EFFECTS OF COMMON H1-ANTIHISTAMINES ON IL-6 AND RANKL EXPRESSION IN OSTEOBLASTS

By

Kriztine Mae U. Chun

Bachelor of Science – Chemistry
University of San Francisco
2014

Doctor of Dental Medicine
University of Nevada, Las Vegas
2021

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science – Oral Biology

School of Dental Medicine
The Graduate College

University of Nevada, Las Vegas
May 2024

UNLV | GRADUATE COLLEGE

Thesis Approval

The Graduate College
The University of Nevada, Las Vegas

March 8, 2024

This thesis prepared by

Kriztine Mae Chun

entitled

Effects of Common H1-Antihistamines on IL-6 and RANKL Expression in Osteoblasts

is approved in partial fulfillment of the requirements for the degree of

Master of Science – Oral Biology
School of Dental Medicine

Brian Chrzan, Ph.D.
Examination Committee Chair

Karl Kingsley, Ph.D.
Examination Committee Member

Charles Hill, DMD
Examination Committee Member

Courtney Coughenour, Ph.D.
Graduate College Faculty Representative

Alyssa Crittenden, Ph.D.
Vice Provost for Graduate Education & Dean of the Graduate College

Abstract

OBJECTIVE

H1-antihistamines are the treatment of choice for prevention and relief of symptoms associated with allergic rhinoconjunctivitis which affects 18.9% of children and 25.7% of adults in the United States. The purpose of this study is to evaluate the expression of RANKL, OPG and IL-6 in human osteoblasts, G-292 osteosarcoma cell line, following treatment with varying concentrations of histamine and common second-generation H1-antihistamines including fexofenadine (Allegra), cetirizine (Zyrtec), and loratadine (Claritin)

METHODS

In this in vitro study, G-292 cells were cultured in 6-well plates with McCoy's medium. The cells were treated with histamine at a concentration of 0.01, 0.1, 1 or 10 µM and then incubated for 24- or 6-hours prior to mRNA isolation. mRNA expression of HRH1, RANKL, OPG and IL-6 were measured using quantitative PCR. To compare the effects of antihistamine treatment on G-292 cells, cell cultures were treated with 1 µM of histamine and fexofenadine, cetirizine and loratadine were added at concentrations of 0.01, 0.1, or 1 µM. mRNA expression of RANKL and IL-6 were measured using quantitative PCR. Luminex Milliplex assay was completed on extracted media from the samples to evaluate protein expression of IL-6. Mann-Whitney U Test was completed to determine statistical significance (P value < 0.05).

RESULTS

Stimulation of G-292 cells with varying concentrations of histamine does not result in a statistically significant change in mRNA expression of HRH1, RANKL or OPG at both treatment times of 6- and 24-hours. The increased expression of IL-6 is statistically significant at higher concentrations of 10 μM after incubation at 24-hours and 1 and 10 μM at 6-hours. The results suggest that there is a positive correlation between histamine concentration and IL-6 expression. Treatment of G-292 cells with histamine and different types and concentrations of fexofenadine, cetirizine and loratadine did not result in statistically significant changes in RANKL mRNA expression. The results suggest that there is a negative correlation between antihistamine concentration and IL-6 mRNA expression for fexofenadine and cetirizine. At the concentration closest to C_{max} for each antihistamine, there is a statistically significant decrease in IL-6 expression for fexofenadine and cetirizine, but no change is observed for loratadine. The data from the Milliplex assay evaluating protein expression of IL-6 supports the qPCR results.

CONCLUSIONS

The results from this in vitro study suggest that loratadine may be a better option compared to fexofenadine and cetirizine with regards to maintaining the rate of orthodontic tooth movement since there were no significant changes in IL-6 expression noted between the treated cells and the positive control.

Acknowledgements

I would like to express my sincere gratitude to Dr. Brian Chrzan for his mentorship and guidance throughout my master's thesis. This research study is of personal interest to me and though I understood that starting a new project would be challenging, Dr. Chrzan has helped and supported me since day one. I would also like to thank Shelley Williams for her time and efforts, from making sure I had the supplies that I needed for my experiments to walking me through the lab procedures. Her kindness and patience are beyond measure, and I am grateful for the opportunity to have worked with her in the research lab. Lastly, I would like to thank my committee members, Dr. Karl Kingsley, Dr. Charles Hill, and Mr. Maxim Gakh for graciously taking the time to support me in this endeavor. Their words of advice and encouragement have been much appreciated throughout this project.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Among its many functions, histamine plays a significant role in immune modulation and allergic inflammation. In allergic conditions, exposure to antigens that do not normally cause an immune reaction result in an abnormal and hypersensitive immune response which includes the release of histamine. It has been proven that allergic diseases are associated with a significant socioeconomic burden and a decreased quality of life with consideration of absences from school or work, cost of drug therapies, decreased productivity and concentration, and learning disorders (Kuna et al, 2016). The prevalence of allergic conditions in both children and adults has been increasing over the years. According to reports from the National Center for Health Statistics in 2021, one in four (27.2%) children in the United States suffer from allergic conditions. Seasonal allergies including hay fever, allergic rhinitis and allergic conjunctivitis were present in 18.9% of children (Zablotsky et al., 2023). Nearly one in three adults (31.8%) over 18 years of age reported having at least one allergic condition with 25.7% reporting seasonal allergies (Ng & Boersma, 2023).

H1-antihistamines are the treatment of choice for prevention and relief of symptoms associated with seasonal and perennial allergic rhinoconjunctivitis such as sneezing, nasal and conjunctival itching, rhinorrhea, erythema, and nasal congestion (Simons & Simons, 2008). The first-generation of H1-antihistamines were non-selective agents and caused nervous, cardiovascular, urinary, and gastrointestinal adverse reactions. New compounds, termed second generation antihistamines, were developed which selectively targets H1-receptors, has low penetration of the central nervous system, and are well tolerated by patients (Kuna et al., 2016)

In addition to its role in immune, gastrointestinal, and nervous systems, previous studies have shown that histamine is also involved in bone metabolism. Studies have shown that histamine induces RANKL expression and osteoclastogenesis (Ikawa et al., 2007). The effects of RANKL in clinical orthodontics have been studied in animal models. Injection of RANKL during orthodontic tooth movement increases osteoclastogenesis and the rate of tooth movement by 130%. Conversely, local RANKL antibody injection reduces the rate by 70% (Jeon et al., 2021).

There have been several studies examining the effects of the H1-antihistamine cetirizine on orthodontic tooth movement, but the results have been inconsistent. Studies by Kriznar et al. (2008) and Meh et al. (2011) showed that cetirizine treatment decreases the rate of tooth movement, however, Sperl et al. (2020) concluded that cetirizine did not have any significant effects. Due to the limited number of experiments studying the effects of H1-antihistamines on orthodontic tooth movement and the conflicting results of the studies, more research is required to determine what, if any, recommendations should be made by the clinician. The purpose of this study is to evaluate the expression of RANKL, OPG and IL-6 in human osteoblasts following treatment with varying concentrations of histamine and common second-generation H1-antihistamines such as fexofenadine (Allegra), cetirizine (Zyrtec), and loratadine (Claritin).

Research Questions and Hypotheses

1. Do G-292 osteoblast cells express the H1-receptor?
    a. Null hypothesis (H_0): G-292 osteoblast cells do not express the H1-receptor.
    b. Alternative hypothesis (H_A): G-292 osteoblast cells express the H1-receptor.
2. Does histamine treatment alter the mRNA expression of RANKL, OPG and IL-6 in G-292 osteoblast cells?
    a. Null hypothesis (H_0): Histamine does not alter the mRNA expression of RANKL, OPG and IL-6 in G-292 osteoblast cells.
    b. Alternative hypothesis (H_A): Histamine alters the mRNA expression of RANKL, OPG, and IL-6 in G-292 osteoblast cells.
3. Does treatment with common H1-antihistamines alter the mRNA expression of RANKL in G-292 osteoblast cells?
    a. Null hypothesis (H_0): H1-antihistamines do not alter the mRNA expression of RANKL in G-292 osteoblast cells.
    b. Alternative hypothesis (H_A): H1-antihistamines alter the mRNA expression of RANKL in G-292 osteoblast cells.
4. Does treatment with common H1-antihistamines alter the mRNA and protein expression of IL-6 in G-292 osteoblast cells?
    a. Null hypothesis (H_0): H1-antihistamines do not alter the mRNA and protein expression of IL-6 in G-292 osteoblast cells.
    b. Alternative hypothesis (H_A): H1-antihistamines alter the mRNA and protein expression of IL-6 in G-292 osteoblast cells.

Chapter 2: Background and Literature Review

Histamine

Histamine is synthesized in the human body from L-histidine, one of the essential amino acids. This is facilitated by histidine decarboxylase (HDC), an enzyme expressed in multiple cells throughout the body including central nervous system neurons, gastric mucosa parietal cells, mast cells, and basophils (Simons & Simons, 2008). Histamine exerts its effects by binding to one of four specific G-protein coupled receptors. The H1-receptor (H1R) and H2-receptor (H2R) can be found throughout the body. The H3-receptor (H3R) is primarily expressed in the brain and the H4-receptor (H4R) on hematopoietic cells (Biosse-Duplan et al., 2009).

Histamine plays a major role in allergic inflammation and immune modulation by increasing cellular adhesion molecule expression and chemotaxis of eosinophils and neutrophils, increasing antigen-presenting cell capacity, stimulation of B-cells and increasing production of inflammatory cytokines. As a neurotransmitter, histamine contributes to energy and endocrine homeostasis, sleep-wake cycle, cognition, and memory. It also increases gastric acid secretion in the stomach by binding to H2R. Through the H1-receptor, histamine plays a role in hematopoiesis, cell proliferation and differentiation, embryonic development, regeneration, and wound healing. The effects of histamine are ubiquitous and include increased pruritis, pain, vascular permeability, vasodilation, tachycardia, bronchoconstriction, flushing, headache, mucus production and nasal congestion (Simons & Simons, 2008).

