Nanotechnology Systems for Drug Delivery, Multi-Modal Targeting and Imaging of Tumors

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ABSTRACT

Cancer is one of the leading causes of deaths (1.6 million deaths annually) in USA. Despite recent advances in chemotherapy, the survival rates in lung cancer patients are unsatisfactory due to limited efficiency of systemic or oral chemotherapy and associated side effects. Oral administration of anti-cancer agents presents a series of advantages for patients. However, most of these agents are hydrophobic and associated with low bioavailability. To overcome this issue we have designed: 1) a unique customized spray gun which allows simultaneously /pulsatile flow of two different liquid systems through single nozzle. This modification allowed us to formulate enteric coated SEDDS (E-SEDDS) of anticancer agents for oral delivery by using SEDDS as one liquid system and enteric coating solution as second liquid system. 2) Tumor homing nanoparticle of bio-imaging agents (D-Luciferin) will target tumor and enhance the time duration of imaging, which can be used for delivering therapeutics.

Keywords: dual channel spray gun, self emulsified drug delivry, multi-model tumor targeting, in-vivo imaging, and bioluminecence

1 INTRODUCTION

Lung cancer is one of the leading causes of deaths (1.6 million deaths annually) in USA. Non-small cell lung cancer (NSCLC) accounts for 85 % of all lung cancers. Despite recent advances in chemotherapy, the survival rates in lung cancer patients are unsatisfactory due to limited efficiency of systemic or oral chemotherapy and associated side effects [1]. Therefore, new targeted drug delivery systems are needed for the treatment of lung cancer. Currently, new approaches in the treatment of lung cancer with novel antiangiogenic drugs have generated clinical interest [2]. Peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists have shown antineoplastic and anti-inflammatory effects [3-5] against a variety of tumor cell lines. Also vascular endothelial growth factor (VEGF) over-expression (61% to 92% of NSCLC) is associated with poor survival. Recently, new approaches in the treatment of lung cancer with novel drugs that selectively inhibit tumor blood supply thus controlling cancer cell survival, proliferation and/or metastasis in combination with conventional anticancer or antiangiogenic drugs have generated clinical interest. Our laboratory has demonstrated that DIM-C-pPhC6H5 (DIM-P), a c-substituted diindolylmethanes is novel anti-cancer agent, has promising anticancer activity and in combination with Docetaxel (Doc) shows additive to synergistic action by activating growth inhibitory and apoptotic pathways [6]. Preliminary studies conducted recently in our laboratory strongly suggest that DIM-P exhibits antiangiogenic activity as indicated by decreased expression of VEGF, CD31, decrease microvessel density and inhibition of tube formation in HUVEC cells. This warrants further investigations into the antiangiogenic role of DIM-P. DIM-P is currently in Phase 1 trials but pharmacokinetic (PK) studies conducted in our laboratory showed that DIM has poor solubility (<1µg/ml), poor oral bioavailability (<20%), and a very short half life (0.8 hr).

The promising anticancer activity of novel PPAR- γ agonists such as DIM-P is intended for oral administration. However, DIM-P has poor oral bioavailability and to overcome this limitation, we have developed a self-emulsified drug delivery system using dual channel spray drying technology (SESD) (Figure 1). The advantage of this technology is that it simultaneously generates microparticles along with a spray of enteric polymer which makes the microparticles formed very uniformly coated. The enteric coating is to prevent the initial drug degradation of DIM-P in the stomach. Conversion of liquid form of the self-emulsifying formulations into solid dosage forms by spray drying technology retains the advantage of self-emulsified systems to improve oral bioavailability and overcome the limitations of liquid self-emulsified formulation delivery aspects. Initial animal pharmacokinetic studies have shown 56% absolute oral bioavailability (40 % more bioavailability than free drug). It is expected that this improved pharmacokinetics will translate into enhanced antitumor activity in tumor models.

This technology has tremendous commercial potential for a variety of reasons: a) DIM-P is a very safe anticancer agent and in the limited toxicity studies done in our and Dr. Safe's laboratory has shown no toxicity in doses as high as 100 mg/Kg. Hence this is a very safe compound which can be pursued for further development as a commercial product b) One of the major problems with DIM-P has been

its poor bioavailability and this has been overcome by the spray dried formulation which our laboratory has developed using a novel dual channel spray drying technology for which a patent has been filed c) The SESD formulation can be made into a commercial product by filing into a capsule using the appropriate excipients so that it can be given to patients and hence has significant potential to be available as a commercial product. This is also supported by the fact that there are several capsule formulations on the market for a variety of drugs and this approach will be easy to accept for future pre-clinical and clinical studies.

