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ORIGINAL ARTICLE BASIC SCIENCE/EXPERIMENTAL

Deoxycholic Acid and the Marginal Mandibular Nerve: A Cadaver Study

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Abstract

Background One of the rare but serious complications observed with deoxycholic acid administration is damage to the marginal mandibular nerve. In this study, we evaluated if deoxycholic acid directly induces histologic damage to fresh cadaveric marginal mandibular nerve.

Methods A segment of marginal mandibular nerve was harvested from 12 hemifaces of 6 fresh cadavers. The nerve specimen was exposed to either 0.9% sterile saline for 24 h, deoxycholic acid (10 mg/ml) for 20 min, or deoxycholic acid (10 mg/ml) for 24 h. The nerve specimens were then fixed in glutaraldehyde for a minimum of 24 h. Toluidine blue stained sections were evaluated for stain intensity using light microscopy and color deconvolution image analysis. Supraplatysmal fat was harvested as a positive control and exposed to the same treatments as the marginal mandibular nerve specimens, then evaluated using transmission electron microscopy.

Results Toluidine blue staining was less in the marginal mandibular nerve exposed to deoxycholic acid when compared to saline. The specimen exposed to deoxycholic acid for 24 h showed less toluidine blue staining than that of the nerve exposed to deoxycholic acid for 20 min. Transmission electron microscopy of submental fat exposed to deoxycholic acid revealed disruption of adipocyte cell membrane integrity and loss of cellular organelles when compared to specimens only exposed to saline.

Conclusions Deoxycholic acid (10 mg/ml) damages the marginal mandibular nerve myelin sheath in fresh human cadaver specimens. Direct deoxycholic acid neurotoxicity may cause marginal mandibular nerve injury clinically.

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Keywords Deoxycholic acid · Submental fat · Marginal mandibular nerve

Introduction

Submental fullness, a common aesthetic concern of patients, does not always respond to exercise and weight loss and may possess a genetic component. A 2016 American Society for Dermatologic Surgery survey found that 73% of the 7322 respondents are somewhat-to-extremely bothered by excess fat under their chin or neck [1]. The perceived submental fullness can result from supraplatysmal fat, subplatysmal fat, prominent submandibular glands, and/or prominent anterior digastric muscles. Surgical options such as cervicoplasty with or without liposuction may address submental fullness, but require significant recovery time that may not be an appealing first option to some patients.

A first in class drug, deoxycholic acid (10 mg/ml), was FDA approved in 2015 for improvement in submental fat

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(SMF). A series of deoxycholic acid (10 mg/ml) injections at 4-week intervals subjectively and objectively reduced SMF in multiple phase III clinical trials [2].

Deoxycholic acid (10 mg/ml) is thought to target the supraplatysmal fat via a detergent physical disruption of adipocyte cell membranes, leading to cell death and reduction in fat tissue [2–5]. As cadaver studies indicate that on average, supraplatysmal fat accounts for 45–70% of total submental fat, deoxycholic acid (10 mg/ml) represents an attractive option for patients interested in non-surgical improvement in submental fullness [6, 7].

Extensive investigations of the pharmacokinetics and safety profile of deoxycholic acid (10 mg/ml) injection have identified common side effects and rare adverse effects [2, 3, 8, 9]. Most patients experience significant pain, bruising, and swelling in the injection area that resolves within monthly treatment intervals, while a minority of patients experience injury to the marginal mandibular branch of the facial nerve (MMN) [3]. The REFINE clinical trials reported marginal mandibular nerve injury in up to 4.3% of patients treated with deoxycholic acid (10 mg/ml) [2, 4]. Although all episodes were temporary, the potential for permanent disfigurement of facial expression and salivary incontinence after this cosmetic intervention still remains. Anecdotal reports of periocular deoxycholic acid (10 mg/ml) to treat lower eyelid steatoblepharon possess particular risks for vision [10].

