



Histomorphometric Changes of the Masseter Muscle of Rats After a Single Injection of Botulinum Toxin Type A

Douglas Massoni Ramos¹ · Raira de Brito Silva¹ · Giancarlo De la Torre Canales^{2,4,5} · Luciana Resende⁵ · Marcelo Augusto Marretto Esquisatto³ · Nádia Cristina Fávoro More Malin Ernberg⁴ · Célia Marisa Rizzatti-Barbosa^{1,5}



Received: 8 June 2023 / Accepted: 19 July 2023 / Published online: 24 August 2023

© Springer Science+Business Media, LLC, part of Springer Nature and International Society of Aesthetic Plastic Surgery 2023

Abstract

Background It has been reported that botulinum toxin type A (BoNT-A) produces structural changes in masticatory muscles. However, not all histomorphometric parameters affected by BoNT-A parameters have been assessed. This study investigated the histomorphometric changes in the masseter muscle of rats after a single injection of BoNT-A. **Methods** Forty-four adult animals were randomly divided into control group ($n = 22$) and BoNT-A group ($n = 22$). Controls received a single dose of 0.14 mL/kg of saline in masseter muscles, and the BoNT-A group received a 7 U/Kg of BoNT-A. The groups received the same volume of injected substances. Animals were sacrificed on 7th ($n = 5$), 14th ($n = 5$), 21st ($n = 5$), 28th ($n = 4$) and 90th ($n = 3$) days post-treatment. Histological masseter tissue slides were obtained from hematoxylin–eosin treatment and analyzed in optical microscopy regarding muscle cross-sectional area, amount of connective tissue and quantity and diameter of myocytes. For statistical analysis,

generalized linear models were used to compare the data (ANOVA). In all test, the significance level of 5% was set. **Results** BoNT-A values of cross-sectional area of the masseter muscle were significantly lower than controls ($p < 0.01$) throughout the study. Regarding myocytes quantity, BoNT-A subgroups presented higher values than controls ($p < 0.0001$) since the 14th day until the end of the study; however, the diameter of myocytes was smaller in all BoNT-A subgroups ($p < 0.0001$) in all assessment points. The amount of connective tissue was higher in BoNT-A subgroups ($p < 0.0001$) throughout the study. **Conclusion** A single injection of BoNT-A altered the structure of masseter muscle of rats, regarding its histomorphometric parameters.

No Level Assigned This journal requires that authors assign a level of evidence to each article. For a full description of these Evidence-Based Medicine ratings, please refer to the Table of Contents or the online Instructions to Authors www.springer.com/00266.

Keywords Botulinum toxin · Masticatory muscle · Myocytes · Connective tissue · Adverse effects

✉ Giancarlo De la Torre Canales
gcanales@egasmoniz.edu.pt

¹ Department of Oral Biology, Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil
² Egas Moniz Center for Interdisciplinary Research (CiiEM), Egas Moniz School of Health & Science, Caparica, Almada, Portugal
³ Araras Dental School, University Center Herminio Ometto, Araras, SP, Brazil
⁴ Department of Dental Medicine, Karolinska Institutet, and the Scandinavian Network for Orofacial Neurosciences (SCON), Huddinge, Sweden
⁵ Ingá University Center Uningá, Department of Dentistry, Maringá, PR, Brazil

Introduction

Botulinum toxin type A (BoNT-A) is one of seven neuromuscular blocking agents produced by the bacterium *Clostridium botulinum*. It has an affinity for motor nerve endings where it inhibits acetylcholine release, exerting its paralytic effect on injected muscles [1–3]. Since the US Food and Drug Administration (US-FDA) approved BoNT-A for the treatment of various muscle disorders such as dystonia and spasticity, it became an accepted and frequently used therapeutic tool to chemically denervate

muscles and disabling of neural transmission [1]. Furthermore, the analgesic effect of BoNT-A which have been demonstrated by several experimental studies led to new potential indications for pain conditions [1–3].

Local application of BoNT-A is considered to be safe by the US-FDA [4]. In addition, a recent systematic review pointed out that self-reported minor adverse effects with a spontaneous resolution such as weakness in neighboring non-target muscles and transient edema were the most prevalent adverse effects [5]. However, it is known that changes in muscle function (BoNT-A muscle-paralytic effect) are followed by structural and morphological changes in the muscles themselves, a diminish of masticatory performance and changes in bone tissue. Considering that the neuromuscular effect of BoNT-A is dose- and muscle-size dependent [6], and because of its time limited action, which leads to performed repeated injections at intervals of 3–4 months, it could be hypothesized that BoNT-A could have deleterious effects on muscle tissue [7, 8].

