The Scientific Journal of University of Benghazi

www.sjuob.uob.edu.ly

The effects of Neostigmine on the Secretory Endpiece Cells of the Sublingual Glands in Female Rabbits

Ahmed H. Elramli[a], Lobna A. Elfrgani[1], Hoda Mansur[1], Bashir A. Saad[1] and Abeer H. Amer[1*]

[1] *Faculty of Medicine, University of Benghazi, Benghazi, Libya*

Received: 3/3/2020; accepted: 9/6/2020

الملخص

تتمثل أهمية اللعاب في المحافظة على سلامة تجويف الفم والقدرة على الكلام، خاصة في الأشخاص الذين يعانون من جفاف الفم. الغدد اللعابية عرضة لمجموعة متنوعة من الأدوية، وكذلك لعدد من الحالات المرضية التي تؤثر على سلامة الافرازات اللعابية وخصوصا الغدة التحت لسانية. كان الهدف من هذا العمل هو وصف آثار النيوستيجمين الذي يستخدم لتقليد تأثير تحفيز الجهاز العصبي السمبتاوي على الغدة التحت لسانية .والذي يمنع عمل إنزيم الكولينستراز ، الذي يدمر مادة أستيل كولين في النهايات العصبية. لتوضيح ملامحها النسيجية ، تمت دراسة الدواء فيما يتعلق بالتغيرات النسيجية التي قد تحدث في الأجزاء الداخلية المفرزة للغدة تحت اللسانية. تم استخدام أربعة وعشرين أنثى أرنب لدراسة تأثير النيوستيجمين. تم استخدام جرعات مختلفة من النيوستيجمين بما في ذلك الجرعة العلاجية والعلاجية المضاعفة والثلاثية . تم حقن نيوستيجمين داخل الصفاق لمدة أسبوعين. في نهاية الوقت تم تشريح الغدة تحت اللسان لكل مجموعة وفحصها تشريحيا مع صبغة الهيماتوكسيلين والايوسين. وتم ملاحظة زيادة كبيرة في قطر الأسيني المخاطي والمظهر الرغوي وفرغات متزايدة في السيتوبلازم في الخلايا حيث زادت بزيادة جرعة النيوستيجمين، وخاصة في الأرانب المحقونة بجرعة العلاجية الثلاثية.

الكلمات المفتاحية:

نيوستيجمين , الغدة التحت لسانية, الأسيني المخاطي.

Abstract

The importance of saliva for the integrity and wellbeing of the oral cavity and speech is well documented, especially in people suffering from xerostomia. Salivary glands are susceptible to a variety of medication, as well as to a number of pathological conditions. The aim of the present work was to describe the effects of neostigmine, a drug used to mimic the effect of stimulation of parasympathetic nervous system on sublingual gland. It inhibits the action of the enzyme cholinesterase, which destroys the substance acetylcholine at nerve endings. To clarify its histological profiles, the drug was studied to investigate possible histological structural changes which might occur in the secretory endpieces of the sublingual gland. Twelve female rabbits were used to study the effect of neostigmine, as parasympathomimetic drug. Different doses of neostigmine were used including the therapeutic, double therapeutic and triple therapeutic dose. Neostigmine was injected intraperitoneally for two weeks. At the end of the time allocated the sublingual gland of each group was dissected and examined histologically with Hematoxylin and Eosin stains. Significant increase in the diameter of the mucous acini and foamy appearance and vacuolation of the cytoplasm of the cells were observed in the experimental group treated with triple therapeutic dose of neostigmine.

Keywords: Sublingual gland, Neostgmine, Parasympathomimetic drug.

1. INTRODUCTION

Sublingual gland is one of the major salivary glands; along with submandibular and parotid glands[1]. It secretes saliva that has a crucial role in providing protection and lubrication for mouth. It also plays an essential role to prevent dental caries[2].

Histologically, the sublingual salivary gland is a branched tubuloacinar gland that consists of a branching duct system and secretory endpiece. Present in the secretory endpiece are two types of cells, mucous which are the prominent cells, serous acini along with myoepithelial cells[3,4]. Salivary glands secretion is a reflex phenomenon controlled by both parasympathetic and sympathetic innervation; stimulation by parasympathetic leads to vasodilation and secretion of copious amount of watery saliva; whereas sympathetic stimulus leads to vasoconstriction and reduced secretion of a few amount of saliva[5]. A parasympathomimetic drug neostigmine, is an anticholinestrase (AChE) inhibitor that increases salivary flow. It acts as a substrate for AChE enzyme and causes an increase in acetylcholine at parasympathetic postganglionic synapses to cause, an increase in the secretion of salivary gland[6].