H1-Antihistamines

According to reports from the National Center for Health Statistics in 2021, the percentage of children with allergic conditions has increased over previous decades affecting over one-quarter (27.2%) of children in the United States. Seasonal allergies including hay fever, allergic rhinitis and allergic conjunctivitis were present in 18.9% of children. Eczema or atopic dermatitis affected 10.8% of children and food allergies were present in 5.8% (Zablotsky et al., 2023). Nearly one in three adults (31.8%) over 18 years of age reported having at least one allergic condition. One in four adults (25.7%) had a seasonal allergy, 7.3% had eczema and 6.2% had a food allergy (Ng & Boersma, 2023). In allergic conditions, exposure to antigens that do not normally cause an immune reaction in most people results in an abnormal and hypersensitive immune response. H1-antihistamines are the treatment of choice for the prevention and relief of symptoms associated with seasonal and perennial allergic rhinoconjunctivitis such as sneezing, nasal and conjunctival itching, rhinorrhea, erythema, and nasal congestion. It is also efficacious in both acute and chronic urticaria by decreasing itching and reducing the number, size and duration of wheals and flares. (Simons & Simons, 2008).

The H1-receptor (HRH1) is a G-protein coupled receptor with 7 transmembrane α-helical segments. Like other G-protein coupled receptors, it exists in an equilibrium between the active or “on” state and the inactive or “off” state and maintains a constitutive level of activity without the presence of a ligand. When histamine binds to the receptor, it stabilizes the active form and causes the equilibrium to swing towards the “on” state. H1-antihistamines are not structurally related to histamine and therefore do not antagonize its binding to the receptor. Instead, H1-antihistamines bind different sites on the receptor to stabilize it in the inactive form to swing

the equilibrium to the “off” state. Thus, H1-antihistamines are inverse agonists and the previously accepted term “H1-receptor antagonist” is no longer used (Church & Church, 2011).

The first-generation of H1-antihistamines were produced initially in 1937 by Staub Bovet. These were non-selective agents which also affected muscarinic, adrenergic and dopaminic receptors leading to nervous, cardiovascular, urinary, and gastrointestinal adverse reactions. Due to its lipophilicity and ability to cross the blood-brain barrier, the adverse effects to the central nervous system such as drowsiness, decreased concentration and reduced ability to learn were significant. Some examples of first-generation antihistamines include diphenhydramine, doxylamine, promethazine and chlorpheniramine. In 1988, new antihistamine compounds were developed which selectively affected H1-receptors and had low penetration of the central nervous system. These drugs also have a wide therapeutic index which decreased the risk of overdose toxicity in comparison to the first-generation antihistamines. These second-generation antihistamines are now recommended as the drug of choice for allergic rhinoconjunctivitis and urticaria (Kuna et al., 2016). Some of the most common second-generation H1-antihistamines available for purchase in the United States include fexofenadine (*Allegra*), cetirizine (*Zyrtec*), and loratadine (*Claritin*). These over-the-counter (OTC) drugs are sold under both the brand name and generic form and will be the antihistamines used in this study.

Fexofenadine hydrochloride is sold under the brand name *Allegra* as 60 mg tablets taken every 12 hours or 180 mg tablets taken every 24 hours. It does not cross the blood-brain barrier and therefore exhibits minimal CNS effects. The mean maximum plasma concentration (C_{max}) following a single dose oral administration of a 60 mg, 120 mg, and 180 mg tablet were 142, 289

and 494 ng/mL respectively. The time to maximum plasma concentration (T_{max}) was 1 hour following oral administration. The primary elimination pathways of fexofenadine are biliary and renal, and care must be observed for use in those exhibiting impairment of those functions. Drug interactions with ketoconazole and erythromycin have been found with these drugs increasing C_{max} and extending T_{max}, however, no adverse effects have been reported. It is not recommended that fexofenadine be taken with antacids as a significant reduction in fexofenadine bioavailability is observed (Sanofi Consumer Health Inc., 2019).

Cetirizine hydrochloride is the active component in *Zyrtec* products. It is sold in tablet form at 5 or 10 mg strengths or in syrup form at a concentration of 1 mg/mL. Cetirizine administered at 10 mg doses exhibited a C_{max} of 311 ng/mL and T_{max} of 1 hour. Cetirizine is minimally metabolized in the body and is eliminated unchanged primarily in urine. Dosing adjustment may be necessary in patients with renal and hepatic impairment. A decrease in clearance of cetirizine is observed with concomitant theophylline administration. Though it is a second-generation H1-antihistamine, somnolence has been reported in patients using cetirizine and caution is recommended for activities requiring mental alertness. (Pfizer Labs)

Loratadine is the active ingredient in *Claritin* products. Like cetirizine, it is also available in tablet form at 5 or 10 mg strengths or in syrup form at a concentration of 1 mg/mL. After administration of the 10 mg dose, the C_{max} was 4.7 ng/mL and T_{max} of 1.5 hours. Loratadine is metabolized in the liver into descarboethoxyloratadine. Dosage adjustments are recommended for patients with hepatic disease but are not required for patients with renal disease. In a double-blind study, loratadine does not appear to impair driving and psychomotor performance either alone or in combination with alcohol (Bayer Inc., 2020).

Table 1: Peak Serum Concentration of H1-Antihistamines

	Dosage for 24-hour relief	C_{max} in plasma (ng/mL)	Molecular Weight	C_{max} in plasma (μM)
Fexofenadine (*Allegra*)	180 mg [a]	494 [a]	501.68	0.985
Cetirizine (*Zyrtec*)	10 mg [b]	311 [b]	388.89	0.800
Loratadine (*Claritin*)	10 mg [c]	4.7 [c]	382.88	0.012

[a]Sanofi Consumer Health Inc. (2019), [b]Pfizer Labs (n.d.), [c]Bayer Inc. (2020)

Bone Remodeling

Bone remodeling is a continuous process of bone resorption by osteoclasts and deposition by osteoblasts to maintain an optimum bone structure adapted to metabolic and mechanical demands. The three vital functions of bone include support and sites of attachment of muscles, protection of vital organs, and storage of calcium and phosphate for metabolism. A disruption in the equilibrium of resorption and deposition leads to disease. Excessive resorption causes a decrease of bone density and is found in conditions such as osteoporosis, Paget's bone disease, arthritis, and periodontitis. Excessive deposition can lead to osteopetrosis wherein bone is abnormally compact and brittle. This delicate balance is tightly controlled by coordinated signaling mechanisms of both local and systemic factors (Feng & McDonald, 2011).

Bone remodeling occurs in a functional structure known as the basic multicellular unit which includes bone-lining cells, osteocytes, osteoclasts, and osteoblasts. The remodeling process occurs in four phases: initiation of bone remodeling, bone resorption by osteoclasts, osteoblast differentiation and osteoid deposition, and mineralization of osteoid. Osteoblasts are derived from mesenchymal stem cells through a multi-step differentiation pathway. Osteoclasts are multinucleated cells that differentiate from the monocyte and macrophage line of cells. The differentiation of these osteoclast precursors occurs upon stimulation by macrophage colony-

stimulating factor (M-CSF) and the receptor activator of nuclear factor κB ligand (RANKL). Studies have shown that osteoclast differentiation and activity are regulated by cytokines such as interleukin (IL) -1, IL-6, IL-7, and tumor necrosis factor (TNF) (Feng & McDonald, 2011).

In the late 1990s, the discovery of the RANKL/RANK/OPG (osteoprotegrin) system has fueled more studies into understanding its role in bone biology and metabolism. RANKL binds to its receptor RANK (receptor activator of nuclear factor κB) which is a member of the TNF receptor superfamily. Cells of the osteoblast lineage, which includes bone-lining cells, stromal cells, osteoprogenitors and osteoblasts, express M-CSF and both membrane-bound RANKL and soluble RANKL. The receptors for M-CSF and RANKL are expressed on osteoclast precursors and stimulate its differentiation. In mature osteoclasts, RANKL stimulates its activation and survival. Osteoprotegrin (OPG), a soluble decoy receptor that is also produced by cells of the osteoblast lineage, antagonizes RANKL function by competing with RANK for binding of RANKL. (Feng & McDonald, 2011).

IL-6 is an inflammatory cytokine that plays a role in bone metabolism. It is produced by immune cells, fibroblasts, tumor cells and osteoblasts. Several of its functions include activation of cytotoxic T-cell generation, induction of acute phase proteins in hepatocytes, induction of differentiation in nerve cells, growth regulation of fibroblasts and as a multi-CSF in hematopoietic stem cells. IL-6 primarily exerts a stimulatory effect on osteoclastogenesis by enhancing the expression of RANKL (Feng & McDonald, 2011).

In addition to its role in immune, gastrointestinal, and nervous systems, previous studies have shown that histamine is also involved in bone metabolism. In the study by Ikawa et al. (2007), histamine was shown to induce osteoclastogenesis in mouse bone marrow culture

(MBMC) in a dose dependent manner. It was also found that histamine induces RANKL expression in both MBMC and MC3T3-E1 osteoblast-like cells. They studied the effects of mepyramine (H1R antagonist) and cimetidine (H2R antagonist) and the results showed that mepyramine inhibited histamine-induced RANKL expression in MC3T3-E1 cells. The authors concluded that drugs which affect histamine levels have some effects on osteoclastogenesis. Further in-vitro and in-vivo studies by Biosse-Duplan et al. (2009) supported the idea that histamine promotes osteoclastogenesis and inhibition was observed with antihistamine treatment.

Fitzpatrick et al. (2003) studied the effects of histamine on bone remodeling and vitamin D metabolism in histidine decarboxylase (HDC) deficient mice. The study established that histamine deficiency increases bone formation and is consistent with other studies that propose bone-protective effects of antihistamines in ovariectomized mice. They hypothesized that people with allergies and illnesses with increased histamine synthesis have increased susceptibility for bone loss. The authors propose that antihistamines or HDC inhibitors can be an accessible treatment option for osteoporosis.