Authors suggest three potential mechanisms of injury to the MMN after deoxycholic acid (10 mg/ml) injection, including sharp injury from the injection needle, diffusion of deoxycholic acid (10 mg/ml) within the tissue surrounding the nerve, and/or post-injection inflammation in the tissue surrounding the nerve [11]. The course of the MMN exhibits significant morphologic variability regarding its distance from the inferior border of the mandible and ranges from 1 to 4 cm inferior to the inferior border of the mandible posterior to the antegonial notch [12, 13]. At the antegonial notch, the nerve traverses the facial artery and nerve superior to the inferior border of the mandible.

To further investigate the mechanism of MMN injury after deoxycholic acid (10 mg/ml), we sought to study its direct histological effects in a human cadaveric model.

Materials and Methods

Twelve hemifaces of 6 fresh human cadavers were evaluated and included for the study. In all 12 hemifaces, the MMN was identified at a variable distance inferior to the inferior border of the mandible posterior to the antegonial notch and dissected anteriorly where it was found to traverse the facial artery and vein approximately 2–3 cm above the inferior border of the mandible (Fig. 1). After careful dissection, 4 cm of the nerve was harvested. The cut ends of the nerve were sutured together using 6-0 polypropylene suture and then the loop of nerve was immersed in a 1.5-ml centrifuge tube containing 1 ml of deoxycholic acid (10 mg/ml) for either 20 min or 24 h, or 0.9% sterile saline for 24 h. This was done taking care that the sutured cut ends of the nerve were not immersed in solution (Fig. 2).

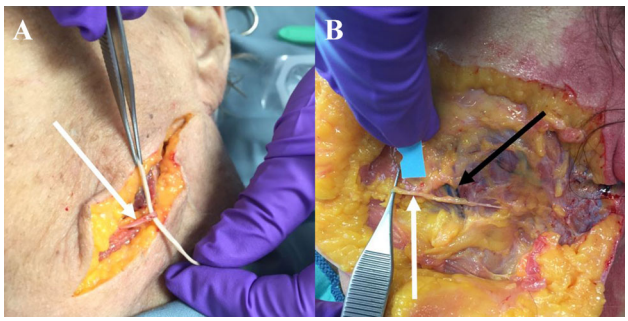


Fig. 1 Harvesting of cadaveric marginal mandibular nerve in a cadaver model. **a** Marginal mandibular nerve (white arrow) tagged. **b** Marginal mandibular nerve (white arrow) shown passing over facial artery and vein (black arrow)

The respective tissues were then fixed in glutaraldehyde for a minimum of 24 h in a refrigerator. Tissue was embedded in plastic, sectioned in a standard way, and stained with toluidine blue. Using color deconvolution image analysis macro in Image Scope (Leica Biosystems, Vista, CA), the intensity of toluidine blue staining of a representative MMN specimen from each treatment group was measured. The color deconvolution algorithm calculates and separates the area and intensity of an individual stain. The percent of weak, medium, and strong toluidine blue positive intensity was recorded.

To evaluate the deoxycholic acid (10 mg/ml) effect on cadaveric fat, supraplatysmal fat was harvested using a

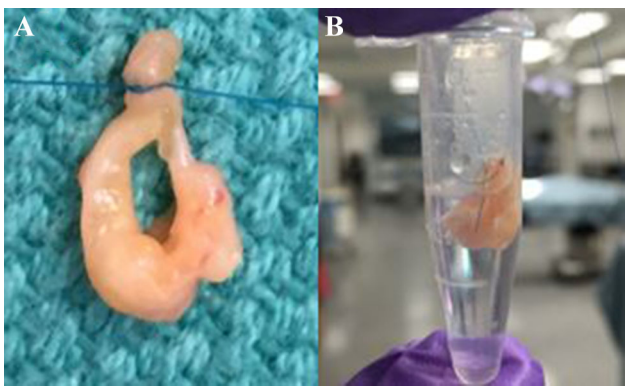


Fig. 2 Marginal mandibular nerve preparation. **a** Marginal mandibular nerve sutured end to end using polypropylene suture. **b** Partial submersion of sutured marginal mandibular nerve in centrifuge tube containing either deoxycholic acid (10 mg/ml) or 0.9% sterile saline

4-mm dermatologic tissue punch from three fresh human cadavers for a total of 18 specimens. The supraplatysmal fat specimens were then exposed to the same conditions (deoxycholic acid (10 mg/ml) for 20 min, deoxycholic acid (10 mg/ml) for 24 h, or normal saline for 24 h) and fixed in glutaraldehyde for 24 h prior to preparation for transmission electron microscopy. This study adhered to the tenets of the Declaration of Helsinki.

