Potential of Liposomes for the Treatment of Lung Cancer- A Review

By

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Declaration

It is here by declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.

2. The thesis does not contain material previously published or written by a third party,except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

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Approval

The project titled "A Review on Potential of Liposomes for the treatment of lung cancer" submitted by Shanzida Mim (18346049) of Summer, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelors of Pharmacy (Hons.) .

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Ethics Statement:

The thesis was completed without doing any unethical acts. This study does not involve with any animal or human trials.

Abstract

Lung cancer is the leading cause of all cancer deaths worldwide among both men and women with symptoms of cough, airway obstruction and hemoptysis. Lung cancer can be divided into two subtypes which are known as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Surgical resection, radiation therapy, chemotherapy, targeted medicines for NSCLC with driver oncogene mutations, and immune checkpoint medications are now available as first-line NSCLC treatments. However, treatment outcomes of such types of cancers is still not adequate and chemotherapy results in more severe systemic adverse effects as well as recurrence of cancers. As a result, new and effective treatment options needs to be researched upon for the treatment of lung cancer, specifically NSCLC.Targeted drug delivery strategies for the treatment of lung cancer are of utmost importance not only as it site-directed treatment targeting the cancer cells but also it has the potential to reduce metastasis and increases the effectiveness of the lung cancer therapy and complements the treatment associated with surgical resection and radiotherapy. In this context, liposomes offer the unique possibility of targeted treatment of lung cancer due to their ability to pass through the leaky tumour blood vessels owing to their small size (100 nm) and accumulate in the cancerous tissue, a phenomenon known as enhanced permeability and retention effect to release the encapsulated drug at the target site.The present review provides an overview of the potential of liposomes in the treatment of lung cancer. Classification of different types of liposomes have been discussed along with their therapeutic applications in the treatment of lung cancer with a direction towards future use of liposomes in lung cancer treatment.

Keywords:, Liposome,Lung cancer, Aerosol Formulation, Inhalation

Dedication

This paper is dedicated to my dear parents.

Acknowledgement

All praises and glory to Almighty Allah (SWT) who has given me enormous courage,knowledge, wisdom and patience to carry out and complete this thesis. Peace and blessing ofAllah be upon last Prophet Muhammad (Peace Be upon Him).First of all, I am so grateful to my supervisor Dr. Zara Sheikh (Asst. Professor, School ofPharmacy, Brac University) for guiding me. I would like to thank her for believing in my abilityto complete my thesis. Throughout this process, I am eternally grateful for her constantguidance, support, wisdom, optimism, and inspiration.I would like to express my heartfelt gratitude to Professor Dr. Eva RahmanKabir (Professorand Dean, School of Pharmacy, Brac University) for her constant guidance, knowledge,patience, and a warm spirit to support me throughout my B.Pharm journey and Professor Dr.HasinaYasmin for her constant support as well. I would like to thank all the faculty membersof the School of Pharmacy, Brac University for guiding me to become a pharmacist.Finally, I would like to express my deepest gratefulness to my loving parents MohammadUllah& Mrs. Shaheda Begum for giving me all the love, support and continuousencouragement all the time.I would like to give thanks everyone involved for their great assistance in this regard.

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List of Acronyms

SUV: Small Unilamellar Vesicles

MLV: Multilamellar Vesicles

LUV: Large Unilamellar Vesicle

HSPC:Hydrogenatedsoybeanphosphatidylco-lineSCLC:Small cell lung cancer

NSCLC:Non-small cell lung cancer

FDA:U.S Food and Drug Adminstration

HSPC:Hydrogenated Soybean Phosphatidyl Co-line

CA :Cyclosporine A

9-NC:9-Nitrocampto Tecin

pMDI:Dose Inhaler(pMDI),

DPI:Dry Powder Inhaler

SMI:Soft Mist Inhalers

MN:Medicated Nebulizers

CFC:Chlorofluorocarbon

DOX:Doxorubicin

5-FU :5-Fluorouracil;

CXR : Chest X-Ray;

PFT : Pulmonary function test;

CBC ; Complete blood count;

BUN : Blood Urea Nitrogen;

AST ::Aspartate transaminase;

ALP : Alkaline phosphatase;

ALT :Alanine transaminase;

LDH : lactate dehydrogenase;

DLCO : Diffusing capacity of the lung for carbon monoxide;

LNP :Lipid-coated nanoparticles;

DPPC :Dipalmitoylphosphatidylcholine;

DLPC :Dilauroylphosphatidylcholine;

DMPC :Dimyristoylphosphatidylcholine;

EPC-Chol : Egg phosphatidylcholine with cholesterol;

PEG :Pegylateddistearoylphosphatidylethanolamine.

