EFFECT OF ANTIOXIDANT GEL ON OXIDATIVE STRESS AND SALIVARY FLOW RATE IN XEROSTOMIC PATIENTS

A Thesis

By

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ABSTRACT

This study was designed to observe the effects of an antioxidant gel on oxidative stress levels and salivary flow rates in xerostomic patients. In this prospective, nested, double blind, crossover, randomized clinical trial, unstimulated whole saliva was collected from 36 subjects, who were randomly divided into two groups: Active-Placebo (n=21) and Placebo-Active (n=15). Subjects either received the active or placebo gel for the first 4 weeks of the study and then underwent a two-week washout period between weeks 4 and 6. Subjects were then given the opposite gel product at week 6 and instructed to use this product for 4 more weeks. Salivary samples were collected at week 0,2,4,6,8,10. Statistical analysis was completed with paired t-test and mixed model analysis. Further analysis of the subset of participants with salivary flow rates below 0.200 mL/min and below 0.100 mL/min was also completed.

Results indicate that oxidative stress levels did not change significantly over the course of the study in either group. Upon evaluation of participants with salivary flow rates below 0.200 mL/min, a statistically significant increase in salivary flow rate was noted in subjects in the active-placebo groups ($P<.05$).

DEDICATION

I dedicate my thesis to my parents, Ruhi and Randhir Kapoor, who have provided unwavering support in my educational pursuits.

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CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Dr. Terry Rees (chair), Dr. Jacqueline Plemons (member), and Dr. Jeffrey Rossmann (member) of the Department of Periodontics, Dr. Yi-Shing Lisa Cheng (member) of the Department of Diagnostic Sciences, Dr. Lynne Opperman (member), and Dr. Kathy Svoboda (member) of the Department of Biomedical Sciences. Statistical analysis guidance was provided by Dr. Elias Kontogiorgos and Dr. Eric Solomon. All other work completed independently by student.

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NOMENCLATURE

8-OHdG	8-hydroxydeoxyguanosine
AO	Antioxidant
A-P	Active-Placebo
OS	Oxidative Stress
P-A	Placebo- Active
ROS	Reactive Oxygen Species

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Saliva and Dry Mouth

Saliva is important to maintain homeostasis within the oral cavity. Its humidifying and lubricating properties allow for speech and swallowing while also preventing mechanical insult to the oral tissues. Whole saliva consists of the secretions from the major and minor salivary glands along with the gingival crevicular fluid.[1, 2] Normal unstimulated salivary flow rates are within the range of 0.3-0.4 mL/min. It is interesting to note that this range includes a large standard deviation, thus making it difficult to predict a "normal" value.[3] Women are noted to have decreased amounts of saliva compared to men. Furthermore, although salivary flow rate may diminish in either sex, women may be more likely to experience decreased salivary output with an increase in age.[4] The actual volume of saliva does not necessarily dictate the subjective feeling of xerostomia as the sensation of oral dryness has been reported by patients with normal salivary flow rates. Studies show that most of these xerostomic patients have a history of use of medications associated with dry mouth, systemic conditions leading to dry mouth, or a habit of mouth breathing.[5-7]

Composition of Saliva

Saliva is composed of 99% water, but the main difference from water is that saliva also contains mucins, which are glycosylated proteins that aid in the

lubrication process allowing for mastication, speech, and swallowing.[8] The main mucins seen in saliva are MUC5B and MUC7. These mucins form the acquired enamel pellicle thus creating a gap between any two surfaces in the mouth and diminishing trauma from abrasion of intraoral surfaces.[9, 10] However, studies have shown that replacing MUB5B or MUC7 does not alleviate dry mouth concerns; rather the combination of both mucins and other proteins creates the acquired pellicle on teeth.[11] Therefore, finding salivary replacement products is a difficult task. Most products are only able to offer palliative relief.

Functions of Saliva

Saliva is important for removal of microorganisms, leukocytes, food debris and desquamated epithelial cells by the process of swallowing. Most important to clear after meals are fermentable carbohydrates that limit the acidic insult to the oral cavity and prevent caries. Unstimulated and stimulated saliva provide the liquid to aid in swallowing during meal times. In patients with reduced salivary levels, the clearance of food particles is reduced thus making these patients more prone to dental caries.[12] Furthermore, saliva exhibits antiviral, antibacterial, and antifungal properties that allow regulation of the oral flora.[13]

Saliva is the hypotonic solution in which food particles can disseminate to the various taste buds and thus allow for taste recognition. Furthermore, saliva contains salivary amylase which aids in the breakdown of food for proper digestion. It also acts as the buffering agent against acidic insult from food or regurgitated acid to protect the oral and oropharyngeal mucosa.[13]

Salivary Gland Hypofunction versus Xerostomia

Lack of saliva can lead to morbid effects on the dentition and the soft tissue of the oral cavity. In order to make a clinical diagnosis, it is important that one is able to distinguish the symptomatic feeling of mouth dryness, or xerostomia, and the clinical measurement of decreased salivary flow or salivary gland hypofunction. The feeling of oral dryness due to a possible decrease in saliva or complete lack of saliva is known as xerostomia.[14]

Xerostomia, or "dry mouth" is the subjective sensation of mouth dryness that is often but not always associated with salivary gland hypofunction.[15] It affects 1 out of every 4 or 5 people, and 40-60% of the population between 60-80 years of age complains of dry mouth. Causes of xerostomia include dehydration, aging, smoking, mouth breathing, certain medications and various physiological or psychogenic conditions. To date, over 500 medications have been associated with mouth dryness.[15, 16]

Salivary gland hypofunction refers to the decrease in measurable saliva production or flow rate and can be the cause of the subjective dryness otherwise known as xerostomia. Quantitatively, salivary hypofunction is defined as secretion volumes at or below 0.100 mL/min.[17] Normal median salivary flow rates are between 0.300-0.400 mL/min when measuring unstimulated saliva.[14] There are several ways that salivary hypofunction can be assessed depending on the variable that is being measured.

Diagnostic tests to determine and evaluate salivary hypofunction include measuring the rate of unstimulated whole salivary output by means of the draining or drooling method, in which a patient's head is tilted forward and saliva is drooled into a sterile container. An unstimulated whole saliva flow rate of less than 0.1 mL/min is suggestive of salivary gland hypofunction. Stimulated whole saliva is collected by challenging the salivary glands through mastication, such as chewing paraffin wax, or through gustatory stimulation by means of citric acid, followed by expectoration into a collection tube. Stimulated whole saliva flow rates below <0.7 mL/minute suggest salivary hypofunction.[15]

Often, patients' response to a health questionnaire in conjunction with a clinical evaluation are used to evaluate xerostomic and low normal salivary output or hypofunction. The subjective findings from questionnaires will elucidate those patients that the sensation of have xerostomia while the clinical quantifiable measurement of salivary output determines salivary hypofunction. [18] In this study, the quantifiable variable of salivary output was assessed as part of the inclusion criteria.

Diagnosis of Xerostomia

A thorough medical and dental history should be taken to properly confirm xerostomia. Questions to ask the patient include:

- Does the amount of saliva in your mouth seem to be too little?
- Does your mouth feel dry when eating a meal?
- Do you sip liquids to aid in swallowing dry food?

- Do you have difficulty swallowing? [19]

Upon confirmation of these symptoms, further questioning of medical history details is integral. Several systemic and medical causes are linked to oral dryness. Temporary hypofunction can be caused by short term drug use (e.g. antihistamines), viral infection (e.g. mumps), dehydration, or psychological dysfunction (e.g. anxiety).[20]

Chronic hypofunction can be caused by the multiple factors shown in Table 1:

Table 1 Chronic Causes of Dry Mouth[20]

Chronically administered drugs	
Autoimmune disorders	Sjögren Syndrome
	Primary Billiary Cirrhosis
Endocrine Disorders	Diabetes Mellitus
	Hypothyroidism
Infections	Human Immunodeficiency Virus
	Hepatitis C
Neurological Disorders	Parkinson's Disorder
	Bell's Palsy
Genetic Diseases	Cystic Fibrosis
	Down Syndrome
	Celiac Disease
Nutritional Deficiencies	
Head and neck radiation	
Graft *versus* Host Disease	
Bone Marrow Transplant	

Several systemic diseases can lead to xerostomia or salivary hypofunction due to the mechanism of the disease process. Mechanisms include infiltration of the immunocompetent cells or granuloma formation which can be

seen in human immunodeficiency virus, graft versus host disease, sarcoidosis, and tuberculosis. Polyuria and dehydration with concomitant dry mouth can be seen in diabetes mellitus and end-stage renal disease. Fibrosis results in xerostomia in conditions such as graft versus host disease and scleroderma. Protein deposition and bacterial infection can also be causes for xerostomia. Identification of the cause can lead to proper diagnosis and treatment.[21]

Furthermore, a head and neck examination should be completed to assess whether or not salivary glands are producing adequate amounts of saliva. The healthcare provider should palpate the glands to assess for any enlargement, tenderness, or masses. [15]

Medication Induced Xerostomia

The use of systemic medications is one of the most frequently reported causes of dry mouth. Such would be expected given the large proportion of the population that is on prescription medications. It is estimated that up to 80% of the population over 60 takes prescribed medications, and 20-30% of all combined age groups do the same. It has been reported that hundreds of drugs can lead to the feeling of xerostomia including analgesics, anorectics, antianxiolytics, antiarrhythmics, anticholinergics, anticonvulsants, antidepressants, antidiarrheals, anti-emetics, antihistamines/decongestants, antihypertensives, antiparkinsonians, antipsychotics, antispasmodics, and diuretics.[22]

Given the broad range of use of these medications, the severity of dry mouth is variable. Furthermore, it is uncertain how much salivary gland hypofunction and decrease in salivary flow can be attributed to medication induced xerostomia as very few objective studies on the effect of medication on salivary output have been completed. [23]

Medications tend not to damage the salivary glands but they may decrease salivary flow rate by targeting certain steps in the salivary reflex both peripherally and centrally. Medications used for irritable bladder or chronic obstructive pulmonary disease block peripheral cholinergic muscarinic receptors on salivary gland acinar cells. Tricyclic antidepressants have targets in both the peripheral and central nervous systems. Adrenergic agonists are known to cause medication-induced xerostomia; however, there is no evidence to show that these medications lead to a decrease in salivary secretion. Beta-blockers are reported to decrease the protein concentration in saliva thus leading to a xerostomic state. Alpha-2 receptor agonists such as clonidine can cause both medication-induced xerostomia and medication-induced salivary hypofunction. Mixed serotonin and noradrenaline reuptake inhibitors may similarly activate alpha-2 receptors with the centrally occurring accumulation of noradrenaline, and thus are also known to lead to medication-induced xerostomia and medication-induced salivary hypofunction. Acting in a similar mechanism, opioids also produce a drying effect in the oral cavity. [23] Medications known to cause xerostomia are listed in Table 2.

Table 2 Medications Known to Cause Xerostomia[14, 24]

Antidepressants	Amitriptyline, Bupropion hydrochloride, Citalopram, Clomipramine, Desipramine, Doxepin, Duloxetine, Escitalopram, Fluoxetine, Fluvoxamine, Imipramine, Mirtazapine, Nortriptyline, Paroxetine, Protriptyline, Reboxetine, Sertraline, Trazodone, Trimipramine, Venlafaxine
Antihistaminic agents	Carbinoxamine, Cetirizine, Clemastine, Desloratadine, Dexchlorpheniramine, Dimenhydranate, Diphenhydramine, Fexofenadine, Hydroxyzine, Levocetirizine, Loratadine, Meclizine, Promethazine
Antiparkinsonian agents	Amantadine, Benztropine Bromocriptine, Carbidopa Entcapone, Levodopa, Pramipexole, Rasagiline, Ropinirole, Selegiline, Trihexyphenidyl
Neuroleptics	Butyrophenone Derivatives of phenothiazine, Thioxanthene
Bronchodilators	B2-adrenomimetics, Inhalatory cholinolytics (ipratropium), Inhalatory glucocorticoids
Cholinolytic agents	Atropine, homatropine, scopolamine
Hypotensive agents	**Angiotensin-converting enzyme (ACE) inhibitors:** Benazepril, Captopril, Enalapril, Fosinopril, Lisinopril, Moexipril, Perindopril, Quinapril, Ramipril, Trandolapril **Alpha-agonists:** Clonidine, Guanabenz, Guanfacine, Methldopa **Beta- blockers:** Acebutolol, Atenolol, Bebivolol, Betaxolol, Bisoprolol, Carvedilol, Esmolol, Labetalol, Metoprolol, Nadolol, Penbutolol, Pindolol, Propranolol, Stalol, Timolol **Diuretics:** Bumetanide, Furosemide, Torsemide **Calcium channel blockers:** Amlodipine, Diltiazem, Felodipine, Isradipine, Nifedipine, Nimodipine, Verapamil
Opioids	Codeine, Methadone, Morphine, Pethidine
Immunostimulants	Interferon-alpha
Appetite suppressants	Siberian
Antimigraine drugs	Rizatriptan 202 M

Additional effects on oral tissues can also be seen. In a study by Leal, dry or cracked lips were more readily seen in patients taking medications with xerostomic potential.[25] The oral mucosa may lose its glistening appearance which is indicative of dried mucous membranes. The oral mucosa may also appear thin and pale, while fissuring and lobulation of the tongue may be seen. These patients can also be more susceptible to fungal overgrowth thus producing angular cheilosis/cheilitis. Evidence of candidiasis can also be seen on the palate and tongue. Dry mouth makes patients more susceptible to denture stomatitis, and the lack of the saliva barrier may also increase susceptibility to dental caries. Saliva tends to appear thicker and stringy as opposed to less viscous and lubricating as seen in non-medicated patients. The salivary glands are often difficult to stimulate to produce more saliva due to lack of water, damage to the glands, or interference in the neuronal control of the glands. Swelling of the salivary glands can also be noted.[17] Multidrug therapy can result in a synergistic decrease in salivary flow and thus make the symptoms of dry mouth even worse.[16] For the purpose of this study, chronic medication use (greater than one year) from the aforementioned categories was considered in the inclusion criteria. In an effort to limit our patient population to medication induced xerostomia, we also excluded patients with certain systemic diseases that are strongly associated with reduced salivary output from the study.

Current Management of Xerostomia

Treatment planning to alleviate dry-mouth symptoms should be tailored to the individual patient. A multidisciplinary model of care for xerostomia and salivary gland hypofunction should include the following components:

- Patient education—a patient-centered process emphasizing daily oral hygiene, regular dental visits, use of topical fluoride, tobacco-use cessation counseling, and other interventions.
- Management of systemic conditions and medication use in consultation with the patient's physician, oncologist, or other health care providers.
- Preventive measures to reduce oral disease and associated complications.
- Pharmacological treatment with salivary stimulants (sialagogues).
- For patients who cannot tolerate sialagogues, palliative measures to improve salivary output, such as use of sugar-free salivary stimulants (for example, chewing gum).[15]

It is important to manage both the subjective and objective symptoms with which these patients present, whether the patients have salivary hypofunction or medication-induced xerostomia. Pharmacological treatment of medication-induced xerostomia includes administration of xylitol, salivary substitutes, and peripherally or centrally acting sialagogues.

