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THE EFFECT OF THE ADMINISTRATION OF TWO DIFFERENT GENERATIONS OF ANTIHISTAMINICS ON LINGUAL PAPILLA AND TASTE BUDS OF RATS' TONGUES

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ABSTRACT

Background: Medications administered to geriatric patients can account for their taste dysfunction. First and second generation antihistamines have been claimed to cause changes in taste perception. Therefore, this study investigated the effect of first and second-generation antihistamines on the ultrastructure of taste buds and lingual papillae of rats' tongues.

Methodology: Twelve adult male albino rats were randomly distributed into three groups. Control group: rats received distilled water daily, Histoloc group: rats received 4.8 mg/kg promethazine hydrochloride in distilled water daily and Zyrtec group: rats received 3 mg/kg of Cetirizine dihydrochloride in distilled water daily. After three weeks, all rats were euthanized and tongues were dissected into two parts, two halves. Specimens from the right halves of the tongue were prepared for Hematoxylin & Eosin stain for histological and histomorphometric evaluation. While specimens from the left halves were used to measure caspase-3 and inducible nitric oxide synthase gene expression by qRT-PCR.

Results: Histological examination of Histoloc and Zyrtec groups revealed distortion of normal fungiform papilla morphology with marked areas of degeneration in the taste bud. Although caspase-3 and inducible nitric oxide synthase revealed a statistically significant increase in gene expression in Zyrtec and Histoloc groups as compared to the control, the difference between Zyrtec and Histoloc was not significant.

Conclusion: First and second-generation antihistamines resulted in various degenerative changes in rats' lingual papillae and taste buds. However, these effects were more pronounced with antihistamines of the first generation than those of the second.

KEYWORDS: Antihistamine, Taste buds, Taste alteration, Histaloc, Zyrtec.

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INTRODUCTION

Several drugs are associated with altered or unpleasant taste sensations which can negatively affect patients' compliance to treatment [1]. It has been reported that 25% of taste disorders in geriatric patients can be attributed to administered medications[2]. The altered taste sensation is commonly produced due to drugs induced changes within the taste transduction pathways, enzymes, and transporters [1]. Drug-associated taste alteration includes bitter or metallic tastes, ageusia, which is the total loss of taste, hypogeusia which is the decreased taste sensation, hypergeusia which refers to heightened sensitivity to taste, in addition to dysgeusia which is distorted taste sensation [3,4].

Several studies demonstrated an association between the intake of first and second-generation antihistaminics and altered taste sensation, where patients reported bitter sensation or altered taste perception associated with histamine intake [1, 5-8]. Histamine is a principal mediator, playing a major role in allergic diseases [9]. Histamine receptors are G protein-coupled receptors, including H1, H2, H3, and H4. Histamine functions mainly by interacting with H1 present in multiple organs [10]. Anti-allergy medications are usually administered for prolonged periods, and chronic use can result in systematic adverse effects [11].

The null hypothesis states that the administration of first and second-generation antihistaminics has no significant effect on the ultrastructure of the lingual papilla and taste buds of rats' tongues. Therefore, the current study was conducted to study the effect of a first-generation antihistaminics promethazine hydrochloride and a second-generation antihistaminics Cetirizine dihydrochloride on the ultrastructure of taste buds and fungiform lingual papillae of rats' tongues via histological and quantitative reverse transcription polymerase chain reaction (mRNA gene expression for inducible nitric oxide synthase (iNOS) and caspase-3).

MATERIALS AND METHODS

Drugs

1- Histaloc®: 25 mg of promethazine hydrochloride (H1 receptor antagonist of the first generation) (Julphar pharmaceutical companies, UAE and Gulf).

2- Zyrtec®: 10 mg of cetirizine dihydrochloride (H1 receptor antagonist of the second generation) (GlaxoSmithKline pharmaceutical company, Egypt).

Sample size calculation:

Based on the previous study [12], a total sample of 12 (4 per each group) rats was found sufficient to detect effect size of 2.5, a power of 0.8, a two-sided hypothesis test, and a significance level of 0.05. Calculation was achieved using PS: Power and Sample Size Calculation Software Version 3.1.2 (Vanderbilt University, Nashville, Tennessee, USA).