DLT:Dose-Limiting Toxicity (DLT)

Chapter 1

Introduction

1.1 Background

Lung cancer refers to epithelial malignancies that develop in the bronchial mucosa and occasionally in the lung parenchyma, which can be seen in the trachea, bronchi, or airways in the lungs(alveoli).Cough, airway obstruction, and hemoptysis common symptoms of lung cancer in both sexes, and it is the most common malignancy to cause death globally.There were almost 200 thousand newly diagnosed cases of lung cancer in 2018, with approximately more than 142,000 deaths. Lung cancer peaks between 73 and 84 years of age and the total less than 10% of people survive five years., mostly because the majority of patients die within that time frame. Smokers are generally more likely to develop lung cancer, which accounts for 24% of all cancer-related fatalities (Xiangwei Xu 1, 17 November 2022).

Lung cancer can be divided into two subtypes which are known assmall cell and non–small cell (NSCLC). However, About 85% of all cases of lung carcinoma are NSCLC, which makes up the majority of lung cancer cases (J. Liposome Res. 2018, 28, 236–248).Surgical resection, radiation therapy, chemotherapy, targeted medicines for NSCLC with driver oncogene mutations, and immune checkpoint medications are now available as first-line NSCLC treatments.However, treatment outcomes of such types of cancers is still not adequate owing to delayed diagnosis, a lack of first-line chemotherapy resulting in reduced survival rates following surgical treatment (Tannock, 2012).Additionally, chemotherapy results in more severe systemic adverse effects as

well as recurrence ofcancers.Furthermore ,chemotherapy is less successful because of the fact thasince lymphoid tissue is widely distributed in the lungs, cancer cells may spread to other places of the body This leads to recurrence of lung cancer, posing a significant obstacle to effective NSCLC treatment. As a result, for the treatment of lung cancer and more specially NSCLC,novel and effective therapy approaches must be investigated (A.c, 2015).

1.2 Rationale and Aim of the Review*:*

In addition site-directed treatments that target the cancer cells,the use of specific drug delivery methods in the treatment of lung cancer may help to lessen metastatic spread.,which, as a result of the cancer's unchecked spread throughout the body, is the major killer worldwide,ranking in the top five for people with lung cancer. Therefore, targeted treatment increases the effectiveness of the lung cancer therapy and complements the treatment associated with surgical resection and radiotherapy (Tannock, 2012).In this context, liposomes offer the unique possibility of targeted treatment for different types of cancer, including lung cancer.

Anticancer drugs such as Paclitaxel have poor water solubility, thus limiting their use for pulmonary drug delivery(Koukis et al., 2013).Additionally, because the pulmonary epithelium is a thin layer, drugs that are targeted to the lung have a shorter residence period there, which could have negative systemic consequences.Distinctive features of liposomes make them potential adverse drug carriers for something like the treatment of several forms of cancer.Since the permeability and retention have been enhanced, liposomes are able to collect in malignant tissue and pass through leaky tumor blood arteries because of their small size (100 nm). This allows the drug to be delivered to the specified location..The lung offers special targeting options because of

its vast surface area, inadequate first-pass metabolism, greater absorption, and increased lung epithelial permeability. Inhaling controlled release systems in the form of liposomes may localize the drug's persistant effect on the lungs, providing a sustained effect, boosting therapeutic benefit of the cancer drug while lessening it's side effects on the body as a whole.

Therefore, the development of targeted anti-cancer drugs to the lungs is fundamental to achieve improved treatment outcomes for lung cancer.In order for the FDA to approve a new molecule, it would take between 10 and 14 years to complete all the clinical trials to ensure the safety and efficacy investigations on a new anti-cancer drug. One practical way of reducing this time to 4 to 5 years while lowering drug development costs is drug repurposing (i.e., researching old medications for their new therapeutic purpose).Medicine repurposing offers a chance to find a new use for an outdated FDA-approved drug(Clancy et al., 2013).

The purpose of this paper is to give a general overview of liposomes' potential for treating lung cancer. Classification of different types of liposomes have been discussed along with their therapeutic applicationswith an eye on the future use of liposomes in the treatment of lung cancer (Olga B. Garbuzenko, 2009).

Chapter 2

Liposomes Types and Methods of Preparation

2.1 Classification of liposome

Liposomes can be classified depending on size and the number of phospholipid membrane layer figure1 illustrates the vesicles can be broken down into three distinct types:Multilamellar(MLV), Small unilamellar(SUV) and Large unilamellar(LUV)(Akbarzadehetal.2013;Pattnietal.2015). Multilameller Vesicles(MLV) are liposomes with an aqueous phase and concentric phospholipid bilayer,typically ranging in size from 1 to 5 m (Figure 1A).

The size of unilamellar vesicles(LUV).The only lipid bilayer in each of these liposomes is encased in an aqueous compartment.These liposomes have a size range of 100-200nm (Figure 1B).SUVs or small uni-lipid vesicles,are liposomes that consist of a single lipid bilayer around an aqueous core(B).Liposomes fall between 20 and 100 nm in size (Figure 1C).