In fact, experimental studies have demonstrated that a single or multiple injections of BoNT-A produced a decrease in muscle mass, histological changes in muscles fibers, expression of apoptotic and muscle atrophy/regeneration markers and fatty infiltration [9–12]. Additionally, clinical studies have demonstrated that multiple or even a single injection of BoNT-A into masticatory muscles diminished masticatory performance and masticatory muscle size [13–15]. Notwithstanding, studies using BoNT-A injection in masseter muscle of rodents have only described a muscle volume reduction by visual inspection [16], by measuring its width [17], by reporting diameter of the fibers [10], without assessing other important parameters such as morphometric effects and the amount of myocytes.

Thus, the aim of this study was to evaluate the structural changes caused by a single bilateral injection of BoNT-A on masseter muscle of rats. Morphometric effects, percentage analysis of substituting connective tissue and the quantity of myocytes were assessed. The null hypothesis was that BoNT-A does not alter the morphometric and histological structures of the masseter muscle.

Methods

Animals

The present study was approved by the Ethics Committee in Animals Research of the State University of Campinas (CEUA/UNICAMP), Sao Paulo, Brazil under protocol n°. 4554-1/2017 and followed the National Council for Control of Animal Experimentation (CONCEA) and the

International Association for the Study of Pain (IASP) guidelines [18].

Forty-four male Wistar rats (300–500 g) aged 60 days were used in this study. Animals were obtained from the University of Campinas Multidisciplinary Center for Biological Research (CEMIB-UNICAMP, Sao Paulo, Brazil) and housed in standard clear polyethylene cages with a maximum of five animals per cage and maintained in a temperature-controlled room (24 ± 1 °C), with a 12-hour light-dark cycle, with food and water ad libitum throughout the experiment. The body mass of all animals was quantified and assessed daily from day 0 (before the intervention) to the end of the experiment (90 days).

Experimental Design

Animals were randomly divided into 2 groups of 22 rats: group 1 (BoNT-A) received one single intramuscular injection of 7 U/kg of BoNT-A (Botox[®], Allergan, Inc. Irvine, CA, USA; 100 units were reconstituted in 2 mL of 0.9% saline solution) in the masseter muscle and group 2 (Control) received a single intramuscular injection of a total doses of 1.4 mL of sodium chloride at 0.9% also in the masseter muscles [19]. Injections were applied bilaterally. The groups received the same volume of injected substances. Then, groups were divided into five subgroups according to the sacrifice period from the experimental days since substance were injected (day 0): 7th day ($n = 5$), 14th day ($n = 5$), 21st day ($n = 5$), 28th day ($n = 4$) and 90th day ($n = 3$). All animals and cages were identified according to their respective subgroups.

Tissue Extraction

Euthanasia of the animals was performed to remove tissues for analyses, according to the experimental design. Briefly, the animals were anesthetized with intraperitoneal injection of a mixture of Dopalen[®] (50 mg/mL) and Rompun[®] (2 g/100 mL), in a 1:1 ratio, at a dose of 0.3 mL/100 g body mass. After presenting signs of general anesthesia, the animals were infused with 60 mL of PBS solution (cardiac perfusion). The masseter muscles of animals were removed, dissected, weighed, and divided into two proportional transverse parts intended for analysis in light microscopy.

Histology and Morphometric Analysis

The muscle slides were prepared for histological and morphometric analysis according to the predefined experimental days of substances application. Briefly, masseter tissue samples were fixed in 20 volumes of 10% formaldehyde in Millonig buffer (pH 7.4) for 24h at 24 °C

Table 1 Cross-sectional area of the masseter muscle fibers (5 μm) in function of the group and the days from BoNT-A application