Animal study and design

This experiment was conducted in the Animal House of the Faculty of Medicine, Cairo University, Egypt, under the guidance and approval of the Institutional Animal Care & Use Committee of Cairo University (CU-IACUC), following the ARRIVE guidelines for in vivo animal research. In the animal house of the Faculty of Medicine at Cairo University, twelve adult male albino rats (Rattus norvegicus albinus, Wistar strain) weighing between 150 and 200 g were purchased and bred. The animals were kept in individual cages and had unlimited access to water and food. The animals were randomly assigned using the Random Sequence Generator program (random.org) into three groups (n=4 per group) based on the treatment used as follows:

Control group: Four rats received distilled water daily via oral gavage for three weeks.

Histoloc group: Four rats received 4.8 mg/kg promethazine hydrochloride in distilled water daily via oral gavage for three weeks.

Zyrtec group: Four rats received 3 mg/kg of Cetirizine dihydrochloride in distilled water daily via oral gavage for three weeks.

Animal sacrifice and tissue preparation

After three weeks, all rats were euthanized by an intra-cardiac overdose of sodium thiopental (80 mg/kg). Tongues were dissected into two parts, two halves. Specimens from the right halves of the tongue were prepared for Hematoxylin & Eosin stain for histological evaluation. While specimens from the left halves were used to measure caspase-3 and iNOS gene expression.

Investigation methods

Light Microscopic Examination

In the Oral Biology department, Faculty of Dentistry, Cairo University, samples were fixed in 10% buffered formalin for 48 hours, dehydrated in ethyl alcohol, cleared in xylol, and embedded in paraffin wax. Sections of about 4-6 um were cut, mounted on glass slides, stained with Haematoxylin and Eosin (H&E) stain then examined under the light microscope (Leica, Switzerland) under magnifications of x400.

Histomorphometric Analysis

Specimens were examined using light microscopy (Leica, Switzerland) under a magnification 400. The data were obtained using Leica Owen 500 image analyzer Computer system Leica (Imaging System Ltd., Cambridge, U.K.). Image J image analysis software (Image J 1.53d) was used to analyze the fungiform papilla width, length, and keratin thickness. For each criterion, four non-overlapping microscopic fields were randomly selected and evaluated.

Quantitative Real Time-PCR (qRT-PCR) Analysis

Following the manufacturer's instructions, a total RNA isolation kit (Qiagen, USA) was used to isolate total RNA from the obtained samples. RNA extracted from the specimens was reverse-transcribed using a cDNA Reverse Transcriptase reagent (Fermentas, USA) according to the protocol included in the kit. The employed Biosystem with software version 3.1 (Step OneTM, USA) was then utilized to amplify and analyze cDNA. Using the comparative CT method, relative mRNA gene expression was normalized relative to the mean critical threshold values of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Table 1 contains the primer sequences (ThermoFisher Scientific, USA) for the inducible nitric oxide synthase (iNOS), caspase-3, and GAPDH genes.

Statistical Analysis:

Data from both the histomorphometric analysis and RT-PCR were expressed as mean ± standard deviation. Normally distributed parametric data were assessed via one-way ANOVA test followed by Tukey's post hoc test for multiple pairwise comparisons in case of statistically significant results. P value <0.05 was considered statistically significant. statistical package (SPSS, version 15.0, Chicago, IL) for social sciences was used for statistical analysis.

TABLE (1) Primer's sequence of all studied genes.

Gene Symbol	Primer sequence (From 5' to 3')	
iNOS	F: 5′- GTTCCCCCAGCGGAGCGATG -3′	NM_012611.3
	R: 5′- ACTCGAGGCCACCCACCTCC -3′	
Caspase-3	F: 5′ CTGGACTGCGGTATTGAG -3′	NM_053304.1
	R: 5′- GGGTGCGGTAGAGTAAGC -3′	
GAPDH	F: 5′- CCATTCTTCCACCTTTGATGCT-3′	NM_017008.4
	R:5′-TGTTGCTGTAGCCATATTCATTGT-3′	

RESULTS

Histological findings

Light microscope examination of Histoloc and Zyrtec groups revealed distortion of normal fungiform papilla morphology. A single taste bud with marked areas of degeneration was evident in examined fungiform papillae. Epithelial covering fungiform papilla showed areas of hyperplasia and areas of increased keratin thickness. Connective tissue papilla revealed areas of degeneration. While control group showed normal fungiform papilla morphology with normal taste bud, even epithelial thickness covered with uniform thin keratin layer (figure 1).

