

Formulation Development of Inhalable Dacomitinib Polymeric Nanoparticles for Non-Small Cell Lung Cancer Treatment

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Summary

Pulmonary delivery of cancer therapeutics has a huge untapped potential for the treatment of lung cancer. Inhalable polymeric nanoparticles are an attractive strategy as they provide localized delivery of drug with minimal systemic adverse effects. In this study, Dacomitinib (DMB), a second-generation tyrosine kinase inhibitor (TKI) used in the treatment EGFR (epidermal growth factor receptor) mutant non-small cell lung cancer (NSCLC), was developed as an inhalable polymeric nanoparticle formulation. Dacomitinib has been successfully encapsulated into nanoparticles (NPs) resulting in a size of 202 ± 32.5 nm with a PDI of 0.183 ± 0.061 , and zeta potential of -19.39 ± 2.50 mV. The NPs exhibited an entrapment efficiency of $55.06 \pm 6.3\%$ and a drug loading of $27 \pm 0.01\%$. With respect to *in vitro* drug release, the DMB-NPs showed sustained drug release with cumulative release of $39.2 \pm 7.5\%$ over 5 days. Moreover, DMB-NPs demonstrated efficient lung deposition with mass median aerodynamic diameter of 3.77 ± 0.17 μ m and fine particle fraction of $80.86 \pm 1.02\%$.

Key Message

This study reported the formulation development of polymeric dacomitinib nanoparticles for NSCLC treatment with optimum particle characteristics and excellent *in vitro* aerosol performance.

Introduction

Lung cancer is the leading cause of cancer related deaths worldwide. Standard treatments such as conventional chemotherapy and radiation are seriously limited by their non-specificity and severe adverse effects. Recent studies have focused on molecular profiling to identify molecular pathways for improving target specificity in cancer treatment. Among various mutations, Epidermal Growth Factor Receptor (EGFR) mutation accounts for 85% of all lung cancer driver mutations and between 10-28% in Non-Small Cell Lung Cancer (NSCLC) sub-type (1). Therefore, EGFR mutation has become a key drug target in NSCLC therapy. Tyrosine kinase inhibitors (TKI) are small molecules that suppress tumor growth by reversible or irreversible binding to EGFR. However, a major challenge with TKI inhibitors is the emergence of drug resistance. Dacomitinib is a second generation, irreversible EGFR-TKI approved for the treatment of NSCLC bearing EGFR "exon 19 deletion" or "exon 21 L858R" substitution mutations. It is given as first line treatment for NSCLC patients resistant to first generation TKIs (2). Currently dacomitinib is marketed as VIZIMPRO® and administered as an oral tablet, this can result in severe systemic side-effects and low drug levels in the lungs (i.e., the primary tumor site). Therefore, effective drug delivery strategies are required to facilitate improved drug concentration in tumor tissue.

Pulmonary delivery of cancer therapeutics provides increased drug levels in the lungs with minimal systemic side effects. It also allows for lower doses, reduced dosing frequency, and overcomes first pass metabolism. Moreover, dacomitinib is characterized by poor water solubility limiting its bioavailability when administered directly into lungs. Hence, development of inhalable polymeric nanoparticle system can be a promising option. Encapsulation of drug into nanoparticles offers distinct advantages such as improved solubility, high drug loading, biocompatibility, and sustained release. The nanoparticles have high stability upon nebulization and facilitate increased penetration into tumor tissue. In this study, we have developed dacomitinib loaded poly-(lactic-co-glycolic-acid) nanoparticles (PLGA NPs) that deliver dacomitinib directly into lungs for NSCLC treatment. Further, we hypothesize that this NP delivery of dacomitinib will improve therapeutic efficacy and lower the systemic side effects.

Experimental methods

Method of preparation of DMB Loaded PLGA Nanoparticles: Dacomitinib NPs (DMB NPs) were prepared by a single emulsion solvent evaporation technique. Briefly, 50 mg of PLGA (Resomer RG 502H acid terminated, Sigma Aldrich, St Louis, MO, USA) and 2.5 mg of DMB (LC Labs, Woburn, MA) were dissolved in 2 mL dichloromethane to prepare the primary organic phase. The organic phase was

then added to 10 mL of 1% w/v polyvinyl alcohol (PVA, Fisher Scientific) solution under probe sonication for 2 min at 40% amplitude to form a uniform primary emulsion. The obtained nanoparticle suspension is subjected to stirring at 500 rpm for 3 hours to remove the solvent and harden the NPs. Further, the colloidal nanosuspension was centrifuged at 4 °C with 21191 g force for 30 minutes to get the final nanoparticulate pellet. The pellet was washed twice with deionized water to remove untrapped drug from the surface of NP. Finally, the nanoparticulate pellet was redispersed in 2 mL of deionized water.