Results

Histopathology confirmed nerve tissue in all 12 specimens. Toluidine blue staining demonstrated a decrease in myelin in MMN samples exposed to deoxycholic acid (10 mg/ml) compared to control (Fig. 3). Color deconvolution image analysis of representative MMN specimens from each treatment group confirmed a decrease in toluidine blue staining in the deoxycholic acid (10 mg/ml) groups (Fig. 4). This was particularly striking when comparing the 24-h saline group to the 24-h deoxycholic acid (10 mg/ml) group. The percent of weak, medium, and strong positive toluidine blue stain intensity for each treatment group is summarized in Fig. 5. Transmission electron microscopy of submental fat exposed to deoxycholic acid (10 mg/ml) revealed disruption of adipocyte cell membrane integrity and loss of cellular organelles when compared to specimens only exposed to saline (Fig. 6). Supraplatysmal fat exposed to deoxycholic acid (10 mg/ml) for 24 h showed further adipocyte disruption than samples exposed to deoxycholic acid (10 mg/ml) for only 20 min.

Discussion

Injury to the MMN can occur after neck liposuction, genioplasty, submandibular gland removal, superficial parotidectomy, oncologic neck dissections, injections of filler to the jowl, facelift surgery, and most recently with deoxycholic acid (10 mg/ml) injection [2, 4, 14–18]. Injury leads to paresis of the ipsilateral depressor anguli oris, mentalis, and labii depressor inferioris muscles and unopposed/over-action of the respective contralateral muscles. This creates a disfiguring asymmetry to the smile where the paretic lip does not depress when smiling and the unopposed contralateral muscles exaggerate the smile contortion. MMN injury can also cause difficulty with swallowing and drinking, as well as salivary incontinence. Treatment for temporary injury consists of active facial exercises or neurotoxin injection to the contralateral depressor anguli oris, mentalis, and/or labii depressor inferioris muscles to improve symmetry and function [16, 19, 20].

In our study, we sought to investigate a potential mechanism of MMN injury related to deoxycholic acid (10 mg/ml) injection—direct damage to the nerve from deoxycholic acid (10 mg/ml). Theoretically this would seem plausible, as deoxycholic acid in vitro destroys cells via a non-specific detergent disruption of cell membranes [5, 21].

We found a dose-dependent decrease in myelin staining after deoxycholic acid (10 mg/ml) exposure at 2 time points compared to control. Toluidine blue staining was significantly reduced in MMN specimens exposed to deoxycholic acid (10 mg/ml), particularly those in the 24-h group where there was negligible staining. Color deconvolution image analysis was performed on each treatment group in only one hemiface because the 24-h deoxycholic acid (10 mg/ml) treatment group showed a 10^4 decrease in strong staining percentage compared to the control and each hemiface displayed similar qualitative staining characteristics. Histologic changes seen in the MMN specimens may be due to the proposed tissue-selectivity of deoxycholic acid. This relates to in vitro and animal studies that show adipocytes are much more likely to lyse when exposed to deoxycholic acid than other cell types of protein-rich tissues with higher interstitial albumin [22]. Transmission electron microscopy of submental fat

