Inhalable Stimuli-Responsive Theranostic Nanoparticles for Targeting Cancer in Deep Lung Tissue

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ABSTRACT

In 2012 lung cancer accounted for 13% (1.83 million) of cancer cases and caused 19% (1.56 million) of cancer deaths worldwide according to the IARC. Despite advances in surgery and drug discovery, lung cancer remains difficult to treat as a result of unavoidable exposure to carcinogens, poor diagnosis and the lack of targeted drug delivery platforms. The aim of this study was to develop a non-invasive, patient convenient platform for the targeted delivery of chemotherapeutics to cancer in deeper lung tissue. This platform is intended to improve the therapy of deep lung tissue cancer through the combinatorial effect of tissue targeting, controlled drug release, and the possible imaging and prognostic values as a result of the USPIONs content.

Keywords: theranostic nanoparticles, stimuli-responsive nanoparticles, USPIONs, deep lung tissue cancer, chitosan

Abbreviations: CS, Chitosan; TPP, Sodium Tripolyphosphate; AA, L-ascorbic acid; FL, Fluorescein; EDAC, N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride; FBS, Fetal Bovine Serum; PBS, Phosphate Buffered Saline; MTT, 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide; DMSO, Dimethyl sulfoxide; EGFR-Antibody or Ab, Monoclonal Anti-Epidermal Growth Factor Receptor Antibody; WGA-AF, Wheat Germ Agglutinin, Alexa Fluor 488 Conjugate; MD, maltodextrin; PVP, polyvinylpyrrolidone.

1. INTRODUCTION

Lung cancer is the deadliest solid tumour [1]. As stated by the International Agency for Research on Cancer (IARC) in 2012, lung cancer accounted for 13% (1.83 million) of cancer cases and caused 19% (1.56 million) of cancer deaths worldwide [2]. Moreover, the World Health Organization estimates that lung cancer will account for 19.35% of all cancer deaths by 2020 [3]. There are two types of lung cancer: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC represents 85% of the lung cancer cases [4].

It is believed that inhalation is the optimal route of administration in the case of NSCLC chemotherapy as it offers a non-invasive route for chemotherapeutic delivery lacking the undesirable attributes of I.V. based approaches in addition to the specific tumour targeting for enhanced patient conveniency. Since most of NSCLCs seem to allocate in the airways [4-5], the development of nanoparticle (NP)/microparticle (MP) carrier system capable of delivering chemotherapeutics to airway tumours is a valid approach.

The aim of this study was to develop a non-invasive, patient convenient platform for the targeted delivery of chemotherapeutics to NSCLC. Tumour cell targeting and localized chemotherapeutic delivery with the provision of a desirable -stimuli responsive- drug release profile will be allowed by the delivery platform.

2. MATERIALS AND METHODS

2.1. Materials

Medium molecular weight CS was purchased from Bio Basic Inc. (Ontario, Canada). TPP was bought from Mistral (Northern Ireland, UK). $FeCl_3.6H_2O$, $NaHCO_3$, AA, FL, EGFR-Antibody, MTT, DMSO, MD and PVP were bought from Sigma-Aldrich (Munich, Germany). Spectra/Por Float-A-Lyzer G2 (MWCO 100 kDa) was supplied from Spectrum Laboratories Inc. (Schwerte, Germany). RPMI 1640 with L-Glutamine, Pen-Strep and L-Glutamine were purchased from Lonza (Basel, Switzerland). A549 and L929 cell lines were obtained from Vacsera Holding Company (Cairo, Egypt) and the American Type Culture Collection (Manassas, VA, USA); respectively. FBS, Hoechst 33258, Pentahydrate (bis-Benzimide) and WGA-AF were supplied by Life Technologies (Carlsbad, CA, USA).

2.2. Methods

2.2.1. NPs formulation:

2.2.1.1. CS NPs:

NPs were prepared by the ionotropic gelation of chitosan (CS) and tripolyphosphate (TPP). CS (0.1 % w/v) was added to TPP (0.1 % w/v) for NPs synthesis.

2.2.1.2. USPIONs:

For synthesis of USPIONs, 0.45 M $NaHCO_3$ was added to 0.3 M $FeCl_3.6H_2O$. Subsequently, 0.3 M Vit C were added. The mixture was then transferred into a steel-lined Teflon tube to be autoclaved.

2.2.2. Encapsulation Efficiency % (EE %) of NPs:

FL was chosen as a model drug. Similarly as CS NPs, drug-loaded NPs were formulated using ionotropic gelation. FL EE % was determined fluorometrically at λ_{ex}= 490 nm and λ_{em}= 519 nm.

2.2.3. Drug Release:

2.2.3.1. Determination of drug release profile from drug-loaded NPs:

FL release rate from NPs was determined using spectra/por float-a-lyzers (MWCO=100 KDa). At assigned time intervals (0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 hrs) aliquots were withdrawn from the release compartment and analyzed for the released drug.

2.2.3.2. Determination of USPIONs effect on drug release upon the application of high frequency magnetic field (HFMF):

USPIONs and FL-loaded CS NPs were used to determine the effect of magnetic field application on drug release profile in comparison to FL-loaded CS NPs. The release profiles of the samples were compared under a constant temperature of 32 °C (thermostat) and under the periodic influence of a HFMF.

2.2.4. NPs-cell interaction:

2.2.4.1. MTT assay:

MTT toxicity assay was performed on A549 and L929 cells with different concentrations of CS NPs, CS-USPIONs and USPIONs. The absorbance was measured using microplate fluorometer at λ= 570 nm.

