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The histologic effects of high doses of botulinum toxin a on the rabbit's salivary gland

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Abstract

The exact mechanism of botulinum toxin A (BTX-A) on submandibular salivary gland (SMG) regarding its function and histology remains unclear. The goal of this work is to clarify the histological effects of BTX-A (at high doses) in SMG in rabbits after one week. Thirty adult male rabbits were used in this study and they arranged as group 1 includes rabbits which received any treatment and kept for one week duration. Group 2 includes rabbits which received 8 units of BTX-A. Group 3 includes rabbits which received 16 units of BTX-A. Animals were euthanized with ether after one week. Specimens of SMG from all rabbits were taken to perform a routine histological preparation and examination. Sections of rabbits of group 2 and group 3 showed evidence of edema that is surrounding striated ducts, congested blood vessels, and even necrosis of both serous and mucous acini. Some sections exhibited features of degeneration of mucous acini. Hemorrhage was noticed in some sections. Injection of either 8 or 16 units of BTX-A induces several alterations in the submandibular glands' histology.

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Introduction

Clinically, botulinum toxin A (neurotoxic protein) has been utilized to manage the increase in the salivary gland secretion, but the exact mechanism of this agent on this gland regarding its function and histology remains unclear (1). In fact, clostridium botulinum bacteria are the source of Botulinum toxin A which was used also in cases of excessive lacrimation, drooling, and hyperhidrosis (2,3). The action of Botulinum toxin A is depending on the inhibitory effect on acetylcholine release at the presynaptic region, as it acts on the parasympathetic nerve terminals (cholinergic nerve terminals) which lead to localized chemical block with neuronal activity loss in the target tissue (4). At 1989, the United States Food and Drug Administration (FDA) firstly give an approval for Botulinum toxin A to deal with blepharospasm beside the strabismus (5). Then this agent was considered to treat drooling resulted from the defects in swallowing by surgical operation that resect of the upper aero digestive tract tumors (6). In addition, it has an effective role in patients with salivary fistulas after oropharyngeal cancer operations, sialadenectomy or where transient ceasing of glandular secretion is mandatory to assist healing (7). In fact, all the previous uses of Botulinum toxin A are accompanied with few adverse effects (8), and make this agent superior to the cholinergic drugs as they were accompanied by several side effects including urinary retention constipation, drowsiness, irritability, fatigue, and possible severe cardiac problems (9,10).

However, the sequel of administration of high doses of Botulinum toxin A are not well clarified. So, investigation whether high doses of this agent induces effects on submandibular salivary gland tissue in rabbits after short exposure (one week) is the aim of this work.

Materials and methods

Ethical approve

The study was performed after obtaining of approval of ethical Committee of Medical Researchers at College of Medicine, University of Mosul UOM/COM/MREC/21-22(38).

Animals

Thirty male rabbits with an average body weight of 1.3 kg with the range of age from 6-8 months were used in this study. They were kept in a standardized animal house conditions with a room temperature of 22- 24 centigrade in in steel cages (1.250×0.5×0.5) meter using 12 hours' day-night cycle. Rabbits were randomly categorized into 3 groups (ten rabbits for each). Free access to food and water was permitted and adaptation of them was done for two weeks prior the beginning of the experiment (11).

The design of the study

Ten animals from each group. Group 1 includes rabbits which were did not received any treatment -Control group and kept for one week duration. Group 2 includes rabbits which were received 8 Unit of botulinum toxin A to hold to for one week duration. Group 3 includes rabbits which were received 16 Unit of botulinum toxin A to hold for one week duration.

Injection of botulinum toxin A

An intramuscular injection of 100mg/kg ketamine in combination with 5mg/kg xylazine was used to induce anesthesia (12). The right submandibular salivary gland was exposed via submandibular incision, then either 0.1 ml saline (control group) or 8 or 16 units of botulinum toxin A (United states of America, Botox®, Allergan Inc.) after reconstitution in saline was injected at the central part of the submandibular salivary gland. The regular housing and feeding regime remained and each group of rabbits were euthanized with over dosage of inhaled ether at the time of one week (5,13).

