

Wonders of Nanotechnology in the Treatment for Chronic Lung Diseases

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Abstract

Application of nanotechnology has significantly increased in different spheres of life including the drug delivery systems and is being considered to be the technology for near future. The plausibility of non-invasive administration of drug via inhalation and avoidance of first-pass metabolism due to direct delivery at the affected site makes respiratory system as an ideal target port for nano-carrier mediated drug delivery systems. The development of several nano-carrier systems (liposomal, solid-lipid nanoparticles, polymeric nanoparticles) offers various potential advantages for respiratory drug delivery with reduced and undesirable side-effects. This review emphasizes the application of several nano-carriers for drug delivery particularly in chronic lung diseases. It summarizes the use of different experimental models currently available (*in vitro* and/ *in vivo*) to study the risk assessment of nano-carriers. Although nano-medicine based studies suggests that drug delivery systems for systemic and/or local treatment of diseases are promising, yet further research is warranted to elucidate long-term toxicity, deposition and clearance of nanoparticle especially following repeated administration.

Keywords: Nanomedicine; Respiratory diseases; Drug delivery; Nano carrier; Toxicity; Chronic lung diseases; Non-invasive

Introduction

Chronic lung diseases (CLDs) like asthma, chronic obstructive pulmonary disease (COPD), lung cancer, pulmonary fibrosis are the leading causes of death worldwide. However the therapeutic options for treating CLDs remain limited particularly due to the lack of cell specific targeting. Lung on one hand serves as the primary port of entry for respiratory toxins including ultrafine and nanoparticles thereby enhancing the occurrence of CLDs, on the other, by virtue of this property lung serves as efficient delivery port for targeted drug delivery systems. Non-invasive application of aerosol inhalation allows direct delivery of the drug to the affected site to treat CLDs. Availability of a huge internal surface area (24-69 m²) of adult human lung offers the possibility of highly effective local drug action due to faster systemic absorption compared to other organs [1]. Additionally direct delivery of drugs to the affected area of lungs (site directed delivery) will result in the use of very low dosage with maximum protective effect due to avoidance of first-pass metabolism. Administration of multiple drugs has several pharmacokinetic limitations, like, (i) difficulties to increase the therapeutic concentration in the blood within optimal time window, (ii) long clearance time and (iii) low solubility of the active compounds etc. All these events ultimately results in undesirable side effects due to high-dose treatments [2,3]. Recent developments in the field of nanomedicine or the drug delivery using nanocarriers/nanoparticles offer ample opportunities to overcome these limitations. Biological properties of nano-carriers (liposomes, micelles and polymeric nanoparticles) can be altered and controlled according to the requirement thereby making them highly efficient for pharmacological and therapeutic purposes [4,5]. Development of nano-medicine or nano-carriers has many advantages, including efficient delivery and accumulation of drug in the affected area even with the physiological condition of compromised vascularization [6,7]. Furthermore, experimental findings have shown that nano-carriers exhibit more efficient tissue penetration thereby resulting in increased tissue specific action of drug compared to the regular drug administration routes. Even though use of nano-carrier system is heavily debated within the respiratory research community, yet this system offers more efficient drug delivery mechanisms in pulmonary disorders [8]. Thus, for

defining novel drug delivery mechanisms in the era of modern medical science, nanoparticles offers an attractive concept for use in respiratory system. This is primarily due to the relatively uniform distribution of the drug tagged with nano-carriers within the alveolar surface along with enhanced solubility and prolonged release. These properties also reduce the frequency of drug administration and improved patient compliance with minimum side effects. In this review we summarized the current knowledge base on the possibilities to explore nano-medicine for its application in pulmonary drug delivery.

Respiratory disease

The term respiratory diseases covers an umbrella of multiple disorders affecting the pulmonary system that consists of upper respiratory tract, trachea, bronchi, bronchioles, alveoli, pleura, pleural cavity, and the nerves and muscles required for breathing. Respiratory diseases range from mild and self-limiting ailments like common cold to life-threatening chronic inflammatory lung diseases like asthma, chronic obstructive pulmonary diseases (COPD) with chronic bronchitis, emphysema; fibrosis, lung cancer as well as restrictive lung diseases which are characterized by restriction in lung expansion due to stiffness of the chest wall, weak muscles, or damaged nerves [9]. Obstructive lung diseases typically results in reduced lung volume, decreased diffusion capacity, increased compliance with exacerbated problem of breathing and inadequate ventilation or supply of oxygen. Infection of the respiratory system and their effect is often specific to either the upper or lower respiratory tract. Infection or inflammation in the upper respiratory tract like tonsillitis, otitis media, pharyngitis