Days	Group		BoNT-A	
	Control		BoNT-A	
	Mean (SD)	Median (min.; max.)	Mean (SD)	Median (min.; max.)
7	75.86 (1.91) Ac	76.06 (72.87; 78.15)	68.71 (1.14) Ba	69.00 (66.89; 69.86)
14	78.79 (1.51) Ab	78.23 (77.36; 80.92)	60.34 (2.66) Bb	61.08 (56.64; 63.20)
21	80.62 (0.69) Ab	80.78 (79.70; 81.52)	54.95 (2.04) Bc	55.34 (52.77; 56.94)
28	82.29 (0.85) Aab	82.27 (81.39; 83.25)	49.41 (1.26) Bd	49.30 (48.32; 50.74)
90	84.39 (0.14) Aa	84.43 (84.23; 84.50)	48.99 (1.00) Bd	48.64 (48.22; 50.12)

Different letters (uppercase in horizontal) represent significant difference ($p \leq 0.05$) between groups

Different letters (lowercase in vertical) represent significant difference ($p \leq 0.05$) among evaluation periods

$p(\text{groups}) < 0.0001$; $p(\text{days from BoNT-A application}) < 0.0001$; $p(\text{interaction}) < 0.0001$

and processed for inclusion of ParaplastTM. Histological slides of cross sections of the samples (5 μm) were stained by the hematoxylin–eosin method for structural observation and morphometric analysis. The following morphometric parameters were evaluated: cross-sectional area of muscle fibers and presence of connective tissue (% per 10,000 μm^2), number (n per 10,000 μm^2) and diameter (μm) of myocyte. Five fields of each of the five sections obtained from the mean region of the tissue collected in the animals of each experimental subgroup/days from substances application were documented. The mean diameter of the fibers was measured using 125 fibers of each experimental group/time ($n = 5/25$ fibers per animal). All images were analyzed in a Leica DM2000 photomicroscope. The measurements were made from the captured images, using the Software Image Leica Measure and Sigma Scan Pro 5.0 (SYSTAT, California, United States).

Statistical Analysis

The G*Power 3.1.9.2 software was used for sample size calculation (Düsseldorf, Germany). Power calculation showed that 22 animals per group would demonstrate more than 90% power when $\alpha = 0.05$ with an effect size of 0.4 for comparisons between two independent means (T-test). Descriptive and exploratory analyses of the data were performed. Descriptive data are presented as mean (SD) and median (Min; Max). Generalized linear models were used to compare the data (ANOVA). Subgroups and days from substances application (day 0) were considered for the statistical analyses, as well as the interaction among them. In all test, the significance level of 5% was set. The analyses were carried out with resources from the R Core Team program (2019) (Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria).

Results

Regarding the weight of the animals, no significant differences were found between BoNT-A subgroups ($p > 0.05$). In the between-group comparisons, values of the weight of the animals were significantly lower in the BoNT-A subgroups compared to the control subgroups ($p > 0.05$).

Cross-sectional Area of the Masseter Muscle Fibers (CSAM)

Considering the CSAM values (Table 1), within-group comparisons showed a significant decrease in CSAM in the BoNT-A subgroups as soon as 7 days after the injection and throughout the study ($p < 0.0001$); however, the control subgroups presented opposite results, showing a significant increase in CSAM ($p < 0.0001$) until the last follow-up. In the between-group comparisons, values of CSAM were significantly lower in the BoNT-A subgroups compared to the control subgroups ($p < 0.01$) throughout the study (Figure 1).

Quantity of Myocyte (MT)

Regarding MT data (Table 2), the within-group comparison showed a significant increase in the quantity of MN in BoNT-A subgroups ($p < 0.05$); however, there were not significant differences between the 21st, 28th and 90th day BoNT-A subgroups. On the other hand, the quantity of MT significantly diminished when the 7th day control subgroup was compared with the other control subgroups ($p < 0.05$). The between-groups comparisons found significant higher values of MT for BoNT-A subgroups when compared with the control subgroups from the 14th day of follow-up until the end of the study ($p < 0.0001$).

