Review

Inhalation of nanoparticle-based drug for lung cancer treatment: Advantages and challenges

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ABSTRACT

Ever since the success of developing inhalable insulin, drug delivery via pulmonary administration has become an attractive route to treat chronic diseases. Pulmonary delivery system for nanotechnology is a relatively new concept especially when applicable to lung cancer therapy. Nano-based systems such as liposome, polymeric nanoparticles or micelles are strategically designed to enhance the therapeutic index of anti-cancer drugs through improvement of their bioavailability, stability and residency at targeted lung regions. Along with these benefits, nano-based systems also provide additional diagnostic advantages during lung cancer treatment, including imaging, screening and drug tracking. Nevertheless, delivery of nano-based drugs via pulmonary administration for lung cancer therapy is still in its infancy and numerous challenges are expected. Pharmacology, immunology, toxicology and large-scale manufacturing (stability and activity of drugs) are some aspects in nanotechnology that should be taken into consideration for the development of inhalable nano-based chemotherapeutic drugs. This review will focus on the current inhalable nano-based drugs for lung cancer treatment.

1. Introduction

Cancer is a leading cause of disease worldwide. Lung, female breast, colorectal and stomach cancers accounted for more than 40% of cancer cases diagnosed worldwide; with the World Health Organization reporting an estimated 14.1 million new cancer cases worldwide in 2012 [1,2]. Among them, lung cancer is one of the most common, with 16.7% of all new cases diagnosed

in men [1]. In Australia alone, lung cancer has accounted for over 11,000 new cancer cases in 2012. Additionally, lung cancer is the most common cause of cancer-related death for men and women and the financial burden to the healthcare system is estimated at >100 million dollars annually in Australia [2]. Importantly, lung cancer has the highest mortality rate of all common cancers and a miserable dismal rate of less than 5 years [2]. Out of the 8.2 million deaths caused by cancer in 2011 globally, mortality from lung cancers contributed the highest,

with 1.3 million deaths alone [2]. Historically lung cancer has been linked to smoking and consequently classified as a social disease with a stigma attached [3]. Contrary to popular belief, lung cancer not only affects smokers but also non-smokers. For example, in women, only 65% of cancer deaths can be attributed to smoking, with lung cancer killing more women than breast, uterine, and ovarian cancers combined in women. Irrespective of cause, mortality from lung cancer is high; with only 15% of lung cancer patients surviving for more than 5 years after diagnosis [4]. Clearly there is a lack of diagnostics and effective treatment regimes.

Surgery, chemotherapy and radiation are standard treatment options for lung cancer depending on the stage of malignancy, resectability and overall performance [5]. Chemotherapy is a first-line treatment for advanced stage of lung cancer in which chemotherapeutic drugs are usually administered intravenously for systemic circulation [6,7]. The use of chemotherapeutic drug is based on the principle of toxic compounds to inhibit the proliferation of cells growing at an abnormal rate. Combination of gemcitabine (FDA approved chemotherapeutic agent) with cisplatin has been widely used for first or second line treatments of patients with advanced or metastatic lung cancer [8]. In addition, common chemotherapeutic drugs such as paclitaxel, docetaxel and gemcitabine, and vinorelbine are widely used in combination with platinum-based drugs to (i.e. cisplatin) improve therapeutic index [8–10]. However, it should be noted that majority of chemotherapy drugs is associated with side effects such as pain, nerve damage and skin allergic reactions. Therefore, minimizing the side effects of chemotherapy drugs remains a challenge in the field of cancer chemotherapy.

2.   Challenges and advantages involved in the delivery of inhalation therapeutic drugs

Lung offers numerous advantages as a delivery route for non-invasive drugs especially for localized therapy, i.e. lung cancer and treatment of airway diseases such as asthma, cystic fibrosis and chronic obstructive pulmonary disease (COPD) [11]. Compared to other delivery methods such as oral or intravenous injection, it is envisaged that the bioavailability of drugs in lung could be enhanced using pulmonary delivery since lung possesses limited intracellular and extracellular drug-metabolizing enzyme activities unlike gastrointestinal tract and liver [12]. On top of that, this option also reduces non-reversible tissue damage caused by drugs' cytotoxicity [12]. In addition, higher absorption rate, reduced drug doses and rapid onset of action are among the advantages of pulmonary administration [11,12]. The bio-barriers existing in the respiratory airway systems such as mucus, ciliated cells and resident macrophages are effective to limit the localization, penetration and adsorption of drugs in the lung [13]. The clearance mechanisms of inhaled drugs are activated depending on the location of deposited drug. Drug localized at the upper airways are removed by ciliated cells in the epithelia region while those in lower airways were protected by resident alveolar macrophages [14,15]. Resident alveolar macrophages detect the presence of foreign particles, followed by engulfment via phago-

cytosis and finally digestion in lysosomal of macrophages. The bioavailability of anti-cancer drugs to cancer cells provides an indirect reflection of success rate of therapy. To achieve this, we should determine the key factors that affect the bioavailability of drug in lungs such as aqueous solubility, dissolution rate, efflux of drugs and drug clearance by alveolar macrophages. Table 1 outlines the factors involved in determining the bioavailability of drugs into tumor cells.

