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Sterically stabilized gelatin microassemblies of noscapine enhance cytotoxicity, apoptosis and drug delivery in lung cancer cells

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ABSTRACT

Noscapine, recently identified as anticancer due to its microtubule-modulating properties. It is presently in Phase I/II clinical trials. The therapeutic efficacy of noscapine has been established in several xenograft models. Its pharmacokinetic limitations such as low bioavailability and high ED_{50} impede development of clinically relevant treatment regimens. Here we present design, synthesis, *in vitro* and *in vivo* characterization of sterically stabilized gelatin microassemblies of noscapine (SSGMS) for targeting human non-small cell lung cancer A549 cells. The average size of the sterically stabilized gelatin microassemblies of noscapine, SSGMS was 10.0 ± 5.1 μm in comparison to noscapine-loaded gelatin microassemblies, GMS that was 8.3 ± 5.5 μm. The noscapine entrapment efficiency of SSGMS and GMS was $23.99 \pm 4.5\%$ and $24.23 \pm 2.6\%$, respectively. Prepared microassemblies were spherical in shape and did not show any drug and polymer interaction as examined by FTIR, DSC and PXRD. *In vitro* release data indicated that SSGMS and GMS follow first-order release kinetics and exhibited an initial burst followed by slow release of the drug. *In vitro* cytotoxicity evaluated using A549 cells showed a low IC_{50} value of SSGMS (15.5 μM) compared to GMS (30.1 μM) and free noscapine (47.2 μM). The SSGMS can facilitate a sustained therapeutic effect in terms of prolonged release of noscapine as evident by caspase-3 activity in A549 cells. Concomitantly, pharmacokinetic and biodistribution analysis showed that SSGMS increased the plasma half-life of noscapine by ~9.57-fold with an accumulation of ~48% drug in the lungs. Our data provides evidence for the potential usefulness of SSGMS for noscapine delivery in lung cancer.

1. Introduction

Despite rapid advances in surgery, and radiotherapy as well as availability of a diverse armamentarium of chemotherapeutic drugs for lung cancer management, its prognosis continues to remain dismal. Although drugs such as gemcitabine, docetaxel, cisplatin, bevacizumab, erlotinib, and gefitinib are currently used in the clinic for the therapy of non-small cell lung cancer (NSCLC) [1], their significant toxicity and emergence of drug resistance limits their potential. Thus there is an ongoing search for new therapeutic approaches that have superior pharmacological profiles and can offset toxicities.

Noscapine, a relatively non-toxic microtubule-modulating anticancer agent is in Phase I/II clinical trials [2]. The efficiency of noscapine as anticancer agent has been established in several xenograft models including breast, lymphoma, prostate, and lung [3–6]. A recent study also showed synergistic activity of noscapine with gemcitabine in inhibiting lung tumor xenografts [7]. The high ED_{50} (300–550 mg/kg body weight) suggests that noscapine can be well-tolerated at higher doses but the therapeutic concentration at the targeted sites cannot be achieved due to poor physicochemical properties of noscapine. Moreover, it gets eliminated from systemic circulation and visceral organs by first-order elimination rate constant, resulting in low drug accumulation in tumor cells [8,9].

Targeting nano- and micro-vesicles to enhance drug uptake in tumor cells while sparing healthy cells is a lucrative strategy and increasingly being investigated [10,11]. The tumor uptake of functionalized biocomposites can be enhanced either through enhanced permeability and retention effect of a variety of ligand-anchored conjugates [12] or by using selective route of administration [13]. In particular, drug delivery to the lungs is amenable due to the large surface area of the alveolar spaces. Employing intravenous administration, the biodistribution of microassemblies in the range of

