

Targeted drug delivery to the virus-infected airway; complications and remedies.

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Abstract

Drug delivery to the airway and lower respiratory tract by aerosol inhalation has become a successful, non-invasive method of preventing and treating local disease of the lung. Consequently, it has been a promising route for clinical trials using highly specific and novel therapies to overcome viral pulmonary infection such as RNA interference, neutralising monoclonal antibodies and microparticle treatments. Yet despite this great potential, this form of delivery has proven somewhat ineffective due to airway remodeling, inflammation and mucus hypersecretion that results from viral symptoms in the respiratory tract. Here we review the research into the delivery technologies available as well as the types of therapeutics used for respiratory virus disease and examine how virus infection-induced airway inflammation modulates its success. We discuss the future of aerosol administration and present potential alternative methods for efficient drug delivery so as to improve post-infection virus control therapies.

Introduction

Growing attention has been given to the potential of the pulmonary route as a non-invasive means of delivery of therapeutic agents ever since the invention of liquid nebulisers around the turn of the 19th century. From the notions of what once were ancient remedies to treat disease, such as inhaling burning leaves or the vapours of aromatic plants, came the first legitimate pharmaceutical therapies. In 1925, inhaled insulin began to be administered in experimental studies of diabetes [1], and in the 1940s, an inhaled formulation of epinephrine was marketed with hand-bulb nebulisers for the treatment of asthma [2]. This was further refined in 1955 when the president of Riker Labs developed a pressurised metered dose inhaler (MDI) at the suggestion of his asthmatic daughter who noticed the mechanism behind perfume spray devices [3]. More recently, advances in molecule design, drug discovery and delivery technologies have facilitated the expansion of various systems, formulations and devices to

administer drugs both locally for the treatment of diseases of the respiratory tract as well as systemically via the lung.

The respiratory system is susceptible to a range of diverse diseases such as asthma, cystic fibrosis, emphysema, pulmonary arterial hypertension and chronic obstructive pulmonary disease. Additionally, due to its location and physiological function, the pulmonary system is the primary site of entry and establishment of many airborne pathogens, particularly viruses. Six viruses are the main causative agents of localised respiratory illness: rhinovirus, respiratory syncytial virus (RSV), influenza virus, parainfluenza virus (PIV), corona virus, and adenovirus. Infections with these respiratory viruses not only cause symptomatic disease, but can also exacerbate pre-existing conditions such as bronchial asthma [4, 5]. The human papillomavirus has also been shown to infect the mucosal surfaces of the upper respiratory tract causing respiratory papillomatosis [6], which in rare cases can lead to airway obstruction [7] or progress to head and neck carcinomas [8]. Furthermore, viruses such as enterovirus, measles and hanta virus are known to cause disseminated disease following entry via the lung. Consequently, these are all prime targets for treatment with pulmonary delivery systems. Despite a number of recent advances showing great promise to treat these viral infections, the pulmonary delivery of peptides and proteins can be impeded by the complexity of the anatomic structure of the human respiratory system. This is further complicated in the diseased state as delivery sites can be obscured by excess mucus secretion in the upper respiratory tract and tissue remodeling from an anti-viral inflammatory response. In this review we will outline the current technologies and molecules used to overcome viral disease, examine the delivery routes and barriers encountered with these therapies during viral infection and offer potential alternatives for efficient drug delivery.

Delivery technologies

The selection of delivery device plays an important role in the formulation

design and overall success of the system. As the required particle/droplet size depends on the specific target location within the respiratory tract, the selected device must be capable of generating and delivering stable compounds of the desired aerosolised particle diameter. The devices most commonly used for respiratory delivery are shown in figure 1 and include nebulisers, aerosolisers, metered dose inhalers (MDIs) and dry powder inhalers (DPIs).