Neostigmine is used in some medical disorders such as oral dryness (Xerostomia); in addition to glaucoma, urinary retention and myasthenia gravis[7]. Furthermore; Davies et al in 2015, suggested the role of parasympathomimetic drugs in treatment of radiation induced salivary gland dysfunction[8].

Aim of the study:

The present study is aimed to shed more light on the histological structural changes on the secretory endpieces of the sublingual gland, in order to study the effects of anticholinestrase (neostigmine).

2. MATERIALS AND METHODS

The present research was conducted on 12 female rabbits, 6 months old, of local mixed breed, weighing around 800-1000mg. The animals were kept under laboratory conditions with unrestricted access to food and water. The rabbits were

*Correspondence:

Dr. Abeer H. Amer
Department of Histology, Faculty of Medicine, University of Benghazi, Benghazi, Libya
abeer.amer@uob.edu.ly

divided into four groups; one control and three drug treated groups with 3 animals in each group respectively. The animals were injected with neostigmine methylsulfate (Neostigmine, ROTEXMEDICA/ Germany) intramuscularly. This drug was available as 0.5 mg injectionvial or ampoules. Neostigmine was stored at room temperature and protected from light. The drug was administrated once a day and the dose used was as following:

Group A: received an injection of therapeutic dose of neostigmine (0.147 mg).

Group B: received an injection of double therapeutic dose of neostigmine (0.294 mg).

Group C: received an injection of triple therapeutic dose of neostigmine (0.441 mg).

Group D: served as a control, and received an equivalent injections of isotonic saline for the same period of time.

The sublingual glands were collected and fixed for 48 hours in 10% neutral buffered formalin. The tissues then were processed for light microscopy. A traditional technique of paraffin embedding was followed. Rotary microtome was used for cutting 3-5 um thick sections for each tissue. The slides were then stained with Harris's Hematoxylin and Eosin[9].

3. RESULTS

Clinical observation:

There was a period excitation of the animals followed by a period of relaxation, weakness and excessive salivation were noticed in the group injected with the therapeutic dose of neostigmine. The symptoms were presented as diarrhea and excessive urination in the group injected with double and therapeutic doses of neostigmine. The group injected with triple therapeutic dose of neostigmine had additional symptom in the form of difficulty in breathing. Nevertheless; the symptoms of the neostigmine treated rabbits was subsided and relieved after two hours.

Histological finding:

The sublingual gland in rabbits of control group was composed of compound tubulo-acinar glands. It is composed of mucous tubular secretory units capped by serous demilunes; it is mixed but mostly mucous. Mucous acini are large in diameter with wider lumen. Mucous cells are arranged in test-tube-shaped tubules, lightly stained. The mucous cells have pale foamy vacuolated cytoplasm, and flattened basal nuclei against the base of the cell as seen it the control group (fig.1). In the animals injected with the therapeutic dose of neostigmine it was noted that the cells of the secretory portion had an increased size and amplified secretion with increased foamy appearance (fig.2). While the sublingual glands of the animals injected with a double therapeutic dose, the size of the mucous cells had increased in size and amplified secretion causing more pronounced vacuolation in the cytoplasm and more foamy appearance in mucous acini with clear cell boundaries. It also showed an appearance of remnants of secretion in the duct system as clarified with the black arrow (fig.3). Whereas for the triple therapeutic dosed animals, the sublingual salivary gland showed larger sized cells and more foamy appearance than the double dosed animals while parts of the cytoplasm had spaces between them. It also showed enlarged size of acini with increased foamy appearance of cell. In addition there was a marked appearance of connective tissue infiltration between the acini as shown by the black arrows in (fig.4), this may indicate increased fibrosis. In all treated sections it was noticed that the nucleus were condensed and flattened to the base of the cell, due to increased secretion.