Fig. 1 Fibers in cross sections of the masseter muscle (HE staining), according to different groups and periods of assessment. Microscopic enlargement: 40x (line = 5 μ m). Control: saline solution; BoNT-A: botulinum toxin type A

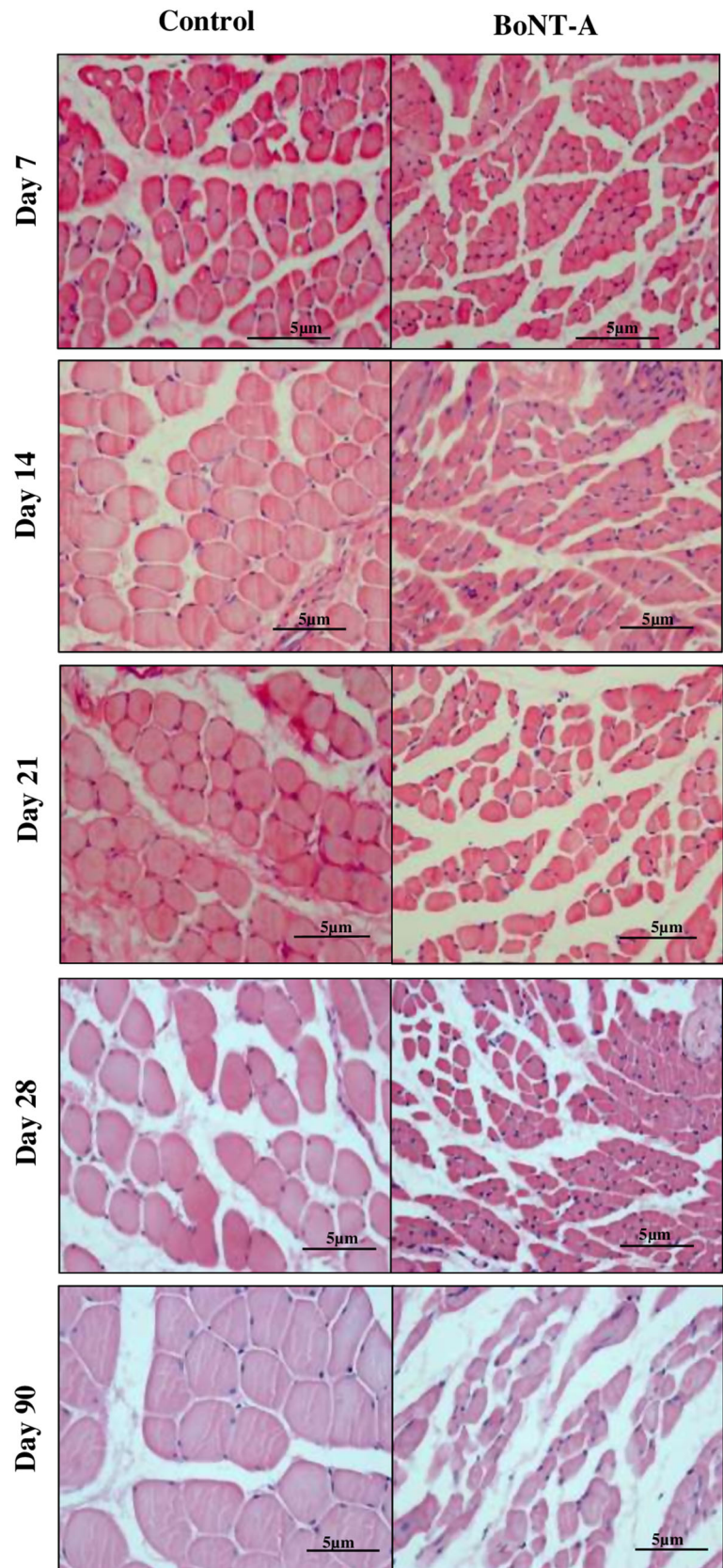


Table 2 Myocyte count (10,000 μm^2) considering group and the days from BoNT-A application

Days	Group		BoNT-A	
	Control		BoNT-A	
	Mean (SD)	Median (min; max)	Mean (SD)	Median (min; max)
7	4.78 (0.74) Aa	4.84 (3.64; 5.52)	5.24 (0.47) Ab	5.44 (4.64; 5.80)
14	3.69 (0.23) Bbc	3.56 (3.48; 4.04)	5.39 (0.68) Ab	5.36 (4.76; 6.52)
21	4.07 (0.21) Bb	4.04 (3.80; 4.28)	6.73 (0.27) Aa	6.68 (6.40; 7.08)
28	4.05 (0.18) Bb	3.98 (3.92; 4.32)	6.45 (0.35) Aa	6.40 (6.08; 6.92)
90	3.28 (0.11) Bc	3.32 (3.16; 3.36)	6.08 (0.59) Aa	6.36 (5.40; 6.48)

