Bioscience Reports (2017) **37** BSR20160618
https://doi.org/10.1042/BSR20160618

PORTLAND PRESS

Review Article

Enhancement of lung gene delivery after aerosol: a new strategy using non-viral complexes with antibacterial properties

Angélique Mottais, Tony Le Gall, Yann Sibiril, Julian Ravel, Véronique Laurent, Frédérique d'Arbonneau and Tristan Montier

"Gene Transfer and Gene Therapy" Team, INSERM UMR 1078; IBSAM; Laboratoire de Génétique Moléculaire et Histocompatibilité, CHRU Brest; UFR Médecine et Sciences de la Santé, 22 avenue Camille Desmoulins, Brest 29238, France

Correspondence: T. Montier (tristan.montier@univ-brest.fr)

The pathophysiology of obstructive pulmonary diseases, such as cystic fibrosis (CF), leads to the development of chronic infections in the respiratory tract. Thus, the symptomatic management of the disease requires, in particular, repetitive antibiotherapy. Besides these antibacterial treatments, certain pathologies, such as CF or chronic obstructive pulmonary disease (COPD), require the intake of many drugs. This simultaneous absorption may lead to undesirable drug interactions. For example, Orkambi® (lumacaftor/Ivacaftor, Vertex), a pharmacological drug employed to treat F508del patients, cannot be used with antibiotics such as rifampicin or rifabutin (rifamycin family) which are necessary to treat Mycobacteriaceae. As far as gene therapy is concerned, bacteria and/or biofilm in the airways present an additional barrier for gene transfer. Thus, aerosol administration of nanoparticles have to overcome many obstacles before allowing cellular penetration of therapeutic compounds. This review focusses on the development of aerosol formulations adapted to the respiratory tract and its multiple barriers. Then, formulations that are currently used in clinical applications are summarized depending on the active molecule delivered. Finally, we focus on new therapeutic approaches to reduce possible drug interactions by transferring the antibacterial activity to the nanocarrier while ensuring the transfection efficiency.

Introduction

Gene therapy is a therapeutic strategy based on gene transfer approaches. They allow the input of nucleic acid constructs inside eukaryotic cells in order to correct a genetic abnormality (e.g. hereditary genetic disorders) or to regulate the expression of genes (e.g. cancers application). In most cases, it is necessary to have a carrier capable of conveying these nucleic acids. In fact, nucleic acid constructs being anionic polymers, cannot, except in some specific cases, interact with the negatively charged plasma membranes. Synthetic vectors are amongst the existing gene transfer systems. In 1987, the first synthetic carrier (DOTMA: N-[1-(2,3-dioleyloxy) propyl]-N,N,N-trimethylammonium chloride), allowing the introduction of DNA into mammalian cells, was developed by Felgner and co-workers [1]. This family of carriers is now used in 4.6% of gene therapy clinical trials (http://www.wiley.com/legacy/wileychi/genmed/clinical/; the journal of gene medicine 2017). Unlike viral vectors, synthesis of chemical vectors is fully controlled and allows for mass production for high incidence pathologies. Moreover, they are, for the most part, neither immunogenic nor very cytotoxic [2,3]. This allows the re-administration of nucleic acid constructs, which is most often required since not only the DNA not integrate into the genome, but the expression of the transgene is a function of the lifetime of the transfected cell as well. Synthetic vectors are mainly cationic molecules that self-assemble with nucleic acids via electrostatic interactions that form polyplexes

Received: 04 August 2017
Revised: 09 October 2017
Accepted: 10 October 2017

Accepted Manuscript Online: 17 October 2017
Version of Record published: 17 November 2017

Bioscience Reports (2017) **37** BSR20160618
https://doi.org/10.1042/BSR20160618

(polymers/nucleic acids) or lipoplexes (liposomes/nucleic acids) [3-6]. In addition to facilitating internalization in the eukaryotic cell, this encapsulation also makes it possible to protect nucleic acids from possible degradation (interaction or degradation by enzymes in the extra or intracellular environment).

Gene transfer systems based on cationic polymers are classified into four different families depending on the nature of the polymer (poly-L-lysine derivatives [7], derivatives of polyethyleneimine (PEI) [8], dendrimers [9], and chitosan [10]). Some other synthetic vectors are bio-inspired from phospholipids that form plasma membranes and are called cationic lipids [11]. These molecules of amphiphilic nature are composed of three parts: a polar head, a spacer, and a hydrophobic domain. Cationic lipids have been classified into four major subfamilies depending on the number of positive charges and the nature of the hydrophobic domain: monocationic, polycationic, cholesterol-derived monocationic, and cholesterol-derived polycationic. In order to improve transfection efficiency, numerous cationic lipids have been synthesized and many formulations have been derived. Phase IIb clinical trial conducted by the U.K. cystic fibrosis (CF) gene therapy consortium showed that the non-viral aerosolization gene therapy approach for CF application was beneficial and allowed CF patients to maintain their respiratory capabilities after an administration per month for a year (forced expiratory volume in 1 s (FEV1) + 3.7%) [12].

The intracellular barriers have been extensively studied in order to better understand how a gene transfer system should behave and know which essential properties are necessary for functional non-viral gene therapy, especially in the respiratory tract [13–16]. Nevertheless, extracellular barriers such as mucus, bacteria, and inflammation are important and decisive primary barriers which determine the extent of the contact between the gene transfer systems and the target cells [17–19]. The nature of bacterial flora in the pulmonary environment has not been taken into account in the evaluation of synthetic vectors nor in the clinical trial carried out by the British CF consortium [12]. Only pulmonary exacerbations were included as a clinical end point. However, some studies have shown that bacteria constitute an extracellular barrier that can oppose gene transfer [20,21]. If the airways do indeed seem to be the natural way to treat respiratory diseases, the effectiveness of the treatment has been slowed by the extra and intracellular barriers. This observation raises the question of both the mode of administration and the barriers faced by gene transfer. For example, the viral envelope of most recombinant vectors have difficulty withstanding the shear forces caused by an aerosol [22]. Then, for viruses still whole, their penetration into the hyperviscous mucus is difficult [23].

Currently, patients with pulmonary infections receive antibiotic therapy frequently. Taking any other treatment simultaneously, such as gene transfer, can create interactions and lead to a decrease in the expected beneficial effects. The new approach proposed in this review consists of developing formulations coupling simultaneously, the properties of gene transfer and the antibacterial effect. Meaning a single treatment will be administered in patients, decreasing the risks of drug interactions and increasing the therapeutic benefits. First, the anatomy of the airways, the mode of administration targetting these pathways, and their limits will be described. Then, the potential benefits of such an approach and the different formulations considered will be explained in terms of their clinical applications.

Direct lung delivery

The respiratory tract

The respiratory tract consists of the upper airways (nasal and oral cavities, pharynx, and larynx) and lower airways (trachea, bronchi, and segmental bronchi). The upper airways allow the filtration, the heating, and the humidification of the incoming air. The primary role of the respiratory system is to ensure gas exchanges between the air and the blood. This exchange is performed at the pulmonary alveoli stage. The lower airways have a tree-like structure (Figure 1). The trachea, corresponding to the trunk (120–150 mm in length with a diameter of 14–15 mm), divides into two main bronchi (right bronchus: 2.5 cm in length for a diameter of 15 mm and left bronchus: 5 cm in length for 11 mm width), which themselves divide into segmental bronchi. Finally, the following bronchioles (diameter less than 1 mm) end with air sacs. The tracheopulmonary tree is divided into three distinct areas according to their function in the transport of oxygen. The conduction area extending from the trachea to the bronchioles allows the air to be conveyed. In this area, there is no air-to-blood gas exchange. Whereas the transition area corresponding to the bronchioles participates in the gas exchange. Finally, the respiratory area comprising all the pulmonary alveoli allows for most of the gas exchange by diffusion. The decrease in respiratory function is often due to repeated aggression on the respiratory tract. For example, in smokers or in chronic obstructive pulmonary disease (COPD) patients, the inhaled toxicants will gradually destroy the cellular layers, leading to a reduction in gas exchange giving rise to an increase in the partial pressure of oxygen. Similarly, in CF patients, repeated cycles of infection and inflammation will lead to fibrosis of the pulmonary parenchyma and a decrease in respiratory function (measured by FEV1).

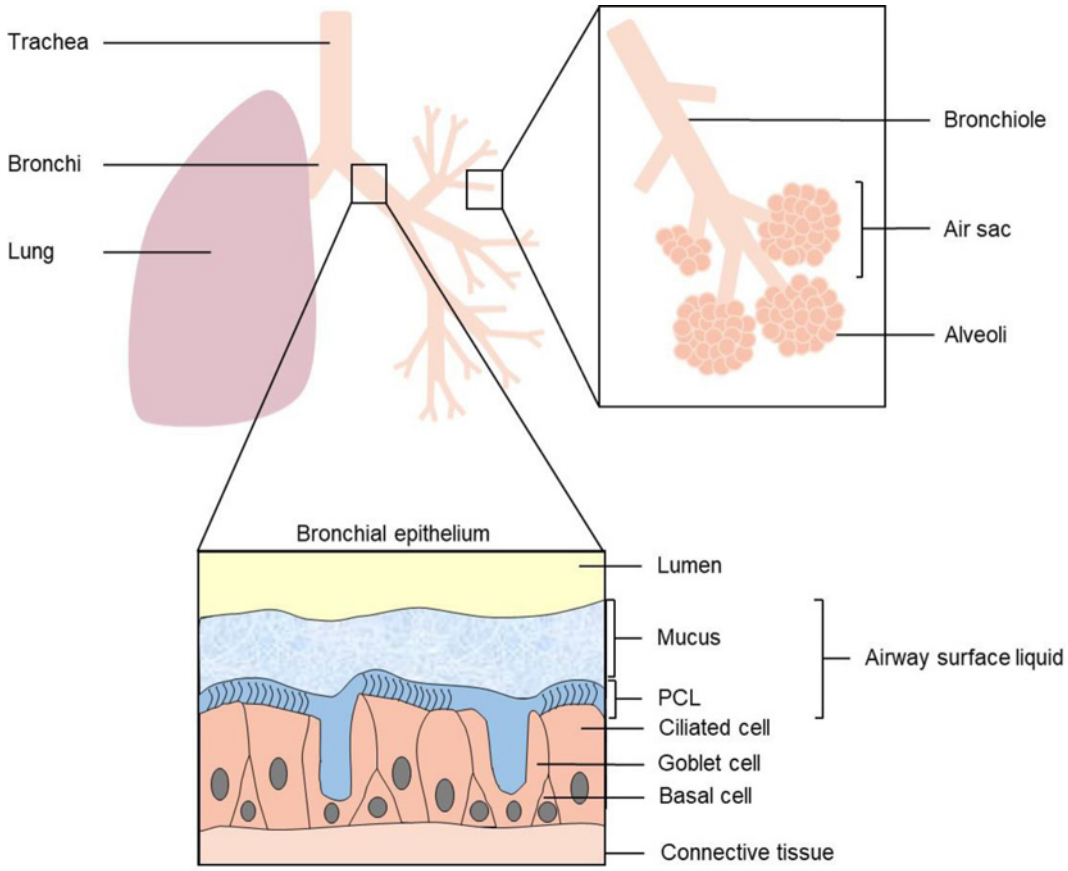


Figure 1. The organization and the structure of the respiratory tract
Abbreviation: PCL, periciliary layer.

