UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

GIANCARLO DE LA TORRE CANALES

EFICACIA TERAPÊUTICA DO TRATAMENTO COM TOXINA BOTULÍNICA NA DOR MIOFASCIAL PERSISTENTE

EFFICACY OF BOTULINUM TOXIN ON THE TREATMENT OF PERSISTENT MYOFASCIAL PAIN

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Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Doutor em Clínica Odontológica, na Área de Prótese Dental.

Thesis presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Clinical Dentistry, in Dental Prosthesis.

Orientadora: Profa. Dra. Célia Marisa Rizzatti Barbosa

Este exemplar corresponde à versão final da tese defendida pelo aluno Giancarlo de La Torre Canales e orientada pela Profa. Dra. Célia Marisa Rizzatti Barbosa

PIRACICABA

2017

Agência(s) de fomento e nº(s) de processo(s): CAPES

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Odontologia de Piracicaba
Marilene Girello - CRB 8/6159

De La Torre, Giancarlo Canales, 1987-
D375e Eficácia terapêutica do tratamento com toxina botulínica na dor miofascial persistente / Giancarlo De La Torre Canales. – Piracicaba, SP : [s.n.], 2017.

Orientador: Celia Marisa Rizzatti Barbosa.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Toxinas botulínicas tipo A. 2. Dor crônica. 3. Síndrome da disfunção da articulação temporomandibular. I. Rizzatti-Barbosa, Celia Marisa,1957-. II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Efficacy of botulinum toxin on the treatment of persistent myofascial pain
Palavras-chave em inglês:
Botulinum toxins, type A
Chronic pain
Temporomandibular joint dysfunction syndrome
Área de concentração: Prótese Dental
Titulação: Doutor em Clínica Odontológica
Banca examinadora:
Celia Marisa Rizzatti Barbosa [Orientador]
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Paulo Henrique Ferreira Caria
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Data de defesa: 29-05-2017
Programa de Pós-Graduação: Clínica Odontológica

UNIVERSIDADE ESTADUAL DE CAMPINAS

Faculdade de Odontologia de Piracicaba

A Comissão Julgadora dos trabalhos de Defesa de Tese de Doutorado, em sessão pública realizada em 29 de Maio de 2017, considerou o candidato GIANCARLO DE LA TORRE CANALES aprovado.

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PROF. DR. PAULO HENRIQUE FERREIRA CARIA

PROF. DR. WANDER JOSÉ DA SILVA

A Ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica do aluno.

DEDICATÓRIA

A **Deus,** por permitir a realização dos meus objetivos.

A meus pais **Pedro De la Torre Dascalachis** e **Rosa Canales de De la Torre**, por todo o sacrifício realizado, abdicando muitas vezes dos seus próprios sonhos para que os meus fossem concretizados.

AGRADECIMENTOS ESPECIAIS

A **Deus,** por cuidar de mim durante estes anos fora de casa e por permitir a realização deste trabalho.

Aos meus pais **Pedro Luis De la Torre Dascalachis** e **Rosa Canales de De la Torre,** por que, graças a vocês, estou conseguindo todos meus objetivos; por todo o suporte que tive desde a decisão de seguir a profissão, até esse momento. Obrigado pela educação e pelos valores transmitidos. Obrigado pelo amor e pelo sacrifício que significa deixar um filho ir para que possa crescer. O estímulo para estudar, a força e a coragem para seguir em frente é a melhor herança que eu poderia ter recebido. Esse momento é de vocês.

Aos meus irmãos **Peter De la Torre** e **Gino De la Torre,** pela amizade, apoio e carinho. Esse momento também é de vocês.

A minha orientadora **Profa. Dra. Célia Marisa Rizzatti Barbosa,** pelo aceite de minha pessoa no Programa de Pós-Graduação em ClÍnica Odontológica da Faculdade de Odontologia de Piracicaba - Unicamp. Agradeço a orientação e apoio a mim dedicados durante estes anos em que trabalhamos juntos.

AGRADECIMENTOS

À Universidade Estadual de Campinas, na pessoa do Magnífico Reitor **Prof. Dr. Marcelo Knobel.**

À Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, na pessoa do seu Diretor, **Prof. Dr. Guilherme Elias Pessanha Henriques**, e Diretor Associado **Prof. Dr. Francisco Haiter Neto**.

À Coordenadora dos Cursos de Pós-Graduação da Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, **Profa. Dra. Cinthia Pereira Machado Tabchoury**.

À Coordenadora do Programa de Pós-Graduação em Clínica Odontológica da Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, **Profª Drª Karina Gonzales Silvério Ruiz**

Aos professores da Área de Prótese Parcial Removível da FOP-Unicamp, **Profa. Dra. Altair Antoninha Del Bel Cury, Profa. Dra. Célia Marisa Rizzatti Barbosa, Profa. Dra. Renata Cunha Matheus Rodrigues Garcia e Prof. Dr. Wander José da Silva,** pelas orientações e ensinamentos.

Aos demais professores do Departamento de Prótese e Periodontia, com quais tive oportunidade de cursar disciplinas do curso.

Aos membros da banca examinadora do exame de qualificação, **Profa. Dra. Maria Beatriz Duarte Gavião, Profa. Dra. Nadia Favaro Moreira e Profa. Dra. Juliana Stuginski Barbosa,** pelas contribuições feitas ao nosso trabalho.

À **Natalia Alvarez Pinzón,** agradeço pela amizade e por todos os momentos compartilhados; mas, acima de tudo, pela nossa parceria neste trabalho. Só nós sabemos o quanto trabalhamos para concluir esta pesquisa. Se houve algum mérito, eu o devo a você.

À **Edmara Tatiely Pedroso Bergamo,** pela amizade, trabalho em equipe e por sempre estar presente quando precisei de alguém. Você fez com que este caminho fosse mais fácil, mesmo estando distante. Muito obrigado.

Ao **Victor Muñoz Lora,** agradeço pela diversão, companheirismo, pelas parcerias nos projetos de pesquisa, pela ajuda partilhada neste trabalho, e pela amizade. Obrigado por me fazer sentir em casa e por sempre estar presente quando mais necessitei de um amigo.

Ao **Alfonso Sanchez Ayala, Daniel Herrera Ramos e Javier Vivanco Purisaca**, agradeço por me ajudarem a chegar neste momento e pela sua amizade e conselhos.

Aos amigos **Mariana Barbosa, Jairo Cordeiro, Olivia Figuereido, Camila de Amaral** e **Louise Dornelas,** por todo o apoio a mim brindado durante estes anos, pela amizade, parcerias em outros projetos de pesquisa, e principalmente por fazerem parte da minha família em Piracicaba. Sem dúvidas a sua amizade foi uma das coisas mais importantes que me levarei da FOP.

Aos companheiros do Laboratório de Prótese Parcial Removível e colegas de Pós-Graduação, **Aline Sampaio, Antônio Pedro Ricomini, Bruna Alfenas, Camila de Amaral, Camila Heitor, Cindy Dodo, Dimorvan Bordin, Edmara Bergamo, Fernando Rigolin, Gabriele Alencar, Giselle Ribeiro, Guilherme Oliveira, Kelly Machado, Louise Dornelas, Luiz Carlos Filho, Marco Aurélio Carvalho, Mariana Barbosa, Martina Bertolini, Mayara Abreu, Olivia Figuereido, Raissa Machado, Rafael Soares, Sales Barbosa, Samilly Souza, Talita Carletti, Victor Munõz, Vinicius Santos, Veber Bonfiim, Yuri Cavalcanti** pela convivência sempre agradável. Muito obrigado a cada um de vocês.

À técnica do Laboratório de Prótese Parcial Removível, **Sra. Gislaine Regiane Alves Piton**, agradeço pelo bom convívio.

À secretária do Departamento de Prótese e Periodontia, **Sra. Eliete Aparecida Ferreira Lima Marim**, pela ajuda e bom convívio.

Às **pacientes** que aceitaram fazer parte desta pesquisa, muita gratidão, porque sua participação ajudou a contribuir com este trabalho.

À **Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP),** pelo financiamento concedido à pesquisa desenvolvida.

À **Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)**, pela bolsa concedida.

A todos aqueles que, em algum momento, estiveram ao meu lado nesta jornada, meus sinceros agradecimentos.

Resumo

A disfunção temporomandibular (DTM) abrange algumas alterações clínicas que comprometem estruturas do sistema estomatognático (SEG). As intervenções terapêuticas conservadoras são as comumente indicadas em seu manejo, onde o aconselhamento e os aparelhos interoclusais planos (AIP) são considerados procedimentos de primeira escolha para este tipo de disfunção, devido ao seu reconhecido índice de sucesso. Atualmente, aplicações de toxina botulínica tipo A (TxBoA) têm sido indicadas para as DTM miogênica com sintomatologia dolorosa aguda. Entretanto, os estudos existentes não confirmam se esta intervenção pode ser eficaz para o controle da dor crônica ligada à DTM; isto devido às características e ação da TxBoA, os quais podem variar substancialmente em função de fatores locais, número e posicionamento das punções realizadas, volume aplicado, concentração da droga, etc. Desta forma, o objetivo deste ensaio clínico, realizado em triplo-cego, randomizado e controlado, foi avaliar a efetividade da TxBoA no controle da dor miofascial persistente relacionada à DTM. Cem voluntárias, classificadas pelo RDC/TMD, foram divididas aleatoriamente em cinco grupos (n=20): um grupo controle, tratado com aconselhamento e aparelho interoclusal plano (SP); um grupo placebo, tratado com aconselhamento e aplicações de solução salina a 0.9% (SS); e três grupos experimentais tratados com aconselhamento e aplicações de TxBoA em três doses distintas: dose baixa (B), dose média (M) e dose alta (A); (30U, 50U e 75U nos músculos masseteres e 10U, 20U e 25U no feixe anterior do músculo temporal, respectivamente). As variáveis dependentes foram: 1) dor, avaliada através do índice subjetivo de dor, mensurado por Escala Visual Analógica (EVA), e através da análise do limiar da dor à pressão (LDP), mensurado por algometria; 2) análise da atividade eletromiográfica (EMG) e da imagem ultrassonográfica (UTS) dos músculos masseter direito e esquerdo (MD, ME) e feixes anteriores dos músculos temporais direito e esquerdo (TD, TE); 3) análise da performance mastigatória das pacientes (PM); 4) análise do volume ósseo do processo coronoide, mediante tomografia computadorizada de feixe cônico (TC); e 5) análise de alguns dados obtidos nos eixos I e II do RDC/TMD. A coleta dos dados foi feita 7 dias antes e até 180 dias após as intervenções terapêuticas. Foram utilizados modelos lineares generalizados para a análise estatística das variáveis EVA, Algometria, EMG e UTS; ANOVA dois fatores para medidas repetidas para PM; correlação de Spearman para PM e UTS; e o teste de Wilconxon para TC. Para todas as análises considerou-se o nível de significância de 5%. Os menores valores encontrados para EVA foram nos grupos SP e TxBoA-B, M e A ($p<0,05$), nas avaliações feitas aos 30, 90 e 180 dias pós-operatórios. Da mesma forma, foram encontrados valores maiores ($p<0,05$) de limiar de dor à pressão nos grupos SP e TxBoA-B, M e A quando comparados ao grupo SS, após 30, 90 e 180 dias das intervenções. Quando considerados os valores EMG, os três grupos

tratados com TxBoA apresentaram uma diminuição significativa ($p<0,05$) nas atividades dos músculos investigados aos 30 dias pós-operatórios, quando comparados aos grupos controle. Exceto na avaliação feita aos 30 dias pós-operatórios, os valores para a PM demonstraram que o grupo TxBoA-B teve comportamento semelhante ao grupo SP, não apresentando diferenças significativas nos períodos subsequentes ($p>0,05\%$). Os dados do US mostraram que o TxBoA-L foi o único grupo tratado que não apresentou diferença quando comparado com o grupo SP após 30 e 90 dias pós interveção ($p>0,05$). Houve correlação negativa entre PM e UTS. Desta forma a TxBoA mostrou-se eficaz no controle da dor miofascial persistente; porém alguns efeitos adversos devem ser considerados na indicação desta intervenção.

Palavras-Chave: Toxina Botulínica; Dor crônica; Disfunção Temporomandibular

Abstract

Temporomandibular dysfunction (TMD) involves some clinical changes that compromises the stomatognathic system. Counseling and splint are the procedures usually considered in TMD handling due to their recognized success. Currently, applications of botulinum toxin type A (TxBoA) have been indicated for acute pain myogenic TMD. However, the literature does not confirm whether this intervention may be effective for the control of persistent TMD-related pain. This is due to the characteristics of BoNTA, which may vary substantially depending on the local factors, the number and position of punctures, the drug volume and concentration, etc. Thus, the objective of this double-blind-randomised-controlled clinical trial was to evaluate the effectiveness of BoNTA in the management of persistent TMD-related myofascial pain. One hundred volunteers, classified by RDC/TMD, were randomly divided into five groups (n = 20): control group, that received counseling and splint (SP); placebo group treated with counseling and 0.9% saline (SS); and three experimental groups treated with counseling and three different doses of BoNTA: low dose (L), medium dose (M) and high dose (H); (30U, 50U and 75U in the masseters and 10U, 20U and 25U in the anterior temporal muscles, respectively). The outcome variables were: 1) pain, assessed through the subjective pain index, measured by Visual Analogue Scale (VAS), and through the pressure pain threshold admeasument, reached by algometry; 2) analysis of the superficial electromyographic signals (EMG) and ultrasound (US) of right and left masseter (MD, ME) and anterior temporal muscles (TD, TE); 3) the patients' masticatory performance (PM); 4) bone volume of bilateral coronoid process, acquired by cone bean computed tomography images (CT), and some data obtained from axis I and II of RDC/TMD. The data were collected 7 days before, and until 180 days after the therapeutic interventions. The statistical analysis of the data obtained for VAS, algometry, EMG and US was done with generalized linear models. Two-way ANOVA was used for MP and RDC/TMD data. Spearman's correlation, was used to correlate MP and US. The Wilconxon test was used for the analysis of the bone density data. The level of significance of 5% was considered in all the analyzes. EVA was lowest to SP and TxBoA-B, M, A ($p < 0.05$) in 30, 90 and 180 postoperative days. Likewise, after 30, 90 and 180 of the interventions, higher values ($p < 0.05$) for pressure pain threshold were found to SP and TxBoA-L, M and H, when compared to SS. The three TxBoA treated groups presented a significant decrease ($p < 0.05$) in EMG muscles at 30 postoperative day, when compared to both control groups. Except for the 30-day postoperative evaluation, PM values showed similarity among TxBoA-B and SP, and did not present alterations over period ($p > 0.05\%$). The US data showed that TxBoA-L was the only treated group that did not present difference when compared to SP after 30 and 90 days post-intervention ($p > 0.05$). There was a negative correlation between PM and UTS.

TxBo-A showed effectiveness to control persistent myofascial pain; however, some adverse effects should be considered in the BoNT-A TMD indication.

Key words: Botulinum Toxin; Chronic pain; Temporomandibular dysfunction

Sumário

1 Introdução

A disfunção temporomandibular (DTM) acomete estruturas do sistema estomatognático (SEG) envolvendo as articulações temporomandibulares (ATM) e a musculatura mastigatória (Okeson, 1998).

As DTMs podem apresentar sinais e sintomas característicos como os ruídos articulares (Okeson, 1998), hiper ou hipomobilidade mandibular (Lam *et al*., 2001), irregularidades e/ou assimetria nos movimentos de abertura e fechamento bucal, cefaléias, mastigação deficiente e distúrbios de fala (Caillet, 1999; Piozzi & Lopes, 2002). Entretanto, a dor localizada na face, na musculatura mastigatória e na região da ATM nem sempre é de fácil diagnóstico, e envolve critério em sua interpretação, qualificação e quantificação (Silveiro *et al*., 1998).

A dor é, sem dúvidas, o sintoma mais presente na DTM (Manfredini *et al*., 2011). Nestes quadros clínicos, ajustes musculares compensatórios são observados durante as situações funcionais, adaptando o SEG a uma nova situação postural para restabelecer a normalidade da mastigação, fala e deglutição, e para diminuir a condição dolorosa (Ciancaglini, *et al*., 2001).

A dor miofascial é a mais comum nos quadros de DTM (Manfredini *et al*., 2011) e, quando se apresenta na condição de dor persistente, pode ser difícil de ser controlada. Isto porque, muitas vezes, o sintoma tem uma forte correlação com os fatores psicossociais, que podem redundar em hiperatividade muscular e fortalecer o ciclo dor/estresse (Silvério *et al*., 1998).

Em função da similaridade com outras disfunções musculoesqueléticas, os tratamentos conservadores são indicados como terapias de eleição (Branco *et al*., 2005). Embora relativamente onerosos, os aparelhos interoclusais planos ainda ocupam um lugar de destaque como intervenção conservadora à DTM devido ao seu alto índice de sucesso (Portero *et al*., 2009; Guarda-Nardini *et al*, 2012). São utilizados com o objetivo principal de redistribuir as forças oclusais, tratar dores nos músculos da mastigação e condicionar as ATM a uma posição postural não patológica (Issa *et al*., 2005). A conscientização e a educação do paciente sobre hábitos mais saudáveis também podem contribuir significativamente para melhorar o resultado ao tratamento proposto (Portero *et al*., 2009). A associação desse aconselhamento ao uso de aparelhos interoclusais tem sido uma boa proposta clínica de intervenção nos quadros de DTM (Canales *et al*., 2017; Conti *et al*., 2012).

