International Journal of Nanomedicine

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REVIEW

Inhaled chemotherapy in lung cancer: future concept of nanomedicine

This article was published in the following Dove Press journal:
International Journal of Nanomedicine
21 March 2012
Number of times this article has been viewed

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Abstract: Regional chemotherapy was first used for lung cancer 30 years ago. Since then, new methods of drug delivery and pharmaceuticals have been investigated in vitro, and in animals and humans. An extensive review of drug delivery systems, pharmaceuticals, patient monitoring, methods of enhancing inhaled drug deposition, safety and efficacy, and also additional applications of inhaled chemotherapy and its advantages and disadvantages are presented. Regional chemotherapy to the lung parenchyma for lung cancer is feasible and efficient. Safety depends on the chemotherapy agent delivered to the lungs and is dose-dependent and time-dependent. Further evaluation is needed to provide data regarding early lung cancer stages, and whether regional chemotherapy can be used as neoadjuvant or adjuvant treatment. Finally, inhaled chemotherapy could one day be administered at home with fewer systemic adverse effects.

Keywords: inhaled chemotherapy, carriers, transducers

Introduction

Lung cancer is responsible for 23% of total cancer deaths.[1] Cancer survival tends to be poorer due to an often advanced stage at diagnosis. Only a minority of patients are eligible for curative surgical treatment. Until now, although new biomarkers have been under development for early diagnosis of lung cancer, early detection of lung cancer has not been achieved. Nonsmall cell lung cancer is the most common type of lung cancer worldwide, and various clinical studies are currently assessing new chemotherapeutic combinations.[2–4] Although targeted and tailored therapies have been introduced for patients according to individual biological tumor characteristics, overall survival rates have failed to demonstrate the expected progression-free survival or overall survival.[5,6] Moreover, acquired resistance to cytotoxic agents has been observed, mainly involving the apoptotic mechanism in nonsmall cell lung cancer cell lines.[7] In small cell lung cancer, five-year survival remains less than 10%, despite use of various drug combinations.[8,9] In addition, acquired resistance has been observed in small cell lung cancer.[10] Therefore, novel therapies are in great demand. The drug concentration reached in solid tumors is a key parameter for successful treatment and, until now, drug concentration at the tumor site has been found to be low after systemic chemotherapy.[11,12]

Nevertheless, drugs already being used for systemic administration have been successfully administered regionally in various types of cancer.[13–23] The concept of local drug delivery is proposed as a method for delivering high drug concentrations to the target site while preventing exposure of vital organs to toxic drug concentrations in the systemic circulation. In this way, systemic side effects are minimized. The respiratory

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http://dx.doi.org/10.2147/IJN.S29997

system has a large surface area, thin alveolar epithelium, rapid absorption, lack of first-pass metabolism, high bioavailability, and the capacity to absorb large quantities of drug, making it an optimal route of drug administration.[24] Aerosol therapy has also been evaluated, and is being used for several other conditions and purposes, such as diabetes mellitus, gene therapy, and vaccination.[25–29] Regarding aerosol chemotherapy administered for lung cancer, a number of drugs have been investigated in vitro, in animal models, and in human trials.[30–64] Several aspects of this treatment modality have been addressed due to the necessity for trials to be conducted, but several parameters remain to be clarified and expanded. The areas of this treatment modality that need to be properly addressed are summarized under the headings: prompt inhalation device; lung airway microenvironment; appropriate molecule-chemotherapy selection; deposition evaluation; protection measures; and disease evaluation. In the current review, these topics will be addressed based on the published literature, and all prior knowledge on the subject will be presented.

We performed an electronic search of the PubMed, Google Scholar, Medscape, and Scopus databases using combinations of the following keywords: "aerolized chemotherapy", "inhaled chemotherapy in lung cancer", "nanoparticles", "aerosol devices", "encapsulation", "inhaled doxorubicin", "inhaled carboplatin", "inhaled cisplatin", "inhaled paclitaxel", "inhaled docetaxel", and "inhaled 5-fluororacil". All types of articles (randomized controlled trials, clinical trials, observational cohort studies, review articles, case reports) were included. Selected references from identified articles were searched for further relevant papers.

Lung anatomy and microenvironment

Airway geometry and humidity

Human lungs have a large (>100 m^2), thin (0.1–0.2 μm), and highly vascular epithelial surface area for absorption. Progressive branching and narrowing of the airways encourages impaction of particles. The lung has a relative humidity of approximately 99.5%. Drug particles are known to be hygroscopic and to grow or shrink in size in high humidity. The increase in particle size above the initial size should affect the amount of drug deposited and, particularly, distribution of the aerosolized drug within the lung.[65,66] Further drug absorption could occur via the lymphatic pathway.[67,68]

Bronchial circulation

The lungs receive the entire cardiac output and represent the most richly perfused organ in the body. However, only the alveolar region is supplied by the pulmonary circulation. Blood flow to the larger airways (trachea, bronchi) is via the systemic circulation, and these airways receive approximately 1% of cardiac output.[69] The endobronchial circulation is recirculated to the peripheral airways and lung parenchyma via the bronchial veins and right atrium. Bronchial blood flow is augmented in diseases such as bronchiectasis, from 1% to as much as 30% of cardiac output.[24] Theoretically, inhaled drugs that are absorbed into the circulation from the tracheobronchial regions can be redistributed downstream and peripherally into otherwise poorly accessible areas of the lung, which may aid in drug effectiveness.[70]

Lung clearance mechanisms

Drug particles deposited in the conducting airways are largely removed by mucociliary clearance. The airway epithelial goblet cells and submucosal glands secrete mucus, forming a two-layer mucus blanket over the ciliated epithelium, ie, a low-viscosity sol layer covered by a high-viscosity gel layer. Insoluble particles are trapped in the gel layer and are moved toward the pharynx (and ultimately to the gastrointestinal tract) by the upward movement of mucus generated via metachronous beating of the cilia.

In the normal lung, the rate of mucus activity varies depending on the airway area and is determined by the number of ciliated cells and their beat frequency. For normal mucociliary clearance to occur, the airway epithelial cells and ciliary structure and activity must remain intact. Further, the depth and chemical composition of the sol layer should be optimal and, finally, the rheology of the mucus must also remain within the physiological range. Mucociliary clearance is impaired in lung diseases such as immotile cilia syndrome, bronchiectasis, cystic fibrosis, and asthma.[71] Lipophilic molecules pass easily through the airway epithelium via passive transport. Hydrophilic molecules cross via extracellular pathways and exocytosis.[72] Particles are absorbed from the submucosal region into the systemic circulation, bronchial circulation, or lymphatic system. Drugs deposited in the alveolar region may be phagocytosed and cleared by alveolar macrophages or absorbed into the pulmonary circulation. Alveolar macrophages are the predominant phagocytic cells for lung defense against inhaled microorganisms, particles, and other toxic agents. There are approximately five to seven alveolar macrophages per alveolus in the lungs of healthy nonsmokers.[73] Macrophages phagocytose insoluble particles that are deposited in the alveolar region and are either cleared by the lymphatic system or moved into the ciliated airways along currents in alveolar fluid and then cleared via

the mucociliary escalator.[74] This process can take weeks to months to complete.[75] Moreover, enzymes are still present in the lungs, so particles can be enzymatically degraded intracellularly (from alveolar macrophages) and/or extracellularly by membrane-associated proteases and peptidases (both epithelial and endothelial).[76]

Lung disease

Bronchoconstriction, inflammation, and airway narrowing alter lung deposition. Respiratory diseases, such as cystic fibrosis and bronchiectasis, change the architecture of the lung. Alterations in bifurcation angles, turbulent flow, and obstruction of the airways due to mucus accumulation modify the deposition and distribution patterns of aerosols. A decrease in the cross-sectional area of the lung caused by obstruction increases air velocities and turbulence in regions where the airflow is usually laminar. Airway obstruction diverts inspired air to unobstructed airways and, thus, remarkably little drug is deposited in obstructed areas. Often the obstructed areas are those that need to be reached in order to achieve the optimal therapeutic effect of a drug.[71,77–80] Inhaled insulin was investigated as to whether it could be administered during an exacerbation and how this situation altered the dosage. It was observed that the drug could be administered and was tolerated by patients, but close glucose monitoring was required because release of the drug into the systemic circulation was insufficiently controlled.[29]

