

Correlation between the expression of the *iNOS*, *caspase-3* and *α-SMA* genes in the parotid glands of albino rats following the administration of two antihistamines from two different generations

Dina Rady^{1,B–F}, Marwa Magdy Abbass^{1,A,B,E,F}, Heba Hakam^{1,A,B,E,F}, Rasha Rady^{2,E}, Iman Aboushady^{3,B–F}

¹ Department of Oral Biology, Faculty of Dentistry, Cairo University, Egypt

² Department of Pediatrics, Faculty of Medicine, Cairo University, Egypt

³ Department of Oral Biology, Faculty of Dentistry, Modern University For Technology and Information, Cairo, Egypt

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online)

Dent Med Probl. 2024;61(3):335–343

Address for correspondence

Dina Rady
E-mail: dina.radi@dentistry.cu.edu.eg

Funding sources

None declared

Conflict of interest

None declared

Acknowledgements

None declared

Received on September 5, 2022

Reviewed on December 9, 2022

Accepted on December 11, 2022

Published online on June 30, 2024

Cite as

Rady D, Abbass MM, Hakam H, Rady R, Aboushady I. Correlation between the expression of the *iNOS*, *caspase-3* and *α-SMA* genes in the parotid glands of albino rats following the administration of two antihistamines from two different generations. *Dent Med Probl.* 2024;61(3):335–343. doi:10.17219/dmp/157346

DOI

10.17219/dmp/157346

Copyright

Copyright by Author(s)

This is an article distributed under the terms of the Creative Commons Attribution 3.0 Unported License (CC BY 3.0) (<https://creativecommons.org/licenses/by/3.0/>).

Abstract

Background. Several medications, including antihistamines, can alter salivary gland function, causing dry mouth or xerostomia. Antihistamines are commonly used for treating allergic rhinitis.

Objectives. The aim of the present study was to compare and correlate the effects of first-generation vs. second-generation H1-antihistamines on the parotid glands of rats.

Material and methods. Twelve adult male albino rats were used; 4 rats served as a control group (group I) and the remaining rats were divided into 2 groups: group II received promethazine hydrochloride; and group III received cetirizine dihydrochloride for 3 weeks. The parotid salivary glands were dissected, and examined histologically and analyzed histomorphometrically for the acinar area percentage. In addition, mRNA gene expression of *iNOS*, *caspase-3* and *α-SMA* was assessed using quantitative real-time polymerase chain reaction (qRT-PCR). Finally, all the obtained data was statistically analyzed.

Results. Histologically, group I showed the typical architecture of the gland. In group II, degenerative changes were noticed, including acinar degeneration and shrinkage with widened connective tissue septa, intracellular vacuolization, and increased inflammatory cell infiltration. In group III, similar histological features were detected as in group II, but to a lesser extent. Histomorphometric results revealed significant differences in the acinar area percentage between various groups. In addition, qRT-PCR results showed a significant increase in *iNOS* expression in both groups II and III as compared to group I, *caspase-3* gene expression was significantly increased in group II, while in group III, it increased non-significantly. Finally, *α-SMA* gene expression non-significantly decreased in both groups II and III. A significant positive correlation was observed between *caspase-3* and *iNOS* gene expression, while an inverse correlation was noticed between *caspase-3* and *α-SMA* gene expression.

Conclusions. The administration of antihistamines resulted in changes in the rat salivary glands, which could be due to the induction of oxidative stress and the resultant apoptotic effect. These changes were suggested to occur mainly through action on muscarinic receptors; yet, action on histamine receptors could not be excluded. However; these effects were less marked with the second-generation antihistamine.

Keywords: nitric oxide synthase, caspase 3, alpha smooth muscle actin, histamine antagonists

Introduction

Xerostomia is a medical condition characterized by oral cavity dryness. It has a multifactorial etiology; most commonly, it is due to systemic diseases like Sjögren's syndrome, head and neck region radiation and the administration of xerostomia-causing medications.¹ Various drugs, including antihistamines, can alter salivary gland function, causing oral dryness, which depends greatly on the number and dosage of the drugs being administered.² The incidence of xerostomia is growing because of the aging population, as older individuals commonly take several medications.³ The occurrence of xerostomia is accompanied by a greater prevalence of fungal infections and dental caries, in addition to difficulties in speech, mastication and deglutition, which consequently affects the patient's oral health and compromise their quality of life.¹

H1-antihistamines are among the most common medications used worldwide. They are structurally unrelated to histamine and act as inverse agonists by stabilizing its inactive conformation.⁴ Moreover, they are functionally classified into 2 broad groups, being first-generation or classical sedating group and the second-generation or non-sedating group.⁵

First-generation H1-receptor antagonists (e.g., promethazine hydrochloride) are lipophilic and can cross the blood–brain barrier. Consequently, they can affect the central histaminic receptors, so they are known for their sedating effect.⁵ Furthermore, promethazine has strong anticholinergic properties, facilitated by blocking the responses to acetylcholine mediated by muscarinic receptors. This atropine-like effect [Please confirm.] is accountable for the most common side effects of antihistamine medicines, including xerostomia (dry mouth), observed in their clinical usage.⁶

Conversely, second-generation antagonists, piperazine derivatives and carboxylated metabolites of hydroxyzine, were developed with more selectivity to peripheral H1-receptors, and with a very poor affinity for muscarinic, adrenergic or serotonergic receptors. Also, second-generation H1-antihistamines can be considered relatively non-sedating, with no anticholinergic effects, as they poorly penetrate through the blood–brain barrier, and are less likely to affect the central histaminic cholinergic or adrenergic receptors.⁵

The antihistaminic activity of cetirizine, a second-generation H1-receptor antagonist, has been reviewed previously, showing no measurable affinity for receptors other than H1 in vitro, in addition to negligible anticholinergic activity in animal models. However, clinically, dry mouth was documented with cetirizine more commonly than in the case of placebo.⁷

The third-generation antihistamines, active metabolites of second-generation agents, possess many of the desirable clinical effects of the first-generation agents with a more tolerable side effect profile.⁸

Despite the negative impact of antihistamines on salivary secretion, which has been documented clinically, the molecular mechanism behind this effect is yet unknown. To better understand the cellular and molecular basis of xerostomia, the current study compared the effects of long-term administration of promethazine hydrochloride, a first-generation H1-antihistamine, and cetirizine dihydrochloride, a second-generation antihistamine.