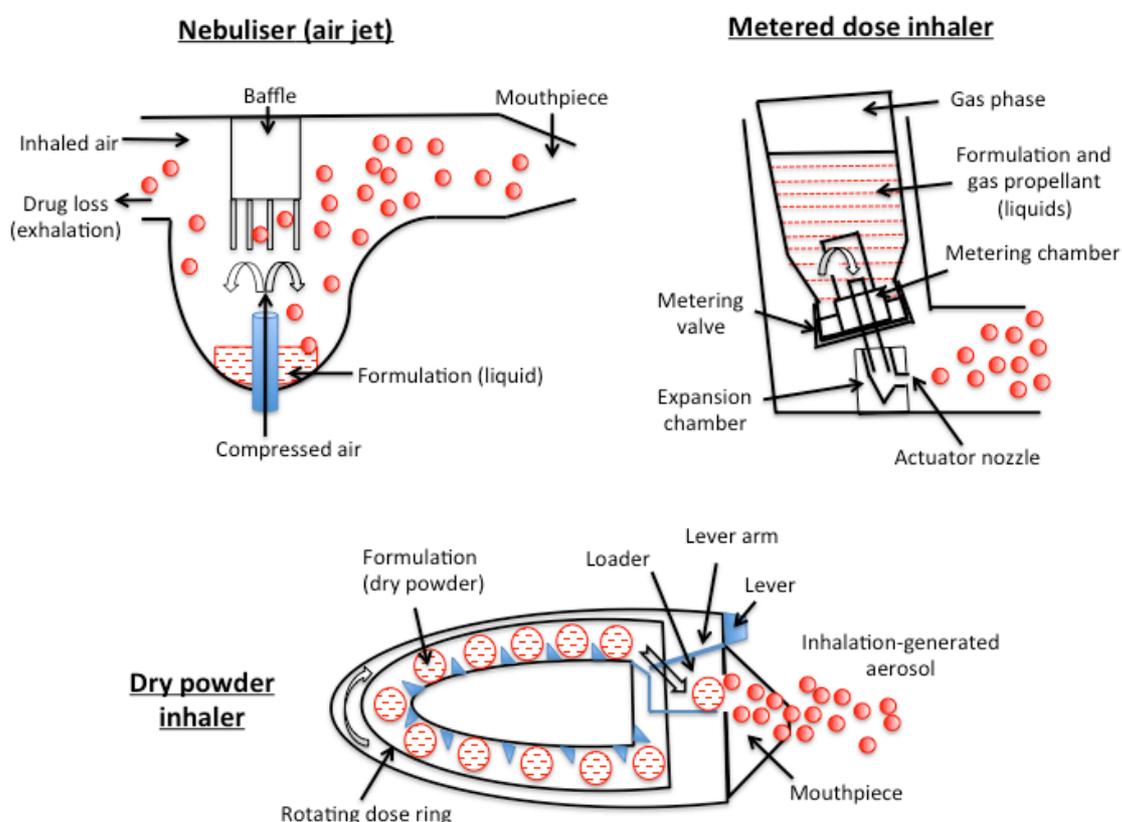


Figure 1. Mechanism of action of various drug delivery technologies used to deliver compounds to the respiratory tract. Pulmonary drugs can be administered using devices that rely on compressed air (nebuliser), propellant (MDIs), or inhalation (DPIs) for delivery.

The majority of anti-viral therapy studies to date have often used conventional jet nebulisers to generate small particle aerosols in the hope of delivering drug compounds directly to the site of viral replication and limit systemic exposure.

One of the first ever anti-viral drugs discovered, ribavirin (or Virazole), was approved in 1986 for the treatment of severe RSV infections by nebuliser delivery [9]. These devices administer atomised, aqueous drug solution by air jet or ultrasonic mechanisms at a continuous rate over multiple inhalations and are often used for critical or ambulatory care. There are several types of nebulisers available such as jet nebulisers that are driven by compressed air, ultrasonic nebulisers that use a transducer to create droplets from a liquid reservoir, or vibrating mesh nebulisers that vibrate to generate fine particles. Similarly, alternative aerosolisers may use propellant to quickly and efficiently administer drug product concentrate directly via spray, often in a manner that has reduced irritation when compared to mechanical application. They have proven successful in delivering a number of therapeutic compounds to the respiratory tract with good efficacy and appear to be well tolerated. Compliance to this form of treatment is usually easily achieved and the direct delivery is especially important to reduce viral load in immunocompromised patients or those with viruses resistant to other forms of therapy such as oral oseltamivir in the case of influenza [10]. Continual development is underway on alternative aerosol delivery devices and this could lead to better distribution, mucous penetration and overall uptake efficiency in pulmonary epithelial cells.

Another device that produces an aerosol from liquid drug formulations is the MDI. These are comparable to nebulisers, but instead expel exact measured amounts of preformed medication mixed with propellant upon activation. This allows for the delivery of accurate doses of the compound, with minimal wastage, from a convenient and portable tool. Due to the ease of self-administration, adherence to treatment regimes can be less than ideal, however monitoring accessories are continually being developed to improve outcomes, such as the SmartTrack device [11]. In the past, MDIs have had more of a role in the treatment of asthma or chronic obstructive pulmonary disease as anti-viral treatments have favoured the use of nebulisers. Consequently, use of these devices tends to have been restricted to testing post-virus treatments, such as administering corticosteroids following RSV infection to improve recurrent wheeze [12]. Recently, MDIs have been instrumental in the development of

combination therapies where identical deposition patterns of separate compounds can be achieved from the one dose [13]. Therefore they may prove useful in delivering anti-viral compounds and steroidal drugs concurrently to ameliorate disease outcomes in the future.

