Neutrophil elastase inhibitors for cystic fibrosis

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For some years, synthetic neutrophil elastase inhibitors have been touted as potential treatments for cystic fibrosis[1]. But despite early optimism, many candidates, for example FK706 (Fujisawa Pharmaceuticals, Osaka, Japan)[2], are no longer in development. Now, a peptide inhibitor of human neutrophil elastase, EPI-HNE-4 (discovered by Dyax Corporation, Cambridge, MA, USA), is being tested in a Phase IIa clinical trial for cystic fibrosis by Debiopharm (Lausanne, Switzerland).

Cystic fibrosis

About 55,000 people in Europe and the US have cystic fibrosis, an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene[3]. Patients produce abnormally thick, sticky mucus that helps to initiate a cycle of lung infection and inflammation. This ultimately leads to respiratory failure in most patients.

Currently, the median survival of US patients with cystic fibrosis is 32.5 years, a great improvement over that of 2 years in 1950 (http://www.cff.org). This improvement is the result of several advances, says respiratory paediatrician Ian Balfour-Lynn (Royal Brompton and Harefield NHS Trust, London, UK). 'The efficient use of pancreatic enzyme supplements and high calorie diets means that patients now maintain better nutrition. Furthermore, a better understanding of the lung disease means that we can use improved and more aggressive antibiotic treatments.' However,

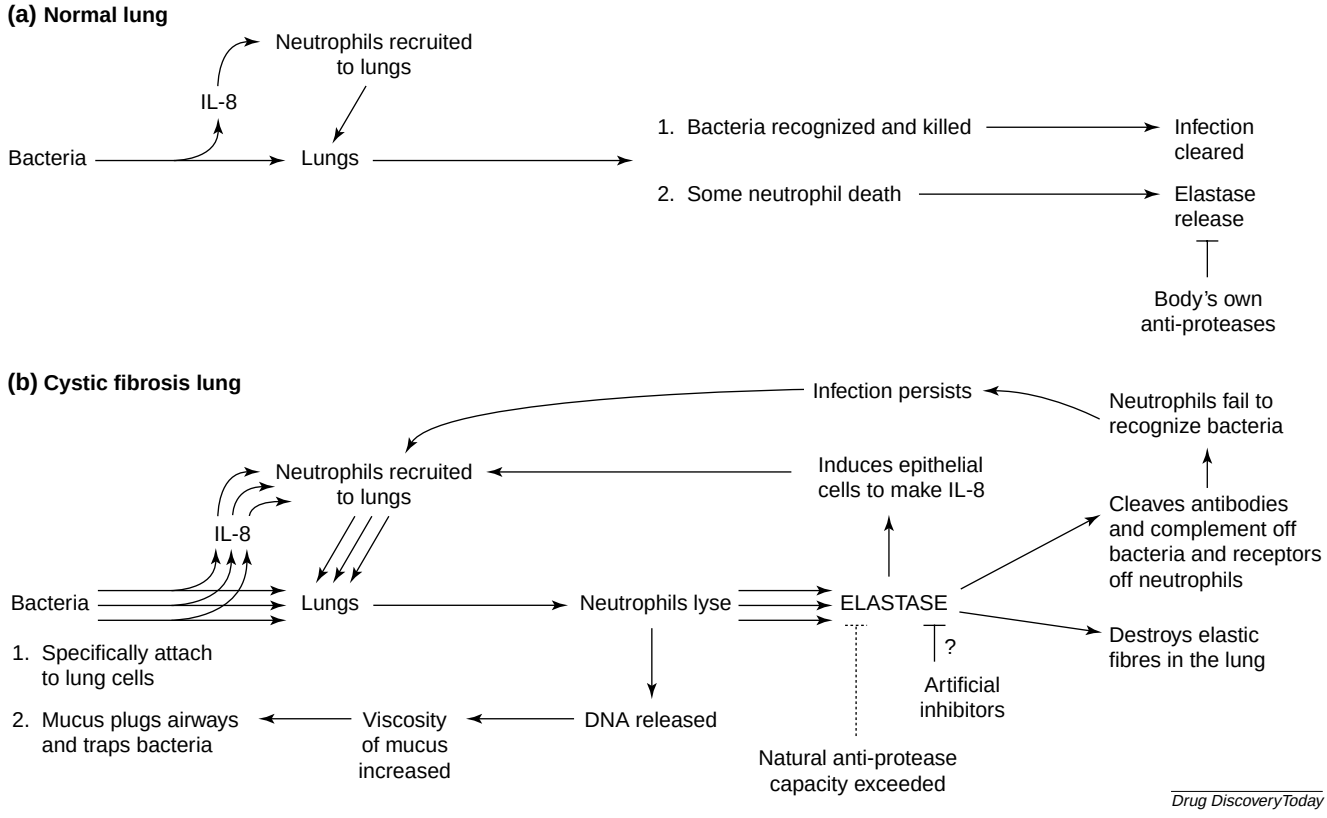

Figure 1. The mechanism by which the body copes with a bacterial infection in **(a)** the normal lung and **(b)** the cystic fibrosis lung.