For an effective drug targeting involving lung, various parameters matter which include: (i) deposition and localization of drugs onto targeted area or cells, (ii) penetrability of drugs through airway mucus, (iii) ability to escape from mucociliary clearance, (iv) transportable across epithelial cells into blood stream for systemic exposure, (v) low entrapment and inactivation of drugs by bacterial biofilms in cases of infections, (vi) tunable phagocytic activity by alveolar macrophages and (vii) minimal host protein–drug molecule interactions [13,23]. In physiological conditions, mucus, a viscoelastic gel, is secreted to protect cellular surfaces and maintain water balance. These mucus layers act as filter to remove pathogens while allowing gas and nutrient exchange within the underlying epithelial cells (extensively reviewed in [24]). Therefore, mucus production by epithelial cells is a physical barrier and rate-limiting step for drug targeting [25]. For instance in CF patients, the over-production of highly viscous mucus effectively limits the bioavailability of drug molecules. Additionally inhaled drugs may be trapped within the mucus and subsequently removed with multiple clearance mechanism. Adhesion interactions usually occur between mucus and drug particles via electro-static, hydrophobic and hydrogen bonding [26]. It is documented that mucus in CF patients is negatively charged and displayed high binding affinities toward positively charged aminoglycosides (tobramycin) [27]. More than 10-fold increases in mucus production were also observed in chronic bronchitis patients compared to healthy subjects. It is therefore useful to manipulate the physicochemical properties of drugs such as surface charges or hydrophobicity to avoid the entrapment of drugs in the mucus and increase the penetration of drugs across the mucus layers. Following the entrapment of deposited drug particles or foreign particles in the mucus is the elimination via

Table 1 – Water solubility and partition coefficient of anti-cancer drugs for lung cancer treatment.

Type of drugs	The partition coefficient (log P)	Water solubility	References
Paclitaxel	3.50	Practically insoluble	[16]
Docetaxel	4.10	Practically insoluble	[17]
Methotrexate	0.54	Practically insoluble	[18]
Cisplatin	−2.19	Soluble	[19]
Doxorubicin (HCl)	0.65	Soluble	[18]
Gemcitabine (HCl)	−1.24	Soluble	[20]
Etoposide	0.60	Sparingly soluble	[19]
5-Fluorouracil	−0.89	Sparingly soluble	[19]
Farnesol	5.31	Very slightly soluble	[21]
Celecoxib	3.68	Practically insoluble	[22]
Nimesulide	2.60	Practically insoluble	[19]

mucociliary clearance (extensively reviewed in [24]). The major roles of mucociliary agents are (i) to act as mechanical filter to entrap particles in the surface liquid onto the airway epithelium and clear by ciliary action. Most insoluble particles, with aerodynamic diameter larger than 6 μm, are eliminated by mucociliary clearance. Meanwhile, nano-sized particles are able to travel faster to reach bronchial epithelial region and escape the action, (ii) to provide antioxidant activities with its surface liquid, and (iii) to provide a surface biological interaction between microorganisms with luminal inflammation cells in order to prevent bacterial migration to airway epithelial cells. The clearance mechanistic is facilitated by hair-shaped structure like cilia, which are present on the topside of epithelial cells. Once foreign particles are trapped in mucus, cilia beat in a coordinated direction (pharynx) to remove the freight either by coughing or swallowing (extensively reviewed in [28] and [29]). Besides mucociliary clearance, phagocytosis is another major mechanism that is involved in the clearance of foreign particles in the lungs [30].

2.1. Formulation aspects: physicochemical properties of anti-cancer drugs

The physiochemical properties of drugs play an important role in therapy index. For that, most anti-cancer drugs are poorly soluble in aqueous physiological condition at pH 7.4. These include taxane-based drugs (paclitaxel and docetaxel) and camptothecin derivatives (9-nitrocamptothecin). The fundamental properties of anticancer drug such as log P and pKa values are important for designing the delivery methods as well as clearance from the lungs. According to Lipinski's rule, the solubility of anti-cancer drug would affect their permeability and potency of the cancer treatment [31,32]. Table 2 provides the water solubility and log P values of the anti-cancer drugs that are used in lung cancer treatment.

