P40 INHIBITION OF PRO-INFLAMMATORY GENES IN CF BRONCHIAL EPITHELIAL CELLS BY MEDICINAL PLANT EXTRACTS

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Innovative pharmacological approaches to control the excessive neutrophil infiltrates into the bronchial lumen of CF patients are thought to be beneficial to reduce the extensive airway tissue damage. The activation of expression of pro-inflammatory genes by *P. aeruginosa* with bronchial epithelial cells is a central mechanism to be targeted with novel therapies. Medicinal plants are attracting a growing interest because of their potential safety, already tested in large scale applications in human diseases. However, due to the presence of different active principles in each plant extract, whose multifunctional effects may even result contradictory, understanding the effect of each component is mandatory to pursue selective and reproducible applications. A panel of medicinal plant extracts have been firstly screened for their capacity to interfere in the binding of nuclear transcription factor proteins (TF) with DNA consensus sequences identified in the promoters of the pro-inflammatory genes, thus for their potential inhibitory action on gene expression. Extracts from *Emblica officinalis* (EO), *Aegle marmelos* (AM), *Polyalthia longifolia* (PL) have been screened for their ability to interfere with the TFs NF-κB, AP-1 and CREB induced by *P. aeruginosa* and have been shown to inhibit TF/DNA interactions, opening the possibility of potential applications to down-regulate expression of pro-inflammatory genes. Extracts from EO, AM, PL were tested in IB3–1 bronchial cells exposed to the *P. aeruginosa* PAO1. EO, AM and PL strongly inhibited the PAO1-dependent transcription of IL-8 in IB3–1 cells. Pyrogallol, one active principle of EO, was tested in IB3–1 cells, where it inhibited the transcription of IL-8, GRO-α and γ, of ICAM-1 and IL-6, similarly to the whole EO extract, whereas a second active principle from EO, namely 5-hydroxy-isoquinoline, had no effect. In conclusion, extracts from plants of the traditional medicine can inhibit expression of pro-inflammatory genes and screening active principles purified from medicinal plants could result useful to identify safe and innovative pharmaceutical molecules to control lung inflammation in the lung of CF patients.

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P41* ANTI-INFLAMMATORY EFFECT OF MIGLUSTAT IN BRONCHIAL CELLS

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The role of CFTR deficiency in promoting inflammation remains unclear. It has been recently reported by Perez et al. that the inhibition of function of w/t CFTR produces an inflammatory profile that resembles that observed in CF patients [Am J Physiol Lung Cell Mol Physiol, 2007], whereas we demonstrated that correction of F508del CFTR function with MPB-07 down modulates the *P. aeruginosa* dependent expression of the pro-inflammatory mediators IL-8 and ICAM-1 in CF bronchial cells [Am. J. Resp. Cell. Mol Biology, 2007]. Since both evidence support a direct link between CFTR function and inflammation, we extended our investigation to other F508del CFTR correctors, such as miglustat [FEBS letters, 2006], which is an approved drug for Gaucher disease, in comparison with an isomer without any correcting effect, namely NB-DGJ. Miglustat significantly reduced the expression of IL-8 and ICAM-1 in CF bronchial cells IB3–1 and in CuFi-1, upon infection by PAO1 or stimulation with TNFα or IL-1β and restored CFTR function. Miglustat had no major effects on overall binding activity of transcription factors NF-*k*B and AP-1, activated by PAO1 in these cell lines. NB-DGJ, which is not a corrector of function of F508del CFTR, down modulated the inflammatory response in CF cells. In addition, both miglustat and NB-DGJ produced the same anti-inflammatory effect in non CF NuLi-1 cells. In conclusion, miglustat is a corrector of F508del CFTR function in CF bronchial IB3–1 and CuFi-1 cells and has an anti-inflammatory effect which is independent of the correction of F508del CFTR, by interfering with the pro-inflammatory signaling downstream the receptors for pathogens and pro-inflammatory cytokines. Since miglustat is already approved for the treatment of Gaucher disease and other glycosphingolipidoses, it could represent an interesting new molecule to ameliorate lung inflammation in CF patients.