2.2.4.2. Effect of NPs concentration and modification on the cells' uptake magnitude:

Different concentrations of tagged and non-tagged CS NPs and CS-USPIONs loaded with FL were used to study NP cellular uptake in A549 cells. Amount of uptaken NPs were determined by fluorometery (λ_{ex}= 495 nm and λ_{em}= 519 nm).

2.2.4.3. NPs Uptake confirmation:

For cellular uptake, purified FL-loaded CS NPs were used. Cells were labelled with Hoechst and WGA-AF.

2.2.5. Spray freeze drying (SFD) and Lung deposition evaluation by next generation impactor (NGI):

2.2.5.1. Incorporation of NPs in PVP: MD MPs:

CS NPs and CS-USPIONs were formulated and suspensions were SFD in the presence of cryopreservatives (5% (w/v) of PVP: MD mixture).

2.2.5.2. Evaluation of aerodynamic properties of MPs using NGI:

MPs were discharged from the dry powder inhaler (DPI) into the NGI passing by seven stages with

aerodynamic diameters cut-offs 9.1, 5.2, 3.3, 1.9, 1.1, 0.6, and 0.4 µm; respectively at rate of 45 L/min. The contents of different stages were determined spectrophotometrically (λ_{es}: 485 nm and λ_{em}: 535 nm) from which the aerodynamic diameter of MPs were calculated.

2.2.6. Statistical analysis:

Statistical analysis of data was performed with one way analysis of variance (ANOVA). *P*-values less than 0.05 were considered significant.

3. RESULTS

3.1. NPs formulation:

3.1.1. CS NPs:

NPs obtained were less than in 150 nm in hydrodynamic diameter and positively charged.

3.1.2. USPIONs:

USPIONs synthesized were of size and surface potential 5 nm and -26.6 mV; respectively. According to the JCPDS-ICDD Card No. 24-0081 the crystal structure was related to cubic γ-Fe_2O_3. They possessed magnetic saturation (Ms), coercivity (Hc) and retentivity (Mr) of 48.4 Am^2/Kg, $9.9x10^{-4}$ T and 0.5 Am^2/Kg; respectively.

3.2. EE % of NPs:

NPs entrapped 23.9 µg/ml of FL with the absence of NP final size alteration. Incorporation of USPIONs into CS NPs reduced concentration of FL entrapped to 13.0 µg/ml.

3.3. Drug Release:

3.3.1. Determination of drug release profile from drug-loaded NPs:

Only 5 and 25 % of FL was released from CS NPs and CS-MNPs; respectively after 168 hrs compared to the control that provided 100 % release during the same period.

3.3.2. Determination of MNPs effect on drug release upon the application of HFMF:

Upon the application of alternating magnetic field (AMF) the rate of release of FL-loaded CS-USPIONs significantly increased. However, for FL-loaded CS NPs the release rate was not affected by AMF application.

3.4. NPs-cell interaction:

3.4.1. MTT assay:

The cytotoxicity of CS NPs, CS-USPIONs and USPIONs were evaluated for A549 and L929 cell lines. At high concentrations for CS NPs and CS-USPIONs (2000 µg/ml) preferential cytotoxicity towards cancer cells is observed. Whereas, at high concentration of USPIONs (200 µg/ml) cytotoxicity was visualized on both cell lines.

3.4.2. Effect of NPs concentration and modification on the cell's uptake magnitude:

Cellular uptake increased with increased NPs concentration while modification of NPs with Abs has lowered the cellular uptake significantly.

3.4.3. NPs Uptake confirmation:

Green fluorescence attributed to FL loaded NPs appeared to be inside the cell in the perinulclear area. The visualized overlap of red (WGA-AF) and green (FL) fluorescence indicated that the green fluorescence seen perinuclearly is in fact owed to NPs rather than noise or released FL.

3.5. SFD and evaluation of Lung deposition by NGI:

3.5.1. Incorporation of NPs in PVP: MD MPs:

MPs obtained are spherical in shape and nearly of a size ~ 3 µm.

3.5.2. Evaluation of aerodynamic properties of MPs using NGI:

Inhaled percentage of MPs (passed the throat) is ~37 % for both NPs. However, ~50 % were deposited past the trachea. Both types of particles have also possessed fine particle fraction (FPF≤ 5.2 µm) of ~40 % indicating successful delivery to deep lung tissue. Processing the NGI data also allowed the determination of the MMAD which corresponded to 6.1 µm for CS-USPIONs and 5.1 µm for CS NPs.

4. CONCLUSION

In conclusion, lung cancer is the deadliest solid tumour among all types of cancer. The number of cases and deaths caused cancer is increasing by time. The optimum route of chemotherapeutic drug delivery was thought to be inhalation since it lacks systemic toxicity

and invasiveness experienced by I.V. route. The combination of initial spike dose of chemotherapy followed by sustained supply of the drug was thought to provide an efficient therapeutic platform for tumour eradication and recurrence prevention. Thus, based on these thoughts a non-invasive, patient convenient platform for the targeted delivery of chemotherapeutic drugs to cancer in deeper lung tissue was developed and studied. MPs containing either CS NPs or CS-USPIONs were developed. USPIONs incorporation has enhanced drug release by 1.7 fold in response to external magnetic field while CS NPs provided a slow sustained release. The prepared MPs had fine particle fraction (FPF≤5.2 μm) of ~40 % w/w and MMAD of 5-6 μm as determined by the NGI. The targeted delivery to the lung cancer using the developed formulation seems to be a promising approach.

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