Xylitol causes the osmosis of water from the tissues to the oral cavity thereby coating the teeth and increasing the salivary pH. Xylitol thus may have an

anticariogenic effect. Currently, the most effective salivary substitutes providing lubrication are glycerin and carboxymethyl cellulose based products. The viscosity of these agents plays a major role in the subjective palliative relief achieved by patients. Those agents that are most similar to normal saliva appear to provide more relief.

Peripheral sialagogues include ascorbic and malic acid. These agents produce an enhanced gustatory response; however, due to their acidic nature, further demineralization of teeth can occur.

Centrally acting sialagogues include pilocarpine and cevimeline. Pilocarpine is a nonselective muscarinic agonist and can thus produce side effects of sweating and gastrointestinal upset. Cevimeline has a higher affinity for the M3R receptor, which is associated with salivary gland secretions. These two medications are contraindicated in patients with uncontrolled asthma and narrow angle glaucoma. [24, 26]

Free Radicals

Free radicals are molecules in which there is one unpaired electron thus making these compounds unstable. Free radicals can be positively or negatively charged or even neutral. There are three chemical mechanisms by which these species are made in nature. First, there can be a breakage in a covalent bond leaving each end-product with an unpaired electron. Secondly, there can be a loss of a single electron from a neutral molecule. Third, an additional electron could be added to the molecule. Creation of free radicals is a normal biological

process and is known as “electron transfer.” The direction of transfer of electrons between molecules depends on the redox potential, which is a measure of the affinity of a substance for electrons, in relation to hydrogen. Molecules more strongly electronegative than hydrogen have positive redox potentials and are termed oxidants. Substances less electronegative than hydrogen have negative redox potentials and are termed reducing agents or antioxidants. Oxidation and reduction reactions always work in pairs and together are termed redox reactions.[27]

An example to consider is molecular oxygen. O_2 is in a triple ground state in which all electrons are paired. Once another electron is added through a reduction process, superoxide is formed ($O_2\cdot^-$). This molecule is a free radical as noted with the extra electron. Further reduction of superoxide forms hydrogen peroxide (H_2O_2). This is an example of a reactive oxygen species. Hydrogen peroxide can readily breakdown into the hydroxyl radical, which is one of the most reactive and harmful free radicals.[28]

Free radicals can also be formed from sources including ultraviolet radiation, trauma, heat, exhaust fumes, ozone, smoking, ultrasound, and various other sources. Endogenous sources of free radicals include leakage from mitochondrial transport pathways forming superoxide and functional generation from immune cells including phagocytes, osteoclasts, and fibroblasts. These endogenous sources seem to be one of the causes for certain chronic diseases and aging.[27]

Reactive Oxygen Species

Reactive oxygen species are important for homeostasis and cell signaling pathways. They are a product of normal metabolic processes and are important for normal physiology. They exist as reactive oxygen radicals or nonreactive radicals. For example, $\cdot O_2^-$ is needed to end the effect of nitric oxide, which is a major compound involved in vascular functions, including regulation of smooth muscle tone and blood pressure, activation of platelets, and vascular signaling.

Cells have several endogenous antioxidants that keep the reactive oxygen species at controlled levels. These compounds include vitamin E, ascorbic acid, and glutathione. In addition, several enzymes such as catalase and superoxide dismutase are involved in reactions with or the breakdown of these reactive species, most often resulting in water as the byproduct. As a consequence of these mechanisms, the levels of reactive oxygen species are fairly low. However, damage can occur when levels of reactive oxygen species increase.[29]

The Problem with Oxidative Stress in Oral Health

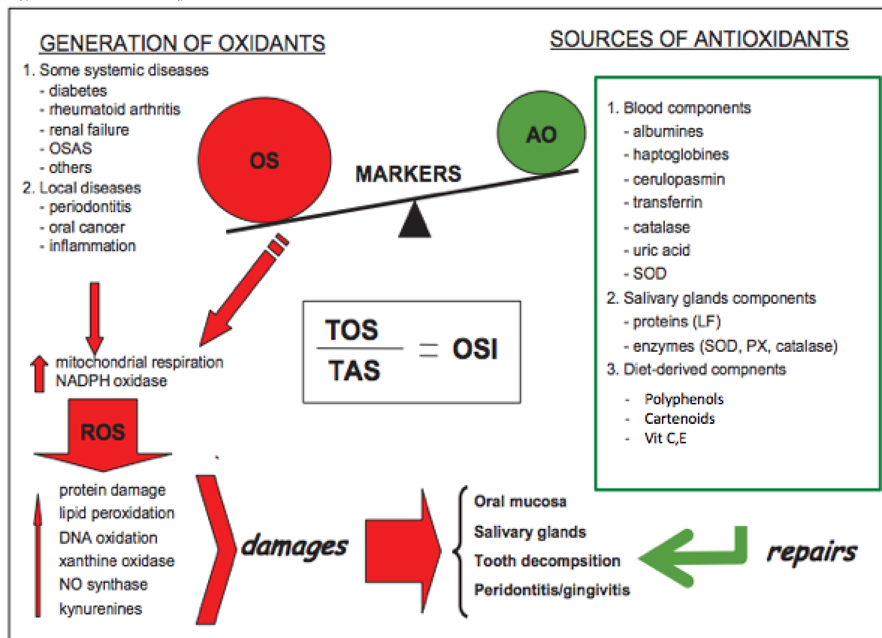
An important aspect of this research is the correlation between inflammation and oxidative markers in saliva. Studies have concluded that part of the problem with low levels of saliva is the resulting low levels of antioxidants.[30] Inflammation and oxidative stress start with a proliferation of free radicals including reactive oxygen species. Free radicals are molecules that have an unpaired electron and attempt to “steal” electrons from other molecules.

Unchecked, the process of “electron theft” can result in deterioration of cell walls and ultimately cell or tissue damage. In the oral cavity, free radicals can result from external sources, such as alcohol, nicotine, and hydrogen peroxide, as well as dental procedures and materials, including veneers, implants, and crowns. Infection due to gingivitis, periodontitis, or even root caries also generates free radicals as part of the inflammatory response.[30]

An overabundance of reactive oxygen species or free radicals results in oxidative stress. Prolonged oxidative stress can lead to a chronic inflammatory state including systemic inflammatory disease.[31] However, oxidative stress is reversed as free radicals are neutralized through the work of antioxidants. These large, complex molecules are able to “donate” electrons to free radicals in order to halt their electron theft. As a portal of entry to the body, the oral cavity is subjected to oxidants at all times which could potentially alter the redox potential and cause damage to oral tissues. Saliva contains several low molecular weight antioxidants to combat these harmful effects of the oxidants. Furthermore, additional sources of antioxidants include red blood cells extravasated from capillary beds. Red blood cells act as sinks for reactive oxygen species. However, hemorrhage can lead to iron catalyzed hydroxyl formation. Thus, red blood cells must remain in a balanced state.[32] Furthermore, the interaction of polyphenol coated red blood cells with low molecular weight antioxidants seems to have a synergistic effect in combating insult to the oral cavity.[32-34]

Normal saliva is rich in antioxidants, including uric acid, albumin, ascorbic acid, glutathione, and antioxidant enzymes. When antioxidant levels in saliva are too low to neutralize the free radicals, the tissues are set up for oxidative stress. In fact, several studies have implicated high levels of oxidative stress markers such as 8-hydroxydeoxy guanosine in oral diseases including periodontal disease, aphthous ulcers, dental caries, and oral cancer.[35-40] Figure 1 below describes the sources of antioxidants, the source of oxidants, the effects of reactive oxygen species, and ultimately the damage that can be induced. Furthermore, the review by Buczko proposes looking at the ratio between oxidants and antioxidants to accurately compare the oxidative stress levels.[41]

Figure 1 Sources of Oxidants and Anitoxidants[41]



Research Supporting the Positive Effects of Antioxidants in the Oral Cavity

Research over the past few years indicates that antioxidants are an important factor in dealing with free radicals and oxidative stress in the oral cavity. Reactive oxygen species have been known to be present in radiation induced salivary hypofunction patients. Studies in the mouse mode have shown that the administration of the antioxidant lecithinized sodiumoxide dismutase inhibits the deleterious dry mouth effects of radiation.[42] Other studies have shown that prophylactic administration of antioxidants and sialagogues before radiation therapy may prove useful in decreasing harmful effects to salivary glands.[43]

The antioxidant gel used in this study contains antioxidants that are flavonoids, specifically phloretin and ferulic acid. Flavonoids function through many different mechanisms such as radical scavenging, terminating lipid peroxidation, iron chelation, sparing vitamin E, and restoration of vitamin C. Published literature supports the topical use of these polyphenols in the mouth to amplify the total antioxidant capacity of saliva and reduce oxidative stress. Polyphenols are naturally found in substances such as nuts, teas, herbs, and vegetables. These compounds have been shown to reduce the incidence of carcinogenesis in the oral cavity, decrease bacterial plaque formation and bacterial adhesion, and inhibit viral replication. The mechanisms by which they act include: direct inactivation of viruses and bacteria, induction of apoptosis of

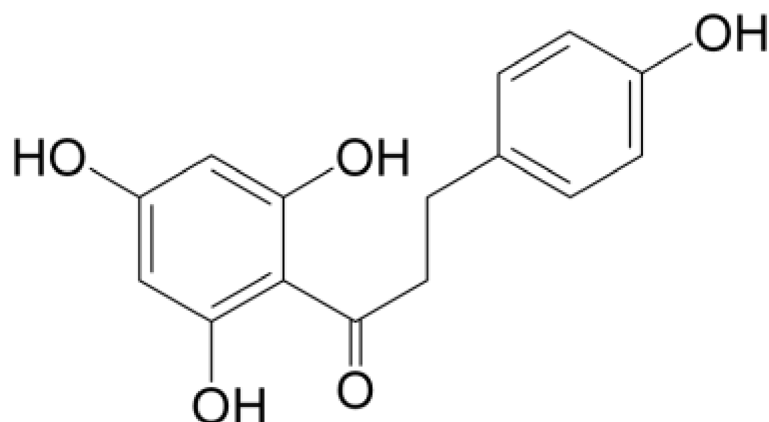
tumor cells, inhibition of enzymatic replication, cytokine activation via monocytes and macrophages, and iodination of neutrophils.[44]

In particular, polyphenols have a chemical structure that allows for attachment to keratinocytes producing a “time release” effect in the mouth working in concert with salivary antioxidants to reduce oxidative stress in the oral cavity.[33] Already, the polyphenolic antioxidants phloretin and ferulic acid have been shown to mitigate the adverse effects on oral fibroblasts caused by reactive oxygen species from nicotine, alcohol, and hydrogen peroxide. These compounds are the active ingredients found in the antioxidant gel that is examined in this study, which has been used for treatment of various oral conditions including oral lichen planus, gingival and periodontal disease.[45, 46]

Phloretin

Phloretin is found in the sap of apples, pears, and other fruits and vegetables. It has been studied for its value in skin-based drug delivery, attenuation of prostaglandins and therefore inflammation, and protection of the skin from UV radiation. Its chemical structure allows for the attenuation of reactive oxygen species (Figure 2), but has been most commonly used to inhibit lipid peroxidation. Specifically, the carbonyl side group is able to attenuate the effect of the radical oxygen species as hydrogen species can be spread over the three oxygen groups. [47]

Figure 2 Phloretin



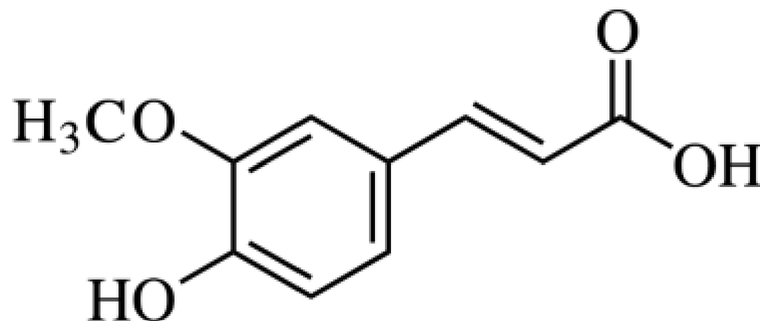
In a study by Yang *et al*, the effect of phloretin on reactive oxygen species was studied in a rat model. This study was able to show successful attenuation of peroxidase compounds by phloretin.[48] This compound has also shown anti-inflammatory effects in lymphoid and myeloid cells.[49] In the presence of phloretin, T cell proliferation can be inhibited by blocking CD69 and CD25 expression.[50] Furthermore, Devi and Das studied the effect of several plant polyphenols on normal and leukemic lymphocyte cells. Their results showed that phloretin was the second most potent polyphenol of those studied in inhibiting growth of the lymphocytic cells and the leukemic cells. Thus, the authors suggest that further evaluation of phloretin is warranted.[51]

Ferulic Acid

Ferulic acid is derived from leaves and seeds and is formed from the metabolism of tyrosine and phenylalanine. It is commonly found in foods such as tomatoes, corn, and rice bran.[52] Due to its phenol base and extended

conjugated side structure, it forms a phenoxy radical that can bond to reactive species and inactivate them (Figure 3). Ferulic acid has been used in lotions as photoprotection from UV radiation. Furthermore, ferulic acid is often used as a food additive as it prevents the peroxidation of lipids. After binding to free radicals, ferulic acid forms a quinone methide intermediate product which is excreted through the bile.[46, 52, 53]

Figure 3 Ferulic Acid



Ferulic acid's prolonged bioavailability allows it to have a long lasting effect. It has been shown to remain in the blood longer than antioxidants like Vitamin C. Ferulic acid has also been shown to control inflammation by blocking COX-2 induction. In addition, ferulic acid has demonstrated antidiabetic, anticancer, hepatoprotective, neuroprotective, radioprotective, pulmonary protective, hypotensive, anti-atherogenic, and antiapoptotic properties.[52]

Combination of Antioxidants

Carefully controlled mixtures of bioactive antioxidants have promoted the proliferation and migration of human oral fibroblasts.[46] A 2009 review reported epidemiologic, animal, and *in vitro* studies with good evidence that directly and indirectly support the potential preventive effect of polyphenols against oral cancer. Consistent studies showing that polyphenols inactivate periodontal pathogens and increase antioxidant capacity of oral fluids further suggest a preventive effect against periodontal disease.[54]