Material and methods

Drugs

The following drugs were used in the study:

- Histaloc®: 25 mg of promethazine hydrochloride (first-generation H1-receptor antagonist) (Julphar Gulf Pharmaceutical Industries, Ras Al Khaimah, UAE); and
- Zyrtec®: 10 mg of cetirizine dihydrochloride (second-generation H1-receptor antagonist) (GlaxoSmithKline, Al Haraneyah, Egypt).

Animals

Based on a previous study conducted by Shahabooui et al., the average difference in the alveolar bone healing between 2 experimental groups of rats was $4 \pm 1.3\%$.⁹ Using a power of 80%, 3 rats were needed in each group. This number had been increased to 4 in each group to compensate for losses during breeding. Sample size calculation was performed using PS: Power and Sample Size Calculation program, v. 3.1.2 (Vanderbilt University, Nashville, USA).

Twelve adult male albino rats (*Rattus norvegicus albinus*, Wistar strain), weighing approx. 150–200 g, were obtained and bred at the Faculty of Medicine, Cairo University, Egypt. They were kept in individual cages, with a light/dark cycle of 12 h, fed a standardized diet and given water ad libitum.

Experimental design

The animals were randomly assigned into 3 groups using the Random Sequence Generator program (<https://www.random.org/sequences>). Four rats served as a control group (group I), where the rats received distilled water daily via oral gavage for 3 successive weeks.

The remaining rats served as experimental groups, and they were randomly divided into 2 groups, 4 rats in each. Groups II and III included rats that received 4.8 mg/kg promethazine hydrochloride and 3 mg/kg cetirizine dihydrochloride, respectively, in distilled water daily via oral gavage for 3 successive weeks.

Investigations

After 3 weeks, rats from all studied groups were euthanized via an intracardiac overdose of sodium thiopental

(80 mg/kg). The parotid salivary glands were dissected and investigated. The assessment steps were carried out by blinded investigators.

Light microscopy examination

In the Department of Oral Biology, Faculty of Dentistry, Cairo University, the samples were fixed in 10% buffered formalin for 48 h, dehydrated in ethyl alcohol, cleared in xylol, and embedded in paraffin wax (El Gomhoureya for Drugs Trade and Medical Supplies, Cairo, Egypt). Sections of 4–6 μm were cut, mounted on glass slides, stained with the hematoxylin and eosin (H&E) stain, and then examined under a light microscope (Leica, St. Gallen, Switzerland), using magnifications $\times 200$ and $\times 400$.

Histomorphometric analysis

The acinar percentage area was assessed in photomicrographs at magnification $\times 400$. Image analysis was done with the use of the ImageJ software, v. 1.53d (<https://imagej.net/ij>). Subsequently, the H&E images were converted by splitting the channels to the requisite color and the thresholds were adjusted specifically for the acinar area percentage. The dimensions were converted from pixels to millimeters by the software. Four non-overlapping microscopic fields were randomly selected and evaluated.

qRT-PCR analysis

In the Microbiology Unit, Department of Biochemistry, Faculty of Medicine, Cairo University, a total RNA isolation kit (Qiagen, Germantown, USA) was used to isolate total RNA from the obtained samples following the manufacturer's instructions. According to the kit protocol, RNA extracted from the specimens was reverse transcribed using a cDNA Reverse Transcription kit (Fermentas, Waltham, USA). cDNA was then amplified and analyzed using the StepOne™ software, v. 3.1 (Applied Biosystems, Waltham, USA). Using the comparative $\Delta\Delta\text{CT}$ method, relative mRNA gene expression was standardized with regard to the mean critical threshold values of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), a house-keeping gene (control gene). The sequences of the applied primers (ThermoFisher Scientific, USA) for inducible nitric oxide synthase (*iNOS*), caspase 3 (*caspase-3*), alpha smooth muscle actin (α -*SMA*), and *GAPDH* genes are listed in Table 1.

Statistical analysis

All data from histomorphometric analysis and qRT-PCR analysis was coded and entered using IBM SPSS Statistics for Windows, v. 25 (IBM Corp., Armonk, USA). Numerical data was summarized and expressed as mean and standard deviation ($M \pm SD$). Comparisons

Table 1. Primer sequences of all studied genes

Gene symbol	Primer sequence (from 5' to 3')	Accession number
<i>iNOS</i>	F: 5'-GTTCCCCCAGCGGAGCGATG-3' R: 5'-ACTCGAGGCCACCCACCTCC-3'	NM_012611.3
<i>caspase-3</i>	F: 5'-CTGGACTGCGGTATTGAG-3' R: 5'-GGGTGCGGTAGAGTAAGC-3'	NM_053304.1
α - <i>SMA</i>	F: 5'-CCGACCGAATGCAGAAGGA-3' R: 5'-ACAGAGTATTTGCGCTCCGAA-3'	NM_001613.4
<i>GAPDH</i>	F: 5'-CCATTCTTCCACCTTTGATGCT-3' R: 5'-TGTTGCTGTAGCCATATTCATTGT-3'	NM_017008.4

between the studied groups were performed using the analysis of variance (ANOVA) with multiple comparisons of Tukey's post hoc tests. The level of significance was set at $p < 0.05$. Correlations between quantitative variables (*iNOS*, *caspase 3* and α -*SMA*) were investigated using Pearson's correlation coefficient.¹⁰

Results

Light microscopy results

Group I

The histological examination of the parotid salivary gland tissues of the control group revealed normal glandular structure. Serous acini were closely packed together with intercalated and striated ducts in glandular lobules, with narrow normal connective tissue septa in between. Small to medium-sized blood vessels could be observed close to striated and excretory ducts (Fig. 1).