Methods enhancing lung deposition

It has been confirmed by plethysmography that addition of 5%–7% CO_2 into the inhalation system enhances the depth of absorption and drug quantity inhaled in every breath by reducing the respiratory rate and increasing the tidal volume by 180%. The subject is forced to breathe slowly and deeply. Nevertheless, it has been observed that if the mixture is enriched with a concentration higher than 7%, adverse effects are observed, with sleepiness, confusion, and severe dizziness being the most common.[81–83]

Tumor size

Tumor size affects the distribution and deposition of the inhaled compound. In previously published studies, the mass median diameter was required to be ≤3–5 cm upon diagnosis, otherwise patients were excluded from trials.[29,40,52,53,84,85] Anticancer drugs penetrate normal tissues by both diffusion and convection,[86] with the net flow of fluid from blood vessels balanced by resorption into the lymphatic circulation. Nevertheless, tumors caused by unstructured neoangiogenesis lack functional lymphatics,[87,88] which can lead to increased levels of interstitial fluid pressure in tumors,[89–91] which in turn reduces convection and inhibits distribution of macromolecules.[92,93] It has been previously demonstrated that some physicochemical properties of drugs, ie, shape, charge, molecular weight, and aqueous solubility, determine the rate of diffusion through tissue.[86] The penetration of a drug is also dependent on its deconstruction, which functions to remove free drug, thereby inhibiting further permeation.[86] Water-soluble drugs distribute most readily in the extracellular matrix and thus diffuse efficiently around and between cells. In contrast, lipid-soluble drugs penetrate lipid membranes, and so can be transported through cells.

Physical properties of drug formulations

Physical properties that have a significant role on the particle size of the inhaled suspension are viscosity ionic strength, osmolarity, and pH. If the values for pH and osmolarity in particular are not in the normal range, bronchoconstriction, coughing, and irritation of the lung mucosa is induced.[94,95]

Drug delivery systems

Optimal particle size

The inhaled drug formulation should consist of a specific particle size, in the range of 1–3 μm, to achieve substantial alveolar deposition.[24] Inhaled molecules of this size becomes trapped in the alveoli and taken up in vesicles by alveolar epithelial cells, so that they can be carried across and released on the opposite side in the narrow interstitial fluid compartment between the epithelial cells. Molecules are then taken up within vesicles by the endothelial cells, transported across the width of these cells, and released into the alveolar capillary bloodstream. This process of particle migration into, across, and out of a cell is known as transcytosis. Other drug formulations given via inhalation, such as corticosteroids and anticholinergics, do not have to be less than 2–3 μm in size, because they act on the larger branches of the bronchial tubes.[96]

Pressurized metered dose inhalers

The pressurized metered dose inhalers (MDIs) use propellants, such as chlorofluorocarbons, which have been recently replaced by hydrofluoroalkanes.[97] The aerosol is emitted through a nozzle at a high velocity of >30 msec). Nevertheless, only 10%–20% of the aerosol emitted from pressurized MDIs is deposited on the lung parenchyma.[98] The main reasons for this can be summarized as inspiratory flow rate and lack of hand-mouth coordination,[99–101] and

the impact of high velocity particles and large particle size (50%–80%) on the oropharynx.[102] In order to maximize the effectiveness of drug absorption from a pressurized MDI, the patient has to breathe slowly by decreasing respiratory frequency and increasing tidal volume. The inhaled volume is increased and the aerosol penetrates deeply into the lung parenchyma.[103,104] To overcome the problem of actuation-inhalation, coordination breath-actuated pressurized MDIs were introduced to the market.[105] Nevertheless, improved peripheral deposition was observed if patients did not hold their breath on completion of inhalation in comparison with pressurized MDIs that are not breath-actuated.[106] In addition, different spacer tubes, valved holding chambers, and mouth piece extensions were developed to reduce deposition in the oropharynx by decreasing particle size and slowing the velocity of the aerosol, and to produce a finer aerosol of smaller mass median aerodynamic diameter.[107]

Dry powder inhalers

Dry powder inhalers were designed to overcome poor actuation-inhalation coordination. There are two basic types on the market, ie, multidose (containing multiple doses) and single-dose capsule dry powder inhalers. Several differences in lung deposition have been observed between the various dry powder inhalers. Approximately 12%–40% of the emitted dose is delivered to the lungs, and about 20%–25% is retained within the device.[108–110] The reduced drug deposition has been attributed to inefficient disaggregation of ultrafine drug particles from coarser carrier lactose particles or drug pellets. Factors affecting disaggregation are high humidity, slow inhalation flow rate, and rapid and large deviations in temperature.[111] Therefore, dry powder inhalers have to be stored in a cool, dry place. In addition, an exhalation maneuver is required before inhalation, because there is the possibility for a patient to exhale into the inhaler nozzle and disperse the dry powder. Pulmonary drug administration is enhanced for the dry powder inhalers because of fast inhalation.[112] This occurs due to different internal resistance to airflow and a range differentiation from low to high resistance.[113,114] Failure to use the device properly is a common error and therefore a dose is not delivered promptly.[115] Dry powder inhalers with high resistance provide increased deposition to the lung parenchyma,[113,116] but the clinical significance of this remains to be clarified. Finally, recent developments, principally in overcoming forced inhalation effort, have produced active dry powder inhalers. This is achieved either by adding a battery-driven propeller that aids the dispersion of the powder or by using compressed air to aerosolize the powder and convert it to an aerosol in a holding chamber where its respiration is independent of the respiratory capability of the patient. Dry powder inhalers that are currently on the market are breath-actuated and still depend on the inhalation flow rate of the patient to achieve maximum drug dose inhalation.[117]

Nebulizers

Nebulizers have been used for many years to treat various respiratory diseases. They work by inhalation through a face mask, and can be used in respiratory distress by the elderly and children younger than 2 years of age. In addition, they can deliver large quantities of solutions and suspensions as small droplets with remarkably little patient coordination required. There are certain parameters of the aerolized solution affecting their efficiency, ie, pH, viscosity, ionic strength, osmolarity, and surface tension. High drug concentration, extremely low pH, and hyperosmolarity or hypo-osmolarity reduce drug output and provoke bronchoconstriction, coughing, and irritation.[94,95] Other additional significant factors can be summarized as the design of the nebulizer chamber, primary drug fill in the reservoir cup, tapping of the nebulizer chamber during nebulization, time taken to nebulize a solution, gas flow and compressor characteristics, and residual volume.[118] Until recently, there were two basic types of nebulizers, ie, jet nebulizers and ultrasonic nebulizers. Jet nebulizers take advantage of the energy provided by compressed gas flow and distribute the liquid substance in the reservoir cup into a fine mist. Jet nebulizers are widely used, but are rather inadequate (50% loss when continuously operated and only 10% deposited to the lungs) in comparison with the newer devices described below.[119] Their performance is largely dependent on the compressor used.[118,120] Newly introduced to the market are the breath-enhanced jet nebulizers and the dosimetric nebulizers. The former delivers drug faster than the conventional jet nebulizers and the latter are breath-actuated, so generate aerosol only by inhalation. They are computer-controlled, and although they contribute by saving aerosol (up to 60%), they remain extremely expensive in comparison with conventional jet nebulizers.[121] The ultrasonic nebulizers use a piezoelectric crystal that vibrates at a high frequency (1–3 mHz) to produce a mist of liquid in the nebulizer. The higher the frequency used, the smaller the droplets produced. Ultrasonic nebulizers nebulize solutions faster than jet nebulizers, but are not suitable for suspensions. In addition, the piezoelectric crystal can heat and inactivate protein-based drugs.[122] The latest nebulizers introduced onto the market are the vibrating mesh nebulizers,