Xerostomia and Oxidative Stress

Saliva has been shown to be the first line of defense against reactive oxygen species. In a decreased saliva state, such as in medication-induced salivary gland hypofunction, reactive oxygen species such as peroxidases can flourish. Oxygen radicals mediate apoptosis thus causing oxidative damage to membrane lipids and proteins, and reducing their function. The lack of antioxidants has been implicated in tissue damage leading ultimately to oral dryness.[55, 56] Oxidative stress is also implicated in some forms of salivary gland hypofunction. Ryo, *et al* report that 8-hydroxydeoxyguanosine levels are markedly increased in Sjögren's syndrome patients, but such is not found in patients suffering solely from dry mouth.[56] A similar finding was found in a study by Norheim *et al*, in which patients with Sjögren's syndrome showed higher levels of certain oxidative stress markers including both protein carbonyl and advanced oxidation protein products. [57] A general nonsignificant trend showing

decreased values of oxidative stress have been seen over time with the use of antioxidant products in addition to increased levels of salivary flow.[56]

Oxidative Stress Marker 8-hydroxydeoxyguanosine

There is extensive experimental evidence that oxidative damage permanently occurs in lipids of cellular membranes, proteins, and DNA. In nuclear and mitochondrial DNA, 8-hydroxy-2′-deoxyguanosine (8-OHdG) or 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) is one of the predominant forms of free radical-induced oxidative lesions, and has therefore been widely used as a biomarker for oxidative stress.[58] Kasai and Nishimura first reported the formation of 8-hydroxy-2-deoxyguanosine by oxygen radicals in 1984. The goal is to trap the reactive mutagens as guanine derivatives when many mutagens react with nucleic acid bases, and particularly guanine. 8-hydroxydeoxyguanosine is a product of oxidative DNA damage following specific enzymatic cleavage after 8-hydroxylation of the guanine base. Singlet oxygen, photodynamic action, or hydroxyl radicals are responsible for the formation of 8-OHdG. The oxidized DNA is continuously modified, and the excised DNAs are excreted in the serum and urine. Thus, the levels of oxidative stress can be measured with this byproduct in serum, urine, and saliva. [59-61]

The 8-hydroxy-deoxy guanosine marker has been used as a marker for oxidative stress in several conditions such as diabetes mellitus, cancer, depression, and periodontal disease. [62, 63,60] With diabetes mellitus, the overproduction of reactive oxygen species from the mitochondria leads to an

increase in advanced glycosylated end-products and therefore increased end organ complications. In mouse models, the reduction of reactive oxygen species appears to mitigate impaired angiogenesis and wound healing effects.[64]

The onset of cancer and its progression has also been linked to damage in DNA from reactive oxygen species via DNA mutations, genome instability, and cell proliferation.[65] A similar mechanism of damage is noted in depressive disorders. Direct damage to neuronal cells of the hippocampus and frontal cortex may explain some of the degeneration associated with psychological disorders.[66]

Furthermore, in the presence of chronic inflammation, such as in periodontal disease, reactive oxygen species can accentuate damage in the tissues. A study conducted by Sezer *et al* examined at the differences between healthy patients with a normal periodontium and those with chronic periodontitis. In this study, normal salivary 8-OHdG levels were reported to be 1.56 +/- 0.12 ng/mL.[67] However, there are other studies that have reported that oxidative stress levels measured with 8-OHdG do not change in saliva.[35] Other studies report that patients with dry mouth as a result of systemic disease such as Sjögren's syndrome will produce an increase in oxidative stress. However, the healthy counterparts do not show any change in 8-OhdG levels. [68]

Based on an extensive review of the literature, there is strong evidence that free oxygen radicals play a detrimental role in the development of salivary gland hypofunction. There is also evidence that diminished levels of saliva are

associated with subtle or obvious inflammation of the oral soft tissues, which is also adversely influenced by free oxygen radicals. Evidence appears to indicate an attenuation of reactive oxygen species and increase in cell viability with treatment of antioxidants.[69] It is not known whether the perceived presence of dry mouth in patients taking medications reported to be associated with xerostomia represents a true reduction in salivary output. Therefore, it was the purpose of this study to evaluate the role of locally applied antioxidant gel therapy in management of medication-induced xerostomia. This study examined changes in salivary flow rate and ROS levels in patients with medication-associated xerostomia before and after use of antioxidant gel.

CHAPTER II

MATERIALS AND METHODS

Overall Strategy

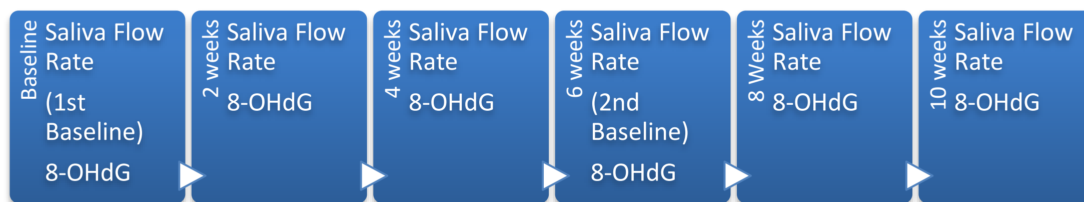
This prospective study was a nested, crossover, randomized, clinical trial testing the effect of a topically applied active antioxidant gel against a placebo in improving the quality and quantity of saliva in patients with medication related dry mouth. Changes in the function (using salivary analysis of oxidative stress marker 8-hydroxy-deoxyguanosine) and flow rates of saliva were determined at baseline, two weeks and four weeks prior to crossover and again at baseline, two and four weeks after crossover (Figure 4).

Methodology

In this study, 43 human patients with a complaint of dry mouth and medication-associated xerostomia were screened. For the purpose of this study, a patient with “medication-associated xerostomia” was defined as a subject who has been using at least one systemic medication for at least a year that has been reported to cause xerostomia as a side effect, and who does not have a history of Sjögren’s syndrome, sarcoidosis, head and neck radiation therapy, and other uncontrolled systemic diseases. Upon enrollment, each subject provided a salivary sample. Each participant was given a gel to use at home, blinded to which is the active gel or inactive placebo. At the initial visit, informed consent was obtained, saliva collected, and instructions given to use a pea sized

amount of the gel three (3) times per day: after their normal oral care regimen in the morning, mid-day after eating, and just before bed. Patients were instructed to apply the gel directly with their finger to the intraoral tissues. Patients returned to Texas A&M University College of Dentistry (TAMUCOD) at 2 weeks and 4 weeks after the initial meeting for saliva sampling. Upon completion of 4 weeks of using the active gel or placebo, all patients stopped using any gel product and continued only with their normal oral care regime for a period of 2 weeks (washout period). At the conclusion of the two-week washout period, patients were required to visit TAMUCOD to provide salivary samples to establish a new baseline at week 6. At that visit, they were given the second gel and instructed to resume the same usage schedule of three (3) times per day. Participants were asked to return to TAMUCOD at 8 weeks and again at 10 weeks to provide saliva samples (Figure 4).

Figure 4 Study Schedule



The gel products were arranged in numerical order from 1 to 43 marked as either A or B. Therefore, the gels were marked as 1A, 1B, 2A, 2B, 3A, 3B and so on. The A, B code was randomized with respect to the active gel versus

placebo such that neither the patient nor investigator knew if a given patient's A or B is the active or placebo gel. Furthermore, it was unknown how many patients had the active gel or placebo during the pre- or post-crossover period. Patients were assigned the number on their product series; e.g., John Smith has product 2A and 2B, so John Smith was referred to as "2" throughout the study to ensure privacy. Patients did not undergo dentist/hygienist dental prophylaxis, debridement, or scaling and root planing during the trial period and were instructed to use their standard oral care regime. Patients were also instructed to continue all medications including those that may cause xerostomia**.** Patients received the following for participating in the study: $15 per visit to cover travel expenses and time from work.

a. *Patient Population*

45 patients were screened for the study. At the initial visit, patients consented to participate in the study and provided a salivary sample for baseline salivary flow rate calculation. Past medical and dental records were reviewed to determine probable cause of xerostomia. Patients with xerostomia likely due to medication were admitted into the study as indicated by their current use of medications known to have dry mouth as a side effect. The saliva sample was used for further evaluation of the oxidative stress marker- 8-hydroxydeoxyguanosine. The selection criteria included:

Inclusion criteria:

- Age: 18-85

- Both men and women of all ethnicities
- Systemic conditions: generally healthy, ASA I or II
- A patient who has been using at least one systemic medication that has been reported to cause dry mouth as a side effect for at least one year

Exclusion criteria:

- Smokers
- Pregnant women
- Patients with history of head and neck radiation treatment or recent chemotherapy
- History of salivary impairments such as salivary stones or previous salivary gland surgeries due to neoplasm or sialolithiasis
- History of:
    - Primary billiary cirrhosis
    - Sarcoidosis
    - Diabetes
    - HIV
    - Sjögren's syndrome
- Patients with an allergy to any of the following ingredients: phloretin, ferulic acid, thyme, sage oil, clove flower oil, xylitol

b. *The Active Gel*

The active gel, branded by PerioSciences as AO ProVantage, contains compounds generally recommended as safe by the FDA, and is currently sold in

dental offices along with an antioxidant-containing toothpaste and mouthwash. The base gel includes water, xylitol, propylene glycol, PEG 12, sorbitol, poloxamer 407, cellulose gum, potassium sorbate, menthol, thymol, spllanthes acmella extract, sodium hyaluronate, caprylic/capric triglyceride, sodium chloride, sodium citrate, disodium EDTA. The active gel additionally contains the antioxidants phloretin and ferulic acid.

The gel was launched as a cosmetic in 2010 after completion of a six-week safety study with 100 patients. While the gel is extremely well tolerated and many practitioners use the gel with xerostomia conditions, no medical claims have been made in marketing the AO ProVantage. Anecdotal evidence provided by dentists indicates that AO ProVantage provides symptomatic relief from dry mouth that is superior to other remedies on the market.

c. *Study Organization*

PerioSciences provided Texas A&M University College of Dentistry the active gel and placebo gel in identical containers. The Thesis Committee conducted regular meetings to monitor progress, solve problems, ensure proper recruitment and retention of patients, ensure proper treatment protocol, address potential adverse events, and review data management.

d. *Patient recruitment plan*

Patients who participated in the study were registered patients of TAMUCOD. Patients who consented to participate in the study physically visited TAMUCOD for evaluation, saliva collection and xerostomia assessment.

Informed consent was obtained from participating patients before admission to the trial. The informed consent document was approved by the TAMUCOD Institutional Review Board (IRB). A fully executed copy of the consent document was provided to the participant and the original maintained by the principal investigator.

e. *Saliva Collection*

Patients were asked to supply a saliva sample during each visit including the initial visit. Patients were asked to provide this sample before eating, drinking, brushing, or rinsing with anything after waking up. The sample was collected without stimulation which took place at the beginning of the visit by having the patient spit into a sterile container until 5 mL of saliva was obtained or 30 minutes of spitting was achieved. Saliva was stored frozen at TAMUCOD, measured to determine salivary flow rate, and tested for 8-hydroxy-deoxyguanosine levels.

f. *Measurement of 8-OHdG*

Each assay kit included a 96 well plate in which two blanks, two total activity, two nonspecific binding, two maximum binding, two sets of standards, and 24 double saliva samples. Fifty µL of DNA/RNA Oxidative Damage AChE tracer and Monoclonal Antibody were added to the saliva samples. The plate was incubated for 18 hours. The plate was then developed with Ellman's Reagent and then read at a wavelength between 405 and 420 nm. The data for

the standard curve was plotted and compared to the sample results to determine 8-OHdG levels between visits.

g. *Gel Usage*

The gel tubes were weighed at every visit to monitor the quantity of the gels throughout the study on a calibrated scale. These values were recorded to ensure compliance and regular usage.

Data Collection and Analysis

a. *Overview*

All saliva specimens were stored frozen in a -80º Celsius freezer at TAMUCOD for 8-hydroxy-deoxyguanosine analysis. The samples were centrifuged at 1250g for 10 min at 4 degrees Celsius prior to freezing.

b. *Data collection*

Forms were organized for each patient visit. Each form had space for the following identifiers: patient number, patient initials, treatment type, and visit number. Treatment/measurement performed was recorded on the form. After completion of a visit, investigators checked each form for accuracy and completeness. The information from the forms was then uploaded into a computer, producing primary and secondary data files. The data entry procedure was designed to allow only codes listed on the form and values in the expected format to be entered. Reports were developed to list completion status, exits, and forms for subsequent statistical analysis.

c. *Confidentiality issues*

Confidentiality of patient data was an important consideration. The biostatistician did not receive the patient's name or any identifier such as medical record number or SSN. Copies of data forms were stored in a locked file cabinet in the office of the Principal Investigator.

d. *Hardware, software, security*

The software platform for the study was Microsoft ® 2000. Programs and data resided on a computer at TAMUCOD. The computer was located in a room with restricted access. In addition to passwords necessary to log into the computer and receive access to the database directory, security limited entry to the database to only specific users via password. The study database was backed up to a zip disk or CD periodically. The computer was protected with an uninterruptible power supply (ups).

e. *Statistical and power analysis*

For this study, 43 patients were recruited, and using a within patient standard deviation of 12 ng/mL power of .8, and a type I error rate of 0.05, a difference between treatments of 36 units was considered significant. As this patient population was considered to be highly motivated, a 20% drop-out rate was assumed, therefore up to 45 patients could be recruited for this study.

f. *Summary of descriptive statistics*

For normally distributed, continuous data, means and standard deviations were used to describe the demographic and clinical characteristics of the patient population participating in the study. Active gel and placebo groups were

compared using a paired t-test. Further analysis of subjects with salivary flow rates below 0.200 mL/min was completed. Analysis for subjects with salivary hypofunction and flow rates below 0.100 mL/min was also completed.

g. *Description of analysis for primary hypothesis*

The primary hypothesis of the study was that the active gel will decrease 8-OHdG in saliva compared to placebo. A linear mixed model statistical analysis was used to examine the outcome variable 8-OHdG. The between factor was the two treatment groups (active gel versus placebo gel), while the within factor was the time measurements made (baseline, 2 week, 4 week, 6 week, 8 week and 10 weeks).

h. *Description of analysis for secondary hypotheses*

The secondary hypothesis was that active gel provides better treatment outcomes than placebo included:

- Salivary flow rate increases with the use of the active gel. In this analysis the time measurements included baseline, week 2, week 4, week 6, week 8 and week 10.

i. *General Statistical Issues*

- IBM SPSS V20 was used to analyze these data.
- All tests, unless otherwise noted, were performed using $p < 0.05$.
- Treatment of missing data- the following procedures were used to account for the effects of missing data on the analysis:

- No variables were included in the analyses that had more than 10% of the values missing. Variables with values missing 10% of the time or more do not occur by chance, and there are systematic causes for missing data at frequencies at this level. Analysis strategies include performing the analysis using the following strategies:
    - Use of MIXED. In MANOVA, ANCOVA, or ANOVA models, missing data have historically caused serious statistical analysis problems. Missing data, when using a mixed models approach (as MIXED in SPSS), is not as serious a problem. In a mixed models approach the patient is considered randomly chosen from a larger group of subjects. These models have been found to be tolerant of missing data as long as the missing data are random.

d) *Check of assumptions*

For all analyses performed, each statistical test was checked to verify that the assumptions for each were satisfied and, if not, appropriate transformations (e.g., log transformations for counts, arcsine square root transformations for proportions, etc.) were performed before analysis. Non-parametric analyses were used where indicated.

e) *Type I error rates*

Multivariate analysis of variance was used, wherever feasible, rather than individual analysis of covariance adjusting for Type I error rates using Bonferroni corrections.

