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Review article

Analysis of nasal potential in murine cystic fibrosis models

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ABSTRACT

The nasal epithelium of the mouse closely mimics the bioelectrical phenotype of the human airways. Ion transport across the nasal epithelium induces a nasal transepithelial potential difference. Its measurement by a relatively non-invasive method adapted from humans allows *in vivo* longitudinal measurements of CFTR-dependent ionic transport in the murine nasal mucosa. This test offers a useful tool to assess CFTR function in preclinical studies for novel therapeutics modulating CFTR activity.

Here we extensively review work done to assess transepithelial transport in the murine respiratory epithelium in the basal state and after administration of CFTR modulators. Factors of variability and discriminative threshold between the CF and the WT mice for different readouts are discussed.

Contents

Abbreviations: ABCC7, ATP binding cassette subfamily C member 7; ATP, Adenosine triphosphate; BALF, Bronchoalveolar lavage fluid; CaCC, Ca^{2+} activated chloride channel; cAMP, 3′,5′-cyclic adenosine monophosphate; CF, Cystic fibrosis; CFTR, Cystic fibrosis transmembrane conductance regulator; ClC, Chloride channel family; CLCN2, Chloride channel protein 2; CNG, Cyclic nucleotide gated; DIDS, Disodium 4,4′-diisothiocyanatostilbene-2,2′-disulfonate; DPC, Diphenylamine-2-carboxylic acid; ENaC, Epithelial sodium channel; FDA, Food and Drug Administration; FVB/NJ, Friend Virus B NIH Jackson; GlyH-101, *N*-(2-Naphthalenyl)-((3,5-dibromo-2,4-dihydroxyphenyl)methylene)glycine hydrazide; HE, Haematoxylin-eosin; CFTR(inh)-172, 5-[(4-Carboxyphenyl)methylene]-2-thioxo-3-[(3-trifluoromethyl)phenyl-4-thiazolidinone; IP3, Inositol 1,4,5-trisphosphate; Iαl, Inter-α-inhabitor; KO, Knock-out; LPC, Lysophosphatidylcholine; NPD, Nasal transepithelial potential difference in humans; OE, Olfactory epithelium; ORN, Olfactory receptor neurons; PKA, Protein kinase A; RE, Respiratory epithelium; RNA, Ribonucleic acid; Rp-cAMPS, Rp-Cyclic 3′,5′-hydrogen phosphorothioate adenosine triethylammonium salt; TEPD, Nasal transepithelial potential difference in mice; UTP, Uridine-5′-triphosphate; V_{TE}, Transepithelial voltage; WT, Wild type.

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1. Introduction

Cystic fibrosis (CF) is an lethal autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator gene *CFTR* (*ABCC7*), located on the seventh chromosome (Rommens et al., 1989). It affects around 1/3500 of Caucasians (Brennan and Schrijver, 2016). This multi-organ disease is associated with pancreatic insufficiency, high sweat salt content and chronic bronchopulmonary disease. Its natural evolution ends in respiratory failure. It is characterised by a dysfunction of the CFTR protein, a cyclic cAMP activated chloride (Cl^-) channel located at the apical membrane of secretory epithelia.

Much knowledge has been gained from the CF mouse model. Genetically modified mice display a wide range of symptoms similar to patients with CF, including distal intestinal obstruction and failure to thrive (Wilke et al., 2011). This animal model is incomplete: unlike humans, the mice do not show bronchial airway plugging and infection (Ratcliff et al., 1993). However, the nasal epithelium of the mouse closely mimics the bioelectrical phenotype of the human airways (Grubb et al., 2009). Ion transport across the nasal epithelium induces a nasal transepithelial potential difference (NPD). Its measurement by a relatively non-invasive method adapted from humans allows *in vivo* longitudinal measurements of CFTR-dependent ionic transport in the nasal murine mucosa. Thus besides allowing the simple characterisation of the model, this test offers a useful tool to assess CFTR function in preclinical studies for novel therapeutics modulating CFTR activity. However, it still needs standardisation, evaluation of its variability and, importantly, validation of diagnostic thresholds, mandatory for the interpretation of CFTR modulator-related changes. This review addresses issues of test interpretation based on an exhaustive literature search.