Different letters (uppercase in horizontal) represent significant difference ($p \leq 0.05$) between groups

Different letters (lowercase in vertical) represent significant difference ($p \leq 0.05$) among evaluation periods

$p(\text{subgroups}) < 0.0001$; $p(\text{days of BoNT-A application}) = 0.0009$; $p(\text{interaction}) < 0.0001$

Table 3 Diameter of myocytes (μm) considering the group and the days from BoNT-A application

Days	Group		BoNT-A	
	Control		BoNT-A	
	Mean (SD)	Median (min; max)	Mean (SD)	Median (min; max)
7	9.50 (0.25) Ac	9.32 (9.32; 9.86)	8.09 (0.41) Ba	7.80 (7.80; 8.67)
14	10.37 (0.06) Ab	10.34 (10.34; 10.47)	7.20 (0.23) Bb	7.07 (7.07; 7.60)
21	10.66 (0.17) Ab	10.54 (10.54; 10.90)	6.64 (0.34) Bc	6.41 (6.41; 7.16)
28	11.00 (0.14) Ab	10.97 (10.88; 11.16)	6.06 (0.22) Bd	5.99 (5.89; 6.36)
90	13.71 (2.69) Aa	14.03 (10.88; 16.23)	6.01 (0.15) Bd	5.92 (5.92; 6.18)

Different letters (uppercase in horizontal) represent significant difference ($p \leq 0.05$) between groups

Different letters (lowercase in vertical) represent significant difference ($p \leq 0.05$) among evaluation periods

$p(\text{subgroups}) < 0.0001$; $p(\text{days of BoNT-A application}) = 0.0013$; $p(\text{interaction}) < 0.0001$

Myocytes Diameter (MD)

Analyses of the MD (Table 3) showed in the withing-group comparisons, a significant decrease in the MD values over time for BoNT-A subgroups ($p < 0.0001$); however, there were no significant differences between the 28th and 90th day BoNT-A subgroups ($p > 0.05$). Differently, a significant increase in MD was found when compared the 7th day control subgroup with the other control subgroups; however, no differences were found between the 14th, 21st and 28th day control subgroups. In the between-group comparisons, the MD was significantly smaller in all BoNT-A subgroups when compared with control subgroups ($p < 0.0001$).

Connective Tissue (CT)

Results of the quantity of connective tissue (% in 10,000 μm^2 , Table 4) showed in the within-group comparisons, a significant increase in the amount of CT in BoNT-A subgroups ($p < 0.05$); however, no significant differences were found between the 28th and 90th day

BoNT-A subgroups ($p > 0.05$). Among the control subgroups, there was a significant decrease in the amount of CT over time ($p < 0.05$). The between-groups comparisons showed, a significant higher quantity of CT in all BoNT-A subgroups when compared with the control subgroups ($p < 0.0001$) throughout the study.

Discussion

The present study demonstrated that a single injection of BoNT-A produced structural changes in masseter muscle tissue which included: a significant decrease in the cross-sectional area of the masseter muscle, an increase in the amount of connective tissue, an increase in the quantity of myocyte nuclei, and a decrease in myocyte nuclei diameter. Importantly, the weight of the animals did not influence the results since there were no significant differences between the BoNT-A subgroups.

It is known that biological tissues have the ability to adapt through numerous functional demands, and muscle tissue is one of the most affected when submitted to a

Table 4 Amount of connective tissue (% in 10,000 μm^2) considering the groups and the days from BoNT-A application

Days	Group		BoNT-A	
	Control		BoNT-A	
	Mean (SD)	Median (min; max)	Mean (SD)	Median (min; max)
7	24.39 (2.13) Ba	24.42 (21.42; 27.09)	31.05 (1.41) Ad	31.00 (29.43; 33.11)
14	21.17 (1.48) Bb	21.77 (19.08; 22.48)	39.61 (2.63) Ac	38.92 (36.80; 43.40)
21	19.37 (0.67) Bc	19.18 (18.48; 20.22)	45.00 (2.08) Ab	44.50 (42.90; 47.23)
28	17.71 (0.85) Bd	17.74 (16.75; 18.61)	50.60 (1.29) Aa	50.70 (49.26; 51.76)
90	15.61 (0.18) Be	15.57 (15.46; 15.81)	51.03 (1.02) Aa	51.40 (49.88; 51.81)