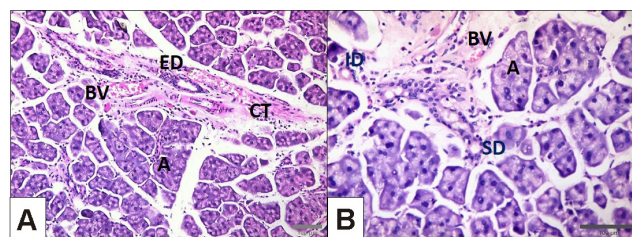


Fig. 1. Photomicrographs of the parotid gland tissues of the control group (group I)

A – normal histological structure, with closely packed acini (A) and connective tissue septa (CT) displaying a blood vessel containing RBCs (BV), as well as an excretory duct (ED) with normal lining; B – serous acini (A) with well-defined outlines, intralobular ducts (intercalated ducts (ID) and striated ducts (SD)) with normal lining, and small blood vessels (BV). RBCs – red blood cells. Scale bar – 100 μm .

Group II

The shrinkage of serous acini with widened connective tissue septa in between could be observed in most areas of the gland. Intracytoplasmic vacuolization was obvious in serous acini and the lining of some striated ducts. In addition, many atrophied and degenerated acini were observed.

Regarding excretory ducts, some of them displayed areas of degeneration and discontinuity in their lining. The degenerated acini were replaced with marked chronic inflammatory cell infiltration in discrete gland regions. Moreover, rare blood vessels were seen near striated ducts, and in connective tissue septa, some blood vessels were dilated, with areas of discontinuity in their endothelial lining, and contained few red blood cells (RBCs) in their lumen (Fig. 2).

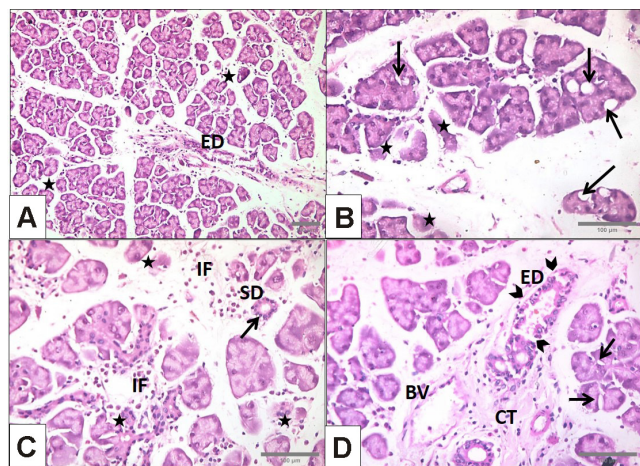


Fig. 2. Photomicrographs of the parotid gland tissues of the Histaloc group (group II)

A – shrinkage of serous acini with widened interlobular spaces (asterisks) and an excretory duct (ED) in connective tissue septa; B – intracytoplasmic vacuolization among serous acini (arrows) and numerous degenerated acini (asterisks); C – degenerated acini (asterisks) with massive chronic inflammatory cell infiltrate (IF) replacing the acinar portions, and cytoplasmic vacuolization in the striated duct (SD) lining (arrow); D – connective tissue septa (CT) displaying an excretory duct (ED) with discontinuity in its lining (arrow heads), and a dilated blood vessel (BV) with discontinuity in its endothelial lining, intracytoplasmic vacuolization (arrows). Scale bar – 100 µm.

Group III

The examination of sections obtained from this group showed enhanced histological features when compared to group II. This was evidenced by the presence of less degenerative changes among serous acini, which were closely packed in many areas of the gland, with narrow connective tissue septa in between. Less intracytoplasmic vacuolization could be detected in acinar cells and some of the ductal cells. Moreover, fewer atrophied and degenerated acini were observed. Intralobular ducts were easily detected with their normal lining and were frequently associated with small blood vessels engorged with RBCs. Connective tissue septa showed few inflammatory cell infiltrates, while excretory ducts showed normal lining in most areas of the gland and congested blood vessels with almost normal lining (Fig. 3).

