Salivary Enhancement: Current status and future therapies

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Abstract:

Saliva provides the principal protective milieu for teeth by modulating oral microbial ecosystems and reversing the initial phases of caries development. Patients with inadequate salivary function are at increased risk for dental decay. Therefore, it is likely that therapies that increase overall fluid output of these individuals will reverse early carious lesions. The most common causes of salivary dysfunction are medication usage, autoimmune exocrinopathy, Sjögren's syndrome, and damage of salivary parenchyma during therapeutic irradiation. For patients with remaining functional acinar tissue, treatment with the parasypathomimetic secretogogues pilocarpine and Cevimeline may provide relief. However, these medications do not benefit all patients. The possibilities of using gene therapy and tissue engineering to develop treatments for those with severe salivary dysfunction are discussed.

Keywords: salivary dysfunction; saliva; xerostomia; Sjögren's syndrome; gene therapy; dry mouth; dental caries

Saliva provides the principal protective milieu for teeth by modulating oral microbial ecosystems, reversing the initial phases of caries development, aiding in the preparation of the food bolus, lubricating oral tissues, and supporting other critical functions.[1] Patients with inadequate salivary function are at increased risk for dental decay, oral and mucosal infections, gastrointestinal complications, and a decreased quality of life.[2,3]

Patients with significantly decreased salivary output have an increased prevalence of dental caries.[4,6] Therefore, therapies that increase overall salivary flow in these individuals are believed to have the potential of reversing early carious lesions. Though this has not been established in humans, studies with rats demonstrated that restoration of salivary function with the cholinergic agonist, pilocarpine, decreased new caries formation in animals whose salivary flow was reduced by partial salivary gland removal.[7]

CAUSES OF SALIVARY DYSFUNCTION

Many systemic diseases are associated with alterations in salivary output. The most pronounced salivary dysfunction occurs in patients taking medications that interfere with salivary secretory processes,[8,9] those who have received therapeutic irradiation to eradicate head and neck malignancie,[9-11] and patients with Sjögren's syndrome (SS; ref. 10-11). Salivary hypofunction secondary to medication usage is likely the most common cause of these conditions.[8,9]

Medications often inhibit cholinergic signaling pathways in salivary tissues and thereby decrease the fluid output of the gland. Interference with other peripheral and central signaling pathways can also reduce salivary output and alter salivary composition. While 300 to 400 medications are believed to interfere with salivary secretion,[8,9] the specific inhibitory

mechanisms are defined only for small subsets of drugs. The permanent impact of prolonged anticholinergic medication usage on salivary tissues still requires definition. Often, the only treatment for these patients is to change the medication to a less-xerogenic type or decrease the medication dose below that leading to oral dryness while maintaining the required therapeutic effect. However, this is not possible in many situations. Further, prescription use in the United States likely will continue to increase.[12,13] At present, elders (> 65 years of age) use an average of about three prescription and two over-the-counter medications per day,[14,15] and salivary flow decreases as the numbers of medications a patient takes daily increases.[16-17] Given that the number of elders is increasing, we can expect an increase in patients with compromised salivary function secondary to systemic diseases and their treatments. The best solution to this problem would be the development of more specific pharmaceutical agents that selectively act on target tissues (such as vessels) while sparing others, such as salivary glands. In addition, clinical studies are needed to determine if salivary flow rates influence a dental restoration's length of service.