Lastly, the DPI devices have been the most popular instrument used to deliver drugs, particularly proteins to the lungs. These devices use micronised medication blended with carrier (but no propellant) and requires the patient to inhale the powder for efficient delivery. The way some blends behave and the interaction of powders with the device have been unclear in the past and computational fluid dynamics have helped to improve design and development of DPI devices. One such study demonstrated how device retention and overall inhaler performance could be improved by altering grid structure and mouthpiece length [14]. These types of improvements have led to a number of successful handheld tools, such as the Diskhaler, which has been widely used to deliver the influenza drug zanamivir (or Relenza). Using this device has demonstrated significant reductions in the median time to alleviate major influenza symptoms by up to 2.5 days compared with placebo in phase III trials [15] and are likely to be used for future anti-viral therapies.

Therapeutic molecules

While a great deal of recent research has focused on the development of novel delivery devices, growing efforts are being dedicated to the various formulations of macromolecular drugs. As the pulmonary membrane is naturally permeable to small molecules, peptides and proteins, many of the first studies attempted direct delivery of naked oligonucleotides or proteins into the respiratory tract. These compounds are thought to be absorbed rapidly into the body as drug efflux transporters and metabolising enzymes are present at low levels, especially when compared to other sites such as the gastrointestinal tract. However, in the case of naked oligonucleotides such as short-interfering RNA (siRNA), the bio-distribution throughout the lung and the uptake mechanism

remains unclear. The negative charge and size of these molecules would suggest that they are not able to bind to the cell surface or cross the cell membrane via passive mechanisms and may explain in part why naked siRNA has not been particularly effective in early clinical trials for RSV [16]. Consequently, effective delivery has been investigated using carrier molecules or vectors to transport drug cargo to the cell (figure 2).

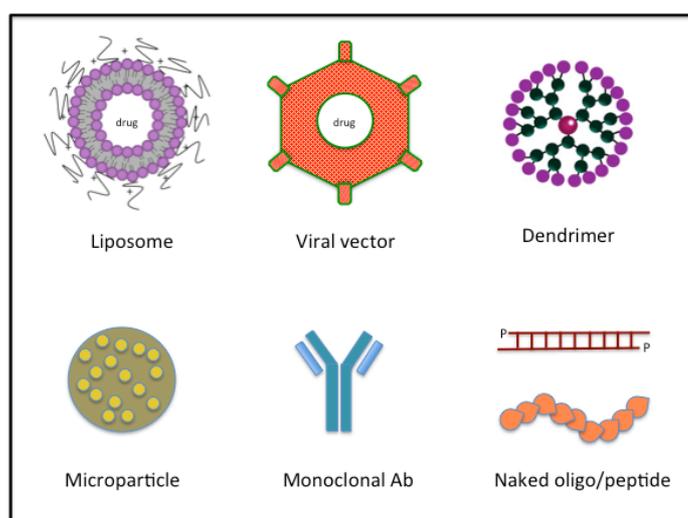


Figure 2. Different therapeutic compounds used for delivery to the respiratory tract. Pulmonary drugs can range from naked nucleic acids and peptides to conjugated carrier particles or viral vectors.

Vaccination therapies have been important in viral prophylaxis and a number of research groups have delivered live attenuated virus to generate viral-specific immunity [17-20]. This idea has been adapted to use viral vectors such as adenoviruses and lentiviruses to deliver therapeutic small RNAs into the respiratory system to treat inflammatory disease [21]. Despite some success due to efficient delivery via viral vectors to target cells in the lung, their immunogenicity limits their broad or repeated use. Innate immune responses to viruses not only impede gene delivery but can also cause severe complications for the patient. In light of these concerns, non-viral delivery has emerged as a promising alternative for the delivery of therapeutic effectors.

The success of drug delivery can be reduced by enzymes in the lung that diminish overall bioavailability, and by the barrier between cells and alveolar air.

Therefore absorption enhancers and carrier vectors have been examined to increase the pulmonary absorption of macromolecular or poorly absorbable drugs [22]. By exploiting their inherent properties, such as hydrophilicity, lipophilicity or net negative charge, these compounds can be complexed to positively charged lipids, fusion proteins or polymers to enhance interactions with the cell surface. Examples of these carrier systems include liposomes, microparticles and dendrimers.

Liposomes are small vesicles consisting of an aqueous core surrounded by one or more natural phospholipids that form a closed bilayer. They can act as carriers for both lipophilic and hydrophilic drugs and have a high degree of biocompatibility and diversity. These structures became a popular tool following the observation that complexing compounds with cationic lipid could improve delivery to cells. Tompkins and colleagues demonstrated protection from lethal infection with influenza virus in mice by transporting unmodified siRNA specific to influenza nucleoprotein and polymerase genes using the cationic lipid Oligofectamine [23]. A similar outcome was achieved against RSV by using siRNAs targeting the phosphoprotein gene that were linked to a commercial transfection lipid [24]. The study also showed an improved outcome over using naked siRNA alone, with pulmonary viral titres being reduced only by 70% of the complexed siRNA treatment in mice.

