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Gene therapy trials for cystic fibrosis

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A new method of gene delivery has been developed that uses compacted DNA nanoparticles to penetrate the nuclear membrane. Researchers have demonstrated the efficacy of the technique in a mouse model of cystic fibrosis (CF), and Phase I/II clinical trials have now begun.

A way into the nucleus

The most effective gene therapy method to date has been to use a modified adenovirus as the vector for delivering genetic material to the cell nucleus. However, viruses can provoke severe reactions and can not be administered repeatedly because of the specific immune response they provoke. An alternative method is to encase the DNA in liposomes that can readily pass through the outer membranes of cells. The main problem with this technique is that the liposomes are usually too big (~100 nm) to pass through pores in the nuclear membrane. This is only possible during cell division, when the nuclear membrane breaks down enough to enable the DNA to pass through. This leads to inefficient uptake and is not practical for slow-dividing cells, such as those lining the lungs. These drawbacks have stimulated the search for alternative methods of gene delivery.

One such alternative is being investigated by researchers from Case Western Reserve University (http://www.cwru.edu/) and Copernicus Therapeutics (http://www.cgsys.com). In their technique, a single DNA molecule is wrapped in a coat of positively charged peptides. The resulting particle has a diameter of only 20–25 nm, small enough to pass through a nuclear pore. Mark Cooper of Copernicus presented data at the *American Society of Gene Therapy* meeting (30 May–3 June 2001; Seattle, WA,USA) [1]. He defined the size constraints that permit compacted DNA to be effective: 'Our compacted DNA nanoparticles robustly transfect post-mitotic human cells, achieving up to a 6900-fold enhancement of gene expression compared to naked DNA.'

The need for a new type of CF therapy

CF is one of the most common genetic diseases, affecting ~30,000 people in the USA alone (http://www.cff.org). A recessive genetic mutation causes deficiencies in the transport of salt across the membranes of secretory cells. This, in turn, causes the accumulation of a thick, sticky mucus in the respiratory and digestive tracts and in the reproductive system, which leads to recurrent lung infections, pulmonary damage and difficulties in obtaining nutrients from food. There is no cure for CF and, until recently, few sufferers survived childhood; however, with modern supportive therapies and careful disease management those affected now often live into their 30s. Gene therapy offers the best hope for curing the disease [2]. However, getting foreign material into the lung lining is particularly challenging, especially when these tissues are coated in thick mucus.

Studies in a mouse model of CF

Pam Davis and co-workers from Case Western applied the nanoparticle technique to mice with CF [3]. Davis explained how the peptide casing, a polylysine chain of >250 residues, results in such a small particle size: 'The negative charges on the DNA phosphate groups interact with the positive charges on the lysines, allowing the atoms to pack closely together, indeed, down to less than twice the minimum theoretical volume based on the size of the atoms alone.' A 17-amino-acid ligand for the serpin-enzyme complex receptor was also conjugated to the particle. This receptor is present on the apical membrane of certain cells and is adapted for the uptake of large molecules into the cell. Attachment of the ligand to the nanoparticle thus facilitated the efficient targeted entry of the particle, which could then proceed to the nucleus.

The gene therapy was successful, with the replacement CF gene being expressed in the cells of the nasal lining [3]. This partially restored function with little immune reaction. However, the airway structure of mice is different to that of humans, so this is no guarantee that that technique would make an effective therapeutic. Davis commented: 'Based on the data in mice, we expect that this reagent will be quite benign, but a mouse is not a man.'

Clinical trials

To address this issue, trials have now begun on 12 people with CF. The trial uses a slightly different form of nanoparticle (Fig. 1). A much shorter polylysine chain is attached via cysteine to polyethylene glycol (PEG). This stabilizes the particle under normal physiological conditions making the nanoparticles more suitable for clinical studies. According to Cooper, it is still unclear how the PEG-conjugated particles enter the cell but they appear to have equal efficacy to those used by Davis, as shown in numerous animal models.