which are divided either into active or passive systems. Their advantages over the previous systems are that they are very efficient, quiet, and portable, and have an extremely low residual volume to prevent drug waste. Moreover, some models provide feedback to the patient regarding dose delivery and patient adherence. Nevertheless, there are a number of disadvantages, in that they are expensive, and need maintenance and cleaning to prevent colonization by pathogens, buildup of deposits, and blockage of the apertures. Finally, although vibrating mesh nebulizers are highly efficient overall, their performance varies according to the drug solution used and, therefore, licensing specific drugs with specific nebulizers is essential. This has no significant clinical importance when bronchodilators are delivered, given that they have a wide therapeutic index, but it is necessary when delivering drug solutions containing liposomes or proteins.[123–126] Facemasks are used for patients with acute respiratory distress and in uncooperative individuals. The facemask is not just a feature connecting the nebulizer to the patient, it also has to prevent face and ocular irritation.[127–130] A mouthpiece is also used, and new mouthpiece designs are currently on the market which enable inhalation by breath actuation, incorporate drug-saving technology, and are environment friendly, protecting medical staff from having to dispose of unwanted solutions.[29,30] Nevertheless, the mouthpiece is not indicated for acute respiratory distress conditions. Kleinstreuer et al[84,131] have presented data regarding the optimal combination of particle size, particle release position, and inhalation waveform that may deliver inhaled drug aerosols efficiently to the desired areas. Micron-sized particles follow trackable trajectories in human lung airways under steady laminar flow conditions. Therefore, the mouth inlet plays a crucial role, because it can affect the dispersion and deposition of the aerosol by backtracking.

Soft mist inhalers

Currently there is only one drug system of this kind available, which is a mechanical achievement of outstanding value. It uses the energy of a spring to force the solution through an extremely fine nozzle system.[132,133] It produces a fine aerosol with relatively high lung deposition.[134–136]

Inhaled particle carriers and strategies

Liposomes

Liposomes have a variety of properties that can be summarized as sustained release with reduced toxicity and less irritation to the lung parenchyma, the possibility to manipulate release and targeting, and improved stability.[137] The amount of drug dose carried by the liposomes, and their release rate and deposition in the lung parenchyma depends on lipid composition, size, charge, drug/lipid ratio, and method of delivery.[138–140] Liposomes are produced from phospholipids, which carry either no charge or a net negative/positive charge.[111,141] Their structure consists of an aqueous volume entrapped by a synthetic lipid single layer or bilayer with or without cholesterol. They are capable of encapsulating either hydrophilic or lipophilic formulations.[142,143] However, formulations of intermediate solubility are poorly retained by liposomes, so they are manipulated to achieve a higher degree of retention.[144] Liposomes are prepared for inhalation either in liquid or dry powder form.[145] During nebulization, an amount of the formulation is lost and hence a manipulation of the lipid composition, and size and operating conditions are necessary to minimize the loss.[146–149] The dry powder liposome formulations are produced by lyophilization followed by milling or by spray-drying.[150,151] The sustained-release capability of liposomes has been observed in several studies using a variety of drugs as aerosol treatment for the lung.[29,50,152] To enhance the sustained-release properties of liposomes further, a polymer surface coating, such as polyethylene glycol (PEG), was developed. This addition, provided a "stealth" shield to the molecule to bypass the body's defense mechanisms[144,153,154] (Table 1).

Microparticles

Microparticles are produced from naturally occurring or synthetic polymers, and their size range is between 0.1 and 500 μm. They are physically and chemically more stable than liposomes, so are capable of higher drug loading. This property makes them an ideal carrier system for proteins and peptides.[155,156] In order to encapsulate a drug, a number of factors, including heat, pH, oxygen, solvents, moisture, and mechanical stresses, must be assessed. Preparation for aerosol delivery can be undertaken using spray-drying, emulsion-solvent evaporation, phase separation, emulsion-solvent diffusion, and supercritical fluid technology.[157–163] Moreover, manipulation of the following parameters will determine drug release: concentration, size, solubility, nature of micromolecular drug, molecular weight, porosity, tortuosity, and uniformity of the polymer. A coating is added to improve the time release characteristics further, and 1,2-dipalmitoylphosphatidylcholine is also added to poly(DL-lactide-co-glycolide) microspheres to decrease uptake by macrophages.[160] When chitosan and hydroxypropylcellulose are added to the particles, their

Table 1 Efficiency enhancement mechanisms

Liposome composition (neutral or anionic lipids)
Phosphatidylcholines (lecithins)
Phosphatidylethanolamines Sphingomyelins
Phosphatidylserines
Phosphatidylglycerols
Phosphatidylinositols
Microparticles
Polylactic acid
Polylactic-co-glycolic acid
Sodium hyaluronate
Calcium phosphate-polyethylene glycol particles
Oligosaccharide derivatives
Oligosaccharide-lipid mix
Lipid-based Pulmosphere
Carbohydrates
Xylitol
Maltitol
Glucose
Sorbitol
Mannitol
Lactose
Cyclodextrins
Pegylation
Biodegradable polymers
Polylactic acid
Oligolactic acid
Mucoadhesive polymer
Bioadhesives
Lectins
Peptides
Antibodies
Heparin
Heparin sulfate
Octa-arginine
Antibodies
Cell-type specific targeting
Alveolar macrophages
Cancer cells
Epidermal growth factor
Folic acid
Low-density lipoprotein
Intracellular targeting
Intracellular trafficking
Endosomal release
Nuclear localization

time residence in the lung parenchyma is increased.[163] It has been widely agreed that the optimal geometric diameter for lung delivery is 1–3 μm, but these particles tend to aggregate[164] and are cleared by alveolar macrophages.[165] Therefore, large porous particles were developed with a geometric diameter of >5 μm, an aerodiameter of <5 μm, and a low density of <0.1 mg/mL.[166] When aerolized, large porous particles deposit homogeneously on the cell surface and, when observed by microscopy, appear nontoxic.[161] Further development of this molecule has led to "Trojan" particles,[165] which have the ability to escape both phagocytic and mucociliary clearance in the respiratory system. They are prepared from nanoparticles, which eventually assemble into a microparticle of low density (<0.1 mg/mL). These particles need to be assessed with a drug load, but published data suggest that they can be aerosolized from dry powder.[157] It has been shown previously that a single cancer cell can ingest one or multiple microparticles. The ingested microparticles are arranged in such a way as to reduce the space occupied inside the cell, and the same occurs with macrophages[44,167] (Table 1, Figure 1).

Carbohydrates

There are currently three carbohydrate formulations approved by the US Food and Drug Administration, ie, lactose (a-lactose monohydrate), glucose, and mannitol (polyol). These carriers contribute to drug flow and dispersability, and also act as stability enhancers. In a recent study, several carriers such as mannitol, sorbitol, maltitol, and xylitol, were assessed and it was concluded that mannitol is the best candidate for dry powder inhaler formulation, given that the others showed limited dispersability.[168] Techniques used to produce a respirable formulation are: supercritical fluid technology, spray-freeze drying, freeze-drying, and lyophilizing followed by milling/jet milling or spray-drying.[169–173] Moreover, it has been observed that lactose enhances the uptake of polylysine into airway cells, and this has been shown to be a method of increasing intracellular localization of proteins and peptides.[174] Two approaches have been developed to improve delivery efficiency and increase drug dispersibility and the respirable fraction. The first approach was mixing fine lactose particles (about 5 μm in diameter) with coarse lactose to improve disaggregation, as well as the fine particle fraction.[175] The second approach was to add a ternary component, such as L-leucine,

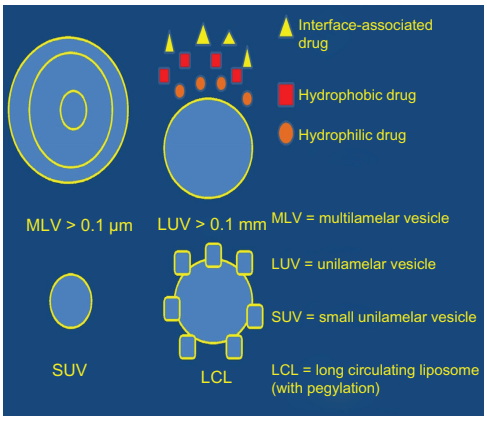

Figure 1 Encapsulation vehicles.

to the formulation.[111] Finally, cyclodextrins, which are cyclic oligosaccharides, have proven to be useful excipients in the respiratory distribution of small molecules.[176] Until recently, their use for protein/peptide delivery was limited due to the need to address penetration enhancement. However, in a recent study, the use of dimethyl-β-cyclodextrin presented increased bioavailability, with increasing concentrations of cyclodextrin[169] (Table 1).