These results have led to a number of further studies including liposomal delivery of peptides, oligonucleotides, chemicals and anti-viral drugs. Vaccine approaches have been developed using liposomes to deliver short, highly conserved influenza-derived peptides to the respiratory tract to elicit potent innate and selective adaptive immune responses [25]. Oligonucleotides such as viral-specific siRNAs [26] and double-stranded RNA immune modulators [27] have been packaged into liposome carriers to be efficiently delivered to cells in order to protect against lethal challenge with influenza virus either by RNA interference or stimulation of innate immunity. The influenza drug oseltamivir (or TamiFlu), which requires large oral doses and is associated with anti-viral resistance, has been investigated for the development of dry powder liposome

formulations for intranasal administration, offering the opportunity to reduce dosing frequency [28]. Liposomal-encapsulated chemicals have also been used as a tool to examine the role of immune cells in viral respiratory disease. As it became apparent that alveolar macrophages played a pivotal role in inflammation and immunostimulation, the intranasal inoculation of clodronate complexed within liposomes has been used to deplete these cells and examine the effects. Mice treated this way before immunisation against RSV showed markedly reduced interstitial inflammation, but also developed lower levels of serum RSV-neutralising antibody and impaired protection against RSV challenge [29]. Alternatively, this treatment prior to infection with the severe acute respiratory syndrome coronavirus (SARS-CoV) showed that severe disease could be overcome as the alveolar macrophages had an inhibitory effect, preventing the development of immune responses [30]. Liposomal delivery this way has allowed localised examination without affecting the level of circulating macrophages and could help to tease out essential mechanisms involved in disease.

Another carrier particle similar to liposomes is micro- or nano-particles. These are hollow, spherical carriers that range from 0.1 to 500 microns in size and instead of lipids, usually consist of a polysaccharide outer layer such as chitosan, which is mucoadhesive, biodegradable, biocompatible and proven safe for human use [31]. Zhang and colleagues demonstrated its application by using the nanochitosan polymer NG042 to deliver a siRNA plasmid targeting the NS1 gene of RSV into the lungs and inhibit infection [32]. It also proved effective in reducing virus titres in the lung and prevented inflammation associated with infection in rats [33].

Another potential carrier used to transport therapeutic compounds is dendrimers. These are hyperbranched structures used to encapsulate peptides or saccharides and tend to be 10 to 100 nanometres in diameter. They have been shown to be efficient as gene delivery vectors [34], with polyamidoamine dendrimer-complexed plasmid DNA producing significant levels of gene expression in the murine lung [35]. Previous work has shown that intranasal

administration of dendrimers carrying sialic acid was able to prevent adhesion of various influenza subtypes in a murine influenza pneumonitis model and inhibit infection [36]. More recently, there have been few examples of this technology used in the treatment of viruses. However, higher generation dendrimers have been shown to be highly biocompatible *in vivo* and more efficient at transfecting cultured cells [37] and so this could lead to effective delivery in the future.

Other than viral vectors and non-viral carrier systems, humanised antibodies have become prevalent as an anti-viral therapy, either alone or in combination with other compounds. However, so far they have only been administered by intramuscular or intravenous injection and have not been adequately tested via inhalation. Prevention of RSV infection was first observed from a prophylactic polyclonal immune globulin and this allowed for the development of palivizumab, a neutralising monoclonal antibody directed against the fusion protein of RSV [38]. It was first seen to produce a 99% reduction of lung RSV titres and has been used clinically to treat serious RSV-related respiratory disease, especially in high-risk infants. After extensive use, it was discovered that a small number of patients were not adequately protected and this has given rise to motavizumab, an affinity-optimized version of palivizumab with improved protection against RSV *in vivo* [39, 40]. The enhanced ability of motavizumab to prevent RSV infection is being evaluated in multiple human clinical trials, but to date, a single intravenous dose of the antibody does not appear to decrease viral load or severity of illness following RSV infection [41].

Neutralising antibodies have also been investigated for the treatment of influenza but due to the hypervariability in the amino-acid sequence of its viral surface antigens, efficacy can be quite low, especially among different groups and subtypes. An antibody isolated many years ago from a mouse immunised with the H2N2 virus was found to cross-neutralise a number of H subtypes [42] yet little progress has been made until recently. In 2011, the passive transfer of a monoclonal antibody selected from plasma cells was shown to recognise all H subtypes and neutralise group 1 and 2 influenza A viruses, conferring protection to mice and ferrets [43]. Further tests are underway for this broad-spectrum

antibody as well as more strain-specific monoclonal antibodies, such as the latest candidate that protects against highly pathogenic avian influenza subtype H7 in mice [44]. Additionally, effective prophylaxis was achieved recently using influenza-specific ovine polyclonal antibodies against homologous influenza virus challenge in a mouse model [45]. The study demonstrated full protection independent of a strong innate immune response and may provide another strategy for prophylactic intervention against influenza A virus.

