Background: The antimicrobial activity of garlic and other alliums are primarily based on allicin, an unstable thiosulphinate present in crushed garlic bulbs but absent in medicinal formulations.

Objectives: To determine if stabilised allicin exhibits antimicrobial properties against multiresistant CF pathogens.

Methods: We investigated 81 isolates comprising the Burkholderia cepacia complex, Stenotrophomonas maltophilia and multiresistant strains of Pseudomonas aeruginosa. Inhibitory activity (MIC and MBC) was determined using concentrations between 5−90 mg/L. Bactericidal activity was measured by a kill curve in the presence of 3*MBC.

Results: All bacterial isolates were inhibited by NAM with MIC values in the range 15−60 mg/L. A kill curve using P. aeruginosa PAO1 at an initial density of 10⁶ CFU/ml showed 100% killing within 60 min. To understand the potency of the chemically complex NAM, mass spectrometry was used to identify the active component(s) and their impact on bacterial protein targets. The potential for synergistic activity between NAM and conventional antibiotics has also been explored.

Conclusion: Unlike other garlic extracts, NAM retains bactericidal properties against B. cepacia complex, S. maltophilia and multiresistant P. aeruginosa. Present therapy against these pathogens is difficult and NAM merits further investigation.

Lynovex®, a novel mucolytic-antimicrobial agent for the treatment of Cystic Fibrosis

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Lynovex® (cysteamine) has been developed as a unique, dual mucolytic-antimicrobial agent for the treatment of cystic fibrosis (CF). With a well established safety profile, Lynovex® has recently been designated as an orphan drug by the European Medicines Agency. The antimicrobial activity of Lynovex® was determined using CLSI method M07-A8 and a microbaldisc system was applied for determining the ability of Lynovex® to disrupt and/or prevent CF-relevant bacterial biofilms. Lynovex® demonstrated broad spectrum antimicrobial activity against P. aeruginosa and other CF bacterial pathogens including antibiotic-resistant strains with a MIC100 of 250 mg/L, and was not sensitive to the altered ionic conditions that typify the CF lung. Importantly, Lynovex® demonstrated antimicrobial synergy with colistin and additivity with ciprofloxacin, tobramycin and gentamicin (up to 32-fold reduction of the antibiotic MIC when combined to Lynovex®). Lynovex® disrupted established bacterial biofilms at 1000 mg/L alone and showed additive activity with tobramycin at 125/2 mg/L (Lynovex®/tobramycin, FIC = 0.63). Lynovex® outperformed all currently available mucolytic agents in its ability to increase the viscosity of isolated mucins. In vivo experiments were performed in murine models of acute P. aeruginosa lung infection in which Lynovex® was administered via intratracheal delivery or nebulisation. In both systems, Lynovex® was very well tolerated and reduced lung P. aeruginosa burden. Lynovex® is a new orphan drug that offers the potential for a step-change in CF treatment. As a co-therapy alongside existing antibiotic regimens, Lynovex® can tackle CF respiratory infections directly whilst at the same time disrupting the mucus and preventing biofilms that facilitate the persistence of these microbes in the CF lung. Clinical trials of this first-in-class drug candidate will commence in 2012.

Evaluation of efficacy of POL7001 against Pseudomonas aeruginosa in lung infection models

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The development and clinical exploitation of antibiotics with new modes of action are a top priority in fighting untreatable chronic infections in CF patients. POL7001 is a Protein Epitope Mimetic (PEM) antibiotic with potent activity against Pseudomonas aeruginosa (PA) (Srinivaset al, Science 2010). To evaluate CF lung infections as potential clinical application of this new antibiotic, the activity of POL7001 was tested in vitro against PA isolated from the onset of infection up to 16 years or until death/lung transplantation in CF patients, and in murine models of acute and chronic lung infection. Comparison to clinically approved antibiotics was included.

MICs for POL7001 ranged between 0.015−0.5 μg/mL with a median of 0.125 μg/mL for all isolates with no difference against mucus, non-mucoid or hypermutable isolates. Over time, many of the CF PA isolates became resistant to antibiotics while remaining sensitive to POL7001. Mice were infected with a multi-drug resistant PA isolate and treated with POL7001 or ciprofloxacin. Subcutaneous administration showed a comparable efficacy of both antibiotics, with more than 1.5 log10 CFU/mL reduction after 24 hrs, while intratracheal administration showed faster killing and better efficacy of POL7001 than ciprofloxacin, with 3 log10 and 1 log10 CFU/mL reduction after 24 hrs, respectively. In a model of chronic lung infection daily subcutaneous treatment showed that POL7001 is more efficacious than ciprofloxacin, with 1 log10 CFU/mL reduction in the BAL. These results represent a step forward in the pre-clinical development of POL7001 to treat CF lung infections.

Use of mannitol to improve antibiotic sensitivity in biofilm-associated persister cells of Pseudomonas aeruginosa

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Lung infection by Pseudomonas aeruginosa is the key mortality factor for Cystic Fibrosis patients. The failure of antimicrobials is partly attributed to the increase in antibiotic tolerance of P. aeruginosa grown in biofilms within the lungs. Therefore, strategies that improve antibiotic sensitivity of tolerant P. aeruginosa cells may reduce disease progression. Mannitol has been shown in Escherichia coli to restore aminoglycoside sensitivity by generating a proton-motive force (Nature, 2011, 473: 216). Here, we show that the commonly used aminoglycoside tobramycin selects for tolerant cells during biofilm growth by P. aeruginosa and incubation with mannitol (10−20 mM) increased tobramycin sensitivity (up to 1,000-fold). In contrast, NaCl (up to 30 mM) had a lower effect on the sensitivity of tolerant cells suggesting that the mannitol effect is not related to only an osmotic change but also to its metabolic properties. At large concentrations (50 and 100 mM), NaCl and mannitol similarly reduced tobramycin tolerance of the biofilm. These results suggest that in addition to improvements in lung function by facilitating mucus clearance in CF, mannitol also affects antibiotic sensitivity in biofilms by at least two distinct and complementary mechanisms. Mannitol alters the tolerance of persister cells through an osmotic effect at higher concentrations and as a substrate increases aminoglycoside sensitivity even at low concentrations.