Historphometric results

Papilla width, papilla length, and keratin thickness

A significant reduction in papilla width was recorded in Histoloc group as compared to the control group. While the difference between Zyrtec and control or between Histoloc and Zyrtec groups was insignificant. Similarly, a significant decrease in papilla length was also observed in both Histoloc and Zyrtec groups as compared to control group. The difference between Zyrtec and Histoloc was insignificant. Additionally, a significant increase in covering keratin thickness was observed in Histoloc group as compared to the control group. While the difference between Zyrtec and control or between Histoloc and Zyrtec groups was insignificant (tables 2).

PCR results

One way ANOVA test of Caspase-3 and iNOS gene expression detected a statistically significant difference between groups. Post-Hoc analysis revealed a statistically significant increase in Caspase-3 and iNOS gene expression in Zyrtec group as compared to the control group. Similarly, a higher gene expression was also detected in Histoloc group as compared to the control. On the other hand, the difference between Zyrtec and Histoloc was not significant (tables 2).

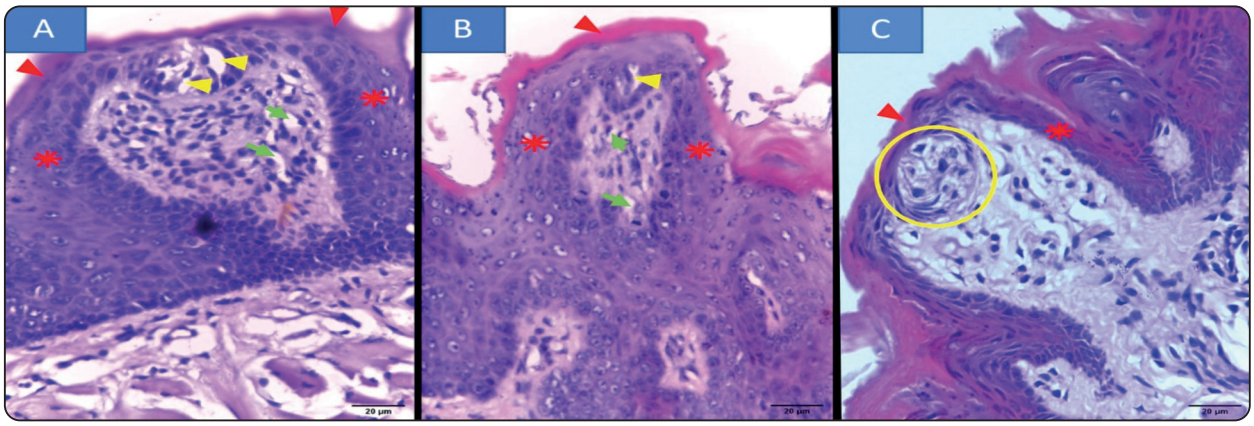

Fig. (1) light microscopic picture displaying, (A) Zyrtec group showing distortion of normal fungiform papilla morphology, areas of epithelial hyperplasia (asterisk), areas of connective tissue papilla degeneration (green arrows), areas of degeneration within the taste bud (yellow arrowheads), and areas of increased keratin thickness (red arrowheads). (B) Histoloc group showing distortion of normal fungiform papilla morphology, areas of epithelial hyperplasia (asterisk), areas of connective tissue papilla degeneration (green arrows), areas of degeneration within the taste bud (yellow arrowheads), and areas of increased keratin thickness (red arrowheads). (C) control group showing normal fungiform papilla morphology, epithelial thickness (asterisk), normal taste bud (yellow arrowheads), and uniform thin keratin layer (red arrowheads) (Scale bar 20μm).

TABLE (2) Descriptive statistics, results for ANOVA test and Tukey's post hoc test for papilla width, papilla length, the thickness of covering keratin, and gene expression of Caspase-3 and iNOS.

