic and inflammatory phases after the second pre-treatment.

| | Group | N | Média | ±SD | Mediana | Min | Max | p†,‡ BoNT/A versus ISS |
|---|---|---|---|---|---|---|---|---|
| Neurogenic phase | | | | | | | | |
| • 24 hours | BoNT/A | 8 | 55.38 | 44.55 | 54 | 0 | 147 | p=0.716† |
| | ISS | 8 | 47.75 | 37.39 | 46.50 | 1 | 112 | |
| • 8 days | BoNT/A | 8 | 75.50 | 18.20 | 72.50 | 47 | 105 | p=0.351† |
| | ISS | 8 | 66.88 | 17.53 | 66.50 | 34 | 88 | |
| • 15 days | BoNT/A | 8 | 51 | 25.86 | 55 | 7 | 83 | p=0.468† |
| | ISS | 8 | 60.25 | 23.67 | 57 | 27 | 90 | |
| • 22 days | BoNT/A | 8 | 41.5 | 21.10 | 38 | 17 | 87 | p=0.624† |
| | ISS | 8 | 47.13 | 23.65 | 40.50 | 14 | 77 | |
| • 29 days | BoNT/A | 8 | 40.13 | 14.83 | 36 | 24 | 62 | p=0.694‡ |
| | ISS | 7 | 49.57 | 28.72 | 40 | 25 | 97 | |
| • 36 days | BoNT/A | 8 | 31.88 | 23.46 | 25 | 5 | 74 | p=0.317† |
| | ISS | 8 | 46 | 30.39 | 39.5 | 20 | 111 | |
| Inflammatory phase | | | | | | | | |
| • 24 hours | BoNT/A | 8 | 121.75 | 79.94 | 89.50 | 49 | 253 | p=0.475† |
| | ISS | 8 | 157.75 | 112.85 | 167 | 31 | 350 | |
| • 8 days | BoNT/A | 8 | 270.75 | 138.79 | 300.50 | 50 | 496 | p=0.673† |
| | ISS | 8 | 302.13 | 152.19 | 301.50 | 79 | 571 | |
| • 15 days | BoNT/A | 8 | 314.50 | 157.27 | 333 | 36 | 527 | p=0.184† |
| | ISS | 8 | 208.75 | 145.38 | 163.50 | 35 | 411 | |
| • 22 days | BoNT/A | 8 | 153.63 | 103.19 | 152.50 | 24 | 328 | p=0.447† |
| | ISS | 8 | 214.88 | 196.16 | 141.50 | 47 | 582 | |
| • 29 days | BoNT/A | 8 | 165.63 | 111.70 | 142.50 | 55 | 390 | p=0.867‡ |
| | ISS | 7 | 189.43 | 204.36 | 183 | 0 | 514 | |
| • 36 days | BoNT/A | 8 | 141.75 | 74.84 | 119.50 | 44 | 291 | p=0.545† |
| | ISS | 8 | 165.38 | 77.63 | 175 | 10 | 275 | |

BoNT/A (botulinum neurotoxin type-A); ISS (isotonic saline solution 0.9%); (Min) minimum; (Max) maximum; • Subgroups; †t-Student test; ‡Mann-Whitney test. Significance p<0.05. Values showed in seconds.

trary to our original hypothesis, the neurogenic phase was not affected at any time and in any subgroup. Our results can be explained by the transient blocking of the vesicular intracellular receptors vanilloids (TRVP1 and TRPA1) by BoNT/A, an effect that peaks at the seventh day[9]. Increased expression of these receptors within the peripheral nociceptors is important to maintain the states of inflammatory hyperalgesia after a neurogenic stimuli[10,11]. Inhibition of these receptors does not allow the expression of nociception in the case of a neural injury. In a model of inflammatory pain sensitization such as the formalin 2.5% test, BoNT/A is able to inhibit or prevent the peripheral sensitization[12].

As described in the literature, the behavior of neurogenic pain was not affected, but an effect of the BoNT/A on the manifestations of the peripheral and central aspects of the neurogenic pain was seen only in the subgroup 8 days, corresponding to the peak of action of these drugs on the vanilloid receptors[9,10]. The results were not replicated at the second treatment suggesting a non-repeated block lead. Perhaps the results were not replicated by the second run by the fact that at the first run the animals were pre-adults while at the second run the animals were adults. In the more aged animals differences in motor skills could be relevant to influence their motor responses. Other explanation for the lack of response at the second block may be the development of an enhanced descending inhibition of the nociceptive neurons, since stimuli may render the primary neuronal pool more sensitive to inhibition[13]. The other mechanism perhaps involved in the lack of response of the second experiment run is the possibility of an immune response to BoNT/A leading to a lesser effectiveness of BoNT/A[14].

In experimental models, BoNT/A may be administered peripherally (subcutaneous route) or centrally (intraventricular route). The administration of BoNT/A by both routes was described to affect the behavioral responses to pain in an experimental model of formalin. The administration of BoNT/A seems not to interfere with the neurogenic phase, but affects similar routes in the in-

flammatory phase of peripheral and central administration[15]. The plantar use of BoNT/A in a formalin test was shown to inhibit peripheral sensitization and the behavioral responses to pain in the inflammatory phase. Further analysis by micro dialysis suggested that these results stem from an inhibition in the release of glutamate[16,17].

Clinical experience with BoNT/A as a treatment for patients with refractory trigeminal neuralgia showed that a reduction in the intensity and frequency of the painful paroxysms may begin from one week up to four weeks after the injection of BoNT/A[18]. In spite of the little effectiveness of BoNT/A on treating episodic migraine[19,20], the use of a BoNT/A in the treatment of chronic migraine have shown positive results[21,22]. In other chronic painful syndromes such as peripheral neuropathy and post herpetic neuralgia, the use of BoNT/A was also described to result in transient antinociceptive effect[23]. BoNT/A also reduces diabetic neuropathic pain, resulting in a secondary improvement in the quality of sleep[24]. All those studies illustrate an analgesic effect of BoNT/A.

The experimental results herein presented add to the previous literature and support a temporary antinociceptive effect of BoNT/A when used as a preemptive treatment, limited to the acute (inflammatory) phase of pain.

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