Dissection of animals and the storing of the specimens

Using euthenisation, animals' dissection was done after the end of agent administration (after 1 week). Specimens of submandibular salivary glands from all rabbits were taken as they are bisected in a mid-sagittal plane. Specimens were immersed in neutral buffer formalin of 10% for 24h for fixation and were prepared for histological technique as they were undergone dehydration in ascending grades of ethyl alcohol, dipped in xylol (for clearing) and embedded later on in paraffin wax, and the sections were cut by 4 µ thickness and placed on a glass slide to be stained with hematoxylin and eosin (H&E) (14,15). Using digital camera (I-Phone) that was firmed into microscope (Leica, Germany), photos of histological sections were taken.

Results

All rabbits remain alive during the period of this study. No signs of general toxicity were noticed among them. The microscopic examination of sections of rabbits of Group 1 (which were received no any treatment) revealed the presence of normal architecture submandibular salivary gland represented by interlobular ducts among lobules of serous acini, and mucous acini. The intralobular duct including intercalated ducts and striated ducts were seen (Figures 1 and 2).

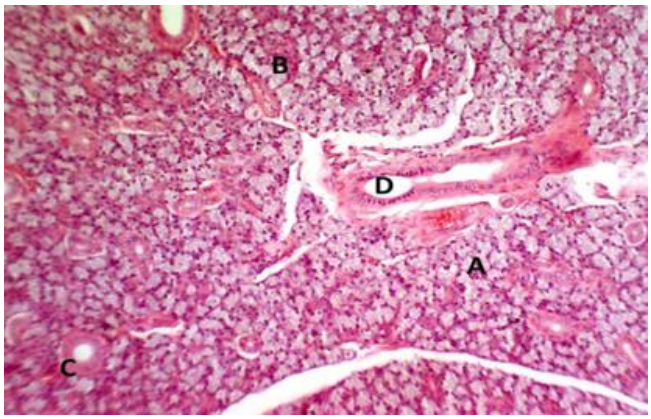

Figure 1: A section from the submandibular salivary gland of a rabbit from Group 1 in a photomicrograph showing normal architecture represented by mucous acini (A), serous acini (B) and intralobular (C) and interlobular ducts (D) is noticed. H&E stain. 100X.

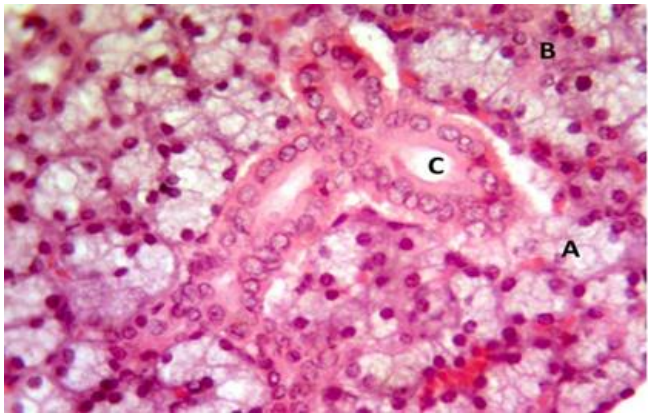

Figure 2: A submandibular salivary gland's section of a rabbit from Group 1 in a photomicrograph with normal features of mucous acini (A), serous acini (B) and striated ducts (C). H&E stain. 400X.

On the other hand, sections of submandibular salivary gland of rabbits of Group 2 (which were received 8 unit of botulinum toxin A) showed evidence of edema that is surrounding striated ducts, congested blood vessels, and even necrosis of both serous and mucous acini. Some sections exhibited loss of spherical pattern, features of degeneration of mucous acini (Figures 3-5). Further, nuclear pyknosis in mucous acini and of hemorrhage were noticed in some of sections of Group 2 (Figure 6).