CHAPTER III

RESULTS

Of the 45 subjects screened, 43 subjects consented to participate in the study. Two subjects were excluded as one did not meet the inclusion criteria and the other denied being interested in participation into the study. Seven subjects were lost to drop out. Three subjects reported scheduling conflicts and were unable to adhere to the scheduled 2-week interval appointments. One subject was excluded as he began smoking cigarettes. One subject reported contracting influenza and was unable to adhere to the 2-week appointment schedule. One subject reported a flare up of lichen planus and discontinued use of the gel. One subject reported an allergic reaction consisting of edema and erythema of the lips. Subject's symptoms subsided immediately after discontinuing use of the gel. Thirty-six subjects completed the study.

After completion of the study, the investigators were informed of the code for the placebo and active gels. At that time, it was determined that 21 subjects began with the active gel and 15 subjects received the placebo gel first. This led to the creation of the A-P (active-placebo) and P-A (placebo-active) groups consisting of 21 and 15 subjects, respectively (Figure 5). The A-P group had 7 males and 14 females with an average age of 57±19.01 years. The P-A group had 4 males and 11 females with an average age of 61.62±10.52 years (Figure 6). The A-P group had an average usage of 11.2 g +/- 5.7 g usage per gel tube.

The P-A group had an average usage of 11.7g +/- 7.3 g. Thus, each group used similar amounts of gel per visit (Table 3).

Figure 5 Study Outline

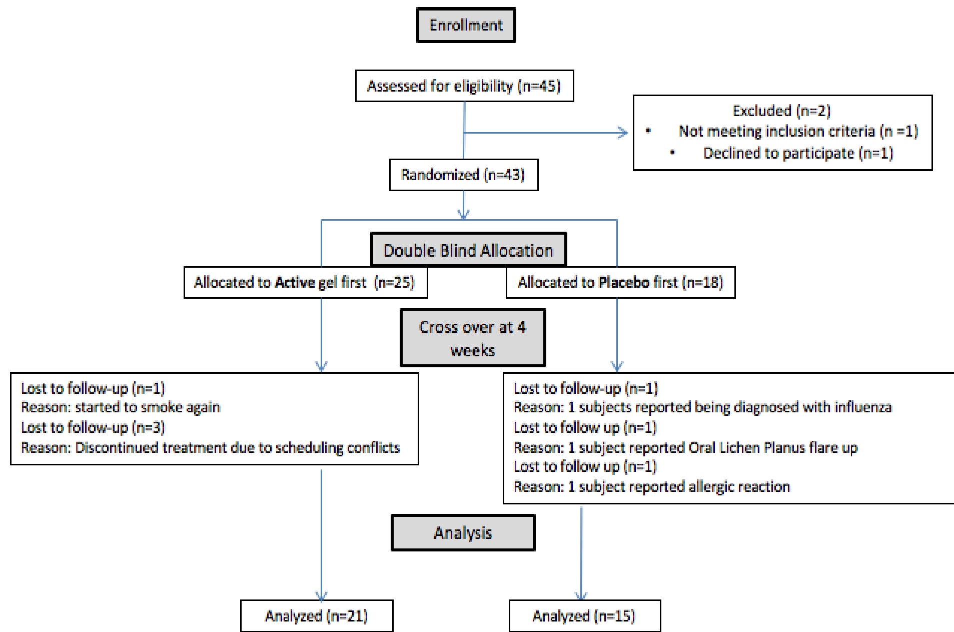


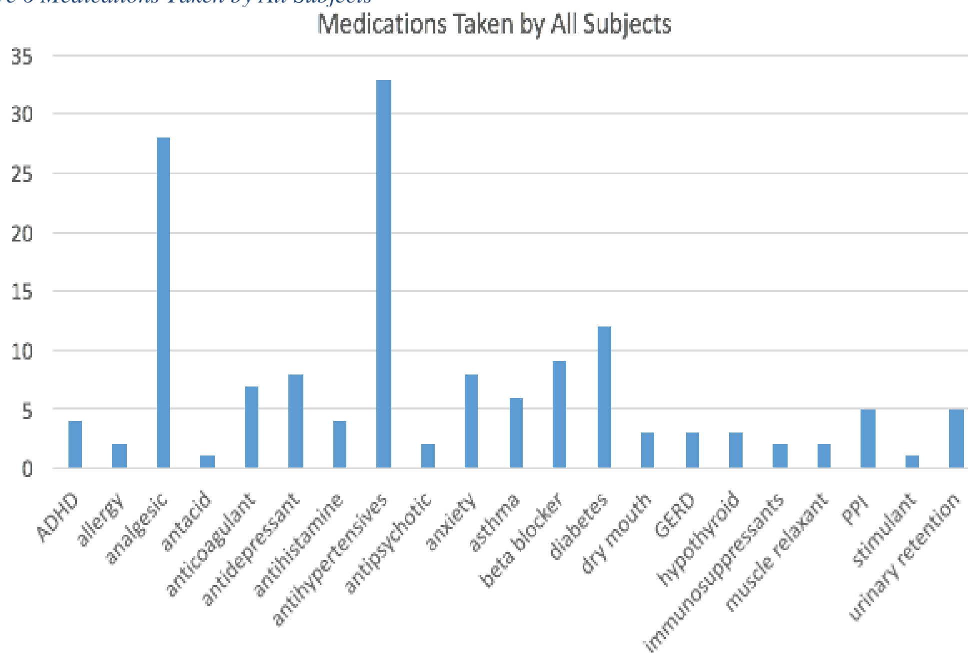
Table 3 Patient Demographics

Parameter	A-P	P-A
No. of patients	21	15
No. of dropouts	4	3
Average Age	57.00±19.01	61.62±10.52
Gender	7 M, 14 F	4 M, 11 F
No. of smokers	0	0
No. of diabetic patients	6	5
Hba1c	6.9%	6.7%

Medications Taken

All medications taken by the subjects were recorded. The largest classes of medications used by these subjects were analgesics and antihypertensives as can be noted in Figure 7. Among the analgesics, the majority of these medications included narcotic analgesics. The antihypertensives mostly contained ACE inhibitors and calcium channel blockers.

Figure 6 Medications Taken by All Subjects



8-hydroxydeoxyguanosine

The mean 8-OHdG values from each visit are listed in Table 4. A percent binding was calculated by determining the sample binding/maximum binding (B/B0) as per manufacturer's guidelines for statistical and analytical purposes. At

baseline, the A-P group has an average value of 23.16±16.91 and the P-A group had an average value of 19.85±12.44. At week 2, the A-P group had an average value of 21.98±17.08 and the P-A group had an average value of 23.87±20.46. At week 4, the average for the A-P groups was 23.16±17.73 and 19.80±15.63 for the P-A group. The time between week 4 and 6 was a washout period where subjects did not receive any gel treatment. Week 6 marks the beginning of the crossover. The average baselines for the A-P and P-A groups at week 6 are 25.14±16.37 and 21.60±18.77, respectively. The week 8 average for group A-P is 21.81±18.00 and the average for group P-A is 22.60±16.07. The week 10 averages for group A-P and P-A are 22.91±19.79 and 22.78±16.41, respectively. There were no statistically significant differences found between the groups' 8-OHdG values over time (Figures 8,9).

Table 4 Average 8-OHdG Measurements

8-OH-dG (B/B_0)	**A-P (21)**	**P-A (15)**
Baseline	23.16± 16.91	19.85±12.44
2 Weeks	21.98±17.08	23.87±20.46
4 Weeks	23.16±17.73	19.80±15.63
6 Weeks	25.14±16.37	21.60±18.77
8 Weeks	21.81±18.00	22.60±16.07
10 Weeks	22.91±19.79	22.78±16.41

Figure 7 Average Oxidative Stress Levels Over Time

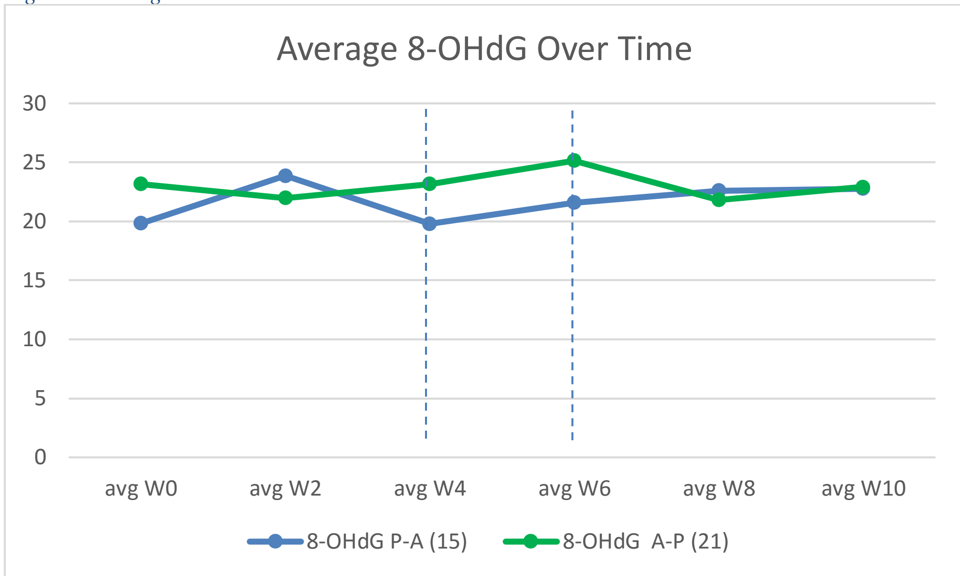
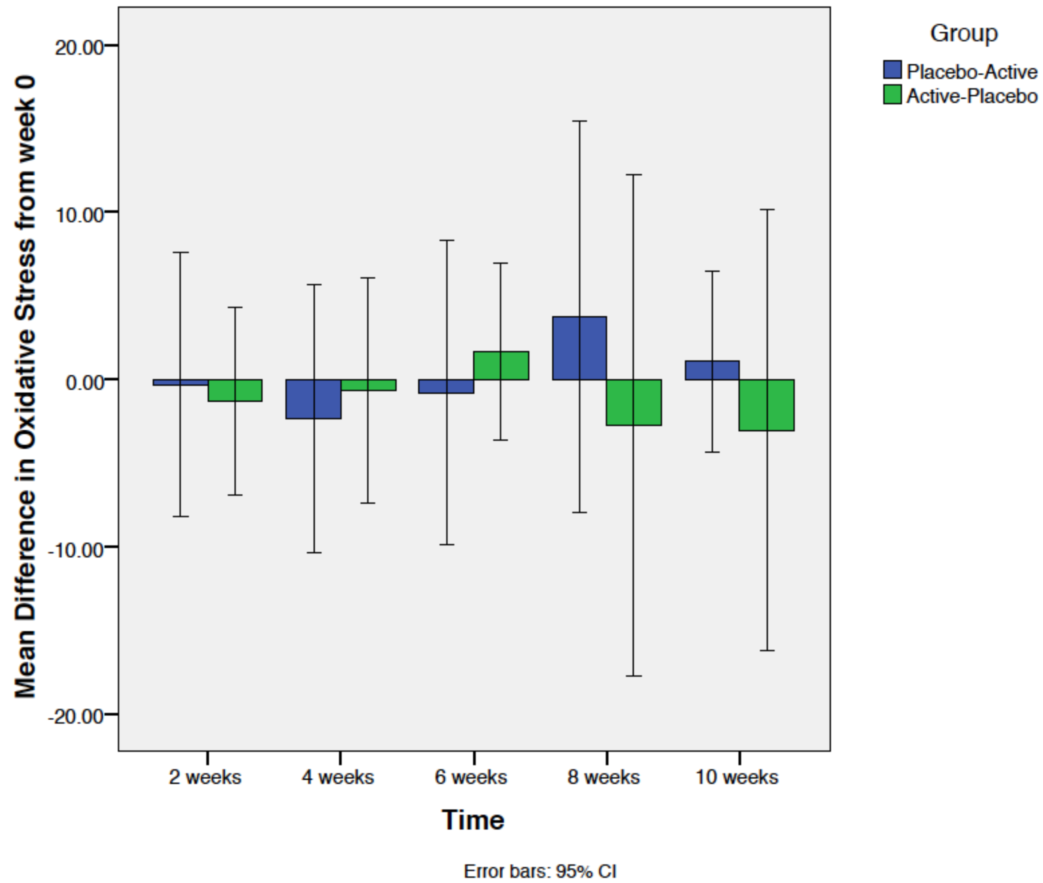


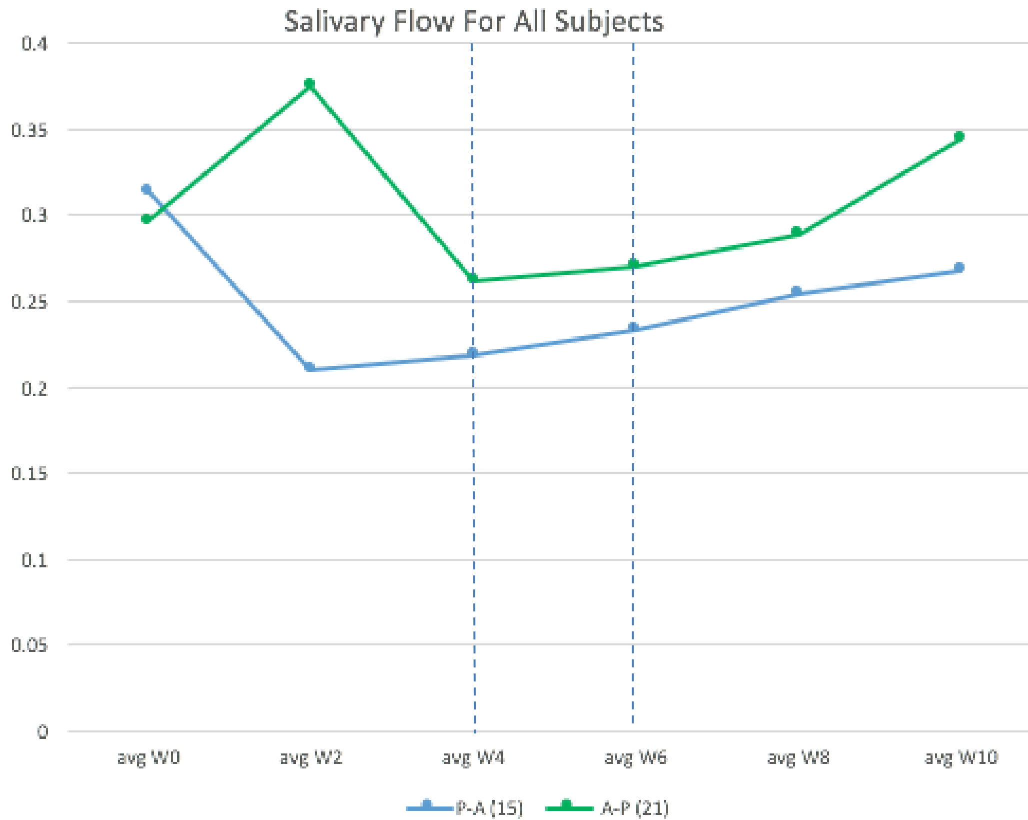
Figure 8 Oxidative Stress Levels



Salivary Flow Rate

Subjects who used the placebo gel first experienced a decrease in saliva and a gradual increase in saliva after switching to the active gel. Subjects who used the active gel first experienced an initial increase in saliva. However, there was a decrease in salivary flow from week 2 to 4. In group A-P, there appears to be an increase in salivary flow with use of the placebo gel. Using mixed model analysis, no statistically significant difference was found (figure 10).