Além destes, diversos tratamentos alternativos têm sido propostos aos portadores de DTM. As aplicações de toxina botulínica tipo A (TxBo-A) nas áreas sensíveis da face têm sido utilizadas com relativo sucesso para a remissão da sintomatologia dolorosa aguda ligada à DTM, principalmente aquelas com compromeperiodnto miogênico. Esta age temporariamente como promotor de analgesia via relaxamento da fibra muscular inflamada (Dressler *et al*., 2005; Matak, 2015), bloqueando a

liberação da acetilcolina (ACh) nas junções neuromusculares (Kok-Yuen *et al*., 2007). A TxBo-A é produzida pela bactéria *Clostridium botulinum* e é constituída por um complexo proteico que contém as neurotoxinas. No passado, foi considerada letal e utilizada como arma química; porém, na atualidade, além de ser usada com finalidade estética, tem sido empregada como instrumento terapêutico de diversas patologias musculares que envolvem a atuação colinérgica. Alguns estudos prospectivos clínicos demostraram a eficácia do uso da TxBo-A particularmente no tratamento de distúrbios neurológicos associados com hiperatividade dos músculos esqueléticos (Dressler *et al*., 2005; Kok-Yuen & Kian-Hian, 2007).

A TxBo-A foi aprovada para uso na Odontologia através da Resolução CFO-112, de 2 de setembro de 2011 no Brasil o que permitiu seu uso terapêutico em procedimentos odontológicos. Atualmente seu uso terapêutico vem sendo amplamente estudado, incorporando diversas possibilidades de uso, como em alguns quadros de sorriso gengival, no controle da sialorréia, em implantodontia, na cirurgia ortognática, no controle da hipertrofia muscular e bruxismo, e no controle da dor relacionada à DTM. Entretanto, os estudos existentes a respeito do assunto não confirmam se esta intervenção nas DTMs pode ser eficaz para alguns quadros de dor específica, como os relacionados à dor persistente (Song *et al*., 2007; Ernberg *et al*., 2011; Guarda-Nardini *et al*., 2012). Isto, provavelmente, devido às características de ação da droga, que podem variar substancialmente em função de sua concentração, de fatores locais, da técnica utilizada e do número de punções realizadas (Dressler *et al*., 2005). Acredita-se que o refinamento destes parâmetros bem como as características dos músculos a serem injetados e a atividade da droga sobre a fibra nervosa, sejam capazes de alterar os efeitos e a eficácia da TxBo-A (Wheeler *et al*., 2001). Isto pode ser hipotetizado tomando-se por base alguns estudos experimentais em animais de laboratório (Cui *et al*., 2004; Matak *et al*., 2013; Lora *et al*., 2016), que demonstraram efeitos antinociceptivos da TxBo-A prévios ao seu período de ação sobre a vesícula colinérgica, e, consequentemente, sobre a fibra muscular. Consequentemente, a utilização da TxBo-A para controlar a dor persistente é uma extensão lógica à sua utilidade clínica, admitindo propor o seu uso no controle da dor miofascial relacionada à DTM (Rizzatti-Barbosa & Albergaria –Barbosa, 2017).

No entanto é importante salientar uma possível associação das aplicações da TxBo-A nos músculos da mastigação com alguns efeitos adversos importantes, como foi demonstrado em alguns estudos em animais (Rafferty *et al*., 2012; Kun-Darbois *et al*., 2015; Matthys *et al*., 2015). Foram observados indícios de osteopenia nos côndilos mandibulares, possíveis alterações no crescimento craniomandibular e diminuição no desempenho mastigatório. Embora contraditórios, existem também dois estudos clínicos sobre as alterações ósseas mandibulares após injeções de TxBo-A (Chang *et al*., 2011; Raphael *et al*., 2014). Tais achados requerem ponderar o uso terapêutico da

TxBo-A e os efeitos adversos que pode promover, tais como as alterações na eficiência mastigatória, possíveis riscos de osteopenia ou mesmo de fraturas mandibulares.

Devido à escassa evidência científica e ausência de consenso sobre um protocolo seguro e padronizado para o uso da TxBo no controle da dor persistente relacionada à DTM buscando minimizar os efeitos adversos, este ensaio clínico propôs analisar a efetividade da TxBo-A em relação à intervenção convencional para o controle da dor miofascial persistente relacionada a DTM (aconselhamento associado ao uso do aparelho estabilizador). Como variáveis dependentes, avaliou-se a presença e intensidade da dor nos diferentes períodos experimentais por análise subjetiva da dor, através da escala visual analógica (EVA), e por análise do limiar de dor à pressão, através do teste de algometria; a atividade eletromiográfica e espessura muscular dos músculos masseter e temporal anterior, através de eletromiografia de superfície e ultrassonografia, respectivamente; o volume ósseo dos processo coronóides mandibulares, através de tomografia computadorizada de feixe cônico; a preformance mastigatória dos sujeitos da pesquisa; e a análise de alguns parâmetros dos eixos I e II do RDC/TMD. Através dos resultados foi possível constatar que a TxBo-A mostrou-se eficaz no controle da dor miofascial persistente relacionada à DTM. No entanto, alguns efeitos colaterais estabelecem correlação com as dosagens utilizadas, e estes fatores devem ser considerados na indicação e uso da TxBo.

2 Artigo

Este trabalho foi realizado no formato alternativo, conforme as normativas definidas pela CCPG/001/2015, da Comissão Central de Pós-Graduação (CCPG) da Universidade Estadual de Campinas.

O artigo intitulado *"Is botulinum toxin type A effective and safe for chronic myofascial TMD pain? A randomised controlled double-blind clinical trial",* foi apresentado neste processo de defesa, e submetido à publicação no periódico *The Lancet*, ISSN: 0140-6736.

Artigo

Is botulinum toxin type A effective and safe for chronic myofascial TMD pain? A randomised controlled double-blind clinical trial

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Abstract

Background. Published data about the management of persistent masticatory muscle pain with Botulinum Toxin A (BoNT-A) has shown mixed results in clinical trials; however the quality of the evidence is low. We compared three different doses of BoNT-A injections for persistent masticatory muscle pain and established an efficient and safe protocol.

Methods. We did a randomised, double-blind, placebo-controlled clinical trial at Piracicaba Dental School-University of Campinas, Sao Paulo-Brazil. Between March 1, 2013 and June 5, 2016, 540 patients were enrolled, and 100 female patients, aged 18-45 years, presenting persistent masticatory muscle pain were randomly assigned into five groups (20 per group): Splint Group (SP), Saline Group (SS), and three TxBo-A groups with different doses (BoNTA-Low/Medium/High). All patients and investigators were masked to treatment assignment. The outcomes over the course of 180 days from the administration were the comparisson of BoNT-A versus control and placebo, measured as the change from baseline in pain intensity and in pressure pain threshold, masseter and anterior temporal muscles ultrasound (US) and eletromyography (EMG), masticatory performance patients (MP), coronoid apophysis bone volume acquired by cone bean computadorized tomography images (TMG), and some data obtained from axis I and II of RDC/TMD. VAS, PPT EMG, UT and RDC/TMD axis I data were analized in the SAS program using the GENMOD procedure. MP data was explored using the IBM® SPSS® Statistics 24 software (NYSE: IBM; Armonk, United States), and all statistical inferences were accomplished with two-tailed trials (α=.05), achieving a statistical power (1-β) of 0.80 (β=.2). The relationship among the variables was verified by Spearman's correlation. For RDC/TMD axis II variable, one way repeated measures analysis of variance was used. For TMG data, Kruskal-Wallis test was used with a significance level of 5% This trial was registered at ReBEC (Universal Trial Number #111111973181).

Findings. BoNT-A reduced pain intensity ($P<0.05$) and increased pressure pain threshold ($P<0.05$) over 180 days compared with placebo. A decrease in MP ($P<0.05$) and EMG in muscle contraction ($P<0.05$) was found as temporary adverse effects of BoNT-A applications. Muscle thickness and coronoid apophysis bone volume were also affected after BoNT-A applications ($P<0.05$).

Interpretation.

BoNT-A is an effective approach to control persistent masticatory muscle pain; however, due to the evident side effects, we suggest BoNT-A low-group (30U/masseter and 10U/temporalis) as the protocol choice for persistent masticatory muscle pain, presenting an efficient analgesic effect with faster adverse-effects recovery period.

Funding. The Sao Paulo Research Foundation (FAPESP) - Brazil (# 2014/15863-7).

Introduction

Myofascial pain syndrome (MPS) is characterized by the presence of myofascial trigger points,[1] and classified as a primary syndrome (non-related to other medical conditions), or as a secondary syndrome (concomitant with other pain conditions)[2,3]. In dentistry, MPS is know as masticatory myofascial pain (MMP), prevalent in 45% of patients with signs and symptoms of Temporomandibular Disorders (TMD)[4,5]. Therefore, the syndrome has a complex pathogenesis, expressed as a multifactorial etiology with various systemic and local risk factors [6] causing the fluctuating and self-limiting nature of this disorder [7].

The uncertainty knowledge on the etiopathogenesis of MMP proposes several treatment approaches[8]. Conservative, multidisciplinary, symptomatic modalities, such as occlusal splints,[9,10] farmacotherapy[11], physiotherapy[12], and behavioral and physical treatments[13] are the most common treatment approaches for MMP. Since the US Food and Drug Administration approved BoNT-A for the treatment of muscle disorders based on its ability to inhibit synaptic exocytosis and, therefore, to disable neural transmission, several studies have suggested that BoNT-A has analgesic activity, independently of the effect over muscle. It may suggests an indication for BoNT-A in analgesia. Thus, because of its muscle-relaxing and analgesic effects, utilizing BoNT-A to treat chronic MMP is a logical extension of its clinical usefullness; however proper doses, efficacy and safety of the administration of this drug for MMP have not yet been investigated.

To date, published data about the management of MMP with TxBo-A has shown mixed results in clinical trials [8,14–17]. Nevertheless, the quality of the evidence is low, because it comes mostly from studies with inappropriate methodological design, makind difficult their use as a guide in the clinical decision. In addition, no study has assessed the efficacy, possible adverse effects and proper doses of administration of TxBo-A for the treatment of MMP, for more than six month after treatment.

Within this premise, we conducted a randomised controlled doble-blind clinical trial to assess the efficacy and possible adverse effects of three different doses of TxBo-A intramuscular injections in patients with masticatory myofascial pain.

Materials and methods

The Ethics Committee in Research of Piracicaba Dental School, University of Campinas, Brazil, approved the research protocol (#114/2013). All subjects signed an informed consent form to participate in the study.

One hundred consecutive patients were selected according to the following inclusion criteria: female gender, age between 18 and 45 years, presence of jaw muscles pain in accordance to group Ia and Ib diagnoses of the Research Diagnostic Criteria for TMD (RDC/TMD) [18] for more than three months without concurrent presence of TMJ pain; complete dentition (except third molars), self-reported pain intensity higher than 50 mm in the visual analog scale (VAS), contraceptive use and all subjects must had received previous treatments for MMP. Patients with a positive history of trauma in the face and neck area, dental pain, systemic diseases (arthritis and arthrosis), major psychiatric disorders, use of drugs acting on neuromuscular junctions, with any contraindication or hypersensitivity to botulinum toxin A, and that recived *anti-tetanus* vaccine at least 3 months before the clinical trial started, were excluded from potential recruitment.

Randomisation and masking

First, patients were submitted to ultrassonography evaluation of masseteres and anterior temporalis muscles, in order to standardize muscle pattern in each group, according to muscle thickness. Patients randomisation was done after ultrassonography asessment, in which each patients was alocated in each group until all groups had the same quantitiy of the similar muscle tickness patients. After, all random process was performed again until each group was completed by the simple random method, in which each subject was invited to remove a small sealed envelope from a larger opaque envelope indicating five treatment groups: Splint Group (SP), treated with counseling/oral splint, Saline Solution Group (SS), treated with counseling/injections of NaCL 0,9%, Botulinum Toxin Low Group (BoNTA-L) treated with counseling/injections of low doses of BoNT-A, Botulinum Toxin Medium Group (BoNTA-M) treated with counseling/injections of medium doses of BoNT-A and Botulinum Toxin High Group (BoNTA-H) treated with counseling/injections of high doses of BoNT-A (Table 1). All patients and investigators were masked to treatment assignment.

Procedures

According to the experimental design, patients were evaluated eight periods during the investigation protocol, as described in Figure 1. This evaluations were performed by a different researcher, who was not involved in any other process of the study .

Counseling (CLS) was applied in all patients. Consisted in educating the patients about the anatomy and physiology of the stomatognathic system, the etiology and possible good prognosis of MMP, and teaching self-care strategies to control parafunction. Information about the improvement of sleep and correct body posture, as weel as the importance of dietary habits, were also given. The same trained clinician performed CSL at the first appointment for all groups.

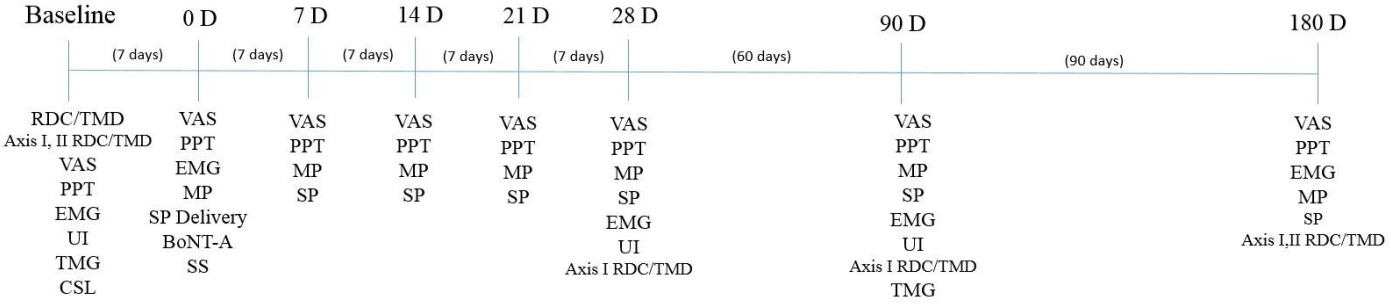


Figure 1. Experimental Chronogram, Treatments and Outcomes

D: day; RDC/TMD: Research Diagnostic Criteria for Temporomandibular Disorders; UI: Ultrasound Imaging; VAS: Visual Analogue Scale; PPT: Pressure Pain Threshold; EMG: Eletromiography; TMG: Computadorized Cone Bean Tomography; CSL: Counseling; MP: Masticatory Performance; SP: Splint adjustment; BoNT-A: Botulinum Toxin Type A; SS: Saline Solution.

Treatments

Splint Group (SP)

The splint (SP) delivered to the patients was a flat occlusal appliance covering all superior teeth, made of transparent thermo-polymerized acrylic resin[10]. The appliance was delivered one week after data collection at baseline. Patients were advised to wear the SP while sleeping, during the six months of therapy. Occlusal adjustment of the SP was performed, if needed, over seven sessions: at the SP delivery, once a week during the first month, after three months, and after six months.

Botulinum Toxin Groups (BoNT-A)

The preparation of drugs and syringes was made by a research who was not involved in any other research procedure. Breafly, BoNT-A (BOTOX®, Allergan, Irvine, California, USA) was supplied as a freeze-dried powder of 100U (99 phials), and was reconstituted with 2 ml of non-preserved saline solution 0.9% to a concentration of 5U/0.1 ml. Doses of BoNT-A were assigned according to the groups distributions (Table 1). BoNT-A was injected in 5 sites of each subject's masseter and anterior temporalis muscles using a 1-ml syringe with a 30-gauge, 13mm needle. In the masseter muscle, the application were done considering 1cm of distance among them; the punctions were placed in the inferior part of the masseter which was the most promient part of this muscle observed when the subjects were asked to clench. In the anterior temporalis muscle, sites were distributed 1cm externally the eyebrow and swith 1cm of distance among them, considering the prominent part of the anterior portion of the temporalis muscle observed when the subject was asked to clench. The injection technique involved inserting the needle into the soft tissue until bone was encountered and then the needle was withdrawn, so that the tip was in the muscle, at which period

the solution was injected after careful aspiration. If aspiration indicated intravascular administration, the needle was slightly moved and aspiration repeated until negative. Another research, handed over the syringe with the drug to be injected to the research investigator to ensure that the patient and the investigator were blinded. The active treatment and placebo solutions were transparent and indistinguishable.

Saline Solution Group

Saline Solution (NaCl 0,9%) was injected bilaterally into each muscle using a 1-ml syrnge with 30-gauge, 13mm needle. The assigned dose is shown in Table 1. Muscles and sites of application as well as the protocol used were the same of the BoNT-A groups.

Injections of BoNTA and SS were done during a single appointment and performed by the same trained clinician.

Experimental Groups	Anterior Temporalis Muscle	Masseter Muscle
BoNTA-L	10U	30U
BoNTA-M	20U	50U
BoNTA-H	25U	75U
SS	20U	50U

Table 1. Distribution of doses according to the experimental groups

BoNTA= Botulinum Toxin Type A; L= Low dose; M= Medium dose; H= High dose; SS= Saline Solution

Outcome Variables (VAS)

Pain, surface electromiography muscle activity, ultrasound imaging, coronoid process bone cone bean tomography, patient's masticatory performance, and I/II RDC/TMD axis were the outcomes variables. Pain was evaluated through Visual Analogue Scale (VAS) and Pain Preassure Threshold (PPT).