2.2. Host related response: efflux proteins

There are five major modalities of anticancer drug resistance: decreased drug influx, increased drug efflux, activation of DNA repair, detoxification and inactivation of apoptosis pathway. ATP-binding cassette (ABC) transporters represent a large family of trans-membrane proteins, which is an ATP-dependent efflux system expelling anticancer drugs from cytoplasm out from the cells. This is a normal defense mechanism from cancer cells to maintain their survival rate as efflux proteins act to pump out or decrease the intracellular concentration of anti-cancer drugs in cancer cell. However, due to their defense mechanism, severe multidrug resistance issue has occurred for cancer treatment. This means that the efflux of the anti-cancer drugs is regulated with transmembrane efflux proteins including P-glycoprotein (P-gp), multidrug resistant associated proteins (MRPs) and breast cancer resistant protein (BRCP) in cancer cells. P-gp is encoded by multidrug resistance-1 (MRP-1) gene and is one of the most studied efflux transporters for drug delivery into lung. It is present on the apical membrane of the bronchial and bronchiolar epithelium, in the endothelial cells of the bronchial capillaries and alveolar macrophages. It is accepted that interaction of anti-cancer drug with drug-binding domains of the transporter resulted in ATP hydrolysis at nucleotide-binding domains of the protein. Such hydrolysis reduced the affinity of drug and stimulated drug expelling from the cancer cells. In addition, treatment of other lung disease such as cystic fibrosis contributed to the up-regulation of P-gp level but this situation was not present for patients with COPD. This information is relatively important for lung cancer treatments, as most of the lung cancer patients are associated with COPD.

2.3. Aerosol drug delivery approaches

Systemic administration of anti-cancer drugs is a commonly used route to treat lung cancer even though this method often results in sub-optimal therapeutic concentration of drugs at tumor regions and damages to healthy cells/organs. Therefore local delivery via inhalation is a suitable alternative to deliver higher local drug concentration to the specific target side. Another advantage of pulmonary delivery is that it enables the delivery of low doses of inhaled drugs to lungs for localized effect while significantly reduces the toxicity of drugs toward organs or healthy cells. To date, four clinically successful aerosol pulmonary delivery systems based on devices include

Table 2 – The advantages and disadvantages of few nanosystems for pulmonary administration of anti-cancer drugs.			
Delivery system	Advantages	Disadvantages	Reference
Polymeric nanoparticles	High drug encapsulation efficiency, sustained release of drug and ability to escape alveolar macrophage clearance.	Owing to the lack of enzymes to break down polymeric materials, the toxicity remains an issue.	[33]
Liposomes	Suitable for delivering hydrophilic drug and enhancing the drug internalization into pulmonary cells via phospholipid interaction between cells and liposome. Highly biocompatible with cells. Alterability of physico-chemical characteristics such as surface charge and particle size is easy.	High production cost. Not suitable for room temperature storage which might cause drug leaking due to the instability of phospholipids.	[34]
Solid lipid nanoparticles	Biocompatibility is not an issue. Minimal induction of pro-inflammation marker expression in alveolar macrophages. Scaling-up friendly and higher stability compared to liposome. Highly suitable for pulmonary delivery as in suspension or dry powder inhalation.	Relatively low drug loading, drug leaking during storage and high water content of dispersions (70–99.9%)	[35]

dry powder inhaler (DPI), nebulizer, pressurized metered dose inhaler (pMDI) and soft-mist inhalers, and have been described in detail elsewhere [36]. pMDI and nebulizers are liquid-based aerosol systems whereby only the former requires the use of a propellant such as hydrofluoroalkanes (HFAs) and released through an orifice as spray upon actuation at a high velocity of 0.30 msec. However, it is reported that 10–20% of emitted aerosol could reach the lung parenchyma probably owing to lack of hand–mouth coordination and inspiratory flow rate. Meanwhile nebulized drug formulation often exists as solution or suspension without propellant and then atomized into droplets.