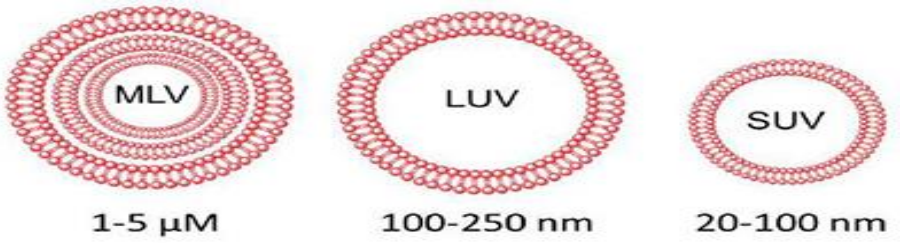

Figure 1- shows the lamellarity-based classification of liposomes.(A) the size of Multilamellar Vesicles (MLV) is between 1 and 5 m and is made up of many lipid bilayers.(B) A single lipid bilayer is present in LUVs or large unilamellar vesicles, are between 100 and 250nm in diameter(C).Small Unilamellar Vesicles(SUV),ranges in size from 20 to 100nm,are made up a single phospholipid bilayer that completely surrounds the liquid**.**

2.2 Preparation techniques for liposomes

A number of methods are available to prepare liposomes namely solvent removal,detergents removal, demulsification injection of ethanol(Laouini et al. 2012; Bozzuto and Molinari 2015).Factors such as medication loading efficacy,size and form stability of liposomes are all influenced by the production procedure. Water or solvent oflipidfilmsremoval method is the most common employed for the manufacture of liposomes (Akbarzadeh et al. 2013; Bozzuto and Molinari 2015).Usually,the fats and oils are combinations of dissolved chloroform and or methanol and the lipid concentration are typically in this range 10-20 mg/mL that is influenced by lipid solubility. Next using a rotary evaporator with lower pressure,the solvent is eliminated resulting in the formation of a thin layer of lipid film.After the thin layer has been separated for the necessary amount of time,hydration comes next.Then,by adding an aqueous solution with a physiologically appropriate osmolarity,dry lipid flims are made to become hydrated.Oncehydrationis complete,Multilamellar vesicles (MLV) are generated in size range from (200to1000) nm (Laouini etal.2012;Akbarzadehet al. 2013).

Soncation is commonly done in a waterbath. The type of ultrasonic device and the water is kept at a temperature that avoids crystallization ,the lipids temperature.Sonic swells causes disruption of

liposomes,culminating in the production of Small Unilameller vesicles with sizes between 20 and 100 nm(Lauinietal.2012.Akbarzadehetal.2013).The amount of sonication of energy is only one factor in how big the liposomes will actually be but depending on many factors,including lipid composition,concentrated and volume of suspension.Extruded liposomes typically have final sizes that are closer to filter pore sizes.The ability to produce large,repeatable unilamellar vesicles(LUVs) are produced by extrusion with 100nm filter pores in general. (Pardis Kalantarian 1, 2010)

2.3 Drug liposomal Encapsulation

The passive loading and the active loading subgroups of drug encapsulation in liposomes can be distinguished.Active loading refers to the loading of drugs after vesicles of formation,whereas passive loading refers to the process of encapsulation when the drug is included during the vesicle creation process (TYAGI et al 2011).

2.3.1 Passive loading

During the liposome manufacturing process,the drug is,"passively loaded,"or encapsulated.In case of the hydrophillic medications, the therapeutic agent is loaded into the liposome's interior by reacting with the hydration buffer used to saturate the lipid bilayer before hand.Drugs that are lipophilic area are combined with other lipid components to generate a thin,dry lipid flim,which is injected into the lipid of bilayer of the liposome.By either gel-filteration chromatography or dialysis; the encapsulated liposomes suspension is filtered to eliminate the drug molecules

(Tyagi et al. 2011; Tyagi et al. 2013).

A measure of liposome formulation effectiveness,encapsulation efficiency depends on a number of variables including lipid content,liposome size,type of lipid choice,etc if passive technique of loading is used,the amount of water volume entrapped in liposomes determines how well hydrophillic medicine that do not interact with lipid are encapsulated (Tyagiet al.2013). Compared to the smaller vesicles, large vesicles have a much higher encapsulationefficiency. On the other hand, drugs with greater lipid solubility (lipophilic drugs) show a much a higher encapsulation efficiency owing to the greater interactions with lipid bilayers of the liposome(Akbarzadeh et al. 2013).

As a result, a number of techniques have been created to increase the encapsulation effectiveness by attaching lipophillic chains to chemical compounds that increase the lipophilicity,allowing for higher partitioning into lipid bilayers(Stradal and Ratful 2014; Bozzuto and Molinari 2015). Another crucial factor that highly influences the efficiency of the liposomes isthechoice of lipid composition.When choosing a cationic lipid to load strongly negatively charged molecules,for instance,the improved drug /lipid interaction will signicantly increase the encapsulation efficency(Bozzuto and Molinari 2015).