Visual analog scale (VAS)

The VAS [19] is a 100 mm horizontal line, anchored by word descriptors at each end. The left end is labeled with the words "no pain," while the right end is labeled "worst pain imaginable." The participants were instructed to mark a point on the line representing the level of their current pain. Patients were instructed to mark at home the daily subjective pain at VAS, therefore the media of the daily VAS was used for data analysis.

Pressure pain treshold (PPT)

The PPT [20] assessment was performed by a single operator (Kappa = 0.89) who did not know the treatments applied to the patients, with a digital algometer (Kratos DDK-20. Sao Paulo, Brazil) with a 1cm^2 circular rod, used to press the muscles. The PPT was assessed bilaterally for the masseter and anterior portion of relaxed-condition temporalis muscles. Pressure was perpendicularly to the surface of the skin at a rate of 0,3kg/cm^2, according to the following sequence: right anterior temporal (RT), right masseter (RM), left masseter (LM), and left anterior temporal (LT) muscle; 5 min later, a second series of pressure followed this order: left anterior temporal (LT), left masseter (LM), right masseter (RM), and right temporal (RT) [20]. Patients were instructed to indicate the moment when the pressure became painful.

Eletromiography (EMG)

To record the electromyographic signal of the evaluated muscles, the ADS 1200 (Lynx Electronic Technology Ltd, Sao Paulo, Brazil) calibrated equipment was handled, using eight channels considering adjusted gain of 1/16,000, with a band-pass filter of 20/500 Hz, and a sampling frequency of 2000 Hz for each channel. The electrodes were placed in the most promient part of the muscle, observed when the subjects were asked to clench in the function test; the neutral electrode was placed on the manubrium of the sternum of the volunteers [21]. The software Lynx AqDa- dos 7.02 and Lynx AqD Analysis 7.0 (Lynx Electronic Technology Ltd, Sao Paulo, Brazil) was used for the acquisition of simultaneous signals and to process the root mean square (RMS) values, expressed in mV. The electrical activity of the muscles was recorded in maximum volunteer contraction (MVC). Three five senconds repetitions of each mandibular position were performed. To avoid muscle fatigue effect [22], a 2-minute period of rest between collections was allowed. To perform MVC, Parafilm M (American National Can, Chicago, IL, USA) was placed bilaterally in the region of the molars. The patients were instructed to clench their jaw to the maximum possible extent and to maintain the same level of contraction for 5 seconds. The RMS value of each acquisition, was obtained in the interval between 2 seconds and 4 seconds. For greater reliability of results, the RMS of the arithmetic mean of the three acquisitions (MVC) was calculated.

An acetate plate was fabricated for each patient in order to keep the algometer and settle the electrodes of electromiography in the same position during the various recording sessions. The acetate plate was perforated at the sites in which BoNT-A and SS were applied, in order to obtain proper reference to place de algometer and the electrodes in the same muscle side of the BoNT-A applications. Perforations were according to anatomic reference lines (external angle of the eye, tragus of the ear, and external angle of the mandible) to warrant reproducibility of future recordings'[23] (Figure 3).

Masticatory performance test (MP)

Condensed silicone (Optosil Comfort®, Heraeus Kulzer, Hanau, Germany) cubes of 5.6 mm edge were obtained using a metal matrix. After initial material setting, the cubes were weighted and stored for 16 h at 60ºC in an oven to perform complete silicone reticulation[24], and disinfected by the use of 2% chlorhexidine solution for 24h[25].

Each subject was asked to chew for 20 chewing cycles in a habitual mode a mouthful of 17 cubes (3.4 g) contained in a plastic pot. Although the subjects were not familiar with the test foods or specified instructions, no pre-test training was performed to obtain the true reproducibility of measures. Also, to prevent imbalance in the conscious/unconscious nature of the chewing process and subsequent fluctuations in bite force and chewing rate, no feedback control was employed [26].

Afterward, the subjects spit out the comminuted particles onto a paper filter placed on a beaker. The oral cavity was rinsed with 200 mL water to recuperate nearly all material (<5% loss). After draining the water and desinfection, the filter with the particles was dried in an oven at 80°C for 25 min. Each sample was sieved through a stack of up to 10 sieves with aperture sizes of 0.50–5.60 mm based on a √2 progression in a shaker (Bertel Indústria Metalúrgica Ltda, Caieiras, Brazil) for 20 min. The particles retained in each sieve were weighed on a 0.001-g balance (Mark 2060; Bel Engineering, Lombardy, Italy). The masticatory performance was calculated using a nonlinear regression $Q_W^-(X) = 1 - 2^{-(X/X_{50})^b}$, considering $\mathbf{Q_W^-}$ the cumulative weight percentage of particles smaller than X or passing through a specific sieve aperture, $\mathbf{X_{50}}$ is the aperture of a theoretical sieve through which 50% of the weight can pass, and **b,** the broadness size distribution of particles [26].

The counting of masticatory cycles, as well as each stage of tests were accomplished by a calibrated examiner (Kappa=0.80).

Ultrasound Imaging (UI)

Real-period imaging of the bilateral masseter and anterior portion of temporalis muscle thickness in a contracted and relaxed condition were performed ultrasonographically (SSA-780 A-APLIO Mx, 38 mm/7-18 MHz; Toshiba Medical System Co., Tokyo, Japan). Muscle thickness was measured directly on the instrument's screen with an accuracy of 0.01 mm. [27].

Computarized Cone Beam Tomography (TMG)

For the measurement of volume of the mandibular coronary process, Cone Beam Computed Tomographies (TMG) were performed using the Picasso Trio 3D® (Vatech, Hwaseong, South Korea). Parameters used to obtain the images were: 85KVp, 5mA, voxel size of 0.2mm and FOV (Field of View) of 12.0cm x 8.5cm, being 2 acquisitions per patient. Thus, three-dimensional images generated from multiplanar reconstructions were obtained using the ITK-SNAP 3.0® segmentation software tools (Cognitica, Philadelphia, PA, USA). Analysis were performed by a single examiner (dental

surgeon and radiologist), who had prior knowledge about the operation of the ITK-SNAP 3.0® software and the tomographic anatomy of the coronoid process, and that was not involved in any other process of the study. Reconstruction of the 3D model was performed with the semi-automatic segmentation mode of the software. The analysed bone structures encompassed beyond the coronoid process itself, in order to have a safety margin in the segmentation. The delimitation of the region of interest was performed with the Snake ROI (Region of Interest) tool.

Research Diagnostic Criteria for Temporomandibular Disorders

The RDC/TMD [18] are standardized diagnostic guide-lines, which provide criteria for a dual-axis assessment, including both physical (axis I) and psychosocial appraisal (axis II). A new version, now called DC/TMD, has been released, but it was not yet available to Brazilian language at the period of this study. Axis I gives information about the physical TMD diagnoses, i.e. muscle disorders, disc displacements, and other joint disorders and clinical findings, while the axis II focuses on the psychosocial symptoms. Axis II comprises an evaluation of the following: chronic pain-related impairment, based on graded chronic pain scale (GCPS) scores (0. no disability; I. low disability, low intensity; II. low disability, high intensity; III. high disability, moderately limiting; IV. high disability, severely limiting); depression levels, based on the Depression Scale (DEP) of the Symptoms-Checklist-90R (SCL-90R) (normal, moderate, severe depression); and non-specific physical symptoms (somatization) levels based on the Somatization Scale (SOM) of the SCL-90R (normal, moderate, severe somatization).

Statistical Analysis

VAS, PPT EMG, UT and RDC/TMD axis I data were analized in the SAS program using the GENMOD procedure. Significance level of 95% was set to all statistical tests. The comparison of the groups and periods of analysis (period), was analized by adjusted generalized linear models considering the gamma distribution (asymmetric), according to a design in repeated measurements for group / period effects and group interaction versus period.

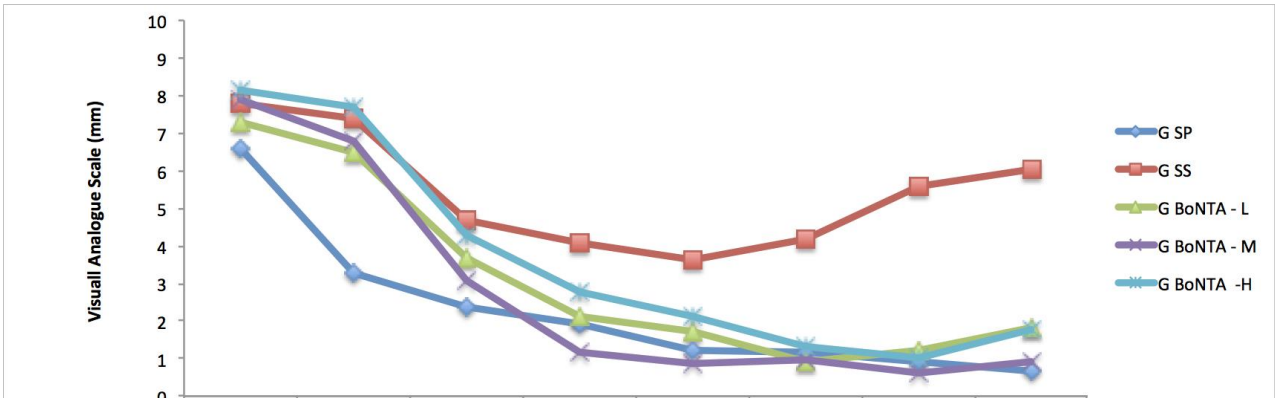
For MP, data was explored used the IBM® SPSS® Statistics 24 software (NYSE: IBM; Armonk, United States), and all statistical inferences were accomplished with two-tailed trials (α=.05), achieving a statistical power (1-β) of 0.80 (β=.2). Assumptions of normality and sphericity were examined through Shapiro-Wilk and Mauchly's (Greenhouse-Geisser correction) tests, respectively. Two-way repeated measures analysis of variance and pairwise comparisons of estimated marginal means using the Fisher's test were performed to find differences in the median particle size assessments. The relationship among the variables was verified by Spearman's correlation. For

RDC/TMD axis II variable, one way repeated measures analysis of variance was used. For TMG data, Kruskal-Wallis test was used with a significance level of 5%

Results

One hundred volunteers (mean age 36.8 ±5.6 years old) were assigned from 540 patients of Piracicaba Dental School (University of Campinas – Brazil), durig the period from 2013 to 2016.

Decrease in VAS was found when considered data baseline and all periods after treatments. It became evident after the 7th day (comparison within-group analysis), specialy for SP group (Figure 2). Moreover, comparison among-groups at different periods showed significant decrease (P<.05) when comparing BoNTA and SP groups with SS group, and the others groups.



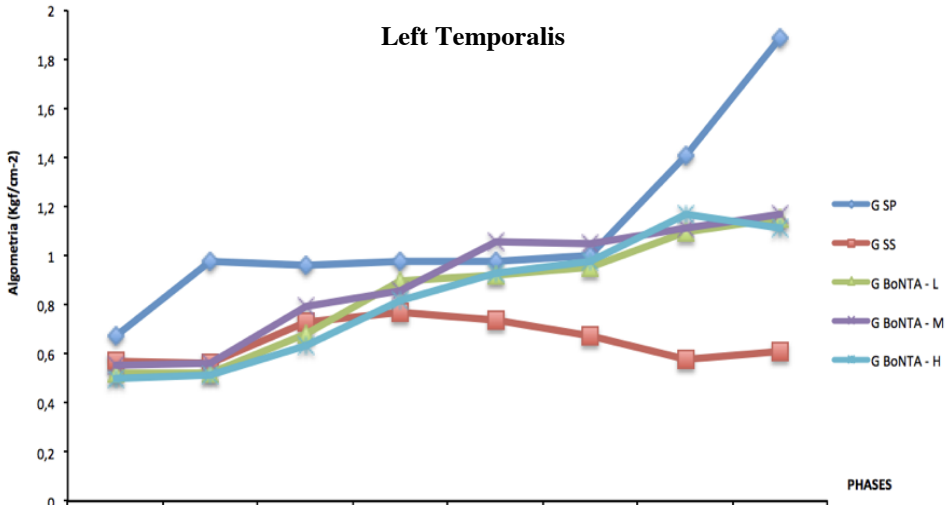
	7 D	0 D	7 D	14 D	21 D	28 D	90 D	180 D
G SP	6,58 C a	3,29 C b	2,35 C bc	1,92 B c	1,24 BC d	1,17 B d	0,94 B d	0,65 C d
G SS	7,78 AB a	7,4 A ab	4,7 A c	4,07 A c	3,63 A c	4,16 A c	5,58 A c	6,05 A bc
G BoNTA - L	7,3 BC a	6,5 B b	3,7 ABC c	2,1 B d	1,7 AB d	0,9 B e	1,2 B e	1,82 B d
G BoNTA - M	7,9 AB a	6,8 AB b	3,09 BC c	1,191 B d	0,87 C e	0,97 B de	0,6 B e	0,91 C e
G BoNTA -H	8,14 A a	7,7 A a	4,3 AB b	2,8 ABc	2,1 A c	1,3 B c	1 B c	1,79 BC c

Phases

Figure 2. Visual Analogue Scale (VAS) data (mm) presented by the groups during experimental periods
Different capital letters in vertical represents statistical differences among groups
Different lowercase letters in horizontal represent differences among periods
G:Group; SP:Splint; SS:Saline Solution; BoNTA:Boulinum Toxin type A; L: Low dose; M: Medium dose; H: High dose; D: Days

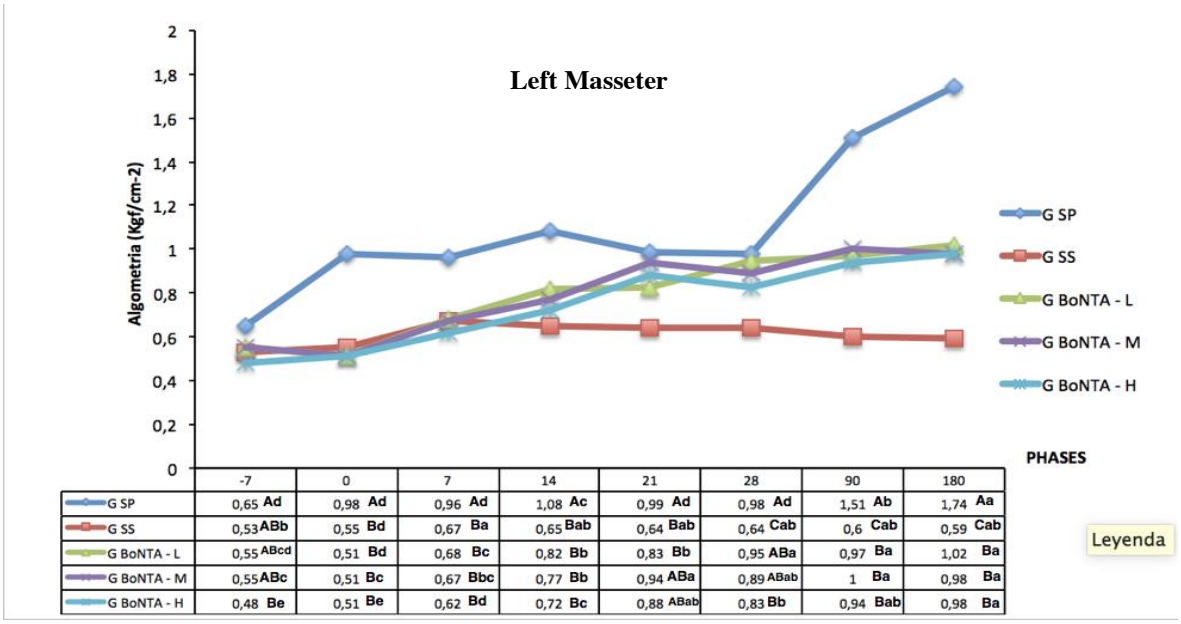
Preassure Pain Threshold (PPT)

Significant increase (P<.05) was found for PPT data for all muscles evaluated in BoNTA and SP groups (within-group analysis) when considering baseline and all post treatment data (Figure 3 a,b,c,d). Aditionally, comparison among groups showed significant PPT increase (P<.05), for LT and LM muscles at the 21st, 90th and 180th days after treatment evaluations. Likewise, significant differences (P<.05) were observed for RT and RM muscles at the 21st, 90th and 180th days in all BoNTA and SP groups (Figure 3 a,b,c,d)