Nebulizers have been used many years in hospital setting to treat respiratory diseases and are feasible for elderly as well as young children (under 2 years of age). This technique is based on the principle of generating aerosol droplets from suspension or solution drugs and does not require specialized inhaled coordination. In contrast to pMDI and DPI, nebulizers can be used in cases where patients are unable to control their breathing or while receiving mechanical ventilation. In the case for treating lung cancer, nebulizers present stronger attractive possibilities for the delivery of chemotherapeutic drugs, especially for drug formulations developed as nano-sized particles in suspensions. Nebulizers are preferred as they could deliver larger amount of aerosolized drug in small droplets continuously over extended time periods. In theory nebulizers should generate high fine particle fraction (FPF) for satisfactory lung deposition, thus ensuring low doses required. In addition an ideal nebulizer should maintain drug stability and does not provoke any changes in formulation during nebulization process [37]. Several parameters have been identified to affect the efficiency of nebulized solution such as solution pH, viscosity, surface tension, drug concentration, and osmolarity. Other factors include the design of the device itself such as emptying (nebulization) rate and aerosol flow rate. Soft-mist inhalers are novel inhalers and are poised to overcome limitations of pMDI, DPI and nebulizers. Soft-mist inhalers are propellant-free, spacer-free and utilize the mechanical energy from the spring to actuate metered drug solution. It has been reported that the optimal aerosol generation by soft-mist inhaler is inspiratory flow rate independent while simultaneously could generate higher FPF compared to pMDI, DPI or nebulizers. In addition, the aerosol generated travelled much slower and had longer duration than aerosol from a pMDI [38].

The development of DPI has originally meant for overcoming poor actuation–inhalation coordination to treat lung diseases such as asthma and COPD. The advantages of DPI include high stability, non-invasive, sustained release profile to achieve modified pharmacokinetics, rapid onset of action, and without the use of propellant. In addition, for delivery of hydrophobic drugs with low tolerance to shear, formulations in the form of dried powder seem to be ideal. Compared to jet nebulizers, DPI is more convenient to use and does not require storage in cold conditions or reconstitution into solution prior to nebulization. A DPI formulation contains either respirable active drug or drugs blended with non-respirable excipients/carrier such as lactose. To date the only FDA-approved non-respirable carrier in the US is lactose. Meanwhile, several non-respirable carriers have been used (outside US) including mannitol, glucose, xylitol, sorbitol, raffinose, sucrose and maltitol. It is expected

that excipient used must possess close resemblance to endogenous substances in lung to evade premature clearance.

3. Rationale of development of inhalable nanoparticle-based drug for lung cancer

3.1. Size of nanoparticles

Although nano-sized particle is believed to be effective for cancer treatment, specific range of particles size is crucial to determine the effectiveness of the treatment, as some of them might be eliminated by organ or engulfed by alveolar macrophage. For example, nanoparticles with the size lower than 10 nm are likely to be eliminated via glomerular capillary by kidney, liver, spleen and lymph nodes, which is also known as reticuloendothelial system (RES). In the lung, alveolar macrophages clear the nanoparticles in the range of more than 100 nm [39].

3.2. Enhanced permeability and retention (EPR)

The EPR effect has an important role to retain drug in tumor tissues using tumor lymphatic drainage. As demonstrated in other published findings, there are some differences in terms of vascular cut-off pore size between tumors and healthy cells. Depending on the type and stage of cancer, the vasculature size of tumors ranged from 10 nm to 1000 nm. Meanwhile normal vascular cells are only permeable to particles less than 2 nm [40]. It has been proven that EPR influenced the accumulation of anti-cancer drug in tumors via passive tumor targeting. A particle size of 10–100 nm was found to be effectively targeted tumor based on animal model [39].

3.3. Surface properties

Surface properties of nanoparticles are known to influence the bioavailability of anti-cancer drug in tumor cells. The coating of hydrophilic polymer layers such as poly(ethylene oxide), poly-ethylene glycol and poloxamine could prevent phagocytosis ingestion by macrophages. Nanoparticles with positive charges could penetrate easily into tumor cells as higher binding activity between tumor cells and particles was noted. On top of that, the stability of nanoparticles in suspension is greatly improved, when the zeta potential values are above ± 30 mV, as the aggregations of particle are minimized [39].

3.4. Challenges for nanoparticle-based drug delivery in lung cancer therapy

The past few decades have witnessed the expedite development of nanoparticle-based medicine such as nanoparticle, liposomes, micelles in applications covering cancer diagnosis, imaging, detection and treatment. The ability to design nanoparticles as personalized medicine is seductive and ideal for lung cancer therapies. Combinational approaches with intricate balance between targeting moieties and anti-cancer agents have been widely documented in recent years. In a nutshell, multicomponent nanoparticle systems are usually

