Review

Pathogenesis and Management of Xerostomia

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Abstract: Xerostomia, or dry mouth, is mainly caused by systemic disease, biological aging, and drug-induced side effect. The innervations underlying the three major salivary glands and numerous minor salivary glands differ substantially. The trafficking of aquaporin-5 (AQP5), which is specifically expressed in salivary glands, is regulated by the autonomic nerves. Upon the stimulation of the cholinergic nerve, AQP5 travels to apical and lateral plasma membranes, nuclei, and saliva. In this review article, I will describe the subcellular localization of AQP5 in relation to the causes of xerostomia. Ways of managing xerostomia, in particular the use of functional foods and salivary secretagogues, are also discussed.

1. Introduction
Saliva has a number of diverse functions in maintaining the integrity of the oral tissues; for example, in protecting teeth from caries, in the tasting, mastication and swallowing of food, in speech, and aiding the tolerance of dentures\textsuperscript{1,2}. Xerostomia, or dry mouth, is caused by a reduction of salivary secretion associated with the dysfunction of salivary glands. This condition is often caused by systemic diseases such as diabetes insipidus, autoimmune diseases, and cardiac failure. Direct damage of the salivary glands by therapeutic irradiation of the head and neck can also lead to xerostomia\textsuperscript{3}. In addition, there are physiological causes of xerostomia, such as anxiety and depression. Salivary gland agenesis also induces xerostomia\textsuperscript{4}. However, the most frequent cause is the use of certain medications, such as anticholinergic drugs, anorectics, antihistamines, antidepressants, antihypertensives, and calcium antagonists\textsuperscript{5}. Xerostomia appears to be particularly prevalent in older individuals who take several xerogenic drugs for treatment of their underlying medical conditions.

Xerostomia may also initiate oral infections such as periodontal disease and dental caries\textsuperscript{6}. Such diseases can aggravate diabetes\textsuperscript{7} and increase the risk of patients developing atherosclerosis and cardiovascular diseases\textsuperscript{8}. Therefore, xerostomia can significantly compromise patients' health and their quality of life.

2. Innervation of salivary glands
Salivary glands can be subdivided into three major types: parotid, submandibular, and sublingual glands. There are also numerous minor salivary glands scattered throughout the oral cavity\textsuperscript{9}. Salivary glands are innervated by autonomic nerves. The cholinergic parasympathetic nerve innervates all salivary glands. The submandibular and sublingual glands are innervated by the parasympathetic nerve that is derived from the superior salivary nucleus. The parotid gland is innervated by the parasympathetic nerve that is derived from the inferior salivary nucleus. Minor salivary glands are innervated by a parasympathetic nerve located in the buccal branch of the mandibular nerve\textsuperscript{10}. However, the adrenergic sympathetic nerve, which is derived from the thoracic spinal cord, innervates the parotid and submandibular glands. In contrast,
the sublingual glands and the minor salivary glands have very few sympathetic nerve fibers.

In non-adrenergic and non-cholinergic nerves, salivary glands are regulated by neuropeptides such as substance P, and nerve activity is regulated by vasoactive intestinal peptide, neuropeptide Y, and neurokinin A.

3. Unstimulated and stimulated salivary secretion

Under acid-stimulated conditions, the parotid glands secrete approximately half of the whole volume of saliva in the mouth; whereas in the unstimulated state, the parotid glands produce very low levels of saliva. In contrast, the submandibular and sublingual glands secrete higher volumes of saliva, even under unstimulated conditions. The contributions of different salivary glands during unstimulated flow are as follows: 20% from the parotid gland, 65% from the submandibular gland, 7% to 8% from the sublingual gland, and less than 10% from minor glands. Under stimulated conditions, the parotid glands contribute significantly higher volumes of secreted saliva, and the submandibular glands contribute 53% of the whole saliva in the mouth.

4. Properties of epithelial cells in salivary glands

Both the salivary acinar and duct epithelial cells are polarized and have characteristic apical and basolateral membrane domains. On the luminal surface of the Golgi apparatus, glycosphingolipid and cholesterol are sorted into apical transport vesicles via an anterograde secretory pathway, and phosphatidylcholine and cholesterol are sorted into basolateral transport vesicles. These vesicles travel to the salivary gland plasma membranes along microtubules. As a result, the apical membrane is enriched in glycosphingolipids and cholesterol and form microdomains called lipid rafts. The glycosphingolipids contain polar head groups that contain one or more carbohydrates. Apical sorting of glycosphingolipids is vital to create cell polarization, and the apical membranes are internalized and replaced within hours of being made.

