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Original Article

Deleterious impact of hyperglycemia on cystic fibrosis airway ion transport and epithelial repair

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Abstract

Background: Cystic fibrosis (CF)-related diabetes (CFRD) is associated with faster pulmonary function decline. Thus, we evaluated the impact of hyperglycemia on airway epithelial repair and transepithelial ion transport, which are critical in maintaining lung integrity and function.
Methods: Non-CF and CF airway epithelial cells were exposed to low (LG) or high (HG) glucose before ion current and wound repair rate measurements.
Results: CFTR and K^+ currents decreased after HG treatments. HG also reduced the wound healing rates of non-CF and CF cell monolayers. Although CFTR correction with VRT-325 accelerated the healing rates of CF cells monolayers under LG conditions, this improvement was significantly abrogated under HG conditions.
Conclusions: Our data highlights a deleterious impact of hyperglycemia on ion transport and epithelial repair functions, which could contribute to the deterioration in lung function in CFRD patients. HG may also interfere with the beneficial effects of CFTR rescue on airway epithelial repair.

Keywords: Airways; Epithelial repair; K^+ channels; CFTR; Cystic fibrosis; Diabetes

1. Introduction

Improvements in the medical management of patients with cystic fibrosis (CF) have led to a gradual increase in median life expectancy. However, this is associated with emerging co-morbidities, including cystic fibrosis related diabetes (CFRD). The prevalence of this complication increases with age, reaching up to 40% in CF adult patients (for review see [1,2]). The progression from normal glucose tolerance to glucose intolerance, and then to diabetes, is accompanied with a marked increase in morbidity and mortality in CF patients and a faster decline in pulmonary function [1–6]. In fact, many lines of evidence indicate that diabetes and hyperglycemia, even in non-CF patients, are associated with reduced pulmonary function compared to control non-diabetic subjects (see review [7]).

The mechanisms underlying the effect of diabetes and hyperglycemia on lung function in diabetic patients have not been clearly defined. However, high blood glucose concentrations, in particular in CF patients, have been associated with elevated levels of glucose in the airway and an increased risk of bacterial infections [8–11]. Accordingly, CFRD has been identified as one of the risk factors for both pulmonary exacerbations and their treatment failure [12–14], suggesting a potential decrease in the pulmonary defense against pathogens. Higher

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levels of oxidative stress and inflammatory mediators may also be responsible for lung tissue injury and remodeling in diabetic patients [15–17]. In fact, it seems that structural changes, including bronchiectasis, airway wall thickening and lung parenchymal histological alterations, have been observed in children with CFRD or impaired glucose tolerance, prior to an associated decline in lung function [18].

Ion transport and epithelial repair, which are crucial for lung function and integrity, may also be impacted by diabetes. An equilibrium between Cl^- secretion and Na^+ absorption is indeed necessary to maintain an adequate periciliary liquid volume and functional mucociliary clearance [19,20]. In addition, K^+ channels, particularly KvLQT1, KCa3.1 and $K_{ATP,}$ also play a role in the control of ion and liquid transport through the airway epithelia [21]. To the best of our knowledge, the impact of hyperglycemia on airway ion transport has not been previously investigated. However, there is evidence that changes in K^+ currents and the expression of different types of K^+ channels is modified after modulation of glucose concentrations in β-pancreatic or kidney embryonic cells [22–25]. Interestingly, K^+ channels are also involved in the regulation of repair processes of several epithelial tissues [26]. Our group has shown that KvLQT1, K_{ATP} and KCa3.1 channels play a role in airway and alveolar cell migration and proliferation as well as wound repair [27–29]. Some evidence also points to the involvement of Cl^- channels [30–32]. Our data showed that CFTR function is crucial for airway epithelial repair and that the basic CFTR defect in CF may be responsible, at least in part, for reduced repair rates in CF airway epithelia [32]. We also showed that a rescue of the CFTR defect with the CFTR corrector VRT-325 led to improved repair rates of CF airway monolayers [32]. However, the impact of hyperglycemia on the repair capacity of non-CF, CF and corrected-CF airway epithelia has not yet been defined.

