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 factors (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 officinale (EO), Aegle marmelos (AM), Polyalthia longifolia (PL) have been screened for their ability to interfere with the TFs NF-kB, 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 from EO, AM, PL extracts in IB3−1 cells exposed to the P. aeruginosa PA01. EO, AM and PL strongly inhibited the PA01-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-a and -g, of ICAM-1 and IL-6, similarly to the whole EO extract, whereas a second active principle from EO, namely 5-hydroxy-isouquinoline, had no effect. In conclusion, extracts from plants of the traditional medicine can inhibit expression different active principles from EO, AM and PL, affected the expression of different pro-inflammatory genes, thus for their potential safety, already tested in large scale applications in human diseases. 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 inflammatory genes have been designed and validated for their ability to interfere with gene transcription. CF bronchial epithelial cells IB3−1 have been exposed to the P. aeruginosa strain PA01. 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 HκK chain NF-κB ODN “decoys” complexed with Lipofectamine, performed 30 hrs before challenge with PA01, was shown previously to inhibit strongly PA01-dependent transcription of IL-8. Hence, our 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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