4-1. Isolation of apical and basolateral plasma membranes

Apical and basolateral plasma membranes are able to be prepared from parotid gland homogenates using differential centrifugation and magnesium chloride precipitation (Fig.1). Alkaline phosphatase and γ-glutamyl transpeptidase (γ-GT) are usually used as apical plasma membrane markers, while K+-activated P-nitrophosphatase (K’NPPase) and Na+/K’-adenosine triphosphatase are used as markers of the basolateral membranes. As shown in Table 1, γ-GT activities in prepared apical and basolateral plasma membranes were 24 and 4 mU/mg protein, respectively.

4-2. Isolation of cell nuclei

Nuclear sphingolipids have important signaling and regulatory roles in the nucleus. Nuclei are able to be isolated from parotid homogenates using differential centrifugation and sucrose density gradients. The ratio of RNA to DNA in isolated nuclei is generally less than 0.25.

5. Aquaporins in salivary glands

In 1988, an integral membrane protein with a molecular mass of 28 kDa was discovered in red blood cell membranes. The protein exhibited a transmembrane channel activity that was specific for water and was therefore named aquaporin (AQP). To date, 13 members of the AQP family have been identified in various mammalian cell types. AQP5 has been cloned from the salivary glands of rats, humans, and mice.
Table 1  Activities of γ-glutamyl transpeptidase and K⁺-activated p-nitrophosphatase in subfractions

<table>
<thead>
<tr>
<th>Fractions</th>
<th>γ-glutamyl transpeptidase (mU/mg protein)</th>
<th>K⁺-activated p-nitrophosphatase (mU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>3.55±0.31</td>
<td>34.47±1.62</td>
</tr>
<tr>
<td>Apical plasma membrane</td>
<td>24.23±1.0</td>
<td>19.56±1.81</td>
</tr>
<tr>
<td>Basolateral membrane</td>
<td>4.00±0.06</td>
<td>109.09±4.11</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>2.11±0.04</td>
<td>15.15±0.61</td>
</tr>
</tbody>
</table>

Parotid tissue

Homogenize in 5 vol. of Buffer II [0.32 mM Sucrose, 3 mM MgCl₂, 1 mM DTT, 5 mM Heps (pH 7.5)] using a glass homogenizer at 1250 rpm for 12 strokes

Filter through a nylon cloth (110 mesh)

Centrifuge at 600 g for 10 min

Sup  Ppt

Suspend in Buffer II

Centrifuge at 600 g for 10 min

Sup  Ppt

Suspend in Buffer II

Add a suitable volume of Buffer III [2.2 mM Sucrose, 3 mM MgCl₂, 1 mM DTT, 5 mM Heps (pH 7.5)] to give a final sucrose molarity of 1.89

Mix thoroughly

Layer over Buffer III

Centrifuge at 75,000 g for 90 min using swing rotor

Sup  Ppt  Nuclei

Fig.2  Scheme for preparation of nuclei.
Sup and Ppt represent the supernatants and precipitates, respectively.

5.1. Trafficking of AQP5 to apical and lateral plasma membranes

Specific interactions of acetylcholine (ACh) and epinephrine with M₁- and M₃-muscarinic acetylcholine receptors (mACHRs) [19] and α₁-adrenoceptors (ARs) [20], respectively, induce rapid AQP5 trafficking to the apical plasma membrane of rat parotid glands. Cevimeline [21] and pilocarpine [22], which are mAChR agonists, induce a rapid trafficking of AQP5 to the apical and lateral plasma membrane via the enhanced Ca²⁺/cyclic guanosine monophosphate (cGMP)/protein kinase G (PKG) signaling pathway. Phenylephrine, an α₁-AR agonist, also induces AQP5 trafficking to the apical and lateral plasma membranes (but not to the basal plasma membrane) via the activation of the Ca²⁺/cGMP/PKG signaling pathway [20].

AQP5 localizes to lipid rafts containing ganglioside GM1, flotillins, and cholesterol in rat parotid salivary glands. Upon the activation of M₁- and M₃-mAChRs and α₁-ARs, AQP5 travels to apical and lateral plasma membranes together with the lipid rafts in acinar cells [24, 25] and duct cells [26, 27]. The expression of AQP5 is higher in parotid acinar cells compared with duct cells [26]. Lipid rafts that have translocated to apical plasma membranes contain higher levels of
cholesterol compared with those isolated from lateral plasma membrane

At apical and lateral plasma membranes, AQP5 moves from lipid rafts to non-raft domains (5).

5-2. AQP5 trafficking to nuclei
Upon M1- and M4-mACHR activation, AQP5, together with cholesterol-poor vesicles, travels to cell nuclei via a retrograde pathway (26). AQP5 may trigger water transport across the nuclear membrane and open the nuclear pore. It has been reported that kidney AQP5, which is barely detectable in control experiments, regulates gene transcription of the histone lysine methyla
transferase, Dot1 (30). These results suggest that AQP5 has dual roles on the nuclear membrane and inside nuclei.