Orthodontic Tooth Movement

In the field of orthodontics, clinicians correct malocclusions and improve dental alignment by applying gentle continuous pressure using appliances such as braces and aligners to move teeth within the alveolar bone. Remodeling of bone around the tooth requires the coordinated action of different cell types including periodontal ligament (PDL) fibroblasts, mesenchymal stem cells, inflammatory cells, osteoblasts, osteocytes, and osteoclasts. During

orthodontic tooth movement (OTM), bone resorption occurs on the compression side and bone deposition on the tension side. On mechanical force loading, the cells sense either compression or tension forces and release multiple cytokines and growth factors which subsequently stimulate a biological response. On the tension side, the PDL is stretched and blood flow is increased, stimulating osteoblastic activity. On the compression side, OTM can be divided into three stages: gradual compression of PDL, hyalinization period characterized by cell death due to the lack of blood flow, and the secondary period characterized by direct bone resorption and tooth movement. The generation of pro-inflammatory cytokines such as M-CSF, IL-1β, IL-6 and TNF-α in the compression side results in a sterile inflammatory response. These cytokines induce osteoclastogenesis by the up regulation of RANKL resulting in bone resorption (Jeon et al., 2021).

The effects of RANKL in clinical orthodontics have been studied in animal models. Injection of RANKL during orthodontic tooth movement increases osteoclastogenesis and the rate of tooth movement by 130%. Other studies have also shown that local RANKL gene transfer in animals accelerates the amount of tooth movement. Conversely, local RANKL antibody injection reduces the rate by 70%. Experimental compressive forces on the periodontal ligament increase RANKL expression 16.7-fold. Taken together, these studies show that RANKL plays a significant role in orthodontic tooth movement (Jeon et al., 2021).

Due to the increasing rate of medication use in the general population and the increasing demand for orthodontic treatment, it is beneficial to understand how medications may affect orthodontic tooth movement. There have been several studies examining the effects of the H1-antihistamine cetirizine, but the results have been inconsistent. In 2008, Kriznar et al.

determined that cetirizine reduced tooth movement in the first stage of OTM in rats, but no significant change is noted in the later stages. The decreased tooth movement on the 7th day coincides with the phase of acute inflammation of the paradental tissues. In the study by Meh et al. (2011), they concluded that cetirizine decreased the amount of tooth movement from day 28 onward which is considered the late stage of OTM. Cetirizine influences bone remodeling primarily through inhibition of bone resorption.

According to Sperl et al. (2020), the previous studies used a higher dose than the clinically applicable dosage. This study investigated both the clinically used human dosage adjusted to rat metabolism of 0.87 mg/kg and the high dose of 3 mg/kg from the previous study. They concluded that there are no significant effects on the rate of orthodontic tooth movement, cranial growth, root resorption or periodontal bone loss and that taking cetirizine at clinically relevant dosages should not have any undesirable effects. Due to the limited number of experiments studying the effects of H1-antihistamines on orthodontic tooth movement and the conflicting results of the studies, more research is required to determine what, if any, recommendations should be made by the clinician. These studies may involve comparison of different types of H1-antihistamines, use of different cell types and cultures, comparison of expression at different time points or randomized controlled trials examining the rate of tooth movement. The purpose of this study is to evaluate the expression of RANKL, OPG and IL-6 in human osteoblasts following treatment with varying concentrations of histamine and common second-generation H1-antihistamines such as fexofenadine (Allegra), cetirizine (Zyrtec), and loratadine (Claritin).

Chapter 3: Materials and Methods

H1-Antihistamines

Histamine and H1-antihistamines were obtained from Thermo Fisher Scientific (Waltham, MA). Histamine was dissolved in ultrapure dH_2O (Invitrogen, Carlsbad, CA) to generate an initial stock of 1 M. Serial dilutions of histamine were completed with H_2O to generate working stocks of 1 mM and 10 µM. Fexofenadine hydrochloride, cetirizine dihydrochloride and loratadine were dissolved in DMSO to generate an initial stock solution of 10 mM. Serial dilutions of H1-antihistamines with PBS (Invitrogen, Carlsbad, CA) were completed to generate working stocks of 100 µM and 10 µM. A 1% dilution of DMSO was also formulated using 10 µL of DMSO and 990 µL of PBS to use in the negative control. The resulting final concentration of DMSO was a maximum of 0.01% in all experiments.

Cell Culture

Human osteosarcoma cells, G-292 clone A141B1 (ATCC CRL-1423), were obtained from the American Type Culture Collection (Manassas, VA). G-292 cells were cultured in McCoy's 5A Medium (Life Technologies, Carlsbad, CA) modified with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO2 in 95% air. Fetal bovine serum, penicillin and streptomycin were obtained from Hyclone Laboratories, Inc. (Logan, UT). Cells were cultured T-25 flasks with 5 mL of growth medium to 70-80% confluency. Each T-25 flask was split into a 6-well plate and a new T-25 flask by removing the media, adding 1 mL of 0.25% Trypsin-EDTA (Life Technologies, Carlsbad, CA) then adding 22 mL of media. Cells were seeded at a density of $0.3x10^6$ in 6-well plates with 3 mL of growth

medium. Media was changed every 1-2 days. All plates were examined using light microscopy to confirm adequate confluency (80-90%) before beginning histamine and H1-antihistamine treatment.

Cell Treatment

To evaluate G-292 cell response to histamine treatment, the cells were treated with histamine at a concentration of 0.01, 0.1, 1 or 10 µM. Histamine was not added to the control. Media in each well was replaced with the treatment media prior to a 6- or 24-hour incubation. To compare G-292 cell response to different H1-antihistamines, cells were treated with 1 µM of histamine and either fexofenadine, cetirizine or loratadine were added at concentrations of 0.01, 0.1, or 1 µM. Negative and positive controls were formulated by adding 100 µL of 1% DMSO to 10 mL of media to a final concentration of 0.01% DMSO. Histamine was added to the positive control sample. All values are reported with respect to the negative control.

mRNA Isolation

Following the 6- or 24-hour incubation, total mRNA was isolated using TRIzol Reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. Assessment of mRNA quantity and quality was confirmed by spectrophotometry at an absorbance of 260 and 280 using a NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). mRNA samples were stored in a -60°C freezer until cDNA preparation is initiated.

cDNA Preparation

Reverse transcription of total mRNA to single-stranded cDNA suitable for quantitative PCR was completed with the High-Capacity cDNA Reverse Transcription Kit following the manufacturer's instructions (Applied Biosystems-Thermo Fisher Scientific, Wilmington, DE, USA). The kit included 10x RT buffer, 10x random primers, 25x dNTP mix and reverse transcriptase enzyme that were combined with 2 µg of total mRNA to complete the reverse transcription reaction at 37°C in a thermal cycler. cDNA samples were stored in a -20°C freezer until qPCR was performed.

Real-Time Quantitative Polymerase Chain Reaction (qPCR)

The relative gene expression of RANKL, OPG, HRH1 (H1-receptor) and IL-6 were determined using qPCR with TaqMan Fast Advanced Master Mix and following manufacturer instructions. All qPCR reagents and primers were obtained from Applied Biosystems. The protocol included the following reaction mixture prepared in triplicate for each treatment: 10 µL of buffer, 1 µL GAPDH primer, 1 µL of primer for the gene of interest, 6 µl of nuclease-free Milli-Q water and 2 µl of cDNA template to a final volume of 20 µl. GAPDH was used as the endogenous control for each experiment. The qPCR was performed with the QuantStudio 3 Real-Time PCR thermocycler (Applied Biosystems-Thermo Fisher Scientific, Wilmington, DE, USA) following the thermal protocol and fast cycling mode with the following steps: enzyme activation at 95°C for 2 minutes followed by 40 cycles of denaturation at 95°C for 5 seconds and annealing-extension at 60°C for 30 seconds.

Table 2: qPCR Primers[a]

Primer	Assay ID	Label or Dye
GAPDH	Hs99999905_m1	VIC
TNFSF11 (RANKL)	Hs00243522_m1	FAM
TNFRSF11B (OPG)	Hs00900358_m1	FAM
HRH1	Hs00185542_m1	FAM
IL-6	Hs00174131_m1	FAM

[a]*Thermo Fisher Scientific (n.d.)*

The comparative (ΔΔCt) method was used for data analysis. The difference between the Ct values (ΔCt) of RANKL, osteoprotegrin (OPG), HRH1 (H1-receptor) and interleukin-6 (IL-6) with GAPDH were calculated for each mRNA sample. The difference in the ΔCt values between the treated and control mRNA samples (ΔΔCt) was then calculated, providing the fold-change in expression of each gene of interest in the samples. Samples from three independent experiments were used to provide mean fold-change ± standard error (SE).

Milliplex Assay (IL-6)

IL-6 levels in the cell culture media samples were analyzed with a MILLIPLEX MAP Kit - Human Circulating Cancer Biomarker Magnetic Bead Panel 1 (Millipore, Billercia, MA) according to manufacturer's instructions. Prior to mRNA isolation with Trizol, media from the wells were removed and transferred into a 15-mL Falcon tube. The tubes were centrifuged at 2,000 RPM for 10 minutes and 500 mL of the supernatant was transferred into microfuge tubes and stored at -20 °C. To prepare the samples for the multiplex assay, cell culture media samples were thawed on ice, vortexed for 30 seconds then placed in the centrifuge at 16,000 rpm for 4 minutes. Samples were diluted to a 1:2 ratio using McCoy's media before adding them to the plate. The sample plate was analyzed on a Luminex 200 (Luminex Corp., Austin, TX). Raw data

was exported from the Luminex 200 and standard curves and unknowns were analyzed using xPONENT software (Luminex Corp., Austin, TX, Version 4.2) with results expressed as pg/mL of IL-6 in media.