Delivery Routes

When considering the pulmonary delivery of any drug, it is important to have a detailed knowledge of the healthy respiratory system, and perhaps more importantly, the various diseased states. The respiratory tract contains two main regions, both of which are susceptible to viral infection (figure 3): the conducting airways consisting of the nasal cavity, sinuses, nasopharynx, oropharynx, larynx, trachea and bronchi and the lower respiratory region which includes the bronchioles, alveolar ducts and alveolar sacs.

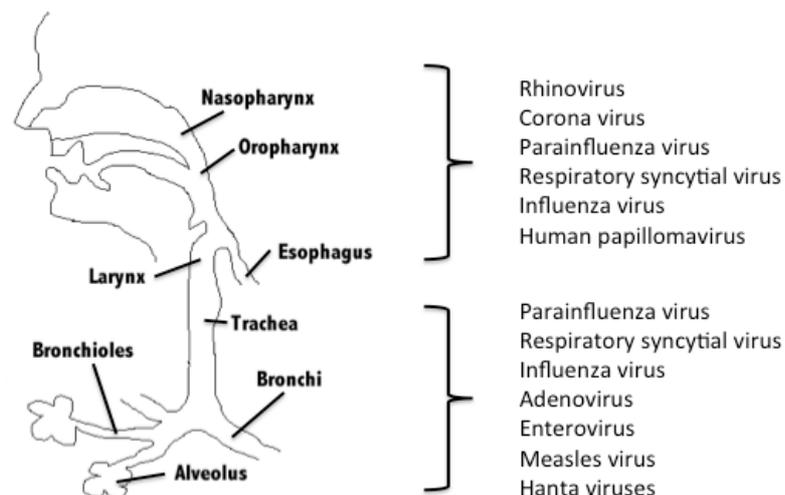


Figure 3. Sites of viral entry and consequent infection in the respiratory tract. A number of viruses enter the conducting airways and cause rhinitis, pharyngitis and laryngitis. Viruses that progress further will infect the lower respiratory tract and can result in tracheitis, bronchitis, pneumonia or disseminated disease.

Consequently, drug systems have been developed to deliver to these areas via certain entry points, each with varying success. Particular formulations have performed extremely well however, progressing through to clinical trials or approved use and these are outlined below (table 1).

Table 1. Anti-viral and vaccine formulations delivered via the respiratory tract that are currently in use or undergoing clinical trials (*).

Drug/compound	Viral Target	Route	Device	Company	Ref
Virazole (ribavirin)	RSV, others	Inhalation	Nebuliser	Valeant Pharmaceuticals	[9]
FluMist	Influenza	Intranasal	Nasal spray	AstraZeneca	[58]
Relenza (zanamir)	Influenza	Inhalation	DPI	Biota, GlaxoSmithKline	[73]
Inavir (laninamivir)	Influenza	Inhalation	DPI	Biota, Daiichi Sankyo	[74]
TMC353121*	RSV	Inhalation	Nebuliser	Johnson & Johnson	[76]
ALN-RSV01*	RSV	Inhalation	Nebuliser	Alnylam	[80]
MEDI-534*	RSV, PIV	Intranasal	Nasal spray	MedImmune LLC	[62]
Carrageenan*	General antiviral	Intranasal	Nasal spray	Marinomed	[64]

Intratracheal instillation

The main advantage of intratracheal (IT) inoculation has been that it is a direct method that delivers the complete drug dose, bypassing the defenses of the upper respiratory tract. Consequently, it has been used in a number of preclinical animal models to analyse delivery efficacy, toxicity and mechanism of action for treatments against various inflammatory diseases and cystic fibrosis [46-48]. It has also had growing interest in gene transfer studies using adenoviral or lentiviral vector systems [49, 50], though the adenoviral backbone has been shown to induce an immune response impeding effective transduction [51].

In regards to viral treatment, there has been limited success with only a handful of studies showing effective outcomes and often only when used in combination with other forms of delivery. DiNapoli and colleagues showed that administering an avian influenza virus vaccine, containing the haemagglutinin protein, by combined IT and intranasal (IN) routes could induce a high level of specific mucosal and serum neutralising antibodies in primates [20]. They later went on

to show a similar result when inoculating a human primate model with a vaccine against SARS-CoV by combined IT/IN routes [19]. When delivery by the IN route alone was attempted, protective immune responses were not elicited suggesting that the IT route may be effective at delivering further into the lower respiratory tract, though this remains unclear. Another study has demonstrated that a recombinant, attenuated measles vaccine can also elicit robust measles-specific immunity but required an IT inoculation followed by an intramuscular injection [18]. This is most likely due to the fact that the attenuated strain could not replicate efficiently in the respiratory tract following IT inoculation in macaque models [52]. Overall, it suggests that IT administration may not be an effective sole route of delivery, at least for vaccine technologies. This also seems to be the case for cationic carriers as encapsulated nucleic acids demonstrated poor delivery to the lungs when compared to intravenous delivery as seen in mice [53]. Combining this poor outcome with the discomfort and non-uniform drug distribution involved, it makes this procedure less preferable and largely impractical for human therapeutics.