The structure of the airway epithelium varies depending on the section. The bronchi are lined with a pseudostratified epithelium, whereas in the bronchioles, the epithelium is simple cylindrical and then cuboidal. The tracheobronchial epithelium (trachea and bronchi) is composed of ciliated cells permitting mucociliary clearance, globlet cells, and basal cells (Figure 1). The ciliated cells allow the elimination of pollutants trapped on the surface's liquid which covers the epithelium. This surface liquid ('airway surface liquid' (ASL)) is composed of periciliary layer (PCL) and mucus layer. Compared with the mucus, the PCL has a low viscosity [24]. The mucus is composed of salts, proteins (glycoproteins, mucins, mucoproteins), and water [25]. It corresponds to the product of secretions from different cells (globlet cells in the trachea and clara cells in the bronchioles). The hydration state of the surface liquid is dependent on the ionic transports (chloride ions and sodium ions in part) [26,27]. Some pathologies, such as CF, induce a defect in regulation or expression of the channels involved in ion transport, resulting in dehydration of the surface liquid and a defect in mucociliary clearance [28]. This hyperviscous mucus becomes a favorable environment for microbial infections' development.

Targetting the respiratory tract by aerosolization

Aerosolization is currently the preferred mode of administration for airway targetting. This technique of administration is non-invasive and induces little stress for patients commonly treated with aerosol. For example, asthmatic patients inhale bronchodilators (terbutaline sulphate, salbutamol sulphate, ipratropium bromide) even before the age of 3 years. Aerosolization allows the passage of a liquid solution in the form of microdroplets. Several types of aerosolization systems exist: jet nebulizers, ultrasonic, or membrane nebulizers. Jet nebulization uses a compressed gas (air or oxygen) to generate microdroplets. With the ultrasonic system of nebulizers, the aerosol is formed by high-frequency vibration of a liquid. The microdroplets of the third type of aerosolization system are obtained after passage of the solution through a membrane. The size of the droplets formed varies according to the aerosolization system used [29]. The choice of the system is important because depositing the aerosol within the respiratory tract is

Bioscience Reports (2017) **37** BSR20160618
https://doi.org/10.1042/BSR20160618

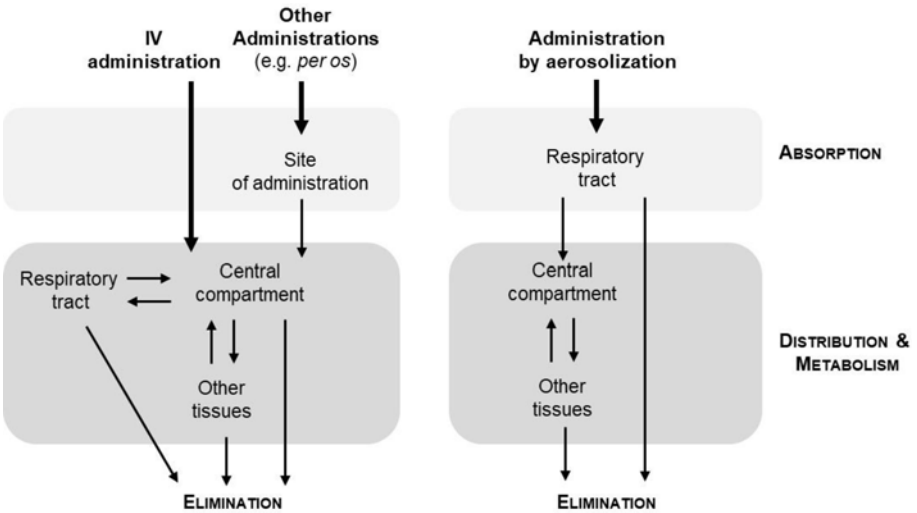


Figure 2. Pharmacokinetics according to the administration used

The aerosolization allows direct targetting of the lungs and thus bypasses the blood circulation.

defined by the size of the droplets formed [30]. Given the variable respiratory flow, the finest particles diffuse deeper at the alveolar level [31]. Three depositing mechanisms can be observed. The impaction phenomenon for droplets larger than 5 μm is due to the respiratory tract structure. Particles between 1 and 5 μm are sedimented, while droplets below 1 μm diffuse by Brownian motion in the bronchioles and the alveoli. The administered fluid volumes are very limited, same as the nasal instillation, because an excess of liquid can lead to drowning.

Pharmacokinetics of nanocomplexes after aerosol delivery

The major advantage of administration by inhalation is its pharmacological properties. Pharmacokinetics and pharmacodynamics indeed determine the therapeutic effect of a drug. Unlike the other major modes of administration, inhalation makes it possible to circumvent the blood circulation, and to avoid the first-pass effect of the liver which may lead to a reduction in the quantity of active principles reaching the targetting tissue and to potential side effects (Figure 2). For example, patients with type 1 Gaucher disease take an inhibitor of glocoyl ceramide synthase orally (eliglustat) [32]. To benefit from this treatment, a cytochrome P450 2D6 genotyping assay is required. The level of enzymatic activity of this cytochrome will determine the rate of metabolism of the drug and therefore the dose administered to the patient. This is also the case with certain classes of antibiotics. Amongst them, the aminoglycosides which bind the rRNAs (16S RNA) and thus block the translation of proteins are described as nephrotoxic and ototoxic agents when administered systemically [33]. The delivery of these antibiotics (example of gentamycin) by aerosolization has reduced these side effects [34]. The elimination of a drug also depends on the route of administration (Figure 3) [35]. If elimination does not occur fully, there are risks of accumulation which cause toxic side effects. When administered by inhalation, a large part of the active principle is eliminated by the mucociliary clearance and by exhalation. Nevertheless, the development of aerosolization systems has reduced the exhalation of treatments. The deposit site will determine the elimination kinetics [36]. The deeper the particles are deposited, the longer will be the elimination time.

Physicochemical constraints due to aerosol protocol

The physicochemical constraints associated with the aerosolization process are important. They may lead to a loss of the expected therapeutic effect (Figure 4). Not every active principle supports this mode of administration, hence the need for a formulation adapted to protect the active molecules. Moreover, depending on the nebulization system employed, the active principle will not react in the same way. For example, the dornase α, used to reduce viscosity of CF patient sputa, is degraded under the effect of heat when using an ultrasonic nebulizer [37]. Finally, research on the development of inhalation immunotherapy has shown that the antibodies poorly tolerate this mode of administration. An aggregation as well as chemical modifications were observed [38]. Once these physicochemical constraints have been overcome, the active principle must pass through various barriers mentioned below.

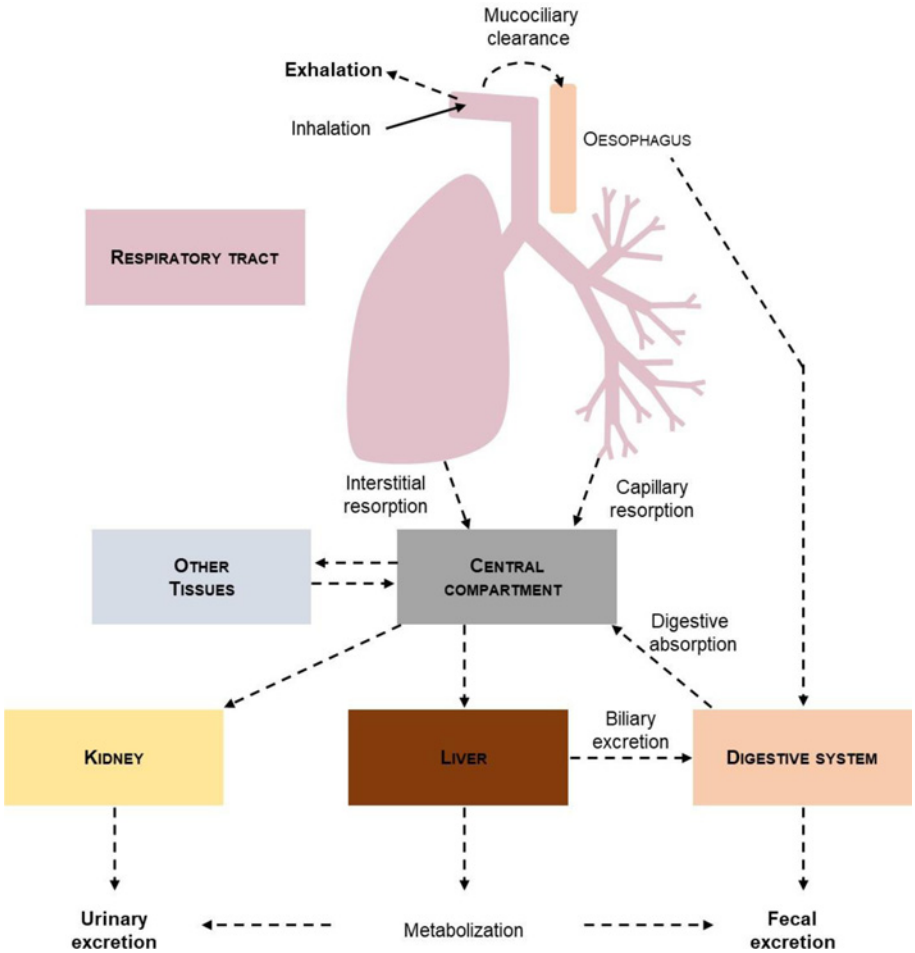


Figure 3. Elimination pathways of an inhaled drug

Some of the inhaled drugs are eliminated by exhalation during breathing. The mucociliary clearance leading to the coughing up of sputum allows the more or less rapid elimination of the active ingredients. Once in the trachea, the active ingredients are swallowed and arrive in the digestive tract. Unlike oral administration, few drugs diffuse into the bloodstream due to the small quantity that reaches the pulmonary alveoli, which is the only point of passage to the blood (modified from [35]).

Extracellular barriers encountered by nanoparticles in the respiratory tract

Treatments targetting the inside of epithelial cells encounter several obstacles during their transit from the upper respiratory tract to target cells. In addition to the mechanical movements of respiration, many extracellular barriers are present in the lungs and are a hindrance for inhalation treatments (Figure 4) [17,39].

The mucus is the first barrier whose role is to purify the air breathed in by the individual by trapping the inhaled particles. The thickness of non-pathological mucus is between 5 and 10 μm [40]. It is replaced every 10–20 min on an average [40]. Due to its composition and rheology, mucus is a key barrier against efficient inhaled therapy [19]. This filtering structure permits the passage of particles having a size of approximately 100–200 nm [41]. In some obstructive diseases such as CF, the mucus is more viscous and the mesh is tighter [42]. In addition to its high mucin concentration, the mucus contains many other anionic molecules such as cell debris, DNA, or actin. The latter, because of their charge, can interact with inhaled drugs and limit their activity [43]. For non-viral gene therapy, this leads to a distinct increase in negative charge concentration which will break apart the nucleic acids/vector complexes.

