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

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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 was compared with the control group. Significant increase in the saliva flow was observed in the neostigmine group. The study suggested that neostigmine could be used as a therapeutic agent in cases where increased saliva flow is desired.

Keywords: Sublingual gland, Neostigmine, Parasympathomimetic drug.

1. INTRODUCTION

Sublingual gland is one of the major salivary glands; along with submandibular and parotid glands. 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. 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. 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. A parasympathomimetic drug neostigmine, is an anticholinesterase (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. Neostigmine is used in some medical disorders such as oral dryness (Xerostomia); in addition to glaucoma, urinary retention and myasthenia gravis. Furthermore, Davies et al in 2015, suggested the role of parasympathomimetic drugs in treatment of radiation induced salivary gland dysfunction.

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 anticholinesterase (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
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The animals were 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 injectionial 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.

**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.
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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 x 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
dialogues drug simulating the effect of parasympathetic
nervous system40 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 malignancies6. 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 trachea11; pancreas12,13. Earlier in
vitro studies on rat parotid gland14 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 secretion15.

In rat sublingual16, 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 secretion17. It was reported that there
were an improvement in compliance of the patients receiving
radiotherapy after the use of neostigmine18. Actually
neostigmine like other parasympathomimetic drugs was
important to induce compound exocytosis to the secretory
granules19, a process that was believed to be radio protective in
preceding study20,21,22,23. In general compound exocytosis is
crucial process in production of excessive amount of saliva by
the parotid salivary gland24. Similar results were attained for
amifostine in experimental studies using rat and rabbit models
and clinical trials25 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
plug26.

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.

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