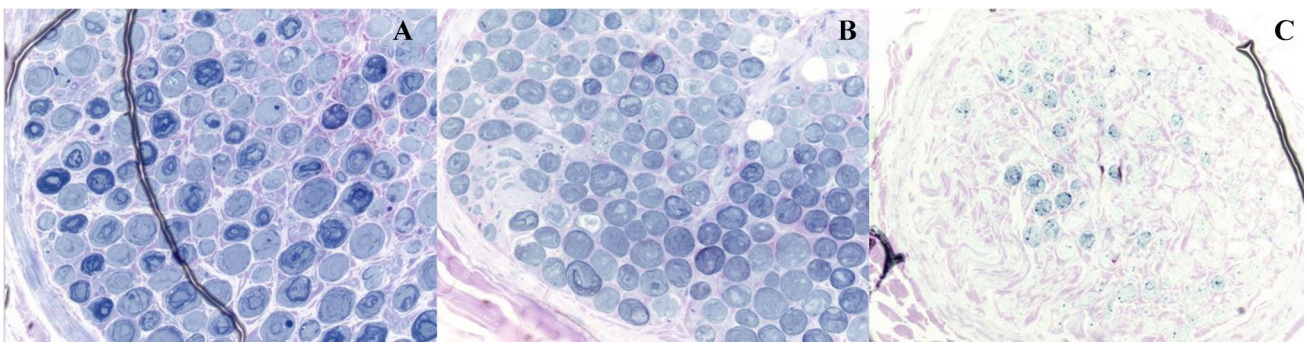


Fig. 3 Marginal mandibular nerve toluidine blue staining after exposure to: **a** saline for 24 h, **b** deoxycholic acid (10 mg/ml) for 20 min **c** deoxycholic acid (10 mg/ml) for 24 h. Magnification of ×400 for all images

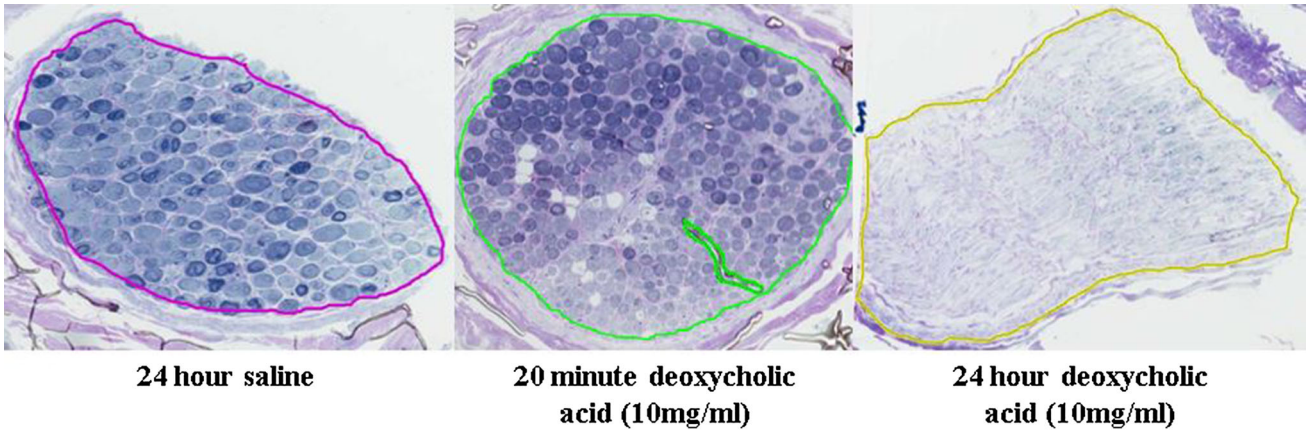
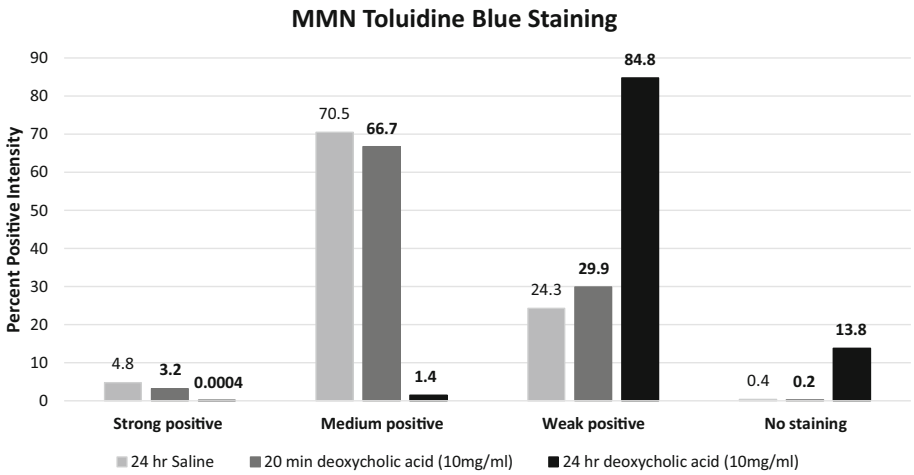