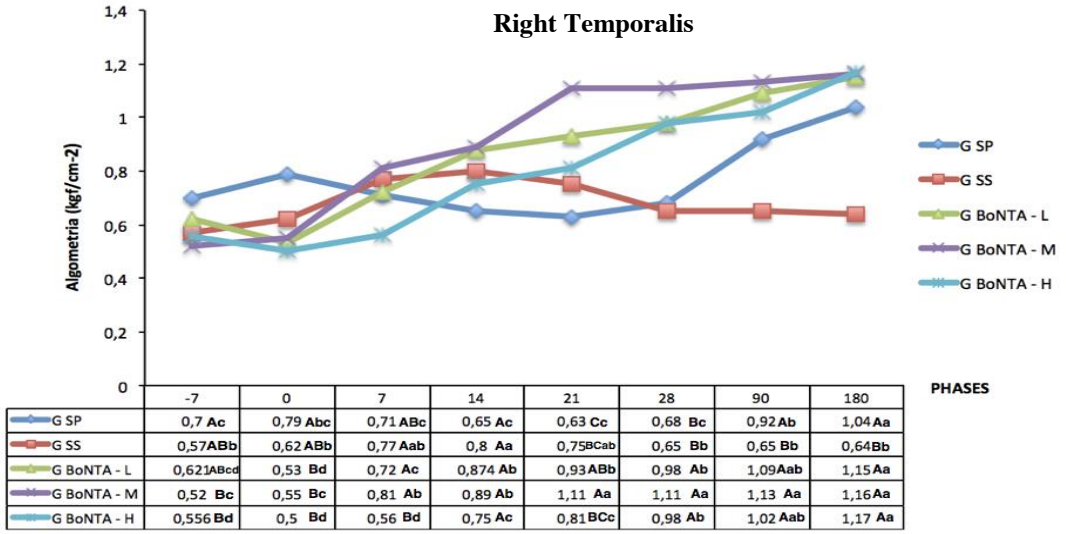
	-7	0	7	14	21	28	90	180
G SP	0,67 Ad	0,98 Ac	0,96 Ac	0,98 Ac	0,98 Ac	1 Ac	1,41 Ab	1,89 Aa
G SS	0,57 ABb	0,56 Bb	0,73 Bab	0,77 Ba	0,74 Ba	0,67 Bab	0,58 Cb	0,61 Cab
G BoNTA - L	0,52 Ad	0,52 Bd	0,68 Bc	0,9 ABb	0,92 ABb	0,95 ABb	1,1 Ba	1,156 Ba
G BoNTA - M	0,55 ABc	0,56 Bc	0,79 Bb	0,86 ABb	1,06 Aa	1,05 Aa	1,11 Ba	1,17 Ba
G BoNTA - H	0,5 Bd	0,51 Bd	0,63 Bc	0,82 ABb	0,93 ABb	0,98 Bab	1,17 ABa	1,11 Bab

Figure 3a.Pressure Pain Threshold (kgf.cm-2) scores among groups in different experimental periods
Different capital letters in vertical represent differences among groups
Different lowercase letters in horizontal represent differences among periods
G= Group; SP= Splint; SS= Saline Solution; BoNTA= Boulinum Toxin type A; L= Low dose; M= Medium dose; H= High dose; D= Days



	-7	0	7	14	21	28	90	180
G SP	0,65 Ad	0,98 Ad	0,96 Ad	1,08 Ac	0,99 Ad	0,98 Ad	1,51 Ab	1,74 Aa
G SS	0,53 ABb	0,55 Bd	0,67 Ba	0,65 Bab	0,64 Bab	0,64 Cab	0,6 Cab	0,59 Cab
G BoNTA - L	0,55 ABcd	0,51 Bd	0,68 Bc	0,82 Bb	0,83 Bb	0,95 ABa	0,97 Ba	1,02 Ba
G BoNTA - M	0,55 ABc	0,51 Bc	0,67 Bbc	0,77 Bb	0,94 ABa	0,89 ABab	1 Ba	0,98 Ba
G BoNTA - H	0,48 Be	0,51 Be	0,62 Bd	0,72 Bc	0,88 ABab	0,83 Bb	0,94 Bab	0,98 Ba

Figure 3b. Pressure Pain Threshold (kgf/cm-2) scores between groups in different periods
Different capital letters in vertical represent differences among groups
Different lowercase letters in horizontal represent differences among periods
G:Group; SP:Splint; SS:Saline Solution; BoNTA: Boulinum Toxin type A; L: Low dose; M: Medium dose; H: High dose; D: Days



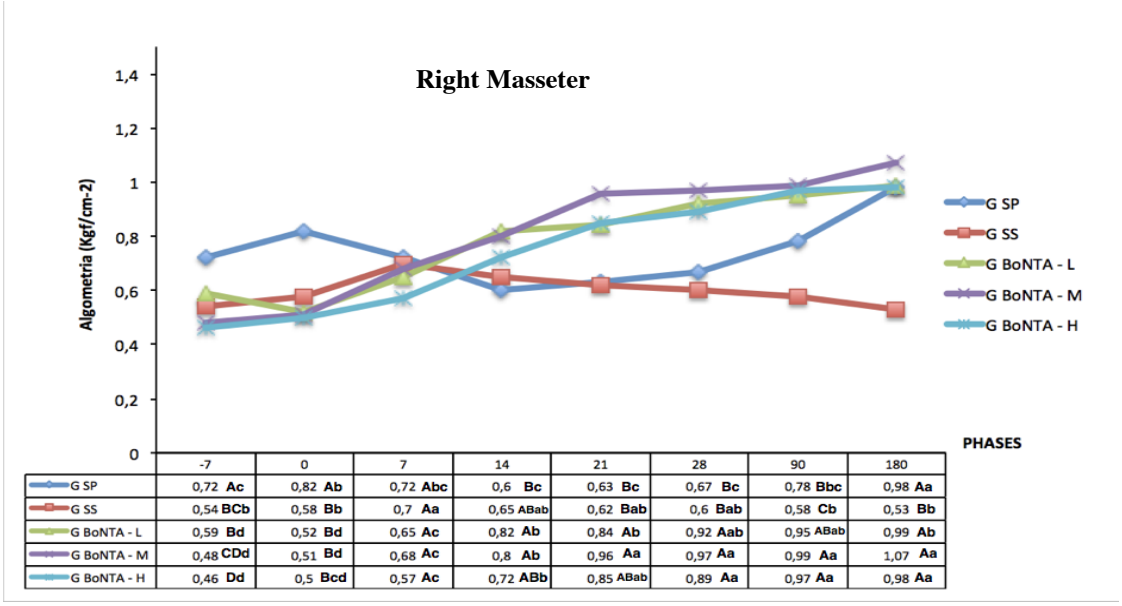
	-7	0	7	14	21	28	90	180
G SP	0,7 Ac	0,79 Abc	0,71 ABc	0,65 Ac	0,63 Cc	0,68 Bc	0,92 Ab	1,04 Aa
G SS	0,57 ABb	0,62 ABb	0,77 Aab	0,8 Aa	0,75 BCab	0,65 Bb	0,65 Bb	0,64 Bb
G BoNTA - L	0,621 ABcd	0,53 Bd	0,72 Ac	0,874 Ab	0,93 ABb	0,98 Ab	1,09 Aab	1,15 Aa
G BoNTA - M	0,52 Bc	0,55 Bc	0,81 Ab	0,89 Ab	1,11 Aa	1,11 Aa	1,13 Aa	1,16 Aa
G BoNTA - H	0,556 Bd	0,5 Bd	0,56 Bd	0,75 Ac	0,81 BCc	0,98 Ab	1,02 Aab	1,17 Aa

Figure 3c. Pressure Pain Threshold (kgf/cm-2) scores between groups in different periods

Different capital letters in vertical represent differences among groups

Different lowercase letters in horizontal represent differences among periods

G:Group; SP:Splint; SS:Saline Solution; BoNTA:Boulinum Toxin type A; L: Low dose; M: Medium dose; H: High dose; D: Days



	-7	0	7	14	21	28	90	180
G SP	0,72 Ac	0,82 Ab	0,72 Abc	0,6 Bc	0,63 Bc	0,67 Bc	0,78 Bbc	0,98 Aa
G SS	0,54 BCb	0,58 Bb	0,7 Aa	0,65 ABab	0,62 Bab	0,6 Bab	0,58 Cb	0,53 Bb
G BoNTA - L	0,59 Bd	0,52 Bd	0,65 Ac	0,82 Ab	0,84 Ab	0,92 Aab	0,95 ABab	0,99 Ab
G BoNTA - M	0,48 CDd	0,51 Bd	0,68 Ac	0,8 Ab	0,96 Aa	0,97 Aa	0,99 Aa	1,07 Aa
G BoNTA - H	0,46 Dd	0,5 Bcd	0,57 Ac	0,72 ABb	0,85 ABab	0,89 Aa	0,97 Aa	0,98 Aa

Figure 3d. Pressure Pain Threshold (kgf/cm-2) scores between groups in different periods

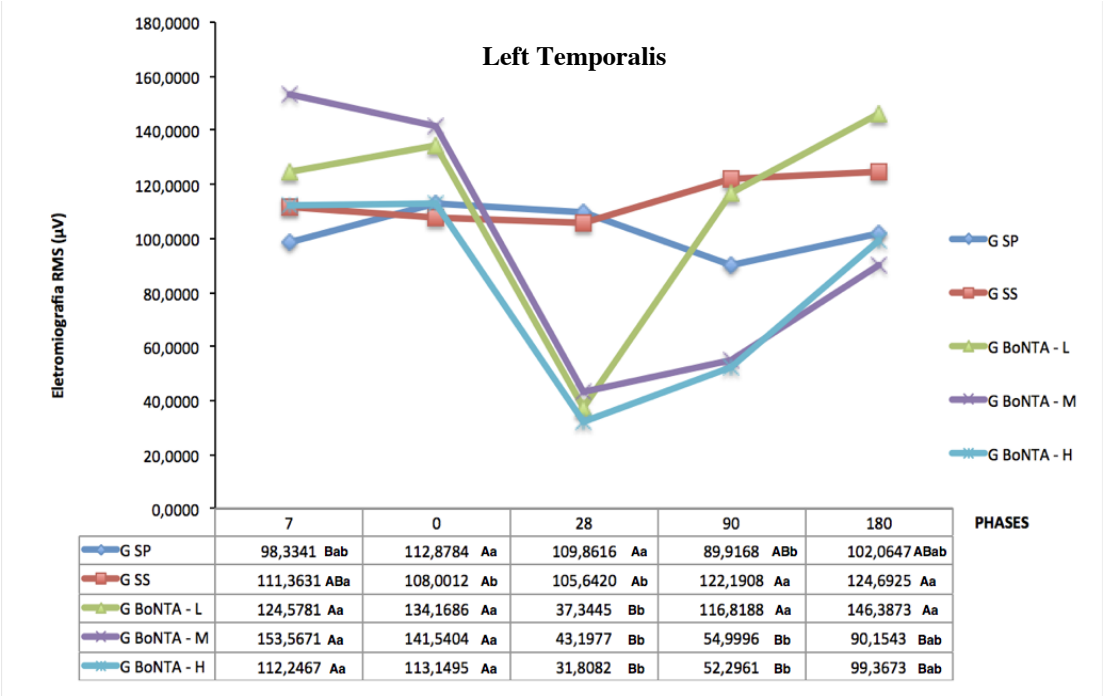
Different capital letters in vertical represent differences among groups

Different lowercase letters in horizontal represent differences among periods

G:Group; SP:Splint; SS:Saline Solution; TxBoA:TxBoulinum Toxin type A; L: Low dose; M: Medium dose; H: High dose; D: Days

Eletromiography (EMG)

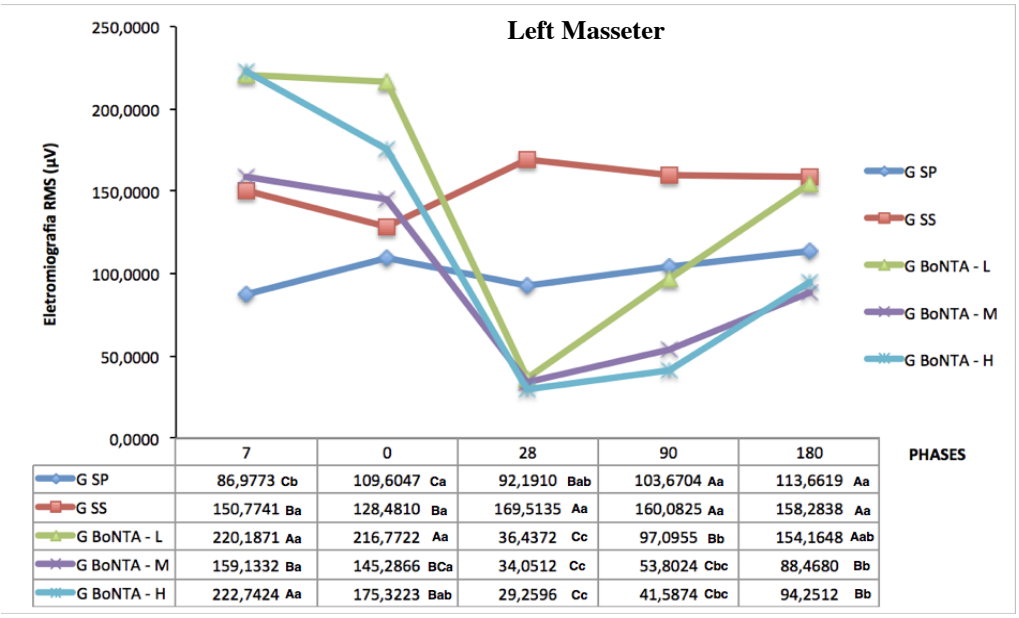
Maximum muscle contraction EMG showed significant dicrease (P<.05) in muscle activity for all muscles for BoNTA groups at the 28th days after treatment (Figure 4 a,b,c,d), when compared with SP and SS. Those data increased for BoNT-L after this period and became normal after 180th days after treatment. That was not observed to the BoNT-M and BoNT-H groups.



	7	0	28	90	180
PHASES					
G SP	98,3341 Bab	112,8784 Aa	109,8616 Aa	89,9168 ABb	102,0647 ABab
G SS	111,3631 ABa	108,0012 Ab	105,6420 Ab	122,1908 Aa	124,6925 Aa
G BoNTA - L	124,5781 Aa	134,1686 Aa	37,3445 Bb	116,8188 Aa	146,3873 Aa
G BoNTA - M	153,5671 Aa	141,5404 Aa	43,1977 Bb	54,9996 Bb	90,1543 Bab
G BoNTA - H	112,2467 Aa	113,1495 Aa	31,8082 Bb	52,2961 Bb	99,3673 Bab

Figure 4a. Root Mean Square scores (RMS µV) in maximum muscle contraction condition among groups in different periods

Different capital letters in vertical represent differences among groups
Different lowercase letters in horizontal represent differences among periods
G:Group; SP:Splint; SS:Saline Solution; BoNTA:Boulinum Toxin type A; L:Low dose; M:Medium dose; H:High dose; D:Days



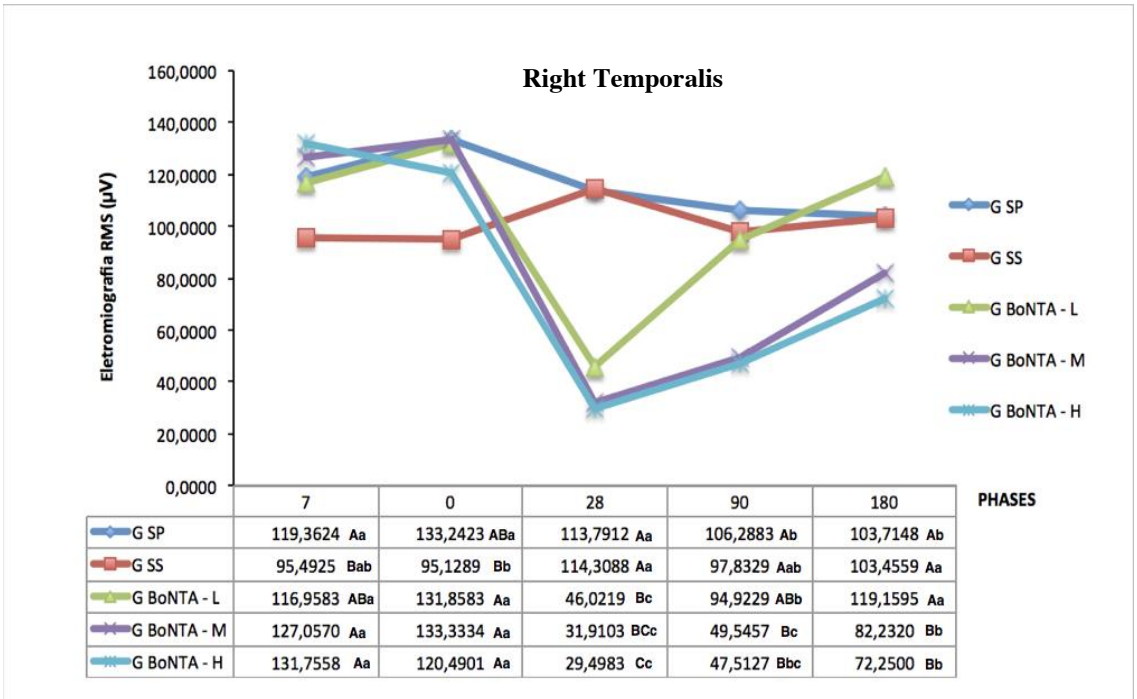
	7	0	28	90	180	PHASES
G SP	86,9773 Cb	109,6047 Ca	92,1910 Bab	103,6704 Aa	113,6619 Aa	
G SS	150,7741 Ba	128,4810 Ba	169,5135 Aa	160,0825 Aa	158,2838 Aa	
G BoNTA - L	220,1871 Aa	216,7722 Aa	36,4372 Cc	97,0955 Bb	154,1648 Aab	
G BoNTA - M	159,1332 Ba	145,2866 BCa	34,0512 Cc	53,8024 Cbc	88,4680 Bb	
G BoNTA - H	222,7424 Aa	175,3223 Bab	29,2596 Cc	41,5874 Cbc	94,2512 Bb	

Figure 4b. Root Mean Square scores (RMS µV) in maximum muscle contraction condition among groups in different periods

Different capital letters in vertical represent differences among groups

Different lowercase letters in horizontal represent differences among periods.