2. Nasal epithelium: a tissue suitable to study cftr protein function in a murine model of cystic fibrosis

2.1. Anatomy

The murine nasal cavity is divided into two symmetric compartments separated by a cartilaginous septum. It includes the nares, the turbinates and the pharynx. There are three pairs of turbinates designated by their anatomic location, from anterior to posterior: naso-, maxillo- and ethmoid turbinate region (Fig. 1 and Fig. 1 Supplemental).

2.2. Histology

The nasal cavity is lined by four different epithelia covering submucosal glands embedded in the connective tissue. The nasal vestibule, lined by the squamous epithelium, is prolonged by a narrow zone of non-ciliated, microvilli-covered surface epithelium, referred to as the nasal transitional epithelium. The respiratory epithelium (RE) covers the dorsal portion of the nasal septum, 2 mm caudal to the nasal apex, and the medial surface of the nasoturbinates (Adams, 1972). It is a pseudostratified epithelium, composed of ciliated, goblet and basal cells. Ciliated cells are the most abundant (about 50%), though much less than in the human nasal epithelium (97%). The olfactory epithelium (OE) prolongs the RE, 9 mm caudal to the nasal apex, and covers about 50% of the nasal cavity. This pseudostratified neuro-epithelium contains sustentacular cells, olfactory receptor neurons (ORNs) and basal cells.

2.3. Cl^- and Na^+ transport across nasal epithelial cells in mice

The murine nasal epithelium displays the highest level of CFTR expression of the entire respiratory tract (Rochelle et al., 2000). CFTR is mainly expressed around gland duct openings in RE, at the apical surface of the OE, and in subepithelial serous gland acini. High levels of ENaC and Na^+ K^+ $2Cl^-$ cotransporter are homogeneously distributed at the surface of both the RE and the OE.

Other Cl^- channels are expressed in the murine nasal epithelium. This is suggested by the fact that the nasal PD in CFTR knock-out mice is not zeroed after ENaC inhibition, and that epithelium hyperpolarisation by low Cl^- solution and cAMP-dependent CFTR activation is inhibited by only one third after CFTR-specific inhibitors (Saussereau et al., 2013). Among these are (i) the CLCN2 channel (as shown by the increase in Cl^- transport in *CFTR* null mice after perfusion of the murine nasal mucosa by the ClC-2 agonist lubiprostone) and (ii) the calcium (Ca^{2+})-activated Cl^- channel, possibly TMEM16A (as reflected by the DIDS sensitive Cl^- secretory response to Ca^{2+} agonist such as ionomycin and UTP in *CFTR* null mice) (Clarke et al., 1994; Rock et al., 2009; Schiffhauer et al., 2013). cAMP and Ca^{2+}-sensitive K^+ channels are also present at the basolateral membrane, as shown by the decreased response to forskolin after application of K^+ channel inhibitors (MacVinish et al., 1998). Evidence for an outward rectifying Cl^- channel stimulated by PKA and ATP is more controversial (Gabriel et al., 1993). However, the suggestion that CLCN2 contributes to the Δ low Cl^- in *CFTR*$^{-/-}$ mice (Schiffhauer et al., 2013) is based on data single paper and is highly questionable, considering the finding of basolateral rather than apical expression of CLCN2 in other epithelial models, the failure of other group to demonstrate lubiprostone activation of recombinant CLCN2/CLC-2 channels, the use of supratherapeutical concentrations (20 μM) of lubiprostone in the nasal perfusions, and the finding that lubiprostone, activates cAMP signaling through EP4 receptors, and therefore also CFTR and basolateral K^+ channels (Bijvelds et al., 2009).