Statistical Analysis

All results are expressed as the mean ± standard error (SE) of three independent experiments with each treatment condition completed in triplicate. The data was analyzed using the Mann-Whitney U non-parametric test to accept or deny the null hypothesis that two populations are equal. All statistical analyses were performed using Microsoft Excel Version 2401. Results were considered significantly different when P-value < 0.05.

Chapter 4: Results

The cells were treated with histamine concentrations of 0, 0.01, 0.1, 1, and 10 µM and incubated for 24 hours prior to mRNA isolation. The relative mRNA expression of RANKL, OPG, HRH1 and IL-6 were determined by quantitative PCR (Figure 1). The results confirm that the H1-receptor is expressed in G-292 osteoblast cells. No significant difference in mRNA expression was observed for RANKL, OPG and HRH1 at different histamine concentrations. There was a statistically significant increase in IL-6 expression at the histamine concentration of 10 µM (4.46 ± 1.42) (Figure 2) compared to the control sample with no histamine added.

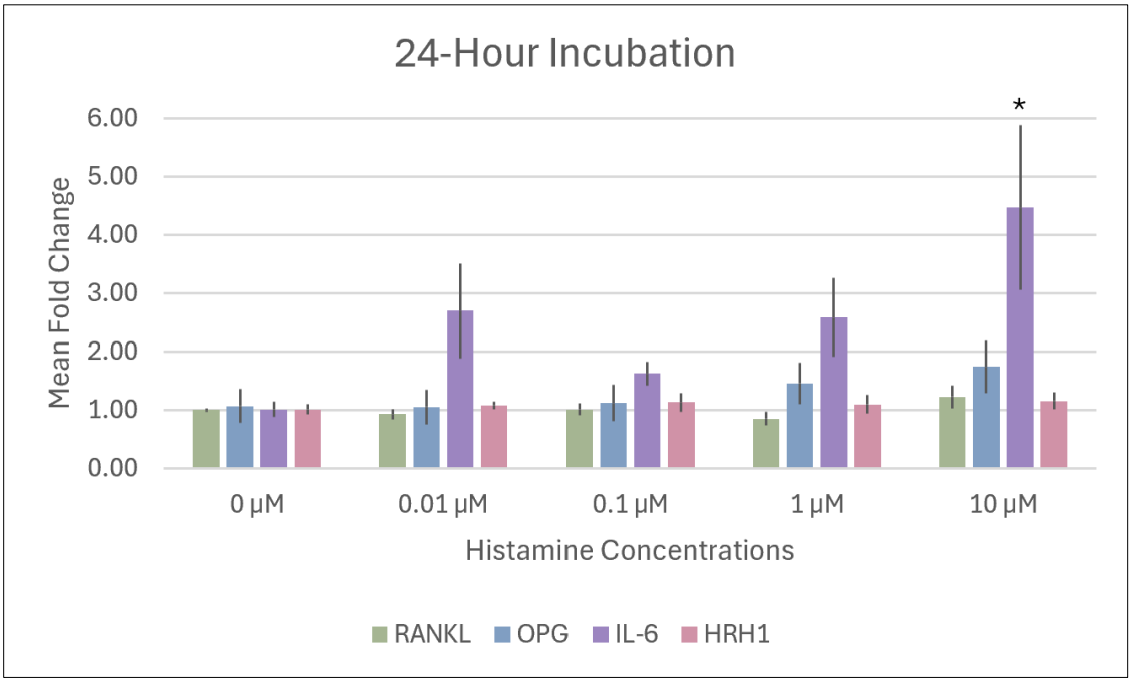

*Figure 1: Quantitative PCR determination of RANKL, OPG, IL-6 and HRH1 relative expression following 24-hour treatment with 0.01, 0.1, 1 and 10 µM of histamine. Data represents mean ± SE of ΔΔCt values derived from samples from three independent experiments. * P < 0.05 indicates a significant difference compared to control.*

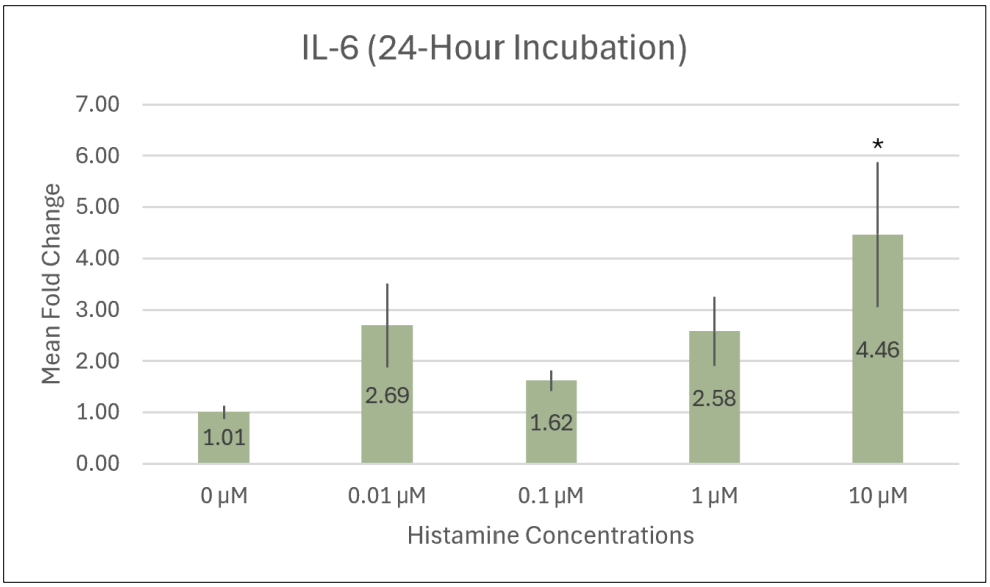

*Figure 2: Quantitative PCR determination of IL-6 relative expression following 24-hour treatment with 0.01, 0.1, 1 and 10 µM of histamine. Data represents mean ± SE of ΔΔCt values derived from samples from three independent experiments. * P < 0.05 indicates a significant difference compared to control.*

The cells were treated with histamine concentrations of 0, 0.01, 0.1, 1, and 10 µM and incubated for 6 hours prior to mRNA isolation. The relative mRNA expression of RANKL, OPG, and IL-6 were determined by quantitative PCR (Figure 3). Similar to the results from the previous experiment where the cells were incubated for 24 hours, there was no significant difference in mRNA expression observed for RANKL and OPG at different histamine concentrations. There was, however, a statistically significant increase in IL-6 expression at the histamine concentration of 1 and 10 µM with a mean fold change of 3.17 $\pm$ 0.50 and 6.96 $\pm$ 0.48 respectively compared to the control sample with no histamine added (Figure 4).

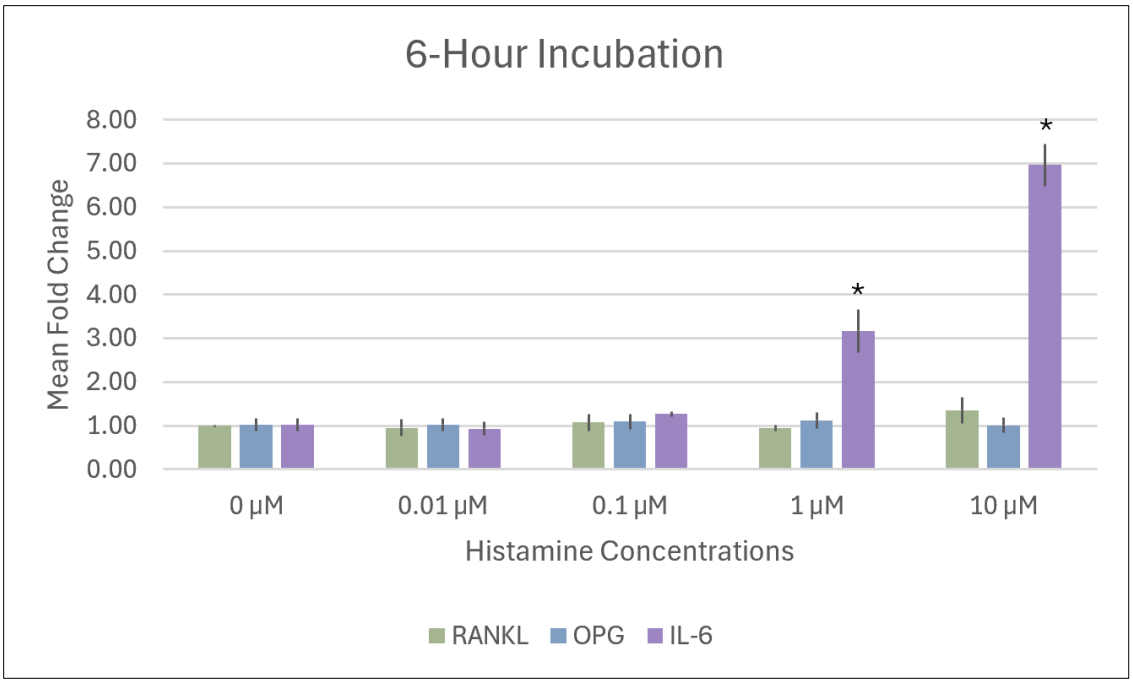

*Figure 3: Quantitative PCR determination of RANKL, OPG, and IL-6 relative expression following 6-hour treatment with 0.01, 0.1, 1 and 10 µM of histamine. Data represents mean ± SE of ΔΔCt values derived from samples from three independent experiments. * P < 0.05 indicates a significant difference compared to control.*