Salivary hypofunction after gland irradiation is very difficult to treat, as salivary parenchyma within the radiation field is damaged permanently.[18,19] Head and neck cancer affects 30,000 - 40,000 new patients each year, most of whom are treated with therapeutic irradiation. These patients are typically middle-aged males and often individuals from economically disadvantaged backgrounds.[20,21] Radiation treatment of oral and pharyngeal malignancies typically includes salivary tissue within the field. At doses above 50 Gy, patients can lose all salivary function if the salivary glands are totally within the radiation field.[22] The reasons for the extreme radiosensitivity of human salivary tissues remain undefined.[18,22]

Sjögren's syndrome affects about one million persons in the U.S., currently estimated to reflect a 9:1, female: male ratio.[23,24] In most patients, the diagnosis is established when the patient is between 40 and 50 years of age. SS is a systemic autoimmune disorder primarily affecting the salivary and lacrimal glands in which clusters of infiltrating lymphocytes replace the parenchyma of the glands.[25] During the last decade, many studies have examined the influences of inflammatory proteins on salivary tissue health and function.[26-27] Autoantibodies were identified in the circulation and saliva of SS patients over forty years ago.[28] Passive transfer of these autoantibodies can reduce salivary output in the nonobese diabetic (NOD) mouse model of SS,[29,30] possibly by blocking cholinergic M3 receptors. Other studies in the last decade have examined autoantibodies to alpha-fodrin[31] and programmed cell death (apoptosis) in SS.[32-35] Still undefined are the events that trigger initial lymphocytic infiltrates and perpetuate the disease.

TREATMENT OF SALIVARY DYSFUNCTION

Both irradiation and Sjögren's syndrome lead to the loss of salivary acinar cells, the only cell type in the glands capable of fluid movement. These conditions exhibit considerable heterogeneity. Some patients experience minimal parenchymal cell loss, while others may have glands entirely replaced by connective tissue and inflammatory cells. Treatment of patients with remaining functional acinar tissue is possible with a parasypathomimetic secretogogue.

The first such drug approved in the U.S. was pilocarpine. Pilocarpine possesses modest, non-specific muscarinic and weak β-adrenergic agonist activity. Its effectiveness in increasing salivary output was demonstrated in several studies of patients with radiation-induced salivary hypofunction and Sjögren's syndrome (Table 1).[36-40] Recently, the FDA approved a second

secretogogue, cevimeline.[41,42] Cevimeline is a more specific drug, with a preference for activation of the primary muscarinic receptor sub-type responsible for fluid flow from salivary glands, the so-called M3 receptor. This medication has not been tested to date in post-radiation patients. In general, secretory agents do not address either the underlying inflammatory processes of SS or the lack of functional acinar cells after radiation therapy, and will have very limited success in those with advanced salivary dysfunction. Likewise, the interaction of a cholinergic agonist with other medications precludes the use of pilocarpine in many patients with medication-induced salivary dysfunction.

Prevention of salivary damage during radiation therapy should be a goal in oncology. Radiation damage to salivary glands can be limited by pre-radiation planning (conformal and static, multi-segmental intensity modulated technique, IMRT) that spares as much salivary tissue as possible from the radiation field.[43] Use of the oxygen radical scavenger amifostine during radiation treatment may decrease damage to glands.[44] Surgically repositioning of submandibular salivary glands to the submental space before radiation has been used to maintain gland function.[45]

Several anti-inflammatory medications have been tested for the treatment of Sjögren's syndrome (Table 2),[46-52] but only interferon alpha and prednisolone irrigation increased salivary output.[51,52] One reason for this generalized treatment failure is that the doses of medications used in most trials were anti-inflammatory, and not immunosuppressive. The increased risk of serious side effects have discouraged investigators and patients from testing more potent regimens of drugs such as prednisone, cylcosporine and azathioprine. However, new anti-inflammatory and immune-mediating agents are continually being tested as treatments for Sjögren's syndrome.

FUTURE TREATMENTS

There is currently no conventional therapy to enhance salivary secretion for patients with extensive gland damage. This circumstance provided the impetus approximately 10 years ago for the application of gene transfer technology to repair irradiation- or autoimmune-damaged salivary glands. The initial goal of these studies was to re-engineer the function of the surviving non-fluid secreting ductal cells in damaged glands to a secretory phenotype.

