Expert Opinion

Pulmonary delivery of nucleic acids

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The lung is an appropriate present and future target for gene therapy approaches designed to treat inherited monogenic diseases, eradicate bronchial tumours, transfer pharmacologically active products to the general circulation, express enzymes to catabolise toxins, manage pulmonary hypertension and lung injury and vaccinate against infection. Despite 35 years of gene therapy research and some significant milestones in molecular biology, the clinical potential of gene therapy has yet to be realised. In pulmonary gene therapy the nucleic acid cargo needs to be delivered to cells in the target region of the lung, and even in cases when these targets are well defined this is severely limited by the pulmonary architecture, clearance mechanisms, immune activation, the presence of respiratory mucus and the availability of a truly representative biological model. The challenge from a drug delivery perspective is to consider the suitability of conventional nebulisers and inhalers for delivering DNA to the lung and design and apply integrated formulation and device solutions specific to nucleic acid delivery.

Keywords: aerosol, DNA, gene delivery, gene therapy, inhaler, lung, pulmonary, respiratory

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1. Introduction

The importance of developing effective aerosol formulations for pulmonary gene therapy has long been recognised. The lung represents an attractive target for gene therapy for a variety of reasons. Localised aerosol delivery facilitates non-invasive ease of access of DNA to a vast localised surface area of tissue, whilst avoiding potentially deleterious interactions with the cellular barriers and serum proteins that are inevitably encountered via more invasive delivery approaches. The respiratory route of delivery has a history of patient acceptability and compliance and benefits from a unique track record of investment in delivery device technologies, thus providing options for delivering the DNA to lung targets. Decisively, a host of pulmonary and related conditions could potentially benefit from gene therapy.

2. Therapeutic targets

The most commonly quoted clinical applications for pulmonary gene therapy remain cystic fibrosis (CF) and α-1 antitrypsin deficiency. In these cases an aberrant gene is replaced by a corrected functional copy. Gene therapy for CF has become a benchmark for pulmonary gene therapy, yet the preclinical and clinical trials conducted so far have not shown irrefutable evidence of functional correction of the disease. The purported reasons for limited clinical response translate to the entire field of pulmonary gene therapy and include debate over the appropriate target region and cell populations, selection of an appropriate gene carrier vector, significant biological barriers to cellular delivery, implications of the disease state, difficulties with repeat administration and poor levels of cellular expression of the exogenous gene [1,2]. The story for α-1 antitrypsin is similar with clinical studies involving injection of the

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gene, with a viral carrier demonstrating proof of concept and lack of significant toxicity, but falling short of functional correction [3]. As Terence Flotte (University of Florida's College of Medicine) comments upon their recent clinical findings '*In one patient we saw evidence for a very brief period that some of the α-1 protein was being produced, but not at a high enough level to be beneficial*' [101].

Nevertheless, progress is being made in these gene replacement approaches and generous funding from the UK Cystic Fibrosis Trust has allowed The UK Cystic Fibrosis Gene Therapy Consortium to develop a lead formulation for CF treatment. The consortium is now embarking on a £20 million clinical programme to determine whether their non-viral vector system is able to demonstrate clinical evidence of improvement in the condition and not just ameliorate symptoms. The initial focus will be to determine the most appropriate surrogate outcome measures for lung function and disease state, with results of the subsequent 100-patient clinical study expected by 2010. As Professor Eric Alton of the Consortium puts it '*I don't think we're going to make Concorde on our first go. It's not going to cure the disease, but we'll see if we can get the plane off the ground and fly it for 50 yards*' [102]. It is fair to say that CF sufferers and the gene therapy research community alike will be willing the plane to soar or at the very least confirm that respiratory gene therapy can become airborne! Given signs of clinical improvement with monogenic diseases, it is hoped that the future may provide gene therapy strategies in the management of polygenic imbalances in chronic diseases such as asthma [4].

However, clinical studies on monogenetic diseases, such as CF, remain in the minority, with two thirds of all gene therapy clinical trials aimed at treating cancer [103]. Indeed, the first approved and marketed gene therapy product is a treatment for head and neck squamous cell carcinoma (Gendicine™ Shenzhen SiBiono GeneTech Co. Ltd). Despite advances in chemotherapy, radiation therapy and surgery, lung cancer causes an estimated 1 million deaths worldwide annually, with a 15% overall survival rate. Gene therapy for lung cancer strategies include blockade of activated tumour-promoting oncogenes, replacement of inactivated tumour-suppressing or apoptosis-promoting genes and stimulation of the immune system to weakly immunogenic tumours. For example, the literature demonstrates that both viral and non-viral mediated *in vivo* delivery of the p53 tumour suppressor gene (the same gene exploited in Gendicine) can lead to significant localised expression of the gene in the lung. Although recent studies demonstrate optimised non-toxic aerosol formulations for p53 administration in preclinical models [5], and it is thought that p53 may prove successful in conjunction with conventional therapies [6], inhalational gene therapy approaches for lung cancer have not yet been successfully demonstrated in the clinic. Interestingly, a relatively new study has shown successful delivery of the cancer-suppressing *FUS1* gene into the tumours of stage four lung cancer patients via an intravenously administered lipid nanoparticle [104].

