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Research paper

Nebulizable colloidal nanoparticles co-encapsulating a COX-2 inhibitor and a herbal compound for treatment of lung cancer

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ABSTRACT

A challenging disease such as lung cancer requires the combination of different modalities to achieve beneficial therapeutic outcomes. In this work, PLGA nanoparticles were chosen as colloidal carrier for two drugs with reported anti-lung cancer activity: naringin and celecoxib. PLGA nanoparticles were prepared and characterized for their particle size, zeta potential, entrapment efficiency, *in vitro* release, stability, morphology, cytotoxicity, as well as aerosolization and nebulization behaviors. Their biodistribution pattern upon pulmonary aerosolization, and safety on healthy lung tissues were determined as well. Results showed that the described system displayed a particle size <260 nm with unimodal distribution, entrapment efficiency for celecoxib and naringin reaching 96% and 62% respectively and a controlled release profile for the two drugs. The selected formula displayed favorable nebulization properties with high drug deposition percentages in lower impinger and impactor stages. It also exhibited higher cytotoxic activity on A549 lung cancer cell lines compared to the free drugs combination, while displaying considerable safety on healthy lung tissues. Biodistribution studies delineated the lung deposition potential of the nanoparticles accompanied with high distribution to the bones, brain and liver which are common metastatic sites of lung cancer, proving their promising nature in the treatment of lung cancer.

1. Introduction

Lung cancer is considered one of the most dangerous cancer types affecting both men and women, and is the most killer cancer type worldwide [1,2]. The prognosis of lung cancer is generally poor, owing to the high risk of metastasis to different body organs such as the lymph nodes, brain, liver, bones and glands [1,3]. Conventional treatment options for lung cancer such as surgery, radiotherapy and chemotherapy suffer from many disadvantages, such as the limitation of surgery to early stages of the disease and small tumor sizes, its diminished efficacy when used alone, in addition to the reported side effects of radiotherapy and chemotherapy [4].

In order to deliver drugs for treatment of lung cancer, both IV and oral routes of administration are commonly used [5]. The use of the pulmonary route as an alternative provides several advantages compared to the former routes owing to its non-invasiveness, higher patient compliance, avoidance of the hepatic first pass metabolism, and availability of direct local therapeutic effect on respiratory tract with possibility of systemic absorption owing to the large surface area, huge vascularization and thin epithelial lining of respiratory tract. The latter advantage would be of uttermost importance in reaching possible lung cancer metastatic sites [5–10].

Drug delivery *via* the lung comprises different methods including dry powder inhalers (DPI), pressurized metered dose inhalers (PMDI), nebulizers and intra-tracheal instillation. Nebulizers are becoming of great interest as they require minimal patient cooperation and ensure deep drug delivery to the lungs. Furthermore, nebulizers can be used conveniently for infants, children and comatose patients in hospitals or emergency departments in addition to being more economic in chronic treatment of diseases such as lung cancer [2,9,11].

The selection of drugs for treatment of cancer is as important as the choice of the route of administration and delivery devices. Therapeutic molecules of herbal origin have been investigated for

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their anticancer potential on several cancer types, including lung cancer. Among these molecules is naringin, which is a natural flavonoid present in citrus fruits that has been reported to exhibit an anticancer effect in lung cancer through different pathways including inhibition of angiogenesis and tumor necrosis factor (TNF) release, antioxidant activity, induction of cancerous cells apoptosis and inhibition of cancer metastatic spread [12–14]. Combination therapy which involves the delivery of two anticancer therapeutic agents with the same nanocarrier delivery system was reported to provide higher therapeutic efficiency and reduce the side effects [15]. This has led us to delineate another drug, celecoxib, in combination with naringin for treatment of lung cancer. Celecoxib is one of the COX-2 inhibitors that has been reported to play a role in targeted cancer therapy either when used alone or in combination with other antineoplastic agents [16–20]. It is considered a multi-functional anti-cancer drug possessing different antineoplastic mechanisms, leading to reduction of tumor growth and induction of cancer cells apoptosis [19,21].

Aerosolizable nanoparticulate systems have shown great promise for the treatment of respiratory diseases [10,22,23]. Polymeric nanoparticles have been extensively used for passive targeting of cancer cells owing to their diverse benefits in this regard, including protection of drugs from enzymatic degradation and macrophagic endocytosis clearance, controlled and targeted drug delivery to the site of action *via* the Enhanced permeability and retention (EPR) effect, and reduction of toxicity and side effects of the delivered drugs [15,24,25]. Poly D,L-lactide-co-glycolic acid copolymer (PLGA) has been the most widely used polymer for synthesis of NPs owing to its biocompatibility and biodegradability [26,27].

To the best of authors' knowledge, no work has been reported on the anticancer potential of herbal-COX 2 inhibitor drugs combination on lung cancer via nebulization till current date. Moreover, only few works have explored the proposed delivery method i.e. nebulization for delivery of PLGA nanoparticles for treatment of lung cancer [28]. Therefore, the objective of our study was to test the hypothesis whether a colloidal biodegradable carrier such as PLGA nanoparticles could be a successful carrier for co-encapsulation of the COX-2 inhibitor drug celecoxib, and the herbal compound naringin for treatment of lung cancer *via* pulmonary nebulization, with emphasis on their anticancer activity on lung cancer cells and their *in vivo* biodistribution behavior.

