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Perspective

Mandibular bone loss: a hidden side effect of botulinum toxin type A injection in masticatory muscles.

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Botulinum toxin type A (BoNTA) is the most potent serotype of a group of neurotoxins produced by obligate anaerobic bacteria *Clostridium botulinum*. This neurotoxin blocks the release of acetylcholine from the motor neuron, uncoupling the neuromuscular junction that results in a reversible paralysis of skeletal muscles that lasts for 4 – 6 months in humans.[1] Commercially available forms of BoNTA (*e.g.* Botox®, Dysport®, Xeomin®) are increasingly used by dentists worldwide.[1] In dentistry, conditions that affect masticatory muscles such as benign masseter hypertrophy and myofascial pain are treated with intramuscular injections of BoNTA,[1,2] However, this neurotoxin is not approved for interventions in the masticatory apparatus, according to the US Food and Drug Administration (Allergan Full Prescribing Information for Botox®); as such, its clinical use is off-label.

The effects of BoNTA in skeletal muscles include paralysis, weakness, and atrophy,[1] but much less studied are the side effects that could affect bone homeostasis. The musculoskeletal system maintains a fine regulation between muscles and bones, in which both tissues sustain a cross-talk through biomechanical (generation of force, tension) and biochemical signals (molecules released by the muscle that contribute to bone homeostasis and *vice versa*).[3] So, what happens to the structure and function of the adjacent bone when muscle paralysis is induced by BoNTA injection?

The intramuscular (IM) intervention with BoNTA promotes bone loss in underlying structures. As demonstrated by Warner *et al* in adult female C57BL/6 mice, IM intervention in the quadriceps and calf muscles with Botox® (0.5U/19.2µl) significantly reduced the trabecular bone volume fraction by 43%, in the distal femoral epiphysis and by 54% in proximal tibial metaphysis after 21 days.[4] In the same model, Aliprantis *et al* described that acute bone loss induced by this intervention occurs as a response to a significant increase of the receptor activator for nuclear factor Kappa Beta ligand (RANKL) in the proximal tibia metaphysis.

RANKL is necessary to differentiate and increase the number of osteoclasts (bone resorption cells). Moreover, the treatment with human recombinant osteoprotegerin (the RANKL physiological antagonist), or conditional deletion of the osteoclastogenesis master regulator "nuclear factor of activated T-cells c1" (NFATc1), prevented the bone loss in this animal model.[5] In addition, direct effects of BoNTA on bone cells were not detected, suggesting that bone loss may be produced by muscle load or signaling after this intervention.[5]

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Considering that the temporomandibular joint requires the activity of masticatory muscles for its development and homeostasis, it is highly relevant to understand the effect of the BoNTA intervention in the masticatory apparatus. Kün-Darbois *et al.* reported trabecular bone loss in alveolar (-20%) and condylar (-35%) areas in adult rats 4 weeks after a unilateral injection of 1U of Botox® in both the masseter and temporalis muscles.[6] The same intervention in masseter muscles of growing mice (5 weeks-old) resulted in a significant decrease in condylar width of the treated side after 4 weeks. Moreover, decreased cell proliferation and increased cell death by apoptosis was observed in subchondral bone of the mandibular condyle.[7] Recently, our group assessed the effect of unilateral injection of Botox® in masseter muscle (0.2U/10µl) of adult BALB/c mice (8 weeks-old) at molecular and microstructural levels.[8] Our results demonstrated a significant increase in the mRNA expression of RANKL in the mandibular condyle of the treated side 2 days after intervention. Also, there was a significant increase in the mRNA expression of both atrophy (atrogin-1/MAFbx and MURF-1) and muscle regeneration markers (myogenin) in the treated masseter muscles after 7 days. These findings, together with the significant reduction in the masseter mass and muscle fibers diameter at the BoNTA-injected side observed after 14 days, support the hypothesis that molecular events precede the onset of BoNTA-induced masseter muscle atrophy. In addition, the bone histomorphometry performed on the mandibular condyles at 14 days post-intervention showed a significant reduction of the trabecular bone (-30%) and trabecular thickness (–55%). Taken as a whole, these results demonstrate that masseter muscle atrophy induced by BoNTA single injections leads to significant microanatomical changes in the masseter muscle and the mandibular condyle of the same side after 14 days, preceded by molecular changes as early as 2 days in bone and 7 days in muscle.[8]

In humans, the bone effects derived from IM injection of BoNTA in masticatory muscles have been much less studied and are more difficult to address. In a pilot qualitative study, cone-beam computed tomography (CBCT) images of mandibular condyles from 7 adult female patients with temporomandibular joint dysfunction (TMJD), receiving two or more BoNTA IM injections for facial pain, were compared with those from 9 demographically matched control patients.[9] Two independent oral and maxillofacial radiologists, blinded to BoNTA exposure conditions, rated bone density patterns in the trabecular region of mandibular heads.

Both radiologists noted a reduction in bone density in all the patients exposed to BoNTA, and in none of the 9 control subjects.[9] In a recent Letter to the Editor, Aziz *et al.* introduced the clinical case of a patient (female, 55 years-old) with a 13-year history of TMJD and a Meige Syndrome diagnosis. Comparing magnetic resonance imaging and computed tomography data within a time span of 15 months, in which the patient admitted she had privately had Botox® injections every three months (140U) into her left masseter, a severe mandibular condyle resorption of the treated side was observed.[10]

Authors are cautious to point out that is difficult to tell if Botox® was the cause of bone resorption, because this is an isolated cause and iatrogenic injury cannot be excluded. However, they highlight the idea that the condylar degeneration may be associated with BoNTA injections. These reports, reinforced by results from experimental studies in animal models, makes it highly recommended for both clinicians and patients to consider the putative bone loss evoked by BoNTA-induced masseter muscle atrophy as a relevant factor prior to a treatment.[10] Therefore, the presented evidence suggests the importance of considering the need to develop follow-up protocols to permanently monitor the associated bone structure by proper imaging techniques after BoNTA intervention in the masseter muscle. This is especially relevant in patients who require multiple successive applications of toxin to maintain the desired effect. Finally, there is not enough evidence to know if BoNTA intervention in the masseter muscle (or other masticatory muscles) predisposes, initiates or perpetuates the TMJD.

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