55 Secretory leukoprotease inhibitor (SLPI) as a novel therapeutic targeting neutrophil chemotaxis in cystic fibrosis

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Cystic fibrosis (CF) is generally diagnosed early in childhood, it is characterised by sustained neutrophil dominated inflammation from a very young age. Conversely, recruited neutrophils are ineffective in bacteria clearance and play a significant role in bronchiectasis development. Identification of agents modulating neutrophil chemotaxis constitutes a relevant goal.

Studies have shown that SLPI is anti-inflammatory in such disorders as atherosclerosis and lung emphysema. This study aimed to enhance knowledge of SLPI's anti-inflammatory effects and in the context of tissue homeostasis, we investigated SLPI's ability to moderate neutrophil chemotaxis.

Neutrophils were purified from whole blood and subcellular fractionation performed using ultracentrifugation techniques. Cell migration was assessed using a *boyden chamber*. The inhibitory effect of SLPI on F-actin polymerisation and talin cleavage post fMLP (10^{-6}M) and IL-8 (10 ng) stimulation was assessed by western blot analysis. Activity of calpain, a cysteine protease involved in cytoskeletal rearrangements required for neutrophil chemotaxis, was determined using a fluorometric assay.

SLPI was localised to the cytosol and secondary granules of resting neutrophils. Experimental results indicate that SLPI is not a physiological substrate for calpain. Instead, SLPI inhibited neutrophil calpain activity in a dose dependent manner (Ki = 70 nM) and prevented fMLP and IL-8 induced chemotaxis, F-actin polymerisation and talin cleavage.

Inhibition of cytoskeletal rearrangements by SLPI may represent a novel anti-chemotactic mechanism, strengthening SLPI's attraction as a potential therapeutic in inflammatory lung disease.

55A Combined approaches to restore airways hydration in cystic fibrosis

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The CF airway disease, characterized by the increased viscosity of mucus, infection and colonization of the lungs, is a consequence of the incapacity of mutated CFTR to secrete Cl^-. This in turn, disrupts the balance between absorption and secretion and leads to a prevalence of Na^+ absorption. We have hypothesized that to restore airway hydration it is necessary either to recover Cl^- secretion from mutated CFTR, to reduce Na^+ absorption or both.

A short-interfering RNA (siRNA) approach targeting the epithelial sodium channel (ENaC) subunits was used to reduce Na^+ absorption, while to increase Cl^- secretion we utilized CFTR potentiators on CF and non-CF bronchial primary epithelial cells. To evaluate the effects of these actions, we measured transepithelial Na^+ and Cl^- short-circuit currents and the height of the Texas Red-coloured surface fluid layer with confocal microscopy.

Our results indicate that (i) siRNA sequences complementary to any of the ENaC subunit are able to reduce ENaC transcripts and Na^+ channel activity but only silencing alpha and beta ENaC subunits at the same time increases the airways surface fluid layer; (ii) the hydration obtained in this way was similar to that measured after maximal CFTR stimulation on non-CF epithelia; (iii) CFTR potentiators increased airway hydration of corrected F508del epithelia.

In conclusion, both CFTR potentiators and ENaC silencing produce a significant and long-lasting increase of the airway hydration *in vitro*.

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