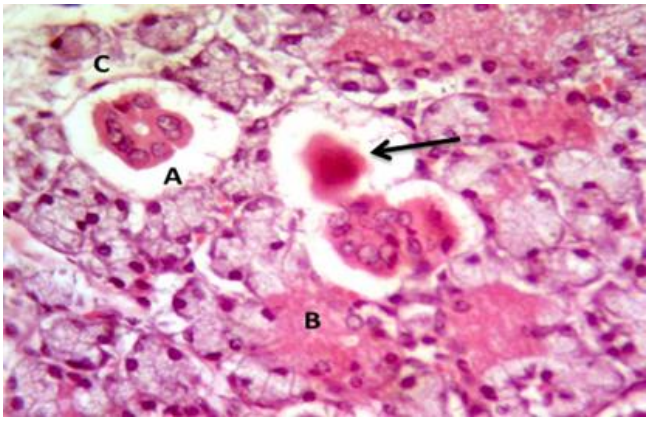

Figure 3: A section of a submandibular salivary gland of a rabbit of Group 2 (which were received 8 U of botulinum toxin A) 1 in a photomicrograph presenting with edema surrounding striated ducts (A) with evidence of necrosis in striated duct (arrow), necrosis of serous acini (B) and degeneration of mucous acini (C). H&E stain. 400X.

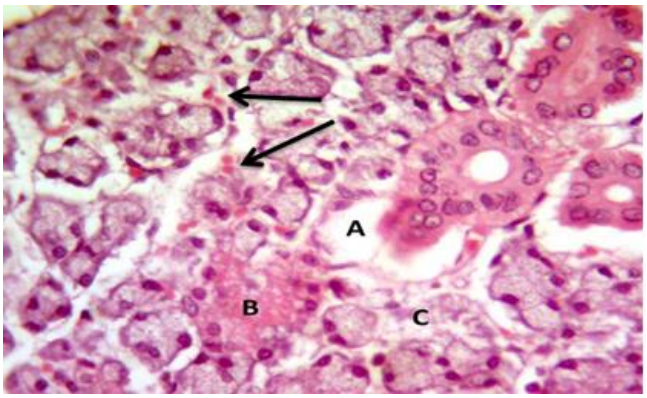

Figure 4: A photomicrograph of a submandibular salivary gland's section of a rabbit of Group 2 (which were received 8 U of botulinum toxin A) with edema surrounding striated ducts (A), loss of spherical pattern of acini, necrosis of serous acini (B) and necrosis of mucous acini (C) with few red blood corpuscles (arrows). H&E stain. 400X.

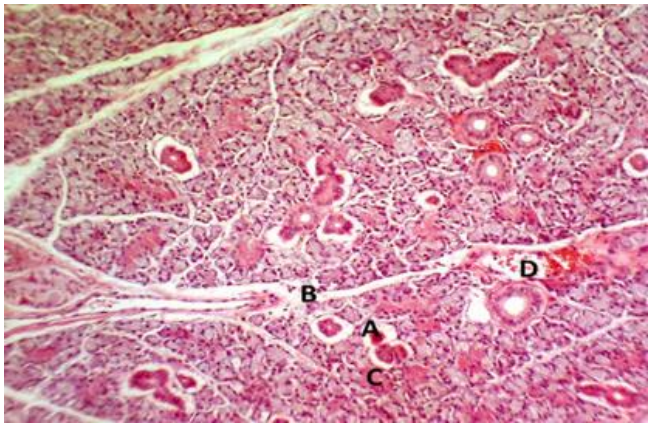

Figure 5: A microphotograph of a submandibular salivary gland's section of a rabbit from Group 2 with edema surrounding straight ducts (A), necrosis of serous acini (B) and necrosis of mucous acini (C) and congestion blood vessels (D). H&E stain. 100X.

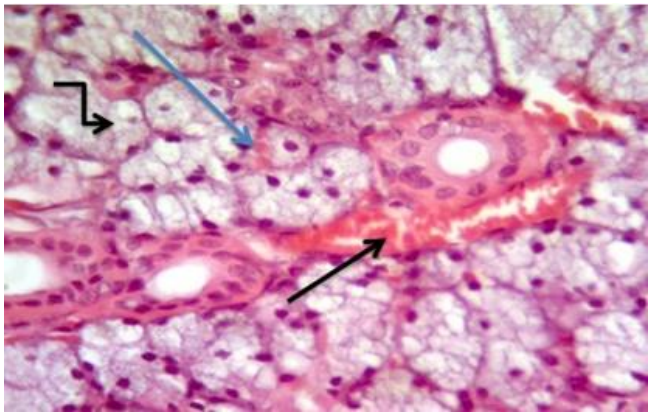

Figure 6: A microphotograph of a submandibular salivary gland's section of a rabbit from Group 2. Features of degeneration of mucous acini and loss of their nuclei and pyknosis (curved arrow), congestion blood vessels (black arrow) and hemorrhage (blue arrow). H&E stain. 400X.