Characterization of DMB PLGA Nanoparticles: The particle size, poly dispersity index (PDI), and zeta potential were determined via dynamic light scattering using Malvern Zetasizer Nano ZS (Malvern Panalytical, Royston, UK). The concentration of dacomitinib in NPs was determined using a high-performance liquid chromatography (HPLC, Waters, Milford, MA USA) equipped with a C18 column (3 µm, 4.6*100 mm) and UV detector. For DMB determination, the HPLC mobile phase was 0.1%v/v orthophosphoric acid /ACN (60:40) with a detection wavelength of 254 nm. The flow rate was 0.5 mL/min. Using this HPLC method, the entrapment efficiency and % drug loading of DMB in PLGA NP was calculated. Approximately 20 µL of NP formulation was subjected to lysis by the addition of 1 mL of a/cetonitrile and 980 µL of deionized water followed by centrifugation at 20,627 g force for 30 min at 4 °C. The supernatant was collected and analyzed using HPLC. The entrapment efficiency and % drug loading was calculated using following equations:

$$\text{Entrapment Efficiency (\% w/w)} = \frac{\text{Amount of drug in DMBNP}}{\text{total amount of DMB added}} \times 100$$

$$\% \text{ Drug Loading} = \frac{\text{Amount of drug in DMBNP}}{\text{total amount of DMB and polymer added}} \times 100$$

In Vitro Release Studies: *In vitro* drug release of the DMB from nanoparticles was performed using dialysis method in phosphate-buffered solution (PBS, pH 7.4) containing 1% w/v polysorbate 80. Briefly, 0.4 mL of the nanoparticle suspension was loaded into the dialysis cassette (7000 MWCO, Thermo Scientific, MA, USA) and immersed in 80 mL of PBS maintained at 37 °C. At each specific predetermined time points, 2 mL of sample was collected from the release medium and replaced with fresh PBS to maintain sink conditions. The amount of drug released was determined by analyzing the collected supernatants by HPLC.

Solid State characterization: The DMB-NPs were freeze-dried (Labconco freeze dryer 4.5, Kansas City, MO, USA) and the drug encapsulation within the NPs was assessed with solid-state characterization studies such as Differential Scanning Calorimetry (DSC) and Powder X-Ray Diffraction analysis (PXRD).

Differential Scanning Calorimetry studies: Differential Scanning Calorimetry studies were performed using DSC Q200 (TA instruments, New Castle, DE USA). Briefly, 4 mg of samples were weighed, sealed in aluminum pans, and analyzed over a temperature range of 10 °C to 210 °C at a heating rate of 10 °C/min against an empty aluminum pan as reference.

Powder X-Ray Diffraction Analysis: Powder X-Ray Diffraction analysis was performed using XRD6000 (Shimadzu, Kyoto, Japan). Uniformly dispersed samples were placed on micro sample glass holder and then analyzed at scan range of 10–80° with scanning speed of 2° (2θ)/min).

In vitro Aerosolization Studies: Pulmonary deposition of DMBNP was performed using a Next Generation Impactor (NGI Model 170, MSP Corporation, Shoreview, MN, USA). Briefly, the NGI plates and adaptor were refrigerated at 4 °C for 90 min prior to use, to minimize the evaporation of nebulized sample. A 15 L/min air flow rate was maintained during the operation of NGI. Two mL of nanoparticle formulation was loaded into the PARI LC PLUS® nebulizer cup and nebulized using Pari FAST-NEB compressor system. The instrument is primed for 30 seconds followed by nebulization for 4 min. Samples from each stage (stages 1–8) including throat and mouthpiece were collected using ACN: Water (50:50) mixture. These samples were centrifuged and then analyzed using HPLC. Parameters such as Fine Particle Fraction (FPF%), Mass Median Aerodynamic Diameter (MMAD), and Geometric Standard Deviation (GSD) were calculated.

Results and Discussion

The DMB nanoparticles were prepared using a single emulsion solvent evaporation method. The prepared suspension displayed a particle size of 202.0 ± 32.5 nm confirming the formation of nanoparticles. The NPs had a P.D.I of 0.183 ± 0.061 , indicating a unimodal distribution. Further, the nanoparticles had a zeta potential of -19.39 ± 2.50 mV. The % entrapment efficiency of dacomitinib in NP was $55.06 \pm 6.3\%$ w/w and the drug loading was 27 ± 0.01 μ g/mg. Solid state characterization studies were performed using DSC and PXRD. Initially, DSC studies were performed to confirm drug encapsulation into NP and identify the form (amorphous or crystalline) of drug. The thermogram of free drug showed sharp endothermic peaks at 160°C and 187°C due to its melting point (Figure 1a) whereas DMB-NP revealed absence of sharp melting point peak suggesting successful encapsulation of drug in NP and likely in an amorphous form. The PXRD spectra of the free drug in Figure 1b exhibited several distinct crystalline peaks of DMB at 10° to 30° in 2θ range. However, the crystalline peaks of DMB were absent in the diffractogram of DMB nanoparticle at 10° to 30° in 2θ range, indicating complete encapsulation of drug into the nanoparticle.