In addition to treating genetic diseases and cancers, the respiratory delivery of genes may offer an opportunity to exploit epithelial cells of the lung to produce, and transfer to the circulation and other tissues, therapeutic gene products such as hormones, clotting factors and enzymes. It may also be possible to use DNA to transfect lung cells to produce enzymes to degrade circulatory toxins associated with genetic diseases (e.g., ornithine-δ-aminotransferase to reduce plasma levels of ornithine that causes blindness in patients with gyrate atrophy) [7]. It has been suggested that pulmonary hypertension, acute lung injury, metastatic disease and medical interventions following lung transplantation are potentially amenable to gene therapy, with strategies including interruption of the cell cycle, gene-directed enzyme prodrug therapy and overexpression of nitric oxide synthase. However, in these cases, sufficient delivery to the target and duration of gene expression are likely to provide serious limitations.

In DNA vaccination, a gene is inserted into a cell to express antigen for prolonged and efficient immune activation [8]. As 53 of the presently available vaccines address pulmonary infections, it seems appropriate to target the pulmonary epithelia with genetic vaccines against conventional immunisation targets (e.g., measles, pneumonia, influenza) and emerging targets (including SARS and bioterrorism agents). Encouragingly, unlike cancer and other therapies, DNA vaccination will not depend on facilitating gene expression in 100% of cell targets, with a low level of gene expression likely to be proficient to stimulate sufficient immune response.

Finally, small interfering (or short inhibitory) RNA (siRNA) therapy using double stranded RNA for effective and specific silencing of malfunctioning genes is receiving increasing attention as an alternative to the aforementioned plasmid-based gene therapy approaches. siRNA has already been used in the lung to suppress haem-oxygenase-1 to study its role in protecting cells following lung injury [9], but this is early-stage research and the clinical opportunities are yet to be fully realised.

3. Method of pulmonary gene delivery

The gene delivery scientist has three basic options when selecting the most appropriate method for administering nucleic acids to the lung: nebulisation of liquid-suspended gene particles, aerosolisation of a dried formulation of the gene vector with carrier particles or pressurised expulsion of DNA from a propellant dispersion. Of these methods, nebulisation remains the most exploited for introducing gene vectors into the lung both in animal and clinical studies. Indeed, in the case of CF, nebulisation is the only pulmonary delivery method presently under consideration in the strategic research plan of the UK CF Gene Therapy Consortium [10]. However, nebulisation of gene therapy formulations can be inefficient: shearing forces, preferential nebulisation of the solute and adhesion to plastic can mean that as little as 10% of DNA in the nebulisation chamber is emitted

through the mouthpiece [11]. These limitations will be of particular importance to expensive gene therapy products, and present research aims to develop more advanced nebuliser technologies and more stable gene delivery vectors. To illustrate (very unscientifically) the relative focus on pulmonary delivery methods, a PubMed search at the time of preparing this editorial reveals 42 hits for 'gene therapy' AND 'nebulis(z)er'. It is perhaps surprising that dry powder inhaler (7 PubMed hits for 'gene therapy' AND 'dry powder') and pressurised metered-dose inhaler (3 PubMed hits for 'gene therapy' AND 'metered dose') devices have not received more attention, particularly as such devices may provide advantages including improved stability, rapid administration and ease of transportation.