designed to encapsulate and stabilize poorly soluble anti-cancer agents while simultaneously anchored with specific targeting moiety on the surface to impart selective targeting at desired sites. However, the translation of nanoparticle-based drug delivery in lung cancer therapy specifically as inhalation medication to clinic is extremely challenging. Firstly, synthesizing nanoparticles designed with specific size distribution and ability to evade clearance as well as residing for sufficient time at targeted site has always been tricky and complicated. Therefore the identification of appropriate physicochemical characteristics (size, surface chemistry and shape) of nanoparticles is imperative to determine the biological behavior of particles in physiological environments. As such, crucial information such as particle–particle interactions, aggregation behavior, adsorption of proteins on nanoparticles and tendency to elicit immunological response in cells would be beneficial to design nanoparticulate systems to achieve the highest drug delivery efficiency. In addition, currently there is lack of established rules and regulations for the testing of nanoparticle-based medicine which include manufacturing, functional testing and safety evaluation. In theory a nanoparticle-based medicine should be evaluated in the same manner of any newly discovered drug that includes optimal design, reproducible manufacturing process, clear analytical methods for characterization, pharmacology, pharmacokinetic and toxicology profiles, and finally efficacy in clinical trial. However it should be noted that unlike conventional anti-cancer drugs which exist as single component, nanoparticles are complex and contain multicomponent. Therefore such variations should warrant the modification of standard testing of nanoparticles.

4. Application of nanotechnology for delivery of inhaled chemotherapeutic drugs

4.1. Polymeric nanoparticles

Poly(lactic-co-glycolic acid) (PLGA), a biocompatible and non-cytotoxic polymer, is the most commonly used either as carrier or excipient in drug delivery to achieve sustained release of drug. In a study by Tomoda and co-workers, nanoparticles-in-microparticles dry powder was prepared to ensure effective drug deposition into deep lung. For this PLGA nanoparticles loaded with anti-cancer drug (TAS-103) with average size diameter of approximately 200 nm were first synthesized as primary particle and subsequently spray-dried in the presence of trehalose as excipient [41]. Higher aerosol performance coupled with sustained release profile was achieved for PLGA nanoparticles loaded with 5% of TAS-103. In comparison, the FPF value for spray-dried formulation was 14.35% while only 0.79% was found for primary TAS-103 loaded PLGA nanoparticles. As expected the powdered formulation was more potent against A549 cells compared to free drug probably owing to enhanced internalization of drug nanoparticles via endocytosis or passive diffusion. It was hypothesized that the dry powder was completely decomposed into nanoparticle dispersion in the presence of cell medium [41]. The in vivo administration of spray-dried TAS-103 loaded PLGA nanoparticles via inhalation to rats demonstrated higher retention of drugs in the lung (approximately

13-times higher) compared to those delivered via intravenous injection [41]. A similar approach was employed to prepare a DPI formulation through incorporation of doxorubicin-loaded nanoparticle into inhalable carrier [42]. Doxorubicin was incorporated into poly(butylcyanoacrylate) nanoparticles using emulsion techniques and coated with both polysorbate 80 and dextran. These purified doxorubicin-loaded nanoparticles with mean particle size of 173 nm were then co-spray freeze-dried with lactose at low temperature to avoid decomposition and loss of drug activity. The mass median aerodynamic diameter (MMAD) of the DPI formulation measured using Andersen Cascade Impactor (ACI) at flow rate of 60 L/min was 3.41 ± 0.22 µm. Similarly, this DPI formulation containing doxorubicin was more cytotoxic against A549 and H460 lung cancer cells compared to free drug [42].

A highly porous PLGA microparticle loaded with doxorubicin was recently synthesized in double w/o/w emulsification method in the presence of ammonium bicarbonate [43]. These particles showed desirable aerosol characteristic with MMAD of 3.6 ± 0.4 µm and were retained in the lungs of C57BL/6 mice up to 2 weeks of administration using a dry powder insufflator. Remarkably, these porous microparticles significantly reduced the masses of B16F10 bearing metastatic lung tumors while exerting negligible toxicity to healthy cells [43]. To increase the selective targeting of cancer cells, the same group attempted to functionalize the surface of porous doxorubicin-loaded PLGA microparticles with Apo2L/TRAIL (tumor necrosis factor (TNF)-related apoptosis-inducing ligand). Apo2L/TRAIL is reported to specifically bind to death receptors such as DR4/TRAIL-R1, which are commonly overexpressed in cancer cells but not healthy cells [44]. The pulmonary administration of these particles resulted in the deposition in mouse lungs in which they remained in situ for a week. Furthermore, the reduction in the volume of BALB/c nu/nu mice bearing H226 metastatic lung cells was more pronounced, thus suggesting a synergistic apoptotic relationship between doxorubicin and Apo2L/TRAIL [44]. Recently the synergistic effect of doxorubicin and paclitaxel incorporated into porous PLGA microparticles was evaluated for treatment of metastatic lung [45]. Combination of doxorubicin and paclitaxel in solubilized form with ratio of 5 to 1 achieved the optimal synergistic therapeutic effect against B16F10 cells in vitro. The in vivo co-delivery of doxorubicin and paclitaxel by PLGA particles using insufflation was far superior in terms of reducing the lesion and volume of lung tumor compared to single therapy. For instance, the average lung weight of melanoma bearing mice treated with both drugs was 284.0 ± 19.49 mg while both mice treated with either doxorubicin or paclitaxel alone were 360 ± 33.17 mg and 420 ± 35.36 mg, respectively [45].

