The Ocular Surface 18 (2020) 595–603

Contents lists available at ScienceDirect

The Ocular Surface

journal homepage: www.elsevier.com/locate/jtos

New insight into lacrimal gland function: Role of the duct epithelium in tear secretion

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ARTICLE INFO

Keywords:
Lacrimal gland duct
Duct epithelium
Tear secretion

ABSTRACT

Tear secretion is a complex process with the involvement of the main and accessory lacrimal glands, corneal and conjunctival epithelial cells and the Meibomian glands. The lacrimal gland is the main source of fluid, electrolytes and proteins in tear fluid. Deficient ion and water secretion results in aqueous deficient dry eye with serious consequences on the integrity of the ocular surface. Functions of acinar cells are widely studied, whereas less information is available about the duct system of the lacrimal gland. Secretory mechanisms of duct epithelium may play an important role in tear production, but only limited studies have tried to elucidate the role of the duct system in tear secretion. Significant progress has been made in the past few years, resulting in new insight into lacrimal gland duct function. New experimental techniques were introduced, which contributed to the exploration of the role of lacrimal gland ducts in more detail. Therefore, the aim of this review is to summarize our present knowledge about the role of ducts in lacrimal gland function and tear secretion, which appears to be the first review with a focus on this topic. Short outline of pancreatic and salivary gland duct functions is also given for the purposes of comparison.

1. Introduction

Appropriate amount and balanced composition of the tear film is essential in maintaining the health of the ocular surface [1]. Tear secretion is a complex process with the involvement of the main and accessory lacrimal glands (LGs), corneal and conjunctival epithelial cells and the Meibomian glands, etc. LG is the main source of fluid, electrolytes and proteins in tear fluid. Deficient ion and water secretion of the LG results in aqueous deficient dry eye with serious consequences on the integrity of the ocular surface [2]. Dry eye can lead to potentially sight-threatening pathologies that can diminish the patient's quality of life. So far there are only very limited treatment options available, rendering the management of this debilitating disease very challenging [3]. Our understanding of the underlying mechanisms of physiological and pathological tear secretion of LG is far from complete, despite its critical importance in developing new treatment strategies [4]. Similarly to other exocrine tubuloacinar glands, LG is mainly composed of three types of cells: acinar, duct, and myoepithelial cells [5,6]. Although functions of acinar cells are widely studied resulting in a broad spectrum of information, much less is known about the secretory function of LG duct cells [7–10]. In the past 3 decades, however, considerable efforts have been made to clarify the involvement of gland ducts in secretory processes in pancreatic and salivary glands [11–14]. These investigations shed light on the molecular and functional identity and regulation of the basolateral and apical transport processes underlying fluid and ion secretion. Until the last 10 years, LG duct cells and their role in tear secretion had received much less attention in contrast to LG acinar cells or other exocrine gland duct function. Only limited number of studies has tried to elucidate the role of duct epithelium in LG secretion, although it was suggested, that secretory mechanisms of duct epithelium may play an important role in tear secretion [15–17]. Significant progress has been made in this area over the last decade, thanks to new technologies and techniques introduced. These results contributed to the exploration of LG duct function in further detail. Considerable amount of new scientific information has become available which has helped to clarify the function of LG duct cells under physiological and pathological conditions. The duct system not only provides a structural framework for the secretory end-pieces, but these epithelial cells also secrete fluid, ions and probably glycoproteins, by modifying the primary acinar fluid while being transported in the duct lumen. This assumption was proposed decades ago, but until the recent years, no definite evidence has been shown regarding the supposed role of the duct cells. Therefore, the aim of this review is to summarize and synthesize the new results and earlier evidences about

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https://doi.org/10.1016/j.jtos.2020.07.002
Received 7 April 2020; Received in revised form 21 June 2020; Accepted 7 July 2020

the functional role of LG ducts.

2. Method for literature review

Systematic literature search was performed on PubMed and Medline for the papers published before June 30, 2019. The following combined search terms were used: “lacrimal gland duct”, “lacrimal gland duct secretion”, “lacrimal gland duct epithelium”, “pancreatic duct epithelium”, “salivary duct epithelium”, “sweat gland duct” and “gland secretion”. Both human and animal studies were included in the outcome evaluation. Correspondences, notes and editorials were excluded. Neither language filter nor limitation of publication time was applied during the literature search. References of the retrieved studies were also reviewed manually to identify relevant articles.

3. Role of duct epithelium in various exocrine glands

Generally, in case of tubuloacinar glands, the contribution of acinar and duct cells to the final glandular product varies on a wide scale. Although the morphology (ie. acinar and duct epithelial cells) can possess considerable similarities among various glands, the functional role of acinar and duct cells differ greatly [18]. For example in the salivary gland, bulk of the fluid is produced by the acinar cells. In case of the pancreas, majority of pancreatic juice volume is produced by the duct cells, while fluid production of the acinar cells is negligible [13,14]. Short outline of pancreatic and salivary gland duct functions is given below, although detailed description of the contributing transporter systems are beyond the scope of this summary.