A second surface liquid called a pulmonary surfactant is also present on the inner surface of the pulmonary alveoli and facilitates respiratory movements. It is secreted by type II pneumocytes. It reduces the air/liquid surface tension on the alveoli facilitating respiration. It also plays a role in immune defense. It consists of 90% lipids (mostly

Bioscience Reports (2017) **37** BSR20160618
https://doi.org/10.1042/BSR20160618

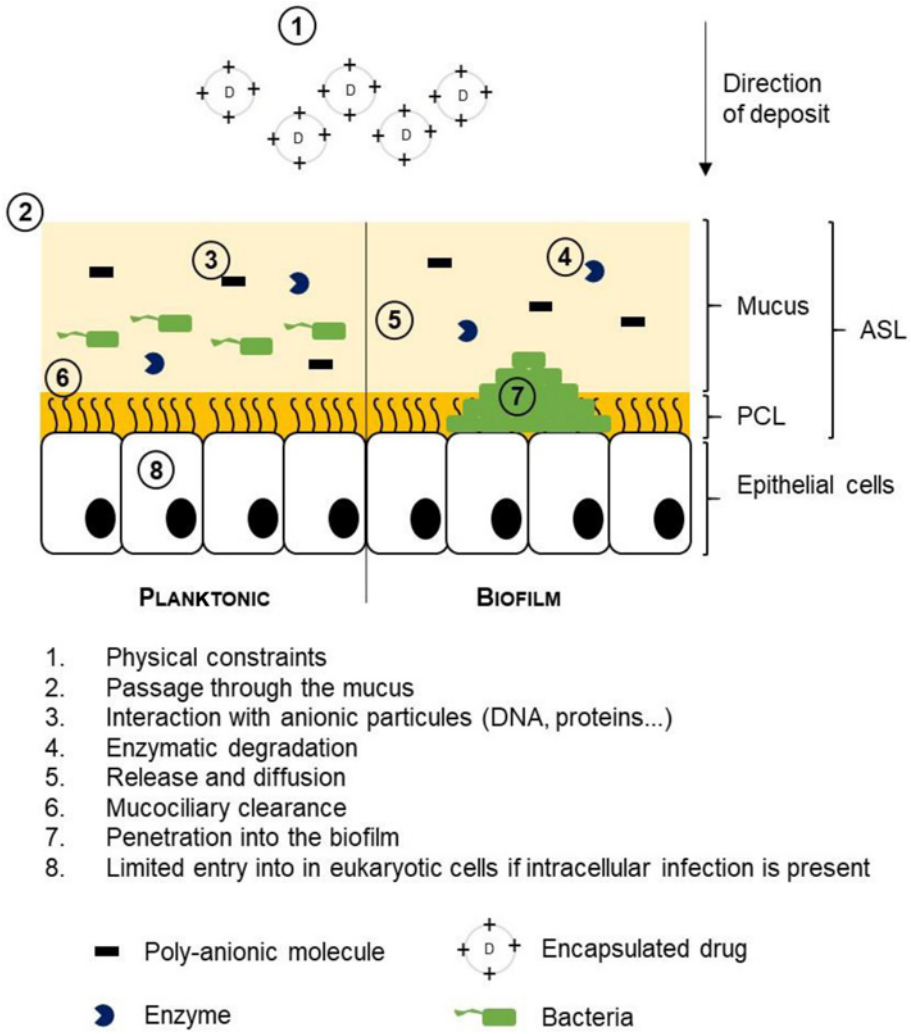


Figure 4. Extracellular factors limiting the therapeutic benefits of an aerosol

Inhaled drugs encounter different physicochemical barriers that can negatively impact their activity. The aerosolization itself is very restrictive for use of drugs. It will determine the size and charge of the aerosolized particles and therefore the deposit site. To interact with eukaryotic cells, particles must penetrate a more or less viscous mucus and limit the interactions with the components and elements trapped in the mucus. ASL contains bacteria, in planktonic form or organized in a biofilm, which can release enzymes capable of degrading the active principle. In addition, bacteria in the form of a biofilm are protected by a very robust exopolyssacharide matrix. Once in contact with eukaryotic cells, the active ingredient must pass through the plasma membrane.

dipalmitoylphosphatidylcholine) and 10% proteins [44]. This surfactant can trap active principles. In newborns, usually premature infants, a deficiency in pulmonary surfactant results in respiratory distress. Similarly in adults, frequent alterations of the pulmonary surfactant are observed. They can occur as a result of drowning and/or acute respiratory distress syndrome. Different exogenous surfactants exist and are administered endotracheally, which is quite invasive [45].

An innate defense system called mucociliary clearance helps eliminate inhaled toxicants (pollutants, microbes etc.) [46-48]. During inhalation, the particles are trapped in the mucus. The cilia present on the surface of the respiratory epithelium beat in a synchronized manner at a frequency of 1000–1500 beats per minute. This ciliary movement moves the mucus up to the trachea. The rate of upward movement of the mucus is between 5 and 20 mm/min. Once in the trachea, the mucus will be eliminated by the digestive tract or by expectoration. As previously stated, mucociliary clearance is a route of rapid elimination for inhaled drugs. It is therefore necessary that these therapeutical drugs must not remain blocked in the mucus which favors their elimination.

Table 1 Example of inhaled drugs

Pharmacological class	INN	Pathology	References
Bronchodilators	Ipratropium bromide	Asthma, COPD	[130]
	Terbutaline	Asthma, COPD	[131]
	Salbumatol	Asthma, COPD	[132]
Corticoids	Budesodine	Asthma	[133]
	Beclometasone	Asthma	[134]
Anti-infective agents	Tobramycin	CF	[135]
	Colistimethate sodium	CF	[136]
	Aztreonam	CF	[137]
	Pentamidine	Immunosuppressed	[138]
Mucolytics	Deoxyribonuclease 1	CF	[139]
Antiplatelet agent	Iloprost	PAH	[140]
Anti-allergic	Sodium cromoglycate	Asthma	[141]
Anesthesic	Lidocaine	Asthma	[142]

Abbreviations: INN, international non-proprietary name; PAH, pulmonary arterial hypertension.

The development of high-throughput sequencing tools has demonstrated the presence of s pulmonary microbiota in the lower respiratory tract which had been long considered sterile, this includes healthy individuals [49]. This flora is present from an early age. It varies from one individual to another, depending on age and health status. Certain pathologies (CF, COPD, asthma) lead to an imbalance of this flora, favoring the progressive development of pathogens [50]. Bacteria responsible for lung infections produce enzymes capable of degrading certain drugs such as antibiotics.

The pulmonary microbiota comprises various bacterial species [49,51-53]. Bacteria can grow planktonically or as biofilm. The passage from planktonic bacteria to bacteria in biofilm leads to an increase in tolerance to treatments [54-56]. In the presence of a biofilm, the penetration of the active ingredient in the mucus is reduced due to the composition of the matrix formed, mainly of polysaccharides, proteins, nucleic acids, and lipids [57]. In a biofilm, the proximity of the bacteria and the presence of nucleic acids favor the dissemination of resistance by horizontal transmission of the genes. Finally, within a biofilm, part of the bacteria are dormant. This state of low active metabolism prevents the activity of certain antibacterial agents [58,59].

Clinical applications for aerosol formulations

In France and most of western industrial countries, several drugs received marketing authorization for administration by aerosolization, their function are diverse: bronchodilators, corticosteroids, antibiotics, antiparasitic, anti-allergic, mucolytic, antiplatelet, and nasal decongestant (Table 1).

Several pathologies benefit from this mode of administration. Currently, the causes of morbidity and mortality of patients with CF (or COPD) are lung damage. In these pathologies, aerosolization is partly used for the administration of antibiotics (Tobi®, Bramitob® Tobramycin; Cayston® Aztreonam lysine; Promixin® Colistimethate sodium; Tobi® Podhaler™ Tobramycin; Colobreathe® Colistimethate sodium; Aeroquin® Levofloxacin) but also for bronchodilators and mucolytics (Bronchitol® Mannitol, Pulmozyme® Dornase α) [37].

On the other hand, this route of administration has also been used in viral or non-viral gene therapy [12,60-64]. To date, clinical trials with viral vectors derived from adenovirus or associated adenovirus were disappointing and did not improve lung function. Alton and co-workers [12] conducted the first randomized clinical trial (phase IIb), as a double-blind (n=135), for non-viral gene therapy for CF (gene drug compared with placebo). The treatment was administered by aerosolization monthly for 1 year. A gain of 3.7% of the FEV1 was observed in treated patients. The results have been a proof of the feasibility of gene transfer by aerosolization with the absence of side effects.

Transfecting formulations with antibacterial effects for gene therapy

Potential benefits for gene delivery

The antibacterial activity of a gene transfer system could be beneficial for transfecting in an infected extracellular environment [20,65]. The benefits of such a combination of different activities are summarized in Figure 5.

Bioscience Reports (2017) **37** BSR20160618
https://doi.org/10.1042/BSR20160618

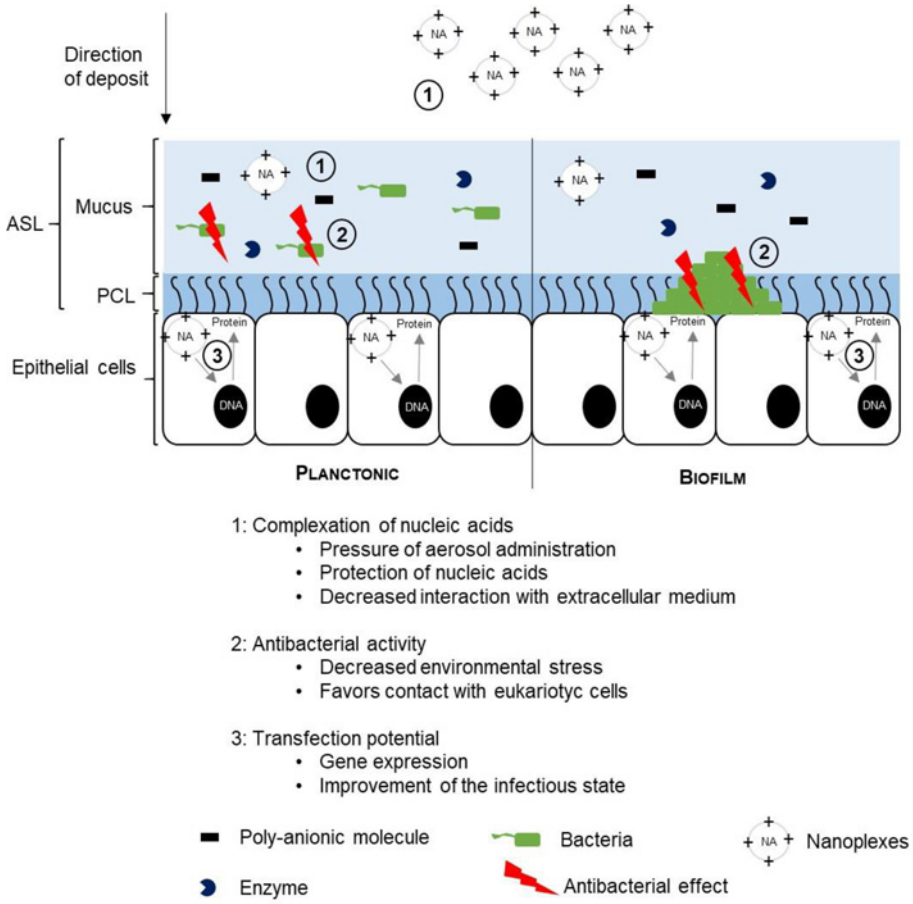


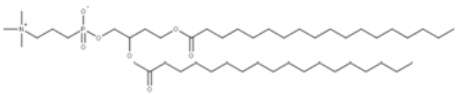
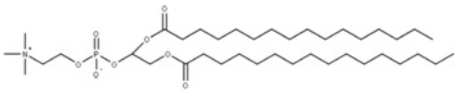
Figure 5. Multifunctional synthetic vectors: an advantage for gene transfer under infectious conditions

The antibacterial activity of a gene transfer system would make it possible to transfect the eukaryotic cells in the presence of bacteria which impair the efficiency of the gene transfer. The production of toxins by bacteria and the induction of an inflammatory response leads to stress or even cell death. The antibacterial effect would eliminate the bacteria on the surface, promoting the transfection process and so the level of expression of the transgene.