Histomorphometric results

Regarding the acinar area percentage, statistically significant differences were observed between the studied groups ($p = 0.021$) (Table 2, Fig. 4). The lowest acinar

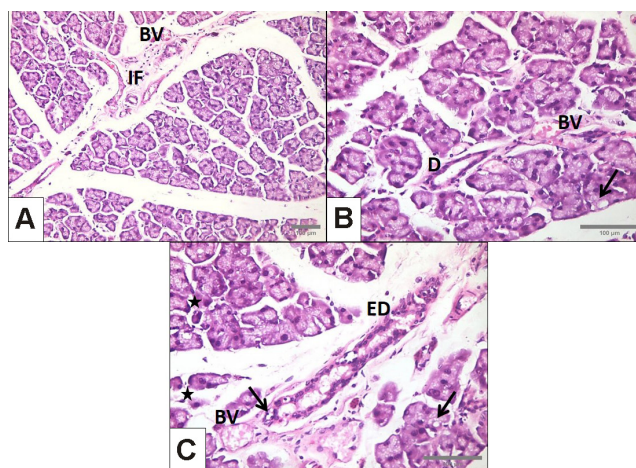


Fig. 3. Photomicrographs of the parotid gland tissues of the Zyrtec group (group III)

A – closely packed serous acini (A) connective tissue septa displaying few inflammatory cell infiltrates (IF), an excretory duct (ED) with normal lining, and a blood vessel (BV) engorged with RBCs; B – acinar vacuolization (arrow), intralobular duct (D) with normal lining, closely related to blood vessels (BV) engorged with RBCs; C – few intracytoplasmic vacuoles in the acinar and ductal cells (arrows), scattered degenerated acini (asterisks), and connective tissue septa displaying an excretory duct (ED) with continuous lining and congested blood vessels (BV) with RBCs. Scale bar – 100 µm.

Table 2. Results of ANOVA for the acinar area percentage [%] in all studied groups

Group	$M \pm SD$	SE	F	p-value
Group I	53.34 $\pm$ 9.06	4.53	6.15	0.021*
Group II	35.95 $\pm$ 2.51 ^a	1.26		
Group III	46.82 $\pm$ 7.88	3.94		

* statistically significant; ^a significant difference vs. group I.

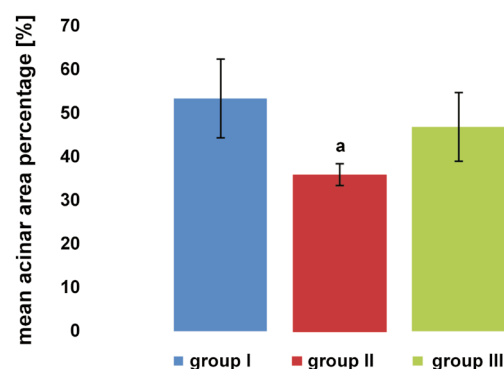


Fig. 4. Column chart comparing the mean acinar area percentage among all studied groups

The letter 'a' denotes a significant difference vs. group I.

percentage area was detected in group II, while the highest was recorded in group I. In addition, a statistically significant decrease in the acinar percentage area was observed in group II when compared to group I ($p = 0.017$). However, the acinar percentage area in group III was decreased as compared to group I and was

increased as compared to group II, although these results did not reach statistical significance ($p = 0.432$ and $p = 0.133$, respectively).

qRT-PCR results

Statistically significant differences were demonstrated between the studied groups regarding *iNOS*, *caspase-3* and α -SMA gene expression ($p < 0.001$, $p < 0.001$ and $p = 0.002$, respectively) (Table 3).

Table 3. Results of ANOVA for the expression of the studied genes [AU] relative to *GADPH* in all studied groups

Gene expression	Group I	Group II	Group III	<i>p</i> -value
<i>iNOS</i>	4.68 ± 0.12	5.49 ± 0.21 ^a	5.33 ± 0.2 ^a	<0.001*
<i>caspase-3</i>	4.31 ± 0.11	6.43 ± 0.41 ^a	4.58 ± 0.28 ^b	<0.001*
α -SMA	5.09 ± 0.14	4.75 ± 0.18	5.41 ± 0.21 ^b	0.002*

* statistically significant; ^a significant difference vs. group I; ^b significant difference vs. group II.

Regarding *iNOS* gene expression, a statistically significant increase was detected in both groups II and III as compared to group I ($p < 0.001$ and $p = 0.002$, respectively), while a decrease was detected in group III as compared to group II ($p = 0.412$). Conversely, *caspase-3* gene expression showed a statistically significant increase in group II as compared to group I ($p < 0.001$), while a non-significant increase was observed in group III as compared to group I ($p = 0.423$). However, *caspase-3* gene expression in group III was significantly decreased when compared to group II ($p < 0.001$). Finally, α -SMA gene expression was decreased in both groups II and III as compared to group I, although this did not reach statistical significance ($p = 0.056$ and $p = 0.071$, respectively), while a statistically significant increase in its expression was observed in group III when compared to group II ($p = 0.001$) (Fig. 5).

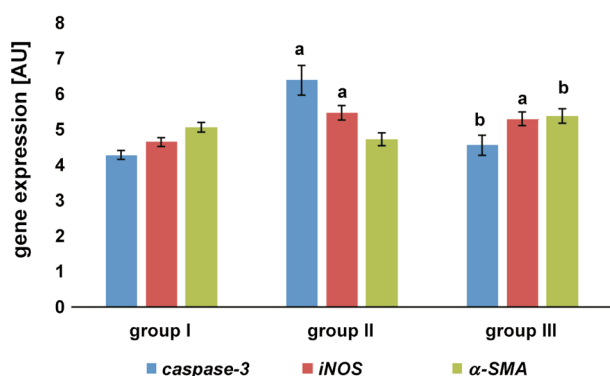


Fig. 5. Column chart comparing the mean expression of different genes among all studied groups

The letter 'a' denotes a significant difference vs. group I, whereas the letter 'b' denotes a significant difference vs. group II.

A significant positive correlation was observed between *caspase-3* and *iNOS* gene expression ($p = 0.046$; $r = 0.585$) (Table 4, Fig. 6). Conversely, the comparison between *caspase-3* and α -SMA gene expression revealed a significant inverse correlation ($p = 0.018$; $r = -0.666$) (Table 4, Fig. 7). Finally, no association was found between *iNOS* and α -SMA gene expression ($p = 0.721$; $r = -0.115$) (Table 4).

