IJAE
Vol. 122, n. 3: 185-191, 2017
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Research Article - Histology and Cell Biology

Study of human Wharton's duct structure and its relationship with salivary flow

Kaori Amano[1,*], Nobuyuki Mitsukawa[2], Tomonori Harada[3], Shin Aizawa[3], Kazuyuki Shimada[3]

[1] Department of Anatomy, Kyorin University School of Medicine, Tokyo; [2] Department of Plastic Reconstructive and Aesthetic Surgery Chiba University Faculty of Medicine, Chiba; [3] Division of Anatomical Science, Department of Functional Morphology, Nihon University School of Medicine, Tokyo; Japan

Abstract

Of all major salivary glands, the human submandibular gland secretes the largest amount of saliva. Along with the sublingual duct, the main duct (Wharton's duct) is known to open into the sublingual caruncula; however, reports regarding this common opening structure are scarce and details unclear. The structure of Wharton's duct opening is quite different from that of parotid duct (Stensen's duct) opening in its overall size and diameter despite what is commonly noted in text books. About 85% of sialolith occurrences in humans is in the submandibular gland and duct, which causes local pain during swallowing in most cases. The details of Wharton's duct's inner structure is relatively unknown, and further investigation is necessary to understand its special characteristics and clinical applications. In this study, we observed the inner structure of the ducts' common opening area by scanning electron microscopy and confirmed a large number of blood vessels present in the connective tissue layer just under the epithelium. In addition, we confirmed the presence of smooth muscle in the same area using smooth muscle actin antibody. These structural findings suggest that Wharton's duct itself is likely responsible for the regulation of salivary flow.

Key words

Submandibular duct, sublingual caruncula, human, SEM, scanning electron microscopy, α-SMA, immunohistochemistry.

Introduction

The submandibular gland is one of the major salivary glands located in the submental triangle area and produces saliva, 60% of which is mixed saliva with mucus being its main component. Submandibular duct runs superior to the oral floor along the medial margin of mandibule up to the mental region at which point it merges into sublingual caruncula, sharing a common opening area with the sublingual duct.

Many cases of typical diseases involving the human submandibular gland have been reported, particularly sialolithiasis, adenoid cyst and cystic carcinoma (Witt, 2005; Saito et al., 2008). Most cases of sialolithiasis occur in the submandibular gland (80-90%), and some common symptoms of the main duct sialolithiasis include swelling of the gland before, during and after meals and severe local pain when saliva flows through the duct (Warden and Adamson, 1995).

---

* Corresponding author. E-mail: kaori26@ks.kyorin-u.ac.jp

http://www.fupress.com/ijae

DOI: 10.13128/IJAE-22978

Infectious diseases are most common in the parotid gland, which is likely associated with the structure and position of the Stensen's duct opening (Lerena et al.. 2007; Harrison, 2009; Amano et al., 2010, 2013). It has been suggested that sialolithiasis, which predominantly occurs in the submandibular gland, is caused by calcium, phosphorus and oral bacteria (streptococcus), which are major components of saliva (Teymoortash et al., 2002, 2003; Witt, 2005; Graziani et al., 2006).

In this study, we conducted scanning electron microscopy observations of the inner structure of adult Warton's duct, particularly of the opening area, and further observed the specimen using immunohistochemistry for the presence of smooth muscle to determine possible functions of the Wharton's duct.

Material and methods

This study was approved by the Ethics Committee of Kyorin University School of Medicine (H21-0197) and conforms to accepted ethical standards formulated in the Helsinki Declaration of 2003. Twenty submandibular ducts of either side were obtained from human adults ranging from age 74-93 from Japanese body donations for medical research at the Department of Anatomy, Kyorin University School of Medicine, Japan.

After removing the skin and platysma, exposing the submandibular and sublingual glands and both ducts up to the submental area of the oral floor, sublingual caruncula with part of the oral floor tissue still attached were removed en bloc along the margin of oral floor from the mandibule. Nylon threads were inserted inside both submandibular and sublingual ducts to label the opening (Fig. 1a, 1b). Specimen consisting of the opening area and part of the duct were cut and fixed in 4% paraformaldehyde for 24 hours (Fig. 1c). They were washed with 0.01 mol/L phosphate buffer (PBS), pH 7.2-7.4 (Wako pure Chemical Industries, Osaka, Japan), then soaked in 30% sucrose solution for 48 hours and prepared as 15 μ frozen sections.