2.3.2 Active Loading

As a result of pH or ion gradiant that are established across the liposomes lipid bilayer,liposomes are capsules into which pharmacological molecules can be inserted that have been performed either actively or remotely using electrochemical potential.When a buffer is used with pH and ion concentration must be maintained,pH or ion gradients are established and then by using dialysis or size exculsion chromatography,the external pH of liposomes is exchanged with a different

buffer having different pH or ion concentration.By combining the drugs with the liposomes at a temperature higher than the lipids phase transition temperature,the pH gradient across the lipid bilayer is established. This is done to make sure that the lipids bilayers of the liposomes are fluid and effective for transport. Inorganic compounds used in pharmaceuticals to get charged via interaction with the ions with the liposomes and as a consequence the charged drug molecules remain enclosed with the lipid core of the liposome. A classic example of the active loading of liposomes liposomal Doxorubicin,Doxil™ , by the method of pH gradient as is shown in Figure 3 (Lasic et al. 1992; Haran et al. 1993). As it is shown in the Figure 3, a pH gradient is developed when citrate buffer liposome gradient is 1000 times greater than to the citrate buffer of themedium.The(Doxorubicin)foundation is somewhat shaky that exists in a state of balance between ionized and non-ionised state and these non-ionised doxorubicin are able to the cross the lipid bilayer of the liposome and thus turns into the ions in the high-proton intra liposome envirnment resulting in highly efficientdoxorubicin accumulation within the liposome (Figure 3).

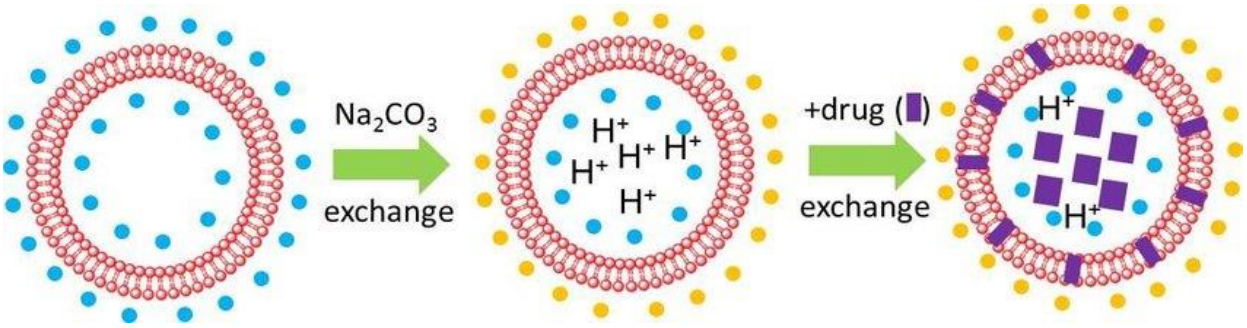

Figure 3-For the pupose of drug loading,liposomes were actively generated by first hydrating in citrate buffer, and exchanging the exterior phase with Na_2CO_3 to provide pH gradient.The neutral version of the medicine given externally can pass through the bilayer,where it will be protonated and get trapped inside the vesicles.

Chapter 3

Liposome's ability to carry drugs safely to the lungs

In the recent years of drug development, liposomes have gained considerable interest in the market for drug delivery to the lungs,because of the medicinal moiety entrapment via liposome vesicles,after inhalation and the localized effect of the drug at the site of action for an extended duration of action, thus providing a sustained effect.According to reports,a drug's efficacy is increased when it is supplied in the form of liposomes and the risk of any systemic side effects is significantly decreased (Clancy et al., 2013; Saari et al., 1999).

In light of the fact that phospholipids with or without cholesterol are used to create liposomes, The compatibility, safety, and effectiveness of liposomes for inhalation medication delivery is further enhanced by the similarity of these components to pulmonary surfactants found in the human lung epithelium due to their natural likeness (Clancy et al., 2013; Saari et al., 1999).Several studies have reinforced the greater extent of compatibility andwhether or not liposomes are biodegradable when utilized as medication carriers in inhaled formulations. In the early days, it was proposed that liposomes could be usedas an alternative for surfactant in the treatment of individuals suffering from acute respiratory distress syndrome.As a result, surfactants for the lung based on phospholipid combinations have recently entered the market (such as Survanta) for the prevention of respiratory distress syndrome in newborns(Paul et al., 2013).

The safety of liposomes for drug delivery to the lungs has been studied widely(Saari et al., 1999)have demonstrated that inhalation of HSPC(Hydrogenated soy phosphatidylcholine)

liposomes has no deleterious effect on the alveolar microphages in animal models(Saari et al., 1999).In animal studies, long-term nebulization of liposome concentration of up to 150mg/ml of either SPC or HSPC, for example, for 30 minutes, has not been seen to cause any issues (the lung of sheep).However, the utilized dose for these studiesshould be considered primarily due to dose –related toxicity variesfor various phospholipids.The safety of inhalation has been demonstrated through numberous research involving human participants. Arikase (amikacin liposome inhalation suspension)is a brand new formulation of an antibiotic that has demonstrated to be safe for pseudomonas and efficacy when used in phase II therapeutic studies with individuals with human cystic fibrosis.Overall, from pulmonary anti-cancer drug deliveryviewpoint,liposomes containing harmful substances ,such as anti-cancer medicationare ideal candidates owing to their established safety and efficacy in human subjects as.The carrier,i.e. liposomes does not cause any harmful consequences, and the therapeutic activity is focused on the lungs and limited to the designated lung region.