4.2. Liposomes

Liposome is one the most successful nano-based drug delivery systems to date with several FDA-approved liposomal formulations in the market [46]. Liposomes are self-assembled carrier with the presence of an outer hydrophobic lipid layer and a hydrophilic core. Liposome can be prepared in different sizes ranging from 50 nm to more than 1000 nm, depending on the composition of phospholipid and cholesterol molecules used. The presence of phospholipid layers makes

liposome an ideal carrier to encapsulate water insoluble drugs, especially most chemotherapeutic drugs such as paclitaxel and doxorubicin [39,47,48].

A multi-component liposomal formulation containing two cytotoxic agents (etoposide and docetaxel) and p53 was recently developed directed toward enhanced effects on A549 and H-1299 cell lines [49]. The enhanced synergistic activity was anticipated owing to increased apoptosis and necrosis following the restoration of p53 apoptotic function, thus leading to sensitization of lung cells toward cytotoxic agents. The liposomal formulation showed an average size of 200–350 nm and released behavior up to 24 h. Using ACI, excellent aerodynamic properties were observed for the liposomal formulation with a FPF and MMAD of 33–37% and 2–4 µm [49].

Paclitaxel liposome consisting of dilauroylphosphatidylcholine (DLPC) at drug-to-lipid ratio of 1:10 (w/w) was prepared in butanol and lyophilized to prepare a DPI formulation [47]. The lyophilized liposomal formulation was reconstituted in sterile water and vortexed to obtain homogenous suspension with particle sizes of 2.0–25.3 mm. Using an ACI at flow rate of 10 L/min and Aeromist jet nebulizer, the generated aerosol demonstrated an MMAD and a GSD of 2.2 µm and 1.9, respectively. The pharmacokinetic studies showed that the AUC in liposomal formulation delivered via nebulization was at least 20-fold higher than that of intravenous administration. Similarly, significant reduction in tumor number and increased survival time was recorded over duration of 2 weeks in aerosolized paclitaxel liposome [47]. The effect of varying drug to lipid ratio (1:7.5 w/w) during drug encapsulation process did not result in appreciable changes of aerosol performance whereby both the MMAD and GSD were 1.6 µm and 2.2, respectively [50]. It was however estimated that chamber aerosol exposure could yield deposition of 6.1 mg/kg paclitaxel liposomes to the lungs. Similar to previous study, the liposomal formulation containing paclitaxel demonstrated significantly lower lung mass and tumor surface areas compared to untreated mice [50].

The presence of cyclosporin A improved the anti-cancer effect of aerosolized paclitaxel liposome in mice. Using a Renca lung metastatic mouse model, BALB/c mice were injected with 100,000 Renca cells for 24 h and were subsequently treated with aerosolized paclitaxel liposome or co-cyclosporin A paclitaxel liposome. The aerosol generated using Aero-Mist jet nebulizer exhibited MMAD of approximately 2 µm. Weight loss was noted in mice with aerosolized co-cyclosporin A paclitaxel liposome by day 22 to 22 and no toxicity following treatment was observed in histopathological examination [50].

The pulmonary administrations of liposomal formulation of 9-nitrocamptothecin using nebulizers have been studied extensively both in vivo and in vitro in regard of the feasibility and therapeutic impact of aerosolized formulations against metastatic lung cancers [51]. Meanwhile, in vivo studies in which rats were exposed to aerosolized 9-nitrocamptothecin liposome or empty liposome showed negligible side effects, thus demonstrating its safety and tolerability. In a study by Knight et al., a liposomal 9-nitrocamptothecin formulation containing dilauroylphosphatidylcholine (DLPC) was administered at doses of less than 200 L g/kg daily, 5 days per week to rat to evaluate the therapeutic efficacy of drugs against various primary cancer xenografts in nude mice [52]. Using Aerotech