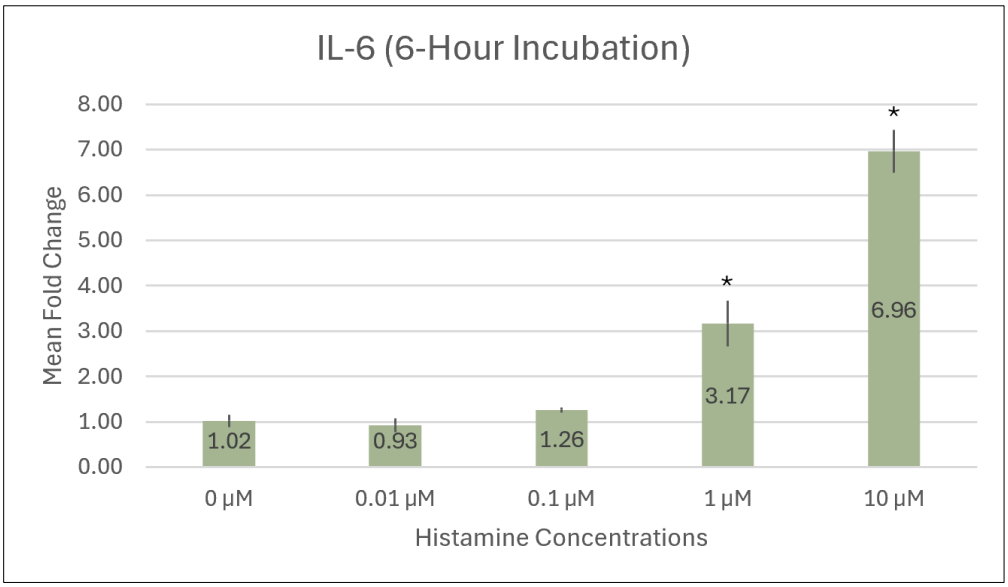

*Figure 4: Quantitative PCR determination of IL-6 relative expression following 24-hour treatment with 0.01, 0.1, 1 and 10 µM of histamine. Data represents mean ± SE of ΔΔCt values derived from samples from three independent experiments. *P < 0.05 indicates a significant difference compared to control.*

The relative mRNA expression of RANKL and IL-6 after treatment with fexofenadine, cetirizine and loratadine were determined by quantitative PCR. The negative control did not have any histamine or antihistamine added. The positive control and all other samples were treated with 1 µM of histamine. The cells were treated with the H1-antihistamines at concentrations of 0.01, 0.1 and 1 µM. All samples were incubated for 6 hours.

Figure 5 is a summary of the relative expression of RANKL between the different concentrations and types of antihistamines expressed as mean fold change compared to the negative control. Statistical analysis was performed to determine if there was a statistically significant change in comparison to the positive control. The results do not show a change between different concentrations of each type of antihistamine.

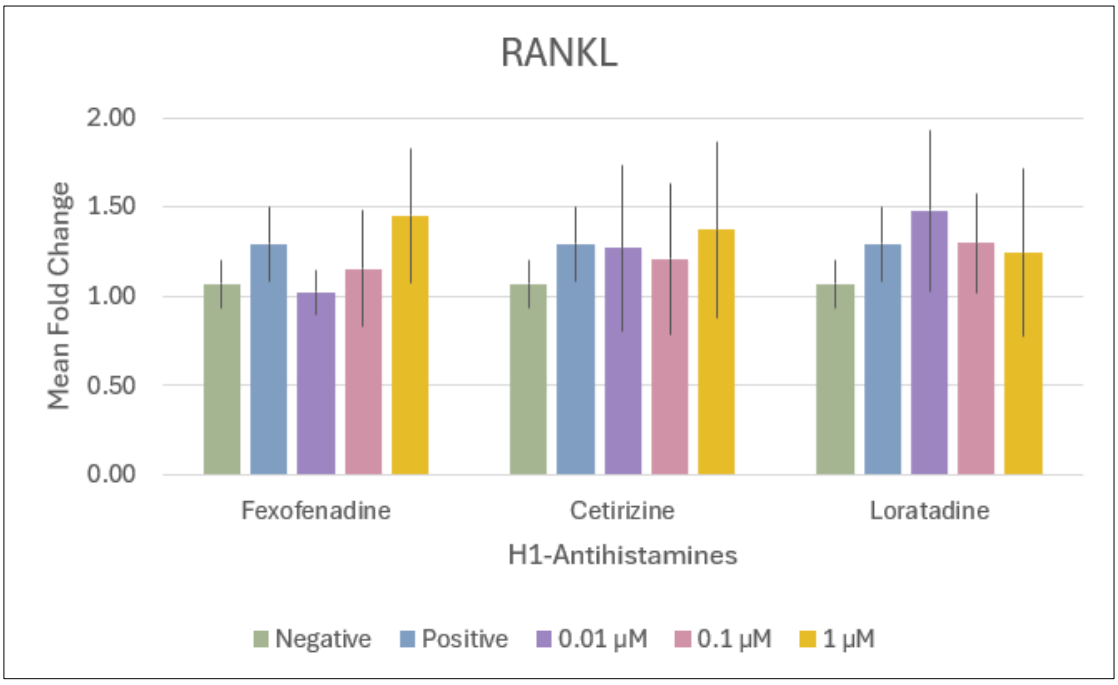

*Figure 5: Quantitative PCR determination of RANKL relative expression following 6-hour treatment with 1 µM of histamine and 0.01, 0.1 and 1 µM of fexofenadine, cetirizine and loratadine. Data represents mean ± SE of ΔΔCt values derived from samples from three independent experiments. * P < 0.05 indicates a significant difference compared to control.*

The peak serum concentrations (C_{max}) of the antihistamines are outlined in table 1. The tested concentrations which were closest to the C_{max} for fexofenadine, cetirizine and loratadine were determined to be 1, 1 and 0.01 µM respectively. There was no statistically significant difference between the mean fold change of RANKL mRNA expression of the different antihistamines at these concentrations (Figure 6).

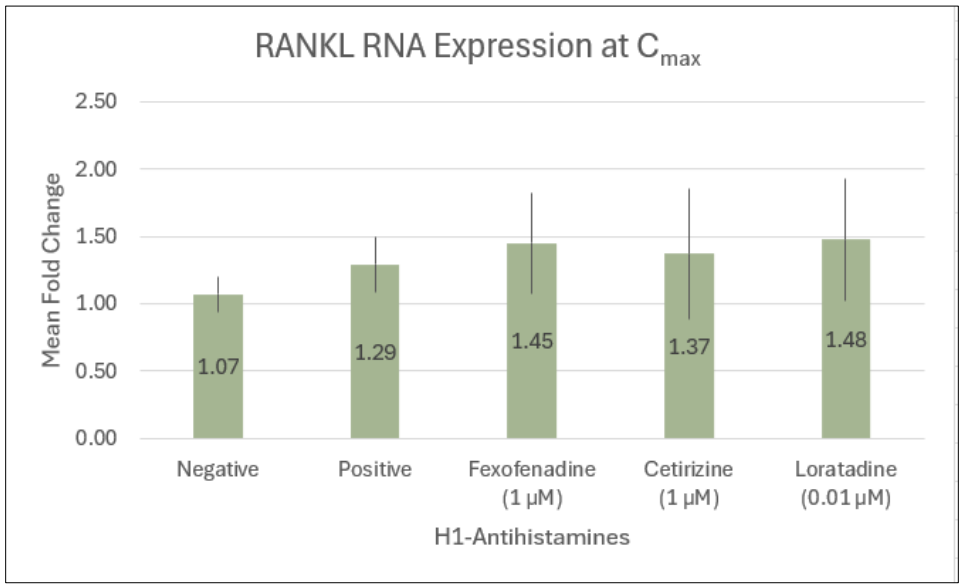

*Figure 6: Quantitative PCR determination of RANKL relative expression following 6-hour treatment with 1 µM of histamine and peak serum concentration (C_{max}) of fexofenadine, cetirizine and loratadine. Data represents mean ± SE of ΔΔCt values derived from samples from three independent experiments. * P < 0.05 indicates a significant difference compared to control.*

Figure 7 is a summary of the relative mRNA expression of IL-6 between the different concentrations and types of antihistamines expressed as mean fold change compared to the negative control. IL-6 expression in the positive control sample increased by 2.03 $\pm$ 0.19. Statistical analysis was performed to determine if there was a statistically significant change in the treatment samples in comparison to the positive control. In the samples treated with

fexofenadine, there was a statistically significant decrease in IL-6 expression at the concentration of 1 µM (1.24 ± 0.29) but not at the concentrations of 0.01 and 0.1 µM. In the samples treated with cetirizine, there was a statistically significant decrease in IL-6 expression at the concentrations of 0.1 (1.3 ± 0.07) and 1 µM (1.3 ± 0.22) but not at the concentration of 0.01 µM. In the samples treated with loratadine, there were no statistically significant changes in IL-6 expression.