Table 4. Correlation between the expression of the studied genes

Gene expression	<i>iNOS</i>	<i>caspase-3</i>	α -SMA
<i>iNOS</i>	<i>r</i>	1	0.585
	<i>p</i> -value	–	0.046*
	<i>n</i>	12	12
<i>caspase-3</i>	<i>r</i>	0.585	1
	<i>p</i> -value	0.046*	–
	<i>n</i>	12	12
α -SMA	<i>r</i>	–0.115	–0.666
	<i>p</i> -value	0.721	0.018*
	<i>n</i>	12	12

* correlation is significant at the level of $p < 0.05$ (2-tailed).

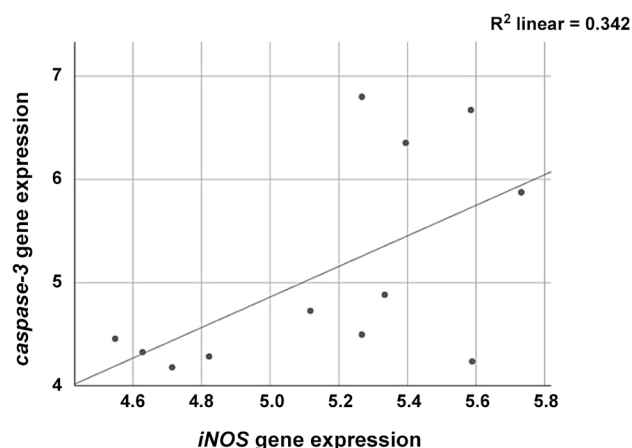


Fig. 6. Scatter chart showing a significant positive correlation between *caspase-3* and *iNOS* gene expression ($p < 0.046$; $r < 0.585$)

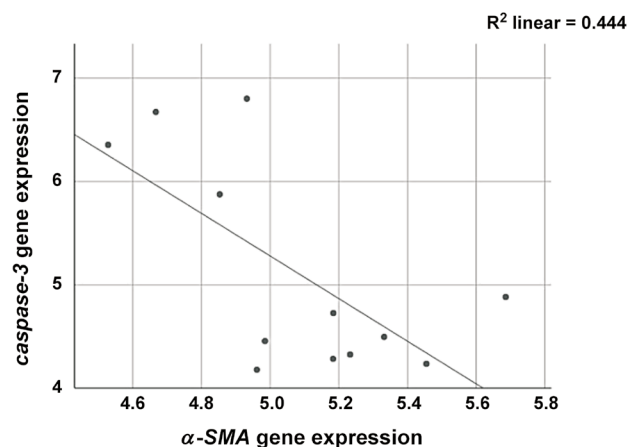


Fig. 7. Scatter chart showing a significant inverse correlation between *caspase-3* and α -SMA gene expression ($p < 0.018$; $r < -0.666$)

Discussion

Several medications can cause xerostomia as a common side effect, including β blockers, antipsychotics, antidepressants, and antihistamines.² Older generations of H1-antihistamines penetrate the blood–brain barrier, thus causing sedation, while second-generation H1-antihistaminic drugs are considered safer, resulting in fewer side effects.⁴

In the literature, cetirizine has been authorized as a potent second-generation H1-antihistamine and a first-choice antihistamine for treating allergic diseases. Cetirizine is normally used with daily dosing, as it is documented to be safe and well-tolerated.¹¹ On the other hand, promethazine is a first-generation H1-antihistamine that possesses anticholinergic properties causing xerostomia and other complications, such as blurred vision, dry nasal passages, dilated pupils, constipation, and urinary retention, and the American Geriatrics Society (AGS) categorizes it as a potentially inappropriate drug for the elderly.¹²

While the adverse effect of antihistamines on salivary secretion has been reported clinically, the underlying cellular mechanism remains unclear. Therefore, the current work investigated the effects of long-term administration of a first-generation H1-antihistamine, promethazine hydrochloride, as compared to second-generation cetirizine dihydrochloride, on the rat's parotid glands to clarify the underlying mechanism at the cellular and molecular levels.

The three-week duration of this study was selected to investigate the effects of long-term administration of both drugs. It has been previously established that in adult rats, every day of the animal's life is approx. equivalent to 34.8 human days (i.e., 1 rat month is comparable to 3 human years).¹³

Following the administration of antihistamines, the following changes were observed in the rat parotid glands: shrinkage and degeneration of serous acini; intracellular vacuolization in acini and the duct system; congested blood vessels; and increased inflammatory cell infiltration. These changes were more prominent following treatment with promethazine hydrochloride (group II) when compared to the cetirizine dihydrochloride-treated group (group III). In addition, histomorphometric results showed that the acinar percentage area was the lowest in group II.