G:Group; SP:Splint; SS:Saline Solution; BoNTA: Boulinum Toxin type A; L:Low dose; M:Medium dose; H:High dose; D:Days



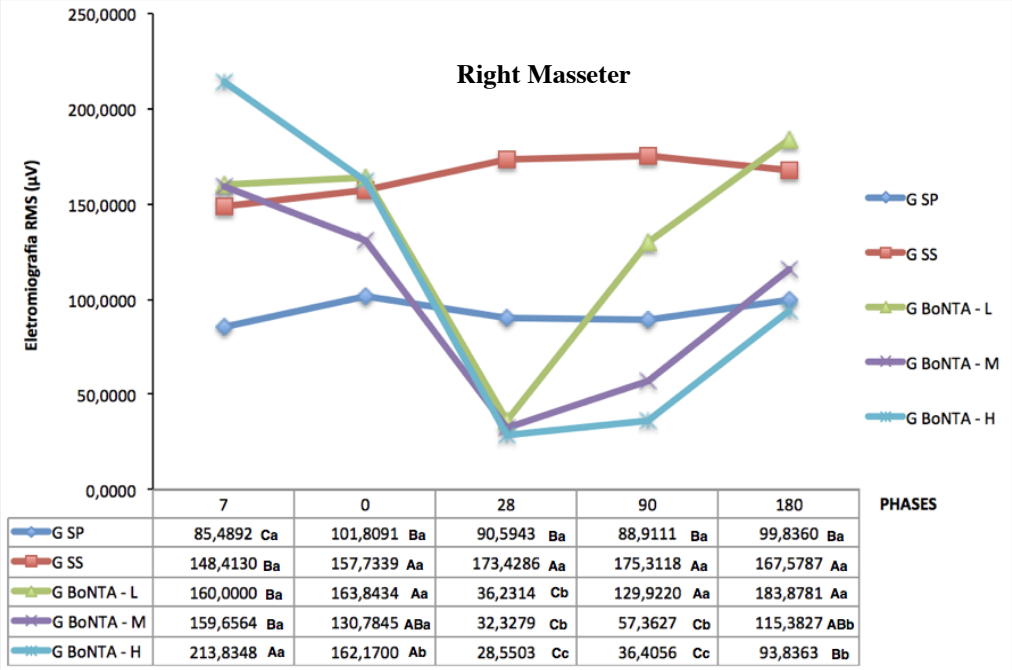
	7	0	28	90	180	PHASES
G SP	119,3624 Aa	133,2423 ABa	113,7912 Aa	106,2883 Ab	103,7148 Ab	
G SS	95,4925 Bab	95,1289 Bb	114,3088 Aa	97,8329 Aab	103,4559 Aa	
G BoNTA - L	116,9583 ABa	131,8583 Aa	46,0219 Bc	94,9229 ABb	119,1595 Aa	
G BoNTA - M	127,0570 Aa	133,3334 Aa	31,9103 BCc	49,5457 Bc	82,2320 Bb	
G BoNTA - H	131,7558 Aa	120,4901 Aa	29,4983 Cc	47,5127 Bbc	72,2500 Bb	

Figure 4c. Root Mean Square scores (RMS µV) in maximum muscle contraction condition among groups in different periods

Different capital letters in vertical represent differences among groups

Different lowercase letters in horizontal represent differences among periods

G:Group; SP:Splint; SS:Saline Solution; BoNTA: Boulinum Toxin type A; L:Low dose; M:Medium dose; H:High dose; D:Days



	7	0	28	90	180
PHASES					
G SP	85,4892 Ca	101,8091 Ba	90,5943 Ba	88,9111 Ba	99,8360 Ba
G SS	148,4130 Ba	157,7339 Aa	173,4286 Aa	175,3118 Aa	167,5787 Aa
G BoNTA - L	160,0000 Ba	163,8434 Aa	36,2314 Cb	129,9220 Aa	183,8781 Aa
G BoNTA - M	159,6564 Ba	130,7845 ABa	32,3279 Cb	57,3627 Cb	115,3827 ABb
G BoNTA - H	213,8348 Aa	162,1700 Ab	28,5503 Cc	36,4056 Cc	93,8363 Bb

Figure 4d. Root Mean Square scores (RMS µV) in maximum muscle contraction condition among groups in different periods

Different capital letters in vertical represent differences among groups

Different lowercase letters in horizontal represent differences among periods

G:Group; SP:Splint; SS:Saline Solution; BoNTA: Boulinum Toxin type A; L:Low dose; M:Medium dose; H:High dose; D:Days

Masticatory Performance (MP)

The changes in MP during 180 days are presented in table 2. The intra-group data showed that MP of all BoNTA groups significantly declined (P<.05), during the first 28 days but fully recovered until the last evaluation (180 days). For SP group, masticatory performance showed no improvement during the first month, but presented a significant recovery in the 90 and 180 days evaluations (P<.05).

The MP inter-groups showed that BoNTA-L group was maintained similar to SS group, showing no significant differences over period (P>.05) with exception of the baseline and 28th days evaluation. BoNTA-M and BoNTA-H groups were significantly lower than SS group (P<.05) in all the evaluations with the exception of the baseline. Also, masticatory performance of BoNTA-L group was significantly higher (P<0.05) than BoNTA-M and BoNTA-H groups in almost all evaluations, with the exception of the baseline and 28th days (P<0.05).

Table 2. Mean and standard deviation of the comminuted median particle sizes (mm) according to period and concentration groups

Groups	Period						
	Baseline	7 D	14 D	21D	28D	90D	180D
BoNT-A							
High	5.9±0.8 Bab	6.4±0.6 Aab	6.6±0.5Aa	6.7±0.6 Aab	6.4±0.7 Aa	6.1±0.7 ABab	5.9±0.9 Ba
Medium	5.9±0.9 Bab	6.8±0.5Aa	6.9±0.6Aa	6.8±0.7 Aa	6.6±0.6ABa	6.3±0.8 Ba	6.2±0.7 Ba
Low	5.5±0.9 Bb	6.1±0.7 Ab	6.1±0.6Ab	6.1±0.8 Abc	6.2±0.4 Aa	5.5±0.7 Bb	5.1±0.8 Cb
SS	6.2±0.7 Aa	5.8±0.7 Ac	5.8±0.8Ab	5.7±0.9 Ac	5.7±0.9 Ab	5.1±1.1 Bc	5.0±1.1 Bb

Uppercase letters in horizontal represent differences among the periods of groups evaluations
Lowercase letters in vertical denote differences among botulinum toxin concentrations and saline solution
BoNT-A: Botulinum Toxin Type A; SS: Saline Solution; D: Day

Ultrasound Imaging (UI)

Table 3 shows the UI results for both condition, relaxation and maximum contraction, of all muscles at baseline, 28th and 90th days. BoNTA-L group significantly decreased muscle thickness in both conditions ($P<0.05$), with exception ME in relaxation and contracted MD in maximum contraction. BoNTA-M group significantly decreases muscle thickness after treatment in maximum contraction and relaxation ($P<0.05$), with the exception of MD in relaxation. BoNTA-H showed a significant thickness decrease in TE and ME muscles in maximum contraction and relaxation ($P<0.05$). In the inter-group analysis, BoNTA-L was the only group with no significant difference when compared with SS group after 28 and 90 days after treatment ($P>0.05$); however it also presented no differences with BoNTA-M and BoNTA-H groups ($P>0.05$); on the other hand, SS group muscle thickness was significantly higher than BoNTA-M and BoNTA-H groups at the 28 and 90 days after treatments in almost all muscles ($P<0.05$), except ME in maximum contraction and ME-MD in relaxation ($P>0.05$).

Table 3 . Mean and standar desviation of muscle thickness evaluated in different periods.

M	T	SS	TxBoA-L	TxBoA-M	TxBoA-H	SS	TxBoA-L	TxBoA-M	TxBoA-H
		CONTRACTED				RELAXED			
	B	2,45 (1,05) Aa	2,32 (0,71) Aa	2,13 (0,88) aA	1,89 (0,82) Aa	1,68 (0,93) Aa	1,65 (0,61) Aa	1,43 (0,73) Aa	1,23 (0,48) Aa
RT	28D	2,45 (1,27) Aa	1,70 (0,65) ABb	1,40 (0,55) Bb	1,54 (0,71) Ba	1,61 (0,86) Aa	1,18 (0,55) ABb	0,94 (0,55) Bb	1,08 (0,58) ABa
	90D	2,57 (1,51) Aa	1,70 (0,59) ABb	1,47 (0,62) Bb	1,41 (0,43) Ba	1,82 (1,21) Aa	1,17 (0,56) ABb	0,99 (0,61) Bb	0,96 (0,40) Ba
	B	11,70 (1,88) Aa	12,37 (1,75) Aa	11,90 (1,55) Aa	12,34 (1,68) Aa	9,33 (1,44) Aa	9,84 (1,68) Aa	9,57 (1,57) Aa	9,91 (1,48) Aa
RM	28D	12,04 (2,11) Aa	11,75 (1,47) ABb	9,88 (1,83) Bb	10,44 (1,26) Ba	9,78 (1,97) Aa	9,62 (1,75)Aa	8,49 (1,62)Aa	8,97 (1,44) Aa
	90D	12,09 (1,79) Aa	11,6 (1,70) ABb	10,49 (1,72) Bb	11,38 (1,67) Ba	10,22 (1,47) Aa	9,61 (1,74) Aa	8,85 (1,86) Aa	9,46 (1,64) Aa
	B	11,51 (1,80) Aa	12,83 (1,44) Aa	12,52 (1,71) Aa	12,39 (1,91) Aa	9,92 (1,65) Aa	10,91 (1,35) Aa	10,64 (1,85) Aa	10,46 (1,90) Aa
LM	28D	11,72 (1,81) Aa	12,08 (2,11) Aa	11,10 (1,77) Ab	10,91 (1,56) Ab	9,64 (1,65) Ab	10,49 (1,89) Ab	9,84 (1,85) Ab	9,46 (1,60) Ab
	90D	11,76 (1,68) Aa	12,01 (1,67) Aa	11,28 (1,64) Ab	11,22 (1,49) Aab	9,89 (1,22) Ab	10,22 (1,55) Ab	9,61 (1,95) Ab	9,66 (1,44) Ab
	B	2,49 (0,99) Aa	2,57 (0,80) Aa	2,26 (0,84) Aa	2,03 (0,57) Aa	1,84 (0,78) Aa	1,71 (0,72) Aa	1,49 (0,71) Aa	1,37 (0,46) Aa
LT	28D	2,44 (0,97) Aa	1,68 (0,62) ABb	1,50 (0,54) Bb	1,59 (0,66) Bab	1,74 (0,81) Aa	1,21 (0,65) ABb	0,98 (0,44) Bb	1,13 (0,53) ABab
	90D	2,58 (0,97) Aa	1,65 (0,64) Bb	1,42 (0,63) Bb	1,41 (0,51) Bb	1,86 (0,88) Aa	1,14 (0,56) Bb	0,9 (0,42) Bb	0,93 (0,39) Bb

Uppercase letters in horizontal represent differences among botulinum toxin concentrations and saline solution
Lowercase letters in vertical denote differences among period groups.
M: Muscle; T:Period; BoNT-A: Botulinum Toxin Type A; L: Low dose; M: Medium dose; H:High dose; SS: Saline Solution; RT: Righ Temporal; RM: Righ Masseter; LM: Left Masseter; LF: Left Temporal; B:Baseline; D: Day

Significant correlation (rho ≈ 0.2, $p < 0.05$) was found between muscle thickness and masticatory performance data for all muscles of BoNT-A groups, when data were grouped (Table 3), escept for the RM and LT in relaxation.

Table 4. Correlation between muscles thickness and masticatory performance

VARIABLE	Correlation Coefficient	P
C-RT	-0,233	0,002*
C-RM	-0,188	0,015*
C-LM	-0,179	0,02*
C-LT	-0,216	0,005*
R-RT	-0,215	0,005*
R-RM	-0,129	0,095
R-LM	-0,17	0,027*
R-LT	-0,115	0,137

*Significant difference (P<.05). C: Maximum Contraction; R:Relaxation RT: right temporal; LT: left temporal; RM: right masseter; LM: left masseter

Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD)

RDC/TMD axis I showed significant increase in spontaneus pain in mouth opening measurements for BoNT-A groups when comparing baseline with the 180th days after treatment evaluation. Moreover, when the analysis was between-group, considering baseline and the 180th days evaluation, significant differences (P<.05) were found among SS group and the other groups, with TxBo-A groups presenting the highest values.

Table 5. Mean and standar desviation of spontaneus pain opening mouth (mm) in different periods.

Groups	Period					
	Baseline		28 D		180 D	
	Mean	SD	Mean	SD	Mean	SD
BoNTA-L	31.9aB	8.7	33.6aB	8.8	38.3abA	7.5
BoNTA-M	32.4aC	9.4	36.0aB	7.7	40.7aA	7.6
BoNTA-H	32.6aB	8.9	35.0aB	9.2	39.0aA	8.1
SS	36.2aAB	6.1	37.6aA	6.6	34.0bB	9.0

Different letters (lowercase in vertical) represent signicant difference (p≤.05) among groups
Different letters (uppercase in horizontal) represent significant difference (p≤.05) among phases
BoNTA: Botulinum Toxin type A; L: low dose; M: medium dose; H:high dose; SS: Saline Solution; D: Days

Table 6. Medium, minimum and maximum values for maximum unassisted and assisted opening in different periods.

	Period								
Unassisted	Baseline			28 D			180 D		
Grupos	Medium	Mn	Mx	Medium	Mn	Mx	Medium	Mn	Mx
BoNTA-L	41.0aB	28.0	59.0	42.0aB	29.0	58.0	44.5aA	31.0	60.0
BoNTA-M	43.0aB	29.0	54.0	43.5aB	30.0	52.0	45.0aA	35.0	60.0
BoNTA-H	41.0aC	26	50.0	45.0aB	25.0	52.0	46.0aA	23.0	55.0
SS	41.5aA	35.0	55.0	42.0aA	50.0	50.0	39.0bB	20.0	45.0
Assisted									
BoNTA-L	43.0aB	30.0	60.0	43.0aB	31.0	58.0	47.0aA	33.0	62.0
BoNTA-M	47.0aAB	30.0	57.0	45.5aB	31.0	55.0	48.5aA	35.0	61.0
BoNTA-H	44.0aB	30.0	55.0	47.0aB	30.0	53.0	50.0aA	31.0	57.0
SS	42.5aA	33.0	56.0	44.5aA	34.0	51.0	41.5bB	22.0	47.0

Different letters (lowercase in vertical) represent signicant difference (p≤.05) among groups
Different letters (uppercase in horizontal) represent significant difference (p≤.05) among phases
BoNTA: Botulinum Toxin type A; L: low dose; M: medium dose; H:high dose; SS: Saline Solution; D: Days

Significant diferences (P<.05) were found between-group analyses for right lateral movement data, with BoNT-A groups presenting higher values than the SS group in the 180th days evaluation.

Table 7. Medium, minimum and maximum values for right (RLM) and left lateral movements (LLM) in different periods.

	Period								
RLM	Baseline			28 D			180 D		
Groups	Medium	Mn	Mx	Medium	Mn	Mx	Medium	Mn	Mx
BoNTA-L	9.5aB	2.0	15.0	9.0aB	3.0	12.0	11.0aA	6.0	13.0
BoNTA-M	9.0aB	5.0	12.0	9.0aA	6.0	14.0	10.0aA	7.0	13.0
BoNTA-H	8.5aB	4.0	14.0	9.5aB	4.0	14.0	10.5aA	5.0	15.0
SS	8.0aB	3.0	11.0	9.0aAB	5.0	14.0	8.5bAB	5.0	12.0
LLM									
BoNTA-B	8.0aB	0.0	13.0	9.5aA	4.0	13.0	9.0aA	4.0	14.0
BoNTA-M	8.0aB	2.0	12.0	9.5aA	6.0	15.0	10.0abA	8.0	15.0
BoNTA-A	9.5aB	4.0	12.0	9.5aB	4.0	15.0	10.0abA	6.0	15.0
SS	8.0aA	3.0	13.0	10.0aA	5.0	15.0	8.5bA	5.0	15.0

Different letters (lowercase in vertical) represent signicant difference (p≤ .05) among groups
Different letters (uppercase in horizontal) represent significant difference (p≤ .05) among phases
BoNTA: Botulinum Toxin type A; L: low dose; M: medium dose; H:high dose; SS: Saline Solution; D: Days

Tenderness palpation showed significant decrease (P<.05) in all muscles for all TxBo-A groups in the 1st and 6th month evaluations. Moreover, when the between-group analysis was considered at the 1st and the 6th month evaluations, significant differences were found between SS groups and the others groups, with TxBo-A groups presenting the lowest values.

Table 8. Medium, minimum and maximum values for right (RT) and left temporal (LT) muscle in different periods.