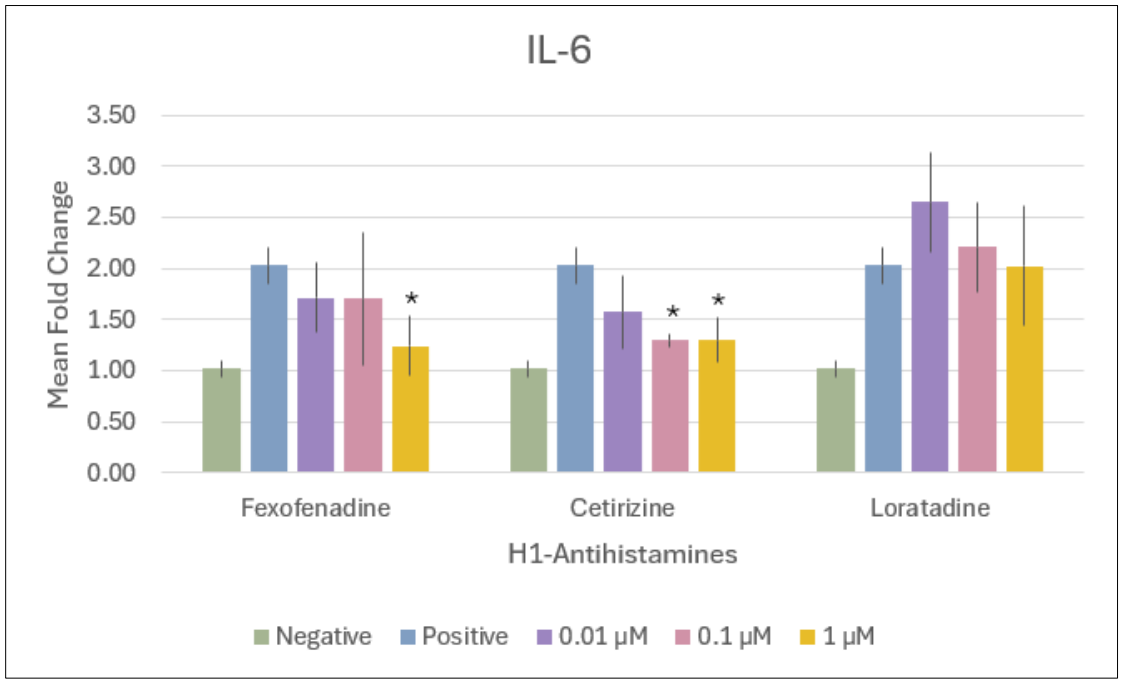

*Figure 7: Quantitative PCR determination of IL-6 relative expression following 6-hour treatment with 1 µM of histamine and 0.01, 0.1 and 1 µM of fexofenadine, cetirizine and loratadine. Data represents mean ± SE of ΔΔCt values derived from samples from three independent experiments. * P < 0.05 indicates a significant difference compared to control.*

Figure 8 shows the relative mRNA expression of IL-6 at the tested concentrations closest to C_{max} for the antihistamines. There was a statistically significant decrease in IL-6 mRNA expression for fexofenadine and cetirizine by 39% (2.03 to 1.24) and 36% (2.03 to 1.30) respectively in comparison to the positive control. There was an increase in IL-6 expression for loratadine at its C_{max} of 0.01 µM but the change was not determined to be statistically significant.

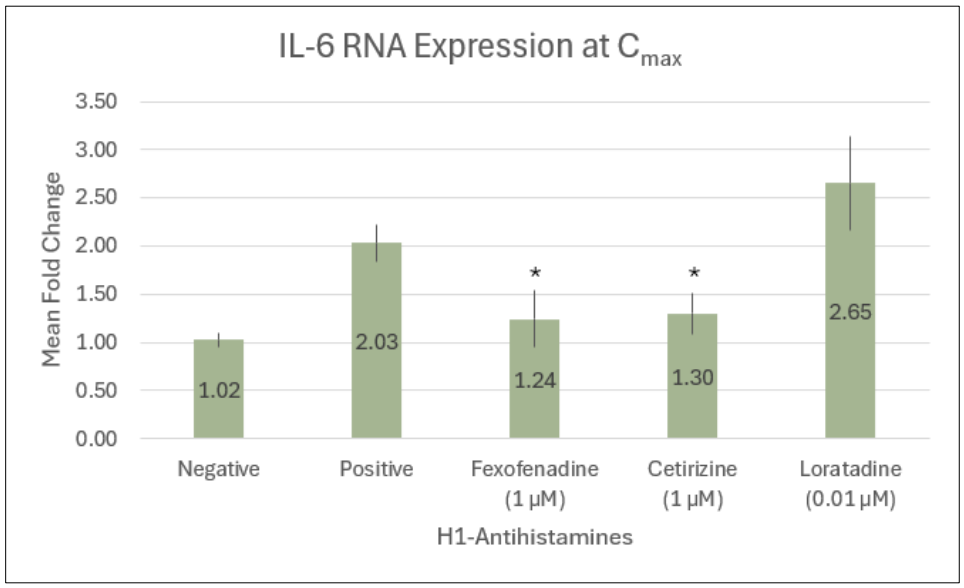

*Figure 8: Quantitative PCR determination of IL-6 relative expression following 6-hour treatment with 1 µM of histamine and peak serum concentration (C_{max}) of fexofenadine, cetirizine and loratadine. Data represents mean ± SE of ΔΔCt values derived from samples from three independent experiments. * P < 0.05 indicates a significant difference compared to control.*

Media was extracted from the sample wells after the 6-hour incubation and prior to mRNA isolation for the second and third replicates of the experiment. Since there are only two sets of samples, statistical analysis could not be performed on the data. The IL-6 protein expression was examined using a Luminex Multiplex assay and the data is reported in Figure 9 as the concentration of IL-6 in pg/mL of media. There was a 625% increase in IL-6 protein expression when the cells were treated with histamine (positive control) compared to the negative control. For each type of antihistamine tested, there appears to be a negative correlation between IL-6 expression and antihistamine concentration with protein expression decreasing as antihistamine concentration increases.

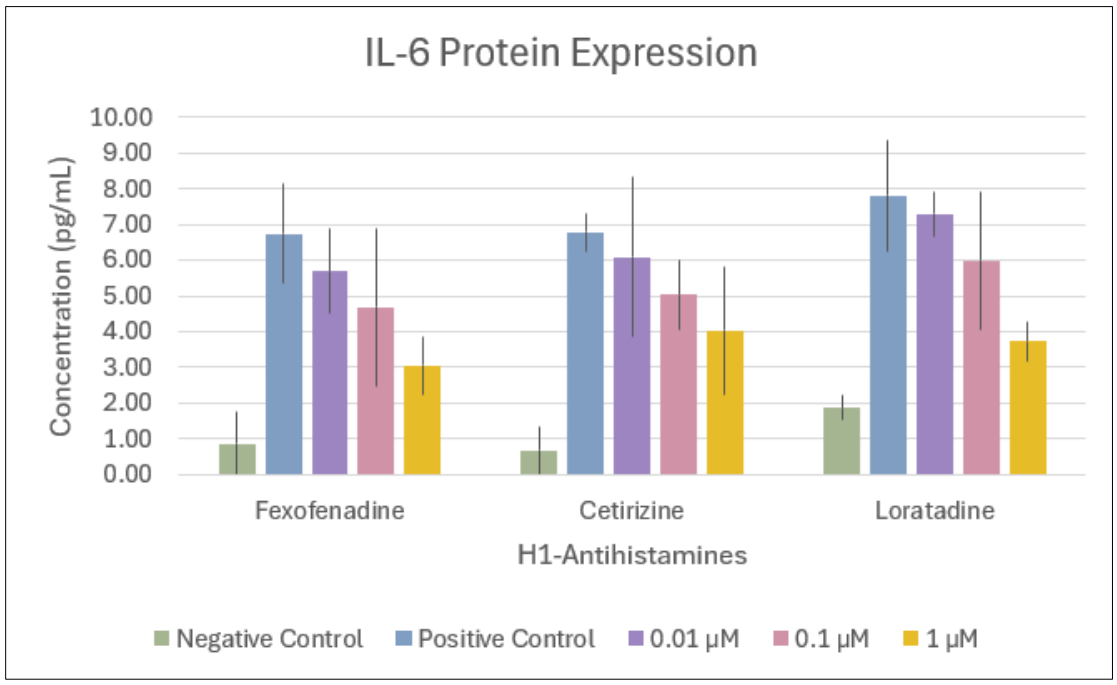

Figure 9: Luminex assay determination of IL-6 protein expression following 6-hour treatment with 1 µM of histamine and 0.01, 0.1 and 1 µM of fexofenadine, cetirizine and loratadine. Data represents mean ± SE of IL-6 protein in pg/mL of media derived from samples from two independent experiments.

Figure 10 shows the protein expression of IL-6 at the tested concentrations closest to C_{max} for the antihistamines. Compared to the positive control, there was a 57% and 45% reduction of IL-6 expression for fexofenadine and cetirizine respectively at the concentration of 1 µM. There was a slight increase of 3% in IL-6 expression for loratadine at its C_{max} of 0.01 µM. The data shows that there was nearly a two-fold reduction in IL-6 expression for fexofenadine and cetirizine but remained relatively the same for loratadine at concentrations closest to C_{max}.

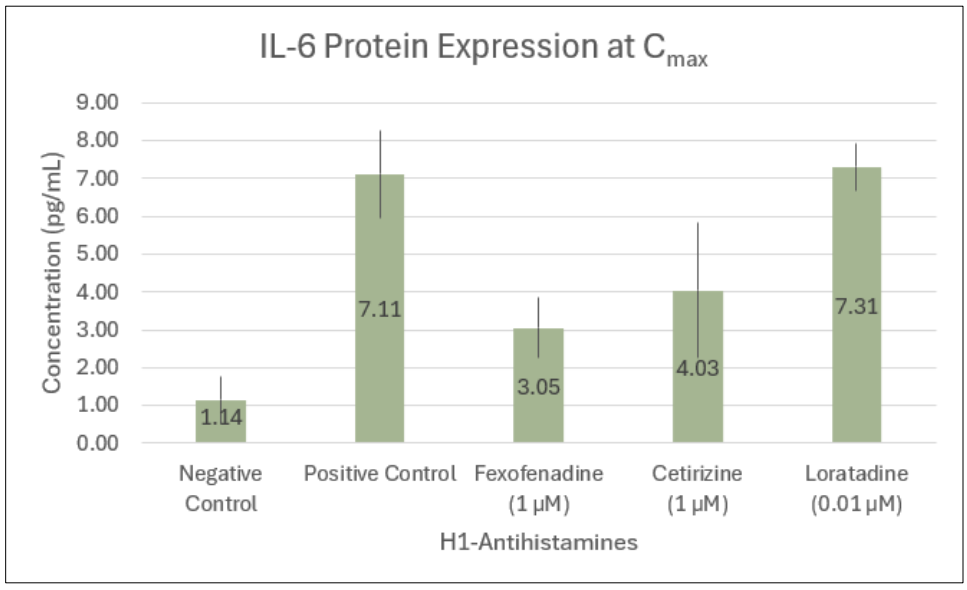

Figure 10: Luminex assay determination of IL-6 protein expression following 6-hour treatment with 1 µM of histamine and peak serum concentration (C_{max}) of fexofenadine, cetirizine and loratadine. Data represents mean ± SE of IL-6 protein in pg/mL of media derived from samples from two independent experiments.