Different letters (uppercase in horizontal) represent significant difference ($p \leq 0.05$) between groups

Different letters (lowercase in vertical) represent significant difference ($p \leq 0.05$) among evaluation periods

$p(\text{subgroups}) < 0.0001$; $p(\text{days of BoNT-A application}) = 0.0013$; $p(\text{interaction}) < 0.0001$

stimuli [20]. Musculoskeletal tissue consists of elongated cells with cytoplasmic filaments, in which myofibrils are responsible for generating cell contraction. When changing their biochemical, mechanical, and structural functions, muscle atrophy is developed, in which a reduction in myofibers number and diameter characterizes the decrease in muscle mass [21]. Our study showed that the area of the CSAM, which expresses the size of muscle fibers, was significantly smaller for BoNT-A than controls ($p < 0.05$) in all days of evaluation. These findings are in line with the results of Balanta-Melo et al, 2018 [10] which reported masseter muscle atrophy, evidenced by Cav3 staining as a reduction in individual muscle fiber cross-sectional area in BoNT-A injected muscles. It is important to mention that our findings could explain the results of clinical studies reporting masseter atrophy by a decrease in masseter thickness and its cross-sectional area of about 18–20% after BoNT-A injection [15, 22].

Many myoprogenitor cells merge and form several peripheral nuclei to form new cells, which gives them great exposure capacity of contractile proteins to form myofibers [23]. Skeletal striated muscle fibers are multinucleated [24] and alterations in the metabolism of these cells induce changes in muscle regeneration and proliferation, as well as in the amount and size of cell nuclei [25, 26]. In our study, we observed, a significant increase in myocyte counts in BoNT-A subgroups from the 14th day of evaluation until the end of the study when compared with controls, whereas the diameter of myocytes significantly decreased in BoNT-A subgroups in all evaluation periods. If we consider the increased myocyte count as an indicator of muscle repair, our data differ from the study of Balanta-Melo et al, 2018 [10], since the latter did not find any indicator of muscle repair, maybe due to the assessment periods performed in the study that were limited to 14 days and related to the injected doses. However, it is important to mention that this finding could be just a consequence of the reduction in the

diameter of the fibers; if they are smaller, there will be a greater number per field. Moreover, it must be considered that muscle tissue has great capacity for regeneration, even when subjected to several cycles of aggression. Myocytes play an important role in this process by proliferating and dividing asymmetrically, to repair or replace damaged muscle fibers as a response to the aggression [27]. Therefore, it was expected that the inactivation promoted by the BoNT-A paralytic effect which constituted a factor of cellular aggression [27–29] would exhibit an evident attempt to repair the inactivated cell tissues by increasing the number of myocytes and their union, thus forming a cluster of cells, which was evidenced over time. However, it must be considered that the methods used in our study to assess the quantity of myocytes before and after BoNT-A injection may have influenced our results. It is known that muscle satellite cells are also activated by injury and are located under the basal lamina of muscle fiber, but separate from the muscle fiber itself [30]. Thus, it could be possible that the increase in the quantity of myocyte reported in our study and could be a mix of muscle satellite cells and myocytes since our methods could not differentiate between these two types of cells.

Interestingly, in addition to the structural changes in the masseter muscle, our study found a significant increase over time of connective tissue infiltrate in the BoNT-A subgroups when compared with controls. These results are in line with the study of Fortuna et al, 2011 [12] which reported infiltration of adipose connective tissue (fatty) after 3 months on the BoNT-A injected side which was even more evident in the 6-month evaluation after repeated injections. However, even though our study did not differentiate the type of connective tissue present in BoNT-A subgroups, we found an increase in connective tissue as soon as 7 days after BoNT-A injection after a single injection. A possible explanation of the proliferation of intramuscular connective tissue after application of BoNT-

A is related to decreased muscle activity. This observation has a strong relationship with the biochemistry of the muscle cell that precedes this effect. When there is no muscle activity, important changes occur in mitochondria and substances related to myocyte physiology, such as the mRNA level of Atrogin-1/MAFbx, Muscle RING finger 1, Muscle atrophy F-box, and Myogenin [10] that may explain what happens in the chemical denervation promoted by BoNT-A. In this way, the decrease in muscle mechanical tension alters growth production and collagen synthesis, while increasing the proliferation of connective tissue. Thus, fibroblasts, which are subject to muscle tensions, both active and passive, have altered their composition and metabolism [31] resulting in an increased quantity of connective tissue.