Chapter 4

Liposomal Drug therapy as a Systemic Therapy for Lung Cancer

The fact that eighty percent or more of lung cancers are resistant to treatment compeletely to chemo therapy is a major roadblock in the treatment of this diseases, resulted in the cancer returning(Gautam et al., 2002).The current treatment plan for the different stages of lung cancer is given inTable 1.One of the limitations of lung cancer treatment is resistance tumour to standard cytotoxic chemotherapy for lung cancer(*Lung Cancer: Diagnosis and Management NICE Guideline,* 2019).High doses of chemotherapeutic medicines are one strategy for overcoming the acquired resistance,which results in dose-related toxicity to healthy organs. Therefore, evidences of the limited treatment outcomes of lung cancer in association with increasingly adverse effects to a high degree,the state of the art in lung cancer therapy demands the exploration of novel approaches for administering medications (Zhou et al., 2013).In this context,the effectiveness of chemotherapy for lung cancer patients may be greatly enhanced by the use of liposomal drug delivery systems,which may show promise for the inhalation of anti-cancer treatment.Table 2 is a summery of the many studies that have looked into the feasibility of liposomal pulmonary delivery of anti-cancer medicine.

To achieve the maximum therapeutic effect for an anticancer drug delivered to the lungs, the concentration of the therapeutic agent (anticancer drug) the lung should be elevated enough to allow access to the tumour site numerous investigations have demonstrated that lower medication concentrations reaching the cancer site following systemic chemotherapy is the main cause of diminished therapeutic efficacy and subsequent treatment failures (Minchinton & Tannock,

2006).This points out the significance of administering chemotherapy drugs straight to the lung in cases of lung cancer if this is the effective course of treatment(Koshkina et al)Liposomal Paclitaxel(PTX; a commonly used anti-cancer medication) was administered intravenously and by inhalation to mice at varying doses to determine its pharmacokinetics and therapeutic efficacy.It was found that pulmonary administration of PTX resulted in longer drug residence time and greater drug concentrations in the lung compared to intravenous administration,resulting in a more potent therapeutic effect (Fig.2).Therefore, high concentration of medicine reaching the site of the tumour after aerosolization thanks to the anti-canccer molecules getting into the lungs accounts for the increased therapeutic efficacy exhibited by PTX aerosol therapy.

How well the liposomes works from the drug used to treat cancer doxorubicin(DOX) delivered to the lungs of mice after intratracheal administration was studied by Garbuzenko (Garbuzenko et al., 2009) and compared to intravenous delivery.The study's result showed that the administration of DOX via the intratracheal route was much more efficient in restricting lung cancer growth with fewer adverse effects on healthy organs.When contrasting aerosol with intra cellular delivery,it is important to consider the following(Hitzman et al., 2006), compared the liposomal formulation for aerosol delivery with intracellular delivery and found highly similar pattern of five-flurouracil release liposomal carriersand liposomal formulation reaching the lower respiratory tract.

IL-2 has shown anti-tumour efficacy and being researched as a potential cancer therapy in the future(Antony & Dudek, 2010). A number of studies involving the incorporation of IL-2 into liposomes have been investigated that has given some promising results.InhalationofIL-2liposomes aerosol formulation demonstrated a 5-fold increase in the production of broncho alveolar lavage leukocytes i n the lungs compared to free IL-2 (Wittgen et al., 2007).Thesignificant increase in leukocytes production following liposomal inhalation formulation of IL-2 suggeststriggering pulmonary immune system

indicating its potentials to be employed as a gauge of an ant-cancer drugs efficacy. Liposome delivery through the lungs has also been shown to be safe and effective for treating dogs with lung cancer that started in the lungs.IL-2 liposomes are not harmful,according to the phase I investigation and anti-tumour efficacy has been noted.To date, there are no randomized clinical trial studies about IL-2 liposomes showing evidence of progression to phase-II trials.Unlike many other diseases,cancer is treated with a combination of different stratigies such as surgery oral/intravenous cthemotherapy , radiotherapy. Recently, aerosolized IL-2 may help prevent cancer from coming back in people with pulmonary melanoma metastasectomy,according to a study.Considering the improvements in inhalation devices for getting drugs to the lungs which enables maximum drug retention with the use of liposomes for inhalation,it could be postulated that IL-2 clinical trials may prove to be a potential anti-cancer drug in form of liposome formulations along with the improvements in inhalation devices for getting drugs to the lung.