Pegylation

PEG added to proteins enables sustained release on the site of deposition. This is achieved by bypassing the defense mechanisms of the respiratory tract, by decreasing degradation of the formulation, and prolonging the half-life in the lungs.[144,154,177] In addition, PEG has been demonstrated to be a safe carrier for inhalational agents[178] (Table 1).

Biodegradable polymers

Polylactic acid has sustained release properties, but is not suitable for pulmonary drug delivery due to its prolonged biological half-life. An oligomer of lactic acid, with a shorter half-life (6–8 days), can be used for drug delivery. The mucoadhesive polymer, hydroxypropyl cellulose, is released over approximately 24 hours and bypasses mucociliary clearance. However, the toxicity profile of hydroxypropyl cellulose has not been established[111,179] (Table 1).

Bioadhesives

Bioadhesives are used to prolong the connection between the carrier-drug and the surface cell in the airway.[180,181] A number of multivalent binding agents have been incorporated in drug-carrier systems, including lectins, peptides, antibodies, octa-arginine, heparin, heparin sulfate, and antibodies[182,183] (Table 1).

Cell targeting

Cell targeting has been the focus of increasing interest in recent years, both from the prognostic and therapeutic points of view. Gene therapy has been widely investigated in cell-selective targeting.[184] Alveolar macrophages are an attractive vehicle by which to deliver a chemotherapeutic agent to the lymph nodes through the lymphatic circulation. Liposomes and microspheres are generally engulfed by alveolar macrophages. Several receptors are overexpressed, such as epidermal growth factor and folic acid, which can be exploited to target specific cells in cancer therapy.[185,186] Low-density lipoprotein has been used for receptor assimilation[187] (Table 1).

Intracellular targeting

Intracellular targeting is an additional strategy to improve the efficiency of a drug. The general concept is to create a potent drug that would reach the proper surface area, but intracellular targeting is essential to take regional therapy a step further.[188,189] In this regard, most chemotherapy regimens interact within the reproductive cell cycle, so this targeting strategy could be further pursued.[190] There are three parameters that are investigated concerning the cell microenvironment and drug-formulation interactions, ie, intracellular trafficking, endosomal release, and nuclear localization (Table 1).

Drug transporters

ATP-binding cassette transporters

ABC transporters are a large family (50 members) of transmembrane proteins, which act as an ATP-dependent efflux system exporting molecules from the cytoplasm to the surrounding cellular environment. There are seven subfamilies, from A to G. ABC transporters prevent accumulation of xenobiotics, so they serve as a defense mechanism in lung tissue.[191] P-glycoprotein, multidrug-resistant proteins, and the breast cancer resistance protein are known to play a role in multidrug resistance, a characteristic observed during the expulsion of chemotherapeutic agents from cancer cells.[192] P-glycoprotein has been extensively studied in the lung. It decreases oral drug absorption, prevents drug entry in the central nervous system, and is responsible for many drug-drug interactions.[193] The transporter is localized based on immunohistochemistry techniques on the apical membrane of the bronchial and bronchiolar epithelium,[194–197] in the endothelial cells of the bronchial capillaries,[198] and in alveolar macrophages.[195,196] Expression of P-glycoprotein in smokers with lung disease versus people with normal lungs has not been adequately investigated. P-glycoprotein and immunohistochemical multidrug-resistant protein analyses are a useful tool for predicting a patient's response to chemotherapy.[199] In one study, mRNA levels in the lung tissue of smokers, nonsmokers, and exsmokers were not found to be significantly different.[194] Several studies have demonstrated directly or indirectly that underlying disease plays a role in regulation of the P-glycoprotein transporter. In addition, pharmaceuticals administered for lung or other disease can upregulate the P-glycoprotein transporter. In cystic fibrosis, due to changes induced by the disease, it has been observed that the P-glycoprotein transporter is upregulated.[200,201] Moreover, it has been reported that toxins released from microorganisms infecting patients with cystic fibrosis also

inhibit P-glycoprotein.[202] In patients with chronic obstructive pulmonary disease (COPD), there are no significant data indicating modification of P-glycoprotein between the disease stages,[203,204] and no relevant data exist for asthma patients. Corticosteroids administered by the inhaled, oral, and intraperitoneal routes upregulate the P-glycoprotein transporter.[205–207] A particularly good example of the importance of transporters in inhaled chemotherapy is the inhibition of P-glycoprotein by lipid nanocapsules, which is a crucial mechanism of resistance for paclitaxel.[208] There are nine multidrug proteins (MRPs). In normal lung tissue, MRP 1 and MRP 5 have been found to be highly expressed. MRP 6 and MRP 7 are moderately expressed, and MRPs 2, 3, 4, 8, and 9 are either low or undetectable.[209,210] MRP 1 and MRP 2 are found in the bronchial and bronchiolar epithelium.[195,211,212] MRP 1 is also found in alveolar macrophages.[195,211] MRP 1 expression and levels are altered in patients with COPD.[203,211] It has been previously shown that smoking downregulates the transporter, and the transporter has a protective role against cell damage.[203,213] Ipratropium, N-acetylcysteine, and budesonide stimulate MRP 1 efflux and activity.[214] Formoterol in combination with budesonide reduces transporter activity, but formoterol on its own does not have an effect on the transporter.[214] In a study of inhaled doxorubicin, MRP 1 and MRP 2 were overexpressed.[51] This information is crucial, because most lung cancer patients are also diagnosed with COPD. Breast cancer resistance proteins were first isolated from breast cancer cell lines. In a recent study, they were found to be highly expressed in human lung tissue using gene microarrays[215] (Figure 2).

Organic cation transporters

Organic cation transporters belong to the greatest facilitator family and comprise five types of carriers, ie, electrogenic OCT 1, OCT 2, and OCT 3, and electroneutral OCTN 1 and OCTN 2. OCT 1–3 are found in the trachea, smooth muscles of the airway, and ciliated bronchial cells, but there are contradictory data in terms of their expression. OCT N1 is expressed in the tracheal epithelium and alveolar macrophages, whereas OCT N2 is expressed in the alveolar epithelium and airway epithelium.[215–218] Published data for animal and in vitro cell lines implicate upregulation or downregulation of OCT transporters upon induced inflammation or drug interactions related to asthma and/or COPD. Nevertheless, these are not clearly associated with a human model[217–220] (Figure 2).