Taking into consideration the results of the present study, our research may contribute with preclinical data to alert about the risk of adverse effects of BoNT-A in masticatory muscles, since this intervention is becoming a common therapy in dentistry for temporomandibular disorders, bruxism, and dystonia [2] and for aesthetics indications such as masseter hypertrophy or even to treat wrinkles. In fact, a randomized clinical trial that assessed different doses of BoNT-A for masticatory myofascial pain found that even a single injection of a median or high doses of BoNT-A produced a decrease in masseter and anterior temporalis muscle thickness and concluded that low doses of BoNT-A must be used in patients that do not get relief from conservative therapies in order to reduce the probabilities of developing adverse effects in muscle and bone tissue [13].

It is important to highlight some limitations of our study. Even though our results are consistent, the staining by the hematoxylin–eosin method could be considered not the most adequate nowadays for this assessment since this method present limitations especially in differentiating structures. Also, we were not able to identify which type of connective tissue increased after BoNT-A injections in our study. We recommend that future studies assess the histomorphometric changes of a single and repeated injections of BoNT-A in masticatory muscles with longer follow-ups, since the paralytic effect of BoNT-A last between 3 and 4 months and muscle contraction could be recovered after this period of time. Finally, the effects of BoNT-A in some muscular proteins such as calsequestrin which plays an important role in muscle contraction should be also evaluated.

Conclusions

The results of this study lead to the conclusion that a single injection of BoNT-A into the masseter muscle of rats produced histomorphometric alterations by diminishing the

cross-sectional area of the masseter muscle fibers, increasing connective tissue formation, lousing contractile tissue, and incrementing the number of myocytes but not its diameter for at least 3 months.

Acknowledgments The authors acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES) for the PhD scholarship of D.M.R.—grant code 001 and the Sao Paulo Research Foundation—FAPESP for the post-doctoral scholarship of G. D. T. C. (grant 2017/21674-0).

Data Availability Datasets related to this article will be available upon request to the corresponding author.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest to disclose.

Informed Consent For this type of study, informed consent is not required.

Institutional Review Board Statement The present study was approved by the Ethics Committee in Animals Research of the State University of Campinas (CEUA/UNICAMP), Sao Paulo, Brazil under protocol n.º. 4554-1/2017 and followed the National Council for Control of Animal Experimentation (CONCEA). All applicable institutional and/or national guidelines for the care and use of animals were followed.

References

- Matak I, Lacković Z (2014) Botulinum toxin A, brain and pain. *Prog Neurobiol* 119–120:39–59
- Muñoz Lora VRM, Del Bel Cury AA, Jabbari B et al (2019) Botulinum toxin type A in dental medicine. *J Dent Res* 98(13):1450–1457
- Matak I, Böleskei K, Bach-Rojecky L et al (2019) Mechanisms of botulinum toxin type A action on pain. *Toxins (Basel)* 11(8):459
- Coté TR, Mohan AK, Polder JA et al (2005) Botulinum toxin type A injections: adverse events reported to the US food and drug administration in therapeutic and cosmetic cases. *J Am Acad Dermatol* 53(3):407–415
- De la Torre CG, Poluha RL, Lora VM et al (2019) Botulinum toxin type A applications for masticatory myofascial pain and trigeminal neuralgia: what is the evidence regarding adverse effects? *Clin Oral Investig* 23(9):3411–3421
- Dressler D, Saberi FA, Barbosa ER (2005) Botulinum toxin: mechanisms of action. *Arq Neuropsiquiatr* 63(1):180–185
- Dutton JJ (1996) Botulinum-A toxin in the treatment of cranio-cervical muscle spasms: short- and long-term, local and systemic effects. *Surv Ophthalmol* 41(1):51–65
- Brin MF (1997) Botulinum toxin: chemistry, pharmacology, toxicity, and immunology. *Muscle Nerve Suppl* 6:S146–S168
- Moon YM, Kim MK, Kim SG et al (2016) Apoptotic action of botulinum toxin on masseter muscle in rats: early and late changes in the expression of molecular markers. *Springerplus* 5(1):991
- Balanta-Melo J, Toro-Ibacache V, Torres-Quintana MA et al (2018) Early molecular response and microanatomical changes in the masseter muscle and mandibular head after botulinum toxin intervention in adult mice. *Ann Anat* 216:112–119