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and laryngitis are mostly caused by the viral (rhinovirus, parainfluenza virus etc) or bacterial (*Streptococcal pharyngitis, Haemophilus influenza*) infection. However, infection in the lower respiratory tract is more severe and is mainly caused by bacteria, particularly *Streptococcus pneumonia* [10]. Viruses and fungi contribute to the development of severe acute respiratory syndrome and pneumocystis pneumonia. Lower respiratory tract is also affected following exposure to ambient ultrafine particles particularly during high pollution episodes as well as smoking. Studies have shown that ultrafine and nano-particles can substantially evade the mucociliary and macrophagic clearance of the airways and lung. This in turn leads to the penetration of ultrafine/nano-particles into the deep lung which ultimately gets deposited in the alveolar region [11-13]. Deposition of particulate matter in the deep lung results in persistent inflammatory reaction locally as well as systemically particularly among individuals with pre-existing complications or low lung function [14-17]. Lungs are also affected by pleural cavity disease and pulmonary vascular diseases. Pleural cavity disease or pleural mesothelioma mainly develops due to inflammation on the pleura triggered by infection, pulmonary embolus, tuberculosis, mesothelioma etc. Pulmonary vascular disease or impairments of pulmonary circulation results from induced arterial pressure in the pulmonary arteries, pulmonary edema or leakage of fluid from capillaries of the lung that also results due to congestive heart failure [9].

Nanomedicine for pulmonary drug delivery: toxic hazards of nanoparticle

Inhalation therapy is in use to treat airway diseases for over 50 years and is being currently under development to treat several other CLDs as well as systemic diseases including diabetes [18,19]. With the revolution of nanotechnology and aerosol generating devices during the last decade it is now feasible to formulate, stabilize and precisely deliver most of the drugs to the lung [5,20-22]. Many therapeutic strategies are investigated for localized and systemic drug delivery through the respiratory system which includes small molecules, protein and/ peptide drug or siRNA targeted to specific genes. Most of these studies were mainly focused for localized application of drugs via nano-carriers to obtain optimal therapeutic outcome for CLDs like asthma and COPD [23]. It is now well accepted that aerosolized delivery of drugs are more convenient and highly efficient in airway treatment primarily because of the large internal surface area of the lung and highly vascularised thin epithelial layer covering the airways. This allows fast absorption and retention of high concentration of drugs in the pulmonary target site [24]. Additionally, the bioavailability of drugs is much higher through this route mainly due to the relatively less

enzymatic activity and this simple yet non-invasive drug application improves patient adherence to the treatment [8].

The other major advantages of nanoparticle mediated drug delivery are attributed to its customized pharmacokinetic and pharmacodynamics properties by modulating physicochemical characteristics of nano-carriers. This specifically allows nano-medicine to be applicable for treating respiratory disorders with: 1: Controlled and slow absorption of drug, 2: Minimum side or toxic effect, 3: Higher retention time and 4: Specific bio-distribution [2,25]. Several nano-carriers are now widely used for pulmonary treatment that includes polymeric nanoparticle, liposomal nano-carriers, and solid lipid nanoparticles. Table 1 summarizes the chemical properties and function of some of the widely used nano-carriers used to develop nanomedicine for specific respiratory disorders. Although nano-particle mediated drug delivery has been significantly optimized during the past few years, however the major issue which needs to be resolved in this research area is the optimal use of drug with highest delivery or uptake in the target organ/cells with least toxicity on the non-targeted tissue by the unused drug [26,27]. Another main challenge in the nano-drug delivery is the deposition or entrapment of the nanomaterials that are used as carrier in the mononuclear phagocytic system. This phenomenon can increase the localized cytotoxicity as well as systemic toxicity following translocation to extra-pulmonary organs. Several researchers have shown that after delivery of nano-particles into the lung by inhalation or intra-tracheal instillation, a portion of nanoparticles is translocated to extra-pulmonary tissues [16,17,28,29]. They reach to other organs (heart, blood, liver, brain etc.) through the circulatory translocation and distribution after crossing the blood/air barrier, which also makes them plausible drug carriers for other chronic diseases like diabetes. However, these events also possess the threat for potential localized and systemic toxicity. Hence cellular/organ toxicity in association with fate of nano-carrier needs to be characterized using multiple approaches [5].