2. Materials and methods

2.1. Materials

Naringin (4′,5,7-trihydroxyflavanone 7-rhamnoglucoside), PVA (polyvinyl alcohol, M. Wt. 31,000), Dimethyl sulphoxide (DMSO), MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide), chloramine-T ($ArSO_2NClNa$ (CAT)) (mol. wt. 227.65 g/mole), methanol (HPLC grade, ⩾99.9%), water (Chromasolv, HPLC grade) and acetonitrile solvent (HPLC grade, ⩾99.93%) were purchased from Sigma Aldrich Co. (Saint Louis, USA). Poloxamer 188 was kindly gifted by BASF Company, Germany. Celecoxib (4-(5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl) benzenesulfonamide) was kindly provided by Amoun Pharmaceutical Industries Co., Egypt. PLGA (poly lactide-co-glycolic acid copolymer) grades Purasorb PDLG 7502 (75:25 DL-lactide/glycolide, 0.2 dl/g, mol. wt. 17,000 g/mole), Purasorb PDLG 7502A (75:25 DL-lactide/glycolide, 0.2 dl/g, mol. wt. 17,000 g/mole, acid terminated), Purasorb PDLG 5002 (50:50 DL-lactide/glycolide, 0.2 dl/g, mol. wt. 17,000 g/mole) and Purasorb PDLG 5002A (50:50 DL-lactide/glycolide, 0.2 dl/g, mol. wt. 17,000 g/mole, acid terminated) were kindly gifted by PURAC Biomaterials company, The Netherlands. Analytical grades acetone solvent, disodium hydrogen phosphate, potassium dihydrogen phosphate and sodium dodecyl sulphate were purchased from El Nasr pharmaceutical company, Egypt. Deionized water was obtained from Direct Q-UV 3 Millipore device, Millipore SAS, France. Spectra/Por dialysis membrane (cutoff Mw 12.000–14.000) was purchased from Spectrum Laboratories Inc., Canada. Phosphate buffer saline (PBS), fetal bovine serum, RPMI 1640, Trypsin/EDTA, HEPES buffer, gentamycin solution and Trypan blue stain were obtained from Life Technologies Inc., Grand Island, NY, USA. Sodium metabisulfite $Na_2S_2O_5$ (M. Wt. 190.107 g/mole) was purchased from the British drug house (BDH) Ltd., England. Radiolabelling sodium iodide $Na^{125}I$ as a carrier free and reductant free solution was purchased from Institute of Isotopes Co., Ltd. (IZOTOP), Budapest, Hungary.

2.2. Methods

2.2.1. Preparation of PLGA nanoparticles co-encapsulating naringin and celecoxib

Eight different PLGA nanoparticulate formulations were prepared using a modified combined nanoprecipitation homogenization/solvent evaporation method, with compositions described in Table 1. An amount of 200 mg of PLGA polymer and 25 mg of each drug with or without poloxamer was dissolved in 10 ml acetone as the organic phase. PVA solution (1% w/v) was prepared in 25 ml deionized water and added to the organic phase [29,30], followed by homogenization (Heidolph DIAX 900, Kelheim, Germany) for 60 s at 21,000 rpm (using ice water bath in case of poloxamer containing formulae). The formulae were magnetically stirred (Yellow Line MAG HS7, IKA®-Werke GMBH and CO., France) till complete evaporation of acetone [31,32].

2.2.2. Determination of particle size, polydispersity index (PDI) and zeta potential of PLGA nanoparticles

The *Z*-average diameter, PDI and zeta potential of the freshly prepared PLGA nanoparticles were measured using photon correlation spectroscopy (Zetasizer Nano ZS 3600, Malvern Instruments Ltd., UK) after proper dilution of the nanoparticulate formulations using deionized water.

2.2.3. Determination of entrapment efficiency (EE%) of drugs in PLGA nanoparticles

In order to separate the free drug fraction from the encapsulated one, formulations were centrifuged using cooling centrifuge (Hermle Labortechnik GmBH, model Z216MK, Germany) for 60 min at a rotation speed of 15,000 rpm and a temperature of 4 °C. The supernatant was collected and the remaining pellet was washed with deionized water. The amount of naringin and celecoxib in both the supernatant and the pellet was quantified after dilution of the supernatant and dissolving the pellet with acetone respectively. Naringin and celecoxib were assayed using HPLC

Table 1
Composition of different PLGA nanoparticulate formulations.

Formulation code[a]	PLGA grade	Poloxamer
P1	PDLG 7502 (75:25)	–
P2	PDLG 7502A (75:25)	–
P3	PDLG 5002 (50:50)	–
P4	PDLG 5002A (50:50)	–
P5	PDLG 7502 (75:25)	25 mg
P6	PDLG 7502A (75:25)	25 mg
P7	PDLG 5002 (50:50)	25 mg
P8	PDLG 5002A (50:50)	mg

[a] All formulae were prepared using 200 mg PLGA polymer, co-loaded with 25 mg of naringin and 25 mg of celecoxib drugs, using 10 ml acetone as organic phase and 25 ml PVA 1% w/v solution as aqueous phase.