II nebulizer at flow rate of 10 L/min, the generated aerosol showed a MMAD of 1.6 µm [52]. The liposomal formulation showed higher efficacy compared to free drug in which tumor growth was greatly suppressed after weeks of treatment [52]. In two follow-up studies, it was found that liposomal 9-nitrocamptothecin formulations could serve as preventive treatment and effective against established lung metastasis [51]. In the first study, liposomal 9-nitrocamptothecin was delivered to C57BL/6 mice immediately after injection of melanoma cells and the treatment was continued for 1 h, 5 days a week for 3 weeks. Fewer lung metastases in treated mice were observed compared to untreated mice, thus showing a preventive effect of drug. In a second model, the aerosol was only delivered after nine weeks of injection of osteosarcoma cells in nude mice [51]. Based on the promising in vivo results, Phase I clinical trial was performed on twenty five patients with either primary or metastatic lung cancer [53]. Aerosols were generated using a jet nebulizer in a HEPA-filtered airborne scavenging tent. This study was performed to evaluate the tolerability and safety of pulmonary administration of liposomal 9-nitrocamptothecin and to determine the recommended Phase II dosages based on an 8-week treatment regime. In the first part, patients received aerosolized liposomal 9-nitrocamptothecin for 5 consecutive days/week for 1, 2, 4, or 6 weeks followed by 2 weeks of rest to determine feasibility. Compared to side effects which occurred using oral route, it was interesting that hematological toxicity was not noted for pulmonary administration. The dose limiting toxicity of aerosolized liposomal 9-nitrocamptothecin was grade 3 chemical pharyngitis. Other side effects include nausea, vomiting, cough, wheezing, chest congestion and sore throat. In addition, lower aerosolized dose was required to achieve similar 9-nitrocamptothecin plasma levels to those seen in oral route. Some patients involved in the trial responded partially to the treatment establishing the therapeutic potential of pulmonary administration of 9-nitrocamptothecin formulated in liposome [53].

Several preclinical studies have nudged that liposomal entrapment of cisplatin did not alter the cytotoxic properties of the drug [54]. The in vitro cytotoxicity and in vivo pharmacokinetic profiles of aerosolized cisplatin loaded into lipid vesicles (Sustained Release Lipid Inhalation Targeting, SLIT) were previously investigated using human tumor cell line (NCI-H460) and Sprague-Dawley rats, respectively [54]. The formulation of cisplatin into lipid-based vesicles showed comparable cytotoxicity activity to free cisplatin. The IC50 values of SLIT cisplatin and free cisplatin were 0.55 and 0.49 µg/mL, respectively. Meanwhile, intratracheal instillation of SLIT cisplatin in rats exhibited higher accumulation in lungs and reduced exposure to kidney, thus minimizing the risk of nephrotoxicity. In addition, no histopathological changes were observed in lungs, kidney or bone marrow following 14 days of inhaled administration of SLIT cisplatin. In terms of anti-cancer potential of SLIT cisplatin, inhaled administration of the formulation dramatically reduced the burden of tumor in metastatic model. A phase I clinical study was set up to investigate the safety profile, maximum tolerated dose and pharmacokinetics of aerosolized SLIT cisplatin in seventeen patients with lung carcinoma [55]. Dose-escalating regime of SLIT cisplatin was aerosolized to patients during 1–4 consecutive days in a 21-day treatment cycle. The

aerosols generated using PARI LC Star nebulizer had a MMAD of 3.7 μm and GSD of 1.9. No dose limiting toxicity was observed at the maximum delivered dose. The common systemic toxicity related to cisplatin such as hematologic toxicity, nephrotoxicity, ototoxicity and neurotoxicity was not observed. The most common side effects were nausea and vomiting as well as a grade 1–2 decrease in FEV_1 [55].

In another study, a phase I study was designed to evaluate the toxicity and suitability of inhaling interleukin (IL)-2 liposomes to patients with pulmonary metastases [56]. The main objective of the study was to determine the maximum doses tolerated which would be biologically active while not exerting toxic effects in an outpatient setting. The liposome containing IL-2 was nebulized using a Puritan twin jet nebulizer for approximately 20 min, 3 times a day. All patients completed at least 8 days of treatment and could perform normal activities during treatment, thus confirming that significant toxicity was not present. Based on the data, the optimal dose and timing of IL-2 liposome via inhalation are 3–6×10^6 IU and 3 times a day [56].

4.3. Gelatin based nanoparticles (GNP)

Gelatin is a denatured protein obtained either through acid or alkaline hydrolysis of animal collagen. The interesting feature of gelatin is that this polypeptide contains cationic, anionic and hydrophobic amino acid groups with ratio of 1:1:1. As such, gelatin polypeptide is ~13% positively charged, ~12% negatively charged and ~11% hydrophobic in nature [57,58]. Gelatin based nanoparticles (GNP) have been studied in detail for the delivery of hydrophilic and hydrophobic anti-cancer drugs such as paclitaxel [59], cisplatin [60], curcumin [61], resveratrol [62] and methotrexate [63]. Similar to other nanotechnology approaches, GNP demonstrated enhanced anti-cancer activity, sustained drug release and exerted very low toxicity to cells [58]. From the economic point of view, the cost of gelatin is low and the good reproducibility of gelatin makes the prospect of future upscaling promising [58]. The superior efficacy of cisplatin-loaded biotinylated-EGF-modified-GNP compared to free cisplatin solution was manifested against A549 lung adenocarcinoma cells in vitro. The IC_{50} was as low as 1.2 μg/mL for cisplatin-loaded GNP compared to free cisplatin (2.54 μg/mL) [60,64]. In addition, the accumulation of cisplatin in lung cancer following inhalation in mouse was much higher for the nanoformulation compared to free cisplatin solution [60]. Analysis of aerosol droplet size following nebulization using DUST monitor revealed that 99% of the particles had MMAD (0.5–5 μm) within suitable range for airway deposition [65].