Intranasal administration

Delivery by the IN route is significantly less invasive than IT administration, but is generally less effective as the compounds can be swallowed or engulfed by the nasal mucosa. Nevertheless, IN delivery of viral therapies has become an important tool, as it can easily deliver anti-virals to affected areas and it provides a safe and needle-free means of vaccination. As vaccines delivered by this route contain live-attenuated virus, they also induce long-lasting mucosal immunity relative to injectable inactivated vaccines. These types of influenza vaccine have long demonstrated an induction of local and systemic antibody production as well as humoral immunity due to the fact that local viral replication mimics the natural infection [54]. Formulations have also shown to produce equivalent serum antibody titers as those delivered by the IM route in rat models [55]. As antigenic variation is an important consideration for influenza vaccination, clinical trials have also been underway using seasonal quadrivalent and potential

pandemic strains to combat infection via the IN route [56-58]. Other vaccine candidates have been developed with this route in mind. Trials using RSV vaccine by IN delivery have shown some potential, however the required multiple doses and the negative impact of vaccine virus replication have deterred further development to date [17, 59]. Phase II clinical trials with a single dose of live-attenuated PIV have been shown to produce an immunogenic response, however there was no significant difference in the occurrence of adverse events compared to placebo in infant patients [60]. Studies have also investigated the combination of IN delivered live-attenuated RSV and PIV bivalent vaccines and have shown similar responses as monovalent therapies [61]. To overcome this, further trials using the recent MEDI-534 bivalent vaccine have been ongoing in children, though difficulties arising from variable serological responses have been observed [62, 63].

The IN route has also been investigated for the delivery of general anti-viral compounds so as to overcome the symptoms generated by a number of respiratory viruses. A prime example of this is the compound carrageenan, which has recently shown promising results against natural cold viruses in clinical trials, significantly reducing viral loads in adult patients [64]. It has also been discovered that the decongestant oxymetazoline, which is used in many intranasal sprays, may also contribute an anti-viral effect against rhinoviruses in patients [65]. While both of these compounds seem to have modest effects, their general mode of action requires multiple, continual doses and so more specialised compounds would be preferable.

Consequently, specific oligonucleotide therapeutics such as siRNA have also been trialled via the direct IN route. One of the first *in vivo* studies using siRNA directed against SARS-CoV in a rhesus macaque model showed that IN delivery of naked siRNA could reduce viral levels while also abating fever and alveoli damage [66]. Alnylam Pharmaceuticals also used a potent siRNA, termed ALN-RSV01, to target a highly conserved region of the RSV nucleocapsid gene. The siRNA was found to be well tolerated, showing limited systemic bioavailability in phase I clinical trials [67], and was then used in phase II trials to treat healthy

individuals infected with RSV. The IN administration of ALN-RSV01 resulted in the reduction of infected participants by 38%, however there was no significant reduction in viral load seen compared to placebo [16]. Other groups continue to strive for successful outcomes using *in vivo* models with IN-delivered siRNA against influenza [23], RSV [24, 68], PIV [24] and other animal viruses such as equine herpesvirus [69]. The latest concepts are also testing combination siRNA therapies such as the silencing of IL-4 expression as well as essential RSV genes to suppress virus growth and related inflammatory responses that exacerbate disease in mouse models [70].

Another specific oligonucleotide therapy demonstrating great promise against disease and viral infection is peptide-bound phosphorodiamidate morpholino oligomers (PPMOs). By interfering with viral protein expression through steric blocking of complementary RNA, PPMOs can specifically target viral proteins essential for replication. The treatment of BALB/c mice prior to RSV infection via the IN route with PPMOs targeting the RSV L gene has shown a significant reduction in viral titres of more than 2 logs in lung tissue [71]. Furthermore, pulmonary inflammation remained attenuated at day 7 post-infection, reducing the severity of disease symptoms. Further development of these compounds and determination of stability, toxicity and effective dose could lead to an effective IN therapy in the future.

Inhalation

Inhalation through aerosolisation of liquids, powders and vector compounds remains the optimal administration route for pulmonary delivery. Although more costly and hard to measure effective doses, inhalation allows more uniform distribution of compounds with greater penetration. A number of anti-virals have been developed for delivery in this way including the influenza neuraminidase inhibitors, which are the most successful drug target against seasonal and pandemic flu. These include the compounds zanamivir [72, 73] and the more recent laninamivir (Inavir), which produces long-lasting anti-viral

activity with a single inhalation dose remaining active in the respiratory tract for several days [74]. Similar anti-viral drugs have been developed for RSV but have been less successful. A prime example is the RSV fusion inhibitor RFI-641, which requires inhalation of the nebulised compound for two hours in an African green monkey model before showing a statistically significant log reduction of RSV titres in bronchoalveolar samples [75]. More recently however, the RSV fusion inhibitor 'TMC353121' has shown promising results in both preclinical mice studies [76] and cotton rat models [77] with marked reductions in virus replication and consequential lung inflammation. Following further evaluation, this small molecular inhibitor could prove to have great potential for clinical development.