Also, recently nanocarriers have generated interest for lung cancer treatment. However, outcome of passively targeted nanocarriers are limited due to reduced efficacy, inefficient tumor cell internalization, and toxicity to normal cells [2]. The current study is to investigating the use of nanostructured lipid carriers (NCs) which are highly versatile drug delivery systems since multiple functions can be built into the particles. NCs can be further targeted to tumor blood supply by linking them using tumor homing pegylated Cys-Arg-Glu-Lys-Ala (CREKA) peptide which will target the clotted proteins and will increase the half life [7-9]. The pegylated CREKA peptide coated NCs containing DIM-P (PCNCs-D) will recognize clotted plasma (see Figure 2) proteins and thus will selectively targeted to tumors by binding to vessel walls and tumor stroma [10].

Based on this preliminary data, the hypothesis of our research were: 1) SESD will show superior pharmacodynamic activity in preclinical lung tumor models which will enable it to be used for future commercial development, 2) Tumor homing pegylated nanolipidcarriers of DIM-P will target tumor blood vasculature and increase plasma half life thereby inhibiting tumor growth by exerting antiangiogenic activity against lung tumors, 3) Tumor homing nanoparticle of bio-imaging agents (D-Luciferin) will target tumor and enhance the time duration of imaging, allowing us in-vivo imaging of tumor progression / tumor vasculature and tracking of nanoparticles.

2 METHODOLOGY

2.1 Dual Channel Spray Dried Formulation

A unique spray gun with two nozzles was designed in the laboratory (Fig.1). we utilized Response surface methodology (RSM) to optimize the SESD. The spray dried product was characterized by Transmission electron microscopy (TEM) and Differential scanning calorimetry (DSC). Evaluation for enteric coating was done by drug release study of spray dried E-SEDDS. Pharmacokinetic parameters were evaluated in Sprague Dawley® rats and data were analyzed by WinNonlin. Efficiency of anti-tumor activity was carried out using orthotopic A549 tumor model in nude mice.

2.2 Multi-Model Targeting and In-Vivo Imaging

Nanoparticles were prepared with DIM-P (NCs-D)/ D-luciferin (NCs-Dl)/XenolightDiR(NCs-Di), Compritol, Miglyol, DOGS-NTA-Ni and sodium taurocholate using high pressure homogenizer (Nano-DeBEE). PCNCs-D and PCNCs-Dl/PCNCs-Di were prepared by conjugating NCs-D and NCs-Dl/ NCs-Di with 6His-PEG2K-CREKA peptide (see Figure 2) and characterized for physical properties, clot binding assay, cytotoxicity and tube formation assay. Pharmacokinetic parameters of formulations, anti-angiogenic activity of PCNCs-D by Matrigel plug assay were evaluated in BALB/c mice and anti-tumor activity using a metastatic H1650 and A549 tumor models in nude mice. In-vivo imaging of tumor and tracking of nanoparticles was carried out with IVIS® Spectrum CT (Caliper life Sciences) by using fluorescent dye (Xenolight DIR) and bioluminescence (luciferin) following intravenous and inhalation delivery of nanoparticles. Molecular pathways involved were studied by western blotting and immunohistochemistry.

3 RESULTS

3.1 Dual Channel Spray Dried Formulation

Preliminary investigations of the process parameters revealed that factors, amount of drug (A), oil (B), and surfactant (C), highly influenced the, particle size, and drug release. Hence factors like, amount of drug (A), oil (B), and surfactant (C), were used for further systematic studies. Using the desirability function, all the measured responses were combined to get one overall response. The optimized batch was identified with a desirability value of 0.97 for SESD. Drug release profile showed efficiency of enteric coating with no drug release at pH less than 5 and 96.98% drug release within 8 hr at pH 5.0 and above. DSC analysis showed there is no drug interaction with excipient or any effects on stability of the formulation. Pharmacokinetic analysis showed increase in AUC from 62.24±19.84 ug.h/ml to 189.89±62.86 for DIM solution to SESD respectively. (Figure 3)

3.2 Multi-Model Targeting and In-Vivo Imaging

Particle size of PCNCs-D was 190-210 nm. The PCNCs-D formulation showed an initial burst release followed by a slow release up to 72 hr (90%). PCNCs-D showed ($p<0.001$) 3 fold higher binding to the clotted plasma proteins compared to NCs-D. PCNCs-D decreased average branching point by 68 ± 5 percent compared to 39 ± 4 percent by DIM-P alone in a tube formation assay suggesting anti-angiogenic activity. Similar results were obtained with matrigel plug assay suggested anti-angiogenic activity of DIM-P, PCNCs-D formulations.