Parameter	Group	Mean	Std. Error	P
Papilla width	Control	45.135±1.451 ^{A}	0.726	
	Histoloc	22.88±9.53 ^{B}	4.76	0.007*
	Zyrtec	35.19±8.65 ^{AB}	4.32	
Papilla length	Control	56.68±4.04 ^{A}	2.02	
	Histoloc	28.06±6.55 ^{B}	3.27	0.000*
	Zyrtec	34.28±7.09 ^{B}	3.55	
Thickness of covering keratin	Control	1.2172±0.1878 ^{B}	0.0939	
	Histoloc	8.41±3.75 ^{A}	1.87	0.004*
	Zyrtec	5.379±0.936 ^{AB}	0.468	
Caspase-3	Control	2.476±0.539 ^{B}	0.270	
	Histoloc	6.080±0.624 ^{A}	0.312	0.000*
	Zyrtec	5.326±0.574 ^{A}	0.287	
iNOS	Control	3.587±0.226 ^{B}	0.113	
	Histoloc	5.359±0.365 ^{A}	0.183	0.000*
	Zyrtec	5.210±0.466 ^{A}	0.233	

Parameter	Difference of levels		95% Confidence Interval	Adjusted P-Value
Papilla width	**Histoloc**	**Control**	(-37.02, -7.49)	0.006*
	Zyrtec	**Control**	(-24.71, 4.81)	0.199
	Zyrtec	**Histoloc**	(-2.46, 27.07)	0.103
Papilla length	**Histoloc**	**Control**	(-40.55, -16.68)	0.000*
	Zyrtec	**Control**	(-34.32, -10.46)	0.001*
	Zyrtec	**Histoloc**	(-5.71, 18.16)	0.355
Thickness of covering keratin	**Histoloc**	**Control**	(2.79, 11.60)	0.004*
	Zyrtec	**Control**	(-0.25, 8.57)	0.064
	Zyrtec	**Histoloc**	(-7.44, 1.37)	0.188
Caspase-3	**Histoloc**	**Control**	(2.458, 4.749)	0.000*
	Zyrtec	**Control**	(1.704, 3.995)	0.000*
	Zyrtec	**Histoloc**	(-1.900, 0.392)	0.212
iNOS	**Histoloc**	**Control**	(1.049, 2.494)	0.000*
	Zyrtec	**Control**	(0.901, 2.346)	0.000*
	Zyrtec	**Histoloc**	(-0.871, 0.574)	0.838

Significance level P<0.05, *significant

Means with different superscript letters are significantly different.

DISCUSSION

Antihistaminics are a class of medications used to treat conditions mediated by histamine. Histaloc (Promethazine) is a first-generation H1 antihistaminics with anticholinergic properties, while Zyrtec (Cetirizine) is a second-generation H1 antihistaminics and first-choice antihistaminics for treating allergic diseases. Cetirizine is administered daily because its safety and tolerability have been established [13, 14]. Although their wide distribution, several research studies reported that patients who took first- or second-generation antihistaminics experienced a bitter taste or a change in their perception of flavors, both linked to antihistaminics usage[1,5-8]. Therefore, to clarify the underlying mechanism on cellular and molecular levels, the current study examined the effect of long-term administration of a first-generation H1 antihistaminics, promethazine hydrochloride, versus a second-generation antihistaminics, cetirizine dihydrochloride, on the lingual papilla and taste buds of rats' tongue.

The histological and histomorphometric Analysis of Histaloc and Zyrtec groups revealed distortion of normal morphological appearance, atrophy of the papilla, in addition to areas of hyperplasia and areas of increased keratin thickness. These alterations were more pronounced in the Histaloc group treated with promethazine hydrochloride than in the Zyrtec group treated with cetirizine dihydrochloride. This could be attributed to the fact that the ability of first-generation H1 antihistaminics to readily traverse the blood-brain barrier and enter the central nervous system is greater compared to second-generation H1 antihistaminics, which lack this capability. The drugs of the first generation exhibit binding affinity towards both central and peripheral histamine-1 receptors, whereas the drugs of the second generation demonstrate selective binding specifically to peripheral histamine-1 receptors. Consequently, this disparity in receptor binding results in distinct side effect profiles [15, 16].

Scully and Bagan (2004) assert that drugs exert their effects by either disrupting the chemical composition or flow of saliva or by altering the function of taste receptors or signal transduction mechanisms [17]. The adverse effect of antihistaminics drugs on salivary glands histology, with subsequent decrease in salivary flow was previously reported. Decreased salivary flow rate could help explain the degenerative changes in taste buds observed in the current study [18, 19].