Foremost, nucleic acids are fragile molecules that cannot tolerate aerosolization. The physicochemical constraints will induce a degradation of the nucleic acids which will be more or less important depending on their size [66,67]. The complexation of the nucleic acids by means of synthetic vectors is therefore required for this mode of administration. Moreover, this complexation will limit the degradation due to the presence of deoxyribonucleases in the extracellular matrix [21]. These enzymes are produced by bacteria such as *Staphylococcus aureus*. In an infected environment, the level of deoxyribonucleases is high. In addition, the formulations based on synthetic vectors are multimodular, which is to say that they can be adapted to the target environment by integrating several compounds enabling the possibility to cross the successive barriers. In example, pegylation (with PEG)) is a frequently used process to reduce surface charge and thus an easier penetration into the ASL [68]. Unlike the mucoadhesive agents which make it possible to increase the retention time of the active ingredient in the mucus, the PEG is a so-called mucopenetrating particle. A study on the delivery of an anti-inflammatory drug (dexamethasone) showed that PEG favored diffusion in the mucus and the release of the drug when compared with mucoadhesive particles such as poly(lactide-co-acid glycolide) (PLGA) [69].

Finally, the encapsulation is beneficial in order to potentiate the effects of the active molecules. Several active drugs (antibacterial, anticancer etc.) have been encapsulated by synthetic vectors. Alipour and co-workers [43] evaluated the antibacterial efficiency of two antibiotics (tobramycin or polymyxin B) encapsulated either by 1,2-dimyristoyl-sn-glycero-3-phosphocholine and cholesterol (DMPC:Chol, molar ratio: 1/2), or by 1,2-dipalmitoyl phosphatidylcholine and cholesterol (DPPC:Chol, molar ratio: 1/2). The antibacterial effect obtained on a strain of *Pseudomonas aeruginosa*, are far greater with the encapsulated form, even in the presence of polyanionic molecules

Table 2 Synthetic vectors used to encapsulate antibiotics

Encapsulated drug	Synthetic vectors chemical structure	References
Tobramycin	 DSPC	[81,143]
Amikacin	 DPPC	[8]

(DNA, actin, lipopolysaccharides, lipoteichoic acids) frequently found in the sputum of CF patients. Similarly, Meers and co-workers [70] encapsulated amikacin (antibiotic) with a liposomal solution of DPPC:Chol (w/w: 2/1). They aerosolized the encapsulated formulation and the free form of the antibiotic in rats infected with *P. aeruginosa*. They found that the free form was ineffective in contrast with the encapsulated one. The lung concentration of bacteria in rat that benefited from the liposomal form was reduced. The aerosolization of the encapsulated form allowed a larger concentration of antibiotics to be present in the lungs for a longer amount of time, thereby limiting the emergence of bacterial resistance [70].

As we have seen, the presence of bacteria in the cellular environment can be harmful for gene transfer. This phenomenon can be accentuated in case of dysbiosis (an imbalance) of the bacterial flora leading to the appearance of infections. Growing bacteria is a very important barrier, which has long been neglected in the context of gene transfer applied to the respiratory tract. As mentioned above, bacteria produce toxins that can induce stress and/or cell death [71,72]. In addition, the infections are accompanied by strong inflammation. Inflammation induces the formation of reactive oxygen species which can lead to cell death [73]. All this will also contribute to limiting the expression of the transgene. The antibacterial activity of a transfecting formulation would eliminate bacteria localized in the cellular environment. Not only this elimination would promote access to eukaryotic cells in the presence of biofilm, it would also decrease the stress induced by the presence of bacteria in the cellular environment. Therefore, gene transfer could be carried out in a more favorable environment. Finally, expression of the transgene could further promote the eradication of infections. This is the case for CF, where the restoration of CFTR expression would induce a reduction in the risk of infection by the progressive fluidification of mucus through the restoration of ionic transports [74-77].

The aim of our strategy is to transfer the antibacterial effect of the active principle to the vector itself so as to be able to transport other active principles (such as nucleic acids for example), which in parallel reduces the risks of side effects generated by drug interactions. In order to obtain formulations with antibacterial and transfecting properties, several options are available. Several antibiotics have been encapsulated by synthetic vectors which have shown transfection capability. However, the antibiotics, which usually find themselves stuck with the complexes, need to be released to be efficient. The combination of antibacterial molecules and nucleic acids encapsulated by synthetic vectors could allow the two activities to be obtained. In addition, some synthetic vectors, which will be described hereinafter, are endowed with their own antibacterial activity. This activity would make it possible not to use the antibiotics which can cause an appearance of bacterial resistance.

Antibiotics encapsulated by synthetic vectors

Attempts have been made to encapsulate antibiotics in order to decrease side effects since the 1980s [78,79]. Due to their fusion capability with plasma membranes and their capability of encapsulation, the synthetic vectors derived mainly from natural phospholipids have been used. These combinations have allowed the production of original formulations which will be described hereinafter.

Currently, two formulations of encapsulated antibiotics are used in Europe for pulmonary infections in aerosol delivery: TOBI® Podhaler™ (Novartis) and Arikace™ (Insmed) (Table 2). TOBI® Podhaler™ corresponds to the encapsulation of tobramycin (aminoside) by a cationic lipid, distearoyl phosphatidylcholine (DSPC). DSPC is used as a co-lipid for the formation of lipoplexes in the context of the delivery of interfering RNA [3,80,81]. This drug is prescribed for treating chronic pulmonary infections with *P. aeruginosa* in CF patients. Arikace™ is an aminoglycoside (amikacin) encapsulated by DPPC and cholesterol. DPPC is a natural lipid commonly used in gene transfer and has shown notable effects toward many different cell types but not for gene therapy in itself [82]. This drug has

Bioscience Reports (2017) **37** BSR20160618
https://doi.org/10.1042/BSR20160618

not received marketing authorization but is used clinically in a regulated context thanks to a transitional exemption. This encapsulated antibiotic can be delivered by aerosolization (eFlow® nebulizer) to CF patients whose pulmonary pathways are chronically infected with *P. aeruginosa* [70,83].

Other anti-infective agents are being evaluated clinically. Polymyxin is a polycationic antibiotic used to control Gram-negative infections. Its systemic administration induces significant side effects (nephrotoxicity, ototoxicity, and neuromuscular blockage). Several studies are searching for ways to encapsulate this antibiotic to limit its side effects and administer it by inhalation. The encapsulation of polymyxin B with DPPC showed better activity compared with the non-encapsulated form on a mouse pneumonia model and limits side effects [84-86].

Pulmaquin™ and Lipoquin™ (Aradigm, Hayward, CA, U.S.A.) are the encapsulated forms of ciprofloxacin with 65.9 mg/ml of hydrogenated phosphatidylcholine (HSPC) and 27 mg/ml of cholesterol. These two forms of liposomal antibiotics are used to treat chronic *P. aeruginosa* infections in immunocompromised patients. Lipoquin™ is also prescribed for CF patients and is administered by aerosol with a jet nebulizer. The release kinetics of ciprofloxacin varies according to the formulation. However, the release of the antibiotic is slower with Pulmaquin™ [87].

Synthetic vectors with antibacterial effects

Some synthetic vectors are endowed with antibacterial and transfecting activities (Table 3).

Antimicrobial peptides

Legendre and Szoka showed that antimicrobial peptides (gramicidin S, tyrocidin) with proved antibacterial activity, had a transfecting capability similar to that observed with cationic lipids [88]. These compounds have the ability to bind DNA by electrostatic interactions. Moreover, the fact that they are amphiphilic makes it possible to permeabilize the membranes [89,90]. These antimicrobial peptides act on Gram(+) and Gram(–) bacteria. The complexation of nucleic acids by antimicrobial peptides does not affect their antibacterial activity [91]. Their broad spectrum of activity makes antimicrobial peptides good candidates for antibacterial and transfecting formulations.

Cationics lipids inspired from antibiotics

Some families of antibiotics such as aminoglycosides have the ability to bind nucleic acids (DNA and RNA) [92]. This observed characteristic, essential to gene transfer agents for correct complexation, was used for the synthesis of novel cationic lipids. Some lipid derivatives' polar heads incorporate an aminoside such as kanamycin (KanaChol) [93,94] or neamine [95], a neomycin fragment. The aminoglycoside polar head makes it possible to condense the nucleic acid constructs and their cholesteryl motif facilitates the entry into the eukaryotic cell. After evaluation of their transfecting activity post-deposit, these lipid derivatives have shown an interesting efficiency for gene transfer in various mammalian cell lines [94,96,97]. In parallel, these amphiphilic derivatives of antibiotics also exhibit an antibacterial activity on *P. aeruginosa* [98–101].

Cationic polymers

On one hand, Wu and Hsu evaluated the cationic polymers' (water-based cationic polyurethanes) antibacterial potency on *Escherichia coli* and *S. aureus* strains which has proved to be potent [102]. On the other hand, high transfection efficiencies were obtained on a renal cell line [102].

Poly-L-lysine is a polymer commonly used for gene transfer [5,68,103,104]. In 2013, Dubois and co-workers [105] studied the antibacterial activity of poly-L-lysine. They found that this polymer made it possible to kill the bacteria such as the *P. aeruginosa* and *S. aureus* species which are frequently isolated from sputum of CF patients [106].

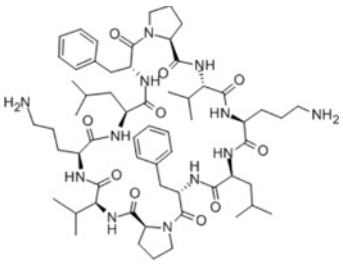
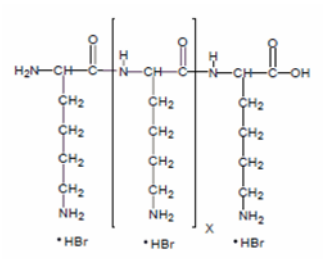
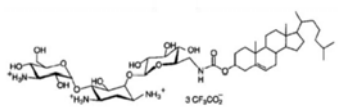
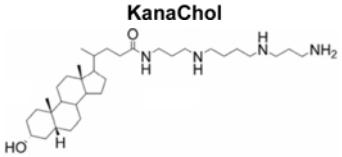
Finally, PEI and its branched or histidinylated derivatives are heavily used for gene transfer. They showed a very good transfection activity [5,107,108] on post-aerosolization on murine and sheep models as well [109-112]. In addition to this gene transfer capability, this family of synthetic vectors exhibited antibacterial (Gram(+) and Gram(–)) and antifungal activity post-deposit [113,114]. No studies evaluated the antibacterial potency of post-aerosolization PEI.