3.1. Role of duct cells in the secretion of pancreatic juice

Most of the pancreatic fluid along with the secretion of HCO_3^- is generated by the duct cells. The unique feature of pancreatic ducts is the secretion of large amount of HCO_3^- into the pancreatic juice with the final concentration of HCO_3^- at times being as high as 140 mM depending on the species [11,12,19–21]. Apical HCO_3^- secretion is largely mediated by cystic fibrosis transmembrane conductance regulator (CFTR) and Cl^-/HCO_3^- exchanger (SLC26A6, member of the SLC26 family, generally referred as “anion exchanger”: AE) which functions as an electrogenic $1Cl^-/2HCO_3^-$ transporter [22,23]. In parallel with the rise of the HCO_3^- concentration of the pancreatic juice the Cl^- concentration reciprocally decreases, as the fluid passes through the duct system. However, the sum of $[HCO_3^-]$ and $[Cl^-]$ in the ductal fluid is constant. In humans, large amount of $NaHCO_3^-$-rich pancreatic fluid is secreted by the proximally located centroacinar, intercalated and small intralobular duct segments, whereas in rodents, bulk of the fluid and bicarbonate is secreted by the interlobular ducts [24,25]. In the distal duct segments, however, most of the HCO_3^- secretion is mediated by CFTR. Although CFTR is more permeable to Cl^- than to HCO_3^-, when the electrochemical gradient for Cl^- secretion is small, the secretory flux through CFTR may be driven mostly by HCO_3^- [20,26]. Taken together, the pancreatic duct secretion is a two-step process. In stage one, the proximal ducts secrete the bulk of pancreatic fluid and HCO_3^-, mediated by the apical Cl^-/HCO_3^- exchanger and by CFTR which recycles the cytosolic Cl^- intraluminally. In the second stage, as the fluid arrives the more distal portions of the duct system, CFTR changes its Cl^-/HCO_3^- selectivity, converting it primarily to a HCO_3^- channel. In the distal duct segments most of the HCO_3^- secretion is mediated by CFTR HCO_3^- conductance as the Cl^--HCO_3^- exchanger approaches equilibrium. Due to HCO_3^- secretion and Cl^- absorption, luminal $[Cl^-]$ falls and $[HCO_3^-]$ rises as the secreted fluid flows through the duct system. HCO_3^- efflux by CFTR thus determines the final HCO_3^- concentration in the secreted fluid [11,12,14,21]. Pancreatic ductal secretion is a tightly coordinated and regulated process, where CFTR acts not only as an anion channel, but also as a key regulator of other apical and basolateral transporters. Many of these interactions occur within macromolecular complexes held together by scaffolding proteins. Paracellular and transcellular water movement driven by the osmotic gradient of the secreted ions determines the fluid secretion of the duct system. The transcellular pathway is mediated by aquaporin (AQP) channels. Although several members of AQP family have been reported to be expressed in the pancreatic ducts, AQP 1 and 5 can be the most determining contributors of fast transcellular water secretion [27,28]. In contrast to the large amount of alkali fluid produced by the duct cells, only small volume of Cl^--rich fluid is secreted by the acinar cells. Thus, the pancreatic duct is not merely a route for delivering digestive enzymes to the duodenal lumen but also secretes large amount of $NaHCO_3^-$-rich fluid to effectively wash out these enzymes to their places of action. The HCO_3^- rich fluid secreted by the duct cells also serves to neutralize gastric acid as it is delivered into the duodenum. In cystic fibrosis and certain cases of chronic pancreatitis, the pancreatic duct epithelium secretes only a small amount of fluid with neutral or even acidic pH value, which causes an obstruction of the duct lumen by clogged viscous secretory material [29,30].

3.2. Function of the salivary gland duct system

Acinar cells are the primary site of fluid and salt secretion in the salivary gland. Acinar epithelial cells also secrete proteins, glycoproteins and digestive enzymes [14,31–33]. The NaCl rich primary acinar fluid is than modified by the duct cells during its passage along the duct system, where most of the NaCl is reabsorbed [34]. $Na^+/K^+/Cl^-$ cotransporter type 1 (NKCC1) located on the basolateral membrane of acinar cells is the main site of coupled Na^+, K^+ and Cl^- entry into the cells. Na^+-H^+ exchanger (NHE) and AE mediate significant amount of Na^+ and Cl^- influx through the basolateral membrane, while Na^+-K^+ pump (Na^+/K^+ ATP-ase; NKA) is responsible to maintain the intracellular Na^+ concentration on a lower level relative to the extracellular-interstitial milieu. Apical chloride secretion is mediated by the Cl^- channel TMEM16A, while Na^+ follows the secreted Cl^- on a paracellular route. This osmotic gradient serves as driving force for water secretion into the acinar lumen. As the primary acinar fluid enters the duct system, brisk reabsorption of Cl^- and Na^+ takes place by the apical Na^+ channel in the striated proximal duct cells [34]. Intracellular Na^+ are than removed by the Na^+ pump across the basolateral membrane of the duct cells. Bicarbonate ions are also crucial component of the saliva and both acinar and duct cells can contribute in their secretion. As a result of HCO_3^- secretion, pH of the salivary fluid is near neutral, which prevent the dissolution of tooth minerals. Tight junctions of duct epithelial cells of salivary glands have greater number of intercellular junctional contacts, making the duct system watertight. In contrast, junctions of acinar cells allow considerable amount of paracellular water movement, while AQP5 channels facilitate transcellular acinar water secretion [35]. Duct cells however do not express fast transcellular water channels. The final saliva is usually hypotonic because of the poor permeability of duct epithelium to water. Greater NaCl reabsorption relative to HCO_3^- secretion also intensifies the hypotonic feature of the excretum. Tight intercellular junctions in the ducts are critical in formation of hypotonic saliva because they prevent passive fluxes of Na^+ and Cl^- from interstitial fluid to lumen via the paracellular pathway. Lack of functionally active ductal AQPs is critical because it minimizes reabsorption of H_2O in response to the lumen-interstitial osmotic pressure difference.