Even though antihistaminics have potent anti-inflammatory [20, 21], and antioxidant properties [22], within the current study, chronic administration of antihistaminics Histoloc and Zyrtec was associated with a statistically significant increase in expression of Caspase-3 and iNOS as compared to the control group.

Reactive nitrogen species, produced by phagocytic cells, are important biological molecules in the inflammatory process[23]. Nitric oxide (NO) belongs to the reactive nitrogen species family, it is implicated in multiple biological processes, including microbiocidal and antitumor activities[24-26]. Additionally, NO is an essential regulatory molecule during cell differentiation and proliferation and can affect cell survival and apoptosis[27-29]. NO is produced in the presence of NO synthetase (NOS), neuronal NOS, endothelial NOS and inducible NOS (iNOS), through L-arginine pathway via multiple cells[27,30,31]. iNOS is produced by phagocytes following inflammatory stimulation by microorganisms or their by-products or by inflammatory cytokines[25,32], iNOS production results in increased local concentration of NO [26, 32]. Detection of iNOS protein expression is a reliable indicator of NO expression by cells [33].

Cellular response to NO depends on its concentration, duration of cell exposure to NO and cell type. At low concentrations NO was reported to inhibit cellular apoptosis, while in higher concentrations and upon chronic cellular exposure to NO, it can induce apoptosis by regulating several enzymes, including caspase proteases [34].

Apoptosis can be defined as the process of programmed cell death. The process of apoptosis involves complex cellular alterations including both cellular morphological and physiological changes. Apoptosis involves shrinkage of the cell, condensation of chromatin and cytoplasm and eventually results in cell fragmentation and formation of apoptotic bodies, which are eliminated via phagocytosis[35-37]. The process of apoptosis is initiated through the activation of caspase proteases[38,39]. Caspases are a group of cysteine proteases present in the cytoplasm in an inactive form called procaspases, their activation results in a cascade of intracellular signaling eventually resulting in apoptosis and cell death[34, 40]. Caspase-3 activation has been implicated in NO mediated cell apoptosis[34, 38, 39].

Through the current study, chronic administration of antihistaminics was associated with increased iNOS, and subsequently increased NO production, caspase-3 activation and cell apoptosis within fungiform papilla taste bud. Similar to findings reported in the current study, H1 receptor antagonists terfenadine and loratadine exerted a dose dependent inhibitory effect on primary neoplastic mast cells proliferation, in addition to increased cellular apoptosis via caspase-3 activation [41].

Similarly, promethazine, H1 receptor antagonists, showed a dose dependent inhibition of colorectal cancer cells proliferation associated with increased apoptosis and increased caspases-3 activation, through Inhibition of the PI3K/ AKT signaling pathway[42]. Cyproheptadine, first generation H1 receptor antagonist, was associated with dose dependent increase in cell apoptosis and increased caspase-3 activation in C6 glioblastoma cells [43]. H1 receptor antagonist diphenhydramine promoted caspase-2 dependent cellular apoptosis in human acute T-lymphocytic leukemia cells[44], and melanoma cell line[45]. Antihistaminics clemastine and desloratadine promoted cutaneous T-cell lymphomas cell line caspase-3 and caspase-7 dependent apoptosis[46]. Azelastine hydrochloride, phthalazinone derivative, demonstrated a dose dependent increase in ROS and caspase-3 and caspase-7 apoptosis in cervical cancer cell line[47]. The antihistaminics deptropine effectively reduced the proliferation of hepatoma cell line and induced caspase-3, caspase-8 and caspase-9 apoptosis[48]. Chronic administration of high dosages of antihistaminics Cetirizine and fexofenadine induced iNOS mRNA expression in aortas of mice models with atherosclerosis[49] .

Therefore, the damaging effect of chronic antihistaminics administration on the taste buds of fungiform papilla observed within the histological and histomorphometric analysis of the current study can be attributed to increased cellular apoptosis within taste bud cells as detected by increased iNOS and caspase-3 expression.

CONCLUSIONS

It can be concluded that the administration of first- and second-generation antihistaminics resulted in various degenerative changes in rats' lingual papillae and taste buds. These alterations may be attributed to increased oxidative stress, leading to an augmented occurrence of apoptosis. However, these effects were more pronounced with antihistaminics of the first generation than those of the second.

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