The first peer-reviewed publication on gene transfer to salivary glands was published in 1994.[53] Since that report, several laboratories have transferred several different genes successfully to salivary glands.[54] Most of these studies have utilized viral vectors, particularly adenoviral vectors, to mediate gene transfer. Viral vectors typically are extremely efficient at transferring genes, but can pose a safety risk and stimulate potent immune responses. An alternative means of gene transfer is to use non-viral methods. Perhaps the most successful form of non-viral gene transfer to salivary glands involves the use of cationic liposomes.[55] While much less efficient than viral vectors, liposomes pose relatively little safety risk. Current research is focusing on ways to optimize this method of gene transfer in salivary glands.

In 1997, a study reported by Delporte *et al.,*[56] described the "correction" of irradiation-induced salivary hypofunction in rats through transfer of the cDNA encoding aquaporin 1, a mammalian water channel (permeability pathway). Gene transfer was accomplished using a replication-deficient, first generation, recombinant adenovirus. Irradiated rats administered a control adenovirus exhibited salivary flow rates approximately 65 percent lower than sham-irradiated animals. Conversely, when animals were administered the aquaporin 1-encoding adenovirus four months after irradiation, salivary flow rates determined three days post-

administration were statistically the same as control levels. The studies were continued in primates[57] and were not as effective as the Delporte *et al.* rat study. Because of the small number of primates used (five), the reasons for the minimal success are not entirely clear and may have been technical. A larger study is now being conducted in pigs that will eventually lead to a clinical trial in humans.

Thus, the specific value of aquaporin 1 gene transfer for irradiated salivary glands must be considered as hypothetical, and is not ready for clinical testing in humans. It is not known whether the simple insertion of a water channel into surviving ductal cells will correct glandular hypofunction. However, gene transfer without question can be readily accomplished *in vivo* in salivary glands and is potentially of considerable clinical value to enhance salivary secretions.

Gene transfer can also be utilized to augment salivary secretions by transferring genes that encode secretory proteins into salivary glands. The proteins are subsequently secreted in an exocrine manner. This was successfully accomplished in animal studies,[58] with the transfer of the human histatin 3 cDNA to rat submandibular glands. Histatin 3, which normally is not secreted in rodent saliva, was secreted at high levels (up to 1 mg/ml) after gene transfer. Another potential clinical use of gene transfer to salivary glands is immunization via DNA vaccination. Kawabata et al[59] showed that delivery of the cDNA for the *P. gingivalis* fimbrial protein into murine salivary glands led to the production of secretory immunoglobulin A specific for this microbial protein. This approach could be used to immunize humans against other oral microbes, such as mutans streptococcus. Gene transfer can also be used to deliver anti-inflammatory proteins, such as Interleukin 10, to salivary glands. This may have potential in the treatment of Sjögren's syndrome.[60]

Gene transfer to repair damaged glands can only be an option if epithelial tissue survives either the irradiation or autoimmune damage. In the absence of any parenchymal cells, when a gland is fully replaced by fibrotic tissue, gene transfer cannot lead to an enhancement of saliva production since no system exists to produce and transport fluid into the mouth. Recently, to address this circumstance, we began initial studies directed at the development of an artificial salivary gland using well-established principles of tissue engineering coupled with genetic engineering.[61]

The prototype design includes a biodegradable substratum shaped as a blind end tube (i.e., like a test tube), coated with a layer of purified extracellular matrix proteins involved in cellular organization, followed by a monolayer lining of polarized epithelial cells capable of unidirectional fluid secretion.[62] Initial feasibility studies have been reported.[63] Although considerably more work is required, based on the success of other groups with developing functional, complex fluid secreting bioartificial organs, notably bladder,[64] it is reasonable to expect that an artificial salivary gland suitable for clinical testing can be achieved in approximately 10 years. The most immediate therapy in this area may be the use of autologous salivary tissues harvested from the individual, expanded *ex vivo,* and then re-implanted in an appropriate matrix to induce regrowth and repair. For example, tissue could be harvested prior to a course of head and neck radiotherapy, and placed back into an individual following radiation and a healing period.