Toxic hazards of nanoparticle: lessons from *in vitro* models

Drug delivery by inhalation route is likely to be absorbed throughout the conducting airway starting from the trachea down to the terminal bronchioles and ultimately in the alveoli. The airway and alveolar epithelium of the lung consisting of different cell types provides barrier capability to drug absorption and thus plays a central role in the therapeutic effect drug [30-32]. Therefore, while designing the drugs for pulmonary delivery, it is important to consider both lung-tissue retention and permeability, irrespective of site of effect. To better understand the drug absorption process in different regions (eg, tracheal, bronchial and alveolar) of the respirable barriers, *in vitro*

Nanocarriers	Properties	Function	Application for respiratory disorder
Polymeric	Composed of biodegradable or biocompatible materials such as poly lactic acid, alginate acid, gelatin and chitosan.	Presence of biocompatible component result in prolonged release of drugs	This nano carriers are used in several pulmonary drugs such as asthma, tuberculosis, pulmonary hypertension etc. [5,26].
Liposomal	Delivered in liquid and dry powder form. Cationic Liposome is also used for gene delivery	Increase the cellular uptake of drug due to presence of several cells penetrating peptide.	Respiratory distress syndrome [48,49].
Solid Lipid nanoparticles	These nanocarriers are composed of solid lipids, surfactants and water. Solid lipid nano carriers are more accepted for drug delivery due to its less or almost no cytotoxic effect than the polymer based carrier.	More tolerable to lungs and major advantages of solid lipid nano particles are the control release of drugs with rapid <i>in vivo</i> degradation.	Mainly used for lung cancer and vaccine Delivery [50,51].
Submicron emulsions	The stable submicron emulsions are promising carriers for DNA vaccines to the lung compare to the commercially available liposomes.	The emulsion system are able to transfect pulmonary epithelial cells, which directly activate dendritic cells, resulting in stimulation of antigen-specific T-cells	The submicron emulsions are used as promising carrier for DNA vaccines (e.g. Mycobacterium tuberculosis) for the pulmonary mucosal Vaccination [52,53].

Table 1: Summarizes the chemical properties and function of some of the widely used nano-carriers used to develop nano-medicine for specific respiratory disorders.

pulmonary cell culture models have been established using many categories of human disease models like cell lines, primary cells, 3D cell models, cell co-cultures, in silico models [9]. The airway epithelial cell lines (A549, BEAS-2B, Calu3, 16HBE) are frequently used for pulmonary toxicity studies since the airway epithelium constitutes the first line of defense against the external stimuli or agonist. A549 is a widely used type II pulmonary epithelial cell line (alveolar pneumocyte) to screen the metabolic and macromolecular mechanisms of drug delivery at the alveolar pulmonary epithelium because of its endocytic ability [33]. Moreover localization of cytochrome P450 systems in pulmonary epithelial cells is largely a function of type II pneumocytes. Similar to A549, BEAS-2B, Calu-3 and 16HBE cell line that represent upper airways (bronchi) are also widely used to test the toxicity of chemicals and biological agents and suitable for mechanistic studies, pathway-mapping [5,9]. Immuno-competent cells (mast cells, neutrophils) are also used for drug targeting assays as they are considered as effector cells that are highly involved in asthma for their capacity to release pro-inflammatory mediators. Moreover, several researcher have shown series of toxicity study following exposure to nano-particle using primary bronchial epithelial cells collected from patient who underwent lobectomy or pulmectomy [34,35]. Hence using primary cells for the drug related toxicity study is another path although *in vitro* system are well established to study the nano-particle mediated drug delivery for respiratory diseases. However there are certain limitations of these models. As for example, Cell lines are not able to give us the fully differentiated phenotypes and function as of the original tissue like mucus secretion, cilia formation etc., thus the physiological relevance is questionable [36,37]. Furthermore, cells in monolayer culture condition behave in a significantly different way compared to *in vivo* conditions due to the lack of cell-cell cross talk. To overcome the limitation concerning physiological relevance of monolayer based *in vitro* systems, three dimensional (3D) *in vitro* models have been developed by mimicking the *in vivo* micro-environment. Among these techniques, cellular matrix scaffold, hang-drop culture, perfusion culture chambers, air-liquid interface (ALI) cultures are well established. However, ALI-3D cultures system are more appropriate in order to simulate the *in vivo* lung conditions as the basal-lateral side of the epithelia is immersed in the culture medium and the apical side is exposed to humidified air with 5% CO₂ [38,39]. ALI-3D model is physiologically relevant, highly convenient and appreciable for most of the experimental applications such as toxicity test, imaging, drug penetration and formulation. Similar to ALI-3D model commercially available and ready-to-use Mucil-air model of human airway epithelium is also suitable to study the molecular mechanism of several respiratory diseases and drug development. The Mucil-air model is morphologically and functionally highly differentiated and possible to maintain in a homeostatic state for a long periods of time [9,40].