In fact, the examination of sections from Group 3 (which were received 16 unit of botulinum toxin A) via light microscopy showed presence of similar features of those in Group 2 including edema surrounding striated ducts, loss of normal architecture, vacuolar /hyaline degeneration in both serous and mucous acini, congestion of blood vessels, pyknosis and necrosis of serous and mucous acini with evidence of hemorrhage. In addition, atrophy of striated ducts and edema of others with loss of basal striation were seen (Figures 7-12).

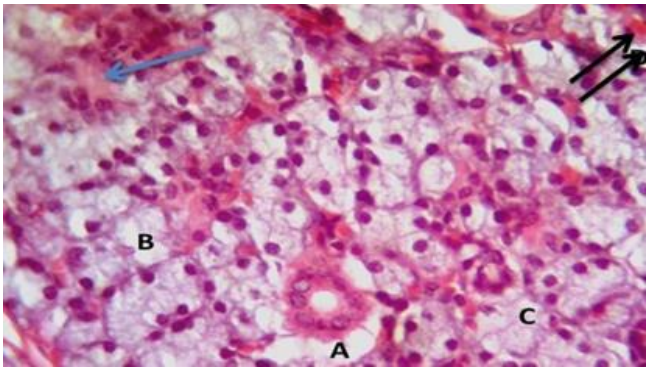

Figure 7: A microphotograph of a submandibular salivary gland's section of a rabbit from Group 3 manifested with edema surrounding striated ducts (A), degeneration (B) and necrosis (C) of mucous acini and serous acini (arrow) with evidence of hemorrhage (double arrows). H&E stain. 400X.

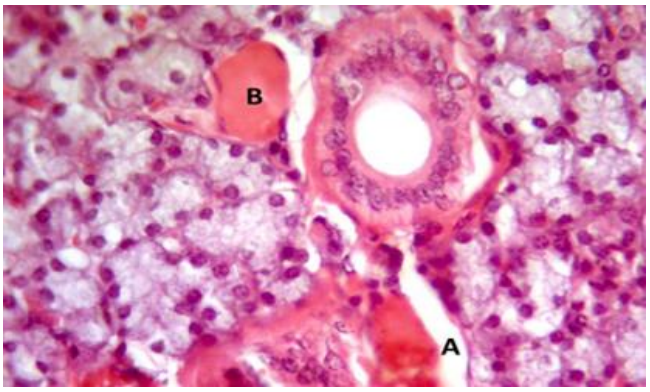

Figure 8: A microphotograph of a submandibular salivary gland's section of a rabbit from Group 3. There is edema surrounding straight ducts (A) and hyaline degeneration of mucous acini (B). H&E stain. 400X.

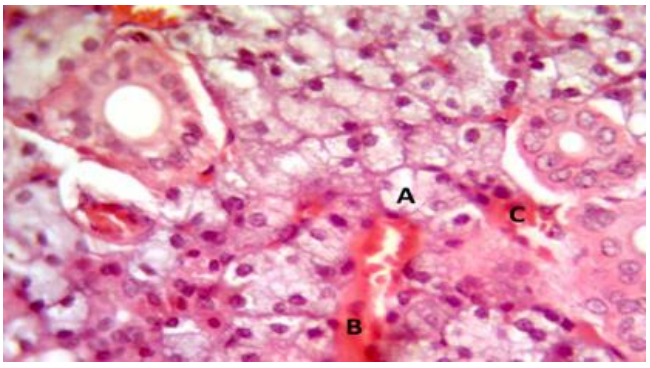

Figure 9: A microphotograph of a submandibular salivary gland's section of a rabbit from Group 3. Degeneration of mucous acini (A), loss of normal architecture of acini with congestion of blood vessels (B) and hemorrhage (C) are seen. H&E stain. 400X.