The current trial is a Phase I/II double-blind, placebo-controlled study in which saline (placebo) or compacted DNA of the *CFTR* gene in saline is applied to the nostrils of CF subjects. The Phase I trial will last 5–6 months and will be followed by a Phase II study in which compacted

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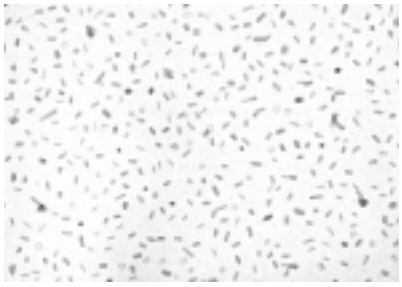

Figure 1. DNA nanoparticles of Copernicus Therapeutics. Electron micrograph of polyethylene glycol (PEG)-stabilized compacted DNA particles (amplification 3×10^4). Figure courtesy of Copernicus Therapeutics (http://www.cgsys.com).

DNA will be administered by a bronchoscope to an isolated bronchial segment. Subsequent trials will administer aerosols of compacted DNA to the whole lung.

Terry Flotte, a researcher of CF gene therapy from the University of Florida (http://www.ufl.edu/), was enthusiastic about the work: 'It is essential that these sorts of therapies continue to be tested in patients because, in the final analysis, there is no other way to move forward toward better therapies for this disease', he commented.

Davis is optimistic about the applicability of the technique to a wide variety of other diseases, including gene therapy to produce blood-clotting factors and surfactant proteins and as a timed response to afflictions such as severe asthma attacks. She also commented on the possible use of the technique as a protection from the side-effects of other therapies: 'It may be possible to protect the lung against known, expected toxicity from radiation therapy or chemotherapy drugs by delivering a protective gene at the right time. This might allow fully effective doses of radiation or chemotherapy to be administered, which might otherwise not be tolerable.'

References

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CDK inhibitor shows promise for inflammatory kidney disease

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An inhibitor of cyclin-dependent kinase (CDK) has been well tolerated in Phase Ia clinical trials and is due to enter Phase Ib trials for glomerulonephritis (GN), a group of inflammatory kidney diseases, in autumn 2002. Details of this were announced by Spiros Rombotis, Chief Executive of Cyclacel (http://www.cyclacel.com), at the *BioEquity Europe 2002* meeting in Zurich, Switzerland (14–15 May 2002).

Glomerulonephritis

Glomerulonephritis is a group of kidney diseases caused by inflammation and cell proliferation that result in gradual, progressive destruction of internal kidney structures (glomeruli). Although rare, GN is the third most common cause of end-stage renal disease (ESRD). This group of diseases causes 20–50% of all renal failures that necessitate kidney dialysis or transplantation. GN also develops in people with certain cancers, liver cirrhosis and some infectious diseases.

Current treatments include high-dosage steroid and cytotoxic drug therapy; however, these are often ineffective and have a high risk of toxic side effects. New therapies directed at specific cytokines and growth factors are under development, as are less toxic forms of immunotherapy [1].

In the developing world, ~700,000 people are affected by GN, and in the USA 330,000 people are on dialysis for ESRD. US$17 billion is spent annually in the USA alone on ESRD.

CYC202

The lead drug from Cyclacel, CYC202, is a small-molecule inhibitor of CDK, which regulates the proliferation of cells. CYC202 is a tri-substituted purine and is a highly specific inhibitor of CDK2/cyclinE activity, inducing selective apoptosis in cancer cells. During glomerulogenesis, visceral glomerular epithelial cells exit the cell cycle and become terminally differentiated. Cell proliferation is under the control of cell-cycle regulatory proteins, such as p21 [2].

The use of CDK inhibitors in the treatment of inflammatory disease of the kidney is to suppress abnormal proliferation of non-cancerous kidney cells that destroy renal function. Cancer treatment using CDK inhibitors emulates the activity

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