For the histological examination, sections of five specimens were stained with hematoxylin and eosin, several of which were stained with van Gieson's elastica stain for observation of elastic fibers. Sections of five different specimens were prepared with monoclonal antibody against alpha-smooth muscle actin (α-SMA; Molecu-

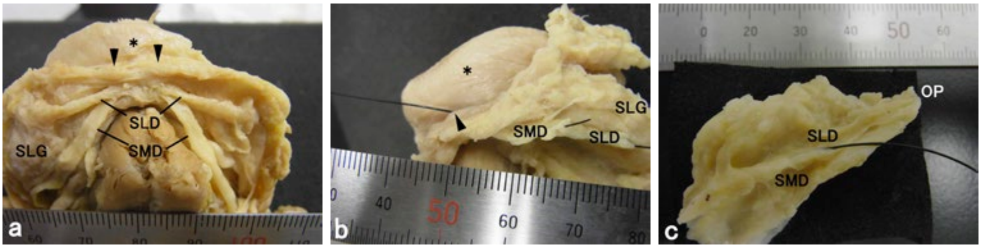

Figure 1. a) Human adult submandibular duct (SMD) and sublingual gland (SLG) duct (SLD). * = tongue. b) Submandibular and sublingual ducts and their common opening (OP); sublingual caruncula (arrow head). * = tongue. c) Inside of the two ducts and their common opening.

lar Probes, Carlsbad, CA, USA), 1:100 overnight at 4°C. They were then washed in PBS, incubated in secondary antibody (Cy3-labeled goat anti mouse IgG; Invitrogen, Carlsbad, CA, USA), 1:400, for 2 hour at 37°C. They were washed in PBS again and mounted with synthetic medium (Vector USA). All specimens were examined under a fluorescence microscope (Keyence BZ-X700, Osaka, Japan).

For scanning electron microscopy observation, ten specimens were fixed with 10% formaldehyde over 24 hours, then fixed again with 2.5% glutaraldehyde for 24 hours. They were cut open from the submandibular duct toward the opening of the oral floor under a microscope. After staining with tannin-osmium (1%) solution, specimens were dehydrated with alcohol, freeze-dried with *t*-butyl alcohol and observed under a scanning electron microscope (Hitachi S-2250N, Ibaraki, Japan).

Results

Scanning electron microscopy observation of the Wharton's duct wall and opening

Using scanning electron microscopy, we observed the inside of adult submandibular duct wall adjacent to the opening area. Under the epithelium in the connective tissue of the duct wall near the opening area, an abundance of blood vessels were present (Fig. 2). Additionally, magnification of the connective tissue layer under the epithelium revealed the presence of smooth muscle fibers (Fig. 3).

Histological and immunohistochemical observation of the Wharton's duct wall

By observing hematoxylin and eosin stained longitudinal sections of the submandibular and sublingual ducts and their common opening area, we found a plethora of blood vessels in the submandibular duct wall (Fig. 4). Observation of specimens

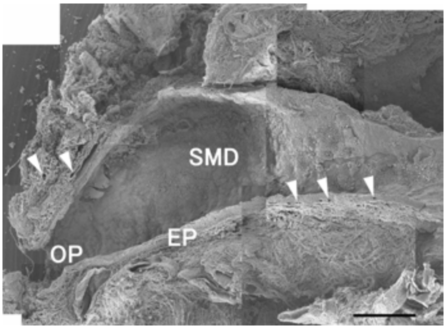

Figure 2. Internal view of the human adult submandibular gland duct (SMD) wall in sagittal section by scanning electron microscopy. Abundance of blood vessels (arrow heads) seen in the connective tissue layer of the duct opening area (OP) under the epithelium (EP). Montage of photomicrographs. Scale bar = 100 µm.