Toxic hazards of nanoparticle: lessons from *in vivo*/animal models

In vivo approaches or animal models for detailed study of respiratory diseases and to develop their protective measure are improving every day. Rodents (mouse and rats) are most commonly used animal models for human respiratory diseases because of their genetic and physiological similarities, well developed molecular biology tools and kits and easier handling [41,42]. However, chicken eggs, rabbits, guinea pig, cats, dogs, sheep; monkeys are also used to study the molecular mechanism and drug development for human respiratory diseases. Mouse and human genomes are almost 97 percent similar. So, once a trait is mapped in mice it can be translated to humans. Therefore, it

becomes easier to compare results of mouse experiments with human. In other words, a mouse with specific disease or alteration can stand as a unique model for human patient with same disease or condition. This allows researchers to study the detailed molecular mechanism of each respiratory complication using *in vivo* models which are technologically as well as ethically challenging to conduct in human. Such studies using *in vivo* models yield the pathway focused lead diagnostic markers along with therapeutic targets [43]. Hence, animal models provide us a connection between patient and the laboratory or in other words bridge the bench to bed side [43,44]. Researchers have developed several animal models specific to different respiratory diseases. As for example the bleomycin mouse model of pulmonary fibrosis is well established [45]. Sepsis is one of the main risk factors for acute respiratory distress syndrome (ARDS), and several animal models of sepsis have been developed to study acute lung injury.

Mouse or animal models are also widely used to study the pharmacokinetics parameters required to develop pulmonary therapeutics after nebulization of colloidal dispersion of drugs. Solid-lipid nanoparticles (SLN) are used widely for drug delivery in lung cancer and also for vaccine delivery. Videira et al. reported the bio-distribution of radio-labeled SLN in adult male Wistar rats after inhalation by acquisition of dynamic, followed by static image collection using gamma camera [46]. Similarly Mizuno et al. have shown that the pulmonary delivery and quantitative gene expression can be also evaluated by indocyanine green (ICG) labeling and the detection of luciferase activity using nondestructive real-time *in vivo* imaging system [47]. Studies on animal models are essential for understanding the molecular patho mechanism of respiratory diseases and development of novel therapeutic strategies for human application. However, caution must be taken while designing and interpreting the data generated from animal models due to the limitations already explained.

Conclusions

To summarize, nanocarrier systems provides the advantage of sustained-drug release in the lung tissue resulting in reduced dosing frequency and improved patient compliance. Local delivery of inhalable nano-carriers is a promising alternative to oral and/or intra venous administration thereby reducing the chances of side effects associated with high drug serum concentration. Consistent with all formulations designed for pulmonary drug delivery, the potential long-term risk of excipient toxicity and nanoscale carriers are an important issue that warrants detailed investigations for successful product development of pulmonary drug delivery systems. Nevertheless, the inherently small size and surface modification properties of nanomaterials offer further opportunities for innovation to attain higher efficiency for controlled drug release and pulmonary cell targeting therapeutic platforms. The integration of nanotechnology and pulmonary drug delivery has therefore the potential to improve targeting, release, and therapeutic effects of drugs and needle-free inhalation vaccines by overcoming the physicochemical and biological hurdles. Although drug delivery studies on nanoparticles for pulmonary application are still in an initial phase, studies performed so far suggest that nanoparticles are an interesting, reasonable as well as feasible option for in the systemic and/or local treatment of respiratory diseases.

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