146 Oxidative stress biomarkers in cystic fibrosis and non-cystic fibrosis bronchiectasis patients

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Objetives: Oxidative stress is believed to play an important role in the pathophysiology of cystic fibrosis (CF). Many factors may contribute to increased oxidative stress in CF, as the disease combines the increased production of reactive oxygen species (ROS) with impaired antioxidant protection. The aim, therefore, of this study was to evaluate oxidative stress using different biomarkers (both plasma and intracellular in peripheral blood leukocytes) in a group of clinically stable adult patients with bronchiectasis (CF and non-CF) and compare the results with a group of healthy controls.
Methods: This cross-sectional study included 90 patients with a diagnosis of bronchiectasis with and without CF, periodically monitored in the adult bronchiectasis/CF unit at a university hospital. A control group comprised 50 healthy subjects, matched with respect to patient sex, age and nutritional status.
The intracellular oxidative stress biomarkers (mitochondrial membrane potential, superoxide anion and hydrogen peroxide and intracellular glutathione) were analyzed in white blood cells as total leukocytes, neutrophils, lymphocytes and monocytes. The plasma oxidative stress biomarkers (total antioxidant capacity, glutathione peroxidase activity, superoxide dismutase activity, catalase activity, 8-iso-prostaglandin F2α, TBARs) were measured.
Conclusions: Cell and plasma markers of oxidative stress were raised in the patients with bronchiectasis as compared with the controls and no differences depending on the cause of the bronchiectasis (CF vs non-CF) were found.

147 Comparative analysis of inflammatory markers in cystic fibrosis and non-cystic fibrosis bronchiectasis

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Bronchiectasis is an airway disease characterized by thickening of the bronchial wall, chronic inflammation and destruction of affected bronchi. Underlying aetiologies include severe pulmonary infection and cystic fibrosis (CF), however in a large number of patients with non-CF related bronchiectasis (NCFB) no cause is found. This study evaluated airway inflammatory markers in patients with CF and NCFB so as to facilitate the identification of common or different treatment options.
Bronchoalveolar lavage (BAL) was performed in patients with CF, NCFB and controls (n = 10). Neutrophil elastase (NE), MMP-2 and MMP-9 activity was quantified by fluorometric assay and zymography. Levels of pro-inflammatory mediators (IL-8 and IL-18) and anti-inflammatory cytokines (IL-4 and IL-10) were measured by ELISA.
Patients with CF had elevated levels of NE ($p < 0.0001$), MMP-2 and MMP-9 activity ($P < 0.01$) when compared to controls, while protease levels in NCFB BAL was at an intermediate level between CF and control samples. Levels of IL-8 were elevated in NCFB BAL when compared to controls ($P < 0.05$) but below concentrations observed in CF ($P < 0.05$). IL-18 levels in CF and NCFB BAL were similar to that of controls. Equal levels of IL-10 and IL-4 cytokine were observed in both CF and NCFB groups which were elevated above control values ($P < 0.05$). This study demonstrates that while similar inflammatory markers are present in the airways of both CF and NCFB, they are also significantly different from each other. Results indicate that appropriate management of the protease burden and the anti-inflammatory response may reduce deterioration of pulmonary function associated with both CF and NCFB.

148 Oxidative stress and inflammation in cystic fibrosis (CF)

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Oxidative stress has a relevant role in the pathogenesis of pulmonary disease in CF. **BAP test** is a validated photometric test on capillary blood samples for the determination of biological antioxidant potential. BAP test normal value is >2200 microvol/L. **d-ROM** test is a validated test wich measures the oxidant ability of a plasma sample. d-ROM test normal value is <300 UCARR. We performed BAP test, d-ROM test and CRP (C-reactive protein) in 35 CF patients (19 male, mean age 18.6 range 43–4, mean FEV1: 59% pr, range 23–120%, st.dev 30). Our results support the condition of high oxidative stress in CF: mean BAP test was 1542 UCARR (range 2304–697, st.dev 509.26), mean d-ROM test was 310 microvol/L (range 132–586, st.dev 95.12). d-ROM was significantly higher in 22 pts with elevated PCR than in 13 pts with normal PCR (mean 355, range 238–586, st.dev 81.09 and mean 234, range 132–351, st.dev 64.6 respectively, p: 0.000). Chi-square test also resulted statistical significance (p: 0.000). The dosage of d-ROM test pre- and post-antibiotic e.v. treatment in 7 pts showed a statistically significant improvement (mean value pre-: 352, range 302–381, st.dev 30, mean value post-: 269, range 182–393, st.dev 73, p: 0.007) according to the reduction in CRP values. BAP test doesn't appear to correlate with CRP in CF even during exacerbations and antibiotic ev therapy. Our results show that, d-ROM and BAP tests may have a role in the oxidative status assessment. The correlation between oxidative stress (d-ROM test) and inflammation (CRP) in CF patients was also shown.

149 Gibberellin induces the NF-κB inhibitor A20 in airway epithelial cells

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Objectives: The zinc finger protein A20 inhibits NF-κB activation in response to LPS. A20 expression is reduced in CF and accompanied by persistent activation of NF-kB and release of inflammatory cytokines [1]. An A20 inducing drug is not currently available. Gibberellin (GA) is a plant diterpenoid involved in the regulation of plant development via induction of A20/ZnF proteins [2]. GA was shown to have anti-inflammatory effects in diabetic mice although the mechanism was not explored [3]. We hypothesise that GA induces A20 and examine its anti-inflammatory properties in airway epithelial cells.
Methods: 16HBE14o– cells (n = 3) and primary nasal epithelial cells (NECs) from healthy subjects (n = 2) were pre-treated with 30 μM GA (Sigma) for 1h prior to the addition of 10 μM LPS (*P. aeruginosa*, Sigma) for 0–24h. A20 induction was determined by qPCR while IL-8 release was measured by ELISA.
Conclusions: A20 mRNA was maximally induced in 16HBE14o– 1h after LPS stimulation. Thereafter, A20 expression fell back to basal levels. However, in 16HBE14o– pretreated with GA, A20 remained at maximal levels 24h after LPS stimulation. The increase in A20 expression was accompanied by reduced IL-8 release in GA treated cells. Reduced IL-8 secretion was confirmed in primary NECs treated with LPS where a 34% reduction was observed with GA pretreatment. Our findings confirm the reported anti-inflammatory effects of GA in airway epithelial cells for the first time. Future studies will explore this mechanism further and will investigate the effect of GA in CF epithelial cells.

Reference(s)

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