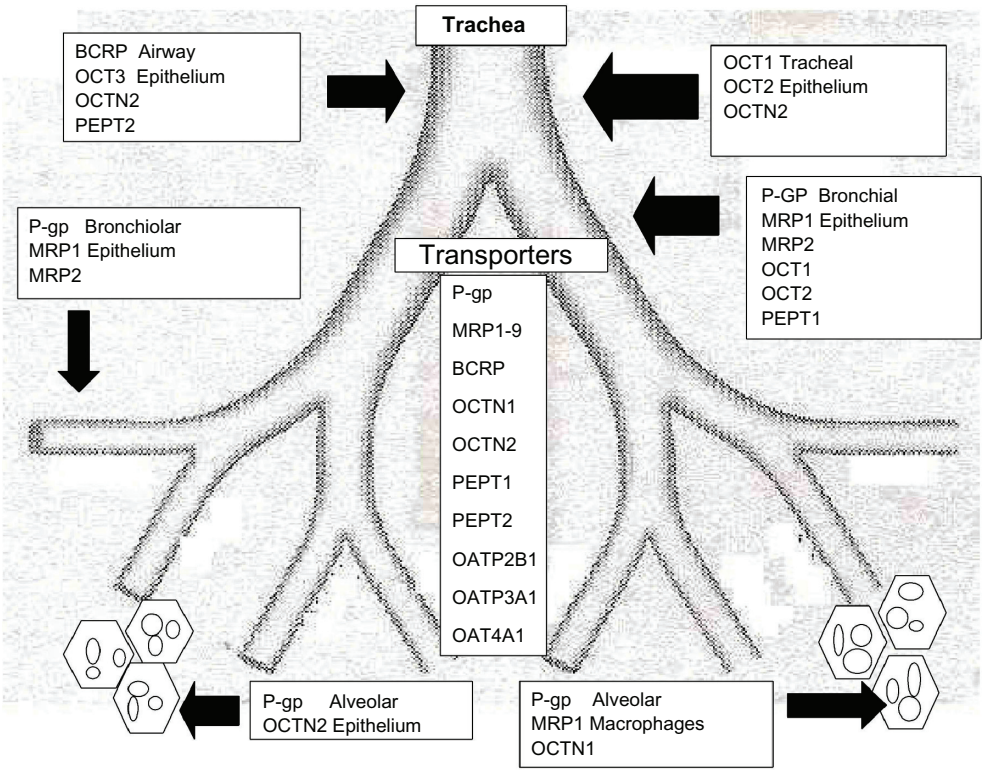

Figure 2 Transporters and their position where they are most highly expressed.
Abbreviations: P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; MRP, multidrug resistance-associated proteins; PEPT, peptide transporters; OCT, organic cation transporters; OCTN, organic cation transporters electroneutral; OAT, organic anion transporters; OATP, organic anion transporting proteins.

Peptide transporters

Peptide transporters are part of the proton-coupled oligopeptide transporter group. The two main transporters are PEPT 1 and PEPT 2, which contribute to the high bioavailability of peptide-like molecules. These peptides can affect the absorption and distribution of several inhaled antibiotic and antiviral drugs.[221] PEPT 2 and more recently PEPT 1 were detected first in the airway epithelium and then in the bronchial epithelium.[222,223] These two transporters have also been found in animals and cell lines.[224,225] However, how they interact with drug formulations and their activity in respiratory diseases[226] has not been fully investigated.

Organic anion transporters

There are six members identified, ie, OAT 1–4, URAT 1, and OAT 5, which are mostly found in the kidneys.[227] Gene microarrays have confirmed their absence in human and murine lungs, but OAT 2 was highly expressed at these sites.[215] In addition, OAT 4 mRNA was highly expressed in the bronchial cell lines Calu-3 and 16HBE14o-.[224]

Organic anion transporting polypeptides

There are 11 human organic anion transporting polypeptides (OATPs), which are divided into six families.[228] Their actual tissue distribution has not been fully investigated.[228] OATP 2B1, OATP 3A1, OATP 4C1, and OATP 4A1 expression has been found in human lungs, animals, and cell lines.[215,224,229]

Inhalation studies

There is a large amount of published data regarding aerosol delivery of chemotherapy in cancer cell cultures, animal models, and Phase I/II human studies (Table 2). These studies are best commented on in terms of the chemotherapeutic agent delivered to the lung parenchyma with additional individual parameters. The first chemotherapeutic agent, investigated almost 30 years ago, was 5-fluorouracil (5-FU).[31] Tatsumura et al[32] presented data for patients treated with inhaled 5-FU and underwent surgery immediately afterwards, who had higher drug concentrations in the tumor than in the surrounding tissues. In addition, high 5-FU concentrations were found for up to 4 hours after administration in the main bronchus and in the lymph nodes around the main bronchus.[32] This observation was confirmed in another study using 5-FU.[60] Moreover, additional formulations of 5-FU with lipid-coated nanoparticles or difluoromethylornithine, an important enzyme in cell proliferation, were devised to achieve sustained drug release and enhance anticancer properties.[60,230,231] In these studies, previously presented for their inhalable system carriers with 5-FU, a step was made forward in using inhaled chemotherapy as an adjuvant treatment. Studies using taxanes either with liposome carriers, nanoparticles, polymeric micelles, or lipid nanocapsules provided evidence of an increased therapeutic index by prolonging regional action in the lung. Further, 5%–7% CO_2 has been used to enhance aerosolized drug deposition.[50,82] However, the data are controversial regarding the safety of taxanes at the lung parenchyma. The data indicate that the mononuclear phagocyte system attacks the colloidal drug, so a combination of pegylated lipid nanocapsules is needed to prolong regional action. In addition, further studies will establish their efficacy in human subjects, after proper alterations/additions to the drug formulation, such as nanoparticles[232] or nanospheres,[233] and when linked to human albumin.[45,50,59,234,235] Moreover, adverse effects, mainly neurotoxicity, was found to be dose-dependent, but also associated with increased tumor burden regression,[50] and addition of cyclosporine A to the paclitaxel aerosol was found to enhance the anticancer effect of the treatment.[55] It was observed that addition of cyclosporine A reversed the resistance of cancer cells to paclitaxel.[55] When a taxane compound was compared with doxorubicin, it was noticed that adverse effects on the lung parenchyma were observed for the doxorubicin group, indicating that taxanes are safer in comparison with doxorubicin regarding the lung region.[34] In addition, severe cardiotoxicity was seen in the doxorubicin group.[34,47] Otterson et al[37,52] created a protocol for inhaled chemotherapy in human subjects, covering all aspects of this treatment modality. Two Phase I and Phase I/II studies demonstrated the adverse effects of aerosol treatment, such as a metallic taste, mild bronchospasm, and moderate reduction of pulmonary function tests. Therefore, bronchodilators were administered before every session and patients rinsed their mouth with water afterwards. It was observed that a significant drawback was the timing of administration of the drug formulation (60 minutes). Aerosol deposition was evaluated by radiolabeling, and remission of pulmonary function tests was observed after every chemotherapy session. In addition, other basic characteristics of this protocol proposal for inhalation chemotherapy were addressed, such as inclusion criteria. It is essential to highlight tumor size, which must not be more than 5 cm in mass median diameter, because this parameter is crucial for drug deposition.[84,131] These issues are analyzed further in the safety section. In another study using nanoparticles with doxorubicin, it was observed that macrophages clear the formulation, so smaller nanoparticles need to be developed.[47] Platinum analogs have also been investigated, and the findings were similar to those for inhaled doxorubicin. Moderate bronchospasm after aerosol inhalation, cough, fever

Table 2 Published studies with inhaled chemotherapy regimens and study investigated parameters