	Period									
RT	Baseline			28 D			180 D			
Groups	Medium	Mn	Mx	Medium	Mn	Mx	Medium	Mn	Mx	
BoNTA-L	2.5abA	0.0	3.0	1.0bB	0.0	2.0	0.0aA	0.0	2.0	
BoNTA-M	2.0bA	0.0	3.0	1.0bB	0.0	2.0	0.0aA	0.0	2.0	
BoNTA-H	2.0aA	0.0	3.0	0.0bB	0.0	3.0	0.5aA	0.0	2.0	
SS	2.0abA	0.0	3.0	2.0aA	0.0	3.0	2.0bAB	0.0	3.0	
Group (P=.071); period (P=.0001); group*period (P=.0139); Source Pr>ChiSq										
*LT										MC
BoNTA-L	2.0	0.0	3.0	1.0	0.0	3.0	0.5	0.0	3.0	b
BoNTA-M	1.5	0.0	3.0	0.0	0.0	3.0	0.0	0.0	2.0	b
BoNTA-H	2.0	0.0	3.0	0.5	0.0	3.0	0.0	0.0	3.0	ab
SS	1.0	0.0	3.0	1.0	0.0	3.0	1.5	0.0	3.0	a
MC		A			B			B		
*Group (P=.0714); period (P<.0001); group*period (P=.3062); Source Pr>ChiSq; MC: Multiple comparison										

Different letters (lowercase in vertical) represent signicant difference (p≤ .05) among groups
Different letters (uppercase in horizontal) represent significant difference (p≤ .05) among periods
BoNTA: Botulinum Toxin type A; L: low dose; M: medium dose; H:high dose; SS: Saline Solution; D: Days

Tabela 9. Medium, minimum and maximum values for right and left masseter muscle in different periods.

	Period								
Right	Baseline			28 D			180 D		
Grupos	Medium	Mn	Mx	Medium	Mn	Mx	Medium	Mn	Mx
BoNTA-B	2.0aA	0.0	3.0	1.0bB	0.0	12.0	1.0bB	0.0	2.0
BoNTA-M	1.0aA	0.0	3.0	0.0bB	0.0	14.0	0.0bB	0.0	3.0
BoNTA-A	2.0aA	0.0	3.0	0.05bB	0.0	14.0	0.0bB	0.0	3.0
SS	2.0aA	1.0	3.0	2.0aA	0.0	14.0	2.0aA	0.0	3.0
Left									
BoNTA-B	1.5aA	0.0	3.0	0.0bB	0.0	2.0	0.5bB	0.0	3.0
BoNTA-M	1.0aA	0.0	3.0	0.0bB	0.0	3.0	0.0bB	0.0	3.0
BoNTA-A	2.0aA	0.0	3.0	0.0bB	0.0	3.0	0.0bB	0.0	2.0
SS	1.0aA	0.0	3.0	2.0aA	0.0	3.0	2.0aA	0.0	3.0

Different letters (lowercase in vertical) represent signicant difference (p≤ .05) among groups
Different letters (uppercase in horizontal) represent significant difference (p≤0,05) among phases
BoNTA: Botulinum Toxin type A; L: low dose; M: medium dose; H:high dose; SS: Saline Solution; D: Days

RDC/TMD axis II findings (Table 10) showed a significant improvement (P<.05) for almost all groups in the graded chronic pain scale. Also, when groups values were compared, significant differences (P<.05) were found in the 180 days evaluations, with the BoNTA-M and H groups and SP groups presenting the lowest values (Table 10).

Significant improvement (P<.05) was found for all treated groups, except for the BoNTA-M group when the between-group analysis was considered at the 180 days evaluation, significant differences were found between SS groups and the others groups, with BoNT-A groups and SP groups presenting the lowest values.

Somatization scale scores showed significant improvement (P<.05) for all BoNT-A groups and SP groups at the 180 days evaluation. When groups were compared, significant differences (P<.05) were found in the 180 days evaluation, among SS groups and the others groups, with BoNT-A groups and SP groups presenting the lowest values.

Table 10. Differences between average scores in axis II findings between groups at the various observation points according to the phase evaluation.

RDC/TMD Axis II	SP	SS	BoNTA-B	BoNTA-M	BoNTA-A
Graded Chronic Pain Scale					
Baseline	2,30aA	2,40aA	2,20aA	2,15aA	2,65aA
180 D	0,50bB	1,75bA	0,85bAB	0,40bB	0,85bAB
Depression Scale					
Baseline	0,90aA	1,20aA	1,30aA	1,05aA	1,55aA
180 D	0,25bB	1,40aA	0,50bB	0,50aB	0,30bB
Somatization Scale					
Baseline	1,35aA	1,60aA	1,60aA	1,30aA	1,75aA
180 D	0,20bB	1,40aA	0,65bB	0,30bB	0,45bB

Different letters (uppercase in horizontal) represent significant difference (p≤0,05) among groups.
Different letters (lowercase in vertical) represent significant difference (p≤0,05) among evaluation phases
SP: Splint; SS: Saline Solution; BoNTA: Botulinum Toxin type A; L: low dose; M: medium dose; H:high dose; D: Days

Computarized Cone Beam Tomography (TMG)

TMG results (Table 11) showed significant reduction (P<.05) of bilateral coronoid apophysis bone volumen in the BoNTA-H group at the 90 days evaluation; however no significant differences were observed in the other groups at the same period (P>.05)

Table 11 . Median of coronoid apophisis bone volume evaluated in different periods

Periods	Right				Left			
	SS	BoNTA-L	BoNTA-M	BoNTA-H	SS	BoNTA-L	BoNTA-M	BoNTA-H
Baseline	217.2aA (±77)	213.6aA (±122.3)	195.6aA (±118)	207.9aA (±108.7)	185.7aA (±25.5)	243.6aA (±184.3)	156.4aB (±76.2)	232.8aA (±86.4)
90D	210.9aA (±50.6)	194aA (±213.8)	164.3aA (±135.9)	189.2bA (±93.8)	171.3aA (±61.1)	236.3aA (±195.4)	158.5aA (±54)	195.1bA (±105.1)

Different letters (lowercase in vertical) represent significant difference (p≤.05) among evaluation periods
Different letters (uppercase in horizontal) represent significant difference (p≤.05) among groups
SS: Saline Solution; BoNTA: Botulinum Toxin type A; L: low dose; M: medium dose; H:high dose; D: Da

Discussion

Our randomised clinical trial (RTL) fulfill the methodological requirements stated in recent systematic reviews. This RCT, as far as the authors know[28], is the first research wich aimed to compared the efficacy and adverse effects of three different doses of BoNT-A on MMP with a control (SP) and placebo (SS) groups. Therefore, the importance of an experimental design considering positive and negative control groups, allows comparing the values within the experimental groups, eliminating placebo effects. Thus, the findings of the present RCT add valuable knowledge.

A significant reduction of subjective pain for BoNT-A groups and SP group was found in our study. All BoNT-A groups presented pain redution since the 7th day after the treatments and last long until 180 days of evaluation. This finding is in line with literature, reporting that when BoNT-A is injected in striate muscle to treat painful muscle disorders, frequently substantial pain relief is achieved [29,30]. So far, this pain relief is attributed to the reduction of the muscle activity due to the blockage of acetylcholine release through the muscle endplate [29,31]. Such as paresis begin after 2-5 days, reaching its maximum effects between the 14th and the 21st day and lasting from 2-3 months before it gradually starts to wear off [29]. Our results pressupose that the decrease of protocol drug doses[14] does not affect subjective pain reduction, and, at the same period, may decrease any possivel side effects and cost treatment, which are the main concerns on literature on BoNT-A use [32].

We observed that clinically, pain relief preceded muscle relaxation and last for a longer period than the described in literature [31]. Probably because BoNT-A treatment may have a pain-relieving effect that is independent of muscle-relaxing variable. Animal studies have shown that BoNT-A peripheral applications inhibited formalin-induced inflammatory pain by preventing the release of glutamate, substance P, and calcitonin gene-related peptide from nociceptive nerve endings, leaving muscle relaxation as a simple secondary effect [33,34]. However, results from human experiments are inconclusive [35,36].

Moreover, BoNT-A groups presented a significant reduction of subjective pain when compared with SS group in VAS evaluation. Our results are incontrast with previous findings [15,32] concernig MMP in which there were no differences between those groups, and in line with two RCTs [14,37] reporting a clear treatment effect of BoNT-A. Lack of clinical protocols, standardization of dosage and different dilution of preparations between the various

commercial brands[38] may contribute to explain the variability in the protocols adopted in the mentioned researches, that certainly influenced the results. Furthermore, the padronization of muscle thickness performed in our study allowed to standarize the sample in a presicely design, inasmuch as literature reports that TxBo-A effects depend on a proportional correlation between muscle size and doses range [29], wich could also alter studies pain results.

Aditionally, significant differences concernig BoNT-A and SP groups in reducing subjective pain in 28th, 90th and 180th evaluation periods, demonstrated that BoNT-A is as effective than oral splints, which is the most used approach for chronic MMP.

We showed a significant increase in PPT values for BoNT-A and SP groups from the 14th day after treatments, remaining higher to SS group only until the 180 days evaluation. Those findings are in contrast with the results of Ernberg *et al* [15] that found no difference in PPT when BoNT-A and splint therapy were compared, or even for BoNT-A applications within different period evaluations. The absence of assessment padronization probably influenced those results. In our RCT, positioning of the measuring tip of the algometer was standardized through marks made on an acetate sheet individualized for each patient, in order to assess them in any evaluation period, and at the same muscle position in which treatment injections were performed, allowing reproductibility of all analyzes**.**

Therefore, BoNT-A positive effects on PPT could be associated to the inhibition on the activation of primary nociceptive afferents, suggesting that BoNT-A was able to inhibit peripheral release of neurotransmitters involved in pain and inflammation processes such as substance P, glutamate and calcitonin gene related peptide, reducing the peripheral sensitization and, indirectly, probably preventing a central sensitization [29,33,34,39,40].

Assessment of muscular electrical activity of the masseter and anterior temporalis muscles was the major concern in our study, due to the paralyzing BoNT-A mechanism of action. EMG MVC values, presented a severe decrease on muscle activity up to the 28st day evaluation in BoNT-A groups, independently of the dose, as it was expected. Our findings are in line with other studies reporting the same tendency in muscular activity after BoNT-A injections [37,41].

Except BoNTA-L group recovered almost all muscle ativity in MVC up to 90th day after treatments, reaching about the same values of baseline, on the opposite of BoNTA-M and BoNTA-H groups, that presented a significant increase in muscle activity even after 180th

day; however this MVC increase was not close by baseline values. It suggests the probable influence of BoNTA spreading in the muscle fibers, making difficult the muscle repairing as a whole [42]; or because the remanescent BoNT-A did not bond to the whole pre-sinaptic vesical once injected due to the large amount of BoNT-A, bonding to the new pre-sinaptic vesicles over period.

Our eletromiographic MVC findings support literature concensous concerning BoNT-A effects under the muscle ativity [29]. When the same patient is treated with identical treatment parameters the action of BoNT-A is usually stable; however as the showed in our study, there is a dose-effect and dose-duration relationship, indicating that higher doses will present more muscle disability and for longer period of period. Additionally, we believe that this variable determines one of the main side effects of BoNT-A when injected into the chewing muscles, considering that EMG activity is directly related to the efficiency and quality of mastication [26].

The most common patient's complaint after BoNT-A injection is the discomfort in chewing, probably due to BoNT-A muscle activity decreasing action [43]. In addition, changes in afferent input after BoNT-A injection into muscles can modify the response of the cortex, alters the motor neuron activity and even decrease muscle activity [44]. Probably, BoNT-A injection into masticatory muscle directely influences chewing by muscle weakness and atrophy, and it indirectly influences mastication by affecting central pattern generator in brainstem through modification of the sensory feedback from masticatory muscle spindle [43].

A significant decrease in MP after BoNT-A injections in masseter and anterior temporalis muscles was found in our study. Mastigatory performance declinee after the 7st day after BoNT-A injections, and remained inefficient until the 28st day evaluation; however MP recovered to baseline values in the 90rd and 180th days evaluations, independently of BoNT-A doses . Our results are in line with studies showing the same pattern in MP [45,46]. It was an expected result because of the decreasing effect of BoNT-A which starts after at 2nd and 5th days, reaching the maximum effects between the 14th and 21st days, gradually starts to wear off, finishing the effect from 2 to 3 months. It could explain the drecrease and recovery patterns found in our study after 180 days.

Moreover, BoNTA M and H groups also presented significant differences in MP when compared with SS group, confirming the undesirible effect of BoNT-A in mastigatory

function. Regarding, BoNTA-L group, even though MP declined also in this group, no significant differences were found when compared with the SS group, demonstrating that low doses of BoNT-A, in MMP patients almost do not affect MP. Considering that subjective pain values were reduced in all BoNT-A groups, this results may suggested that the doses used in BoNTA-L group may be considered as the ideal doses to control chronic MMP, due to its positive effect over subjective pain and the absence of adverse effects in MP.

Similarly, a significant decrease in muscle thickness was found in all BoNT-A groups in UI contraction position until the 90 days for almost evaluated muscles. Our results are in line with previous studies reporting decrease in muscle thickness [47–49] after BoNT-A use. Atrophy, shortening of muscle fibers and consequently loss of muscle mass may be the causes of those effects.

In aestethic field this result could be seen as benefical, due to the countering face effect, owing to the hypertrophy reducing effect of BoNT-A; however, in dentistry, it could be considered an important adverse effect. Decrease in muscle mass and strength have been reported in the literature after masticatory muscles BoNT-A injections [50–52] the associated structural changes for single or repeat injections must to be considered in dental activities. Physiological cross section, muscle mass is influenced by factors including the content of non-contractile fibrotic or fatty tissue, which accumulates after BoNT-A injection[53]. Atrophy of muscle mass stop after 3 months BoNT-A injections [54]. However, even though BoNTA-L group muscle tickness declined in UI, we did not observe significant differences with the SS group, demonstrating that low doses of BoNT-A do not affect muscle thickness.

A significant relationship between muscle thickness from almost evaluated muscles and the masticatory performance values was found independently of the doses and period. This fact confirms the importance of muscle size and strength in mastication performance, which was the main side effect reported in our study, even after a single injection of TxBo-A.

In addition, muscle mass and strength decrease, could lead to other important side effects such as bone loss at alveolar bone, condyles and coronoid process of the mandible due to the lack of loading in bone tissues [55–57]. We found a significant decrease in both coronoid process bone volumen for the TxBoA-H group at the 90th evaluation. This finding may alert towards the need for an assessment of a possible increased risk of osteopenia or coronoid fractures. It is important to remark that this side effect have been reported in possible

association with repeated BoNT-A in other studie [55], however we showed that this adverse effect could appeare even after a single injection of BoNT-A. Therefore, again, low doses of BoNT-A can be effective to control pain and the prefered protocol to prevent bone or other adverse effects.

On the other hand, the improvement in clinical parameters such as mouth opening, lateral movements and palpation tenderness evaluated with the RDC/TMD axis I in all BoNT-A groups, strengthen the indication of this therapy for MMP. In the same way, the improvement observed in GCPS, DEP and SOM evaluated with the RDC/TMD axis II, in patients allocated in all BoNT-A groups, demonstrated the possitive effects of BoNT-A treatment in the psychological imparment of TMD patients, that usually is affected due to the presence of pain.

Few limitations in this study were addressed. Even though this is the largest double-blind placebo study concerning BoNT-A in chronic MMP, follow ups for longer periods should be performed in order to assess long term effects of a unique injection of BoNT-A.

As a final remark we enphasize that our study if the first RCT evaluating the efficacy and possivel adverse effects of three different doses of BoNT-A, and that compared those groups with two controls: the splint and saline. Base on our findings, we considerd BoNT-A as an effective approach to control chronic myofascial masticatory pain; however due to the evident side effects on muscle contraction, masticatory performance, muscle thickness and in mandibular bone, which are not reported from conservative treatments, we speculate that patients for whom conservative treatment gives inadequate pain relief, low doses of TxBo-A might be a benefical approach as an adjunct.

Acknowledgments

The Sao Paulo Research Foundation (FAPESP) - Brazil (# 2014/15863-7), supported this study.

Conflict of interest statement

The authors do not have any financial relationships that might lead to a conflict of interest.

References

1 Simons DG. Clinical and Etiological Update of Myofascial Pain from Trigger Points. *J Musculoskelet Pain* 1996; **4**: 93–122.

2 Ballyns JJ, Shah JP, Hammond J, Gebreab T, Gerber LH, Sikdar S. Objective sonographic measures for characterizing myofascial trigger points associated with cervical pain. *J Ultrasound Med* 2011; **30**: 1331–40.

3 Xu YM, Ge HY, Arendt-Nielsen L. Sustained nociceptive mechanical stimulation of latent myofascial trigger point induces central sensitization in healthy subjects. *J Pain* 2010; **11**: 1348–55.

4 Manfredini D, Guarda-Nardini L, Winocur E, Piccotti F, Ahlberg J, Lobbezoo F. Research diagnostic criteria for temporomandibular disorders: A systematic review of axis i epidemiologic findings. *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology* 2011; **112**: 453–62.

5 Lora VRMM, Canales GD la T, Gonçalves LM, Meloto CB, Rizzatti-Barbosa CM. Prevalence of temporomandibular disorders in postmenopausal women and relationship with pain and HRT. *Braz Oral Res* 2016; **30**: e100.

6 Greene CS. The etiology of temporomandibular disorders: implications for treatment. *J Orofac Pain* 2001; **15**: 93-105-16.

7 Ohrbach R, Dworkin SF. The Evolution of TMD Diagnosis. *J Dent Res* 2016; 22034516653922.

8 Guarda-Nardini L, Manfredini D, Salamone M, Salmaso L, Tonello S, Ferronato G. Efficacy of botulinum toxin in treating myofascial pain in bruxers: A controlled placebo pilot study. *Cranio - J Craniomandib Pract* 2008; **26**: 126–35.

9 Dao TT, Lavigne GJ. Oral splints: the crutches for temporomandibular disorders and bruxism? *Crit Rev Oral Biol Med* 1998; **9**: 345–61.

