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Journal of Cystic Fibrosis 15 (2016) 681 – 688

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Original Article

Advanced glycation end products are elevated in cystic fibrosis-related diabetes and correlate with worse lung function

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Received 29 May 2015; revised 9 November 2015; accepted 14 December 2015
Available online 23 January 2016

Abstract

Background: The onset of cystic fibrosis-related diabetes (CFRD) exacerbates lung function decline and increases mortality. One pathway that may worsen the lung dysfunction associated with CFRD is that of the receptor for advanced glycation end products (RAGE) and its ligands.
Methods: Human plasma was obtained from age-matched healthy, CF and CFRD patients. Plasma RAGE ligands (i.e. advanced glycation end products, S100A12, and high-mobility group protein B1) and soluble RAGE (sRAGE) levels were measured.
Results: CFRD patients had elevated plasma levels of AGEs and S100A12. Soluble RAGE, a RAGE ligand decoy receptor, was not significantly different between groups. Plasma AGE levels and S100A12 levels had significantly negative correlations with FEV_1.
Conclusions: AGEs are significantly elevated in CFRD and correlate negatively with FEV_1. CFRD patients did not have significant increases in the decoy sRAGE, suggesting there may be heightened binding and activation of RAGE in CFRD exacerbating activation of proinflammatory pathways.

Keywords: Cystic fibrosis-related diabetes; Advanced glycation end products; Receptor for advanced glycation end products; Cystic fibrosis

1. Introduction

Cystic fibrosis (CF) is the most common inheritable lethal disorder amongst Caucasians, with a current average life expectancy of only 40.7 years [5]. While CF is primarily characterized by a progressive obstructive pulmonary disease, loss of functional CFTR can manifest with other multiple organ system dysfunctions [33]. The most common co-morbidity associated with CF, and perhaps the most devastating, is cystic fibrosis-related diabetes (CFRD). The onset of CFRD at any age is associated with an accelerated rate of pulmonary decline as well as increased frequency of pulmonary exacerbations [23,29]. Despite considerable increases in morbidity and mortality associated with CFRD, relatively little is known about the pathobiology of the disease. An important receptor pathway that may have a significant effect on CFRD-associated pulmonary decline and morbidity is that of the receptor for advanced glycation end products (RAGE).

RAGE is a multi-ligand receptor in the MHC class III immunoglobulin superfamily [35]. It has been implicated in conditions of chronic inflammation, micro- and macrovascular disease associated with non-CF diabetes, wound healing, and cancer progression [15,37,38,40]. There are relatively high levels of RAGE expression within the lung epithelium, which may position the receptor as an important component involved

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http://dx.doi.org/10.1016/j.jcf.2015.12.011

in lung homeostasis and pathology [2,4,10]. As its name implies, it is a receptor for advanced glycation end products (AGEs), although RAGE is capable of binding a number of other ligands [6,16,17,46].

Few studies have explored RAGE signaling in CF. Utilizing flow cytometry, RAGE expression was discovered to be significantly elevated in CF airway neutrophils compared to their blood counterparts [27]. Foell and coworkers found that levels of S100A12, a potent RAGE ligand released by neutrophils, were elevated in lung tissue specimens of patients with end-stage CF disease [13]. Sputum levels of high-mobility group protein B1 (HMGB-1), another RAGE ligand, were found to be significantly elevated in CF sputum both at baseline and during acute pulmonary exacerbations [11,34]. More recently, heightened expression of RAGE was demonstrated in lung tissue from CFTR knockout mice and antagonizing RAGE signaling attenuated inflammation in this model [19]. Only one prior study has explored this receptor or its various ligands in CFRD**.** Mulrennan and coworkers found ratios of S100A12 to soluble RAGE were significantly elevated in sputum from patients with CFRD and this correlated with worsening lung function [30]. However, the study was limited by its small size. Additionally, its cohort of CFRD patients had an average body mass index consistent with obesity (30.72 ± 21.13), which is very atypical for this patient population suggesting that this cross-sectional cohort may not be an accurate sample of the larger CFRD population [44]. As such, the present study set out to further explore components of the RAGE system in relation to the additional stress of CFRD.