Author	Inhaled chemo	Main adverse effects	Synthesis	FEV_1	FVC	DLCO	6MIN	TLC	Evaluation	Subjects	Inhalation device	Protection	Reference
Zarogoulidis et al[29]	CARBO	Cough	–	√	√	√	√	–	Thorax HRCT, RECIST, Blood samples	Human	Nebulizer	HEPA	30
Tatsumura et al[31]	5-FU	Glottitis	–	–	–	–	–	–	Radiological, blood samples	Human	Supersonic Nebulizer	Protected room	31
Tatsumura et al[32]	5-FU	–	–	–	–	–	–		Bronchoscopy, HPLC, histopathology, blood samples	Human	Nebulizer	Protected room	32
Wattenberg et al[54]	5-FU	Weight loss	–	–	–	–	–	–	Histopathological	Animal	Nebulizer	Hood	54
Hitzman et al[60]	5-FU	–	Lipid-coated nanoparticles	–	–	–	–	–	HPLC, blood samples	Animal	Ultrasonic Atomizer	Plexiglass chamber	60
Hitzman et al[60]	5-FU	–	Lipid-coated nanoparticles	–	–	–	–	–	HPLC, blood samples	In vitro Animal	Ultrasonic Atomizer	Plexiglass chamber	230
Hitzman et al[60]	5-FU	–	Liposomes, microspheres, Lipid-coated nanoparticles	–	–	–	–	–	Microdialysis	–	–	–	231
Hershey et al[34]	PTX DOX	Cough, upper airway toxicity, pneumonitis/ fibrosis	PEG	–	–	–	–	–	Chest x-ray V/Q, blood samples	Animal	Nebulizer	Controlled environment	34
Koshkina et al[35]	9-NX PTX	–	Liposome	–	–	–	–	–	HPLC, histopathological, blood samples	Animal	Nebulizer	Plastic cage	82
Koshkina et al[35]	PTX	Neurological toxicity, aggressiveness	Liposome	–	–	–	–	–	HPLC, histopathological	Animal	Nebulizer	Plastic cage	50
Knight et al[55]	PTX CYS A	Weight loss	Liposome	–	–	–	–	–	HPLC, histological	Animal	Nebulizer	Sealed plastic cage	55
Hureaux et al[48]	PTX	–	Lipid nanocapsules	–	–	–	–	–	V/Q, HPLC	In vitro	Mesh nebulizer	Controlled environment	48
El-Gendy et al[58]	PTX CIS	–	Nanoparticle loaded	–	–	–	–	–	HPLC, TEM DSC, TGA	In vitro	Manually	–	58
Anderson et al[49]	CIS	–	a-TEA, Liposome	–	–	–	–	–	Histological, HPLC, Ki-67, TUNEL	Animal	Nebulizer	Plastic cage	49
Wittgen et al[42]	CIS	–	liposome	–	–	–	–		HEPA filter,	Human	Nebulizer	HEPA	42

Wittgen et al[42]	CIS	Nausea, fatigue, dyspnea, vomiting hoarseness, bronchitis bronchial wall thickening	Liposome	√	√	√	–	–	Blood samples, HRCT	Human	Jet nebulizer	Negative pressure room Protective clothing equipment	53
Selting et al[41]	CIS	Cough, pneumonitis, fibrotic lesions	–	–	–	–	–	–	Blood samples, chest x-ray, histopathological urine analysis	Animal	Nebulizer	Intracorporal catheter	41
Tseng et al[45]	CIS	Weight loss	Biotinylated EGF gelatin	–	–	–	–	–	Blood samples, histopathological	Animal, In vitro	Nebulizer	–	45
Gagnadoux et al[242]	GEM	Pulmonary edema	–	–+	–	–	–	–	Chest x-ray, V/Q, histopathological	Animal	Microsprayer	Hood	242
Koshkina and Kleinerman[62]	GEM	–	–	–	–	–	–	–	HPLC, Immunohistochemistry, FasL pathway, histopathological	In vitro Animal	Jet nebulizer	Plastic cage	62
Gagnadoux et al[241]	GEM	–	–	–	–	–	–	–	V/Q, filter system, histological, HPLC	In vitro, Animal	Nebulizer	Inhalation cabin	241
Min et al[244]	GEM	Acute lung injury	–	–	–	–	–	–	Blood samples, TNF, BALF	Animal	Nebulizer	Controlled environment	244
Lemarie et al[243]	GEM	Cough, dyspnea, vomiting, severe bronchospasm	–	–	–	–	–	–	V/Q, blood samples	Human	Nebulizer	Protective chamber	243
Rodriguez et al[61]	GEM	Vascular connective tissue into the airway lumina	–	–	–	–	–	–	Histological, Immunohistochemistry chest x-ray, TUNEL, blood samples, FasL	Animal	Minimate compressor nebulizer	Controlled environment	61
Azarmi et al[59]	DOX	–	Nanoparticle loaded	–	–	–	–	–	XTT CLSM	In vitro	Spray freeze-drying	Hood	59
Otterson et al[52]	DOX	>20% drop in PFTs Necessary steroid use wheezing, chest pain hypoxia	–	√	√	√	–	–	Thorax CT, RECIST, HPLC, V/Q, blood samples	Human	Nebulizer	HEPA	52

(*Continued*)

Table 2 (*Continued*)

Author	Inhaled chemo	Main adverse effects	Synthesis	FEV_1	FVC	DLCO	6MIN	TLC	Evaluation	Subjects	Inhalation device	Protection	Reference
Garbuzenko et al[51]	DOX	Alveolar hemorrhage, Peribronchial inflammatory cells	Liposome	–	–	–	–	–	Bioluminescent image, ultrasound, histological	Animal	Nebulizer	Nose only chamber	51
Otterson et al[37]	DOX	> 20% drop in PFTs Necessary steroid use	–	√	√	√	–	–	Thorax CT, RECIST, V/Q	Human	Nebulizer	Controlled environment	37
Roa et al[47]	DOX	Cardiac toxicity, weight loss	Effervescent/ noninhalable nanoparticles	–	–	–	–	–	HPLC, MRI, histopathological	Animal	DP-4M insufflator	Controlled environment	47
Koshkina et al[63]	9NC	–	Liposome	–	–	–	–	–	HPLC, histological, Blood samples	Animal	Nebulizer	Plastic cage	63
Knight et al[240]	9NC	–	Liposome	–	–	–	–	–	HPLC, histopathological	Animal	Nebulizer	Plastic cage	240
Verschraegen et al[56]	9NC	Bronchitis, pharyngitis, cough	Liposome	√	√	√	–	√	HRCT, blood, BAL, urine analysis	Human	Nebulizer	HEPA	56
Lawson et al[57]	9NC	–	a-TEA, Liposome	–	–	–	–	–	Histological, HPLC, Ki-67, TUNEL, CD31	Animal	Nebulizer	Plastic cage	57
Lawson et al[238]	a-TEA	–	Liposome	–	–	–	–	–	Histopathological, TUNEL	Animal	Nebulizer	Plastic cage	238
Sharma et al[64]	Review	Review	Review	Review	Review	Review	Review	Review	Review	Review	Review	Review	64
Khanna et al[39]	Review	Review	Review	Review	Review	Review	Review	Review	Review	Animal	Review	Review	39
Gagnadoux et al[40]	Review	Review	Review	Review	Review	Review	Review	Review	Review	Review	Review	Review	40

Abbreviations: DOX, doxorubicin; CIS, cisplatin; PTX, paclitaxel; 9NC, 9-nitro-camptothecin; CARBO, carboplatin; GEM, gemcitabine; 5-FU, 5-fluorouracil; CYS A, cyclosporin; CD31, endothelial antigen also referred to as PECAM1 (platelet-endothelial cell adhesion molecule), is an indicator of small capillaries in primary tumor tissue; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling, is a method for detecting DNA fragmentation by labeling the terminal end of nucleic acids; Ki-67, antigen Ki-67, also known as MKI67, is a protein that in humans is encoded by the MKI67 gene, it is a nuclear protein that is associated with and may be necessary for cellular proliferation; A-TEA, alpha-tocopherol ether analog; HPLC, high-performance liquid chromatography or high-pressure liquid chromatography, is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify, and purify the individual components of a mixture; FasL pathway, Fas ligand or CD95 L is a type II transmembrane protein that belongs to the TNF family, its binding with its receptor induces apoptosis, Fas ligand/receptor interactions play an important role in the regulation of the immune system and the progression of cancer; BALF, bronchoalveolar lavage fluid; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; 6MIN, 6-minute walking test; TLC, total lung capacity; TNF, tumor necrosis factor-alpha is a cytokine involved in systemic inflammation and also stimulates the acute phase reaction; V/Q, ventilation/perfusion ratio; XTT, colorimetric assay for cell proliferation, viability, and cytotoxicity; CLSM, confocal laser scanning microscopy, a technique for obtaining high-resolution optical images with depth selectivity; TEM, transmission electron microscopy, a technique that a beam of electrons is transmitted and converted to a sensor charged coupled device, photographic film or fluorescent screen; DSC, differential scanning calorimetry, a thermoanalytical technique; TGA, thermogravimetric analysis is a type of testing performed on samples that determines changes in weight in relation to change in temperature.