10 Conti PCR, De Alencar EN, Da Mota Corrêa AS, Lauris JRP, Porporatti AL, Costa YM. Behavioural changes and occlusal splints are effective in the management of masticatory myofascial pain: A short-term evaluation. *J Oral Rehabil* 2012; **39**: 754–60.

11 Rizzatti-Barbosa CM, Martinelli DA, Ambrosano GMB, De Albergaria-Barbosa JR. Therapeutic response of benzodiazepine, orphenadrine citrate and occlusal splint association in TMD pain. *Cranio* 2003; **21**: 116–20.

12. Nicolakis P, Erdogmus B, Kopf A, Nicolakis M, Piehslinger E, Fialka-Moser V. Effectiveness of exercise therapy in patients with myofascial pain dysfunction syndrome. *J Oral Rehabil* 2002; **29**: 362–8.

13 De Laat A, Stappaerts K, Pappy S. Counseling and Physical Therapy as Treatment for Myofascial Pain of the Masticatory System. *J Orofac Pain* 2003; **17**: 42–9.

14 von Lindern JJ, Niederhagen B, Bergé S, Appel T. Type A botulinum toxin in the treatment of chronic facial pain associated with masticatory hyperactivity. *J Oral Maxillofac Surg* 2003; **61**: 774–8.

15 Ernberg M, Hedenberg-Magnusson B, List T, Svensson P. Efficacy of botulinum toxin type A for treatment of persistent myofascial TMD pain: A randomised, controlled, double-blind multicenter study. *Pain* 2011; **152**: 1988–96.

16 Sidebottom AJ, Patel AA, Amin J. Botulinum injection for the management of myofascial pain in the masticatory muscles. A prospective outcome study. *Br J Oral Maxillofac Surg* 2013; **51**: 199–205.

17 Connelly ST, Myung J, Gupta R, Tartaglia GM, Gizdulich A, Yang J, Silva R. Clinical outcomes of Botox injections for chronic temporomandibular disorders: do we understand how Botox works on muscle, pain, and the brain? *Int J Oral Maxillofac Surg* 2016; **46**: 322–7.

18 Dworkin SF, LeResche L. Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. *J Craniomandib Disord* 1992; **6**: 301–55.

19 Ferreira-Valente MA, Pais-Ribeiro JL, Jensen MP. Validity of four pain intensity rating scales. *Pain* 2011; **152**: 2399–404.

20 Isselée H, De Laat a, Bogaerts K, Lysens R. Short-term reproducibility of pressure pain thresholds in masticatory muscles measured with a new algometer. *J Orofac Pain* 1998; **12**: 203–9.

21 Pope GD. Introduction to Surface Electromyography. *Physiotherapy* 1998; **84**: 405.

22 De Felício CM, Ferreira CLP, Medeiros APM, Rodrigues Da Silva MAM, Tartaglia GM, Sforza C. Electromyographic indices, orofacial myofunctional status and temporomandibular disorders severity: A correlation study. *J Electromyogr Kinesiol* 2012; **22**: 266–72.

23 Grillo CM, Canales GT, Wada RS, Alves MC, Rizzatti-Barbosa CM, Berzin F, de Sousa ML. Could Acupuncture Be Useful in the Treatment of Temporomandibular Dysfunction? *J Acupunct Meridian Stud*. 2015; **8**: 192-9

24 Omar SM, McEwen JD, Ogston SA. A test for occlusal function. *Brit J Orthod* 1987; **14**: 85-90.

25 Campos SS, Pereira CV, Zangerônimo MG, Marques LS, Pereira LJ. Influence of disinfectant solutions on test materials used for the determination of masticatory performance. *Braz oral res* 2013; **27**: 238–44.

26 Sánchez-Ayala A, Vilanova LSR, Costa MA, Farias-Neto A. Reproducibility of a silicone-based test food to masticatory performance evaluation by different sieve methods. *Braz Oral Res* 2014; **28**: 01–8.

27 Castelo PM, Gavião MBD, Pereira LJ, Bonjardim LR. Evaluation of changes in muscle thickness, bite force and facial asymmetry during early treatment of functional posterior crossbite. *J Clin Pediatr Dent* 2010; **34**: 369–74.

28 Chen YW, Chiu YW, Chen CY, Chuang S-K. Botulinum toxin therapy for temporomandibular joint disorders: a systematic review of randomised controlled trials. *Int J Oral Maxillofac Surg* 2015; **44**: 1018–26.

29 Dressler D, Saberi FA, Barbosa ER. Botulinum toxin: mechanisms of action. *Arq Neuropsiquiatr* 2005; **63**: 180–5.

30 Matak I, Stracenski I, Lacković Z. Comparison of analgesic effects of single versus repeated injection of botulinum toxin in orofacial formalin test in rats. *J Neural Transm* 2013; **120**: 141–4.

31 Colhado OCG, Boeing M, Ortega LB. Botulinum Toxin in Pain Treatment. *Brazilian J Anesthesiol* 2009; **59**: 366–81.

32 Nixdorf DR, Heo G, Major PW. Randomised controlled trial of botulinum toxin A for chronic myogenous orofacial pain. *Pain* 2002; **99**: 465–73.

33 Cui M, Khanijou S, Rubino J, Aoki KR. Subcutaneous administration of botulinum toxin a reduces formalin-induced pain. *Pain* 2004; **107**: 125–33.

34 Lora VRMM, Clemente-Napimoga JT, Abdalla HB, Macedo CG, Canales G de la T, Rizzatti-Barbosa CM. Botulinum toxin type A reduces inflammatory hypernociception induced by arthritis in the temporomadibular joint of rats. *Toxicon* 2017; **129**: 52–7.

35 Blersch W, Schulte-Mattler WJ, Przywara S, May A, Bigalke H, Wohlfarth K. Botulinum toxin A and the cutaneous nociception in humans: A prospective, double-blind, placebo-controlled, randomised study. *J Neurol Sci* 2002; **205**: 59–63.

36 Gazerani P, Pedersen NS, Staahl C, Drewes AM, Arendt-Nielsen L. Subcutaneous Botulinum toxin type A reduces capsaicin-induced trigeminal pain and vasomotor reactions in human skin. *Pain* 2009; **141**: 60–9.

37 Kurtoglu C, Gur OH, Kurkcu M, Sertdemir Y, Guler-Uysal F, Uysal H. Effect of botulinum toxin-A in myofascial pain patients with or without functional disc displacement. J. *Oral Maxillofac*. *Surg*. 2008; : 1644–51.

38 Ramirez-Castaneda J, Jankovic J, Comella C, Dashtipour K, Fernandez HH, Mari Z. Diffusion, spread, and migration of botulinum toxin. *Mov*. *Disord*. 2013; **28**: 1775–83.

39 Aoki KR. Evidence for antinociceptive activity of botulinum toxin type A in pain management. *Headache* 2003; **43 Suppl 1**: S9-15.

40 Matak I, Lacković Z. Botulinum toxin A, brain and pain. *Prog*. *Neurobiol*. 2014; **119**–**120**: 39–59.

41 Freund B, Schwartz M, Symington JM. Botulinum toxin: new treatment for temporomandibular disorders. *Br J Oral Maxillofac Surg* 2000; **38**: 466–71.

42 de Paiva A, Meunier FA, Molgó J, Aoki KR, Dolly JO. Functional repair of motor endplates after botulinum neurotoxin type A poisoning: biphasic switch of synaptic activity between nerve sprouts and their parent terminals. *Proc Natl Acad Sci U S A* 1999; **96**: 3200–5.

43 Kim KS, Byun YS, Kim YJ, Kim ST. Muscle weakness after repeated injection of botulinum toxin type A evaluated according to bite force measurement of human masseter muscle. *Dermatol Surg* 2009; **35**: 1902–6.

44 Soboļeva U, Lauriņa L, Slaidiņa A. The masticatory system-an overview. *Stomatologija* 2005; **7**: 77–80.

45 Park HU, Kim BI, Kang SM, Kim ST, Choi JH, Ahn HJ. Changes in masticatory function after injection of botulinum toxin type A to masticatory muscles. *J Oral Rehabil* 2013; **40**: 916–22.

46 Ahn KY, Kim ST. The change of maximum bite force after botulinum toxin type a injection for treating masseteric hypertrophy. *Plast Reconstr Surg* 2007; **120**: 1662–6.

47 Kim JH, Shin JH, Kim ST, Kim CY. Effects of two different units of botulinum toxin type a evaluated by computed tomography and electromyographic measurements of human masseter muscle. *Plast Reconstr Surg* 2007; **119**: 711–7.

48 To EW, Ahuja AT, Ho WS, King WW, Wong WK, Pang PC, Hui AC. A prospective study of the effect of botulinum toxin A on masseteric muscle hypertrophy with ultrasonographic and electromyographic measurement. *Br J Plast Surg* 2001; **54**: 197–200.

50 Herzog W, Leonard TR. Force enhancement following stretching of a skeletal muscle. *J Exp Biol* 2002; **202**: 1275–83.

51 Longino D, Butterfield TA, Herzog W. Frequency and length-dependent effects of Botulinum toxin-induced muscle weakness. *J Biomech* 2005; **38**: 609–13.

52 Dodd SL, Selsby J, Payne A, Judge A, Dott C. Botulinum neurotoxin type A causes shifts in myosin heavy chain composition in muscle. *Toxicon* 2005; **46**: 196–203.

53 Rafferty KL, Liu ZJ, Ye W, *et al*. Botulinum toxin in masticatory muscles: Short- and long-term effects on muscle, bone, and craniofacial function in adult rabbits. *Bone* 2012; **50**: 651–62.

54 Fortuna R, Aurélio Vaz M, Rehan Youssef A, Longino D, Herzog W. Changes in contractile properties of muscles receiving repeat injections of botulinum toxin (Botox). *J Biomech* 2011; **44**: 39–44.

55 Raphael KG, Tadinada A, Bradshaw JM, *et al*. Osteopenic consequences of botulinum toxin injections in the masticatory muscles: A pilot study. *J Oral Rehabil* 2014; **41**: 555–63.

56 Kun-Darbois JD, Libouban H, Chappard D. 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* 2015; **77**: 75–82.

57 Matthys T, Ho Dang HA, Rafferty KL, Herring SW. Bone and cartilage changes in rabbit mandibular condyles after 1 injection of botulinum toxin. *Am J Orthod Dentofac Orthop* 2015; **148**: 999–1009.

3 DISCUSSÃO

A abordagem terapêutica mais comum para dor miofascial (DM) dos músculos mastigatórios é baseada em modalidades reversíveis, conservadoras e multidisciplinares (Dao & Lavigne, 1998; Nikolakis *et al*., 2002; De Laat *et al*., 2003). Da mesma forma, desde que a Food and Drug Administration dos EUA aprovou a TxBo-A para o tratamento de vários distúrbios musculares tais como espasticidade e doenças musculares hipertônicas, a TxBo-A ganhou muito interesse além de seu uso cosmético, sendo utilizada para a dor miofascial, devido à sua atividade sobre a musculatura estriada (Qerama *et al*., 2010). Revisões sistemáticas sobre o uso da TxBo-A na dor miofascial relacionada à DTM, afirmam que não há evidência cientifica suficiente para apoiar o uso de TxBo-A no tratamento da DM, uma vez que há poucos trabalhos randomizados, controlados e duplo-cego e com homogeneidade metodológica (Soares *et al*., 2009; Antonia *et al*., 2013; Chen *et al*., 2015; Sandrini *et al*., 2017)

Somado à falta de evidência científica do uso da TxBo-A, devemos mencionar os possíveis efeitos adversos desta terapia. Um dos efeitos secundários mais frequentemente relatados após tratamento com TxBo-A quando injetada nos músculos mastigatórios, é a presença de enfraquecimento muscular, o que leva ao desconforto mastigatório, principalmente de alimentos consistentes (Ahn and Kim, 2007; Kim et al., 2009; Park et al., 2013;). A maioria dos estudos mostra uma correlação positiva entre o enfraquecimento muscular e força de mordida máxima após aplicações de TxBo-A, indicando uma diminuição na força de mordida durante o primeiro mês, que se recupera ao seu estado normal após três meses da aplicação (Ahn and Kim, 2007; Kim et al., 2009; Park et al., 2013;). Em contrapartida, embora seja verdade que a espessura dos músculos mastigatórios esteja relacionada à força de mordida e à eficiência mastigatória, ainda não existem estudos associando o desempenho mastigatório e diferentes doses de TxBo-A. Estes se limitam a avaliações clinicas sem preocupação comparativa com grupos controle positivo e negaivo simultaneamente (Tsai et al., 2009; Rafferty et al., 2012;).

O presente trabalho clínico randomizado, controlado e duplamente-cego cumpre os requisitos metodológicos estabelecidos nas revisões sistemáticas supra-mencionadas. Ademais, é a primeira pesquisa que visou comparar os efeitos da TxBo-A na dor miofascial crônica, utilizando doses diferentes e comparando estes resultados com dois grupos controle.

Com o objetivo de estudar os benefícios e possíveis efeitos adversos desta terapia, buscou-se investigar os efeitos de diferentes doses desta intervenção sobre a dor subjetiva e objetiva, a atividade muscular, seus efeitos sobre estruturas ósseas mandibulares, alguns parâmetros relacionados aos movimentos mandibulares e psicossociais, e ainda correlacionando a espessura muscular e performance mastigatória.

Uma redução significativa da dor subjetiva para os grupos de TxBo-A foi encontrada nos pacientes dos grupos tratados. A diminuição da dor ocorreu desde a primeira semana de tratamento até a avaliação de 180 dias . Esse achado condiz com a literatura, que relata o efeito da TxBo-A sobre a dor miogênica. Freqüentemente um alívio substancial da dor é observado (Matak *et al*., 2013). Esse alívio tem sido atribuído à perda de efeitividade na contração das fibras musculares devido ao bloqueio da liberação de acetilcolina na placa motora (Colhado *et al*., 2009). Tal paresia muscular começa por volta de 2 a 5 dias pós tratamento, atingindo seu efeito máximo entre o 14 º e 21 º dias, perdurando por 60 a 90 dias, após o que reduz gradualmente até desaparecer (Dressler *et al*., 2005). Este comportamento foi observado em nosso experimento, em todos os períodos de observação da variáveis da EVA e do LDP. Observou-se ainda que as alterações na dose do fármaco não afetou a redução subjetiva da dor.

Nossos resultados diferem, dos reportados em estudos prévios (Nixdorf *et al* ., 2002; Ernberg *et al*., 2011), onde não houve diferenças significativas entre grupos que utilizaram TxBo-a e placas de desoclusão.. Entretanto, nosso estudo corrobora com os resultados de dois estudos clínicos, randomizados (von Lindern *et al*., 2003; Kurtoglu *et al*., 2008) nos quais foi relatado um efeito positivo do tratamento com TxBo-A. Observa-se que existe grande diferença entre as metodologias experimentais utilizadas nos experimentos que avaliama ação da TxBo-A. E ainda a ausência de padronização no emprego dos protocolos clínicos, na padronização da dosagem e homogeneidade no processo de diluição entre as diversas marcas comerciais, dentre outros. Estes fatores, a nosso ver, podem contribuir com diferenças observadas nos resultados, bemcomo pode a explicar a variabilidade nos resultados dos estudos mencionados. Ademais, o delinemento metodológico inadequado, incluindo tempo de avaliação insuficientes (Nixdorf *et al* ., 2002) tamanho inadequado da amostra (Nixdorf *et al* ., 2002; Ernberg *et al*., 2011), falta de padronização dos pontos de aplicação, presença de

tratamentos concomitantes de DTM durante o experimento (Ernberg *et al*., 2011) e expectativas dos pacientes devido ao delineamento cruzado (Ernberg *et al*., 2011), indubitavelmente, afetam os resultados de um estudo clínico. Nosso experimento buscou padronizar a maior parte de variáveis que pudessem interferir nos resultados. Buscamos estabelecer parâmetros reprodutíveis para o uso da TxBo-A em outros experimentos. Uma de nossas preocupações foi a padronização da espessura dos músculos estudados, já que a literatura relata que os efeitos da TxBo-A dependem da proporção entre o tamanho do músculo e a dose de TxBo-A utilizada (Dressler *et al*., 2005).

Nosso estudo não encontrou diferenças significativas entre os grupos TxBo-A e grupo SP nos valores da EVA, nas avaliações de 30, 90 e 180 dias, demonstrando que a eficácia da TxBo-A é quase igual à dos AIP para o controle da dor crônica miofascial relacionada a DTM. Observou-se ainda um aumento significativo nos valores do LDP para os grupos de TxBo-A e para o grupo SP a partir do 14º dia pós tratamento, valores que foram superiores aos do grupo SS até a avaliação de 180 dias. Nossos achados diferem com os resultados de Ernberg *et al* (2005) que não demonstraram diferenças significativas nos valores de PPT para os grupos que receberam TxBo-A e que usaram placa de desoclusão. Observou-se que nesse estudo, houve ausência de padronização das avaliações entre os grupos, o que pode ter influenciado os resultados. Em nosso estudo, o posicionamento da ponta de medição do algômetro foi padronizado através de marcas feitas em uma folha de acetato individualizada para cada paciente, a fim de posicionar, em qualquer avaliação, a mesma porção muscular na qual as injeções de tratamento foram realizadas, permitindo a reprodutibilidade das análises, em todos os períodos experimentais. Adicionalmente, é importante salientar o tamanho reduzido na amostragem investigada por Ernberg *et al* (2005), o que pode ter afetado os resultados do estudo.