Anti-viral siRNA therapies have also been administered via inhalation to try and improve the effective delivery of compounds to target areas. Conventional or older methods of aerosolisation can have high shear forces that decrease the efficiency of naked nucleotide delivery. This has generally been overcome by complexing to polyethylenimine [78], or packaging into carrier molecules like biodegradable polyester nanoparticles [79]. Alternatively, newer aerosol devices may also improve delivery via the oral inhalation route. In an attempt to achieve a better response with ALN-RSV01, Alnylam Pharmaceuticals altered their delivery device to an investigational eFlow nebulizer system (PARI Pharma) in a new, small phase IIa trial [80]. Treatment of twenty-four lung transplant patients with three doses of ALN-RSV01 this way showed that the well-tolerated regime resulted in a significant reduction of the incidence of new or progressive clinical sequelae from RSV infection after 90 days. A larger phase IIb clinical trial in lung transplant patients also suggested promising results and further studies for the compound are in discussion.

Challenges for delivery to the virus-infected airway

Due to the morphology of each region of the respiratory tract, the amount of drug delivered in each area can be quite different. While the lower respiratory

tract has a large surface area and is directly connected to the systemic circulation via the pulmonary circulation making it an ideal delivery area, factors such as the complex branched nature, the presence of alveolar macrophages and the coverage of pulmonary surfactant can pose substantial obstacles. Moreover, the major components of pulmonary surfactant, that is phospholipids, proteins and mucins, have all been shown to decrease transfection and delivery efficiencies [81, 82]. Before reaching the lungs, drug compounds must also first travel through the upper airways, overcoming mucociliary and cough clearance mechanisms. Delivery to cells in this passageway is limited due to a smaller surface area, lower regional blood flow and a high capacity to filter out foreign objects, including drugs. Transport is also inhibited as epithelial cells are coated with a mucus layer that traps inhaled substances, while ciliated cells constantly propel the mucus away from the lower respiratory tract.

During viral infection, this can be further compounded by activation of an innate cytokine response. Infection can induce mucosal edema in mice by increasing the vascular endothelial permeability through increased levels of IL-1 β , IL-6, TNF α and trypsin [83], while the viral-induced inflammasome can become activated, producing airway inflammation and mucus hyperplasia [84, 85]. Basophils and mast cells have also been reported to produce pro-inflammatory cytokines and chemical mediators such as histamine upon infection and this results in further mucus secretion and bronchoconstriction [86, 87]. This cascade of events can create an impermeable barrier to current therapies and so smarter design or alternative delivery routes may need to be considered to effectively deliver to an already infected airway.

Alternative methods for delivery

Modifications to existing compounds

As penetration of the airway mucus barrier is required for any kind of drug delivery in the viral-infected lung, closer examination into this layer is necessary.

Currently, translocation of particulate matter through the barrier is not completely understood, however particle adhesion and diffusion are key indicators of successful delivery. By atomic force microscopy and quantification of penetration velocities, Kirch and colleagues were able to investigate particle-mucus interactions and the consequent adhesion properties of some widely used carriers [88]. They discovered that differences in particle adhesion to mucus components were strongly dependent on the particle coating, with polyethylene glycol (PEG) exhibiting mucoinert properties while chitosan coatings showing a strongly increased adhesion to mucus. This would suggest that unlike polysaccharide nanoparticles, which are efficient at mucosal binding in the uninfected lung, small PEG-ylated liposomes may be more likely to avoid being trapped in the mucus layer and instead diffuse to the cells underneath. This has been further demonstrated with IN administration of PEG-coated DNA nanoparticles carrying a plasmid encoding the cystic fibrosis transmembrane conductance regulator gene that resulted in uniform airway distribution and enhanced lung delivery in mouse models [89]. Consequently, polymer composition appears to play a crucial role in the deposition pattern within the respiratory tract and these modifications may improve the penetration of anti-viral compounds.

Another alternative might be the use of pulmonary surfactant to improve viral-mediated gene transfer or inactivated viral vaccine delivery. Previous work has shown that IT instillation of therapeutic virus in rabbits can spread more effectively into distal lung tissue when combined with surfactant [90]. This was also seen more recently when recombinant adenovirus became ten-fold more effective at reducing tumours in a KRAS mutation-bearing lung cancer mouse model when administered in the presence of surfactant [91]. A further adaptation of this work has also used non-ionic surfactants in combination with mucoactive agents to improve nasal delivery of poorly absorbed hydrophilic compounds in rats [92]. Mucoactive medications include mucolytics that depolymerise polymers of mucin or DNA/actin in pus, mucokinetic agents that increase cough clearance and mucoregulatory medications, which decrease abnormal mucus (reviewed in [93]). These latter compounds especially could be

useful in reducing mucus hypersecretion, as well as stimulating a potent anti-inflammatory effect. This consequence has been suggested for rhinovirus infections, where pre-treatment with the mucoactive drug carbocysteine has shown a reduction in cytokine production in primary tracheal cell culture upon infection [94]. Combining these approaches with efficient drug carriers may then allow diffusion through the otherwise dense barrier and result in more effective delivery of therapeutic agents.