(three days in some cases), and a metallic taste were observed. Bronchodilators were administered before every session. Pulmonary function tests were also performed according to the American Thoracic Society/European Respiratory Society guidelines[53,56,236,237] along with a high resolution CT scan of the thorax.[30,53] Mild reduction in pulmonary function tests was observed immediately after the chemotherapy session, but regressed until the next cycle.

In a study by Tseng et al[45] a biotinylated epidermal growth factor-modified gelatin nanoparticle carrier was investigated, and found to enhance the anticancer activity of the formulation. In addition, it paved the way for targeted inhaled chemotherapy. Cancer cells with epidermal growth factor overexpression show increased uptake of this formulation. An additional benefit is reduced nephrotoxicity, because more cisplatin remains regionally in the lung cancer cells, and fewer carriers with cisplatin are delivered to the systemic circulation. Anderson et al[49] administered a novel nonhydrolyzable ether-linked acetic acid analog of vitamin E (a-TEA) with intraperitoneal administration of cisplatin. This treatment is mentioned due to the positive effect observed in reducing lung metastasis to the lungs.[49,238,239] The a-TEA could be used as a molecule additional to the aerosol formulation, in order to augment apoptosis of cancer cells and to decrease cancer cell proliferation.[49,57,239] Again, safety issues are discussed in the safety section. The anticancer effect of 9-nitro-camptothecin as an aerosol has been established.[56,57,240] Several formulations using carrier systems have been used to deliver sustained-release 9-nitro-camptothecin. Feasibility and effectiveness was established, and in addition to encapsulation with carriers, pegylation added a "stealth" property, as previously mentioned. The macrophages did not recognize the formulation and so did not attack, but cancer cells ingested the molecules due to the cationic charge (PEG technology).[44] However, the polystyrene microparticles used in this study cannot be used in human subjects because they cannot be eliminated from the body. Therefore, an initiative to develop biocompatible aggregated nanogel particles has been started using additional PEG technology.[44] In a study by Verschraegen et al[56] aerosol therapy was taken to a level never previously achieved. After effectiveness and safety had been observed, patients were educated to receive their therapy at home. This study provides evidence that, if safety and effectiveness of such a system is confirmed, patients can receive any chemotherapy agent without having the side effects that are usually observed with these agents.

Gemcitabine, a well known chemotherapeutic agent, has been evaluated in dogs and baboons as an aerosol formulation. The formulation was radiolabeled and blood samples were collected until 360 minutes.[43,241,242] The efficacy of this treatment modality on lung metastasis due to osteosarcoma was investigated, along with Fas/FasL expression. The Fas ligand is a type II transmembrane protein that belongs to the tumor necrosis factor family. Fas expression in metastatic foci was increased compared with that in lung metastases before treatment, and at even higher levels than in the primary tumor. The results of these studies indicate that aerosolized gemcitabine treatment is effective against metastatic osteosarcoma lesions.[61] Gemcitabine has been administered to patients with lung cancer, either as an aerosol or instilled into the lung parenchyma, and the data have demonstrated efficacy.[243,244] The safety of gemcitabine in humans is discussed in the safety section.

Safety and protection measures

Both patients and medical staff should be considered concerning safety (Table 2). The question that needs to be answered is whether interstitial lung disease is induced due to inhalation chemotherapy. Several agents have been observed to induce this kind of damage after intravenous administration.[245–251] On high resolution CT, a ground-glass pattern, linear opacities, interlobular or intralobular thickening, and alveolar shadows were observed. Different histopathological appearances are also observed. Determining the background of pulmonary infiltrates in patients who develop pulmonary severe involvement while receiving chemotherapeutic agents can be problematic. There are several factors that could produce this type of appearance, including pulmonary edema, alveolar hemorrhage, involvement of background disease, and radiation. Moreover, a confirmatory biopsy or bronchoalveolar lavage is often not possible to undertake due to respiratory distress or severity of the underlying condition. Because several chemotherapeutic agents may induce interstitial lung disease by intravenous administration, a through safety evaluation has had to be performed for the aerolized formulations. Regarding the safety of medical personnel, the aerolized studies were performed with certain protection measures, including special plastic cages for animals.[49,57] Other measures for protection were the design of the delivery system or method. In a number of studies, the drug was delivered through a special nose-only chamber[51] and using an intracorporeal catheter.[41] An evaluation of whether these systems had sufficient environment safety was performed in several cases and the measures were adequate.[34]

Evaluation of pulmonary toxicity was observed by post mortem histological examination and radiological

investigations, ie, x-ray, magnetic resonance image, bioluminescent image, and V/Q scan.[41,43,47,51] The most severe side effects were cough, weight loss, neurotoxicity, cardiotoxicity, alveolar interstitial pattern (radiological findings), moderate fibrosis (histopathological findings), and death as a result of pulmonary edema.[34,41,43,50,54] One death was reported in a Phase I study by Otterson et al[52] after doxorubicin aerosol administration, but autopsy revealed focal hyaline membrane deposition and obstructive pneumonia due to tumor burden. Staphylococcus and *Acinetobacter baumannii* were isolated from blood cultures, two bacteria commonly found in intensive care units, where the patient had been hospitalized due to severe respiratory distress. Death was attributed to disease progression. Selting et al[41] demonstrated clearly that when administered repeatedly to a specific part of the respiratory airway, platinum analogs moderate the fibrosis and an alveolar interstitial pattern occurs. Nevertheless, these findings are dose-associated and depend on the time interval of treatment. When bronchospasm occurred and there was a drop in pulmonary function tests, additional steroid treatment reversed these adverse effects.[34,37,52] These lesions did not appear using liposomal paclitaxel in studies which included histopathological evaluation.[55] In a study by Tseng et al[45] less nephrotoxicity was observed with biotinylated epidermal growth factor-modified gelatin nanoparticle carriers with a platinum analog, in comparison with a free circulating platinum analog. Regarding human subjects, studies used either a mouthpiece[30] or a facial mask[42,53,56] under a high efficiency particulate air system; the drug formulation which escaped (if any) was evaluated, and no toxic effects were found. In one study, the drug delivery system and formulation were evaluated and found to be efficient and safe enough that the patients were instructed to administer the drug formulation at home.[56] Methods of evaluating the pulmonary parenchyma and respiratory capacity in human subjects have included pulmonary function tests with forced expiratory volume in one second (FEV_1), forced vital capacity, carbon monoxide diffusing capacity (DLCO), the 6-minute walking test, and high resolution CT or CT scan.[30,37,52,53,56] The patients included in these studies did not have any known collagen disease in order to be certain whether interstitial disease findings, if observed, were due to the inhaled compound. The adverse effects most commonly observed were coughing, mild bronchoconstriction, fever, nausea, pharyngitis, thickening of the bronchial wall (high resolution CT finding),[53] focal hyaline membrane deposition (post mortem finding),[52] and reduction in pulmonary function tests that responded to corticosteroids or resolved after termination of treatment.[30,37,53] In a study by Garbuzenko et al[51] alveolar hemorrhage and bronchial accumulation of chronic inflammatory cells were observed in histopathological specimens after aerosol administration of liposomal doxorubicin. Moreover, large bronchi were surrounded by aggregates of chronic inflammatory cells, including lymphocytes, macrophages, and plasma cells.[51] In three studies, a mild reduction of FEV_1, forced vital capacity, and DLCO was observed after aerosol administration and therefore bronchodilators and inhaled corticosteroids were administered before every treatment.[30,37,52,53] Gemcitabine in an aerosol formulation did not induce fibrotic lesions in the lung parenchyma and does not contain any chemical ingredients incompatible with aerosol delivery.[43,62] Nevertheless, in an animal model, death from pulmonary edema occurred after aerosol administration of gemcitabine.[43] Studies with 9-nitro-20(S)-camptothecin did not report fibrotic lesions