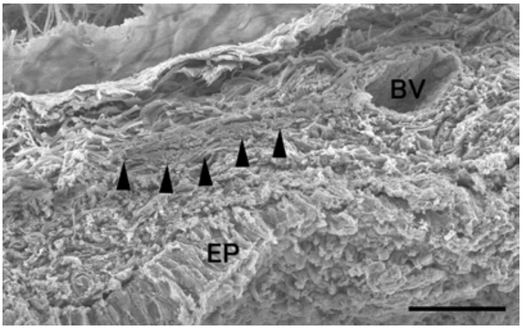

Figure 3. Highly magnified view of the human adult submandibular duct in sagittal section. Muscle fibers (arrow heads) were present in the connective tissue layer of the duct wall under the epithelium EP. BV = blood vessels. Scale bar = 50 µm.

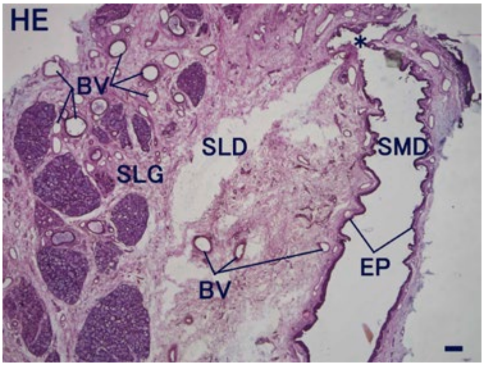

Figure 4. Light microscopy of a sagittal section of the submandibular (SMD) and sublingual duct (SLD) opening area (asterisk). EP = epithelium; BV = blood vessels. Hematoxylin and eosin. Scale bar = 200 µm.

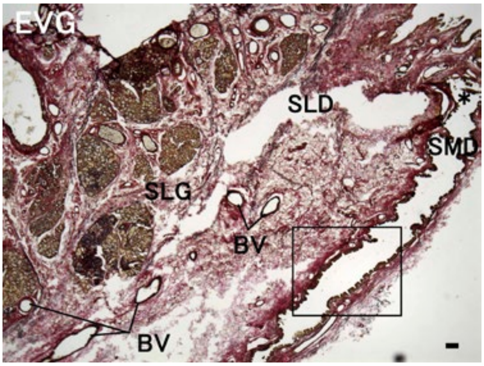

Figure 5. Light microscopy of a sagittal section of submandibular and sublingual duct opening area (asterisk). SMD = submandibular duct; the boxed area is enlarged in figure 6. SLD = sublingual duct. EP = epithelium; BV = blood vessels. Van Gieson elastica staining. Scale bar = 200 µm.

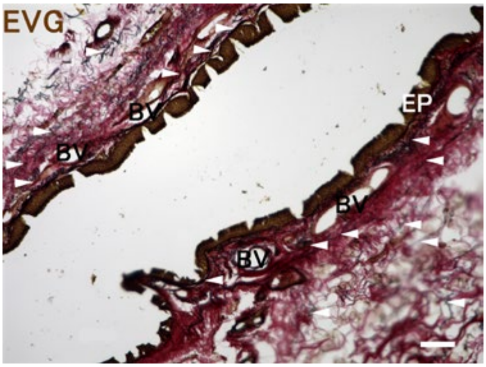

Figure 6. Magnification of the boxed area of figure 5. Elastic fibers (arrow heads) are abundant in the connective tissue layer under the epithelium. BV = blood vessels; EP = epithelium. Van Gieson elastica stain. Scale bar = 200 µm.

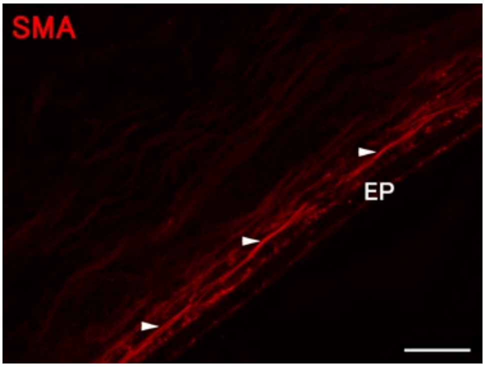

Figure 7. Sagittal section of submandibular duct immuno-stained for α-smooth muscle actin (α-SMA). Smooth muscle fibers are present in the connective tissue layer under the epithelium. EP = epithelium. Anti α-SMA indirect immunohistochemistry. Scale bar = 100 µm.

stained for elastica also confirmed an abundance of elastic fibers in both submandibular and sublingual duct walls (Fig. 5, 6). Furthermore, specimens stained in the same area against anti α-SMA actin consistently showed smooth muscle fibers in the connective tissue layer under the epithelium (Fig. 7).