4. Morphology and functional role of the duct system in the LG

4.1. Search for the function of the duct system: earlier studies

Possible role of LG ducts in tear production was suggested decades ago. In 1972, Alexander and coworkers were the first pioneers who proposed the potential role of duct system in LG secretion, by using micropuncture and catheterization techniques [15]. They suggested

that LG duct system can modify the composition of the primary acinar fluid and therefore can determine the final composition of tears, which have much higher K^+ and Cl^- concentrations compared to the primary (i.e. acinar) fluid. In 1981, Dartt and colleagues showed high NKA density in LG duct cells by using auto-radiographic method [16]. Duct cells contained the heaviest density of NKA sites among the component cells of LG. They suggested that although the amount of duct cells is much lower, compared to acinar cells, duct cells could secrete a significant portion of lacrimal gland fluid. This notion was further supported by electronmicroscopy studies demonstrating secretory vesicles in duct cells, suggesting their role also in protein secretion beside ion and water production. Role of duct cells in the secretory function of LG was also suggested by the authors. These results strongly suggested that LG duct cells greatly contribute to the production of LG fluids analogous to their counterparts of pancreatic duct cells that play a critical role in the formation of pancreatic juice. LG secretion was postulated as a two-step process: acinar production of primary LG fluids followed by secretion and/or modification by duct cells into final LG fluids. Thus, ion and water components of LG fluids could be a mixture of plasma-like primary fluids secreted by acinar cells and K^+-rich fluid produced by duct cells. The intensity of NKA pump sites was later confirmed quantitatively by Okami et al., who demonstrated that expression of NKA is three to five times higher on the basolateral membranes of the duct cells compared to the acinar cells [36]. Mircheff and colleagues hypothesized that transepithelial electrolyte transport in the ducts differs from that of acini by producing Cl^-- and K^+- rich fluids by the duct cells [37]. They also estimated that duct cells could contribute to as much as 30% of final LG fluid production, even though they represent only ~15% of LG mass. Later studies have continued to support the role of duct cells in LG secretion in a variety of aspects. Existence of the intermediate conductance Ca^{2+}-activated K^+ channel ($IK_{Ca}1$) in rat LG duct cells was identified by Thompson-Vest et al., Ding and colleagues reported strong α_1 adrenergic staining on these cells in mouse, while vasoactive intestinal polypeptide receptor subtypes (VIPR I and II) and P_2X_7 purinergic receptors were identified in duct cells from rat LG by Hodges et al. [38–42]. Walcott and co-workers demonstrated NKCC1 immunoreactivity on the basolateral membrane of the duct cells from mouse LG [43].

While these studies provided insights and suggestions for the role that duct cells may play in LG tear production, most of these data were descriptive in nature, and/or failed to provide definite and direct evidence.

4.2. Tracing the molecular background of epithelial transport mechanisms: protein and gene expression studies

4.2.1. Protein and gene expression patterns under physiological condition

Thanks to modern technology and techniques, investigation of LG ducts has progressed significantly in recent years. Laser capture microdissection, a state-of-the-art technology has been used extensively in other tissues/organs [44]. With the introduction of laser capture microdissection into LG research by Ubels and colleagues, gene expression profile of LG acinar and duct cells could be analyzed separately [45]. Their gene expression and immunofluorescence examination results demonstrated that both duct cells and acinar cells express numerous ion transporters/channels and AQPs, with varied expression intensity. Distribution of those transporters is of particular interest: NKCC1, NKA and AE were located on the basolateral membrane of duct cells, while K^+/Cl^- cotransporter type 1 (KCC1), $IK_{Ca}1$, CFTR, and chloride channel type 3 (ClC3) were localized apically. Their data provided evidence of polarized expression of transporters and ion channels on LG duct membranes. These results were consistent with the hypothesis that duct cells secrete the relatively high K^+ found in tears.

Ding et al. recently established a nomenclature for the lacrimal duct system in rabbits [46]. By analyzing the anatomical, histological, and gene expression patterns, it was confirmed, that LG duct system could be separated into distinct segments. The smallest branches of the duct system are the intercalated ducts, which merge into the intralobular ducts. Interlobular, intralobar, interlobar, and main collecting ducts are the further segments of the duct tree. The gene expression profile of these various duct segments was analyzed using laser capture microdissection and real-time RT-PCR. mRNA levels of NKA α and β subunits; AE3; NHE; KCC1 and ClC2 varied significantly among various duct segments. Significant levels of CFTR mRNA were detected in cells from each duct segments, but not in acini while distribution of NKCC1 mRNA levels exhibited an opposite pattern with the highest levels in acini and less abundance in the duct cells. The level of AQP4 mRNA was relatively low in the acini and high in the intralobular, intralobar, and interlobar ducts, while the levels of AQP5 mRNA showed the opposite pattern. Live cell imaging demonstrated the presence of CFTR-mediated Cl- transport across the apical membrane of duct cells [47]. The marked differences measured in the examined transport protein mRNA levels among distinct duct segments and acini strongly suggest their differing role in the secretory processes.

4.2.2. Protein and gene expression alterations in LG ducts originated from experimental dry eye models

Two different animal models of dry eye disease were introduced recently to study LG pathology. Rabbits with induced autoimmune dacryoadenitis (IAD), a model of Sjogren's syndrome was developed in the University of Southern California [48–50]. These animals exhibit many of the dry eye symptoms and LG pathology characteristics of Sjogren's syndrome and therefore has been used extensively to study the etiology of the disease. Another animal model suitable for the investigation of dry eye disease is the pregnant rabbit model. Dry eye is closely related to changes in sex hormones, thus altered hormone profile during pregnancy may play a role in the development of dry eye [51].