One of these most often utilized medications in today's lung cancer therapies is cisplatin(Table 1).However,nephrotoxicity and peripheral neuropathy are linked to its dose-limitng toxicity when administered systemically.and totoxicity.Therefore, in this context liposomal formulation of cisplatin could overcome the possibility of these side-effects for anti-cancer drug delivery. Safety and efficacy of aerosolized liposomal cisplatin was shown in Phase-I studies for primary or metastatic lung carcinoma treatment(Wittgen et al., 2007).Outcome of the Phase I studies showed very few side-effects with no dose-limiting toxicity when the majority of patients(80% of the patients) received the maximum administered dose,demonstrating the liposomal formulation's safety and effectiveness in comparison to intravenous drug delivery. The low drug decomposition in the target in this Phase I research was one of its primary drawbacks,lung region which could possibly due to the fact that the study was caaried out in absence of a 5% CO_2,as revealed in a different

investigation,concentration in the nebulizer (Koshkina et al., 2001). The drug cisplatin liposome (SLITCisplatin) was created and byTransaveInc.(the same company who created the Arikase liposomal amikacin formulation).Insmed bought Transave Inc.in 2010,took Arikase to more advanced stages of development,and "handed over" Cisplatin to Eleison Pharmaceuticals LLC for more clinical research .

Camptothecin,9-NC and its analogue have shown solubility issues with reduced bioavailabilityand dose limited toxicity, posing significant problems to the development of an effective anti-cancer therapy, thus limiting its uses a potential anti-cancer treatment (Chen et al., 2013). As a consequence,Several studies using animal models and clinical trials have looked into the possibility of using aerosolized liposomal 9-NC formulations to address these concerns. These studies have collectively shown that the use of inhalation liposomal formulationsof 9-NC demonstrated improved efficacy along with reduced toxicity compared to other conventional formulations using other modes of drug delivery. Specifically, animal studies using liposomal 9-NC on lung tumour xenograft shave reported that these clinical efficacy of liposomal 9-NC aerosolized particles in reducing tumor size,with markedly lower toxicity profiles.These results are particularly in advantages of local aerosol administration as it provides concentrations that are similar to parenteral drug administration with reduced side effects compared to the widespread use of 9 –NC.A clinical experiment using aerosolized liposomal 9 NC(Phase I revealed) that founded one out of six patients with primary lung cancer experienced partial remission,while three others saw their tumors stabilize. The initial dose is 13.3g/kg day was determined to be better tolerated and safe for all patients based on these Phase I investigation and was therefore advised for phase II trials.Very mild side-effects were reported in this study such as pharyngitis effects like feeling sick,throwing up,being tired and coughing. Overall,a faster absorption profile with minimum

adverse effects were reported in these clinical trial.Importantly,no haematological toxicity was associated with studies done,the use of liposomal 9-NC instead of systemic 9-NC delivery is better by Tedescoetal(Tedesco et al., 2005).

A number of many studies have been reported by combining,9-NC in liposome formulations with the same effect as vitamin E,it is possible to stop the growth of lung cancer(Lawson et al., 2004).The combination treatment has resulted in enhanced drug bioavailablity and inhibited the P-glycoprotein multi-drug resistant transporter(Lawson et al., 2004). Furthermore, the dual therapy of Vitamin E with 9-NC demonstrated a decrease in secondary metastases at non-aerosol-targeted-location. So, drug combination in inhaled liposome formulations can increase the death of tumor cells by apoptosis by a large amount and may cause fewer side-effects than single drugs to the therapy with a lone medication.Evidence suggests that when liposomes are inhaled through the lungs,they can lower doses of anti-cancer drugs given in combination therapy had fewer side effects, which might be attributed to the combined effect of the different mechanisms of actions utilizing various different pathways of the various types of anticancer drugs (Lawson et al., 2004). Phase I research on 9-NC liposomes showed encouraging results(Tedescoetal.,2005); however,further development depends on an interplay of factors,incorporating formulation toxicity in relation to the exiting treatment method (koshkina et al.2004) proposed that co-administration of PTX and (dilauroylphosphatidylcholine-ciclosporin A) CA in single liposomal formulation,etc.CA has strong binding affinity for p-glycoprotein and block enzymatic degradation of other medicines in tumor cells(Gottesman & Pastan, n.d.). Comparing to people who get CA and PTX at the same time or PTX alone,The results showed that inhaling CA before giving PTX and continuing to do so during PTX therapy greatly reduced the tumor lesions and their size (Garbuzenko et al. 2010) liposomes were made with DOX and oligonucleotides against

MRP1 and BCL2 and tested.Protein expression markers MRP1 and BCL2 are linked into tumor cell resistance(Hsia et al., 2002). Thestudy came to the conclusion that combination therapy caused apoptosis and signifcantly slowed the growth of lung cancer tomonotherapy.Antisen oligonucleotides are responsible for this phenomenon because they inhibit the protein expression.

Plan of care for individuals with lung cancer,Table 1;(adapted from NICE,2011)[77]