Discussion

Sialolithiasis is a common disorder in humans known to occur in the submandibular gland or duct in over 80% of cases. Outbreaks in the parotid gland or the sublingual gland are extremely rare. The main reason for its frequent occurrence in this area is due to a higher mucus content of the submandibular saliva, greater degree of alkalinity, and greater concentrations of calcium and phosphate salts compared with saliva of the parotid and sublingual glands. Additionally, the extended length of the submandibular duct and the position of the gland causes saliva to flow more against gravity than in any other duct. Generally, 15 mm stones are classified as giant (Gupta et al., 2013), but in rare cases 50 mm stones have been reported (El Gehani et al., 2010; Oteri et al., 2011). Perforation of the oral floor due to giant stones is unusual (Rauso et al., 2012). The fact that giant stones can temporarily remain inside of the duct and even grow can be explained by the extraordinary elasticity of its structure.

From our observation of elastic fibers in the connective tissue under the epithelium of the Wharton's duct wall, we can speculate that its elasticity is high. Also considering the presence of abundant elastic fibers inside the parotid duct wall (Amano et al., 2013), we suggest that both parotid and submandibular ducts are structured similarly to withstand extensive external pressure considering their locations and involvement in oral movements.

Sialolith has been reported to occur predominantly in the main duct of the submandibular gland (Wharton's duct). In some cases, stones accumulated around the opening area are excreted naturally; however, with regards to ordinary stones in the duct area, surgical removal seems to be the mainstream treatment, in some cases exeresis of the entire gland is recommended depending on the location of the outbreak (Kraaij et al., 2014). Although it is possible to leave them, stone removal is necessary in most cases to relieve pain associated with swallowing during meals. Among the many surgical procedures which have been reported, Nahlieli et al. (2007) have recommended removal of the salivary gland and ductal stretching.

Intercalated and striated ducts of the submandibular gland are fewer in numbers compared to those of the parotid gland, but their structure is known to be very similar between these glands (Antonio, 2003). In comparison to the parotid duct, the main duct of the submandibular (Wharton's) was smaller in diameter; however, its inner appearance was strikingly similar to that of the parotid duct when cut open along the sagittal direction. From the presence of a large number of blood vessels in the connective tissue layer of the main duct under the epithelium, similar to results found in the parotid duct, we suggest that these walls provide some kind of defense mechanism. Additionally, the parotid duct wall in the opening area consisted of muscle fibers derived from the surrounding buccinator forming a sphincter-like structure for the duct, in lieu of smooth muscle fiber presence (Amano et al., 2013). On the contrary, the Wharton's duct wall was very thin in the opening area, almost half the thickness of the parotid duct wall connective tissue layer under the epithelium, with the presence of evenly spread smooth muscle fibers.

The bile duct of the digestive system, known for its frequent stone accumulation, similarly opens into the major duodenal papilla, and its structure resembles that of the duct of major salivary glands in many ways. For instance, bile duct, submandibular and sublingual ducts share common opening areas with another duct and are susceptible to calculus formation. Severe pain associated with stones in the bile duct is caused by an increase of internal pressure when excretion of bile and pancreatic fluids stop. In many reported cases of chronic biliary pain and recurrent pancreatitis associated with sphincter of Oddi dysfunction, the sphincter's constriction of the opening raises the internal pressure of the bile duct, which limits its ability to discharge pancreatic fluid and bile. For this reason, the structure of the sphincter has clinically important implications (Corazziari, 2003; Bristriz and Bain, 2006; Kang et al., 2015).

Similar in structure to the buccinator muscle penetrated by the parotid duct around the opening area and regulating salivary flow, the sphincter of Oddi, which is formed by smooth muscle in the major duodenal papilla (the opening area), also plays a major role in regulating the secretion of bile and pancreatic fluids (Amano et al., 2010, 2013). On the other hand, unlike the smooth muscle of the sphincter of Oddi which surrounds the duct's opening, the smooth muscle in the submandibular duct is dispersed throughout. From these results, we suggest that although it may not have such a major function as regulating the amount of saliva through the submandibular duct, smooth muscle may have a small role in the salivary flow adjustment, supported by the notion that when elastic fibers and smooth muscle fibers of the submandibular duct wall diminish due to aging, the duct can no longer maintain its lumen form, thereby raising the internal pressure of the duct and restricting its salivary flow.