2. Materials and methods

2.1. Human subjects

Patient-derived samples were purchased from the Emory + Children's Center for Cystic Fibrosis and Airways Disease Research Biospecimen Registry (CFBR). All patients willingly gave consent and were enrolled utilizing the informed consent process in accordance with the policies and regulations set forth by the Emory University Institutional Review Board (IRB#00,042,577). Samples were obtained from three separate categories: healthy controls, cystic fibrosis patients with normoglycemia and a normal oral glucose tolerance test (OGTT), and cystic fibrosis patients diagnosed with CFRD by two previous abnormal OGTTs. Individuals with impaired glucose tolerance as determined by an OGTT (plasma blood glucose ≥ 140 mg/dL but < 200 mg/dL 2 h after oral glucose challenge) were not included in this study. When samples were provided to the CFBR, consent was obtained for chart review in order to link clinical data to the patient samples. Baseline demographic and clinical characteristics were obtained from the patient's chart on the day of the sample acquisition. Healthy controls had no known history of diabetes or any chronic disease, including lung disease, and therefore are missing certain clinical values (e.g. hemoglobin A1c (HgbA1c) values) as these tests were not relevant to their clinical visit.

2.2. Plasma

Whole blood was collected via venipuncture in a K_2 EDTA blood tube and spun at 1300 × *g* at 21 °C for 10 min. Plasma was extracted, aliquoted, and stored at −80 °C until use.

2.3. ELISAs

Plasma sRAGE was measured utilizing the human RAGE Quantikine ELISA kit (R&D Systems, Minneapolis, MN) following the manufacturer's instructions. Plasma levels of RAGE ligands including AGEs, S100A12, and HMGB-1 were measured with commercially available ELISA kits: OxiSelect AGE ELISA Kit (Cell Biolabs, San Diego, CA), Circulex S100A12/EN-RAGE ELISA kit (BML, Woburn, MA), and human HMGB-1 ELISA Kit (MyBiosource, San Diego, CA), as per the manufacturers' directions.

2.4. Statistical analysis

Data are expressed as means ± SD. Statistical analyses were performed with SigmaPlot 12.0 software (Jandel Scientific). Comparisons between groups were performed by Kruskal–Wallis one-way analysis of variance (ANOVA) based on ranks with pairwise multiple comparison procedures utilizing the Dunn method. Statistically significant differences between groups were pre-defined as $p < 0.05$. Correlations were determined using Spearman's rank order correlation.

3. Results

3.1. Patient characteristics

Subjects' baseline demographics and clinical characteristics from three defined groups (1, healthy controls; 2, CF patients with normoglycemia; or 3, CF patients with confirmed CFRD) are shown in Table 1. Clinical data are linked to the patient's samples through chart review. As such, healthy controls—individuals with no known lung disease or diabetes—did not routinely have lab results or procedures that are commonly performed for patients with CF (e.g. HgbA1c levels or pulmonary function tests). All three groups were well matched for age, with an average age throughout the entire cohort of 29.16 ± 8.25 years. Individuals with CFRD had significantly higher levels of HgbA1c consistent with their known history of hyperglycemia, though the average HgbA1c in the CFRD patients was modestly elevated at 6.28% ± 0.66%. Consistent with the known worsening decline in pulmonary function associated with the onset of diabetes, absolute values for forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were significantly lower in CFRD patients (2.75 ± 0.95 L and 1.73 ± 0.75 L, respectively) compared to normoglycemic CF patients (4.14 ± 1.3 L and 3.10 ± 1.03 L, respectively). This difference in PFT values between normoglycemic CF and CFRD patients remained when percent predicted volumes were compared. CFRD patients had a significantly lower FEV_1 percent predicted at 58.0% ± 20.8% compared to normoglycemic CF patients with an average FEV_1