Coinciding with the present findings, similar age-related degenerative changes have been observed in human labial salivary glands, as demonstrated by the presence of acinar atrophy and fibrosis, together with a diffuse inflammatory cell infiltrate.¹⁴ In addition, Tatar et al. demonstrated acinar and ductal vacuolization in the rat's submandibular salivary glands following the administration of an antihistamine, namely desloratadine.¹⁵ More recently, Mohammed et al. histologically evaluated the rat's parotid salivary gland tissues following treatment with desloratadine.¹⁶ The authors demonstrated remark-

able cytoplasmic vacuolization and the atrophy of serous acini, as well as increased interstitial spaces between parenchymal elements and mononuclear cell infiltrations.¹⁶ The existence of vacuolization and consequent alteration in the content of secretory granules is associated with diminished salivary secretion.¹⁷ Similarly, Choi et al. reported diminished salivary flow rates in association with atrophy and vacuolization in the salivary glands.¹⁸

The demonstrated histological changes following the administration of antihistamines could be attributed to the induction of oxidative stress. This assumption was based on previous findings by Tatar et al., who linked the effect of an antihistamine, desloratadine, with the induced mitochondrial oxidative stress and the resultant intra-mitochondrial reactive oxygen species (ROS),¹⁵ thus bringing an equilibrium between oxidants and antioxidants.¹⁹ Reactive oxygen species have various physiological roles and are normally created as by-products resulting from oxygen metabolism.²⁰ The nitric oxide synthase (NOS) family is significantly important in different physiological and pathological processes. Three isoforms for NOSs have been recognized, namely endothelial, inducible and neuronal NOSs (eNOS, iNOS and nNOS, respectively).²¹ Generally, eNOS and nNOS are constantly generated at low concentrations by relevant cells; however, iNOS, the inducible type, has an important role in immunity and inflammation. This isoform is enhanced in inflammation, leading to exacerbated inflammatory effects.²² In the present study, *iNOS* gene expression was significantly increased in both antihistamine groups as compared to the control group. Moreover, this expression was non-significantly decreased in group III when compared to group II. These results support the histological alterations observed in the antihistamine-treated groups (groups II and III), which were more obvious in group II rather than in group III.

Excess cellular ROS could damage proteins, lipids, nucleic acids, and membranes, eventually activating cell death processes, such as apoptosis. Apoptosis is a tightly controlled process necessary for the survival of living organisms.²³ Apoptotic caspases include upstream initiators (caspases 2, 8, 9, and 10) and downstream effectors (caspases 3, 6 and 7).²⁴ Herein, the gene expression of *caspase-3* was significantly increased in the promethazine hydrochloride group (group II) as compared to the untreated control, while it was non-significantly increased in the cetirizine dihydrochloride group (group III) as compared to the control group. This could be related to the observations made by Olanas et al., who investigated the effects of an acetylcholine receptor agonist, carbachol, on the interferon- β -induced apoptosis of a human neuroblastoma cell line.²⁵ The authors found that carbachol inhibited mitochondrial cytochrome c release, the activation of caspases 3, 7 and 9, as well as DNA destruction. They added that this antiapoptotic effect of carbachol was mediated through M3 muscarinic receptors.²⁵

Based on these findings, it could be deduced that muscarinic receptor antagonists, like antihistamines, may be involved in enhancing apoptosis.

Our findings further support several recent in vitro and clinical studies confirming the pro-apoptotic and antitumor properties of antihistamines, with regard to both first- and second-generation drugs. For example, a first-generation antihistamine, cyproheptadine, caused a dose-dependent elevation in apoptosis in the C6 glioblastoma cell line, suggesting a potential anticancer effect of this drug.²⁶ In addition, second-generation drugs, clemastine and desloratadine, induced apoptosis in cutaneous T cell lymphoma cell lines.²⁷ In the present investigation, *caspase-3* gene expression was significantly decreased in group III when compared to group II. Thus, it could be deduced that promethazine hydrochloride exerted a more pronounced apoptotic effect than cetirizine dihydrochloride, which may be related to enhanced *iNOS* gene expression in the promethazine hydrochloride-treated group. This assumption was based on the correlation results of the current study, which revealed a significant positive correlation between the gene expression of *iNOS* and *caspase-3*.

This positive correlation could be attributed to the association between antihistamine-induced apoptosis and enhanced oxidative stress. For example, Trybus et al. demonstrated that azelastine, a second-generation antihistamine, induced a significant concentration-dependent increase in cytoplasmic vacuoles, an increase in ROS, increased oxidative stress, and DNA damage, and obviously promoted apoptosis in human cervical adenocarcinoma cells through the stimulation of caspases 3 and 7, and the inhibition of Bcl-2 protein.²⁸ The authors suggested the contribution of ROS in causing DNA damage, which may, in turn, trigger apoptosis.²⁸

The muscarinic M3 receptor was assumed to be related to smooth muscle mass development, as M3 receptor-deficient mice displayed lower levels of alpha smooth muscle actin in airway arteries and bronchial tissues.²⁹ In normal salivary glands, α -SMA is expressed by the periacinar myoepithelial cells, in salivary ducts and normal blood vessels.³⁰ Herein, we revealed a non-significant decrease in α -SMA gene expression in the antihistamine groups II and III as compared to the control group. However, the regulatory effect of cetirizine dihydrochloride on α -SMA expression was significantly less than that of promethazine hydrochloride. These findings could be attributed to antagonizing the action of histamine, which is essential for α -SMA expression. The importance of histamine in α -SMA expression was demonstrated by Tibbo et al., who evidenced a significant reduction in α -SMA at both the protein and gene expression levels, following the treatment of a rabbit model of arthrofibrosis with ketotifen, a second-generation antihistamine, when compared to the untreated control group.³¹

In our study, the correlation analysis of *caspase-3* and α -SMA gene expression revealed a significant inverse relationship. This finding supports the demonstrated pro-apoptotic role of antihistamines in the present work, and the decreased α -SMA expression could result from the apoptosis of myoepithelial cells. Additionally, since the M3 receptor has been suggested to be critical in smooth muscle mass development³⁰ and consequent α -SMA expression, antihistamines as potential antimuscarinics acting on M3 receptors could result in decreased α -SMA expression. Since myoepithelial cells are essential for conveying saliva from the acini and supporting the parenchyma during secretion, any alterations in these cells could lead to defective salivary secretion, eventually causing xerostomia.