4. Expert opinion

Given the clearly identified opportunities for pulmonary gene therapy and the relentless advances in molecular biology, is it surprising that viable treatments have not progressed into the clinic? Perhaps not if one considers the issues of delivering the nucleic acid of interest to the cell and through the cell, and the limitations on testing candidate genes. Although the lung is generally perceived to be an easily accessible target organ for localised gene therapy, it is clear that this is not necessarily the case. Specifically, the intricate pulmonary architecture, active clearance processes and effective immune responses all intend to prevent the invasion of foreign material into the lung. In addition, the presence of respiratory mucus provides a significant physical and chemical barrier to the delivery of gene therapy vectors to the cellular surface. It is also debatable whether biological models are predictive of efficacy in man. It is generally accepted that there is little if no correlation between *in vitro* and *in vivo* gene expression studies and this can be particularly true in the lung when comparing results obtained in a cell monolayer versus an intact differentiated airway epithelium. This is a significant problem, as the choice of the gene vector, be it viral or non-viral, is influenced by *in vitro* data. Secondly, with specific reference to animal studies, The American Society of Human Genetics Statement on Gene Therapy states that the promise of gene therapy '*will only be achieved through continued rigorous research on the mechanisms underlying gene delivery in animals. Clinical trials should be undertaken only after solid evidence of both safety and efficacy has been obtained in an appropriate animal model...*' [12]. The question must then be: is there an appropriate animal model? In fact, can any model accurately reflect the architecture, clearance mechanisms, immunity, mucus and surfactant coverage and cellular biology of the human lung? Will it ever be possible to completely answer certain questions outside of the clinic – such as, what is the effect of the disease state on gene delivery and expression, where are the most appropriate cell targets and what is the level of gene expression needed to observe a clinical effect?

In addition to the aforementioned physiological issues, pharmaceutical factors relating to an appropriate formulation and delivery device require further investigation. None of the conventional aerosol-generating devices for pulmonary administration was designed for delivering large, fragile molecules such as DNA into the lung. It is essential, therefore, for drug delivery scientists to interface with gene therapists to ensure that the exciting advances in molecular science translate to the clinic through robust and efficient formulation design and selection or design of more appropriate pulmonary delivery devices.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

1. FERRARI S, GEDDES DM, ALTON EWFW: Barriers to and new approaches for gene therapy and gene delivery in cystic fibrosis. *Adv. Drug Deliv. Rev.* (2002) **54**:1373-1393.
2. GRIESENBACH U, GEDDES DM, ALTON EWFW: Gene therapy progress and prospects: cystic fibrosis. *Gene Ther.* (2006) **13**:1061-1067.
3. BRANTLY ML, SPENCER LT, HUMPHRIES M *et al.*: Phase I trial of intramuscular injection of a recombinant adeno-associated virus serotype 2 α1-antitrypsin (AAT) vector in AAT-deficient adults. *Hum. Gene Ther.* (2006) **17**:1177-1186.
4. WANG LC, LEE JH, YANG YH, LIN YT, CHIANG BL: New biological approaches in asthma: DNA-based therapy. *Curr. Med. Chem.* (2007) **14**:1607-1618.
5. ZOU Y, TORNOS C, QIU X, LIA M, PEREZ-SOLER R: p53 aerosol formulation with low toxicity and high efficiency for early lung cancer treatment. *Clin. Cancer Res.* (2007) **13**:4900-4908.
6. SWISHER S, ROTH JA: p53 gene therapy for lung cancer. *Curr. Oncol. Rep.* (2002) 4:334-340.
7. ENGELHARDT JF: The lung as a metabolic factory for gene therapy. *J. Clin. Invest.* (2002) **110**:429-432.
8. BIVAS-BENITA M, OTTENHOFF THM, JUNGINGER HE, BORCHARD G: Pulmonary DNA vaccination: concepts, possibilities and perspectives. *J. Control. Rel.* (2005) **107**:1-29.
9. ZHANG X, SHAN P, JIANG D *et al.*: Small interfering RNA targeting heme oxygenase-1 enhances ischemia – reperfusion-induced lung apoptosis. *J. Biol. Chem.* (2004) **279**:10677-10684.
10. ALTON EWFW: Use of nonviral vectors for cystic fibrosis gene therapy. *Proc. Am. Thorac. Soc.* (2004) **1**:296-301.
11. BIRCHALL JC, KELLAWAY IW, GUMBLETON M: Physical stability and *in vitro* gene expression efficiency of nebulised lipid–peptide–DNA complexes. *Int. J. Pharm.* (2000) **197**:221-231.
12. BOARD OF DIRECTORS OF THE AMERICAN SOCIETY OF HUMAN GENETICS: Statement on gene therapy, April 2000. *Am. J. Hum. Genet.* (2000) **67**:272-273.

Websites

101. http://www.sciencedaily.com/releases/2006/11/061121232101.htm
Gene therapy for hereditary lung disease advances, November (2006).
102. http://www.24dash.com/health/13829.htm
UK trials for 'breakthrough' cystic fibrosis treatment, December (2006).
103. http://www.wiley.co.uk/genmed/clinical/
Gene therapy clinical trials worldwide, provided by the Journal of Gene Medicine, July (2007).
104. http://nanotechwire.com/news.asp?nid=4565&ntid=190&pg=2
Intravenous nanoparticle gene therapy shows activity in stage IV lung cancer, April (2007).

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