We are presently experiencing a time of unparalleled biomedical scientific progress. The human genome has been sequenced, and functions are being identified for these newly discovered genes. The challenge will be managing the great volume of information to make meaningful interpretations in real time that apply to therapy. For example, current European

studies are examining the genetics of SS by studying the presence of SS autoantibodies and different HLA alleles.[65,66] Understanding the genetic basis of Sjögren's syndrome should lead to earlier and more accurate diagnosis of this condition. Major progress in cellular, developmental and molecular biology also will result in treatments of oral biological problems. Enhancing saliva will be but one of many such examples.

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Table 1. Randomized, placebo-controlled trials evaluating pilocarpine treatment

Author	Design	Patient diagnosis	Number (n)	Dose	Effect on salivary Flow (statistically)	Comments
Fox et al.(36)	Placebo 1 month, pilocarpine 5 months	Post-radiation (PR) Sjögren's (SS) Idiopathic xerostomia (IX)	PR = 12 SS = 21 IX = 6	5 mg, tid, 5 months	↑ unstimulated parotid and submandibular flows	
Johnson et al. (37)	Placebo vs 5 or 10 mg pilocarpine	PR	207	5 or 10 mg, tid 12 weeks	↑ unstimulated whole salivary flow rates	Increased incidence of side effects with 10 mg dose
Leveque et al. (38)	Placebo vs fixed dose or dose titration	PR	162	2.5 mg, 4 weeks; 5.0 mg, 4 weeks; 10 mg, 4 weeks	↑ unstimulated whole and parotid salivary flow rates	Best results with continuous treatment > 8 weeks at doses > 2.5 mg tid
Rieke et al. (39)	Placebo vs fixed dose or dose titration	PR	369	2.5 mg, 4 weeks; 5.0 mg, 4 weeks; 10 mg, 4 weeks	↑ unstimulated whole salivary flow rates	Best results with continuous treatment > 8 weeks at doses > 2.5 mg tid
Vivino et al. (40)	Placebo vs 2.5 or 5.0 mg pilocarpine	SS	373	2.5 or 5.0 mg qid, 3 months	↑ unstimulated whole salivary flows	

Table 2. Salivary flow response of patients with Sjögren's syndrome to treatment with immune modulating agents (controlled trials)

Treatment	Design	Number (n)	Dose	Effect on salivary Flow (statistically)	Comments
Cyclosporin A (46)	Placebo vs active drug	10 each group	5 mg/kg body weight, 6 months	No change	
Nandrolone decanoate (47)	Placebo vs active drug	10 each group	100 mg biweekly, 6 months	No change	
Hydroxy-Chloroquine (48)	2 yr, cross-over trial (all had drug and placebo)	19	400 mg/day, 12 months	No change	
Piroxicam or Prednisone (49)	Placebo vs active drug	8 each group	30 mg qod (prednisone) or 20 mg qd (piroxicam) for 6 months	No change	
Azathioprine (50)	Placebo vs active drug	13 azathioprine 12 placebo	1 mg/kg body weight	No change	6 patients, all taking azathioprine, withdrew 2° to side effects
Prednisolone irrigation (51)	Saline irrigation followed by active treatment	28	2 mg / parotid gland, 1x / week for 3 weeks	↑ stimulated whole salivary flow	Saline Tx for 8 weeks, then prednisolone Tx
Interferon alpha lozenge (52)	Placebo vs four doses of interferon alpha	Placebo = 22; 150 IU* qd = 21 150 IU tid = 22 450 IU qd = 23 450 tid = 21	150 IU qd or 150 IU tid or 450 IU qd or 450 tid Tx for 12 weeks	↑ stimulated whole salivary flow only in the 150 IU tid group	

* IU = International Units