Exploring Polymeric Nanocrystals as Drug Delivery System for Lung Cancer Therapy

Thesis submitted in partial fulfilment for the Award of Degree

Doctor of Philosophy

By

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LIST OF ABBREVIATIONS

ACI	: Anderson cascade impactor
BET	: Brunner-Emmett-Teller
BTB	: Bosutinib
BSA	: Bovine serum albumin
C6	: Coumarin-6
Chol	: Cholesterol
Cmab	: Cetuximab
DLS	: Dynamic light scattering
DMAP	: N, 4-Dimethylaminopyridine
DMEM	: Dulbecco's Modified Eagle Medium
DPIs	: Dry powder for inhalation
DSC	: Differential scanning calorimetry
ED	: Emitted dose
EDC	: 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EPR	: Enhanced permeation and retention effect,
FBS	: Fetal Bovine Serum
FP	: Fucoidan
FPD	: Fine particle dose
FPF	: Fine particle fraction
FTIR	: Fourier Transform Infrared Spectroscopy
H-33342	: Hoechst 33342 dye
HEC	: Hydroxyethylcellulose
HR-TEM	: High-Resolution Transmission Electron Microscope

HSPC : Hydrogenated Soyphosphatidylcholine

i.v. : Intravenous

IC50 : Half maximal inhibitory concentration (IC50)

Inh. : Inhalation

MPS : Mononuclear phagocytic system

MTT : (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide)

NCs : Nanocrystals

NSCLC : Non-small cell lung cancer

PBS : Phosphate Buffer Saline

PI : Propidium iodide

PDI : Polydispersity index

PTX : Paclitaxel

RES : Reticuloendothelial system

RF : Respirable fraction

ROS : Reactive Oxygen Species

SEM : Scanning electron microscope

SLF : Simulated lung fluid

Sol : Soluplus

SPM : Scanning probe microscope

ss-NMR : Solid-state NMR

TKIs : Tyrosine kinase inhibitors

TPGS : Tocopherol polyethylene glycol succinate

USP : United States of Pharmacopeia

XPS : X-ray photoelectron spectroscopy

XRD : Powder X-ray diffraction

PREFACE

Lung cancer stands as the third most common cancer and the main cause of cancer-related death worldwide (mainly in the United States). According to the American Cancer Society, the chances of overall survival for 5 years is only 16-25% (Distant stage cancer only 6%). Lung cancer has been reported to have the highest mortality rate (31.8%) compared to other cancers. Among lung cancers, 80-85% are non-small cell lung cancer (NSCLC). First-line treatment of lung cancer includes surgical removal or radiation therapy. However, complete eradication is seldom achieved. Therefore, the chemotherapy is followed. However, among approved anticancer drugs, 65% of drugs exhibit poor aqueous solubility and low permeability, thus not achieving potential therapeutic outcomes. This results in high dose requirements and frequent administration. Non-selective drug distribution to different organs also reduces the drug availability at the target site and thus results in systemic toxicity or severe adverse effects with poor therapeutic effects. Therefore, strategies to improve the cellular bioavailability of anticancer drugs are required to obtain better therapeutic effects. Currently, various nanoformulations have been developed to address the above issues and improve drug delivery to cancer sites. Design of "Drug Nanocrystals" is one such approach that can be utilized for improving the drug delivery of anticancer drugs with poor aqueous solubility and low permeability.

Drug nanocrystals were invented in 1999 with the first marketed product in 2000. Drug nanocrystals have been reported with sub-micron size, high drug loading, and greater structural stability. Drug nanocrystals can be formulated using synthetic polymers as well as natural biopolymers as stabilizers. The hydrophilic and amphiphilic stabilizers are favored over surfactants due to their higher stabilizing potential and safety. Nanocrystals depending on size and shape may provide a better antitumor effect via

enhanced permeation and retention in the vicinity of the tumor. Nanocrystals can be formulated as dispersible dry powder for intravenous as well as pulmonary administration. Pulmonary delivery of drug nanocrystals as dry powders has gained popularity for both local as well as systemic action. Drug nanocrystals offer high drug dissolution, excellent aerosolization performance, enhanced mucus penetration, good lung tissue distribution, and minimal drug clearance, on pulmonary administration. Drug nanocrystals can escape the clearance mechanism by their size and hydrophilic surface, resulting in higher dissolution in lung fluid and mucus/epithelial permeation in its free or intact form to reach the tumor microenvironment. Nanocrystals have extra edges over nebulizers due to their higher physical stability towards aerosolization pressure, portability, and ease of use. However, nanocrystals can release drugs rapidly owing to their higher dissolution velocity, as predicted by the Ostwald-Freundlich and Noyes-Whitney principles. This may result in off-target distribution of drugs to all major organs. Therefore, to control the drug release and to achieve prolong systemic circulation, and also to maintain higher drug availability at the target site, surface modification of nanocrystals can be done. Nanocrystals stabilized using hydrophilic and amphiphilic polymers provide drug crystals with hydrophilic surfaces. Such nanocrystals can be utilized for surface coating with lipid materials. Lipid-coated nanocrystals have dual advantages of nanocrystals as well as lipid nanoparticles, providing high payload, superior aerosolization stability along with better membrane fluidity and control over drug release via increasing the drug diffusion path causing improved systemic circulation when administered intravenously and increased drug retention in the lung on intrathecal administration. Lipid-coated nanocrystals can further be utilized to obtain a ligand-functionalized shell with NCs core for selective delivery of

drug to the target site, thereby avoiding off-target distribution and minimizing systemic side effects related to anticancer drugs.