5-3. AQP5 in exosomes
Exosomes have been identified in body fluids such as saliva, breast milk, blood, urine, semen, amniotic fluid, and ascites fluid (60). Exosomes are small membrane vesicles (40-100 nm in diameter) of endocytic origin that are secreted by most cell types upon fusion of multivesicular bodies with the plasma membrane (41). Exosomes participate in intercellular communication (42, 43), immune regulatory functions (44), transport of morphogens and RNA (45-47), and tumor metastasis (48).
Ogawa et al. identified two types of exosomes in whole human saliva: exosome I (84 nm mean diameter) and exosome II (40 nm mean diameter) (49). Exosomes I and II contain AQP5, alpha-amylase and proline-rich proteins. Exosomal proteome studies are listed in the table described previously (50). Salivary exosomes have been shown to be useful in detecting diseases, such as Sjögren’s syndrome (51), as well as cancers of the head and neck (46).

6. AQP5 distribution in salivary glands under aging and diseases
The stimulatory effect by acetylcholine on AQP5 levels in the apical plasma membrane was found to decrease with aging (52). Salivary AQP5 levels were observed to decrease in parallel with salivary volume in an aging-dependent manner (26). Salivary AQP5 is therefore a useful biomarker for the diagnosis of aging-related xerostomia.

In patients with diabetes mellitus, salivary AQP5 levels also decrease concomitantly with salivary secretion (54). In diabetic rat parotid glands, AQP5 mRNA is increased but AQP5 protein levels are decreased (55). Upon mACHRs activation in normal rat parotid glands, AQP5 travels to apical plasma membrane together with lipid rafts and then moves from lipid rafts to non-rafts on the apical plasma membrane. In contrast, AQP5 does not travel to the apical plasma membrane nor does it move to non-rafts in diabetic rat parotid glands.

In salivary or lacrimal glands of patients with Sjögren’s syndrome, the expression of bone morphogenetic protein 6 (BMP6) is increased (56). Increased BMP6 expression results in decreased expression of AQP5 in mice with Sjögren’s syndrome (57). In addition, abnormal distribution of AQP5 has also been reported (58), suggesting that a decrease of AQP5 in the apical plasma membranes leads to decreased salivary secretion.

7. Functional foods for restoring salivary glands
The oral administration of whey, a co-product of cheese manufacturing, over a long-term (4-week) period has been found to prevent and restore the age-dependent decrease of salivary volume and protein concentration, and atrophy of the salivary glands (59). Therefore, cow’s whey, especially whey sourced from Jersey cattle, can serve as a functional food to restore the age-dependent functional decline of salivary production (50).
Polyphe
nols, resveratrol (50) and epigallocatechin-3-gallate (51) improve salivary dysfunction in a non-obese diabetic rodent model of Sjögren’s syndrome. The administration of epigallocatechin-3-gallate has been shown to prevent and delay the onset of type 1 diabetes caused by autoimmune disease in murine submandibular and pancreatic exocrine glands (52).

8. Salivary secretagogues
Cevimeline or (±)-cis-2 methylspiro [1,3-oxathiolane-5,5'-quino
culidine] monohydrochloride hemihydrate (Evoxac, SNI-2011 or AF102B) is a partial agonist of mACHRs (63). Cevimeline induces a persistent increase of AQP5 levels in the apical plasma membranes of parotid glands (26). Therefore, cevimeline administration stimulates long-lasting salivation, with its effects lasting 2-fold longer than pilocarpine (60). In patients with Sjögren’s syndrome, cevimeline augments not only the salivary flow rate but also the secretion rate of digestive and/or infection defense factors, which is beneficial in preventing oral infections and other serious sequelae (60).
Pilocarpine (Salagen) is a partial agonist of mACHRs (60). In systemic diseases (67) including Sjögren’s syndrome (68), pilocarpine is valuable as a secretagogue.
Side effects from the administration of cevimeline and pilocarpine include sweating; increased secretion from gastric, pancreatic, and intestinal glands; and diarrhea (67).

9. Conclusion
Xerostomia is a complex condition that is debilitating for many individuals. Salivary glands, which are involved in saliva secretion, are innervated by autonomic nerves. All
salivary glands are innervated by cholinergic parasympathetic nerves. In contrast, the sympathetic nerves innervate the parotid and submandibular glands but not the sublingual and minor salivary glands. AQPs play a major role in saliva secretion. Parasympathetic and sympathetic nerves regulate the trafficking of AQPs under stimulated conditions in parotid and submandibular glands. In diseased salivary glands, the lack of trafficking of AQPs leads to xerostomia. Administration of cevimeline or pilocarpine together with functional foods is a valuable treatment for xerostomia. Whey, resveratrol, and epigallocatechin-3-gallate are functional against the effects of aging, Sjögren's syndrome, and type 1 diabetes, respectively.

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