Chapter 5: Discussion

The purpose of this in vitro study was to evaluate the expression of RANKL, OPG and IL-6 in human osteoblasts following treatment with varying concentrations of histamine and common second-generation H1-antihistamines including fexofenadine (Allegra), cetirizine (Zyrtec), and loratadine (Claritin). G-292 osteosarcoma cells were chosen for this study since they are considered to be a valid experimental model for primary human osteoblasts (Bradford et al., 2000). According to the study by Deyama et al. (2002), the effect of histamine on expression of RANKL in MC3T3-E1 cells (mouse osteoblast cells) was transient with a peak at 6 hours and returned to baseline levels at 24 hours. The first part of this study was done to evaluate H1-receptor (HRH1) expression in G-292 osteoblast cells and to compare RANKL, OPG and IL-6 mRNA expression after treatment with varying concentrations of histamine for 24 and 6 hours (figure 1). From our initial experiment, we confirmed the alternative hypothesis that the H1-receptor is expressed in G-292 osteoblast cells, however, there was no change observed in HRH1 mRNA expression at different concentrations of histamine.

As discussed previously, the RANKL/RANK/OPG system plays a major role in bone remodeling. Osteoblast cells express RANKL when stimulated by other factors, which then binds to RANK on osteoclast precursors to stimulate differentiation. OPG is a soluble decoy receptor that competes with RANK for binding of RANKL. When the ratio of RANKL to OPG increases, osteoclastogenesis is upregulated and favors the resorption of bone. Although we hypothesized that histamine treatment affects RANKL mRNA expression, the results of our experiments show that there was no statistically significant change in expression for both the 6- and 24-hour

incubation (figure 1 and 3). The results also show that there is no significant change in mRNA expression of OPG in both experiments.

Biosse-Duplan et al. (2009) studied the effects of histamine on osteoclastogenesis. They investigated the effect of histamine on RANKL and OPG mRNA expression in primary osteoblasts in the presence and absence of 1,25-dihydroxycholecaliferol (calcitriol). Calcitriol is the active form of Vitamin D and plays an important role in bone metabolism. In the presence of 0.01 µM of calcitriol, they noted an increase in RANKL expression in cells treated with 1 µM of histamine compared to the control. The expression of OPG was not modified by the addition of histamine but the RANKL/OPG ratio still shifted in favor of osteoclastogenesis. When calcitriol was not added to the samples, both RANKL and OPG expression did not change. The author proposed that calcitriol regulates the subtype and distribution of histamine receptors and increases H1-receptors in osteoblasts, thus modifying the effects of histamine on the cell. Deyama et al. (2002) also showed that there was an increase in RANKL expression in osteoblastic cells in the presence of calcitriol. The synergestic effects of calcitriol and histamine may explain why we did not see any changes in the expression of RANKL in our experiments since calcitriol was not added to the media. In this experiment, we accept the null hypothesis that there is no significant change in mRNA expression of RANKL and OPG after treatment with histamine for 6- and 24-hours.

IL-6 is an inflammatory cytokine that plays a role in bone metabolism and is produced by immune cells, fibroblasts, tumor cells and osteoblasts. The study by Ishimi et al. (1990) showed that IL-6 is produced by osteoblasts in response to local bone-resorbing agents, and it induces bone resorption both alone and in combination with other bone-resorbing agents. IL-6-induced

osteoclast differentiation depends on signal transduction mediated by the IL-6 receptor expressed on osteoblast cells but not on osteoclast progenitors (Udagawa et al., 1995). Because IL-6 is an inflammatory cytokine that has been shown to stimulate osteoclastogenesis, an increase in its expression may be linked to increased bone resorption in vivo.

When cells were incubated for 24-hours, there appeared to be a positive correlation between IL-6 expression and histamine concentration except at the concentration of 0.01 μM where the mean fold change was measured at 2.69 $\pm$ 0.81 (figure 2). The increase in IL-6 expression at 0.01, 0.1 and 1 μM was not determined to be statistically significant compared to the control. At the concentration of 10 μM, there was a statistically significant change in IL-6 expression (4.46 $\pm$ 1.42). When the cells were treated with histamine for 6 hours, the positive correlation between IL-6 expression and histamine concentration is more evident (figure 4). There is a statistically significant change compared to the control sample at the concentrations of 1 and 10 μM at a mean fold change of 3.17 $\pm$ 0.5 and 6.96 $\pm$ 0.48 respectively. This data supports our alternative hypothesis that histamine treatment alters mRNA expression of IL-6 in G-292 cells.

To my knowledge, this is the first study to evaluate changes in RANKL and IL-6 mRNA expression after 6-hour treatment of osteoblasts with histamine and different H1-antihistamines. Due to the large difference in peak plasma serum concentration (C_{max}) between the drugs, we decided to test three concentrations that encompassed these values (0.01, 0.1 and 1 μM). We chose the histamine concentration of 1 μM since we noted a significant change at this concentration, and this concentration was also used in the studies by Deyama et al. (2002) and Ikawa et al. (2007). Since osteoclastogenesis is a crucial part of orthodontic tooth

movement, it is of interest to determine if antihistamine treatment decreases the expression of RANKL and IL-6, and if there is less of a change observed in one drug compared to the other.

In the experiment, there was no statistically significant change in RANKL mRNA expression noted between the different types of antihistamines and between the different concentrations within each type (figure 5). Based on our initial experiments using different concentrations of histamine and the lack of changes in RANKL expression, the same results were expected when studying antihistamine treatment. The null hypothesis that H1-antihistamine treatment does not alter RANKL mRNA expression is accepted. Future research could be done to evaluate whether the addition of calcitriol to the media will result in a change in RANKL expression in G-292 osteoblast cells.

When the cells were treated with histamine only, the mRNA expression of IL-6 doubled with a mean fold change of 2.03 $\pm$ 0.19 (figure 7). When the cells were treated with fexofenadine, the difference in mean fold change was not statistically significant at the concentrations of 0.01 and 0.1 µM compared to the positive control. At the concentration of 1 µM, however, the mean fold change decreased from 2.03 in the positive control to 1.24 $\pm$ 0.29. This reduction of 39% was determined to be statistically significant. When the cells were treated with cetirizine, the mean fold change was not statistically significant at the concentration of 0.01 µM. The decrease of 36%, from 2.03 in the positive control sample to 1.3 $\pm$ 0.07 and 1.3 $\pm$ 0.22 at the concentrations of 0.1 and 1 µM respectively, was determined to be significant. There were no statistically significant changes in IL-6 mRNA expression at all concentrations of loratadine. At the concentration closest to C_{max} for each antihistamine (figure 8), there is a significant decrease in IL-6 expression for fexofenadine (1 µM) and cetirizine (1 µM) but no

change is observed for loratadine (0.01 µM). Our alternative hypothesis is, therefore, partially accepted for fexofenadine and cetirizine but rejected for loratadine.

After completing the first replicate of the antihistamine treatment experiment, we wanted to know if protein expression of IL-6 followed the same pattern as mRNA expression. We collected the media from the two remaining replicates of the experiment and analyzed protein expression using the Luminex Multiplex Assay (figure 9). Since there were only two sets of samples, a statistical analysis could not be completed on the Luminex Milliplex data. The results of the protein assay support the findings from the qPCR experiments evaluating mRNA expression. There is a 625% increase in IL-6 expression when the cells are treated with histamine (positive control) compared to the negative control. For each type of antihistamine tested, there appears to be a negative correlation between IL-6 expression and antihistamine concentration with protein expression decreasing as antihistamine concentration increases. The data supports our alternative hypothesis that antihistamine treatment alters IL-6 protein expression in G-292 cells. At the concentration closest to C_{max} for each antihistamine (figure 10), there is a decrease in IL-6 expression for fexofenadine by 57% (1 µM) and cetirizine by 45% (1 µM) but a very slight increase of 3% is observed for loratadine (0.01 µM) when compared to the mean values of the positive control.

In this in vitro study, the data shows that there is a reduction in both mRNA and protein expression of IL-6 when G-292 cells are treated with fexofenadine and cetirizine at concentrations closest to C_{max} but not when treated with loratadine. Since IL-6 is one of the inflammatory cytokines that contribute to osteoclastogenesis, a decrease in its concentration may reduce the rate of tooth movement. The results suggest that loratadine may be a better

option with regards to maintaining the rate of orthodontic tooth movement since there were no significant changes in IL-6 expression noted between the treated cells and the positive control.

A systematic review and meta-analysis of randomized controlled trials by Hong et al. (2023) compared the efficacy of different oral H1-antihistamine treatments on allergic rhinitis. They concluded that loratadine 10 mg was the least effective among the antihistamines that were studied. Since loratadine is not as effective in reducing symptoms of allergic rhinitis and inflammation, it is understandable that it also does not reduce the expression of IL-6 in our experiment. The lack of symptom relief from choosing loratadine over the other medications may outweigh the potential benefits of maintaining the rate of orthodontic tooth movement. Further studies will be needed to determine if clinical recommendations can be made. As mentioned previously, it would be beneficial to test for RANKL expression in the presence of calcitriol. It would also be of interest to test the expression of other cytokines and inflammatory mediators such as IL-1β, TNF-α, and M-CSF. More in vivo experiments on the effects of different types of antihistamines on orthodontic tooth movement will be valuable as there are not many studies that have been done.