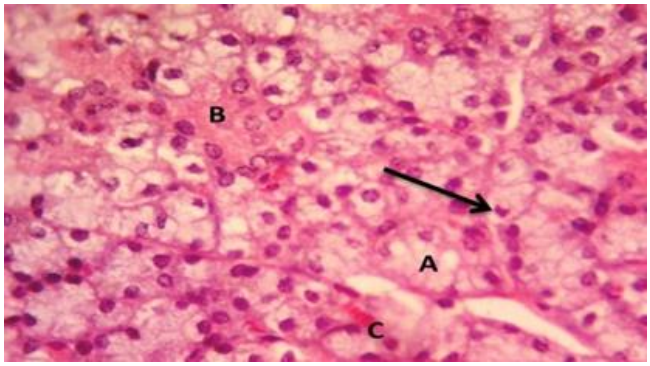

Figure 10: A microphotograph of a submandibular salivary gland's section of a rabbit from Group 3. Vacuolar degeneration of epithelial cells of mucous acini (A), necrosis of epithelia cells of serous acini (B) and hemorrhage (C) are noticed with features of pyknosis (black arrow). H&E stain. 400X.

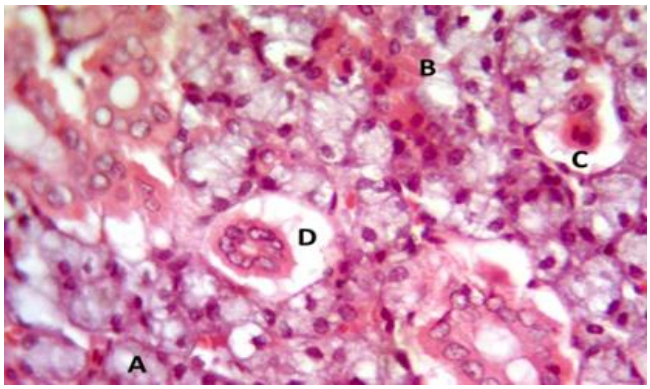

Figure 11: A submandibular salivary gland' section of a rabbit from Group 3 in a microphotograph with features of vacuolar degeneration of epithelial cells of mucous acini (A), and epithelial cells of serous acini (B), atrophy of striated ducts (C) and edema of others with loss of basal striation (D). H&E stain. 400X.

Discussion

Various diseases may be accompanied by hypersalivation and resulted a marked burden on patient physically and psychosocially beside causing the discomfort (16-20). There is sometime a need to do a temporary treatment of sialorrhea. Intra-glandular injection of botulinum toxin in submandibular salivary glands has been utilize for management of sialorrhea as it has minimal invasive characters. Further, it has an accidental use throughout the cosmetic procedure of platysma muscle facial lines. The clarification of the histological effects of intraglandular injection of high doses on submandibular salivary glands is important (5,15).

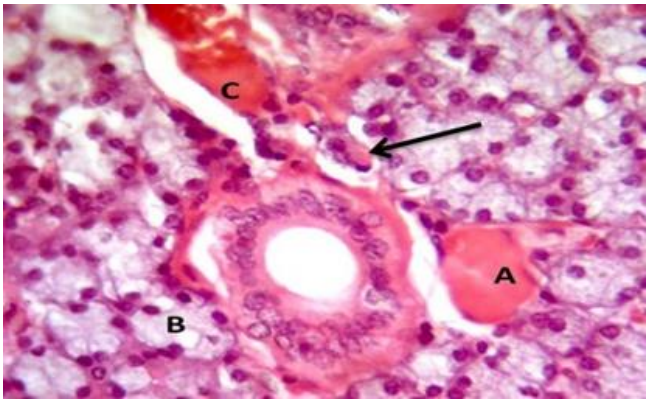

Figure 12: A microphotograph of a submandibular salivary gland's section of a rabbit from Group 3. Hyaline epithelial cells' degeneration of mucous acini (A), and vacuolar degeneration of others (B) with congestion of blood vessels (C) with necrotic acini (arrow). H&E stain. 400X.

On the other hand, the submandibular salivary gland's sections of rabbits which were injected with 8 unit of botulinum toxin A for one week exhibited several and significant morphological alterations including presence of striated ducts with surrounding edema, congested blood vessels, necrosis of both serous and mucous acini with features of degeneration of mucous acini in some sections and hemorrhage. Similar features were noticed in sections from Group 3 (which were received 16 unit of botulinum toxin A for one week). These findings indicated that there is an effect of this agent on submandibular salivary gland. In fact, the clinical effect of this agent starts within one to two days (5). In addition, as reported by other authors, the changes induced by botulinum toxin A were similar to that in this work including loss of globe shape fashion, serous acini with cytoplasmic vacuoles, striated ducts' degeneration (loss of basal striations), presence of small acini, and defective borders were observed (21).