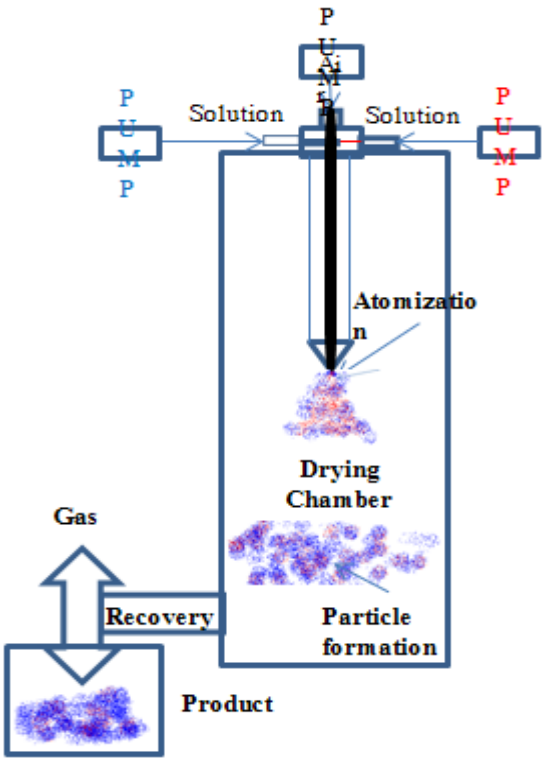

Figure 1: Schematic diagram of dual channel spray dryer.

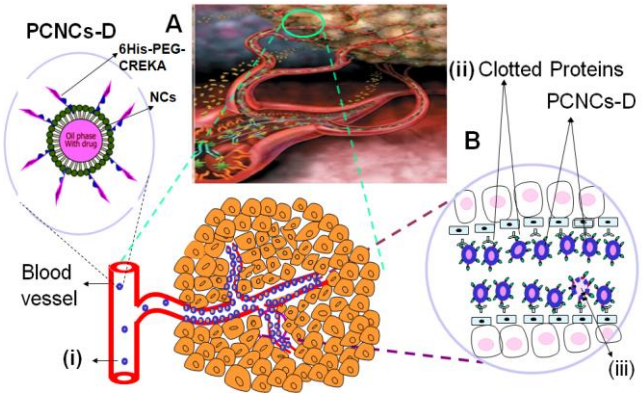

Figure 2 : Proposed model for tumor blood vessel targeted CREKA peptide coated NCs system. A) Tumor infiltrated with blood vessels; i) administration of targeted PCNCs-D by i.v; B) Expanded view of PCNCs-D targeting/ accumulation at tumor blood vessels; ii) clotted plasma proteins on tumor blood vessels; and iii) NCs binding to tumor blood vessel following penetration of NCs by enhanced permeation and retention effect (EPR) effect. YKA (nonspecific peptide) coated NCs unable to attach to plasma clot in tumor blood vessels.

Pharmacokinetic parameters showed that PCNCs-D increased plasma half life of DIM-P from 0.83 ± 0.26 hr to 2.34 ± 0.39 hr. PCNC-Dl/Di allowed us to track and visualize migration in tumors of these nanoparticles by use of IVIS system. In-vivo imaging following exposure of PCNCs-Dl/PCNCs-Di demonstrated their targeting to the tumor vasculature (Figure 4), where the PCNCs-Di were found to migrate more in newly formed blood vessels and total radiant efficiency [p/s] / [µW/cm²] was $2.1*10^{12}$ ± $0.5*10^{12}$ over the period of 0.5 hr to 3h. NCs-Di didn't show any specific migration to tumors confirming the specific targeting of PCNCs-Di and total radiant efficiency [p/s] / [µW/cm²] was $0.6*10^{11}$ ± $0.18*10^{11}$. PCNCs-D showed significantly higher tumor reduction compare to other treatment groups. DIM-P and PCNCs-D showed inhibition of VEGF and Sp proteins as well as down regulation of micro-vessel density (CD31).

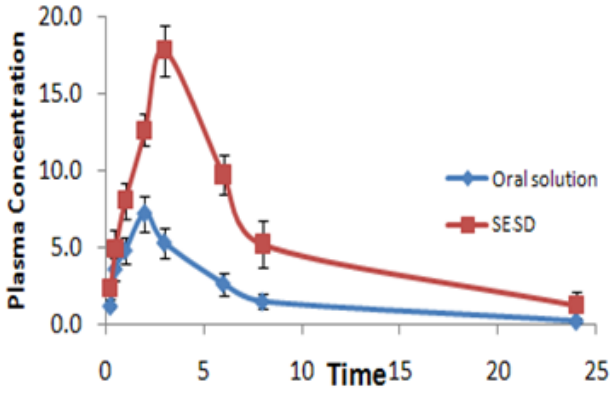

Figure 3 : Plasma concentration Vs Time profile of self-emulsified spray dried formulation of DIM-P (SESD).