According to the systematic review from Makrygiannakis et al. (2018), many commonly used medications have been shown to affect the rate of orthodontic tooth movement. Some drugs such as diazepam, pantoprazole and vitamin C may increase the rate and others, such as atorvastatin, losartan, metformin, famotidine, and cetirizine, may decrease it. While they consider the quality of evidence to be low, they still recommend that any possible implications of medications should be considered in the treatment plan. If patients are taking medications that have potential to slow the rate of orthodontic tooth movement, it may be prudent for the

clinician to consider extending the time between appointments or using lighter forces. Given the prevalence of H1-antihistamine use and the demand for orthodontic treatment, it is in our best interest to continue studying the effects of antihistamines on bone metabolism and the rate of orthodontic tooth movement.

Chapter 6: Conclusion

Human osteoblast-like cells (G-292) express the H1-receptor (HRH1) and respond to histamine treatment. Stimulation of G-292 cells with varying concentrations of histamine does not result in a statistically significant change in mRNA expression of HRH1, RANKL or OPG at both treatment times of 6- and 24-hours. The increased expression of IL-6 is statistically significant at higher concentrations of 10 µM after incubation at 24-hours and 1 and 10 µM at 6-hours. The results suggest that there is a positive correlation between histamine concentration and IL-6 expression.

Treatment of G-292 cells with histamine and different types and concentrations of fexofenadine, cetirizine and loratadine did not result in statistically significant changes in RANKL mRNA expression. Future research could be done to evaluate whether the addition of calcitriol to the media will result in a change in RANKL expression in G-292 cells. The results suggest that there is a negative correlation between antihistamine concentration and IL-6 mRNA expression for fexofenadine and cetirizine. There is no significant change observed when the cells were treated with loratadine. At the concentration closest to C_{max} for each antihistamine, there is a significant decrease in IL-6 expression for fexofenadine and cetirizine, but no change is observed for loratadine.

Evaluation of IL-6 protein expression shows that there is a negative correlation between antihistamine concentration and IL-6 protein expression for all three types of H1-antihistamines tested. At the concentration closest to C_{max}, however, there is a decrease in IL-6 expression for fexofenadine and cetirizine, but a slight increase is observed for loratadine. The data from the Milliplex assay evaluating protein expression of IL-6 supports the qPCR results.

The results from this in vitro study suggest that loratadine may be a better option compared to fexofenadine and cetirizine with regard to maintaining the rate of orthodontic tooth movement since there were no significant changes in IL-6 expression noted between the treated cells and the positive control. Given the prevalence of H1-antihistamine use and the demand for orthodontic treatment, it is in our best interest to continue studying the effects of antihistamines on bone metabolism and the rate of orthodontic tooth movement.

References

Bayer Inc. (2020, April 30) Claritin product monograph. https://www.bayer.com/sites/default/files/claritin-pm-en.pdf

Biosse-Duplan, M., Baroukh, B., Dy, M., de Vernejoul, M. C., & Saffar, J. L. (2009). Histamine promotes osteoclastogenesis through the differential expression of histamine receptors on osteoclasts and osteoblasts. *The American Journal of Pathology*, *174*(4), 1426–1434. https://doi.org/10.2353/ajpath.2009.080871

Bradford, P. G., Maglich, J. M., Ponticelli, A. S., & Kirkwood, K. L. (2000). The effect of bone morphogenetic protein-7 on the expression of type I inositol 1,4,5-trisphosphate receptor in G-292 osteosarcoma cells and primary osteoblast cultures. *Archives of Oral Biology*, *45*(2), 159–166. https://doi.org/10.1016/s0003-9969(99)00122-3

Church, M. K., & Church, D. S. (2013). Pharmacology of antihistamines. *Indian Journal of Dermatology*, *58*(3), 219–224. https://doi.org/10.4103/0019-5154.110832

Deyama, Y., Kikuiri, T., Ohnishi, G.i, Feng, Y. G., Takeyama, S., Hatta, M., Yoshimura, Y., & Suzuki, K. (2002). Histamine stimulates production of osteoclast differentiation factor/receptor activator of nuclear factor-kappaβ ligand by osteoblasts. *Biochemical and Biophysical Research Communications*, *298*(2), 240–246. https://doi.org/10.1016/s0006-291x(02)02440-3

Simons, F. E., & Simons, K. J. (2008). H1 antihistamines: current status and future directions. *The World Allergy Organization Journal*, *1*(9), 145–155. https://doi.org/10.1186/1939-4551-1-9-145

Feng, X., & McDonald, J. M. (2011). Disorders of bone remodeling. *Annual Review of Pathology*, *6*, 121–145. https://doi.org/10.1146/annurev-pathol-011110-130203

Fitzpatrick, L. A., Buzas, E., Gagne, T. J., Nagy, A., Horvath, C., Ferencz, V., Mester, A., Kari, B., Ruan, M., Falus, A., & Barsony, J. (2003). Targeted deletion of histidine decarboxylase gene in mice increases bone formation and protects against ovariectomy-induced bone loss. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(10), 6027–6032. https://doi.org/10.1073/pnas.0934373100

Hong, D., Weng, J., Ye, M., & Liu, Y. (2023). Efficacy of different oral H_1 antihistamine treatments on allergic rhinitis: a systematic review and network meta-analysis of randomized controlled trials. *Brazilian Journal of Otorhinolaryngology*, *89*(4), 101272. https://doi.org/10.1016/j.bjorl.2023.03.009

Ikawa, Y., Yonekawa, T., Ohkuni, Y., Kuribayashi, M., Fukino, K., & Ueno, K. (2007). A comparative study of histamine activities on differentiation of osteoblasts and osteoclasts. *The Journal of Toxicological Sciences*, *32*(5), 555–564. https://doi.org/10.2131/jts.32.555

Ishimi, Y., Miyaura, C., Jin, C. H., Akatsu, T., Abe, E., Nakamura, Y., Yamaguchi, A., Yoshiki, S., Matsuda, T., & Hirano, T. (1990). IL-6 is produced by osteoblasts and induces bone resorption. *Journal of Immunology (Baltimore, MD: 1950)*, *145*(10), 3297–3303.

Jeon, H. H., Teixeira, H., & Tsai, A. (2021). Mechanistic insight into orthodontic tooth movement based on animal studies: A critical review. *Journal of Clinical Medicine*, *10*(8), 1733. https://doi.org/10.3390/jcm10081733

Kriznar, I., Sprogar, S., Drevensek, M., Vaupotic, T., & Drevensek, G. (2008). Cetirizine, a histamine H1 receptor antagonist, decreases the first stage of orthodontic tooth movement in rats. *Inflammation Research: Official Journal of the European Histamine Research Society*, *57 Suppl 1*, S29–S30. https://doi.org/10.1007/s00011-007-0615-1

Kuna, P., Jurkiewicz, D., Czarnecka-Operacz, M. M., Pawliczak, R., Woroń, J., Moniuszko, M., & Emeryk, A. (2016). The role and choice criteria of antihistamines in allergy management - expert opinion. *Advances in Dermatology and Allergology*, *33*(6), 397–410. https://doi.org/10.5114/pdia.2016.63942

Makrygiannakis, M. A., Kaklamanos, E. G., & Athanasiou, A. E. (2018). Does common prescription medication affect the rate of orthodontic tooth movement? A systematic review. *European Journal of Orthodontics*, *40*(6), 649–659. https://doi.org/10.1093/ejo/cjy001

Meh, A., Sprogar, Š., Vaupotic, T., Cör, A., Drevenšek, G., Marc, J., & Drevenšek, M. (2011). Effect of cetirizine, a histamine (H1) receptor antagonist, on bone modeling during orthodontic tooth movement in rats (Vol. 139, Issue 4, p. e323). Elsevier BV. https://doi.org/10.1016/j.ajodo.2009.11.013

Meh, A., Sprogar, S., Vaupotic, T., Cör, A., Drevenšek, G., Marc, J., & Drevenšek, M. (2011). Effect of cetirizine, a histamine (H(1)) receptor antagonist, on bone modeling during orthodontic tooth movement in rats. *American Journal of Orthodontics and Dentofacial Orthopedics*, *139*(4), e323–e329. https://doi.org/10.1016/j.ajodo.2009.11.013

Ng, A. E., & Boersma, P. (2023). Diagnosed allergic conditions in adults: United States, 2021. *NCHS Data Brief,* (460), 1–8.

Pfizer Labs (n.d.). Zyrtec (cetirizine hydrochloride) [package insert]. U.S. Food and Drug Administration website. https://www.accessdata.fda.gov/drugsatfda_docs/label/2002/19835s15,%2020346s8lbl.pdf

Sanofi Consumer Health Inc. (2019, November 7) Allegra product monograph. https://products.sanofi.ca/en/allegra.pdf

Sperl, G., Gattner, J., Deschner, J., Wolf, M., Proff, P., Schröder, A., & Kirschneck, C. (2020). Effects of histamine receptor antagonist cetirizine on orthodontic tooth movement. *Biomedicines*, *8*(12), 583. https://doi.org/10.3390/biomedicines8120583

Thermo Fisher Scientific (n.d.). TaqMan Real-Time PCR Assays. Retrieved February 23, 2024 from https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays.html

Udagawa, N., Takahashi, N., Katagiri, T., Tamura, T., Wada, S., Findlay, D. M., Martin, T. J., Hirota, H., Taga, T., Kishimoto, T., & Suda, T. (1995). Interleukin (IL)-6 induction of osteoclast differentiation depends on IL-6 receptors expressed on osteoblastic cells but not on osteoclast progenitors. *The Journal of Experimental Medicine*, *182*(5), 1461–1468. https://doi.org/10.1084/jem.182.5.1461

Zablotsky, B., Black, L. I., & Akinbami, L. J. (2023). Diagnosed allergic conditions in children aged 0-17 Years: United States, 2021. *NCHS Data Brief*, (459), 1–8.

Curriculum Vitae

Kriztine Chun, D.M.D.

Contact Information

School of Dental Medicine
University of Nevada, Las Vegas
1700 W Charleston Blvd Building D
Las Vegas, NV 89102
kriztineu@gmail.com

Education

University of Nevada, Las Vegas
Doctor of Dental Medicine, 2021

University of San Francisco
Bachelor of Science in Chemistry, 2014