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new therapeutic approaches are badly needed. Approaches under investigation include gene therapy (see Box 1), correction of the abnormal protein and improvement of ion transport (for examples, see the Cystic Fibrosis Foundation website at http://www.cff.org and Ref. 3). In addition, ways are being sought to reduce lung inflammation.

Box 1. A new vector for gene therapy in cystic fibrosis?

Ever since *CFTR* was cloned in 1989, researchers have been trying to introduce non-mutated *CFTR* efficiently into the lungs of patients with cystic fibrosis. Existing gene therapy vectors (for example, adenovirus) have been partly successful but poor transduction efficiencies, short expression durations, inflammatory responses, or combinations of problems have limited progress.

Now, Gary Kobinger (Institute of Human Gene Therapy, University of Pennsylvania Health System, Philadelphia, PA, USA) and colleagues report that an HIV-based virus carrying Ebola virus envelope proteins enables 'efficient transduction of airway epithelia to a degree and for a period of time' not previously achievable. They show that their virus can transduce intact human airway epithelium from the apical surface *in vitro* and *ex vivo*, that the virus can infect mouse tracheal airway *in vivo*, and that marker expression persists for up to 63 days. Pseudotyped lentiviral vectors, they conclude, could hold promise for the treatment of cystic fibrosis[4].

Breaking the inflammatory cycle

Lung inflammation in cystic fibrosis results from host responses to chronic bacterial infection (Fig. 1). 'In normal lungs, any elastase released by neutrophils is controlled by the body's own anti-proteases, such as α-1 antitrypsin', explains Melvin Berger (Case Western Reserve University, Cleveland, OH, USA). 'In cystic fibrosis lungs, neutrophil numbers may be a million-fold higher and elastase concentrations several thousand-fold higher, but natural protease inhibitor levels are increased only 4–5-fold in response to the elastase increase.'

Two approaches aim to redress this protease–antiprotease imbalance[1]. The first aims to use natural anti-proteases. For example, PPL Pharmaceuticals (Roslin, UK) has completed Phase II trials of human recombinant α-1 antitrypsin in cystic fibrosis and hopes to start Phase III trials in 2003. The second approach (that taken by Dyax and Debiopharm) uses synthetic elastase inhibitors.

Phage display for drug discovery

'Our elastase inhibitor EPI-HNE-4 was discovered by the use of phage display technology', explains Shirish Hirani, Senior Vice-President of Product Development at Dyax. A phage library was constructed in which each phage expressed a variant of the Kunitz domain of inter-α-trypsin inhibitor on its surface. Kunitz domains are very small, rigid structures that have important anti-protease activity, explains Hirani. The library was then screened for binding to human neutrophil elastase and phages that inhibited this enzyme selectively were chosen[5]. After further tests of activity and selectivity, the best inhibitor, EPI-HNE-4, was taken into development. 'This small polypeptide is a very potent (picomolar), reversible and selective inhibitor of human neutrophil elastase', notes Hirani. 'And importantly, given that it has to work in the high oxidative environment of the lung, EPI-HNE-4 is very resistant to oxidation.'

Preclinical and clinical studies

In 1997, Dyax linked up with Debiopharm to continue the preclinical and clinical development of EPI-HNE-4. Debiopharm has now formulated the inhibitor for nebulization and has demonstrated pharmacodynamic activity and lack of toxicity in animals. In addition, the company has shown that EPI-HNE-4 inhibits elastase *ex vivo* in sputum from patients with cystic fibrosis and *in vivo* in rats' lungs.

Moving into clinical studies, in a Phase Ia study, explains Debiopharm's Program Director François Saudubray, 38 healthy human volunteers were given a single inhaled dose of EPI-HNE-4, the magnitude of which was increased stepwise as the trial progressed. No clinical or biological side effects were observed, he says. In a subsequent Phase Ib study, 12 volunteers were given repeated daily doses of the inhibitor for two weeks. 'Again, there were no clinical or biological side effects and we have evidence, from bronchoalveolar lavages, that the inhibitor reaches the lower airways and is active', says Saudubray.

Encouraged by these results, Debiopharm has now started a Phase IIa trial. In this, 24 adults with cystic fibrosis will be given repeated doses of EPI-HNE-4 over a three-week period. The safety of the molecule will be monitored and pharmocodynamic and pharmacokinetic measurements will be taken. Both Dyax and Debiopharm hope that the Phase IIa trial will yield positive data by the end of 2001 that will enable further development of their inhibitor.

References

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