Alternate administration routes

Due to the numerous physical barriers presented upon delivery via IN, IT or local inhalation and the further obstacles faced by drastic changes in the microenvironment resulting from viral infection, other routes have been proposed as successful workarounds for efficient delivery. Oral agents have been widely used such as amantadine, rimantadine and oseltamivir for influenza, which have worked well to both prevent and treat infection [95-97]. Indeed, oseltamivir (as well as the inhaled alternative zanamivir) is still recommended by the Centre of Disease Control and Prevention for influenza treatment today. Similar trials have also followed suit and include two drugs in clinical trials: pleconaril and T-705 (or favipiravir). Pleconaril is a specific oral inhibitor of picornaviruses and has demonstrated therapeutic efficacy against community-acquired colds caused by rhinoviruses [98, 99], while T-705 is an experimental broad-spectrum RNA virus inhibitor [100]. Continuing studies on these compounds may provide further efficacious anti-virals to complement inhaled agents.

Even though the main alternative for inhaled respiratory drugs has been via the oral route, intravenous or systemic administration has also made great strides in facilitating delivery of compounds to target cells in the lung. Initially, the systemic application of naked oligonucleotides was found to be complex as rapid renal clearance, digestion by endogenous nucleases and poor uptake due to inherent qualities resulted in limited therapeutic benefit [101-103]. However

this was soon remedied by employing similar carrier molecules that were used to deliver via the respiratory tract. Development of these liposomes, polymers and nanoparticles permitted protection from serum exonucleases and excretion, but also were modified to avoid systemic toxicity and travel through the circulatory system to deposit in capillary beds of first-pass organs. As approximately 30% of all endothelial cells are found in the large surface area of the pulmonary vasculature, many delivery vectors will track to the lung and deliver any therapeutic cargo.

A number of recent studies have also shown that efficient delivery of siRNA can be achieved via intravenous inoculation of lipoplexes. McCaskill and colleagues demonstrated that targeted gene and protein knockdown could be achieved using liposome-encapsulated siRNA directed against the non-essential lamin structural gene in the murine lung [104]. Following a single intravenous dose, 45% of epithelial cells were positive for uptake showing efficient biodistribution of the therapeutic compound. Similarly, a single dose of DACC siRNA lipoplexes administered by bolus injection or by infusion was shown to sufficiently silence genes expressed in pulmonary endothelial cells such as CD31, Tie-2, VE-cadherin, or BMP-R2 [105]. Additionally, incorporation of a surface-modified multifunctional peptide into nanoparticles may further increase delivery to pulmonary epithelia by targeting sialic acid on the cell surface, analogous to the influenza virus [106]. Lastly, siRNA against avian influenza has also been shown to significantly localise to the lungs when linked to a fusion protein based on an anti-haemagglutinin antibody fragment providing evidence of targeted delivery in this manner [107].

Apart from intravenous administration, other systemic routes could be used such as intramuscular administration. However, as they are far removed from the target site, these locations may only serve as a basis for vaccine or gene therapies against viral infection. A prime example of this has shown that intramuscular administration of liposome-DNA complex adjuvant is able to enhance the cross-protective efficacy of influenza A vaccine in mice, generating anti-viral protection for over a year without waning [108]. Furthermore,

liposome-encapsulated constructs encoding for recombinant antimicrobials have been successfully used to inhibit influenza A virus replication following injection into the muscle [109]. These observations suggest that recombinant proteins and vaccines could be delivered efficiently via the muscle and provide another avenue for virus prevention and treatment.

Conclusion

There have been a number of significant developments in not only the technologies used to deliver anti-viral drugs to the respiratory tract, but also the formulations, providing biocompatible, biodegradable, non-immunogenic and non-toxic drug compounds that can be delivered efficiently to target cells of the lung. Unfortunately, due to the exacerbation and intensification of a number of physiological and immunological barriers encountered during respiratory virus infection, many superb *in vitro* or prophylactic therapies have become less effective after viral symptoms have been initiated. Further developments into diffusion and penetrative capability of carrier molecules should facilitate more efficient delivery by the respiratory route during infection, and this is likely to be complemented by clever targeting mechanisms utilised by compound therapies delivered systemically.

Conflicts of Interest

The authors confirm that this article content has no conflicts of interest.

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