Figure 9 Salivary Flow Rates in All Subjects



Analysis Related to Reduced Salivary Flow Rates

Further analysis using mixed models was completed to study the effect of the gel on patients with reduced salivary flow rates less than 0.200 mL/min. 20 subjects had salivary flow rates below 0.200 mL/min. 12 of these subjects were in the active-placebo group and 8 subjects were in the placebo-active group. Figure 11 displays the change in salivary flow from the initial baseline visit over the course of the study. Subjects who began using the active gel as the initial treatment had increased flow rates over the course of the study.

Patients who used the placebo had a decrease in salivary flow. After using the active gel, salivary levels increased but were not able to reach the initial baseline levels.

Salivary flow decreased during the washout period after use of the placebo gel.

This data suggests that the active gel may have a sustained release effect as salivary flow rate levels did not decrease with placebo use. Figure 11 further demonstrates the difference between the active-placebo group and placebo-active group. The cumulative difference in salivary flow rates in the two groups was statistically significant ($p<.05$).

Figure 10 Difference in Salivary Flow Rates from Baseline

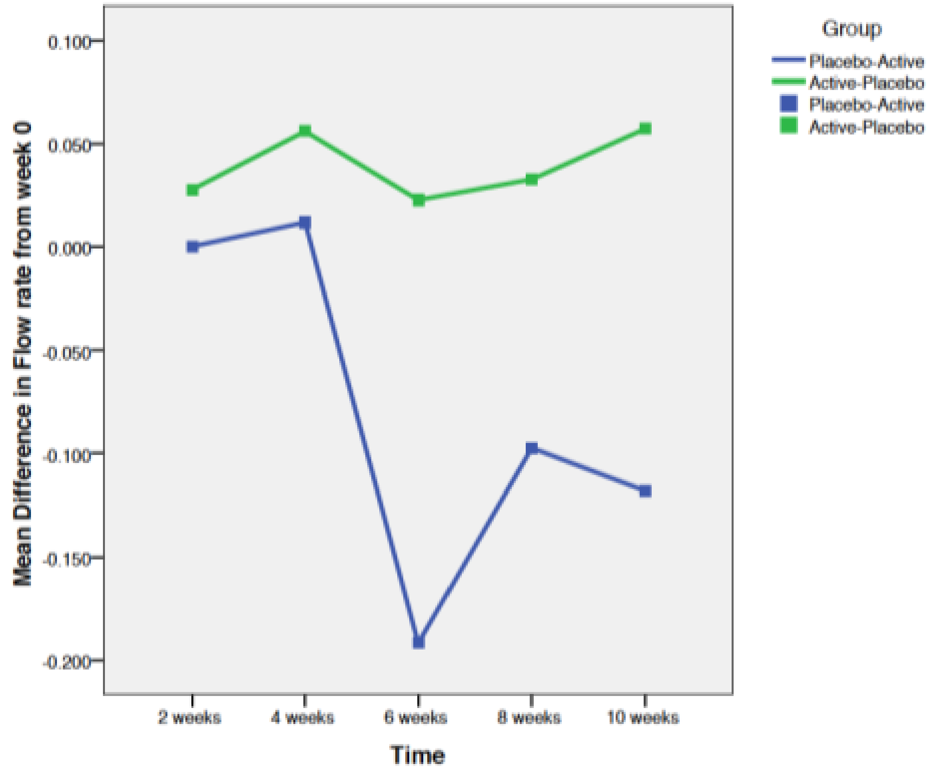


Figure 11 Overall Differences in Flow Rates

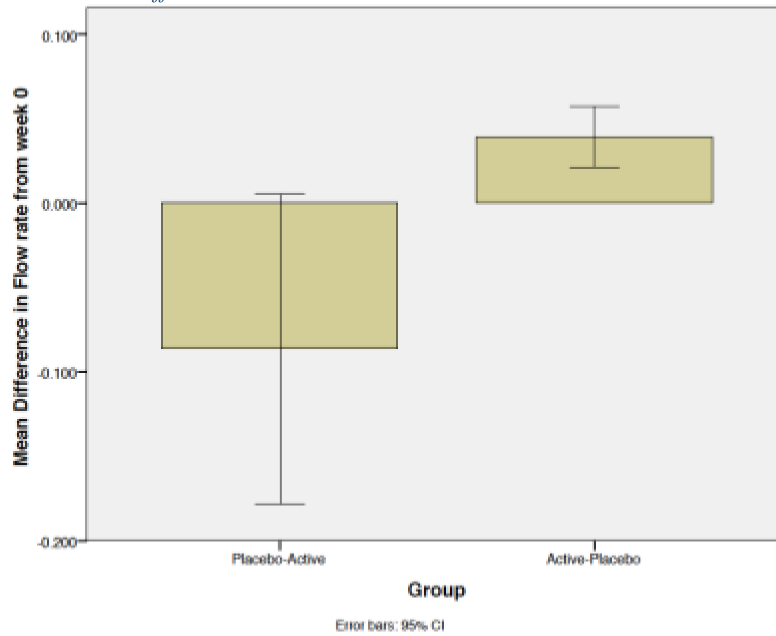
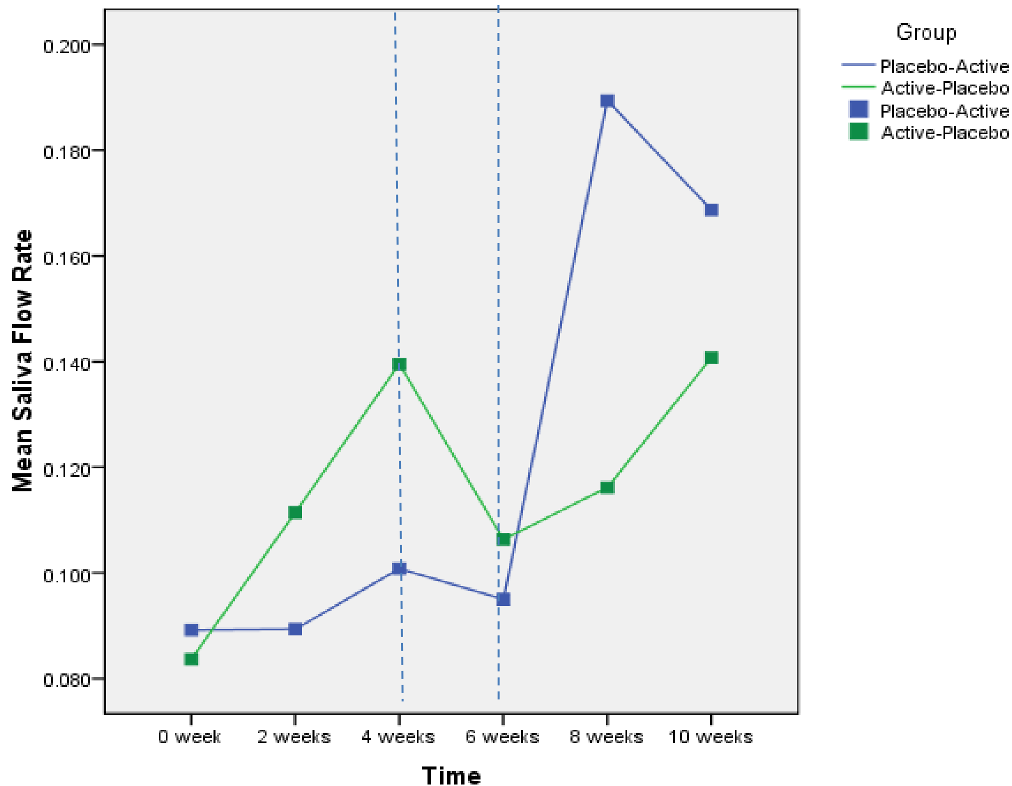


Figure 12 displays the average flow rates as opposed to the difference from baseline as in previous graphs. Subjects who used the active gel first had a steep increase in salivary flow, but decreased during the washout phase. Use of the active gel may have had a lingering effect thus producing an increase in salivary flow with placebo use. Subjects who used the placebo first did not show

much of a change in salivary flow. However, once the subjects crossed over to the active gel, salivary flow rates increased.

Figure 12 Mean Salivary Flow Rates Over Time



Furthermore, baseline measurements were compared in this reduced salivary flow subset as seen in Figure 13 and Table 5. The first baseline was measured at week 0 and the second baseline was measured at week 6 after the 2-week washout period. There was an increase of 0.023 mL/min in the active-placebo group and an increase of 0.04 mL/min in the placebo-active group.

Therefore, subjects had 5.75 times more saliva at their second baseline visit versus their initial baseline visit after using the active gel first.

Figure 13 Comparing Difference in Salivary Flow Baselines in A-P and P-A Subjects

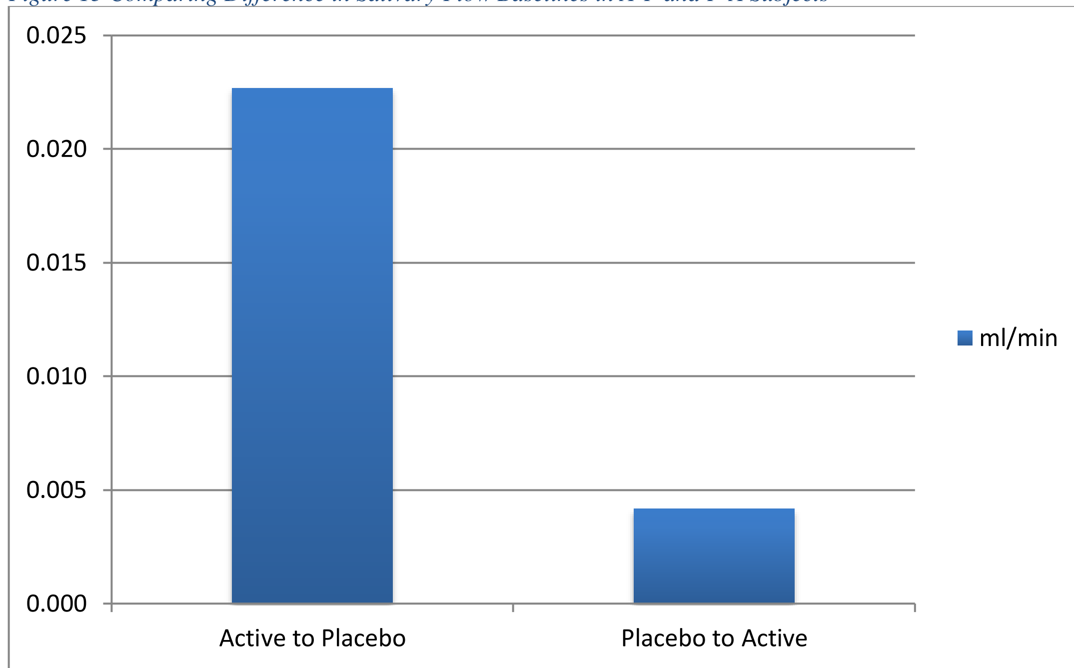


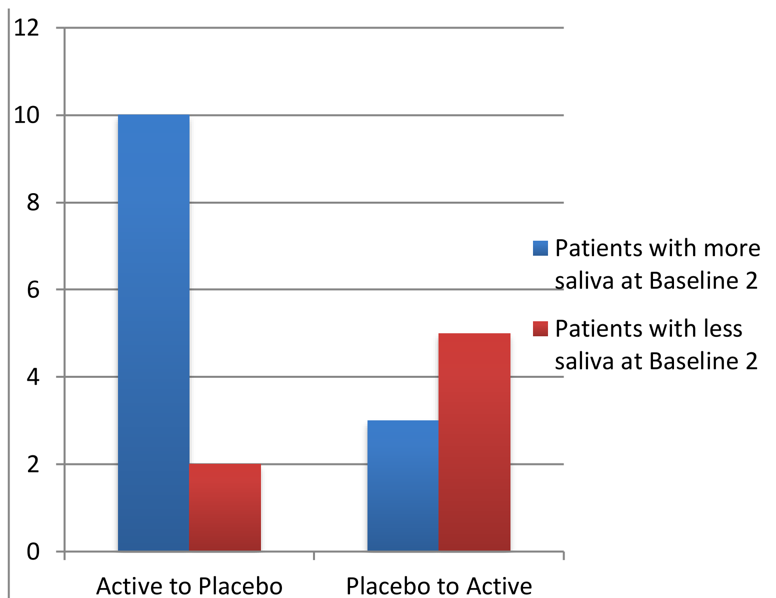
Table 5 Change in Baseline Salivary Flow Rate Values

	Active to Placebo	Placebo to Active	
Average saliva measurment at first baseline	0.084	0.089	ml/min
Average saliva measurment at second baseline	0.106	0.093	ml/min
Increase/decrease from 1st to 2nd baseline	0.023	0.004	ml/min
Percent Increase / decrease	27.1%	4.7%	

Upon evaluation of the difference in baselines, there were more subjects in the active-placebo group that had increased second baselines at week 6.