By using laser capture microdissection and real time RT-PCR, substantial changes of numerous ionic transporters/channels and AQPs were reported in both acinar and duct cells in rabbit with IAD [52,53]. Data from laser capture microdissection samples collected from rabbits with IAD indicated that ducts were generally less abundant with AQP4 mRNA than control (healthy) rabbit samples. Expression of NKA subunits in the LGs of rabbits with IAD were significantly lower in many duct segments compared to the control results obtained from healthy animals. Western blot studies, however, showed opposite results, i.e., the expression levels of all subunit proteins were significantly higher in the rabbits with IAD. Whether the increased expressions of NKA subunits in rabbits with IAD was a primary or secondary consequence of IAD is unclear. Increased expression of NKA subunit proteins might be a counter regulatory response to decreased ion secretion and fluid production although increased efficiency of translation or decreased rate of protein degradation may also play some role. Significant alterations were detected in the expression of different Cl^- channels in LG duct cells from IAD rabbits compared to healthy animals [52]. The reduced levels of NKCC1 and CFTR mRNA in duct cells derived from diseased animals suggest the active contribution of these transporters in lacrimal fluid secretion as the decreased expression of their genes comes along with the reduced tear production.

As pregnant rabbits exhibit many symptoms of dry eye, LGs from these animals can demonstrate the effect of the altered hormonal milieu. Ding and colleagues reported that the expression patterns of AQP4 and AQP5 in the LGs from pregnant rabbits undergo significant changes at both gene and protein levels in both acini and specific duct segments [54]. AQP4 mRNA levels were significantly decreased while AQP5 mRNA level increased in the duct cells derived from pregnant animals. AQP4-immunoreactivity in ducts from pregnant animals however did not differ significantly from ducts from controls. Interestingly, only minimal AQP5-immunoreactivity was detected in duct cell membranes of control animals, while significant AQP5-immunoreactivity was observed in ducts of pregnant rabbits. These results

were in accordance with findings from LG ducts from IAD rabbits. Significant changes of AQP in LG have been suggested to play a role in dry eye [53,54].

In the pregnant rabbit model, real time RT-PCR results demonstrated changes of mRNA for NKA subunits in both acini and ducts as compared to control rabbits [55]. Western blot also indicated that the expressions of all three β subunits was increased during pregnancy. The predominant presence of NKA in duct cells and the changes of its subunits during pregnancy suggest active Na^+ and K^+ transport in the duct cells and their potential contribution to pregnancy-related LG secretion changes.

Epithelial Na^+ channel (ENaC) is a class of ion channels that is located in the apical membrane of polarized epithelial cells mediating Na^+ transport across the membranes [56,57]. The first molecular evidence of the presence of ENaC subunits in the rabbit LG was provided by Wang et al. [58]. Although the expression of mRNA for ENaC subunits was extremely low in LG from rabbits, immunofluorescence confirmed the presence of proteins of all three subunits in duct cells, but no immunoreactivity was detected in acinar cells. In LGs from rabbits with IAD, levels of mRNA for both α and γ subunits were significantly decreased. In LGs from pregnant rabbits, protein expressions of α and γ subunits were significantly lower, albeit no difference was detected at mRNA level. These results suggest that decreased expressions of ENaC may also play some, but probably not dominant role in the altered LG secretion in dry eye disease.

Although these studies explored substantial changes in the expression of various transport proteins in LG duct cells at the molecular level and strongly suggested, that duct cells play a key role in LG secretion, functional relevance of these alterations remained unknown. Changes in mRNA abundance and protein expression may not always correspond to each other and do not necessarily reflect the functional activity of the given transporter. The reported discrepancies in the results of mRNA and protein expression studies further highlights the limitations of data interpretations without the exploration of the function. Furthermore, it should be noted that differences in protein expressions may not always correspond with the function therefore only direct functional studies can provide the definite evidence of functional activity of the structure.

4.3. Physiological and pharmacological investigation of LG duct function: introduction of isolated duct model

Argent et al. [59] introduced an isolated pancreatic duct model for physiological and pharmacological experiments. The isolated LG duct segment model was developed by Tóth-Molnár and colleagues with the adaptation of the pancreatic duct method [60]. Similarly to the pancreatic tissue, viable duct segments can be isolated from freshly dissected LGs with enzymatic digestions and mechanical micro-dissection, followed by short-term culture (Fig. 1). Ultrastructural examinations revealed that the isolated duct segments contained numerous microvilli in the apical regions, tight junctions, secretory granules, mitochondria, and infoldings of the basolateral cell membranes. Cells were relatively rich in vesicles and secretory granules [60]. These isolated LG duct segments turned out to be useful tools for physiological and pharmacological investigation of duct function. Microfluorometry combined with the use of pH or Ca^{2+}-sensitive fluorescent dyes enabled the examination of intracellular mechanisms of LG duct epithelia.