Fig. 4 Color deconvolution image analysis of toluidine blue staining

Fig. 5 Marginal mandibular nerve toluidine blue staining intensity



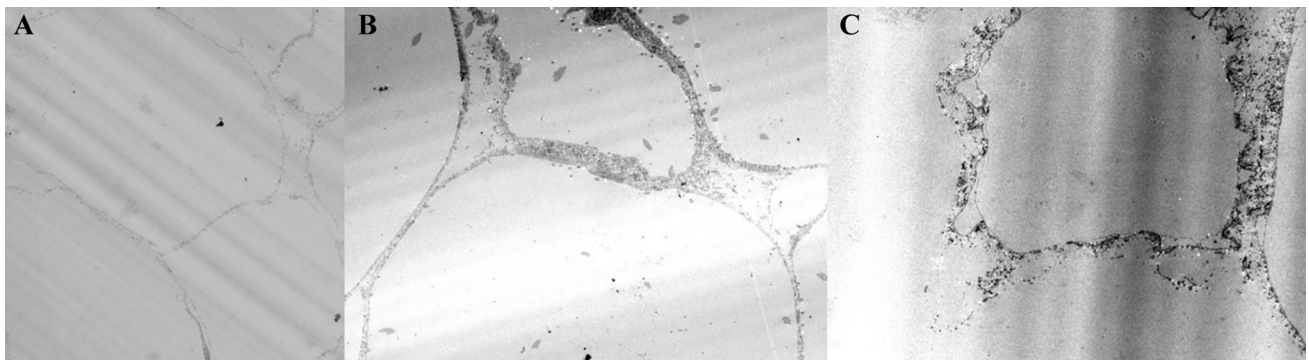


Fig. 6 Transmission electron microscopy photomicrographs of supraplatysmal cadaver fat: **a** exposed to saline for 24 h. **b** Exposed to deoxycholic acid (10 mg/ml) for 20 min. **c** Exposed to deoxycholic acid (10 mg/ml) for 24 h

exposed to deoxycholic acid (10 mg/ml) showed significant damage to adipocyte cell membranes, similar to those seen in prior studies of ex vivo human adipose tissue [23].

Myelin is high in phospholipids and may share similar vulnerability to deoxycholic acid. Therefore, our study may point more to direct MMN myelin injury, rather than from the needle during injection or intense local post-injection inflammation. Further molecular studies evaluating cellular organelle or DNA damage are needed to better characterize the exact mechanism of injury. Although not evaluated in this study, injection-related mechanical damage and/or local inflammation still may play a role in the transient MMN injury seen clinically.

Overall, sound knowledge of the biochemical properties of deoxycholic acid (10 mg/ml) combined with an understanding of submental/cervical anatomy are critical when treating SMF with deoxycholic acid. This study, while limited by fresh cadaveric nerve, points to the need for further in vivo animal studies to better understand the mechanism of MMN injury after deoxycholic acid (10 mg/ml) injection. These results should also lend strong caution to providers considering the use of deoxycholic acid (10 mg/ml) to treat periocular fat, such as lower eyelid steatoblepharon, due to risk of damage to nearby neurovascular structures.

Conclusion

In summary, deoxycholic acid (10 mg/ml) damages the marginal mandibular nerve myelin sheath in fresh human cadaver specimens. Direct deoxycholic acid neurotoxicity may cause marginal mandibular nerve injury clinically.

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Compliance with Ethical Standards

Conflict of interest None of the authors have a proprietary interest in this study or any conflicts of interest to disclose.