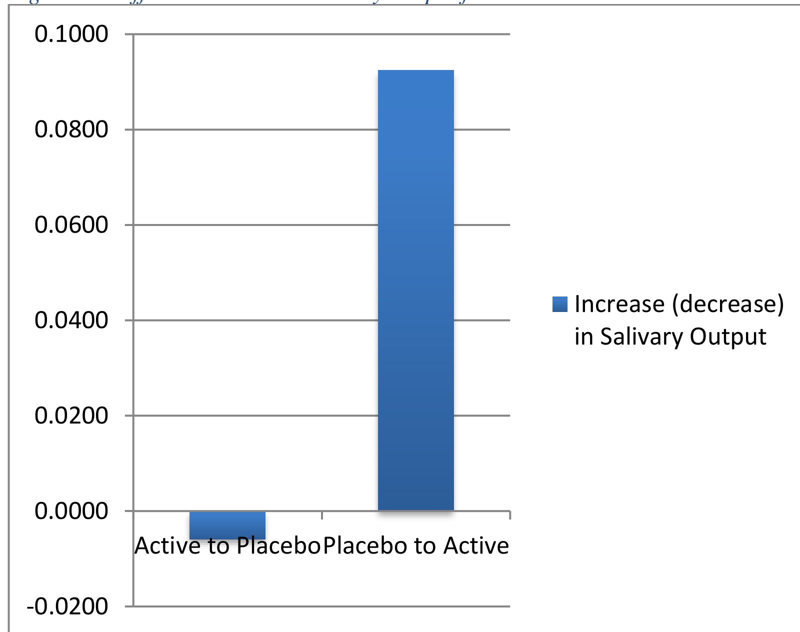
There were 10 subjects in the A-P group with increased baselines and 2 subjects with decreased second baselines. The placebo-active group had 3 subjects with increased baselines and 5 subjects with decreased baselines as depicted in Figure 14.

Figure 14 Comparing Baselines of Subjects in Groups A-P and P-A



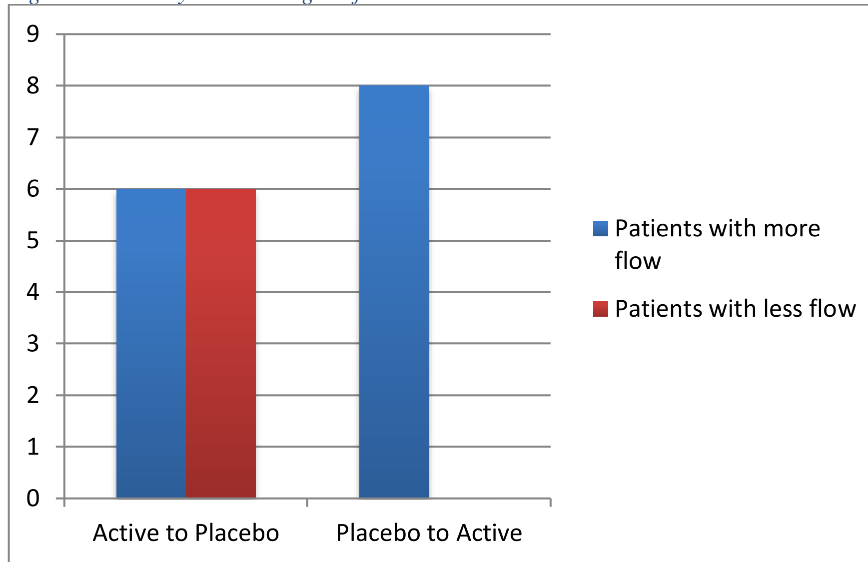
When evaluating the salivary output for weeks 8 and 10, subjects who began with the active gel during the first half of study had an average decrease of 0.0059 mL while using the placebo gel. However, subjects who first received the placebo gel had an average increase of 0.0924 mL after switching to the active gel as seen in Figure 15.

Figure 15 Difference Between Salivary Output for Active and Placebo Gel Use



When comparing total salivary output (p1 + p2) during the active gel phase compared to total saliva output (p1 + p2) during the placebo phase, all 8 patients that used the placebo first had more saliva in the active phase. However, when the active was given first 6 of the 12 patients (50%) had less salivary output and 6 subjects had increased salivary output in the placebo gel phase as seen in Figure 16.

Figure 16 Salivary Flow Changes After Crossover



The data was then split into data sets consisting of the two groups: active and placebo. A data set is defined as a base line saliva sample followed by a period 1 (week 2 or week 8) sample and a period 2 sample (week 4 or week 10). Each patient provided two data sets- one with active gel use and the other with placebo gel use. Figure 17 shows the number of data sets exhibiting varying percent changes from baseline salivary flow rates (week 0 or week 6) to the period 1 sample. One active gel data set had an increase of 1120% from baseline. There were 17 active and 13 placebo data sets that had an increase in salivary flow rate. There were 7 data sets in both the active and placebo groups that exhibited decreased salivary flow rates from baseline. The range of change

in salivary flow rate for the active data sets is -0.099 to 0.307 mL/min and -0.082 to 0.104 mL/min for the placebo data sets in period 1.

Figure 17 Percent Change in Salivary Flow from Baseline to Period 1

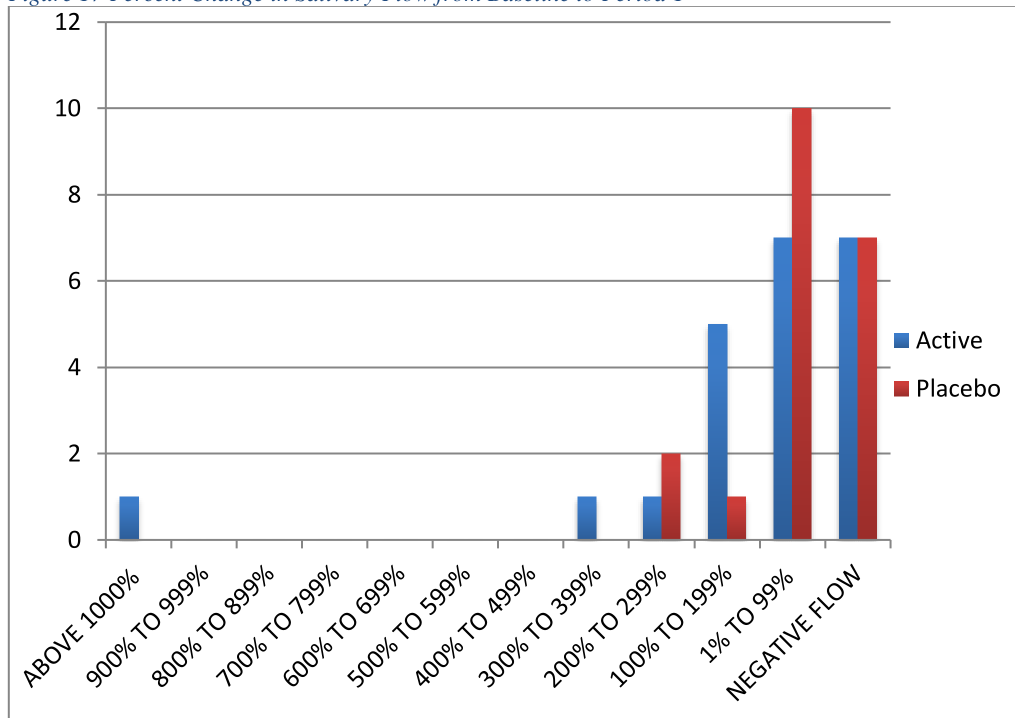
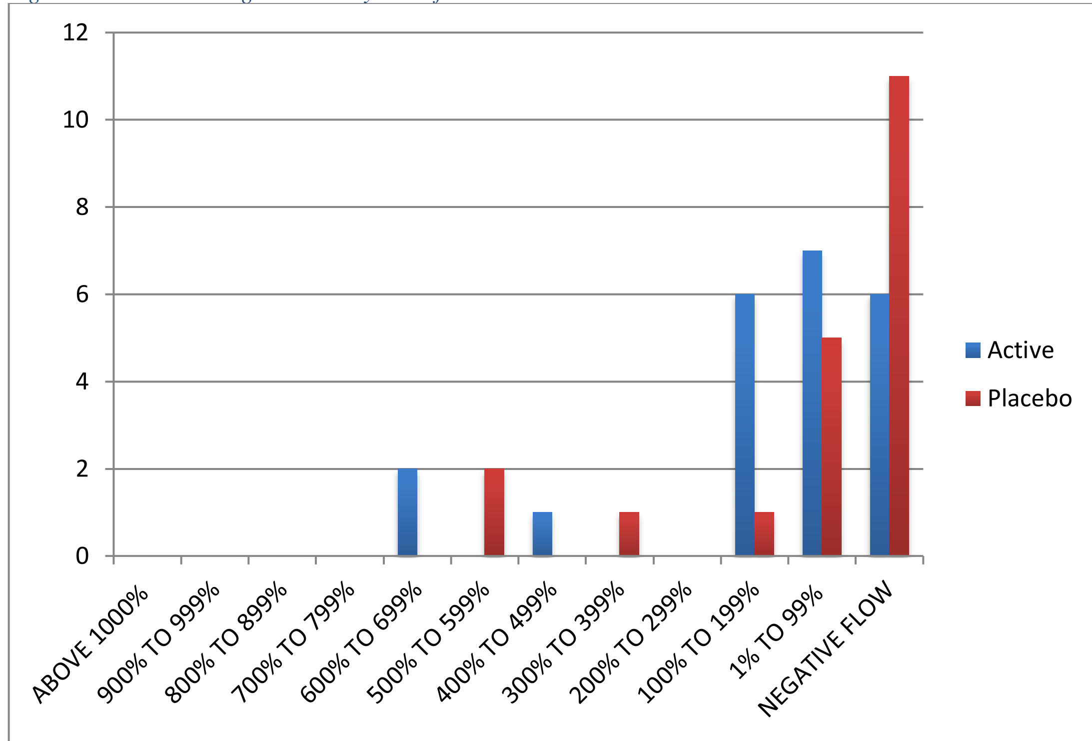


Figure 18 shows the number of data sets exhibiting the varying percent changes from baseline to the period 2 measurement. 16 active data sets had an increase in salivary flow rate. 9 placebo data sets had an increase in salivary flow rates. 6 active data sets had a decrease in salivary flow rate from baseline and 11 placebo data sets had a decrease in salivary flow rate. The range of the salivary flow rate changes for period 2 for the active data sets is -0.027 to .369

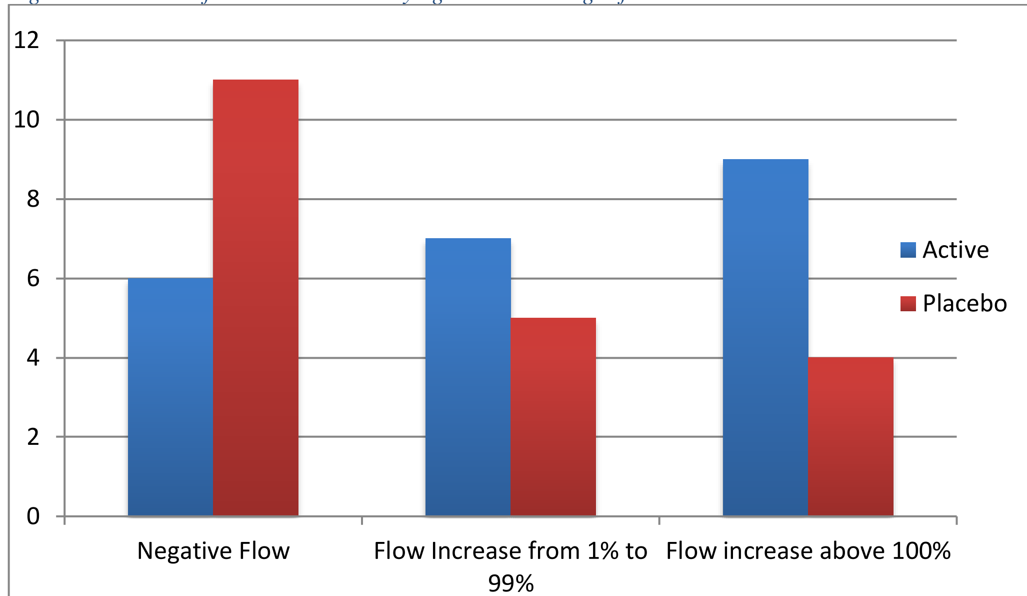
mL/min. The range of salivary flow changes for the placebo data sets in period 2 is -0.095 to 0.198 mL/min.

Figure 18 Percent Change in Salivary Flow from Baseline to Period 2



As seen in Figure 19, with long term use (period 2 over baseline), there were 6 active data sets and 11 placebo data sets with decreased flow rates from baseline to period 2. There were 7 active data sets and 5 placebo data sets with a salivary flow increase between 1 and 99%. Lastly, there were 9 active data sets and 4 placebo data sets that had salivary flow rate increases greater than 100%.

Figure 19 Number of Data Sets With Varying Percent Changes from Baseline to Period 2



Analysis of Patients with Salivary Hypofunction

Further analysis was completed for subjects having baseline salivary flow rates below 0.100 mL/min, otherwise known as salivary hypofunction. 12 subjects qualified to be in this subset. 5 of 12 were in the Placebo-Active group and 7 were in the Active-Placebo group. The findings mimicked those from the analysis of the subjects with salivary flow rates below 0.200 mL/min.

Upon evaluating the baselines of the A-P and P-A groups, subjects had 4.54 times (0.029/0.006) more saliva at their second baseline measurement compared to the first baseline measurement after using the active gel compared to use of placebo gel as seen in Figure 20 and Table 6.