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P42 "DECOY" MOLECULES FOR NUCLEAR TRANSCRIPTION FACTORS AND REGULATION OF EXPRESSION OF PROINFLAMMATORY GENES

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Massive infiltrates of neutrophils in the mucosal wall and lumen of the conductive airways of CF patients contribute to the progressive lung function decline by releasing different proteases responsible for the progressive airway tissue damage. Bacterial products and pathogens themselves within the mucopurulent material of the airway surface fluid induce the activation of transcription factors such as NF-kappaB, AP-1, Sp1, NF-IL6, NF-AT, Elk-1, CREB resulting in expression of chemo/cytokine genes driving the recruitment of leukocytes inside the bronchial lumen. We are exploring the transcription factor (TF) "decoy" strategy, in which oligodeoxynucleotides (ODN) mimicking the consensus sequences for the TFs proteins identified in the promoters of different chemo/cytokines are delivered inside the cell in order to interfere with gene transcription. CF bronchial epithelial cells IB3–1 have been exposed to the *P. aeruginosa* strain PAO1. Transcription of genes involved in innate immune response has been quantified by real-time (RT) PCR. Transcription factor "decoy" ODNs directed against the consensus sequences identified in the promoters of different genes have been designed and validated by testing their interference in TF protein/DNA binding assays. Transfection of IB3–1 cells with HIV-1 LTR and IgK chain NF-kB ODN "decoys" complexed with Lipofectamine, performed 30 hrs before challenge with PAO1, was shown previously to inhibit strongly PAO1-dependent transcription of IL-8. Therefore other TF "decoy" ODNs have been also tested: (a) ODN for NF-kB from IL-8 promoter inhibited IL-8, GRO-gamma and IL-6; (b) ODN for Sp1 from HIV-1 genome inhibited IL-6; (c) ODN for AP-1 from IL-8 promoter inhibited both IL-8 and GRO-gamma. In conclusion, transcription of cytokines in CF bronchial epithelial cells *in vitro* can be inhibited with different efficiency and selectivity by TF "decoy" molecules. These results provide useful hints for a gene-targeted anti-inflammatory approach and add further information on the regulation of expression of pro-inflammatory genes in bronchial epithelial cells.

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P43 EZRIN PHOSPHORYLATION AND ACTIVATION OF RHOA PLAY A ROLE IN THE RESCUE OF ΔF508CFTR IN CFBE41O− CELLS BY NHERF1

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We have demonstrated an important role for NHERF1 in regulating CFTR localization and stability on the apical membrane of normal cell monolayers [1]. NHERF1 over-expression in CFBE41o– (CFBE) cells induces a significant increase both of CFTR expression at the apical membrane and of the PKA-dependent activation of chloride efflux. We hypothesize that this rescue of ΔF508CFTR-dependent chloride secretion induced by NHERF1 over-expression could be driven by the interaction of the actin cytoskeleton with the ezrin-NHERF1-CFTR multiprotein complex. Indeed, confocal analysis demonstrated that normal 16HBE14o– (HBE) cells have a well-organized actin cytoskeleton, while CFBE cells have a substantial disassembly of actin filaments. Importantly, over-expression of NHERF1 in the CFBE cells led to an F-actin cytoskeleton reorganization into well organized stress fibers on the apical side that closely resemble the normal cells. These results suggest that an organized cytoskeleton is critical for apical membrane CFTR expression and/or CFTR activity and that NHERF1 has an essential role in regulating these mechanisms, possibly through its interaction with cytoskeleton proteins such as ezrin. As only active ezrin is able to bind to NHERF1, we analyzed the distribution of active P-T567-ezrin with respect to total ezrin. Confocal analysis demonstrated that active ezrin colocalized with NHERF1 at the apical membrane in HBE monolayers and in CFBE monolayers over-expressing NHERF1 while it was mislocalized at basolateral membrane in CFBE cell monolayers. These results were confirmed by differential detergent extraction experiments where the ratio active ezrin/total ezrin was increased in CFBE cells transfected with NHERF1 with respect to CFBE cells. To investigate how the association of CFTR with the multiprotein complex NHERF1-ezrin-actin is regulated we focused our attention on the small GTPase RhoA, as RhoA has been shown to activate ezrin. Constitutive active RhoA induced a rescue of CFTR activity in CFBE cells while having no effect in either HBE cells or in CFBE