In light of the evidence that diabetes and hyperglycemia impair the wound-repair capacity of other epithelial tissues [33–35] and affect ion currents and/or channel expression in different cell types [22–25,36], we hypothesized that these crucial functions of the respiratory epithelia may be affected by high glucose levels. To address this, we investigated the effect of exposure of non-CF and CF airway cells to normal glucose (5 mM, low glucose, LG) or 25 mM glucose concentrations (high glucose condition, HG), which has been commonly used to mimic diabetic-like challenge in many studies [23–25,33,35]. We then compared the Cl^- and K^+ currents as well as the repair rates of non-CF and CF airway cell monolayers under both conditions. Our data indicated that high glucose treatment impairs ion transport as well as the ability of airway epithelial monolayers to repair after mechanical injury. This deleterious effect on two crucial functions of the airway epithelia could contribute to the decline in pulmonary function in diabetic patients.

2. Materials and methods

2.1. Cell culture

CFBE41o- airway epithelial cells [37], transduced with wt-CFTR (CFBE-wt) or ΔF508-CFTR (CFBE-ΔF508) [38], were maintained for 8 days in EMEM (Life Technologies, Burlington, ON) supplemented with 10% FBS (Life Technologies), 2 mM L-glutamine (Invitrogen) and 100 U/ml of penicillin-streptomycin (Life Technologies) in Petri dishes or for 2 to 4 weeks on permeant filters (Costar Transwell, Corning Inc., Corning, NY) coated with 1 mg/ml BSA (Invitrogen), 0.05 mg/ml bovine collagen I (Invitrogen) and 1 mg/ml human fibronectin (VWR, Mont-Royal, Canada), in LHC medium (Life Technologies Inc., Burlington, ON), as previously described [32]. EMEM (containing 5 mM glucose) was supplemented with 20 mM glucose (for a total of 25 mM glucose, high glucose, HG) or 20 mM mannitol (control condition with identical osmotic change, low glucose, LG) 24 h, 48 h or 7 days before experiments.

Primary human airway nasal cells were recovered after polypectomy procedures from CF patients with various genotypes (four homozygous ΔF508/ΔF508, two ΔF508/N1303, one ΔF508/undefined and one ΔF508/A455E, median age 21.5 yrs, mean FEV1 of 83.1 ± 6.1%), according to protocols approved by the CHUM ethical committee and with written informed consent by the patients. After dissection, polyps were digested overnight, at 4 °C, under gentle agitation in MEM (Invitrogen) supplemented with 7.5% $NaHCO_3$ (Sigma-Aldrich, Oakville, ON), 2 mM L-glutamine, 10 mM HEPES (Fisher, Ottawa, Canada), 0.05 mg/ml gentamycin (Sandoz, Boucherville, QC), 25 U/ml penicillin–streptomycin, 0.25 μg/ml Fungizone (Invitrogen), 0.1% pronase E (Sigma-Aldrich) and 10 μg/ml DNAse (Sigma-Aldrich). After pronase-DNAse neutralization, the cells were gently scraped off the remaining tissue. Cells were cultured in petri dishes coated with Purecol (Cedarlane, Burlington, Canada) and maintained in complete SAGM (LHC basal medium supplemented with the SAGM kit (Clonetics, Walkersville, MD)) and 25 ng/ml EGF (Sigma-Aldrich), 100 U/ml of penicillin-streptomycin, 0.07 μg/ml phosphorylethanolamine (Sigma-Aldrich), 1.86 ng/ml ethanolamine (Sigma-Aldrich), 0.05 nM retinoic acid (Sigma-Aldrich), stock 11, stock 4 and trace elements [32].

2.2. Wound-healing assays

Airway epithelial cells cultured on plastic support were injured mechanically with a pipette tip following a highly reproducible technique [29,32,39]. Marks under the wells allowed us to photograph the wounds exactly at the same place at time 0 after injury and after 6-h of wound-closure (Fig. 3A). The wound repair rates ($\mu m^2/h$) were then measured individually in each wound with ImageJ software, by measuring the wound area at time 0 (μm^2) minus the wound area at time 6 h (μm^2), this value corresponding to the repaired area (μm^2), which is then divided by the duration (6 h). The same procedure was repeated 12 times (i.e. 6 wounds/well, 2 wells/condition, per experiment (n)) and the mean wound repair rates (among the 12 measurements) were calculated. This method allowed us to correct for variations in the initial wound area. It should be emphasized that our method is highly reproducible and the initial wound area were very similar in size. This commonly employed "wound-healing assay" enables the study of the early mechanisms triggered after injury, i.e. cell