Considering all the above facts, the nanocrystals of anticancer drugs with poor aqueous solubility and low permeability were investigated. Paclitaxel (PTX) and bosutinib as anticancer drugs belonging to BCS class IV with low aqueous solubility and poor permeability, were used as model drugs. Paclitaxel is usually given through *i.v.* injection and works by stabilizing the microtubules of cells and inhibiting late G2 or M phases of the cell cycle, causing cells to die. PTX has been formulated using Cremophor EL (brand name "Taxol") consisting of castor oil and dehydrated ethanol to overcome solubility issues, however showed serious adverse effects. While, bosutinib can inhibit ACK1 (Activated CDC42 kinase 1 as oncogenic kinase) which attenuates the migration and invasion of KRAS mutant NSCLC. Bosutinib can also inhibit Src (Src-family tyrosine kinase protein) overexpressed in NSCLC to improve patient survival. Therefore, to overcome the solubility and permeability issue as well as minimize systemic toxicity associated with non-selective drug distribution, both drugs were chosen for designing their nanocrystals.

The nanocrystals were prepared using hydrophilic marine biopolymer (fucoidan) and amphiphilic polymer (Soluplus) as stabilizers. Fucoidan is a Hydrophilic sulfated polysaccharide with intrinsic antioxidant and antitumor activity. Fucoidan is biodegradable, biocompatible, and non-immunogenic and has been employed in the formulation of anticancer drug carriers. Fucoidan being anionic can provide electrostatic stabilization to nanocrystals. Soluplus was another stabilizer selected for nanocrystal formulation. It is a triblock copolymer of Polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol. It is amphiphilic and biodegradable with high dilution stability and outstanding solubilization properties for poorly soluble APIs. It can act as a novel

crystallization inhibitor for nanocrystal formulation. Based on their role in forming stable nanocrystals, both polymers were selected.

The nanocrystals were further coated with lipid materials consisting of hydrogenated Soy phosphatidylcholine (HSPC), Cholesterol (Chol), stearylamine, and tocopherol polyethylene glycol succinate (TPGS). HSPC was the phospholipid used to coat the hydrophilic surface of nanocrystals, Chol stabilized the lipid coating, stearylamine aided lipid coating over anionic nanocrystal's surface, while TPGS provided a stealth layer. This resulted in nanocrystals with better control over drug release, prolonged systemic circulation, broaden lung accumulation, and caused higher retention in the lung. In another study, lipid nanocrystals were then surface-functionalized with Cetuximab as a targeting moiety to specify the drug delivery to cancer cells. The nanocrystals were evaluated for particle characteristics (particle size, zeta potential, PDI, and drug content), solid-state characteristics (FTIR, XRD, DSC, XPS, solid-state NMR, and BET), *in vitro* studies (wettability, saturation solubility, drug release, mucus permeation, powder flow, and aerosolization performance), *In vivo* studies (pharmacokinetics, organ distribution, lung cancer treatment, and survival analysis), and *In vitro* cell line studies (MTT, morphology change, cellular uptake, and cell cycle). Based on the objectives, studies were conducted to investigate nanocrystal potential in pulmonary delivery as well as intravenous administration. The nanocrystals size ranged from 100-1000 nm with high homogeneity and was stable during storage. Nanocrystals were cubic and rod-shaped for bosutinib and paclitaxel nanocrystals, respectively as confirmed by microscopy (SEM, AFM, and TEM). The solid-state characterization confirmed the conversion of crystalline drugs to nanocrystals with amorphous nature and their subsequent coating with phospholipids. *In vitro* studies confirmed significantly improved solubility and drug release from nanocrystals as compared to coarse drugs.

Nanocrystals evaluated for their role in pulmonary administration showed higher mucus permeation, excellent flowability, and good aerosolization performance, indicating their suitability in administration as dry powder for inhalation (DPIs). *In vivo* pharmacokinetic and distribution results proved the potential of nanocrystals in improving half-life, prolonging mean retention time, minimizing off-target drug distribution, and improving drug availability in lungs. The *in vitro* cell line studies confirmed the nanocrystals role in inducing cancer death in the lung cancer cell line (A549) by improving the cellular internalization of the drug. In addition to the role of bosutinib and paclitaxel in lung cancer therapy when used alone, their combination was also investigated. The combination of bosutinib and paclitaxel demonstrated synergistic antitumor efficacy at substantially lower concentrations. Furthermore, the nanocrystals of both drugs and their combination produced similar effect even with their reduced dose as compared to pure drugs alone and combination. Overall, nanocrystals of paclitaxel and bosutinib, and their combination can be a novel approach for the effective treatment of lung cancer.