Figure 20 Difference in Baselines (week 0 and week 6)

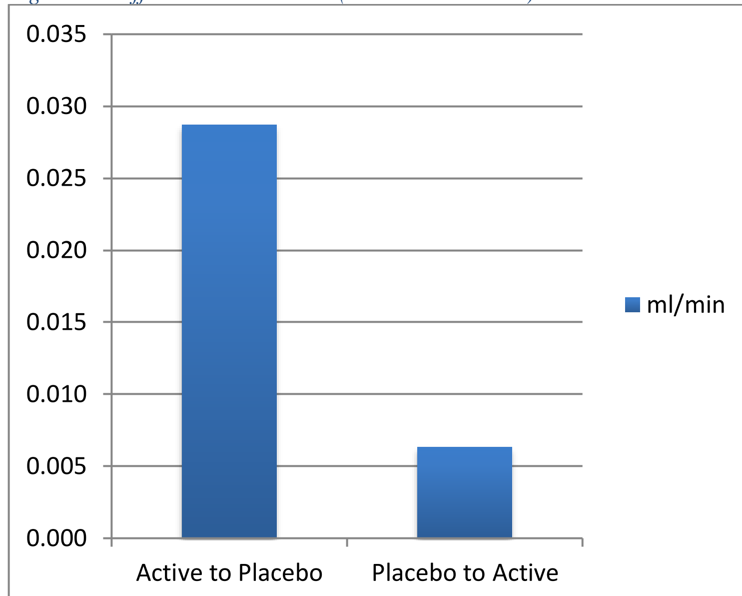
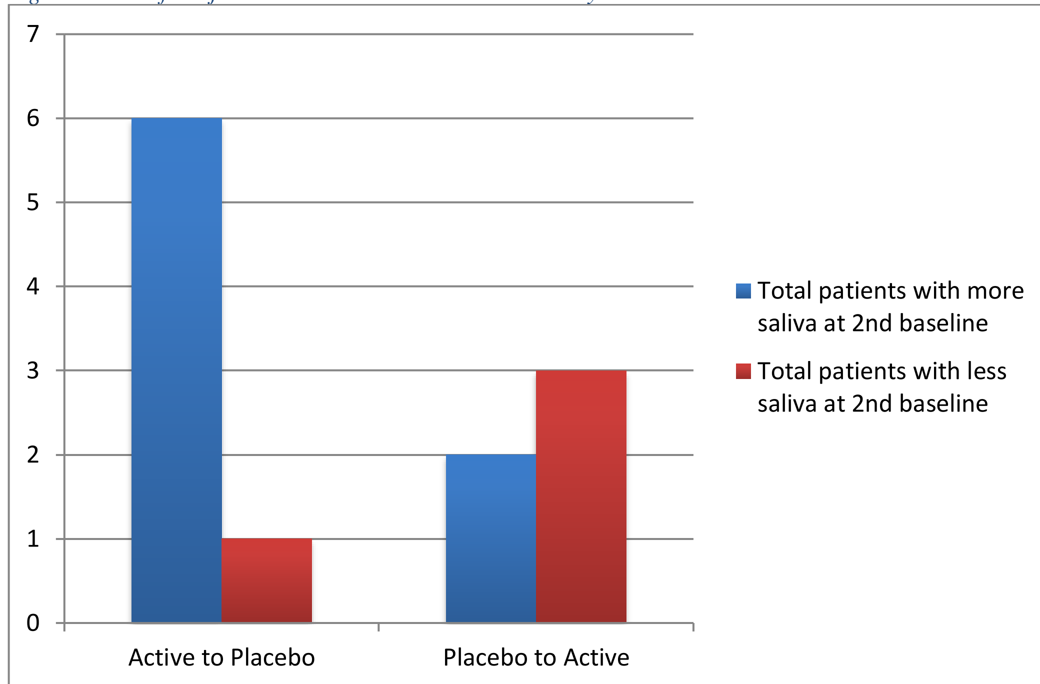


Table 6 Change in Salivary Flow Rate Baseline Values

	A-P	P-A	
Average saliva measurement at first baseline	0.044	0.048	ml/min
Average saliva measurement at second baseline	0.073	0.054	ml/min
Increase/decrease from 1st to 2nd baseline	0.029	0.006	ml/min
Percent Increase / decrease	65.1%	13.2%	

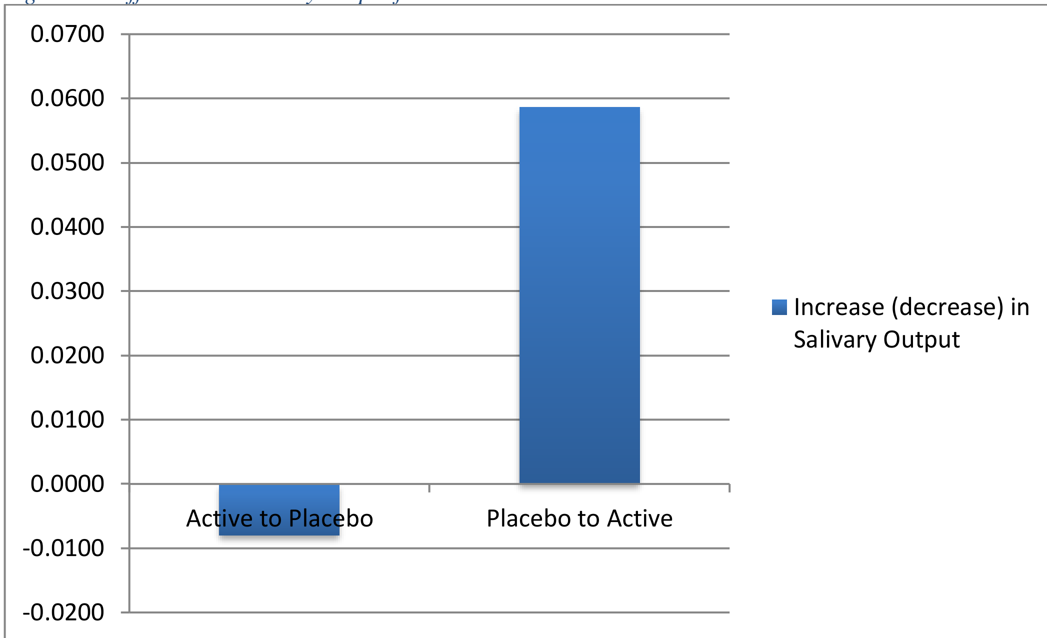
Specifically, 6 of the 7 Active-Placebo subjects had an increase in salivary flow rates from their initial baseline while one subject had a decrease in salivary flow rate between baseline measurements. In the Placebo-Active group, two subjects had an increase between baseline measurements and three subjects had a decrease between baseline visits as seen in Figure 21.

Figure 21 No. of Subjects with Increased/Decreased Salivary Flow rates at B2



When evaluating the salivary output for weeks 8 and 10, subjects that began with the active gel during the first half of study had an average decrease of 0.01 mL while using the placebo gel. However, subjects who first received the placebo gel had an average increase of 0.06 mL after switching to the active gel as seen in Figure 22.

Figure 22 Difference in Salivary Output from Active Gel to Placebo Gel



CHAPTER IV

DISCUSSION

The results of this study fail to support the hypothesis that oxidative stress levels are reduced with the use of the active antioxidant gel product. There does not appear to be a relationship with oxidative stress levels measured with 8-OHdG and use of the active gel or placebo gel. Results indicate an increase in salivary flow rate with the use of the active antioxidant gel. There appears to be a lingering effect of the active gel in subjects even after the wash out period as seen in the elevated second baseline values. Upon evaluation of the nested subgroup of participants with salivary flow rates below 0.200 mL/min, a statistically significant difference was found between the active-placebo and placebo-active groups. The active-placebo group had significantly increased salivary flow rates compared to the placebo-active group.

Currently, another study examined quality of life parameters associated with xerostomia symptoms in the same patient pool. A visual analog scale was used to determine the levels of difficulty speaking, difficulty swallowing, decreased saliva in the mouth, dry mouth, and dry throat on a scale of 1 to 10 in which 1 equates with rare occurrence of these symptoms and 10 means constant awareness of these symptoms. Qualification of xerostomia is a subjective finding, and thus utilization of such surveys allows monitoring of the xerostomia symptoms.[24] Additionally, a gel satisfaction survey was used with a

Likert scale to determine the patient perceived effectiveness of the gel. Patients were asked to rate the following parameters as very satisfied, satisfied, neutral, unsatisfied, and very unsatisfied:

- Time it took to feel relief after using the dry mouth gel.
- Confidence in breath after using the dry mouth gel.
- Ability to eat after using the dry mouth gel.
- The soothing effect in the mouth after using the dry mouth gel.
- The ability to sleep through the night after using the dry mouth gel.

These qualitative parameters allowed for an assessment of the xerostomia conditions in the subjects. The only parameter that was statistically significant over time was the soothing effect of the gel. Patients from both the placebo-active and active-placebo groups reported a sooth effect of the gel over time. All other qualitative parameter differences were not statistically significant. The current study focuses on the quantitative analysis of salivary flow and oxidative stress levels in this xerostomic subject pool.

8-hydroxydeoxyguanosine

This study was unique in that it looked at the oxidative stress levels in relatively healthy subjects in that none of the participants reported uncontrolled systemic diseases. Salivary levels of 8-OHdG have been studied in subjects with a history of head and neck radiation, Sjögren's, diabetes, periodontitis, and several other diseases.[35-40] Oxidative stress levels are elevated in the

aforementioned disease states. However, studies have shown that healthy controls do not display an increase in oxidative stress levels.[67] Such was the case seen in our study. There was great variability in the values and trends in the data were not found. 8-OHdG levels were reported in the results section according to manufacturer's guidelines as a percentage of sample binding/maximum binding. Research studies report the raw values of this assay. Dede reports oxidative stress levels in chronic periodontitis as 605.5 pg/ml ± 139.1 and 550.52 pg/ml ± 150.28 in healthy patients.[35] Raw data from this study produced baseline (week 0) values of 374.23±141.79 pg/mL for group P-A and 407.81±155.03 pg/mL in the A-P group. Reasons for this difference could be a difference in the inflammatory process associated with chronic periodontitis. Subjects in this study did not necessarily have inflamed tissue or signs of erythema. The range of the 8-OHdG values within 1 standard deviation found for healthy individuals in Dede's study is within the same range of oxidative stress values found in the A-P group in this study. The P-A group started out with a slightly lower oxidative stress baseline value. However, the range within one standard deviation for both the A-P group and P-A group overlaps so there is not a significant difference between these two groups. To the best of our knowledge, this was the first study to look at oxidative stress levels in medication induced xerostomic patients, thus comparative values are those from other studies reporting "healthy" individuals.

As per manufacturer's guidelines, assay values should be reported as a percentage of sample binding/ maximal binding. Most values in the literature are reported as raw values, however inter-assay claims should not be made in this metric. We observed lower levels of oxidative stress in this subject pool when evaluating the raw values. However, other studies reporting oxidative stress levels in saliva do not report the percent binding of their sample thus fair comparisons are difficult to make.

Furthermore, studies measuring salivary oxidative stress have reported that salivary oxidative stress levels do not necessarily change between healthy and test groups. Rather, serum levels may prove to be more reliable markers. This finding may also explain the lack of difference found in the groups over time.[35] Future studies observing the serum oxidative stress levels may prove useful.

The lack of a significant difference in oxidative stress could also be explained by the lack of significant statistical differences in the qualitative parameters noted in the xerostomia visual analog scale. Subjects reported a statistically significant difference from baseline to week 10 regarding the satisfaction with the soothing effect of the gel with both the placebo and active gels. However, there was not a statistically significant difference between both groups.

After analyzing the qualitative data for subjects with reduced salivary flow rates (<0.200 mL/min, n=20), there does not appear to a be a correlation with

XVAS values and second baselines values. Subjects who had elevated second baseline values did not necessarily report less occurrence of xerostomia symptoms. This same analysis was completed for subjects with salivary flow rates >0.600 mL/min (n=7). No significant correlation was found between xerostomia visual analog scale values and salivary flow rates. Three subjects in this group showed an increase in salivary flow rates with use of the active gel, and four subjects showed a decrease in salivary output with use of the active gel.

A potential weakness in this study includes the need for stricter exclusion criteria. The only social habit accounted for in the exclusion criteria was tobacco use. Subjects were not asked to halt alcohol or caffeine use. Most subjects reported social drinking and intake of some tea or coffee daily. Changes in levels of consumption of these could alter the salivary flow rates.[17] Also, periodontal examinations were not completed for the study participants. Periodontal disease is known to increase oxidative stress levels.[27] Furthermore, any ulcerations or erythematous lesions could increase oxidative stress levels.[70] Thus, variability in the data could be explained if active disease was present in any of the participants. The initial chief complaint of the majority of the study subjects was the occurrence of dry mouth and secondary symptoms related to the dryness.

This study was conducted from Fall 2015 to Summer 2016. Several subjects informed the investigators regarding seasonal allergies occurring during the change of seasons. Patients using antihistamines for relief from seasonal

allergies such as cetirizine are known to have dry mouth as a side effect. However, use of this medication or other similar medications was not grounds for exclusion in the design of this study. Such circumstances are out of the control of the investigators but may explain some variability in the measurements.

Other oxidative stress measures could have been used to observe the effect of the use of the active and placebo gels. Other studies have reported use of malondialdehyde and 4-hydroxy-2-nonenal to assess levels of lipid peroxidation. Values of advanced glycosylated end products can act as another biomarker for oxidative stress levels. However, great variability has been seen in all of these markers.

Salivary Flow Rates

This study is a nested, double blind, placebo controlled, crossover, randomized clinical trial. This study design has some limitations that must be addressed when interpreting the results. As was the case in this study, crossover studies allow subjects to be exposed to both the active and placebo products. However, it does not allow a comparison with the gold standard of therapy. In this study, the subjects and investigators were blinded to the mode of therapy to avoid introduction of a bias that would compromise the validity of the study. Comparing the active gel to another product such as a sugar free gum or an oral sialagogue would be easily discernable as they have different vehicles of administration. This could potentially introduce bias into the study from either the

participants, investigators, or both. Furthermore, there is a lack of evidence recommending one treatment modality over another, thus there is not a gold standard for treatment of xerostomia.[15, 71]

Reduced Salivary Flow Rates

A lingering effect was apparent after the use of the active gel, specifically, in the participants with salivary flow rates below 0.200mL/min. The use of a placebo can introduce the placebo effect and thus increase the half-life of the active product in a cross over study design. Research shows that verbal, contextual, and social cues can elicit responses that cause the subject to remember the sensations from previous events and create an expectancy that for what is likely to be experienced in the current situation.[72] To apply this behavior in the current study, one could expect that the subjects who received the active gel first create an expectation that gel application, regardless of its ingredients, will alleviate dryness. The process of gel application can actually stimulate a response in the central nervous system that produces physiologic change without the active stimulus. Furthermore, a “nocebo” effect has also been described in the literature in which subjects, who believe they have received the placebo first, assume that there are no active effects and can develop negative results.[73]

Another behavioral modifier that could have altered the outcomes in this study is the Hawthorne effect, in which people tend to alter their behavior when they are being observed. Subjects may perform more positively due to an

increased interest in their actions. [74] This could potentially explain the increased second baseline salivary flow rates in the group of subjects who received the active gel first. However, it is difficult to know if the Hawthorne effect or even the placebo effect could cause physiologic changes such that salivary flow rates would increase over the course of the study.

Another limitation in the study design may be the length of the study. Perhaps a longer wash out period was needed to return to initial baseline levels or greater amount of time was needed in between visits to observe the effects of the antioxidants. Current knowledge does not indicate the length of time antioxidants are retained in tissue thus further studies are needed to investigate this molecular interaction.

One of the major strengths of this study is that subjects were appointed for salivary measurement almost 10 hours after their most recent gel applications. Subjects were instructed to use the gel three times a day: morning, after lunch, and before going to bed. Subjects reported to the clinic for their appointments in the morning before eating, drinking, or brushing. Thus, their last application would have been from the previous evening. Finding statistically significant differences in salivary flow rates after that long span may provide merit to the finding of increased salivary flow rate.

