Secretory leukoprotease inhibitor: a native antimicrobial protein presenting a new therapeutic option?

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Abstract

Secretory leukoprotease inhibitor (SLPI) is a low molecular weight serine protease inhibitor found on various mucosal surfaces and has been ascribed an important role in maintaining the protease-antiprotease balance of the airways. Recent scientific evidence has suggested that SLPI may also have a broad spectrum antibiotic activity that includes antiretroviral, bactericidal, and antifungal activity. Given the unpropitious development of drug resistance to infectious micro-organisms in the human population, the need for therapeutic alternatives in the treatment of infectious diseases has become clear. SLPI may prove valuable in the prophylaxis and future treatment of infectious diseases, yet the clinical efficacy of SLPI remains largely to be elucidated.

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expression, at least in vitro, whereas lipopolysaccharide (LPS) has recently been shown to induce SLPI production by macrophages which may antagonise LPS induced signalling and secretion.³ A recombinant form of SLPI (rSLPI) has been produced in *Escherichia coli* and was found to be identical to the naturally occurring molecule in terms of structure and function. rSLPI retains its function when aerosolised in vitro or when given to experimental animals, and limits the development of LPS mediated experimental pulmonary emphysema.⁴ Indeed, inhaled rSLPI may be beneficial in the treatment of a number of inflammatory lung disorders including cystic fibrosis, emphysema, ARDS, and chronic bronchitis.¹

Recent findings have shown that SLPI may kill micro-organisms and may limit viral spreading. These observations offer completely new insights into the factors that constitute the mucosal defence against human pathogens and, in addition, may open new windows to the future treatment of infectious diseases in man.

Over the past decades viral, bacterial and fungal infections in clinical patients have increased dramatically, primarily as a consequence of the widespread use of immunosuppressive agents and the AIDS pandemic. Antimicrobial therapy may have various adverse side effects such as drug related toxicity. However, the emerging resistance of clinically important pathogens to various antibiotics has posed a serious problem in recent years that imperils successful treatment so the need for new antimicrobial agents has become evident.

Secretory leukoprotease inhibitor (SLPI or antileukoprotease (ALP)) is a relatively small and cationic antiprotease found in mucous secretions of the respiratory and genital tracts. In partnership with α_1 -proteinase inhibitor (α_1 -PI), SLPI comprises the antineutrophil elastase (NE) shield in the lung, limiting NE-induced inflammation.¹ In the lung SLPI is produced locally and the concentration in the epithelial lining fluid of the upper airways may increase with chronic bronchial infection.² In addition, neutrophil elastase and pro-inflammatory cytokines (IL-1, TNF α) may induce SLPI mRNA

Scientific basis

ANTI-HIV ACTIVITY

The first report on the antimicrobial activity of SLPI came from McNeely and co-workers⁵ who showed that infection with adherent primary monocytes with HIV-1 was significantly suppressed in the presence of human saliva. Of the proteins present in the saliva only SLPI was found to have antiretroviral activity at physiological concentrations. SLPI also partially inhibited HIV-1 infection in proliferating human T cells, although this was not confirmed in later studies.⁶ No direct interaction of SLPI with, or downregulation of, the CD4 antigen on T cells was found, nor any interaction of SLPI with viral proteins. The mechanism of the antiretroviral activity of SLPI is therefore probably related to an interaction of the molecule with one or more host cell associated molecule(s).

BACTERICIDAL ACTIVITY

Prompted by the observation of antimicrobial activity of a newly isolated polypeptide from

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Figure 1 Schematic representation of the possible modes of antifungal activity of secretory leukoprotease inhibitor (SLPI) against *Aspergillus fumigatus* (Af) in the mucosal defence of the airways. Impaction of *A. fumigatus* spores on the mucosal surface may result in local germination of fungal spores and growth of *A. fumigatus* hyphae. Release of fungal proteases may result in epithelial cell detachment and production of pro-inflammatory cytokines by these cells, followed by the recruitment and activation of inflammatory cells that may further damage the epithelium. By causing cell detachment, fungal spores may adhere more readily to basement membrane proteins and serum components and gain ready access to the underlying lung parenchyma. SLPI may interfere in this process by killing *A. fumigatus* spores and by inhibiting fungal protease activity.