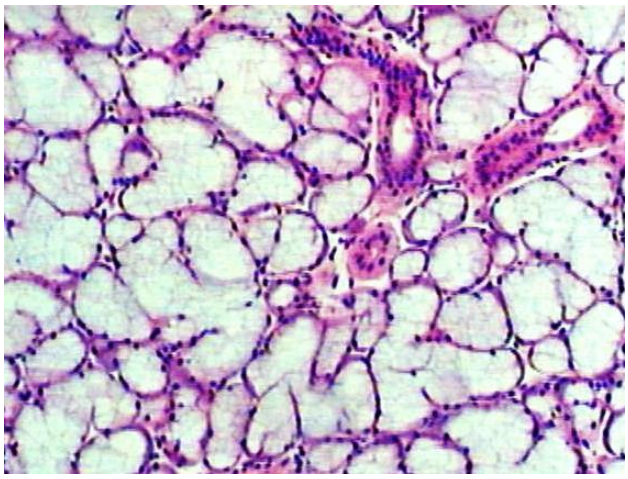

Figure 1. The photomicrograph from the sublingual gland of the control group, showing a normal structure and appearance of mucous acini and duct system. Original magnification × 200.

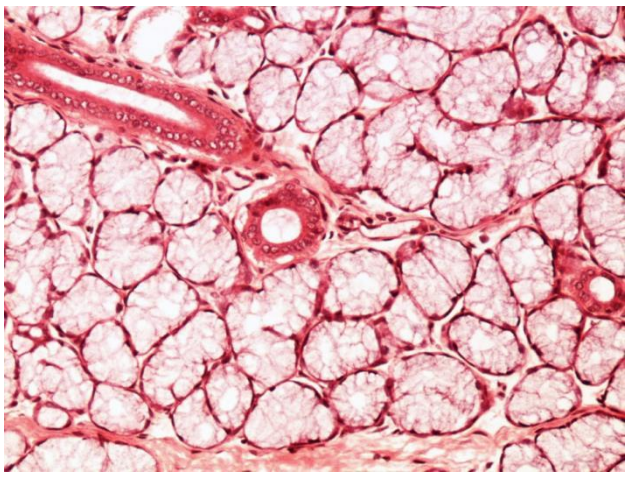

Figure 2. The photomicrograph from the sublingual gland of the therapeutic dose group shows normal appearance of duct system with increase in size and secretion of mucous cells with increased foamy appearance in the cytoplasm. Note the presence of vacuolation in the cytoplasm. Original magnification × 200.

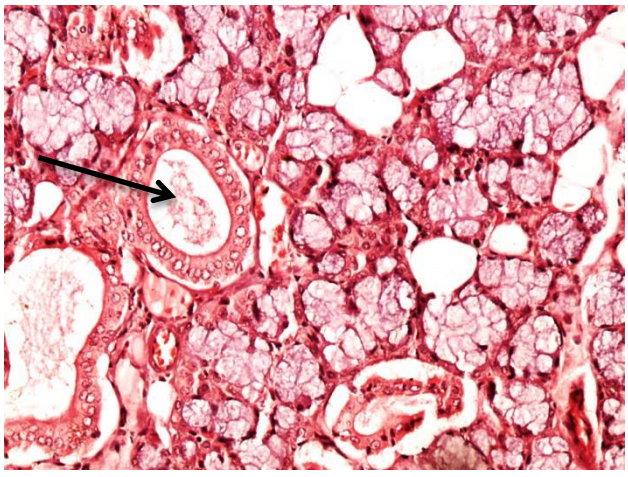

Figure 3. The photomicrograph from the sublingual gland of the double therapeutic dose group shows increased size of mucous cells and amplified secretion causing more pronounced vacuolation in the cytoplasm. Also, shows appearance of remnants of secretion in the duct system as clarified with the black arrow. Original magnification × 200.

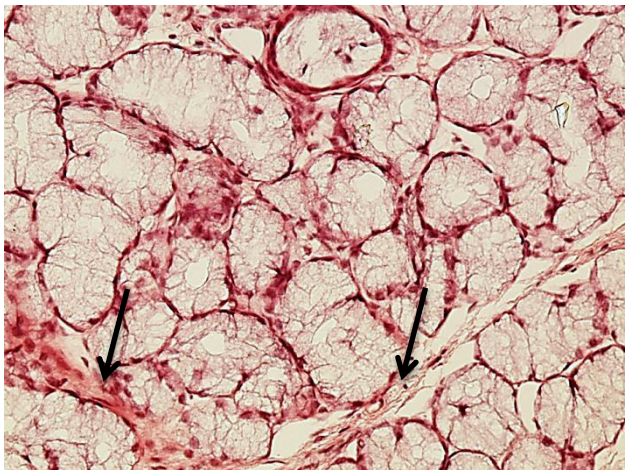

Figure 4. The photomicrograph from the sublingual gland of the triple therapeutic dose group shows larger sized mucous cells and more foamy appearance than the mucous cells of the double therapeutic dose group with parts of the cytoplasm with spaces between them. In addition there was a marked appearance of connective tissue infiltration between the acini as shown by the black arrows. Original magnification × 200.