Table 3 Summary of inhaled chemotherapy in lung cancer

- Although there is a variety of inhalation devices available on the market, each one with advantages and disadvantages, administration of an inhaled chemotherapy formulation is currently feasible through a nebulization system.
- The aerosol compound time release can be enhanced by either adding a carrier which provides sustain release, or by adding 5%–7% CO_2 to the inhalable aerosol.
- Aerosol chemotherapy studies previously published provide conclusions with safety and feasibility of this treatment modality. Nevertheless, more trials are needed with patients of early stages to present long term data regarding adverse effects to the lung parenchyma. In addition, more single-agent or double-agent trials for the aerosol are needed to present indisputable data regarding the safety and effectiveness of this treatment modality in comparison with intravenous administration.
- A question remains whether this treatment modality is proper for early lung cancer stages or as neoadjuvant/adjuvant, since tumor size is a limitation for patients to be candidates.
- A new methodology of manipulating the aerosol deposition site according to cancer lesions has been proposed and developed, but is still under investigation.
- Inhaled chemotherapy has been evaluated in a number of studies (high efficiency particulate air system) and the results indicate that certain drug administration systems are efficient enough to eliminate diffuse/spilling of the aerosolized agent to the environment. The next step of aerosol chemotherapy agent administered inhouse has also been tested successfully with proper education and use by patients.
- Administration of inhaled bronchodilators, corticosteroids, and N-acetylcysteine could prevent and protect the lung parenchyma from adverse effects.
- The crucial question of whether such a treatment modality should be pursued will remain unanswered if further studies are not performed. The concept of a treatment modality for cancer patients free of systemic side effects is very tempting.
- This treatment modality in order to have widespread acceptance, solid data regarding the safety and feasibility needs to be pursued.

in the lung parenchyma, although a reversible reduction in pulmonary function tests and mild adverse effects from the aerosol compound were observed (bronchial irritation, sore throat, pharyngitis).[56] In studies performed in cancer cell lines, administration of the aerosol was conducted inside hoods, so medical personnel were safe from the toxic compounds. Assessment of efficacy was made by observing the decrease in the population of cancer cells.[58,59]

Conclusion

Inhaled chemotherapy is a feasible treatment modality. Nevertheless, the pulmonary side effects of this treatment have to be assessed further. Until now, inhaled chemotherapy was administered for advanced lung cancer and, therefore, although some chemotherapeutic agents provided immediate evidence of dose/time-related toxicity, others did not due to the limited duration of administration. Early-stage lung cancer studies did not administer inhaled chemotherapy for more than one session.[31,32] Therefore, studies involving early-stage lung cancer are needed to provide time/dose-related safety data. Moreover, studies providing doublet inhaled chemotherapeutic agents need to be performed because doublet chemotherapy is the cornerstone of treatment for lung cancer. However, these trials should not be performed until sufficient data regarding the safety profile in the lung parenchyma are available. Regarding efficacy, regional studies have demonstrated positive results, but there are limitations to use of drug locoregionally according to tumor size, penetration of the drug at the tumor site, the chemical characteristics of the drug, and its biological effects. Regional therapy does not necessarily mean that higher drug levels reach the tumor. However, this concept contradicts the fact that, due to blood circulation in the lung[52,53] and the lymphatic circulation, drug concentrations have been found in the systemic circulation and surgically resected lymph nodes.[31,32,84,131,230,231,238] In addition, a high percentage of lymph nodes in the aerosol treatment groups did not show any metastasis in several studies, so it is possible that this route of administration destroys tumor cells and prevents them from trafficking from the primary subcutaneous tumor to the lungs and lymph nodes via the lymphatic system.[31,32,49,57] Nevertheless, in many of these studies, an additional intravenous chemotherapy regimen was administered, so clear conclusions cannot be drawn. Nanoparticles have the ability to evade macrophages and transport them into lung tissues other than the alveolus and into the general circulation.[47] However, macrophages are a defense mechanism that is also responsible for clearing the nanoparticles and, thus, reduce the anticancer effect of inhaled chemotherapy. Furthermore, an evaluation has to be made of whether inhaled chemotherapy is appropriate for early-stage lung cancer, or as neoadjuvant or adjuvant treatment.[57,60,61,230,231]

Because lesions are of smaller size, drug deposition is not prevented by the large mass median diameter of the tumor.[131] Current drug delivery systems have demonstrated an ability to achieve sustained release locoregionally, but further improvement is welcomed.[40,48,252] Nevertheless, the timing of the drug administration has to be shortened.[30,48] Additional modifications were made to the molecules, to include targeted therapies, such as the epidermal growth factor receptor.[45] In another study by Garbuzenko et al[51] additional pump and nonpump suppressors were added to inhaled doxorubicin. This concept was based on the observation that there are two main mechanisms responsible for cancer cell chemotherapy resistance, ie, pump and nonpump.[253–255] Pump resistance is caused by membrane efflux pumps that decrease the anticancer drug concentration inside cells. Nonpump resistance is primarily attributed to the activation of antiapoptotic cellular defense, and Bcl-2 is also a key parameter in this defense. Similar to MRP 1, expression of Bcl-2 protein increases significantly after treatment with anticancer drugs.[253,255] a-TEA could be used as a molecule additional to the aerosol formulation to augment apoptosis and decrease cancer cell proliferation.[49,57,239] Nebulizers have also demonstrated efficiency for delivery and deposition of inhaled chemotherapeutic regimens.[30,37,52,53] Additional modifications have been shown to improve deposition further by modifying respiratory rate and tidal volume.[82] Delivery systems have been evaluated for safety regarding environmental release of toxins and it has been found that certain systems have the ability to deliver their entire drug cargo to patients, without any loss to the environment.[30,42] Drug transporter gene expression appears to be high in the lungs.[215] However, interaction of these genes with inhaled drug formulations, any alterations due to underlying respiratory disease, and their role in drug deposition are not fully explored.[226] The transporters are less likely to influence absorption of inhaled drugs than they are to exert an effect in the gastrointestinal tract; nevertheless, efflux pumps can be exploited to prolong drug retention in situ.[226] Transporter properties can be fully exploited if a formulation is designed for distribution to specific sites in the respiratory tract where they are highly expressed in combination with the appropriate drug carrier. In addition to the transporters, several other carriers have been investigated in combination with chemotherapy agents, either in order to make feasible

their administration to the lung parenchyma for toxicity reasons, or to add a sustained-release capability to the lung formulation.[34,39,40,47–51,53,55–60] Transporters could be used in addition as a prognostic factor.[199] Moreover, regarding carriers, liposomes would appear to require dosing three or four times a day and would be expected to be well tolerated within the lung following repeated dosing. Microspheres and lipid-coated nanoparticles could be given once or twice daily, depending on particle residence time at the site of action. However, issues of particle accumulation within the alveoli may be encountered with repeated dosing. Due to the composition of lipid-coated nanoparticles in comparison with microspheres, there would be less of a concern with toxicity using lipid-coated nanoparticles.[231] The optimal formulation for the clinician would be once-a-day delivery of a chemotherapy regimen, with the additional effect of pegylation for the "stealth" properties previously mentioned.

Finally, the next step in delivering inhaled chemotherapy at home has already been made after proper patient education, and the concept is intriguing.[56] Monitoring of patients can be done using peak flow measurements at home between restaging, and addition of a combination of inhaled bronchodilators and corticosteroids before inhaled chemotherapy could help prevent adverse effects, such as bronchoconstriction. Addition of N-acetylcysteine could be used as a protective measure.[93,214] Finally, inhaled chemotherapy regimens when administered alone demonstrate fewer systemic cytotoxic effects, making the concept of safe inhaled chemotherapy the next challenge in the treatment of lung cancer. Nevertheless, the safety and efficacy of such formulations have yet to be fully and completely evaluated (Table 3).

Disclosure

The authors report no conflicts of interest in this work.

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