The stage of lung cancer	The treatment	Type's of chemotherapy	Regimen
NSCLC **Stage 1**	Pneumonectomy or Lobectomy Radiotherapy 1 Pneumonectomy/ Lobectomy Radiotherapy 1 Chemotherapy 2	Cisplatin-based on the combination chemotherapy	Cisplatin plus single 3rd-generation drug(DOX,gemcitabine,PTX or vinorelbine)
Stage 2	Chemotherapy, Radiotherapy, Pneumonectomy	Platinum-based on these combination chemotherapy	Platinum drug (carboplatin or cisplatin) generation drug (DOX,plus single third-gemcitabine,PTX or vinorelbine)
Stage 3	Chemotherapy	Platinum-based on combination chemotherapy	Carboplatin or cisplatin,single third generation medication (DOX,gemcitabine or vinorelbine) and platinum drug
Stage 4 **SCLC** **Limited stage Disease**	Chemotherapy and radiotherapy are administered after a Lobectomy	Cisplatin-based/s combination/s chemotherapy	Cisplatin + one medication of the 3rd generation (DOX,gemcitabine,PTX or vinorelbine) and platinum therapy
Illness in advanced stage	Chemotherapy brain Radio treatment	Platinum-based/s combination/s chemotherapy	Cisplatin+one medication of the third generation (DOX,gemcitabine,PTX or vinorelbine) and platinum therapy

w

Small cell lung cancer(SCLC) and **Non-small cell lung cancer(NSCLC)**

1 .Surgery can be substituted by radiotherapy if there are any health issue.

2 .If the tumour is entirely eliminated,cisplatin-based combination chemotherapy may be recommended to reduce the likelihood of the cancer returning.

3. Chemotherapy can be used if there are any cancer cells are discovered in the lymph nodes during surgery.

4.If there are any health issues,radiotherapy or chemotherapy can be used in place of surgery.

5 .People whose lung cancer reduces the chemotherapy of treatment may be offered radiotherapy since cancer has typically migrated to the brain.

Table 2.Aerosolized liposomal formulas and how they work;in vivo animal and human investigations for lung cancer

Therapeutic agent	**Delivery system**	**Utilized liposome type**	**Subject**	**Study's phase**	**Adverse outcomes**	**Dosage and routine**	**Tracking of the tumour**	**References**
Cisplatin	Star jet Nebulizer PARI LC	DPPC	Human	Phase I	Symptoms of dyspnea tiredness, Nausea, vomiting and hoarness DLT	Every one to three weeks,the dosage is increased from 1 to5mg/m2 To DLT for one to 4 hour Consecutive days	Clinical evaluation Common Blood and urine tests ,PFT,CXR and thoracic CT	86

					wasn't reached			
9-NC	Aero Mist nebuliser	DLPC	Human	Phase1/2	Tiredness, anemia, neutropenia bronchial discomfort,nausea, vomiting and cough DLT reversible Grade two neurotoxicity,grade Three non-myelosuppressive toxicity, and grade three or grade four Haematological toxicity	One mg/m2/day for twenty-five days throughout an eight-week period	Daily pulse oximeter Readings,CBC Testing every week, blood chemisty tests every month and urine analysis.At baseline and before each course,tumor Markers and chest computer assisted tomography	32,108

							Scan were collected. Lung volumes,simple spirometry and DLCO Before and the after the first aerosol exposure	
IL-2	Puritan Bennett twin jet nebulise	DMPC	Human	Phase I	No major Negative impact	Three times a day for eight Eighty-four days,1.5,3.0, and 6.0 106 IU of IL-2	CXR, CBC, Electrolyte, BUN,Creatinine,AST, ALP,Bilirubin,LDH,DLCO and PET are all part of the physical examination	31
IL-2	Pious Bennet Jet Nebulizer twin	DMPC	Animal/s (dogs)	-	Mild cough that develops Right after	D106 IU of IL-2 twice per Day for fifteen days then Fifteen days of 106 IU of IL-2 three times per	Physical examination,CBC serum biochemical measureme	84

					Aerosolization treatment	day or 30 days of 1 106IU of IL-2 Twice per day	nts (such as albumin, total Protein, ALT,ALP, AST,total Bilirubin,BUN, Creatinine and electrolytes and concentratios) urine tests and biopsy	
9-NC	A mouse Exposure Chamber Using an AeroTech II nebulizer Soly for Nasal exposure	DLPC	Animal (mice)	-	Weight loss And skin lesions	0.1 to 1.0mg/kg daily,administered five days per week for 36 to 49days	Calipers used to measure the size or volume of a tumor	90
9-NC	Aero Mist nebuliser	DLPC	Animal (mice)	-	Not recorded	Exposure to aerosols for one to two hours five times	Lung weight	76

						A week for sixteen to twenty one days Total dosage deposited 2.3-3.7 mg/kg		
9-NC and polyethyleneimine -p-^{53} DNA (PEI-p^{53})	Aero Tech II nebuliser	DLPC	Animal (mice)	-	Not captured	0.5mg/1ml twice a week for two weeks and two mg Plasmid/10ml once a week For two weeks	Lung mass	76
9-NC	AeroTech II nebuliser	DLPC	Animal (mice)	-	Not captured	Exposure to aerosols for one to two hours five times A week for 16-17days Total dosage deposited 2.3-3.7mg/kg	Lung mass	89
9-NC	Nebulizer Aero Mist	DLPC	Animal (mice)	-	Not captured	Exposure to aerosols for one to two hours five a week for 16-21 days Deposited sum	Lung mass	91
An analog of viamin E (a-Tea) and 9-NC	Aerosolizer AeroTech II	DLPC	Animal (mice)	-	Not captured	Treatment lasted for three	Every other day tumors were	