4. DISCUSSION

In the current work the effects of different doses of Neostigmine on the histological structural changes of rabbit sublingual have been examined. The histological changes were shown as an increase in the diameter of the mucous acini and the vacuolation of the cytoplasm of the cells with increasing dose of neostigmine injection. The evaluation of neostigmine as sialagogues drug simulating the effect of parasympathetic nervous system[10] is very essential. In 2015, Davies et al, support the use of neostigmine and other parasympathomimetic drugs for the treatment of salivary gland dysfunction due to radiotherapy in head and neck malignancies[8]. The clinical approaches of the latter author were in consistent with the histological findings of the current research. There has been understanding that vacuolation, after the use of parasympathomimetic drugs, can take place at episode in exocrine serous cells of the trachea[11]; pancreas[12,13]. Earlier in vitro studies on rat parotid gland[14] illustrated that stimulation with muscarinic and adrenergic agonists effected progress of water and vacuole development. It was believed that vacuole formation is a necessary part of water secretion[15].

In rat sublingual[16], prolonged strong parasympathetic stimulation has amplified the affinity for acinar vacuolation as seen in triple dose treated rabbits of the current work.

In addition, we have examined the super sensitivity of the mucous cells in rabbits which manifested by increase the rate of salivation. That replicates the findings of sensitization of the serous acini by parasympathetic stimulation which was a necessary condition for secretion[17]. It was reported that there were an improvement in compliance of the patients receiving radiotherapy after the use of neostigmine[18]. Actually neostigmine like other parasympathomimetic drugs was important to induce compound exocytosis to the secretory granules[19], a process that was believed to be radio protective in preceding study[20,21,22,23]. In general compound exocytosis is crucial process in production of excessive amount of saliva by the parotid salivary gland[24]. Similar results were attained for amifostine in experimental studies using rat and rabbit models and clinical trials[25] which favor such treatment. The endogenous saliva production is very significant to patient equally for its convenience and the importance of natural saliva to oral function. The artificial saliva does not replace the many macromolecules vital to protective and other functions of saliva. Stimulation of gland function also may help prevent ascending infection of salivary glands and delay the formation of mucous plug[26].

5. CONCLUSION

The structural histological alterations noticed in this study confirm the use of the drug neostigmine in cases of xerostomia. The production of endogenous saliva is of greatest benefit to patient both for its convenience and the importance of natural saliva to oral function. Neostigmine could be used as prophylactic agents in patients receiving radiotherapy with head and neck malignancies, showing diminished salivary gland output.