Monocationic lipids derived from phospholipids

Similarly, other molecules not derived from antibiotics and which bind to DNA, such as spermine, have also shown transfection activity as well as an antibacterial effect on Gram(+) bacteria (*Bacillus subtilis*) and Gram (–) bacteria (*E. coli*) [115].

Fein and co-workers [20] are interested in the antibacterial and transfection properties of two steroid-derived cationic lipids called dexamethasone spermine (DS) and disubstituted spermine (D2S). These two compounds have

Table 3 Example of synthetic vectors with an antibacterial activity

Synthetic vector family	Chemical structure	Antibacterial effect	References
Antimicrobial peptide	 Gramicidine S	Gram+ Gram–	[88]
Polymer	 Poly-L-lysine	Gram+ Gram–	[124]
Aminoside derivative	 KanaChol	Gram+	[98–101]
Sterol derivative cationic lipid	 5-choleryl-spermide-3ß-ol	Gram+ Gram–	[20,115]
Lipophosphoramidate	 KLN47 DODAB	Gram+	[21]

been studied individually and as co-formulation. The evaluation of the various lipoplexes by direct deposit in the extracellular medium, revealed a good transfection activity on cell line A549 (epithelial cells derived from pulmonary carcinoma). Antimicrobial activity on Gram(–) bacteria (*E. coli* MG1655 and *P. aeruginosa* PAO1) and Gram(+) *B. subtilis* was obtained with D2S at low concentrations (5 μM). Given the chemical structure of D2S, they hypothesize that the antibacterial activity is due to the amphiphilic structure resembling antimicrobial peptides such as cathelicidin LL-37 which would favor destabilization of the bacterial membrane [20].

Subsequently, novel cationic derivatives of steroids containing other glucocorticoids (flumetasone, budesonide, and beclometasone) have been developed. Anti-inflammatory, antibacterial, transfectant, and cytotoxic activities were then evaluated [116]. These compounds showed antibacterial effects (a few μM depending on strain) on different strains of *P. aeruginosa* and methycillin-resistant *S. aureus* (Xen30). The transfection capability was evaluated in parallel by direct deposit on BAECs and A549 cell lines. Some compounds have levels of transfection similar to those

Bioscience Reports (2017) **37** BSR20160618
https://doi.org/10.1042/BSR20160618

obtained with Lipofectamine® 2000, a commercial transfer agent which has no antibacterial effect (Thermo Fischer Scientific).

Dodecyltrimethylammonium bromide (DODAB) can complex the DNA and thus allow the gene transfer [117,118]. Different studies have shown that quaternary ammonium compounds have antibacterial and antifungal activities [119-121]. Some geminis with two quaternary ammonium heads have shown good transfection efficiency due to their strong DNA interaction [122,123]. In addition, they exhibit antibacterial activity on Gram(+) bacteria (*E. coli* and *P. aeruginosa*) [124].

In 2013, our study confirmed the presence of antibacterial activity on some synthetic vectors originally designed for gene transfer [21]. After structure-activity analysis of a series of cationic lipophosphoramidate, it has been found that the nature of the polar head and aliphatic chains are the key elements of antibacterial potency. Contrary to trimethylammonium lipophosphoramidates, only a few cationic lipids with a trimethylarsonium or trimethylphosphonium polar head exhibit an antibacterial activity on different strains of *S. aureus*. The best antibacterial activity was obtained with arseno compounds. Furthermore, the structure and length of the aliphatic chains would affect the antibacterial activity. The degree of unsaturation and the length of the aliphatic chain permit the improvement of the antibacterial activity of trimethylarsonium lipophosphoramidate. This antibacterial activity was observed for relatively low concentrations, which are close to those used for the transfection of eukaryotic cells. This is an important point to simultaneously study both activities. To explore this hypothesis, liquid co-cultures of bacteria and human bronchial epithelial cells were used. In the present study, it was shown that the antibacterial activity of the cationic lipid makes it possible to obtain, in the presence of a bacterial infection, a transfection activity equivalent to that observed in the absence of bacteria.

Synthetic vector based formulations

To broaden the spectrum of activity, previously studied silver compounds [125] were introduced into the formulation and experiments showed that the antibacterial activity was extended to Gram(–), which are problematic in CF, and that this activity was retained post-aerosolization (currently being submitted). To our knowledge, only one other study has combined molecules of different activities in order to obtain multifunctional transfer systems. Peng and co-workers [126] combined a gold nanoparticle (AuP) with an antimicrobial peptide (PEP 'peptide sequence from lactoferrin'). This combination allowed Peng and co-workers [126] to efficiently transfect mesenchymal stem cells. The gold nanoparticles are known for their transfection power [127] as well as for their antibacterial activity on Gram(+) and Gram(–) bacteria [128,129]. However, these activities have not been tested by aerosolization.

Conclusion

Antibiotics encapsulated in a formulation must be released in order to be available and come into contact with bacteria. Transferring the antibacterial effect directly to the vector would allow a more immediate effect. Multimodular vectors are a major asset to overcome the different barriers encountered and to act according to therapeutic targets, which are, not only the bacteria for the antibacterial effect at the extracellular level, but also the nucleus for the gene transfer. Besides gene transfer, many other applications such as administration of anticancer drugs, anti-inflammatory reagents, or various other molecules such as insulin can be considered. The formulations will be adapted to the constraints related to the inhaled administration and its environment. Finally, in order to combat the rapid increase in bacterial resistance, the antibacterial activity of the vector coupled with the antibacterial activity of the encapsulated antibiotic would allow the introduction of bi-antibiotic therapy. Thus, the targets will be more numerous and will allow the better treatment of the infections that are still difficult to treat today, such as nosocomial diseases which can infect immunosuppressed patients.

Acknowledgements

We thank Prof Pierre Lehn for his constant scientific support.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

This work was supported by the 'Association Française contre les Myopathies' (AFM, Evry, France) [grant number XXXX]; the 'Vaincre La Mucoviscidose' (Paris, France) [grant number XXXX]; the 'Association de transfusion sanguine et de biogénétique

Gaétan Saleün' (Brest, France) [grant number XXXX]; the 'Région Bretagne' [grant number XXXX]; the Brest Métropôle [grant number XXXX]; and the Mr Michel Caugant [grant number XXXX].

Abbreviations

ASL, airway surface liquid; BAEc, Bovine Aortic Endothelial cells; CF, cystic fibrosis; CFTR, cystic Fibrosis Transmembrane conductane Regulator; COPD, chronic obstructive pulmonary disease; DPPC, 1,2-dipalmitoyl phosphatidylcholine; DSPC, distearoyl phosphatidylcholine; D2S, disubstituted spermine; LL-37, cathelcidin antimicrobial peptide; FEV1, forced expiratory volume in 1 s; F508del, deletion of three nucleotides (position 508) of cftr gene; PCL, periciliary layer; PEI, polyethyleneimine; PEP, Peptide sequence from lactoferrin.

References

1 Felgner, P.L., Gadek, T.R., Holm, M., Roman, R., Chan, H.W., Wenz, M. et al. (1987) Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7413–7417
2 Ibraheem, D., Elaissari, A. and Fessi, H. (2014) Gene therapy and DNA delivery systems. *Int. J. Pharm.* **459**, 70–83
3 Yin, H., Kanasty, R.L., Eltoukhy, A.A., Vegas, A.J., Dorkin, J.R. and Anderson, D.G. (2014) Non-viral vectors for gene-based therapy. *Nat. Rev. Genet.* **15**, 541–555
4 Mintzer, M.A. and Simanek, E.E. (2009) Nonviral vectors for gene delivery. *Chem. Rev.* **109**, 259–302
5 Li, L., Wei, Y. and Gong, C. (2015) Polymeric nanocarriers for non-viral gene delivery. *J. Biomed. Nanotechnol.* **11**, 739–770
6 Oliveira, C., Ribeiro, A.J., Veiga, F. and Silveira, I. (2016) Recent advances in nucleic acid-based delivery: from bench to clinical trials in genetic diseases. *J. Biomed. Nanotechnol.* **12**, 841–862
7 Wu, G.Y. and Wu, C.H. (1987) Receptor-mediated *in vitro* gene transformation by a soluble DNA carrier system. *J. Biol. Chem.* **262**, 4429–4432
8 Boussif, O., Lezoualc'h, F., Zanta, M.A., Mergny, M.D., Scherman, D., Demeneix, B. et al. (1995) A versatile vector for gene and oligonucleotide transfer into cells in culture and *in vivo*: polyethylenimine. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 7297–7301
9 Chaplot, S.P. and Rupenthal, I.D. (2014) Dendrimers for gene delivery–a potential approach for ocular therapy. *J. Pharm. Pharmacol.* **66**, 542–556
10 Mansouri, S., Lavigne, P., Corsi, K., Benderdour, M., Beaumont, E. and Fernandes, J.C. (2004) Chitosan-DNA nanoparticles as non-viral vectors in gene therapy: strategies to improve transfection efficacy. *Eur. J. Pharm. Biopharm.* **57**, 1–8
11 Montier, T., Benvegnu, T., Jaffrès, P.-A., Yaouanc, J.-J. and Lehn, P. (2008) Progress in cationic lipid-mediated gene transfection: a series of bio-inspired lipids as an example. *Curr. Gene Ther.* **8**, 296–312
12 Alton, E.W.F.W.x, Armstrong, D.K., Ashby, D., Bayfield, K.J., Bilton, D., Bloomfield, E.V. et al. (2015) Repeated nebulisation of non-viral CFTR gene therapy in patients with cystic fibrosis: a randomised, double-blind, placebo-controlled, phase 2b trial. *Lancet Respir. Med.* **3**, 684–691
13 Kitson, C., Angel, B., Judd, D., Rothery, S., Severs, N.J., Dewar, A. et al. (1999) The extra- and intracellular barriers to lipid and adenovirus-mediated pulmonary gene transfer in native sheep airway epithelium. *Gene Ther.* **6**, 534–546
14 Lechardeur, D. and Lukacs, G.L. (2002) Intracellular barriers to non-viral gene transfer. *Curr. Gene Ther.* **2**, 183–194
15 Gottfried, L.F. and Dean, D.A. (2013) Extracellular and intracellular barriers to non-viral gene transfer. . http://www.intechopen.com/books/novel-gene-therapy-approaches/extracellular-and-intracellular-barriers-to-non-viral-gene-transfer
16 Montier, T., Delépine, P., Pichon, C., Férec, C., Porteous, D.J. and Midoux, P. (2004) Non-viral vectors in cystic fibrosis gene therapy: progress and challenges. *Trends Biotechnol.* **22**, 586–592
17 Sanders, N., Rudolph, C., Braeckmans, K., De Smedt, S.C. and Demeester, J. (2009) Extracellular barriers in respiratory gene therapy. *Adv. Drug Deliv. Rev.* **61**, 115–127
18 Schuster, B.S., Kim, A.J., Kays, J.C., Kanzawa, M.M., Guggino, W.B., Boyle, M.P. et al. (2014) Overcoming the cystic fibrosis sputum barrier to leading adeno-associated virus gene therapy vectors. *Mol. Ther.* **22**, 1484–1493
19 Duncan, G.A., Jung, J., Hanes, J. and Suk, J.S. (2016) The mucus barrier to inhaled gene therapy. *Mol. Ther.* **24**, 2043–2053
20 Fein, D.E., Bucki, R., Byfield, F., Leszczynska, K., Janmey, P.A. and Diamond, S.L. (2010) Novel cationic lipids with enhanced gene delivery and antimicrobial activity. *Mol. Pharmacol.* **78**, 402–410
21 Le Gall, T., Berchel, M., Le Hir, S., Fraix, A., Salaün, J.Y., Férec, C. et al. (2013) Arsonium-containing lipophosphoramides, poly-functional nano-carriers for simultaneous antibacterial action and eukaryotic cell transfection. *Adv. Healthc. Mater.* **2**, 1513–1524
22 Griesenbach, U., McLachlan, G., Owaki, T., Somerton, L., Shu, T., Baker, A. et al. (2011) Validation of recombinant Sendai virus in a non-natural host model. *Gene Ther.* **18**, 182–188
23 Hida, K., Lai, S.K., Suk, J.S., Won, S.Y., Boyle, M.P. and Hanes, J. (2011) Common gene therapy viral vectors do not efficiently penetrate sputum from cystic fibrosis patients. *PLoS ONE* **6**, e19919
24 Button, B., Cai, L.-H., Ehre, C., Kesimer, M., Hill, D.B., Sheehan, J.K. et al. (2012) A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia. *Science* **337**, 937–941
25 Lai, S.K., Wang, Y.-Y., Wirtz, D. and Hanes, J. (2009) Micro- and macrorheology of mucus. *Adv. Drug Deliv. Rev.* **61**, 86–100
26 Tarran, R., Loewen, M.E., Paradiso, A.M., Olsen, J.C., Gray, M.A., Argent, B.E. et al. (2002) Regulation of murine airway surface liquid volume by CFTR and Ca2+-activated Cl- conductances. *J. Gen. Physiol.* **120**, 407–418
27 Boucher, R.C. (2004) New concepts of the pathogenesis of cystic fibrosis lung disease. *Eur. Respir. J.* **23**, 146–158
28 Munkholm, M. and Mortensen, J. (2014) Mucociliary clearance: pathophysiological aspects. *Clin. Physiol. Funct. Imaging* **34**, 171–177
29 Davies, L.A., Nunez-Alonso, G.A., McLachlan, G., Hyde, S.C. and Gill, D.R. (2014) Aerosol delivery of DNA/liposomes to the lung for cystic fibrosis gene therapy. *Hum. Gene Ther. Clin. Dev.* **25**, 97–107