4.3.1. Filtration permeability and fluid secretion of LG ducts

Determination of the osmotic water permeability of rabbit LG duct epithelium by means of calculation of filtration permeability (P_f) was recently reported [61]. Value of P_f describes the velocity of water molecule movement across the epithelial layer. P_f of LG ducts (~60 μm/s) proved to be lower than the highly water permeable pancreatic duct epithelium (~160 μm/s) and very similar to the distal airways of the guinea pig, while the salivary gland duct epithelium is watertight [62,63]. The reported P_f value of rabbit LG ducts was clearly sufficient

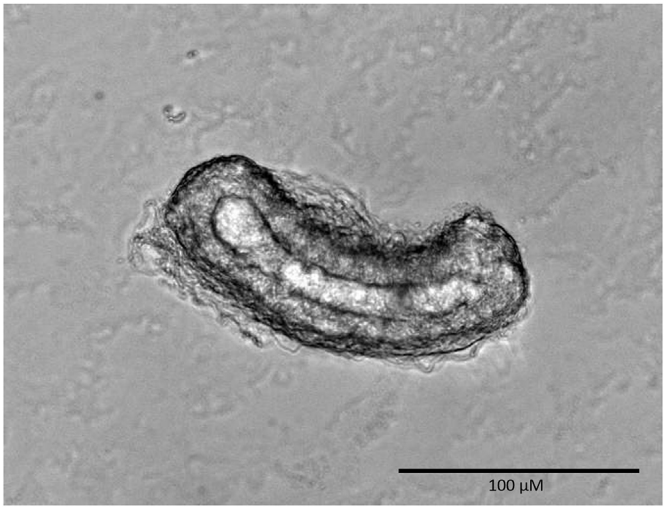

Fig. 1. Bright-field micrograph of isolated mouse lacrimal gland duct segment. Interlobular duct segments of mouse lacrimal gland were isolated and cultured overnight. The ends of ducts seal during incubation forming a closed luminal space.

to support the process of fluid secretion, and therefore the value of P_f provided indirect evidence that rabbit LG duct epithelium could be able to secrete fluid. Video-microscopic technique was adapted by Tóth-Molnár et al. to investigate fluid secretion of isolated LG duct segment [61]. The method was originally developed by Fernandez-Salazar et al. for the measurement of pancreatic duct fluid secretion [64]. As a result of epithelial cell proliferation, ends of isolated LG duct segments seal during overnight incubation, forming a closed luminal space. Therefore, secretion of duct cells into the closed intraluminal space results in swelling of the ducts as the luminal space fills with the secreted fluid. The volume of secretion can be analyzed by measuring changes in the intraluminal space. Fluid secretory effect of elevated cytosolic cAMP and Ca^{2+} signaling on ductal fluid secretion was investigated with the video-microscopy technique using isolated duct segments. Representative video recording of these experiments is displayed as supplementary video of reference 61. Elevation of cytosolic cAMP resulted in brisk fluid secretion which was almost unaffected by inhibition of HCO_3^- transport mechanisms, but was completely abolished when basolateral Cl^- uptake was blocked by bumetanide. This suggests the predominant role of Cl^- transport mechanisms over HCO_3^- secreting processes in lacrimal duct fluid secretion in rabbit. Cholinergic stimulation resulted in a biphasic secretory response with a faster initial and a plateau second phase in these experiments. Parasympatholytic atropine abolished the stimulatory effect of carbachol, suggesting the involvement of muscarinic cholinoceptors. The secretory effects of cAMP were remarkably higher, compared to the response of elevated cytosolic Ca^{2+}. These experiments provided the first direct evidence of the fluid secretory capability of LG duct epithelium and strongly supported the hypothesis that the duct system is actively involved in lacrimal fluid secretion.

4.3.2. Transporters on the basolateral membrane

The isolated duct segment model was found to be suitable to study the functional activity and relevance of basolateral transport mechanisms in LG duct cells. Using the pH sensitive fluorescent dye BCECF-AM, microfluorometric studies confirmed the functional presence of a Na^+ dependent proton efflux mechanism (NHE) on the basolateral membrane of rabbit LG ducts [60]. Amiloride partially inhibited this Na^+/H^+ exchange mechanism. Given the fact, that NHE1 and NHE2 are the most sensitive to amiloride inhibition, while NHE3 and NHE4 are amiloride resistant, these results indicated, that most of the functionally active NHEs are NHE1 and NHE2 isoforms. Functionally active Cl^--

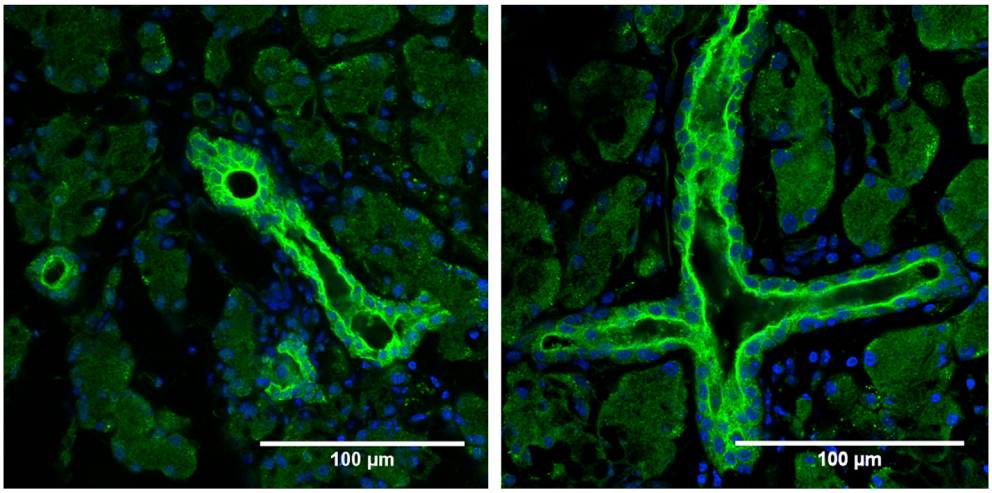

Fig. 2. Immunofluorescence staining of CFTR in histological sections of mouse lacrimal gland. Staining was most prominent in the apical membranes of duct cells showing the localization of CFTR. Hoechst was used to stain nuclei as blue.

dependent HCO_3^- efflux mechanism was characterized on the basolateral membrane of duct cells from rabbit LG. Since H_2DIDS - the classic and defining inhibitor of SLC4 family AE1-AE4 - strongly inhibited this Cl^--dependent HCO_3^- efflux mechanism, it could be considered as evidence of the functional presence of AE on the basolateral membrane of duct cells. Only marginal role of active Na^+-dependent HCO_3^- transport mechanisms could be verified in these experiments demonstrating that Na^+/HCO_3^- cotransporter (NBC), if present, play only a minor part in pH_i regulation of LG duct cells.