References

1. ASDS survey: seven out of ten consumers considering cosmetic procedures. https://www.asds.net/2017-consumer-survey/. Accessed 27 Jan 2018
2. Dayan SH, Humphrey S, Jones DH et al (2016) Overview of ATX-101 (deoxycholic acid injection): a nonsurgical approach for reduction of submental fat. Dermatol Surg 42(Suppl 1):S263–S270
3. Fagien S, Mcchesney P, Subramanian M et al (2016) Prevention and management of injection-related adverse effects in facial aesthetics: considerations for ATX-101 (deoxycholic acid injection) treatment. Dermatol Surg 42(Suppl 1):S300–S304
4. Jones DH, Carruthers J, Joseph JH et al (2016) REFINE-1, a multicenter, randomized, double-blind, placebo-controlled, phase 3 trial with ATX-101, an injectable drug for submental fat reduction. Dermatol Surg 42(1):38–49
5. Rotunda AM, Suzuki H, Moy RL et al (2004) Detergent effects of sodium deoxycholate are a major feature of an injectable phosphatidylcholine formulation used for localized fat dissolution. Dermatol Surg 30(7):1001–1008
6. Renaut A, Orlin W, Ammar A et al (1994) Distribution of submental fat in relationship to the platysma muscle. Oral Surg Oral Med Oral Pathol 77(5):442–445
7. Larson JD, Tierney WS, Ozturk CN et al (2014) Defining the fat compartments in the neck: a cadaver study. Aesthet Surg J 34(4):499–506
8. Walker P, Fellmann J, Lizzul PF (2015) A phase I safety and pharmacokinetic study of ATX-101: injectable, synthetic deoxycholic acid for submental contouring. J Drugs Dermatol 14(3):279–287
9. Walker P, Lee D (2015) A phase 1 pharmacokinetic study of ATX-101: serum lipids and adipokines following synthetic deoxycholic acid injections. J Cosmet Dermatol 14(1):33–39
10. Taban M (2017) Lower blepharoplasty in eyelids previously injected with hyaluronic acid gel filler. Podium presentation, ASOPRS spring scientific symposium vancouver, BC
11. Kenkel JM, Jones DH, Fagien S et al (2016) Anatomy of the cervicomental region: Insights from an anatomy laboratory and roundtable discussion. Dermatol Surg 42(Suppl 1):S282–S287
12. Baker DC, Conley J (1979) Avoiding facial nerve injuries in rhytidectomy. Anatomical variations and pitfalls. Plast Reconstr Surg 64(6):781–795
13. Dingman RO, Grabb WC (1962) Surgical anatomy of the mandibular ramus of the facial nerve based on the dissection of 100 facial halves. Plast Reconstr Surg Transpl Bull 29:266–272
14. Ramirez OM (2008) Advanced considerations determining procedure selection in cervicoplasty. Part one: anatomy and aesthetics. Clin Plast Surg 35(4):679–690
15. Wider TM, Spiro SA, Wolfe SA (1997) Simultaneous osseous genioplasty and meloplasty. Plast Reconstr Surg 99(5):1273–1281
16. Infante-Cossio P, Prats-Golczer VE, Lopez-Martos R et al (2016) Effectiveness of facial exercise therapy for facial nerve dysfunction after superficial parotidectomy: a randomized controlled trial. Clin Rehabilit 30(11):1097–1107
17. Moller MN, Sorensen CH (2012) Risk of marginal mandibular nerve injury in neck dissection. Eur Arch Otorhinolaryngol 269(2):601–605
18. Batstone MD, Scott B, Lowe D et al (2009) Marginal mandibular nerve injury during neck dissection and its impact on patient perception of appearance. Head Neck 31(5):673–678
19. Chen CK, Tang YB (2007) Myectomy and botulinum toxin for paralysis of the marginal mandibular branch of the facial nerve: a series of 76 cases. Plast Reconstr Surg 120(7):1859–1864
20. Krohel GB, Cipollo CL, Gaddipati K (2005) Contralateral botulinum injections improve drinking ability and facial symmetry in patients with facial paralysis. Am J Ophthalmol 139(3):540
21. Gupta A, Lobocki C, Singh S et al (2009) Actions and comparative efficacy of phosphatidylcholine formulation and isolated sodium deoxycholate for different cell types. Aesthet Plast Surg 33(3):346–352
22. Thuangtong R, Bentow JJ, Knopp K et al (2010) Tissue-selective effects of injected deoxycholate. Dermatol Surg 36(6):899–908
23. Hübner NF, Horch RE, Polykandriotis E et al (2014) A histopathologic and immunohistochemical study on liquification of human adipose tissue ex vivo. Aesth Plast Surg 38(5):976–984