30 Yhee, J.Y., Im, J. and Nho, R.S. (2016) Advanced therapeutic strategies for chronic lung disease using nanoparticle-based drug delivery. *J. Clin. Med.* **5**, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5039485/, doi:10.3390/jcm5090082
31 Resnier, P., Mottais, A., Sibiril, Y., Gall, T.L. and Montier, T. (2016) Challenges and successes using nanomedicines for aerosol delivery to the airways. *Curr. Gene Ther.* **16**, 34–46
32 Becquemont, L. (2017) Maladie de Gaucher de type 1 (CYP2D6-éliglustat). *Thérapie* **72**, 319–322
33 Saleh, P., Abbasalizadeh, S., Rezaeian, S., Naghavi-Behzad, M., Piri, R. and Pourfeizi, H.H. (2016) Gentamicin-mediated ototoxicity and nephrotoxicity: a clinical trial study. *Niger. Med. J.* **57**, 347–352
34 Murray, M.P., Govan, J.R.W., Doherty, C.J., Simpson, A.J., Wilkinson, T.S., Chalmers, J.D. et al. (2011) A randomized controlled trial of nebulized gentamicin in non-cystic fibrosis bronchiectasis. *Am. J. Respir. Crit. Care Med.* **183**, 491–499
35 Kuzmov, A. and Minko, T. (2015) Nanotechnology approaches for inhalation treatment of lung diseases. *J. Control. Release* **219**, 500–518
36 Weers, J., Metzheiser, B., Taylor, G., Warren, S., Meers, P. and Perkins, W.R. (2009) A gamma scintigraphy study to investigate lung deposition and clearance of inhaled amikacin-loaded liposomes in healthy male volunteers. *J. Aerosol Med. Pulm. Drug Deliv.* **22**, 131–138
37 Dubus, J.-C. and Ravilly, S. (2008) Inhaled therapies in cystic fibrosis. *Rev. Mal. Respir.* **25**, 989–998
38 Respaud, R., Vecellio, L., Diot, P. and Heuzé-Vourc'h, N. (2015) Nebulization as a delivery method for mAbs in respiratory diseases. *Expert Opin. Drug Deliv.* **12**, 1027–1039
39 d'Angelo, I., Conte, C., La Rotonda, M.I., Miro, A., Quaglia, F. and Ungaro, F. (2014) Improving the efficacy of inhaled drugs in cystic fibrosis: challenges and emerging drug delivery strategies. *Adv. Drug Deliv. Rev.* **75**, 92–111
40 Lai, S.K., Wang, Y.-Y. and Hanes, J. (2009) Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. *Adv. Drug Deliv. Rev.* **61**, 158–171
41 Schuster, B.S., Suk, J.S., Woodworth, G.F. and Hanes, J. (2013) Nanoparticle diffusion in respiratory mucus from humans without lung disease. *Biomaterials* **34**, 3439–3446
42 Kim, N., Duncan, G.A., Hanes, J. and Suk, J.S. (2016) Barriers to inhaled gene therapy of obstructive lung diseases: a review. *J. Control. Release* **240**, 465–488
43 Alipour, M., Suntres, Z.E., Halwani, M., Azghani, A.O. and Omri, A. (2009) Activity and interactions of liposomal antibiotics in presence of polyanions and sputum of patients with cystic fibrosis. *PLoS ONE* **4**, e5724
44 Hidalgo, A., Cruz, A. and Pérez-Gil, J. (2017) Pulmonary surfactant and nanocarriers: toxicity versus combined nanomedical applications. *Biochim. Biophys. Acta* **1859**, 1740–1748
45 Speer, C.P., Sweet, D.G. and Halliday, H.L. (2013) Surfactant therapy: past, present and future. *Early Hum. Dev.* **89** (Suppl. 1), S22–S24
46 Knowles, M.R. and Boucher, R.C. (2002) Mucus clearance as a primary innate defense mechanism for mammalian airways. *J. Clin. Invest.* **109**, 571–577
47 Button, B.M. and Button, B. (2013) Structure and function of the mucus clearance system of the lung. *Cold Spring Harb. Perspect. Med.* **3**, doi:10.1101/cshperspect.a009720
48 Bustamante-Marin, X.M. and Ostrowski, L.E. (2017) Cilia and mucociliary clearance. *Cold Spring Harb. Perspect. Biol.* **9**, a028241
49 Faner, R., Sibila, O., Agustí, A., Bernasconi, E., Chalmers, J.D., Huffnagle, G.B. et al. (2017) The microbiome in respiratory medicine: current challenges and future perspectives. *Eur. Respir. J.* **49**, doi:10.1183/13993003.02086-2016
50 Marsland, B.J. and Gollwitzer, E.S. (2014) Host-microorganism interactions in lung diseases. *Nat. Rev. Immunol.* **14**, 827–835
51 Morris, A., Beck, J.M., Schloss, P.D., Campbell, T.B., Crothers, K., Curtis, J.L. et al. (2013) Comparison of the respiratory microbiome in healthy nonsmokers and smokers. *Am. J. Respir. Crit. Care Med.* **187**, 1067–1075
52 Sze, M.A., Hogg, J.C. and Sin, D.D. (2014) Bacterial microbiome of lungs in COPD. *Int. J. Chron. Obstruct. Pulmon. Dis.* **9**, 229–238
53 Hoppe, J.E. and Zemanick, E.T. (2017) Lessons from the lower airway microbiome in early CF. *Thorax*, doi:10.1136/thoraxjnl-2017-210030
54 Olson, M.E., Ceri, H., Morck, D.W., Buret, A.G. and Read, R.R. (2002) Biofilm bacteria: formation and comparative susceptibility to antibiotics. *Can. J. Vet. Res.* **66**, 86–92
55 Ehsan, Z. and Clancy, J.P. (2015) Management of *Pseudomonas aeruginosa* infection in cystic fibrosis patients using inhaled antibiotics with a focus on nebulized liposomal amikacin. *Future Microbiol.* **10**, 1901–1912
56 Rukavina, Z. and Vanić, Ž. (2016) Current trends in development of liposomes for targeting bacterial biofilms. *Pharmaceutics* **8**, doi:10.3390/pharmaceutics8020018
57 Ciofu, O., Tolker-Nielsen, T., Jensen, P., Wang, H. and Høiby, N. (2015) Antimicrobial resistance, respiratory tract infections and role of biofilms in lung infections in cystic fibrosis patients. *Adv. Drug Deliv. Rev.* **85**, 7–23
58 Walters, M.C., Roe, F., Bugnicourt, A., Franklin, M.J. and Stewart, P.S. (2003) Contributions of antibiotic penetration, oxygen limitation, and low metabolic activity to tolerance of *Pseudomonas aeruginosa* biofilms to ciprofloxacin and tobramycin. *Antimicrob. Agents Chemother.* **47**, 317–323
59 Lewis, K. (2008) Multidrug tolerance of biofilms and persister cells. *Curr. Top. Microbiol. Immunol.* **322**, 107–131
60 Porteous, D.J., Dorin, J.R., McLachlan, G., Davidson-Smith, H., Davidson, H., Stevenson, B.J. et al. (1997) Evidence for safety and efficacy of DOTAP cationic liposome mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis. *Gene Ther.* **4**, 210–218
61 Bellon, G., Michel-Calemard, L., Thouvenot, D., Jagneaux, V., Poitevin, F., Malcus, C. et al. (1997) Aerosol administration of a recombinant adenovirus expressing CFTR to cystic fibrosis patients: a phase I clinical trial. *Hum. Gene Ther.* **8**, 15–25
62 Perricone, M.A., Morris, J.E., Pavelka, K., Plog, M.S., O'Sullivan, B.P., Joseph, P.M. et al. (2001) Aerosol and lobar administration of a recombinant adenovirus to individuals with cystic fibrosis. II. Transfection efficiency in airway epithelium. *Hum. Gene Ther.* **12**, 1383–1394
63 Aitken, M.L., Moss, R.B., Waltz, D.A., Dovey, M.E., Tonelli, M.R., McNamara, S.C. et al. (2001) A phase I study of aerosolized administration of tgAAVCF to cystic fibrosis subjects with mild lung disease. *Hum. Gene Ther.* **12**, 1907–1916