Using the isolated duct segment model, functional role of NKCC1 with ammonium-pulse technique was investigated in rabbit LG ducts by Vizvari et al. [65]. NKCC1 activity can be determined by the rate of intracellular acidification caused by NH_4^+ entry into the cells via this transport mechanism on abrupt application of NH_4Cl [66]. The theoretical background of this technique is the competition between NH_4^+ and K^+ uptake as NKCC1 can accept NH_4^+ at its K^+ binding site. Generally, NKCC1 activity can be characterized functionally during NH_4^+ pulse as bumetanide-sensitive, Na^+ and K^+-dependent NH_4^+ enter into the cells. Results of these experiments confirmed the existence of the pathway with these characteristics in the duct cells confirming the functional involvement of NKCC1 in basolateral ion uptake. Various factors that may influence the activity of NKCC1 in LG duct cells were also investigated. Low cytosolic Cl^- level caused a significant increase in the activation of NKCC1. Activation of NKCC1 by low intracellular Cl^- level can result in enhanced Cl^- entry into the cell through the basolateral membrane to restore cytosolic Cl^- homeostasis. It is widely demonstrated that NKCC1 has a key role in volume regulation of cells, that is, hyperosmolarity-related cell shrinkage can be a potent signal of its activation. In these experiments, hyperosmolarity of bath media proved to be a potent activator of NKCC1 in LG duct cells. NKCC1 could also be activated by elevated cytosolic cAMP level, but cholinergic stimulation seemed to play only a minor role. Regulation of NKCC1 activity proved to be consistent with the regulation of ductal fluid secretion demonstrated by the video-microscopic experiments [61]. These experiments provided direct evidence of the important role of this transporter in the coupled Cl^- and K^+ uptake on the basolateral membrane of rabbit LG duct cells.

4.3.3. Transporters on the apical membrane

In contrast to pancreatic ducts, the isolated LG duct model doesn't allow direct investigation of the apically located transporters. Cannulation of these narrow and fragile structures cannot be implemented properly, therefore the luminal space cannot be reached in sealed ducts. Transgenic mouse models carrying function loss genetic defects in transport proteins located on the apical membranes of the duct cells allows direct functional examination of these transporters [67–69]. It is widely accepted that the apically located CFTR plays a critical role in the transmembrane transport of chloride in many secretory epithelia including pancreas, salivary glands, sweat glands and airway epithelium. The defect of CFTR may cause cystic fibrosis (CF), the most common genetic disease among Caucasians [29,34,70–73]. Previous investigations demonstrated the predominant expression of CFTR in the duct cells from rat and rabbit LG [45–47]. Although several clinical studies reported dry eye symptoms in CF patients [74–76], which further strengthened the potential importance of CFTR in altered tear secretion, little is known about the role CFTR may play in LG function. In contrast, considerable attention was paid earlier to the exploration of the role of CFTR in other components of the ocular surface system such as the cornea and the conjunctiva. It has been reported that CFTR was found on the apical membrane of conjunctival and corneal epithelial cells [77–80]. Levin and Verkman found high capacity of CFTR-facilitated Cl^- transport at the ocular surface in mice [81]. The unknown functional relevance of CFTR in LG secretion was investigated in CFTR knockout (KO) mice model, introduced in LG research by Berczeli et al. [82]. Immunofluorescence confirmed the predominant presence of CFTR in the apical membrane of duct cells in this study (Fig. 2). Lack of cAMP-stimulated fluid secretion in ducts isolated from LG of CFTR KO mice suggested the important role of CFTR in LG duct secretory process. These results also demonstrated that CFTR may be the only cAMP-dependent transporter on the luminal surface of duct cells in mouse LG. In contrast to the findings revealed from mouse LG duct experiments, pancreatic ducts isolated from CFTR KO mice had significant secretory capacity for forskolin stimulation [83]. Considering the strong predominance of CFTR protein in LG ducts, CFTR may play a key role in LG secretion through modification of LG fluids while being transported in the ducts. Taking together, CFTR may play a critical role in LG secretion and disorders of its function may contribute to LG deficiency and subsequent ocular surface abnormalities.

4.3.4. Synopsis of electrolyte secretion mechanism underlying fluid secretion in LG interlobular duct epithelial cells

Fig. 3 illustrates the schematic summarized model of LG duct secretion based on the channels and transporters identified by Dartt [16], Mircheff [37], Ubels [45], Ding [46] and Tóth-Molnár [60,61,82]. Both elevated cytosolic cAMP levels and intracellular Ca^{2+} signaling can activate Cl^- and K^+ secretion through different Cl^- and K^+ selective channels located on the basolateral and apical membranes. Parasympathomimetic stimulation will activate NHE, followed by activation