equine neutrophils (eNAP-2) and its sequence homology to SLPI, Hiemstra and coworkers investigated the *in vitro* antibacterial activity of rSLPI against *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive).⁷ A dose dependent bactericidal activity of rSLPI towards both organisms was found but, with *E. coli* as a target organism, the activity of rSLPI was found to be lower than the cationic antimicrobial polypeptides, lysozyme and defensins, on a molar basis. The mechanism of the SLPI mediated bactericidal activity is largely unknown but may include binding of the protease inhibitor to bacterial mRNA and DNA.⁸ Hiemstra, however, proposed that this mechanism pertained to bacteria endogenously producing rSLPI but may not fully explain the observed antibacterial activity of rSLPI added exogenously to these cells.⁷ The SLPI molecule comprises two largely identical domains; the carboxy terminal domain contains the protease inhibitory site whereas the function of the amino terminal domain is largely unknown. Remarkably, the bactericidal activity was found to be located primarily in the amino terminal domain of rSLPI.⁷

ANTIFUNGAL ACTIVITY

We have recently completed studies on the antifungal activity of rSLPI against human isolates of the pathogenic fungi *Aspergillus fumigatus* and *Candida albicans*⁹ and found a statistically significant and dose dependent killing of *A. fumigatus* conidia (50% fungicidal activity at 5.8 µM) provided that the conidia were pre-incubated in a watery solution to induce metabolic activity of the fungal cells (fig 1). The

airborne (metabolically quiescent) type of *A. fumigatus* conidia were totally resistant to the action of rSLPI. *Candida albicans* yeast cells were also killed in a dose dependent fashion by rSLPI (50% fungicidal activity at 10.0 µM) and, in addition, the growth rate of a *C. albicans* inoculum in culture medium was inhibited significantly.⁹ As with the antibacterial activity described by Hiemstra,⁷ the antifungal activity was mainly localised in the amino terminal domain while the concentration at which rSLPI displays bactericidal and antifungal activities *in vitro* (>2 µM) appears to be relevant to the *in vivo* situation in the lung. The bactericidal and antifungal activity may be related to the cationic nature of SLPI^{7,9} as was previously described for other cationic peptides such as defensins. In other studies¹⁰ we also found a partial inhibition of fungal protease activity by rSLPI (fig 1), a putative virulence factor of *A. fumigatus*. Following the inhibition of fungal protease activity, rSLPI also inhibited the induction of the pro-inflammatory cytokine response in cultured human airway epithelial cell lines (fig 1).¹⁰

Therapeutic potential

rSLPI has proved to be active in physiological concentrations against some pathogenic fungi and bacteria *in vitro* and HIV. It is available for therapeutic use and is, in fact, being considered for the treatment of inflammatory lung diseases.¹ The potential clinical importance of SLPI in limiting airway inflammation was shown by McElvaney and Crystal¹¹ in the early 1990s in patients with cystic fibrosis. rSLPI in a dose of 100 mg was administered to these

patients twice daily for one week and resulted in a significant reduction in both elastase activity and IL-8 levels in epithelial lining fluid. Probably as a result of decreased IL-8 levels, a marked reduction in neutrophil number in the epithelial lining fluid was also found, thereby limiting airway inflammation in these patients. The same group also showed that inhalation of a single 100 mg dose resulted in a significant increase in SLPI in the epithelial lining fluid at one hour, diminishing gradually over time.¹²

rSLPI has so far been used in only a limited number of clinical trials which have shown that, upon inhalation, SLPI may be retained in the lung for 24 hours and an increase in SLPI concentration may result from even a single dose of rSLPI. Furthermore, rSLPI may exert biological activity in vivo without any adverse effects on the patient. However, although rSLPI may prove therapeutically useful, proteases released from colonising micro-organisms in cystic fibrosis may potentially inactivate rSLPI as has been shown with *Staphylococcus aureus* and *Pseudomonas aeruginosa* proteases in vitro.¹³

Conclusions

Given its broad spectrum of antimicrobial activity rSLPI may prove a valuable therapeutic option in the future treatment or prevention of infectious diseases. Its lack of organism specificity may, in fact, be an important characteristic. rSLPI may prove useful in patients with multiple infections or in those patients where the nature of the organism causing the infection is unclear. Additional in vitro studies will be required to determine whether other clinically important human pathogens are also vulnerable to the antimicrobial activity of rSLPI, while its clinical efficacy in preventing or treating infection remains to be established. By inhibiting antimicrobial protease activity

and killing the organisms involved, rSLPI may prove to be a useful agent in supporting the native host defence, supplying the patient with the necessary tools to combat pulmonary infection.

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