64 Moss, R.B., Rodman, D., Spencer, L.T., Aitken, M.L., Zeitlin, P.L., Waltz, D. et al. (2004) Repeated adeno-associated virus serotype 2 aerosol-mediated cystic fibrosis transmembrane regulator gene transfer to the lungs of patients with cystic fibrosis: a multicenter, double-blind, placebo-controlled trial. *Chest* **125**, 509–521
65 Myint, M., Limberis, M.P., Bell, P., Somanathan, S., Haczku, A., Wilson, J.M. et al. (2014) In vivo evaluation of adeno-associated virus gene transfer in airways of mice with acute or chronic respiratory infection. *Hum. Gene Ther.* **25**, 966–976
66 Lentz, Y.K., Worden, L.R., Anchordoquy, T.J. and Lengsfeld, C.S. (2005) Effect of jet nebulization on DNA: identifying the dominant degradation mechanism and mitigation methods. *J. Aerosol Sci.* **36**, 973–990
67 Catanese, D.J., Fogg, J.M., Schrock, D.E., Gilbert, B.E. and Zechiedrich, L. (2012) Supercoiled Minivector DNA resists shear forces associated with gene therapy delivery. *Gene Ther.* **19**, 94–100
68 Ryan, G.M., Kaminskas, L.M., Kelly, B.D., Owen, D.J., McIntosh, M.P. and Porter, C.J.H. (2013) Pulmonary administration of PEGylated polylysine dendrimers: absorption from the lung versus retention within the lung is highly size-dependent. *Mol. Pharm.* **10**, 2986–2995
69 Schneider, C.S., Xu, Q., Boylan, N.J., Chisholm, J., Tang, B.C., Schuster, B.S. et al. (2017) Nanoparticles that do not adhere to mucus provide uniform and long-lasting drug delivery to airways following inhalation. *Sci. Adv.* **3**, e1601556
70 Meers, P., Neville, M., Malinin, V., Scotto, A.W., Sardaryan, G., Kurumunda, R. et al. (2008) Biofilm penetration, triggered release and *in vivo* activity of inhaled liposomal amikacin in chronic *Pseudomonas aeruginosa* lung infections. *J. Antimicrob. Chemother.* **61**, 859–868
71 Rada, B. and Leto, T.L. (2013) Pyocyanin effects on respiratory epithelium: relevance in *Pseudomonas aeruginosa* airway infections. *Trends Microbiol.* **21**, 73–81
72 Adam, D., Perotin, J.-M., Lebargy, F., Birembaut, P., Deslée, G. and Coraux, C. (2014) Regeneration of airway epithelium. *Rev. Mal. Respir.* **31**, 300–311
73 Rosanna, D.P. and Salvatore, C. (2012) Reactive oxygen species, inflammation, and lung diseases. *Curr. Pharm. Des.* **18**, 3889–3900
74 Johnson, L.G., Olsen, J.C., Sarkadi, B., Moore, K.L., Swanstrom, R. and Boucher, R.C. (1992) Efficiency of gene transfer for restoration of normal airway epithelial function in cystic fibrosis. *Nat. Genet.* **2**, 21–25
75 Davies, J.C., Stern, M., Dewar, A., Caplen, N.J., Munkonge, F.M., Pitt, T. et al. (1997) CFTR gene transfer reduces the binding of *Pseudomonas aeruginosa* to cystic fibrosis respiratory epithelium. *Am. J. Respir. Cell Mol. Biol.* **16**, 657–663
76 Biffi, A., Sersale, G., Cassetti, A., Villa, A., Bordignon, C., Assael, B.M. et al. (1999) Restoration of bacterial killing activity of human respiratory cystic fibrosis cells through cationic vector-mediated cystic fibrosis transmembrane conductance regulator gene transfer. *Hum. Gene Ther.* **10**, 1923–1930
77 Hisert, K.B., Heltshe, S.L., Pope, C., Jorth, P., Wu, X., Edwards, R.M. et al. (2017) Restoring CFTR function reduces airway bacteria and inflammation in people with cystic fibrosis and chronic lung infections. *Am. J. Respir. Crit. Care Med.*, doi:10.1164/rccm.201609-1954OC
78 Lagacé, J., Dubreuil, M. and Montplaisir, S. (1991) Liposome-encapsulated antibiotics: preparation, drug release and antimicrobial activity against *Pseudomonas aeruginosa*. *J. Microencapsul.* **8**, 53–61
79 Omri, A., Beaulac, C., Bouhajib, M., Montplaisir, S., Sharkawi, M. and Lagacé, J. (1994) Pulmonary retention of free and liposome-encapsulated tobramycin after intratracheal administration in uninfected rats and rats infected with *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **38**, 1090–1095
80 Zimmermann, T.S., Lee, A.C.H., Akinc, A., Bramlage, B., Bumcrot, D., Fedoruk, M.N. et al. (2006) RNAi-mediated gene silencing in non-human primates. *Nature* **441**, 111–114
81 Kulkarni, J.A., Myhre, J.L., Chen, S., Tam, Y.Y.C., Danescu, A., Richman, J.M. et al. (2016) Design of lipid nanoparticles for *in vitro* and *in vivo* delivery of plasmid DNA. *Nanomed. Nanotech. Biol. Med.*, doi:10.1016/j.nano.2016.12.014
82 Manosroi, A., Thathang, K., Manosroi, J., Werner, R.G., Schubert, R. and Peschka-Süss, R. (2009) Expression of luciferase plasmid (pCMVLuc) entrapped in DPPC/cholesterol/DDAB liposomes in HeLa cell lines. *J. Liposome Res.* **19**, 131–140
83 Clancy, J.P., Dupont, L., Konstan, M.W., Billings, J., Fustik, S., Goss, C.H. et al. (2013) Phase II studies of nebulised Arikace in CF patients with *Pseudomonas aeruginosa* infection. *Thorax* **68**, 818–825
84 Omri, A., Suntres, Z.E. and Shek, P.N. (2002) Enhanced activity of liposomal polymyxin B against *Pseudomonas aeruginosa* in a rat model of lung infection. *Biochem. Pharmacol.* **64**, 1407–1413
85 Alipour, M., Halwani, M., Omri, A. and Suntres, Z.E. (2008) Antimicrobial effectiveness of liposomal polymyxin B against resistant Gram-negative bacterial strains. *Int. J. Pharm.* **355**, 293–298
86 He, J., Abdelraouf, K., Ledesma, K.R., Chow, D.S.-L. and Tam, V.H. (2013) Pharmacokinetics and efficacy of liposomal polymyxin B in a murine pneumonia model. *Int. J. Antimicrob. Agents* **42**, 559–564
87 Cipolla, D., Blanchard, J. and Gonda, I. (2016) Development of liposomal ciprofloxacin to treat lung infections. *Pharmaceutics* **8**, doi:10.3390/pharmaceutics8010006
88 Legendre, J.Y. and Szoka, F.C. (1993) Cyclic amphipathic peptide-DNA complexes mediate high-efficiency transfection of adherent mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 893–897
89 Kichler, A., Bechinger, B. and Danos, O. (2003) Antimicrobial peptides as efficient DNA vectors. *Méd. Sci.* **19**, 1046–1047
90 Kichler, A., Leborgne, C., Savage, P.B. and Danos, O. (2005) Cationic steroid antibiotics demonstrate DNA delivery properties. *J. Control. Release* **107**, 174–182
91 Kichler, A., Leborgne, C., März, J., Danos, O. and Bechinger, B. (2003) Histidine-rich amphipathic peptide antibiotics promote efficient delivery of DNA into mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1564–1568
92 Walter, F., Vicens, Q. and Westhof, E. (1999) Aminoglycoside-RNA interactions. *Curr. Opin. Chem. Biol.* **3**, 694–704
93 Belmont, P., Aissaoui, A., Hauchecorne, M., Oudrhiri, N., Petit, L., Vigneron, J.-P. et al. (2002) Aminoglycoside-derived cationic lipids as efficient vectors for gene transfection *in vitro* and *in vivo*. *J. Gene Med.* **4**, 517–526