Conversely, we found no association between α -SMA and *iNOS* gene expression in the current work. On the contrary, Brennan et al. reported that a significant positive correlation existed between *iNOS* and α -SMA gene expression in pleomorphic adenoma cases.³⁰ However, this difference could be attributed to the difference in tissue types, as we investigated this correlation in normal and not cancerous salivary glands.

The more advanced degenerative effects in the first-generation (promethazine hydrochloride) group in comparison with the second-generation (cetirizine dihydrochloride) one in the present work agree with a previous study that compared both generations.³² That study demonstrated that older antihistamines resulted in prominent side effects in the central nervous system (CNS) in addition to anticholinergic effects, including xerostomia, while the newly developed antihistamines (H1 receptor-specific antagonists) were related to a significantly lower incidence of xerostomia.³² Similarly, Liu and Farley, demonstrated that second-generation antihistamines were more selective for histamine than first-generation drugs, with cetirizine having the highest potency toward histamine receptors.³³ Besides, cetirizine did not affect the concentration–response relationship for acetylcholine, suggesting a minimum or lack of interaction with M3 receptors. This could clarify why in our study, cetirizine dihydrochloride, as a second-generation antihistamine, exerted a less inhibitory effect on α -SMA gene expression than a first-generation antihistamine, promethazine hydrochloride. Despite the muscarinic-dependent xerostomic effect of antihistamines,³³ this effect could also be mediated through H1 receptors present in the salivary glands. This could explain why the new-generation antihistamines, with very low antimuscarinic effects, could still lead to xerostomia via their antagonistic effect on H1 receptors.¹²

Conclusions

We conclude that the administration of both first- and second-generation antihistamines induced several degenerative alterations in the rat parotid salivary glands,

which could be mediated through enhanced oxidative stress, with subsequent increased apoptosis and diminished α -SMA gene expression, eventually leading to xerostomia, a well-known adverse effect of antihistamines. However, these effects were more marked following the administration of the first-generation rather than the second-generation antihistamine. Despite the unfavorable risk–benefit profile of first-generation antihistamines, they continue to be overconsumed because of their over-the-counter status and long history of use. Additionally, the significant pro-apoptotic effect of first-generation H1 receptor antagonists is promising in treating cancer diseases, particularly salivary gland carcinomas. Within the limitation of the current study, it is suggested to monitor the evidence of the superior safety of new-generation antihistamines. Thus, third-generation antihistamines are recommended to be investigated in future studies, using a bigger sample size.

Ethics approval and consent to participate

The experiment was carried out according to the recommendations of the ethics committee on animal experimentation at the Institutional Animal Care and Use Committee (IACUC), Cairo University, Egypt. This research was done in compliance with the ARRIVE guidelines and regulations (<https://arriveguidelines.org>).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Dina Rady  <https://orcid.org/0000-0002-9672-6935>
 Marwa Magdy Abbass  <https://orcid.org/0000-0002-6455-7516>
 Heba Hakam  <https://orcid.org/0000-0002-2427-1695>
 Rasha Rady  <https://orcid.org/0000-0003-1155-6306>
 Iman Aboushady  <https://orcid.org/0000-0003-3284-1878>