4.4. Others

An interesting concept involving the use of effervescent molecules as excipient during formulations of doxorubicin nanoparticles was published recently [66]. Effervescent technology is based on the concept of employing carbonated or citric-based molecules which form gas bubbles when in contact with aqueous solutions, thus increasing the phase transition from gas to liquid and hereby achieving a more rapid action. Roa and co-workers evaluated the effectiveness of using effervescent components in their formulations containing inhalable doxorubicin nanoparticles. Tumor bearing mice treated with effervescent nanoparticles survived longer compared to those administered with non-effervescent nanoparticles [66]. Meanwhile, Al-Hallak and co-workers demonstrated that effervescent nanoparticles containing doxorubicin achieved deep lung deposition, were distributed primarily in the lung and negligible accumulation in other tissues or organs [67].

Pulmonary instillation of free doxorubicin had been shown to be completely absorbed within minutes. To prolong and liberate the exposure of lung cancers to chemotherapeutic drugs, Kaminskas and co-workers recently explored the possibility of using PEGlated polylysine dendrimer in conjugation with doxorubicin [68]. After intratracheal instillation to rats, an average of 60% dendrimers was cleared from the lungs within 24 h followed by a slower clearance phase. The bioavailability of the dendrimer-conjugated doxorubicin was in the range of 10–13% and approximately 15% of drugs remained in the lungs after a week of administration. A comparison between the efficacy of administered route demonstrated that dendrimer-conjugated doxorubicin delivered via pulmonary led to a >95% reduction in lung tumor burden after 2 weeks. Meanwhile, only 30–50% reduction was observed in rats receiving drugs via intravenous administration [68].

As mentioned, apoptotic TRAIL protein exhibits high affinities toward over-expressed death receptors in abnormal cells. In a recent study, hydrophobic self-assembled nanoparticles consisting of doxorubicin, octyl aldehyde and human serum albumin (HSA) were functionalized with TRAIL protein to increase synergistic apoptotic effect [69]. Using an aerosolizer, TRAIL-doxorubicin-HSA conjugates were well deposited in mouse lung and demonstrated gradual release of drug over 3 days [69]. Recently, Taratula and co-workers explored the feasibility of mesoporous silica nanoparticles (MSN) as carrier for inhalation therapy with specific targeting to lung cancer [70]. The formulation contained five main components: MSN, chemotherapeutic drugs (cisplatin and doxorubicin), suppressors of pump (MRP1) and non-pump (BCL2) drug resistance and targeting ligand (LHRH peptide) specific for lung cancer. The simultaneous delivery of chemotherapeutic drugs and suppressor proteins via inhalation to A549-bearing NCR nude mice led to higher accumulation of nanoparticles in the lung. In addition, the delivery route preserved the activities of both chemotherapeutic drugs and siRNA, which in turn resulted in enhanced apoptotic actions in lung cells [70,71].

In another study, the encapsulation of COX-2 inhibitor, celecoxib into nanostructured lipid carriers using triglycerides such as compritol and miglyol, was reported via high pressure homogenization method [72]. The suspension of celecoxib-loaded nanoparticles showed an average diameter of 217 ± 20 nm and demonstrated controlled release kinetics in vitro for extended period of time. The aerosol performance of the formulation was determined using ACI with Pari LC Star jet nebulizer. The nebulized droplets presented an FPF, MMAD and GSD of 75.6%, 1.6 μm and 1.2, respectively [72]. In addition, nebulization of celecoxib loaded nanoparticles demonstrated 4-fold higher area-under-curve (AUC) in lung tissues, and thus improving its bioavailability in vivo [72].

The in vivo pharmacokinetics of 5-fluorouracil lipid coated nanoparticles (LNP) in hamster following inhalation was investigated to determine its feasibility for lung cancer therapy

[73]. Approximately 30 mg LNP/kg body weight (1.5 mg/kg 5-fluorouracil) was delivered using atomizer at 1.7 MHz ultrasonic drive. It was found that the nanoparticles were mainly deposited in the respiratory tracts, specifically in the lung, trachea and larynx. The nanoparticles were cleared away from respiratory tract within 24 h [73].

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