						weeks,seven days a week	measured using calipers	93
PTX	Aero Mist nebuliser	DLPC	Animal (mice)	-	Not captured	The amount of PTX deposited in the lungs ranged from 1.4 to 7.8mg/kg(dose schedule:3Times per week for 3 weeks)	Lungs were resected and weighed	102
PTX with cyclosporine A combined	Aero Mist nebuliser	DLPC	Animal (mice)	-	Loss of weight	(Dosage Regimen:3 times per week for 3 weeks)A total of 1.4-7.8 mg/kg of PTX and 1.1-6.1 mg/kg of CA were deposited in the lungs	The weight and resection of the lungs	102
PTX	Aero Mist nebuliser	DLPC	Animal (mice)	-	Aggressiveness	A total of 5 mg/kg were given over the course of 30 minutes	The weight and resection of the lungs	80,104
DOX	Collisonnebuliser connected to four-	DLPC	Animal (mice)	-	Alterations of normal pulmonar	2.5mg/kg for single inhalation every third day	Tumour Growth was monitored	

	port,nose-only Exposure chambers				y parenchyma characterised by alveolar haemorrhage	for twenty four days	by biolumines cent IVIS Vevo 2100(VisualSonices)Ultrasound and (Xenogen) imaging	104

Table 2:(continued)

DOX with Antisense oligonucleotides together	Connected To four-port nose only exposure Chambers is a Collison nebulizer	DLPC	Animal (mice)	-	None	Every third day for 24days,2.5 mg/kg of DOX was inhaled together with0.125 mg/kg of antisense oligonucleotides	Bioluminescent IVIS (Xenogen) and ultrasound Vevo2100 (Visual Sonices) Imaging devices were used to track the progression of the tumour	104
Camptothecin	Nebulizer Aerotech II	DLPC	Animal (mice)	-	Not captured	Thirty minutes at 81g/kg of inhalation only	Resected and weighed lungs	109
DOX-Liposomes	Nebulizer With a collison jet	EPC-/sChol,/s/s DSPE/s PEG	Animal (Mice)	-	Compared to free Medication formulation very restricted	Inhalation of fourteen g/kg Combined with intravenous Administration of 2.5g/kg. Comparing this to an intravenous injection alone	Using the cell death plus ELISA kit, Apoptosis induction in various organs (including the liver,kidney, Spleen,heart and brain) was assessed	107
Encapsulation Of DOX in PEG liposomes with Transferrin conjugation	Catheter for intracorporeal nebulization		Animal(naked Rowett rat with athymus)	-	Not captured	0.2-0.4mg/kg	Rate of animal survival	106

5-FU stands for 5-Fluorouracil;CXR stands for chest X-Ray; PFT stands for pulmonary function test;CBC stands for complete blood count;BUN stands for Blood Urea Nitrogen;AST stands fo Aspartate Transaminase;;ALP stands for alkaline phosphatase;ALT stands for alanine transaminase;LDH stands for Lactate dehydrogenase;DLCO stands for diffusing .

Chapter 5

Conclusion & Future perspective

5.1 Conclusions and Future Perspectives

Comparing liposomal drug delivery systems to parenteral administration, studies have indicated that liposomal delivery systems increase the delivery of chemotherapy drugs,when dealing with lung cancer patients and avoiding the spread of disease.It has been demonstrated that local administration of these pharmaceuticals at therapeutic concentrations to the lung considerably reduces the side effects and toxicity associated with anticancer medications.Although more study has must be improved if the chemotherapeutic liposomal aerosols are to be more effective, the ability to specifically target the lung when using liposomes shows the promise of liposomes as medication combinations rather than monotherapy techniques.

A balance of advantages and disadvantages in comparison to other tried-and-true therapeutic modalities is necessary for the creation of inhalable anticancer compositions using liposomal carriers.Fundamental factors in the development of vaporized anti-cancer liposomes include formulation stability, nebulization mechanism, aerosol targeting to cancer cells, minimizing of deposition in the oropharyngeal area, and enhanced aerosol delivery systems.Arikace®, a nebulisable liposome formulation, has successfully completed phase II clinical research, and these findings were used to assess the future of this field of drug administration.Overall, once liposomes were successful in treating lung infections with inhalable carriers exemplified byArikace® , a phase II clinically tested formulation of neubulizable liposomes,developing inhaled liposomes carriers for the therapy of lung malignancies is anticipated to receive increasing attention from research and development.To enhance the development of efficient aerosolization devices and

more precisely targeted liposomes may be crucial to the future success of inhalable anti-cancer therapies in terms of both therapeutic efficacy and the avoidances of undesireable side-effects,both of which may occur as a result of the therapy's adminstration route.

It should be noted that the diseased condition of the lungs can operate as a barrier, preventing the appropriate deposition of pulmonary clearance of drugs means of liposomal aerosol.For instance, liposomes may only deposit partially in the peripheral airways of people with severe asthma, according to some reports.

Further research is needed to determine whether the presence of malignant tissue in the lung hinders the efficient deposition and clearance patterns of lung cancer patients and healthy persons may be different.

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