6. REFERENCES

1. Igbokwe CO, Neba PC, Bello UM. (2015). Comparative histology and histochemistry of the major salivary glands in the giant pouched-rats (Cricetomys gambianus) and greater cane rats (Thryonomys swinderianus) Indian J. Anim. Res., 49 (4): 451-460.
2. Sakr, S. Gehan A. Elba, Samia S. Omar, Sahar S. Karam. (2017). Effect of Neostigmine Administration On The Ultrastructure Of The Parotid Salivary Gland In Rats With Induced Diabetes. Alexandria Dental Journal. 42:243-247.
3. Ross H. M., Kaye G.I., and Powlina W. (2006). Histology- a text and atlas with cell and molecular Biology, 4th edition: 454-461.
4. Janqueira L.C. and Carneiro J. (2005). Basic Histology, Text and atlas, 10th edition, Organ associated with digestive tract-salivary gland: 317-322.
5. Wilson K. J.W. and Waugh A. (1998). Ross and Wilson Anatomy and physiology in health and Illness, 8th edition, Digestive system: 290-291.
6. ELRamli A., Yasear A., Sultan A. (2013). Structural histological changes in the parotid salivary gland of rabbit treated with neostigmine. J Basic Med Allied Sci. 1:1-15.
7. Andera A. (2005). Pharmacology notes- Autonomic nervous system- Cholinergic agent- Neostigmine. A unique dental hygiene- Amyrdh.com. 1998-2005.
8. Davies AN, Thompson J. (2015). Parasympathomimetic drugs for the treatment of salivary gland dysfunction due to radiotherapy. Cochrane Database Syst Rev. (10):CD003782.
9. Wail A. Elhawari, Akram Y. Yasir, Manal F. Yaya and Abeer H. Amer. The Antioxidant Effect of Vitamin C on The Liver and kidney tissue of Albino Mice Treated with Chromium Hexaoxide. Journal of Basic Medical and Allied Sciences 2018: 2 (1). 1-7.
10. Katzung B. (2004). Basic and clinical pharmacology, 9th edition (middle east edition): 101-107.
11. Mills J.W. and Quanton P.M. (1981). Formation of stimulus induced vacuoles in serous cells of tracheal submucosal gland. Am.J.physiology cell Biology. 241: C18-C24.
12. Watanabi I., Seguchi H., Oxada T., Kobayashi T., Jin Q.S. and Jiang X.D. (1996). Fine structure of the acinar and duct cell component in the parotid and submandibular glands of rat: TEM, SEM, and HR SEM study. Histolo. Histopatho. II: 103-110.
13. Hammel I., Shor-Hazan O., Elder T., Amihhai D., and Lew S. (1999). Morphometric studies of secretory granule formation in mouse pancreatic acinar cells, Dissecting the early structural changes following pilocarpine injection. J. Anat. 194: 51-60.
14. Schramm M. and Slinger Z. (1974). The function of α- and β- adrenergic receptors and a cholinergic receptor in the

secretory cells of rat parotid gland. In Advances in Cytopharmacology 2: 29-32.
15. Garrett J.R. (1987). The proper role of nerves in salivary secretion. a-review. J. Dent. Res. 66 (2): 387-397.
16. Garrett J. and Kyriacou, K. (1988). Paralytic secretion after parasympathectomy of rabbit submandibular gland includes a cholinergic Q. J . Exp. Physiol.; 73:737-746.
17. Ikeno T., Ikeno K. and Uno T. (1988). Relationship between serum amylase activity and intra ductal pressures in the rat parotid and submandibular salivary glands after administration of pilocarpine or isoprenaline. Arch. Oral biolo. 33 (6): 403-406.
18. Hakim S.G., Kosmhl H., Lauer I., Nadrowitz R., Wedel T., and Sieg P. (2005). A comparative study on the protection profile of lidocaine, amifostine and pilocarpine on the parotid gland during radiotherapy. Cancer res.; 65: 10486-10493.
19. Gravenmade E. J., Roukema P.A. and Panders A.K. (1974). The effect of mucin containing artificial saliva on sever xerostomia. Int. J. oral surg. 3: 435-439.
20. Roesink J.M., Konings A.W.T., TerHaard C. H., Battermann J. J., Campinga H. H. and Coppes R. P. (1999). Preservation of the rat parotid gland function after radiation by prophylactic pilocarpine treatment: radiation dose dependency and compensatory mechanisms. Int. Radiat. Oncol. Biology and physiology 45: 483-489.
21. Coppes R. P., Zeilstra L. J., Vissink A. And Konings A. W.T. (1999-b). Muscarinic receptor stimulation increases tolerance of rat salivary gland function to radiation damage. Int. J. Radiat. Biolo. 72: 240-247.
22. Mateos JJ, Setoain X, Ferre J, (2001). Salivary scintigraphy for assessing the protective effect of pilocarpine in head and neck irradiated tumors. Nucl Med Commun;22:651–656.
23. Coppes R. P, Zeilstra L. J, Kampinga H. H, Konings A. W. (2001). Early to late sparing of radiation damage to the parotid gland by adrenergic and muscarinic receptor agonists. Br J Cancer;85:1055–1063
24. Nanci A. (2003), Ten Cate's oral Histology-Development, structure and function 6th edition, Salivary gland: 299-328.
25. Pratt NE, Sodicoff M, Liss J, Davis M, Sinesi M, (1980). Radioprotection of the rat parotid gland by WR-2721: morphology at 60 days post-irradiation. Int J Radiat Oncol Biol Phys;6:431–435.
26. Fox P.C., Vander Ven P.F., Baum B.J. and Mandel I.D. (1986). Pilocarpine for the treatment of xerostomia associated with salivary gland dysfunction. Oral surg. Oral med. and Oral path. 61: 243-245.