An ultrastructural work of Younis *et al.* (22) which used intra-glandular injection of two units of botulinum toxin type A into the parotid gland of rats and concluded a decrease in the acinar volume with wide differences in secretory granules with presence of numerous coarse intracellular vacuoles. However, these findings are not in accordance with those of (1), and that may be due the difference in the given dose of this agent. Botulinum toxin A blocks selectively the acetylcholine (Ach) releasing from the cholinergic nerve end plates or junctions and lead to thus leading to glandular chemical or pharmacological innervation and muscular inactivity and leads to the reduction in the secretion of saliva (5,23).

In fact, botulinum toxin type A was included as one of the safe and sufficient medications via national institutes of health (4), however, the data on its histological effects on submandibular salivary glands is limited. On the other hand, beside the action of botulinum toxin type A on acetylcholine, there is a report on its effects on neuronal nitric oxide synthase as suggested by immunohistochemical techniques. Nitric oxide which is a neurotransmitter that has a potential vascular neuro-modulatory action in specific secretory processes' regulation in the upper proximal aero-digestive tract (24,25). Among crucial marker of nerve terminals in salivary glands is the enzyme neuronal nitric oxide synthase (26-30). The findings of the present study revealed that the action of botulinum toxin type A is dose dependent with similarity to those of (31-39).

This work revealed presence of edema in submandibular salivary glands' structure after exposure to botulinum toxin type A for a week, a previous work on parotid glands of rats suggested that this may be due to a retention of excretory material (4,22). On the other hand, this study showed a feature of atrophy in the striated ducts, these findings are similar to those of (1,4). Pykosis was noticed in sections of submandibular glands after exposure to 16 units of botulinum toxin type A. These features indicated that this agent at high doses leads to cell injury. These findings are going with those of a recent work showed that there is a reduction in the proliferative activity of the acinar epithelium with apoptosis in sections of submandibular salivary glands after exposure to botulinum toxin type A in rabbits (40).

Conclusions: This study concluded that injection of 8 units or 16 units of botulinum toxin type A for one week induces several alterations (which may be irreversible) in the submandibular gland's histology in a dose dependent manner, so the adjustment of the dose of this agent is recommended in the clinical practice.

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Conflict of interest

None.

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التأثيرات النسيجية للجرع العالية من سم البوتيلينيوم أ على الغدة اللعابية لدى الأرانب

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الخلاصة

تبقى الألية المحددة وبشكل دقيق لسم البوتيلينيوم أ على وظيفة ونسيجية الغدة اللعابية تحت الفكين غير واضحة. تهدف الدراسة الى توضيح التأثيرات النسيجية للجرع العالية لسم البوتيلينيوم في الغدة اللعابية تحت الفكين لدى الأرانب بعد أسبوع من التعرض له. تم استعمال ثلاثون أرنبا من الذكور بعمر يتراوح من ستة الى ثمانية أشهر في هذه الدراسة ونظموا كالاتي: المجموعة الأولى شملت أرانب لم يستلموا أي علاج وحفظوا لمدة أسبوع. أما المجموعة الثانية فقد شملت أرانب استلموا ثماني وحدات من سم البوتيلينيوم أ وشملت المجموعة الثالثة أرانب استلموا ست عشر وحدة من سم البوتيلينيوم أ. تم إجراء القتل الرحيم للأرانب باستعمال الايثر بعد أسبوع واحد. تم اخذ العينات من الغدد اللعابية تحت الفكين من كل الأرانب لإجراء التحضير والفحص النسيجي الروتيني. أن الشرائح العائدة للأرانب في المجموعتين الثانية والثالثة شهدت وجود وذمة حول القنوات المخططة مع احتقان في الأوعية الدموية وحتى تنكس في العنيبات المصلية والمخاطية. بعض الشرائح أظهرت علامات للاضمحلال في العنيبات المخاطية. وأيضا، لوحظ نزف في بعض الشرائح. إن حقن سم البوتيلينيوم أ أما بثماني وحدات أو بست عشر وحدة ولمدة أسبوع أحدث بضع تحويرات في نسيجية الغدة اللعابية تحت الفكين.

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