A avaliação da atividade elétrica muscular dos músculos masseter e temporal anterior foi uma grande preocupação em nosso estudo, devido ao mecanismo de ação da TxBo-A sobre a atividade muscular. Os valores de repouso eletromiográficos mostraram uma tendência de manutenção da tonicidade muscular em todos os períodos de avaliação, e em todos os grupos que receberam TxBo-A. No entanto, os valores de máxima contração voluntária apresentaram uma severa diminuição na atividade muscular desde o 14º dia pós tratamento, até os 30 dias de avaliação, nos grupos TxBo-A. Nossos achados corroboram resultados apresentados por

outros estudos relatando a mesma tendência da diminuição na atividade muscular após injecções de TxBo-A (Freund *et al*., 2000; Kurtoglu *et al*., 2008). A recuperação da atividade muscular ocorreu ao longo do experimento.

Apenas o grupo TxBoA-B recuperou quase toda a atividade muscular na adquisição de máxima contração voluntária (MCV) após 180dias do tratamento, atingindo os mesmos valores do baseline. Embora os grupos TxBoA-M e TxBoA-A tenham apresentado valores maiores da EMG após 90 dias do tratamento, este aumento não atingiu os valores do base line, mesmo após os 180 dias pos tratamento. Por outro lado, os valores de MVC não foram alterados no grupo SP e SS em todos os períodos experimentais.

Nossos resultados da EMG suportam o consenso já existente na literatura sobre os efeitos da TxBo-A na atividade muscular (Dressler *et al*., 2005). Entretanto, como foi demostrado em nosso estudo, há uma relação dose-efeito e dose-duração da droga, indicando que doses mais altas apresentam maior índice de paresia muscular, por um período de tempo mais longo. O mecanismo de ação da TxBo-A consistem em inibir a liberação da acetilcolina na fenda sináptica mioneural, através da inibição das proteínas do complexo SNARE (*Soluble Methylmaleimide-Sensitive Factor Attachment Protein Receptor*), e, em função deste processo, inibem a contração da fibra muscular. Acreditamos que este aspecto seja o principal determinante dos efeitos colaterais do TxBo-A quando injetada nos músculos da mastigação, considerando que a atividade muscular está diretamente relacionada à eficiência e qualidade da mastigação. Neste contexto, ponderamos que os dados obtidos neste ensaio clínico podem orientar a indicação de doses menores, mas efetivas, para o controle da dor crônica relacionada à DTM.

Ainda em função da hipoatividade muscular, as perdas óssea nas regiões condilares e alveolares da mandíbula e as possíveis alterações no crescimento craniomandibular podem ser alguns efeitos colaterais a serem considerados como possível correlação ao uso da TxBo-A (Rafferty *et al*., 2012; Kun *et al*., 2015; Matthys *et al*., 2015). Com relação aos estudos clínicos, há apenas dois estudos contraditórios sobre as alterações ósseas mandibulares após a injeção de TxBo-A (Chang *et al*., 2011; Raphael *et al*., 2014). Tais achados podem alertar quanto à necessidade de um possível risco de osteopenia ou fratura mandibular, nos casos em que doses altas e/ou frequentes forem empregadas nos músculos da mastigação.

Nosso estudo encontrou ainda uma diminuição significativa da performance mastigatória após injeção de TxBo-A. O desempenho mastigatório diminuiu drasticamente desde oo 7º dia pós traamento até as avaliações de 30 dias. Contudo os valores voltaram para os dados basais após os 90 a 180 dias, independentemente das doses utilizadas. Nossos resultados corroboram outros estudos mostrando o mesmo padrão de PM após aplicações de TxBo-A (Park et al., 2013; Ahn e Kim, 2007). Este foi um resultado esperado em virtude da denervação química que a TxBo-A promove sobre a fibra muscular. Este efeito se inicia após 2 a 5 dias, atingindo seus efeitos máximos entre o dia 14 e ou 21 dias, que perduram por 60 a 90 dias, desaparecendo gradualmente. Estes períodos no qual a TxBo-A atinge seu potencial máximo explica o padrão de diminuição e recuperação da PM observado em nosso estudo. O brotamento das fibras nervosas e a conseqüente reativação da liberação de acetilcolina através da placa neuromotora a partir de 90 dias após a aplicação de TxBo-A pode explicar a reabilitação da função de contração muscular e mastigação.

Embora a PM tenha diminuido também no grupo TxBoA-B, não foram encontradas diferenças significativas quando este grupo experimental foi comparado com o grupo SS, demonstrando que doses baixas de TxBo-A, em pacientes com dor miofascial crônica relacionada a DTM não afetam significativamente a PM .

Nosso estudo também evidenciou, através do US, uma diminuição significativa da espessura dos músculos tratados, em todos os grupos de TxBo-A, até à avaliação de 90 dias após os tratamentos. Atrofia, encurtamento das fibras musculares e conseqüentemente perda de massa muscular podem ser as causas do efeito observado. Estudos prévios (Kim *et al*., 2007, Kim *et al*., 2009), relataram diminuição semelhante à apresentada em nosso estudo; porém o nosso estudou mostrou que a diminuição na espessura muscular, também foi observada no grupo TxBoA-B, que foram as mesmas observadas nos grupos SP e SS, demonstrando que doses baixas de TxBo-A não afetam significativamente a espessura muscular.

Este resultado pode considerado benéfico no campo da estética dos musuculos da mímica, devido ao efeito da TxBo-A na redução de rítides de expressão. No entanto, este poderia ser um efeito adverso importante quando a toxina for utilizada nos úsculos da mastigação. Este fato ficou bem evidenciado em nosso estudo, quando demonstramos a

correlação direta entre a espessura dos músculos tratados e os valores do desempenho mastigatório, independentemente das doses utilizadas e do tempo experimental. Este fato por si, confirma a importância do volume muscular e da força muscular no desempenho da mastigação. Consideramos este um dos efeitos colaterais mais relevantes a ser ponderado na indicação da TxBo-A para o controle da dor nas DTM miogências..

Os resultados apresentados neste estudo, não devem ser extrapolados para situações nas quais múltiplas injeções de TxBo-A forem realizadas. Portanto, sugerimos que mais estudos, incluindo injeções múltiplas de TxBo-A devem ser realizados a fim de esclarecer a possibilidade de efeitos colaterais irreversíveis ao SEG.

Embora este seja o maior estudo duplo-cego randomizado e placebo referente ao tratamento com TxBo-A na DTM miofascial crônica, algumas limitações neste estudo precisam ser abordadas. Acompanhamento por períodos mais longos devem ser realizados para avaliar os efeitos a longo prazo de uma injeção única de TxBo-A, bem como estudos com amostragens maiores e pacientes com perfis difeentes aos aqui investigados poderiam contribuir na obtenção de parâmetros e protocolos que correlacionemm dose/efeito ao uso da TxBo-A sobre os músculos da mastigação.

4 CONCLUSÃO

Com base nos achados do presente estudo e dentro das limitações apresentadas, é possível confirmar que a TxBo-A é uma abordagem eficaz para controlar a DTM miofascial crônica; no entanto, devido aos efeitos colaterais observados sobre o desempenho mastigatório, a espessura muscular e sobre estruturas ósseas mandibulares, sugerimos que doses menores devem ser consideradas como primeira escolha quando a TxBo-A for utilizada sobre os músculos elevadores da mandíbula. Desta forma, sugerimos que, nos quadros de DTM miogência com dor crônica nas quais as terapias convencionais não proporcionarem alívio adequado à dor, a TxBo-A em baixas doses pode ser uma abordagem adjuvante à intervenção.

REFERÊNCIAS

1. Antonia DM, Oliveira Netto RM, Sanches ML et al. Jaw muscles myofascial pain and botulinum toxin. *Rev*. *Dor São Paulo* 2013; 14:52–57.
2. Branco CA, Fonseca RB, Oliveira TRC, et al. Acupuntura como tratamento complementar nas disfunções temporomandibulares: Revisão de Literatura. *Rev Odontol Unesp*. 2005; 34: 11-16.
3. Caillet R. *Dor: mecanismos e tratamento*. Porto Alegre:Artmed; 1999. 312 p.
4. Chang CS, Bergeron L, Yu CC et al. Mandible changes evaluated by computed tomography following botulinum toxin a injections in square-faced patients. *Aesthet Plast Surg*. 2011; 35:452–455 .
5. Chen YW, Chiu YW, Chen CY et al. Botulinum toxin therapy for temporomandibular joint disorders: a systematic review of randomised controlled trials. Int. *J*. *Oral Maxillofac*. *Surg*. 2015; 44:1018–1026.
6. Ciancaglini R, Radaelli G. The relationship between headache and symptoms of temporomandibular disorder in the general population. *J Dent*. 2001; 29:93-8.
7. Colhado OCG, Boeing M, Ortega LB. Botulinum Toxin in Pain Treatment. *Braz J*. *Anesthesiol*. 2009; 59:366–381.
8. Conti PC, de Alencar EM, da Mota Corrêa AS et al. Behavioural changes and occlusal splints are effective in the management of masticatory myofascial pain: a short-term evaluation. *J Oral Rehabil*. 2012; 39:754-760
9. Cui M, Khanijou S, Rubino J, Aoki KR. Subcutaneous administration of botulinum toxin a reduces formalin-induced pain. Pain 2004; 107:125–133.
10. Dao TT, Lavigne GJ. Oral splints: the crutches for temporomandibular disorders and bruxism? *Crit*. *Rev*. *Oral Biol*. *Med*. 1998; 9:345–361.
11. De Laat A, Stappaerts K, Papy S. Counseling and Physical Therapy as Treatment for Myofascial Pain of the Masticatory System. *J*. *Orofac*. *Pain*. 2003; 17:42–49.
12. Canales GT, Manfredini D, Grillo CM et al. Therapeutic effectiveness of a combined couseling plus stabilization appliance treatment for myofascial pain of jaw muscles: a pilot study. *Cranio*. 2017; 35:180-186.
13. Dressler D, Saberi FA, Barbosa ER. Botulinum Toxin Mechanisms of action. *Arq Neuropsiquiatr*. 2005; 63: 180-185

14. Ebrahim S, Montoya L, Busse JW et al. The effectiveness of splint therapy in patients with temporomandibular disorders: a systematic review and meta-analysis. *J Am Dent Assoc*. 2012; 143:847-857.
15. Ernberg M, Hedenberg-Magnusson B, List T et al. Efficacy of botulinum toxin type A for treatment of persistent myofascial TMD pain: A randomised, controlled, double-blind multicenter study. *Pain*. 2011; 152:1988–1996.
16. Freund B, Schwartz M, Symington JM. Botulinum toxin: new treatment for temporomandibular disorders. *Br*. *J*. *Oral Maxillofac*. *Surg*. 2000; 38:466–71.
17. Guarda-Nardini L, Manfredini D, Salamone, M et al. Efficacy of Botulinum Toxin in Treating Myofascial Pain in Bruxers: A Controlled Placebo Pilot Study. *J*. *Craniomand*. *Pract*. 2008; 28:126-135
18. Guarda-Nardini L, Stecco A, Stecco C. et al. Myofascial Pain of the Jaw Muscles: Comparison of Short-Term Effectiveness of Botulinum Toxin Injections and Fascial Manipulation Technique. *J*. *Craniomand*. *Pract*. 2012; 30:95-102.
19. Issa JPM, Silva AMB, Miani PK et al. Avaliação clínica no uso de placas oclusais em pacientes portadores de disfunção temporomandibular-uma revisão. *Rev Dor*. 2005; 6:450-453.
20. Ho KY, Tan KH. Botulinum toxin A for miofascial trigger point injection: A qualitative systematic review. *Eur J Pain*. 2007; 11:519-527.
21. Kün-Darbois JD, Libouban H, Chappard D. 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*. 2015; 77: 75–82
22. Kurtoglu C, Gur OH, Kurkcu M et al. Effect of botulinum toxin-A in myofascial pain patients with or without functional disc displacement. *J*. *Oral Maxillofac*. *Surg*. 2008; 66: 1644–1651.
23. Lam DK, Lawrence HP, Tenenbaum HC. Aural symptoms in temporomandibular disorder patients attending a craniofacial pain unit. *J*. *Orofacial Pain*. 2001; 15: 146-57.
24. Lora VRMM, Canales GT, Gonçalves LM et al. Prevalence of temporomandibular disorders in postmenopausal women and relationship with pain and HRT. *Braz*. *Oral Res*. 2016; 30(1): e100.

25. Manfredini D, Guarda-Nardini L, Winocur E et al. Research diagnostic criteria for temporomandibular disorders: A systematic review of axis I epidemiologic findings. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2011;112: 453-462.

26. Matak I, Lacković Z. Botulinum neurotoxin type A: Actions beyond SNAP-25? Toxicology 2015;335:79–84.

27. Matak I, Stracenski I, Lacković Z. Comparison of analgesic effects of single versus repeated injection of botulinum toxin in orofacial formalin test in rats. J. Neural Transm. 2013;120:141–144.

28. Matthys T, Ho Dang HA, Rafferty KL et al. Bone and cartilage changes in rabbit mandibular condyles after 1 injec- tion of botulinum toxin. *Am J Orthod Dentofac Orthop*. 2015; 148: 999–1009

29. Nicolakis P, Erdogmus B, Kopf A et al. Effectiveness of exercise therapy in patients with myofascial pain dysfunction syndrome. *J. Oral Rehabil*. 2002;29:362–368.

30. Nixdorf DR, Heo G, Major PW. Randomised controlled trial of botulinum toxin A for chronic myogenous orofacial pain. *Pain* 2002; 99: 465–473.

31. Okeson JP. *Dores bucofaciais de Bell*. 5. Ed. São Paulo: Quintessence; 1998. 500p

32. Piozzi R, Lopes FC. Desordens Temporomandibulares – Aspectos Clínicos e Guia para a Odontologia e Fisioterapia. *J Bras Oclusão, ATM e Dor Orofac*. 2002; 2: 43-47.

33. Portero PP, Kern R, Kusma SZ et al. Placas oclusais no tratamento da disfunção temporomandibular (DTM). *Rev Gestão e Saúde*. 2009; 1: 36-40.

34. Qerama E, Fuglsang-Frederiksen A, Jensen TS. The role of botulinum toxin in management of pain: an evidence-based review. *Curr. Opin. Anaesthesiol*. 2010; 23: 602–610.

35. Rafferty KL, Liu ZJ, Ye W et al. Botulinum toxin in masticatory muscles: short- and long-term effects on muscle, bone, and craniofacial function in adult rabbits. *Bone*. 2012; 50: 651–662

36. Ramirez-Castaneda J, Jankovic J, Comella C et al. Diffusion, spread, and migration of botulinum toxin. *Mov. Disord*. 2013; 28: 1775–1783.

37. Raphael KG, Tadinada A, Bradshaw JM et al. Osteopenic consequences of botulinum toxin injections in the masticatory muscles: a pilot study. *J Oral Rehabil*. 2014; 41:555–563.

38. Rizzatti-Barbosa CM, Albergaria-Barbosa JR. *Uso da toxina Botulínica em Odontologia*, Rio de Janeiro: Elsevier, 2017. 219p.

39. Sandrini G, De Icco R, Tassorelli C et al. Botulinum neurotoxin type A for the treatment of pain: not just in migraine and trigeminal neuralgia. *J. Headache Pain* 2017;18(1):38.

40. Silverio KC, Gil IA, Rizzatti-Barbosa CM et al. Multidisciplinary approach to chronic pain from myofascial pain dysfunction syndrome: a four-year experience at a Brazilian center. *Cranio*. 1998; 16: 17-25.

41. Soares A, Regis AB, Atallah N et al. Botulinum toxin myofascial pain syndrome in adults. *Cochrane Database Syst. Rev. Protoc*. 2009 Issue 1 John Wiley Sons, Ltd Chichester, UK

42. Song PC, J Schwartz J, Blitzer A. The emerging role of botulinum toxin in the treatment of temporomandibular disorders. *Oral Diseases*. 2007; 13: 253–260.

43. von Lindern JJ, Niederhagen B, Bergé S, Appel T. Type A botulinum toxin in the treatment of chronic facial pain associated with masticatory hyperactivity. *J Oral Maxillofac Surg*. 2003; 61: 774-778.

44. Wheeler H, Goolkasian P, Gretz S. Botulinum toxin A for the treatment of chronic neck pain. *Pain*. 2001; 94: 255-260.

Anexo 1 - Certificado de aprovação do Comitê de Ética em Pesquisa da Faculdade de Odontologia de Piracicaba

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"A eficácia do tratamento conservador e do tratamento com toxina botulínica em indivíduos portadores de Disfunção Temporomandibular (DTM) com dor crônica"**, protocolo nº 114/2013, dos pesquisadores Giancarlo de La Torre Canales e Célia Marisa Rizzatti Barbosa, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 02/04/2014.

The Ethics Committee in Research of the Piracicaba Dental School - University of Campinas, certify that the project **"Efficacy of conservative treatment and botulinum toxin treatment in subjects with temporomandibular disorders with chronic pain"**, register number 114/2013, of Giancarlo de La Torre Canales and Célia Marisa Rizzatti Barbosa, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee on Apr 02, 2014.

Prof. Dr. Felippe Bevilacqua Prado
Secretário
CEP/FOP/UNICAMP

Profa. Dra. Lívia Maria Andaló Tenuta
Coordenadora
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.