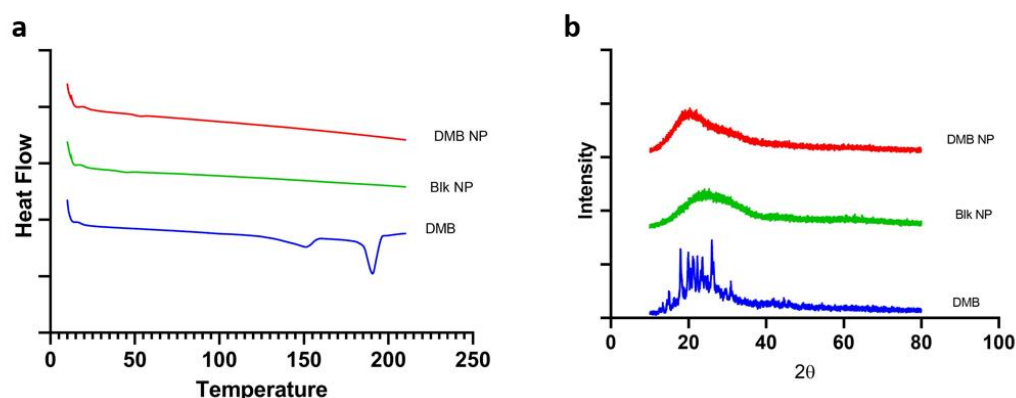


Figure 1 Solid state characterization of Dacomitinib (DMB), Blank NP (Blk NP), and Dacomitinib PLGA nanoparticles (DMB-NP): a) DSC thermograms and. b) PXRD patterns.

In vitro drug release of the optimized DMBNP formulation was performed in PBS, pH 7.4 at 37°C . The data in Figure 2a showed a rapid drug release of about $10.21 \pm 0.43\%$ in 8 h with a gradual increase in release up to 72 h. This was then followed by slower and sustained release with a cumulative release of $39.2 \pm 7.5\%$ after 5 days (4). Sustained release of the drug can be due to effective encapsulation of drug into the polymeric core of nanoparticle.

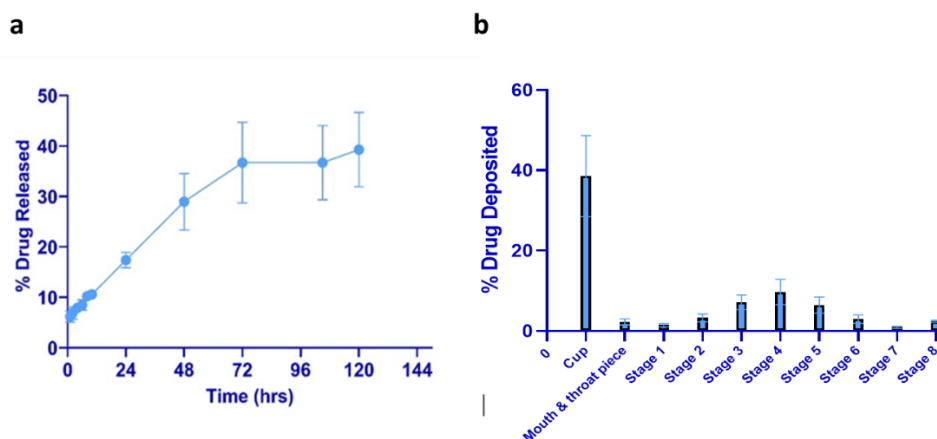


Figure 2: 2a) *In vitro* release profile for DMB NP in phosphate buffer saline, pH 7.4. 2b) *In vitro* aerosol deposition profile of DMB-NP represented as percent drug deposited on each stage of the Next Generation Impactor (NGI). Data represented as Mean $\pm$ SD, n = 3.

The therapeutic efficacy of nebulized dosage form depends mainly on MMAD and FPF. Hence, various *in vitro* aerosolization parameters like FPF (%), MMAD, and GSD (Figure 2b) were calculated to assess the respirable capacity of nanoparticle formulation. A MMAD less than $5\ \mu\text{m}$ is essential for effective

lung deposition of particulate therapeutics (5). After nebulization of DMBNP's, the MMAD and GSD values were calculated to be $3.77 \pm 0.17 \mu\text{m}$, and 2.23 ± 0.10 respectively, indicating suitability for inhalation. Further, the NPs displayed a fine particle fraction (FPF) of $80.86 \pm 1.02\%$ suggesting that this could result in efficient deposition in the bronchoalveolar region of the lungs. From NGI studies the formulated DMB-NPs for inhalable drug delivery of DMB can be considered as efficient and well proven.

Conclusions

Our studies have shown that dacomitinib has been successfully encapsulated into NPs resulting in a size of 200 nm and charge of -20 mV . The solid-state characterization studies (DSC and PXRD) revealed effective drug encapsulation in NPs in an amorphous form. In vitro aerosolization studies also demonstrated good inhalable properties with MMAD of $3.77 \pm 0.17 \mu\text{m}$ and FPF of $80.86 \pm 1.02\%$. Moreover, our ongoing and future studies will include performing MTT assay, *in vitro uptake*, and 3D spheroid (as they better mimic tumor microenvironment) studies to evaluate DMB-NP efficacy in comparison to free drug in suppressing tumor growth.

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