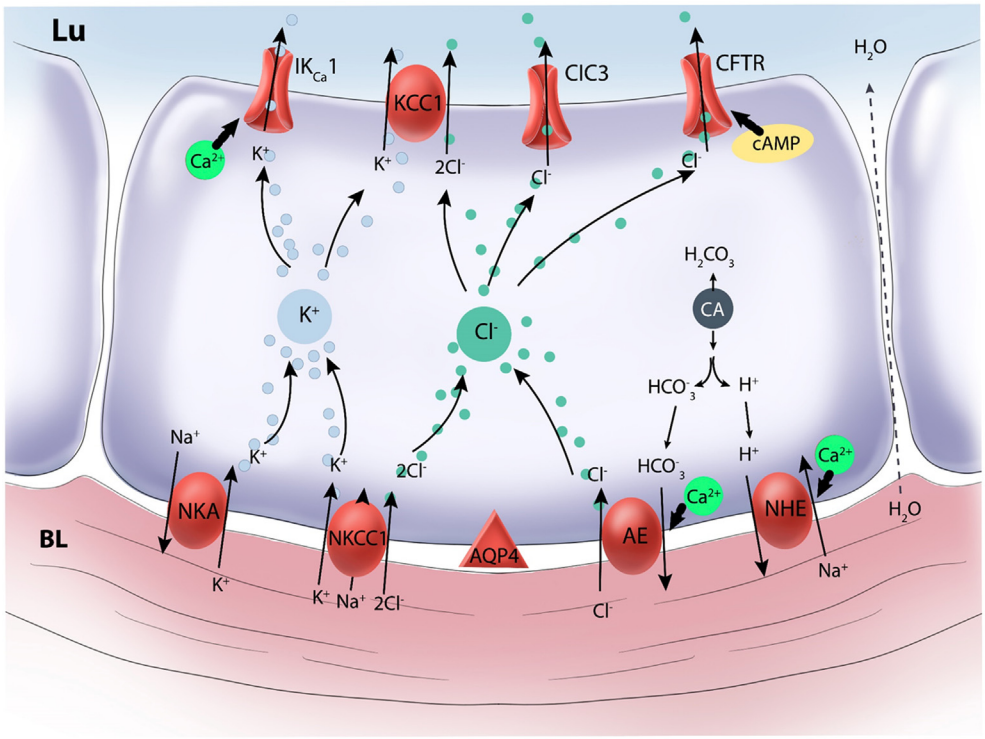

Fig. 3. Schematic model of intracellular mechanisms underlying electrolyte secretion in lacrimal gland duct epithelial cells. Summarized actions of depicted ion channels and transporters of duct epithelial cells result in intraluminal flux of Cl^- and K^+. Lu, luminal side; BL, basolateral side; NKA, Na^+/K^+-ATPase; NKCC1, $Na^+/K^+/2Cl^-$ cotransporter type 1; AQP4, aquaporin 4; AE, anion exchanger or Cl^-/HCO_3^- exchanger; NHE, Na^+/H^+ exchanger; CA, carbo-anhydrase; $IK_{Ca}1$, intermediate conductance Ca^{2+}-activated K^+ channel; KCC1, K^+/Cl^- cotransporter type 1; ClC3, chloride channel type 3; CFTR, cystic fibrosis transmembrane conductance regulator.

of AE on the basolateral membrane through Ca^{2+} signaling that drive Na^+ and Cl^- into ductal cells. Coupled influxes of Na^+, K^+ and Cl^- are mediated by bumetanide-sensitive NKCC1 located on basolateral membrane, whereas elevated Na^+ can be exchanged for K^+ through NKA. The basolateral electrogenic NKA is expressed in the basolateral membrane of ducts and is essential in establishing the electrochemical gradients for active anion secretion. The elevated intracellular Ca^{2+} concentration can also activate $IK_{Ca}{}^{2+}$, and ClC3, an apically located Cl^- channel. Elevated cytosolic cAMP level activates Cl^- secretion through apically located CFTR. Cl^- secretion through CFTR may be a major contributor to the transmembrane electrochemical gradient and subsequent electrolyte and water movements. Therefore, defects in CFTR may significantly compromise Cl^- and water secretion from LG ducts. Summarized actions of Cl^- and K^+ selective channels located on the basolateral and apical membranes of the duct cells result in intraluminal flux of Cl^- and K^+. Elevation of intraluminal Cl^- concentration is the main determinant of lumen-negative transepithelial voltage difference, which is the driving force of ductal fluid secretion. Overall actions of basolaterally and apically located ion transporters produce an osmotic gradient, which determine the direction of water flow. Water passively follows secreted ions depending on the osmotic gradient. It should be noted that the proposed model is based on the available data we have so far. Therefore, it is subject to further modification as new information becomes available about the known pathways, new mechanisms or synergisms between pathways.

5. Future perspectives in LG duct research

Electrophysiological studies with patch-clamp technique provided evidences of the existence and functional involvement of various transmembrane transporters in isolated LG acinar cells [84–86]. Although isolation of single cells from lacrimal duct segments holds a great promise to investigate various transporters with cellular electrophysiology methods, only limited amount of experimental results is available so far [87–89]. Implementation of these experiments needs the development of an advanced cell isolation technique combined with a solid identification of duct cells. Furthermore, it must be taken into account during the interpretation of the results of these electrophysiology experiments that duct cells lose their polarity following isolation. Despite that, patch-clamp investigation of duct cells provides a great chance of characterization of ion channels involved is LG duct fluid secretion.

In addition to the array of ion transporters and channels, AQPs are another important group of molecules that can be essential to the normal function of secretory epithelia [90–93]. Although AQP4 was found in the basolateral membranes of rabbit LG duct cells, whereas AQP5 was present predominantly in acinar cells [46], and changes in their ductal and acinar expression were demonstrated in experimental dry eye [53,54] the functional relevance of AQPs in LG secretion has to be clarified. It has been suggested that when secretory rate is low, AQPs are not required at physiologic conditions [94]. However, AQPs may play substantial role under pathologic conditions, and their changes may contribute in the altered LG secretion and ocular surface changes in Sjögren's syndrome and pregnancy [95]. Therefore, the role AQPs may play in LG secretion and their functional involvement in LG duct secretion needs to be clarified.