94 Sainlos, M., Hauchecorne, M., Oudrhiri, N., Zertal-Zidani, S., Aissaoui, A., Vigneron, J.-P. et al. (2005) Kanamycin A-derived cationic lipids as vectors for gene transfection. *Chembiochem* **6**, 1023–1033
95 Le Gall, T., Baussanne, I., Halder, S., Carmoy, N., Montier, T., Lehn, P. et al. (2009) Synthesis and transfection properties of a series of lipidic neamine derivatives. *Bioconjug. Chem.* **20**, 2032–2046
96 Desigaux, L., Sainlos, M., Lambert, O., Chevre, R., Letrou-Bonneval, E., Vigneron, J.-P. et al. (2007) Self-assembled lamellar complexes of siRNA with lipidic aminoglycoside derivatives promote efficient siRNA delivery and interference. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 16534–16539
97 Mével, M., Sainlos, M., Chatin, B., Oudrhiri, N., Hauchecorne, M., Lambert, O. et al. (2012) Paromomycin and neomycin B derived cationic lipids: synthesis and transfection studies. *J. Control. Release* **158**, 461–469
98 Baussanne, I., Bussière, A., Halder, S., Ganem-Elbaz, C., Ouberai, M., Riou, M. et al. (2010) Synthesis and antimicrobial evaluation of amphiphilic neamine derivatives. *J. Med. Chem.* **53**, 119–127
99 Bera, S., Zhanel, G.G. and Schweizer, F. (2010) Antibacterial activity of guanidinylated neomycin B- and kanamycin A-derived amphiphilic lipid conjugates. *J. Antimicrob. Chemother.* **65**, 1224–1227
100 Ouberai, M., El Garch, F., Bussiere, A., Riou, M., Alsteens, D., Lins, L. et al. (2011) The *Pseudomonas aeruginosa* membranes: a target for a new amphiphilic aminoglycoside derivative? *Biochim. Biophys. Acta* **1808**, 1716–1727
101 Sautrey, G., Zimmermann, L., Deleu, M., Delbar, A., Souza Machado, L., Jeannot, K. et al. (2014) New amphiphilic neamine derivatives active against resistant *Pseudomonas aeruginosa* and their interactions with lipopolysaccharides. *Antimicrob. Agents Chemother.* **58**, 4420–4430
102 Wu, G.-H. and Hsu, S.-H. (2016) Synthesis of water-based cationic polyurethane for antibacterial and gene delivery applications. *Colloids Surf. B Biointerfaces* **146**, 825–832
103 Choi, Y.H., Liu, F., Kim, J.-S., Choi, Y.K., Park, J.S. and Kim, S.W. (1998) Polyethylene glycol-grafted poly-l-lysine as polymeric gene carrier. *J. Control. Release* **54**, 39–48
104 Fajac, I., Allo, J.C., Souil, E., Merten, M., Pichon, C., Figarella, C. et al. (2000) Histidylated polylysine as a synthetic vector for gene transfer into immortalized cystic fibrosis airway surface and airway gland serous cells. *J. Gene Med.* **2**, 368–378
105 Dubois, A.V., Midoux, P., Gras, D., Si-Tahar, M., Bréa, D., Attucci, S. et al. (2013) Poly-L-Lysine compacts DNA, kills bacteria, and improves protease inhibition in cystic fibrosis sputum. *Am. J. Respir. Crit. Care Med.* **188**, 703–709
106 Shima, S., Matsuoka, H., Iwamoto, T. and Sakai, H. (1984) Antimicrobial action of epsilon-poly-L-lysine. *J. Antibiot. (Tokyo)* **37**, 1449–1455
107 Bertrand, E., Gonçalves, C., Billiet, L., Gomez, J.P., Pichon, C., Cheradame, H. et al. (2011) Histidinylated linear PEI: a new efficient non-toxic polymer for gene transfer. *Chem. Commun. Camb. Engl.* **47**, 12547–12549
108 Shi, B., Zheng, M., Tao, W., Chung, R., Jin, D., Ghaffari, D. et al. (2017) Challenges in DNA delivery and recent advances in multifunctional polymeric DNA delivery systems. *Biomacromolecules*, doi:10.1021/acs.biomac.7b00803
109 Davies, L.A., McLachlan, G., Sumner-Jones, S.G., Ferguson, D., Baker, A., Tennant, P. et al. (2008) Enhanced lung gene expression after aerosol delivery of concentrated pDNA/PEI complexes. *Mol. Ther.* **16**, 1283–1290
110 Davies, L.A., Hyde, S.C., Nunez-Alonso, G., Bazzani, R.P., Harding-Smith, R., Pringle, I.A. et al. (2012) The use of CpG-free plasmids to mediate persistent gene expression following repeated aerosol delivery of pDNA/PEI complexes. *Biomaterials* **33**, 5618–5627
111 Ma, Z. and Sun, W. (2014) The effect of aerosol polyethylenimine/interferon-γ plasmid complexes on expression of inflammatory cytokines in mouse lung. *J. Aerosol. Med. Pulm. Drug Deliv.* **27**, 117–124
112 Kolte, A., Patil, S., Lesimple, P., Hanrahan, J.W. and Misra, A. (2017) PEGylated composite nanoparticles of PLGA and polyethylenimine for safe and efficient delivery of pDNA to lungs. *Int. J. Pharm.* **524**, 382–396
113 Azevedo, M.M., Ramalho, P., Silva, A.P., Teixeira-Santos, R., Pina-Vaz, C. and Rodrigues, A.G. (2014) Polyethyleneimine and polyethyleneimine-based nanoparticles: novel bacterial and yeast biofilm inhibitors. *J. Med. Microbiol.* **63**, 1167–1173
114 Barros, J., Dias, A., Rodrigues, M.A., Pina-Vaz, C., Lopes, M.A. and Pina-Vaz, I. (2015) Antibiofilm and antimicrobial activity of polyethylenimine: an interesting compound for endodontic treatment. *J. Contemp. Dent. Pract.* **16**, 427–432
115 Randazzo, R.A.S., Bucki, R., Janmey, P.A. and Diamond, S.L. (2009) A series of cationic sterol lipids with gene transfer and bactericidal activity. *Bioorg. Med. Chem.* **17**, 3257–3265
116 Myint, M., Bucki, R., Janmey, P.A. and Diamond, S.L. (2015) Synthesis and structure–activity relationships of novel cationic lipids with anti-inflammatory and antimicrobial activities. *Bioorg. Med. Chem. Lett.* **25**, 2837–2843
117 Hsu, W.-L., Chen, H.-L., Liou, W., Lin, H.-K. and Liu, W.-L. (2005) Mesomorphic complexes of DNA with the mixtures of a cationic surfactant and a neutral lipid. *Langmuir* **21**, 9426–9431
118 Silva, J.P.N., Oliveira, A.C.N., Gomes, A.C. and Oliveira, M.E.C.D.R. (2012) Development of dioctadecyldimethylammonium bromide/monoolein liposomes for gene delivery. http://www.intechopen.com/books/cell-interaction/development-of-dioctadecyldimethylammonium-bromide-monoolein-liposomes-for-gene-delivery
119 Ahlström, B., Chelminska-Bertilsson, M., Thompson, R.A. and Edebo, L. (1997) Submicellar complexes may initiate the fungicidal effects of cationic amphiphilic compounds on *Candida albicans*. *Antimicrob. Agents Chemother.* **41**, 544–550
120 Martins, L.M.S., Mamizuka, E.M. and Carmona-Ribeiro, A.M. (1997) Cationic vesicles as bactericides. *Langmuir* **13**, 5583–5587
121 Campanhã, M.T., Mamizuka, E.M. and Carmona-Ribeiro, A.M. (1999) Interactions between cationic liposomes and bacteria: the physical-chemistry of the bactericidal action. *J. Lipid Res.* **40**, 1495–1500
122 Kirby, A.J., Camilleri, P., Engberts, J.B.F.N., Feiters, M.C., Nolte, R.J.M., Söderman, O. et al. (2003) Gemini surfactants: new synthetic vectors for gene transfection. *Angew. Chem. Int. Ed. Engl.* **42**, 1448–1457
123 Ahmed, T., Kamel, A.O. and Wettig, S.D. (2016) Interactions between DNA and Gemini surfactant: impact on gene therapy: part I. *Nanomedicine (London)* **11**, 289–306

124 Obląk, E., Piecuch, A., Guz-Regner, K. and Dworniczek, E. (2014) Antibacterial activity of gemini quaternary ammonium salts. *FEMS Microbiol. Lett.* **350**, 190–198
125 Berchel, M., Gall, T.L., Denis, C., Hir, S.L., Quentel, F., Elléouet, C. et al. (2011) A silver-based metal–organic framework material as a "reservoir" of bactericidal metal ions. *New J. Chem.* **35**, 1000–1003
126 Peng, L.-H., Huang, Y.-F., Zhang, C.-Z., Niu, J., Chen, Y., Chu, Y. et al. (2016) Integration of antimicrobial peptides with gold nanoparticles as unique non-viral vectors for gene delivery to mesenchymal stem cells with antibacterial activity. *Biomaterials* **103**, 137–149
127 Ghosh, P., Han, G., De, M., Kim, C.K. and Rotello, V.M. (2008) Gold nanoparticles in delivery applications. *Adv. Drug Deliv. Rev.* **60**, 1307–1315
128 Lima, E., Guerra, R., Lara, V. and Guzmán, A. (2013) Gold nanoparticles as efficient antimicrobial agents for *Escherichia coli* and *Salmonella typhi*. *Chem. Cent. J.* **7**, 11
129 Shamaila, S., Zafar, N., Riaz, S., Sharif, R., Nazir, J. and Naseem, S. (2016) Gold nanoparticles: an efficient antimicrobial agent against enteric bacterial human pathogen. *Nanomaterials* **6**, 71
130 Li, X., Obeidat, M., Zhou, G., Leung, J.M., Tashkin, D., Wise, R. et al. (2017) Responsiveness to ipratropium bromide in male and female patients with mild to moderate chronic obstructive pulmonary disease. *EBioMedicine* **19**, 139–145
131 Bjermer, L., Gauvreau, G.M., Postma, D.S., O'Byrne, P.M., van den Berge, M., Boulet, L.-P. et al. (2017) Methacholine challenge tests to demonstrate therapeutic equivalence of terbutaline sulfate via different Turbuhaler(®) devices in patients with mild to moderate asthma: appraisal of a four-way crossover design. *Pulm. Pharmacol. Ther.* **44**, 1–6
132 Kerwin, E.M., Ferro, T.J., Ariely, R., Irwin, D.E. and Parikh, R. (2017) Real-world health care utilization in asthma patients using albuterol sulfate inhalation aerosol (ProAir (®) HFA) with and without integrated dose counters. *J. Asthma Allergy* **10**, 171–179
133 Lewis, A., Torvinen, S., Dekhuijzen, P.N.R., Chrystyn, H., Melani, A., Zöllner, Y. et al. (2017) Budesonide + formoterol delivered via Spiromax(®) for the management of asthma and COPD: The potential impact on unscheduled healthcare costs of improving inhalation technique compared with Turbuhaler(®). *Respir. Med.* **129**, 179–188
134 Profita, M., Riccobono, L., Bonanno, A., Chanez, P., Gagliardo, R., Montalbano, A.M. et al. (2013) Effect of nebulized beclomethasone on airway inflammation and clinical status of children with allergic asthma and rhinitis: a randomized, double-blind, placebo-controlled study. *Int. Arch. Allergy Immunol.* **161**, 53–64
135 Wang, X., Koehne-Voss, S., Anumolu, S.S. and Yu, J. (2017) Population pharmacokinetics of tobramycin inhalation solution in pediatric patients with cystic fibrosis. *J. Pharm. Sci.*, doi:10.1016/j.xphs.2017.06.010
136 Greenwood, J., Schwarz, C., Sommerwerck, U., Nash, E.F., Tamm, M., Cao, W. et al. (2017) Ease of use of tobramycin inhalation powder compared with nebulized tobramycin and colistimethate sodium: a crossover study in cystic fibrosis patients with pulmonary *Pseudomonas aeruginosa* infection. *Ther. Adv. Respir. Dis.* **11**, 249–260
137 Heirali, A.A., Workentine, M.L., Acosta, N., Poonja, A., Storey, D.G., Somayaji, R. et al. (2017) The effects of inhaled aztreonam on the cystic fibrosis lung microbiome. *Microbiome* **5**, 51
138 Audag, N., Liistro, G., Van der Linden, D., Smets, F., Leal, T. and Reychler, G. (2017) *In vitro* and *in vivo* comparison of two nebulizers used for inhaled pentamidine delivery. *Arch. Bronconeumol.*, doi:10.1016/j.arbres.2017.05.001
139 Tarrant, B.J., Le Maitre, C., Romero, L., Steward, R., Button, B.M., Thompson, B.R. et al. (2017) Mucoactive agents for chronic, non-cystic fibrosis lung disease: a systematic review and meta-analysis. *Respirol. Carlton. Vic.* **22**, 1084–1092
140 Theodoraki, K., Thanopoulos, A., Rellia, P., Leontiadis, E., Zarkalis, D., Perreas, K. et al. (2017) A retrospective comparison of inhaled milrinone and iloprost in post-bypass pulmonary hypertension. *Heart Vessels*, doi:10.1007/s00380-017-1023-2
141 Netzer, N.C., Küpper, T., Voss, H.W. and Eliasson, A.H. (2012) The actual role of sodium cromoglycate in the treatment of asthma–a critical review. *Sleep Breath.* **16**, 1027–1032
142 Slaton, R.M., Thomas, R.H. and Mbathi, J.W. (2013) Evidence for therapeutic uses of nebulized lidocaine in the treatment of intractable cough and asthma. *Ann. Pharmacother.* **47**, 578–585
143 Yin, H., Kanasty, R.L., Eltoukhy, A.A., Vegas, A.J., Dorkin, J.R. and Anderson, D.G. (2014) Non-viral vectors for gene-based therapy. *Nat. Rev. Genet.* **15**, 541–555