References

- Kapourani A, Kontogiannopoulos KN, Manioudaki AE, et al. A review on xerostomia and its various management strategies: The role of advanced polymeric materials in the treatment approaches. *Polymers (Basel)*. 2022;14(5):850. doi:10.3390/polym14050850
- Wolff A, Joshi RK, Ekström J, et al. A guide to medications inducing salivary gland dysfunction, xerostomia, and subjective sialorrhea: A systematic review sponsored by the World Workshop on Oral Medicine VI. *Drugs R D*. 2017;17(1):1–28. doi:10.1007/s40268-016-0153-9
- Anil S, Vellappally S, Hashem M, Preethanath RS, Patil S, Samaranayake LP. Xerostomia in geriatric patients: A burgeoning global concern. *J Investig Clin Dent*. 2016;7(1):5–12. doi:10.1111/jicd.12120
- Church MK, Church DS. Pharmacology of antihistamines. *Indian J Dermatol*. 2013;58(3):219–224. doi:10.4103/0019-5154.110832
- Van Schoor J. Antihistamines: A brief review. *Prof Nurs Today*. 2012;16(5):16–21. <https://hdl.handle.net/10520/EJC127059>. Accessed September 4, 2022.
- Cantisani C, Ricci S, Grieco T, et al. Topical promethazine side effects: Our experience and review of the literature. *Biomed Res Int*. 2013;2013:151509. doi:10.1155/2013/151509
- Portnoy JM, Dinakar C. Review of cetirizine hydrochloride for the treatment of allergic disorders. *Expert Opin Pharmacother*. 2004;5(1):125–135. doi:10.1517/14656566.5.1.125
- Ellis AK, Day JH. Second-and third-generation antihistamines in the treatment of urticaria. *Dermatol Ther*. 2008. doi:10.1046/j.1529-8019.2000.00034.x
- Shahabooei M, Razavi SM, Minaiyan M, et al. A histomorphometric study of the effect of doxycycline and erythromycin on bone formation in dental alveolar socket of rat. *Adv Biomed Res*. 2015;4:71. doi:10.4103/2277-9175.153895
- Schober P, Boer C, Schwarte LA. Correlation coefficients: Appropriate use and interpretation. *Anesth Analg*. 2018;126(5):1763–1768. doi:10.1213/ANE.0000000000002864
- Corsico AG, Leonardi S, Licari A, et al. Focus on the cetirizine use in clinical practice: A reappraisal 30 years later. *Multidiscip Respir Med*. 2019;14:40. doi:10.1186/s40248-019-0203-6
- Kim JH, Park SH, Moon YW, et al. Histamine H1 receptor induces cytosolic calcium increase and aquaporin translocation in human salivary gland cells. *J Pharmacol Exp Ther*. 2009;330(2):403–412. doi:10.1124/jpet.109.153023
- Sengupta P. The laboratory rat: Relating its age with human's. *Int J Prev Med*. 2013;4(6):624–630. PMID:23930179. PMCID:PMC3733029.
- Sørensen CE, Larsen JO, Reibel J, et al. Associations between xerostomia, histopathological alterations, and autonomic innervation of labial salivary glands in men in late midlife. *Exp Gerontol*. 2014;57:211–217. doi:10.1016/j.exger.2014.06.004
- Tatar A, Parlak SN, Yayla M, Ugan RA, Polat E, Halici Z. Effects of allergic rhinitis and desloratadine on the submandibular gland in a rat allergy model. *Int Forum Allergy Rhinol*. 2015;5(12):1164–1169. doi:10.1002/alr.21589
- Mohammed IJ, Sarhat ER, Wade SA, Al-Shiakhani SM. Histological and biochemical evaluation of the effect of desloratadine drug in parotid gland tissues. *Anb Med J*. 2021;17(2):72–77. doi:10.33091/amj.2022.171074
- Şimşek G, Gürocak S, Karadağ N, et al. Protective effects of resveratrol on salivary gland damage induced by total body irradiation in rats. *Laryngoscope*. 2012;122(12):2743–2748. doi:10.1002/lary.23609
- Choi JS, Park IS, Kim SK, Lim JY, Kim YM. Analysis of age-related changes in the functional morphologies of salivary glands in mice. *Arch Oral Biol*. 2013;58(11):1635–1642. doi:10.1016/j.archoral-bio.2013.07.008
- Sarhat ER. Altered serum marker of thyroid profile and antioxidant enzymes in individuals with Alzheimer's disease. *Int Res J Pharm*. 2019;10(1):56–60. doi:10.7897/2230-8407.100110
- Pizzino G, Irrera N, Cucinotta M, et al. Oxidative stress: Harms and benefits for human health. *Oxid Med Cell Longev*. 2017;2017:8416763. doi:10.1155/2017/8416763
- Costa ED, Rezende BA, Cortes SF, Lemos VS. Neuronal nitric oxide synthase in vascular physiology and diseases. *Front Physiol*. 2016;7:206. doi:10.3389/fphys.2016.00206
- Papi S, Ahmadizar F, Hasanvand A. The role of nitric oxide in inflammation and oxidative stress. *Immunopathol Persa*. 2019;5(1):e08. doi:10.15171/ipp.2019.08
- Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim Biophys Acta*. 2016;1863(12):2977–2992. doi:10.1016/j.bbamcr.2016.09.012
- Vakifahmetoglu-Norberg H, Norberg E, Perdomo AB, et al. Caspase-2 promotes cytoskeleton protein degradation during apoptotic cell death. *Cell Death Dis*. 2013;4(12):e940. doi:10.1038/cddis.2013.463
- Olianas MC, Dedoni S, Onali P. Protection from interferon- β -induced neuronal apoptosis through stimulation of muscarinic acetylcholine receptors coupled to ERK1/2 activation. *Br J Pharmacol*. 2016;173(19):2910–2928. doi:10.1111/bph.13570

26. Kacar S, Hacıoglu C, Kar F, Sahinturk V, Kanbak G. Cyproheptadine causes apoptosis and decreases inflammation by disrupting thiol/disulfide balance and enhancing the levels of SIRT1 in C6 glioblastoma cells. *Toxicol In Vitro*. 2021;73:105135. doi:10.1016/j.tiv.2021.105135
27. Döbbeling U, Waeckerle-Men Y, Zabel F, Graf N, Kündig TM, Johansen P. The antihistamines clemastine and desloratadine inhibit STAT3 and c-Myc activities and induce apoptosis in cutaneous T-cell lymphoma cell lines. *Exp Dermatol*. 2013;22(2):119–124. doi:10.1111/exd.12086
28. Trybus E, Król T, Trybus W. The multidirectional effect of azelastine hydrochloride on cervical cancer cells. *Int J Mol Sci*. 2022;23(11):5890. doi:10.3390/ijms23115890
29. Kistemaker LE, Bos ST, Mudde WM, et al. Muscarinic M₃ receptors contribute to allergen-induced airway remodeling in mice. *Am J Respir Cell Mol Biol*. 2014;50(4):690–698. doi:10.1165/rcmb.2013-0220OC
30. Brennan PA, Umar T, Zaki GA, et al. Are myoepithelial cells responsible for the widespread expression of inducible nitric oxide synthase in pleomorphic adenoma? An immunohistochemical study. *J Oral Pathol Med*. 2000;29(6):279–283. doi:10.1034/j.1600-0714.2000.290607.x
31. Tibbo ME, Limberg AK, Salib CG, et al. Anti-fibrotic effects of the antihistamine ketotifen in a rabbit model of arthrofibrosis. *Bone Joint Res*. 2020;9(6):302–310. doi:10.1302/2046-3758.96.BJR-2019-0272.R2
32. Scully C, Bagan JV. Adverse drug reactions in the orofacial region. *Crit Rev Oral Biol Med*. 2004;15(4):221–239. doi:10.1177/154411130401500405
33. Liu H, Farley JM. Effects of first and second generation antihistamines on muscarinic induced mucus gland cell ion transport. *BMC Pharmacol*. 2005;5:8. doi:10.1186/1471-2210-5-8