Acknowledgments

We thank Ms. Miki Matsuo for her involvement in proofreading and formatting the manuscript; and Ms. Chikako Okada (Division of Systems Physiology) for preparations and assistance with the experiments.

References

Amano K., Moriyama H., Shimada K., Matsumura G. (2010) Morphological study of human fetal parotid duct and buccinator muscle and the relationship to salivary secretion. Clin. Anat. 23(6): 642-648.

Amano K., Moriyama H., Shimada K. Matsumura G. (2013) Study of human duct in the area penetrating through buccinator muscle and their functional relationship as a sphincter. Ital J Anat. Embryol. 118(1): 6-18.

Antonio N. (2003) Ten Cate's Oral Histology Development. Structure and Function. 6th Edn. Mosby, St. Louis. Pp. 299-328.

Bistritz L., Bain V.G. (2006) Sphincter of Oddi dysfunction: managing the patient with chronic biliary pain. World J. Gastroenterol. 12(24): 3793-3802.

Corazziari E. (2003) Sphincter of Oddi dysfunction. Dig. Liver Dis. 3(suppl. 3): S26-29.

El Gehani R., Krishnan B., Shehoumi M.I. (2010) Submandibular giant sialoliths: report of two cases and review of the literature. Ear Nose Throat J. 89(6): E1-4.

Graziani F., Vano M., Cei S., Tartaro G., Mario G. (2006) Unusual asymptomatic giant sialolith of submandibular gland: a clinical report. J. Craniofac. Surg. 17(3): 549-552.

Gupta A., Rattan D., Gupta R. (2013) Giant siloliths of submandibular gland duct: Report of two cases with unusual shape. Contemp. Clin. Dent. 4(1): 78-80.

Harrison J.D. (2009) Causes, natural history, and incidence of salivary stones and obstructions. Otolaryngol. Clin. North. Am. 42(6): 927-947.

Kang H.L., Sang E.L., Jae W.J., Sang Y.J. (2015) Spinal cord stimulation for intractable visceral pain due to sphincter of Oddi dysfunction. Korean J. Pain 28(1): 57-60.

Kraaij S., Karagozoglu K.H., Forouzanfar T., Veerman E.C., Brand H.S. (2014) Salivary stones: symptoms, aetiology, biochemical composition and treatment. Br. Dent. J. 217(11): E23.

Lerena J., Sancho M.A., Cáceres F., Krauel L., Parri F., Morales L. (2007) Salivary calculi in children. Cir. Pediatr. 20(2): 101-105.

Nahlieli O., Shacham R., Zagury A., Bar T., Yaffe B. (2007) The ductal stretching technique: An endoscopic-assisted technique for removal of submandibular stones. Laryngoscope 117(6): 1031-1036.

Oteri G., Procopio R.M., Cicciù M. (2011) Giant salivary gland calculi (CGGC): report of two cases. Open Dent. J. 5:90-95.

Rauso R., Gherardini G., Biondi P., Tartaro G., Colella G. (2012) A case of a giant submandibular gland calculus perforating the floor of the mouth. Ear Nose Throat J. 91(6): E25-27.

Saito M., Nishiyama H., Maruyama S., Oda Y., Saku T., Hayashi T. (2008) Adenoid cystic carcinoma of sublingual gland involving the submandibular duct. Dentomaxillofac. Radiol. 37(7): 421-424.

Teymoortash A., Buck P., Jepsen H., Werner J.A.(2002) Sialolith crystals localized intraglandularly and in the Wharton's duct of the human submandibular gland: an X-ray diffraction analysis. Arch. Oral. Biol. 48(3): 233-236.

Teymoortash A., Ramaswamy A., Werner J.A. (2003) Is there evidence of a sphincter system in Warton's duct? Etiological factors to sialolith formation. J. oral. Sci. 45(4): 233-235.

Warden P.J., Adamson D.N. (1995) Sialolithiasis: Literature review and case report. Gen. Dent. 43(6): 518-520.

Witt R.L. (2005) Histology and pathology of sialolithiasis. In: Zurhellen O. Salivary Gland Diseases, Surgical and Medical Management. Thieme, New York. Pp. 72-78.