11. Tsai CY, Lin YC, Su B et al (2012) Masseter muscle fibre changes following reduction of masticatory function. *Int J Oral Maxillofac Surg* 41(3):394–399
12. Fortuna R, Vaz MA, Youssef AR et al (2011) Changes in contractile properties of muscles receiving repeat injections of botulinum toxin (Botox). *J Biomech* 44(1):39–44
13. De la Torre CG, Alvarez-Pinzon N, Muñoz-Lora VRM et al (2020) Efficacy and safety of botulinum toxin type A on persistent myofascial pain: a randomized clinical trial. *Toxins (Basel)* 12(6):395
14. Shome D, Khare S, Kapoor R (2019) Efficacy of botulinum toxin in treating Asian Indian patients with masseter hypertrophy: a 4-year follow-up study. *Plast Reconstr Surg* 144(3):e390e–e396
15. Park HU, Kim BI, Kang SM et al (2013) Changes in masticatory function after injection of botulinum toxin type A to masticatory muscles. *J Oral Rehabil* 40(12):916–922
16. Kün-Darbois JD, Libouban H, Chappard D (2015) Botulinum toxin in masticatory muscles of the adult rat induces bone loss at the condyle and alveolar regions of the mandible associated with a bone proliferation at a muscle enthesis. *Bone* 77:75–82
17. Moon YM, Kim YJ, Kim MK et al (2015) Early effect of Botox-A injection into the masseter muscle of rats: functional and histological evaluation. *Maxillofac Plast Reconstr Surg.* 37(1):46
18. Zimmermann M (1983) Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 16(2):109–110
19. Lora VR, Clemente-Napimoga JT, Abdalla HB et al (2017) Botulinum toxin type A reduces inflammatory hypernociception induced by arthritis in the temporomandibular joint of rats. *Toxicol* 129:52–57
20. Turner NJ, Badylak SF (2012) Regeneration of skeletal muscle. *Cell Tissue Res* 347(3):759–774
21. Cao RY, Li J, Dai Q et al (2018) Muscle atrophy: present and future. *Adv Exp Med Biol* 1088:605–624
22. Kim HJ, Jeon BS, Lee KW (2000) Hemimasticatory spasm associated with localized scleroderma and facial hemiatrophy. *Arch Neurol* 57(4):576–580
23. Shenkman BS, Turtikova OV, Nemirovskaya TL et al (2010) Skeletal muscle activity and the fate of myonuclei. *Acta Naturae.* 2(2):59–66
24. Dumont NA, Bentzinger CF, Sincennes MC et al (2015) Satellite cells and skeletal muscle regeneration. *Compr Physiol* 5(3):1027–1059
25. Ito R, Higa M, Goto A et al (2018) Activation of adiponectin receptors has negative impact on muscle mass in C2C12 myotubes and fast-type mouse skeletal muscle. *PLoS ONE* 13(10):e0205645
26. Addison WN, Hall KC, Kokabu S et al (2019) Zfp423 regulates skeletal muscle regeneration and proliferation. *Mol Cell Biol.* <https://doi.org/10.1128/MCB.00447-18>
27. Zammit PS, Golding JP, Nagata Y et al (2004) Muscle satellite cells adopt divergent fates: a mechanism for self-renewal? *J Cell Biol* 166(3):347–357
28. Rantanen J, Hurme T, Lukka R et al (1995) Satellite cell proliferation and the expression of myogenin and desmin in regenerating skeletal muscle: evidence for two different populations of satellite cells. *Lab Invest* 72(3):341–347
29. Ten Broek RW, Grefte S, Von den Hoff JW (2010) Regulatory factors and cell populations involved in skeletal muscle regeneration. *J Cell Physiol* 224(1):7–16
30. Morgan JE, Partridge TA (2003) Muscle satellite cells. *Int J Biochem Cell Biol* 35(8):1151–1156
31. Hwang JH, Ra YJ, Lee KM et al (2006) Therapeutic effect of passive mobilization exercise on improvement of muscle regeneration and prevention of fibrosis after laceration injury of rat. *Arch Phys Med Rehabil* 87(1):20–26

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.