Also, compliance was strictly enforced by measuring gel samples at each visit to ensure usage of gel. Subjects were thoroughly instructed on gel application to avoid confusion of the application protocol.

Furthermore, during analysis of the subject pool in this study, it was noted that subjects taking analgesics were most common in the reduced salivary flow rate group. Studies have noted that subjects taking narcotics are 2.4 times more likely to report xerostomia. Studies have also shown that antidepressants and anxiolytics are also responsible for increased reports of xerostomia.[75] Greater communication with the physicians prescribing these medication and with the patients taking these medications may help in managing the side effect xerostomia.

At this time, it is unknown how long antioxidants can be retained in the oral tissues. Future studies examining the absorbance mechanism may prove useful to understand the cell biology associated with antioxidants in the oral cavity. There is evidence to suggest the lipophilic polyphenols need blood cells, platelets, and salivary proteins to increase the solubility of the antioxidants in whole saliva.[34] However, due to the lack of quantity of saliva in some of the xerostomic subjects, there may be less antioxidant retention over time. This may explain why subjects with extreme dryness may not respond as well to antioxidant therapy as there is insufficient solvent for the antioxidants. Further studies evaluating the binding mechanism of antioxidants with the quality of saliva may provide more insight.

CHAPTER V

CONCLUSION

Within the limits of this study, subjects receiving the active antioxidant gel first had similar levels of salivary 8OHdG before and after treatment compared to the subjects who received the placebo gel first. The subjects receiving the active antioxidant gel first had an increase in salivary flow rate over time, however this was not statistically significant. Nonetheless, subjects who had low salivary output levels at the initial baseline especially in the active-placebo group experienced a significant increase in salivary output that demonstrated a sustained positive effect throughout the washout and placebo phases of the study. Despite the fact that there were limitations to this study such as large variability in the oxidative stress data and the potential placebo effect, there may be a place clinically for the use of this topical combination antioxidant gel in the treatment of xerostomia. Future research should further investigate the efficacy of longer use and perhaps a heavier dosage of antioxidants.

REFERENCES

1. Edgar WM. Saliva: its secretion, composition and functions. Br Dent J 1992;172(8):305-12.
2. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent 2001;85(2):162-9.
3. Wainwright HBW. Human saliva XIII. Rate of flow of resting saliva of healthy individuals. J Dent Res 1943;22(5):391-96.
4. Heintze U, Birkhed D, Bjorn H. Secretion rate and buffer effect of resting and stimulated whole saliva as a function of age and sex. Swed Dent J 1983;7(6):227-38.
5. Narhi TO. Prevalence of subjective feelings of dry mouth in the elderly. J Dent Res 1994;73(1):20-5.
6. Fox PC, Busch KA, Baum BJ. Subjective reports of xerostomia and objective measures of salivary gland performance. J Am Dent Assoc 1987;115(4):581-4.
7. Longman LP, Higham SM, Rai K, Edgar WM, Field EA. Salivary gland hypofunction in elderly patients attending a xerostomia clinic. Gerodontology 1995;12(12):67-72.
8. Tabak LA. In defense of the oral cavity: structure, biosynthesis, and function of salivary mucins. Annu Rev Physiol 1995;57:547-64.
9. Gibbins HL, Proctor GB, Yakubov GE, Wilson S, Carpenter GH. Concentration of salivary protective proteins within the bound oral mucosal pellicle. Oral Dis 2014;20(7):707-13.
10. Siqueira WL, Zhang W, Helmerhorst EJ, Gygi SP, Oppenheim FG. Identification of protein components in in vivo human acquired enamel pellicle using LC-ESI-MS/MS. J Proteome Res 2007;6(6):2152-60.
11. Yakubov GE. Lubrication. Monogr Oral Sci 2014;24:71-87.
12. Dawes C. Salivary flow patterns and the health of hard and soft oral tissues. J Am Dent Assoc 2008;139 Suppl:18S-24S.
13. Dawes C, Pedersen AM, Villa A, et al. The functions of human saliva: A review sponsored by the World Workshop on Oral Medicine VI. Arch Oral Biol 2015;60(6):863-74.
14. Tanasiewicz M, Hildebrandt T, Obersztyn I. Xerostomia of various etiologies: a review of the literature. Adv Clin Exp Med 2016;25(1):199-206.
15. Plemons JM, Al-Hashimi I, Marek CL, American Dental Association Council on Scientific A. Managing xerostomia and salivary gland

hypofunction: executive summary of a report from the American Dental Association Council on Scientific Affairs. J Am Dent Assoc 2014;145(8):867-73.

16. Scully C. Drug effects on salivary glands: dry mouth. Oral Dis 2003;9(4):165-76.

17. Sreebny LM, Schwartz SS. A reference guide to drugs and dry mouth--2nd edition. Gerodontology 1997;14(1):33-47.

18. Navazesh M, Kumar SK, University of Southern California School of D. Measuring salivary flow: challenges and opportunities. J Am Dent Assoc 2008;139 Suppl:35S-40S.

19. Navazesh M, Ship, II. Xerostomia: diagnosis and treatment. Am J Otolaryngol 1983;4(4):283-92.

20. Glore RJ, Spiteri-Staines K, Paleri V. A patient with dry mouth. Clin Otolaryngol 2009;34(4):358-63.

21. Mortazavi H, Baharvand M, Movahhedian A, Mohammadi M, Khodadoustan A. Xerostomia due to systemic disease: a review of 20 conditions and mechanisms. Ann Med Health Sci Res 2014;4(4):503-10.

22. Thomson WM, Chalmers JM, Spencer AJ, Slade GD. Medication and dry mouth: findings from a cohort study of older people. J Public Health Dent 2000;60(1):12-20.

23. Proctor GB, Carpenter GH. Regulation of salivary gland function by autonomic nerves. Auton Neurosci 2007;133(1):3-18.

24. Turner MD. Hyposalivation and xerostomia: etiology, complications, and medical management. Dent Clin North Am 2016;60(2):435-43.

25. Leal SC, Bittar J, Portugal A, et al. Medication in elderly people: its influence on salivary pattern, signs and symptoms of dry mouth. Gerodontology 2010;27(2):129-33.

26. al-Hashimi I, Taylor SE. A new medication for treatment of dry mouth in Sjogren's syndrome. Tex Dent J 2001;118(3):262-6.

27. Chapple IL, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. Periodontol 2000 2007;43:160-232.

28. Battino M, Bullon P, Wilson M, Newman H. Oxidative injury and inflammatory periodontal diseases: the challenge of anti-oxidants to free radicals and reactive oxygen species. Crit Rev Oral Biol Med 1999;10(4):458-76.

29. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. J Toxicol 2012;2012:645460.

30. Fordham JB, Naqvi AR, Nares S. Leukocyte production of inflammatory mediators is inhibited by the antioxidants phloretin, silymarin, hesperetin, and resveratrol. Mediators Inflamm 2014;2014:938712.

31. Rahal A, Kumar A, Singh V, et al. Oxidative stress, prooxidants, and antioxidants: the interplay. Biomed Res Int 2014;2014:761264.

32. Ginsburg I, Kohen R, Shalish M, et al. The oxidant-scavenging abilities in the oral cavity may be regulated by a collaboration among antioxidants in saliva, microorganisms, blood cells and polyphenols: a chemiluminescence-based study. PLoS One 2013;8(5):e63062.

33. Ginsburg I, Kohen R, Koren E. Saliva: a 'solubilizer' of lipophilic antioxidant polyphenols. Oral Dis 2013;19(3):321-2.

34. Ginsburg I, Koren E, Shalish M, Kanner J, Kohen R. Saliva increases the availability of lipophilic polyphenols as antioxidants and enhances their retention in the oral cavity. Arch Oral Biol 2012;57(10):1327-34.

35. Dede FO, Ozden FO, Avci B. 8-hydroxy-deoxyguanosine levels in gingival crevicular fluid and saliva in patients with chronic periodontitis after initial periodontal treatment. J Periodontol 2013;84(6):821-8.

36. Greabu DMM. Oxidative Stress - A possible link between systemic and oral diseases. Farmacia 2011;59(3):329-37.

37. Kesarwala AH, Krishna MC, Mitchell JB. Oxidative stress in oral diseases. Oral Dis 2016;22(1):9-18.

38. Saral Y, Coskun BK, Ozturk P, Karatas F, Ayar A. Assessment of salivary and serum antioxidant vitamins and lipid peroxidation in patients with recurrent aphthous ulceration. Tohoku J Exp Med 2005;206(4):305-12.

39. Hershkovich O, Shafat I, Nagler RM. Age-related changes in salivary antioxidant profile: possible implications for oral cancer. J Gerontol A Biol Sci Med Sci 2007;62(4):361-6.

40. Bahar G, Feinmesser R, Shpitzer T, Popovtzer A, Nagler RM. Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. Cancer 2007;109(1):54-9.

41. Buczko P, Zalewska A, Szarmach I. Saliva and oxidative stress in oral cavity and in some systemic disorders. J Physiol Pharmacol 2015;66(1):3-9.

42. Tai Y, Inoue H, Sakurai T, et al. Protective effect of lecithinized SOD on reactive oxygen species-induced xerostomia. Radiat Res 2009;172(3):331-8.

43. Nagler RM, Baum BJ. Prophylactic treatment reduces the severity of xerostomia following radiation therapy for oral cavity cancer. Arch Otolaryngol Head Neck Surg 2003;129(2):247-50.

44. Sakagami H, Oi T, Satoh K. Prevention of oral diseases by polyphenols (review). In Vivo 1999;13(2):155-71.

45. Ha C, Rees T, Abraham C, Cheng Y-SL, Jordan L, Plemons J. The use of anti-oxidants in the treatment of persistent, non-responsive oral lichen planus: A randomized control clinical trial. J Implant & Adv Clin Dent 2015;7(6):17-27.

46. San Miguel SM, Opperman LA, Allen EP, Svoboda KK. Use of antioxidants in oral healthcare. Compend Contin Educ Dent 2011;32(9):E156-9.

47. Rezk BM, Haenen GR, van der Vijgh WJ, Bast A. The antioxidant activity of phloretin: the disclosure of a new antioxidant pharmacophore in flavonoids. Biochem Biophys Res Commun 2002;295(1):9-13.

48. Yang YC, Lii CK, Lin AH, et al. Induction of glutathione synthesis and heme oxygenase 1 by the flavonoids butein and phloretin is mediated through the ERK/Nrf2 pathway and protects against oxidative stress. Free Radic Biol Med 2011;51(11):2073-81.

49. Lu XY, Zeng YY, Ye YX, et al. Anti-inflammatory and immunosuppressive effect of phloretin. Yao Xue Xue Bao 2009;44(5):480-5.

50. Chang WT, Huang WC, Liou CJ. Evaluation of the anti-inflammatory effects of phloretin and phlorizin in lipopolysaccharide-stimulated mouse macrophages. Food Chem 2012;134(2):972-9.

51. Devi MA, Das NP. In vitro effects of natural plant polyphenols on the proliferation of normal and abnormal human lymphocytes and their secretions of interleukin-2. Cancer Lett 1993;69(3):191-6.

52. Srinivasan M, Sudheer AR, Menon VP. Ferulic Acid: therapeutic potential through its antioxidant property. J Clin Biochem Nutr 2007;40(2):92-100.

53. Graf E. Antioxidant potential of ferulic acid. Free Radic Biol Med 1992;13(4):435-48.

54. Petti S, Scully C. Polyphenols, oral health and disease: A review. J Dent 2009;37(6):413-23.

55. Battino M, Ferreiro MS, Gallardo I, Newman HN, Bullon P. The antioxidant capacity of saliva. J Clin Periodontol 2002;29(3):189-94.

56. Ryo K, Takahashi A, Tamaki Y, et al. Therapeutic effects of isoflavones on impaired salivary secretion. J Clin Biochem Nutr 2014;55(3):168-73.

57. Norheim KB, Jonsson G, Harboe E, et al. Oxidative stress, as measured by protein oxidation, is increased in primary Sjogren's syndrome. Free Radic Res 2012;46(2):141-6.

58. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2009;27(2):120-39.

59. Kasai H. Analysis of a form of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutat Res 1997;387(3):147-63.

60. Takane M, Sugano N, Iwasaki H, et al. New biomarker evidence of oxidative DNA damage in whole saliva from clinically healthy and periodontally diseased individuals. Journal of Periodontology 2002;73(5):551-54.

61. Tothova L, Kamodyova N, Cervenka T, Celec P. Salivary markers of oxidative stress in oral diseases. Front Cell Infect Microbiol 2015;5:73.

62. Maes M. The cytokine hypothesis of depression: inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. Neuro Endocrinol Lett 2008;29(3):287-91.

63. Robertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. J Biol Chem 2004;279(41):42351-4.

64. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010;107(9):1058-70.

65. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med 2010;49(11):1603-16.

66. Michel TM, Pulschen D, Thome J. The role of oxidative stress in depressive disorders. Curr Pharm Des 2012;18(36):5890-9.

67. Sezer U, Cicek Y, Canakci CF. Increased salivary levels of 8-hydroxydeoxyguanosine may be a marker for disease activity for periodontitis. Dis Markers 2012;32(3):165-72.

68. Ryo K, Yamada H, Nakagawa Y, et al. Possible involvement of oxidative stress in salivary gland of patients with Sjogren's syndrome. Pathobiology 2006;73(5):252-60.

69. San Miguel SM, Opperman LA, Allen EP, Zielinski J, Svoboda KK. Bioactive polyphenol antioxidants protect oral fibroblasts from ROS-inducing agents. Arch Oral Biol 2012;57(12):1657-67.

70. Avci E, Akarslan ZZ, Erten H, Coskun-Cevher S. Oxidative stress and cellular immunity in patients with recurrent aphthous ulcers. Braz J Med Biol Res 2014;47(5):355-60.

71. Furness S, Bryan G, McMillan R, Birchenough S, Worthington HV. Interventions for the management of dry mouth: non-pharmacological interventions. Cochrane Database Syst Rev 2013(9):CD009603.

72. Colagiuri B, Schenk LA, Kessler MD, Dorsey SG, Colloca L. The placebo effect: From concepts to genes. Neuroscience 2015;307:171-90.

73. Reeves RR, Ladner ME, Hart RH, Burke RS. Nocebo effects with antidepressant clinical drug trial placebos. Gen Hosp Psychiatry 2007;29(3):275-7.

74. Trudeau T. The Hawthorne study revisited. Hosp Top 1982;60(6):17.

75. Murray Thomson W, Chalmers JM, John Spencer A, Slade GD, Carter KD. A longitudinal study of medication exposure and xerostomia among older people. Gerodontology 2006;23(4):205-13.