Beside water and ion secretion, LG duct epithelia may play an important role in production of other components of tear fluid. Duct cells possess numerous secretory granules, just as seen in acinar cells, albeit with varied size and density. The presence of these granules suggests the possible role of these cells in protein secretion of LG [16,60,96]. LG duct cells of rabbit are mucous or seromucous cells and those cells might be capable of secreting mucin into LG fluids [97,98]. However, the proteins and glycoproteins lacrimal duct cells secrete have not been fully identified [99–101].

Autonomic regulation of the LG duct transport processes is not fully understood. Parasympathetic pathways are considered the main regulatory machinery of the LG, sympathetic stimulation of LG function has been assumed to play an indirect role in lacrimal secretion through the regulation of blood flow [102]. There are increasing evidences, however, that sympathetic stimulation - apart from the hemodynamic effects – play a direct role in the protein secretion of the LG [39,40,103–105]. All earlier studies investigated the secretory capacity of the acinar cells or lacrimal gland pieces, but the effect of adrenergic

stimulation on duct cells is completely unknown.

6. Pharmacological modification of duct secretion: new perspectives in the treatment of dry eye?

Pharmacological modification of duct secretion may promise a novel potential in the treatment of aqueous deficient dry eye. Recent gene and protein expression studies identified and located several ion transporters and channels in LG duct cells. Function of these transmembrane structures seem to be substantial in the secretion process of LG ducts. Contrarily to the important role of the known ion channels, there is no ion channel modifying compound in the accessible dry eye treatment armory. Lack of such kind of medications is especially notable in the light of the fact that at least 15% of our presently available human medications act on ion channels [106]. Results from recent functional studies may facilitate the conception of novel dry eye therapies on this path: precise functional and pharmacological characterization of the transmembrane ion channels and transporters of LG ducts can help the understanding of their exact role under physiological and pathological conditions. This information may eventuate later the identification of some of them as possible new drug targets. Research activities focusing on LG duct function must include the investigation of human LG tissue characteristics since our present data originated solely from animal experiments and these animal data might be different from human parameters [107]. Therefore, the presently available information derived from animal experiments can strengthen the basis of the needed drug development but may not safely fulfil the requirement of such a process.

Compounds capable to modify the activity of the possible target transporters and ion channels of LG ducts may open a new horizon and reveal a scope of the causal therapy in the treatment of dry eye. One of these targets is CFTR located in the apical membrane of LG duct cells. Recent functional studies by employing the isolated duct segment model suggested that CFTR plays a pivotal role in the fluid secretion of LG duct system. Further studies are needed to clarify whether modification of CFTR function may serve as a potential target to stimulate LG secretion and therefore can be an option in treating aqueous deficient dry eye. In recent studies by Flores et al. and Lee et al., small-molecule CFTR activators increased tear secretion in a LG-ablated mouse model of dry eye [108,109]. Since LG was absent in this animal model, the enhanced Cl^- -driven fluid secretion could be from the conjunctival and corneal epithelial cells. However, CFTR affects not only corneal and conjunctival epithelial cell function, but also LG duct fluid secretion, modification of channel activity can be a potential pharmacological target.

Pharmacological investigation of LG duct transmembrane transporters may also reveal the possible harmful effect of different drugs, which may cause or aggravate tear secretion problems. This later kind of knowledge can help to improve the ophthalmologic safety of the current pharmacological therapy used for the treatment of different ocular and non-ocular diseases or may result in even new drugs with a better safety profile.

7. Summary

Dry eye is the most common eye disease for which patients seek care from eye-care professionals in the industrialized countries, with particularly high percentage in seniors and women. The etiology of dry eye remains largely unknown. Therefore, clinicians are severely limited by the therapeutic options available, translating into enormous societal and economical losses from this debilitating disease [110–113]. Dry eye often results from decreased production of LG fluids. While there has been significant progress in our understanding regarding the cellular and molecular mechanisms of tear production from LG duct cells, our information is far from complete. Better understanding of the mechanisms underlying LG function is critical to understand the pathology of dry eye and development of novel therapeutic modalities. Our notion is that LG fluid is initially produced in acini as plasma ultrafiltrate followed by modification during its passage through the duct system. Several ion transporters and channels play important role in the function of the polarized duct cell: NHE, AE on the basolateral membrane and CFTR, $IK_{Ca}{}^{2+}$, and ClC3 on the apical membrane. Different duct segments play different roles in LG fluid secretion. It was demonstrated recently that gene and protein expressions vary significantly in different duct segments, although their functional relevance remain to be investigated.

Further efforts are needed for more understanding of LG secretion at the cellular and molecular levels under physiological and pathologic conditions, information that may contribute to the development of targeted pharmacological interventions in order to improve deteriorated LG functions during dry eye. Recent advances in our knowledge regarding the LG duct system, in addition to what we've known about the acinar cells, will likely promise new frontiers for researchers to develop novel approaches by targeting duct cells, particularly specific duct segment, for developing novel approaches to manage dry eye.

Funding

The work was supported by the NKFIH NN 115611, the EFOP-3.6.1-16-2016-00008 and the SZTE ÁOK-KKA No 2018/Tóth-MolnárE research grants.

Declaration of competing interest

There is no conflict of interest for all authors.

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