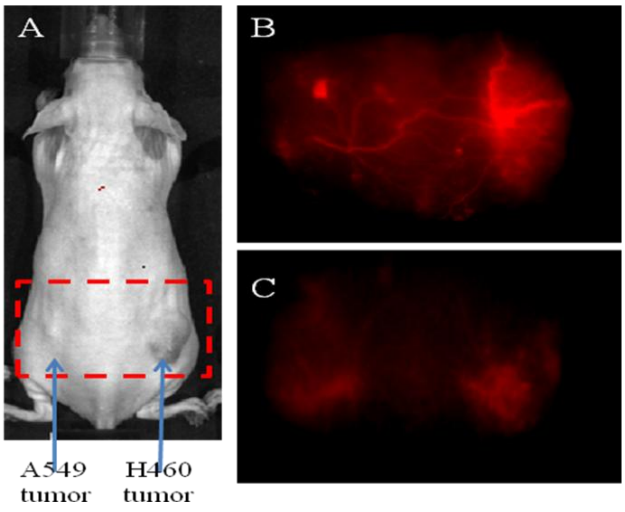

Figure 4: In-Vivo Imaging; A) A549 and H460 lung cancer cell tumor bearing Mouse in in-vivo imaging system and Spectrally Unmixed Image of Vasculature with B) NCs-Di and C) DiR

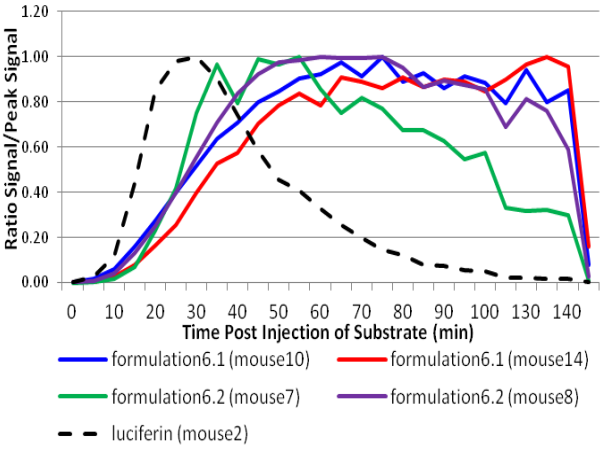

Figure 5: Kinetic Luciferase Activity Using Nanoluciferins and Luciferin as Substrates in 4T1-luc2 Orthotopic Tumor Model. (Data Normalized to Peak Signal)

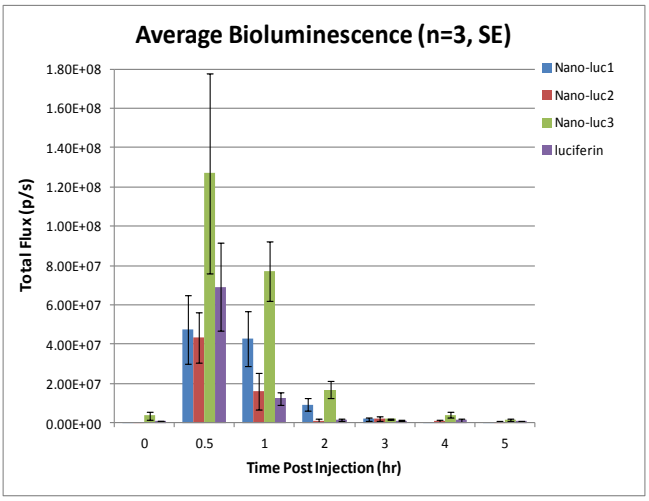

Figure 6: Kinetic Luciferase Activity Using Nanoluciferins (15 mg/kg) and Luciferin (150 mg/kg) as Substrates in H460-luc2 Orthotopic Tumor Model. (Data Normalized to Peak Signal)

4 CONCLUSION

The results emanating from these studies demonstrate potential use of dual channel spray dried enteric coated self-emulsifying drug delivery system for enhanced oral absorption and thus increased anti-cancer activity and the role of NCs-D as an effective tumor homing imaging systems for cancer. The results emanating from these studies demonstrate the potential of nanoparticles for increasing the duration of substrate activated luminescence and the role of PCNCs as effective tumor homing imaging/drug delivery systems for cancer treatment.Also luciferin containing nanoparticles could be formulated which had a longer residence time in tumors.

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