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7–12 μm can be attained efficiently in the lung due to the trapping effect of pulmonary blood vessels that improves therapeutic indices [14]. Gelatin microparticles also increase the risk of embolization and its consequences like stroke or pulmonary embolism. But these risks are generally associated with particles in the range of 40–120 μm [15]. In addition to size related biodistribution pattern, it has been well established that microassemblies are quickly recognized by the reticuloendothelial system and phagocytosed. Several physical and chemical properties of microassemblies such as particle size, lipophilicity and zeta potential significantly dictate their extent of phagocytosis by macrophages and influence their *in vivo* biodistribution. Hydrophobic microassemblies with positive zeta potential are highly receptive to macrophages compared to hydrophilic microassemblies with negative surface charge [16,17]. Therefore, docking anticancer drugs to tailored stealth microassemblies is an attractive approach for tumor-targeted therapies. Essentially, steric stabilization involves covalent attachment of poly(ethylene)glycol (PEG) polymer chain to a protein or drug that shields the injected biocomposite from the host's immune system and prolongs its circulatory time by reducing renal clearance. PEG also precludes adsorption of proteins on the shell of composite system due to its steric repulsion effect, thus preventing opsonization [18].

Our laboratory is actively engaged in the design and development of oral, parenteral and topical biocomposites for targeted delivery of known anticancer agents [19–23]. We have previously reported the synthesis of PEG-implanted gelatin nanoshells to improve the plasma half-life of noscapine [20]. Several organ distribution studies of gelatin and PEG-grafted gelatin nanoparticles (<200 nm) injected intravenously have indicated liver and spleen as the major sites of delivery, with negligible concentration in lungs, brain and kidney [24,25]. Here we present synthesis and optimization of sterically stabilized gelatin microassemblies of noscapine (SSGMS) that were examined for their lung targeting potential *in vitro* and *in vivo*.

2. Materials and methods

2.1. Chemicals and reagents

Noscapine (~98% purity, free base), gelatin (Type B; bloom-strength ~225; 100–115 mM of free carboxylic acid/100 g protein with isoelectric point of 4.7–5.2 and average molecular weight 40–50 kDa) and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) were from Sigma (St. Louis, MO). Monomethoxy-polyethylene glycol (monomethoxy-PEG) with a molecular weight of 5 kDa was from Fluka. All other chemicals were of analytical grade and used without further purification.

2.2. Cells and reagents

Human non-small cell lung cancer cell line (A549) was maintained in 95% air and 5% CO_2 atmosphere at 37 °C using Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum [26].

2.3. Preparation of sterically stabilized gelatin microassemblies of noscapine

Noscapine was encapsulated in SSGMS and gelatin alone (GMS) for comparing drug release kinetics and antiproliferative activity. Microassemblies of unmodified gelatin (GMS–GMS7) and sterically stabilized gelatin (with 35% of available amine groups modified using 0.6 g PEG-epoxide to 1.0 g of gelatin) [20] (SSGMS) were prepared by coacervation phase separation technique utilizing temperature change (Table 1) [27]. Briefly, gelatin or sterically stabilized gelatin (2.0–6.0 g) was dissolved in 10 mL of pre-warmed distilled water (50 °C). Noscapine (500 mg) was dispersed in the solution while stirring and aqueous phase was poured drop-wise in 125 mL of sunflower oil containing span 20 (0.5 mL) and the emulsion was heated to 50 °C. The dispersion was stirred at 600 rpm for 5 min to ensure uniform dispersion followed by cooling to 10 °C to effect phase separation. The dispersion was stirred for 2 h in an ice bath at 10 °C. After the first hour, glutaraldehyde (25% aqueous solution, 1.2×10^{-1} M to 4.5×10^{-1} M) was added to the dispersion and stirring was continued for the next 1 h. After 2 h, the stirring was stopped, and the resulting emulsion refrigerated at −5 °C for 24 h to ensure rigidization of microassemblies. After 24 h, the slurry was filtered and the microassemblies were collected, washed with ice-cold isopropyl alcohol to make them free of oil and residual glutaraldehyde and air-dried for 24 h.

2.4. Characterization of microassemblies

2.4.1. Particle size distribution and zeta potential analysis of gelatin microassemblies

Particle size distribution and zeta potential analyses of microassemblies were done by using a zetasizer, HAS 3000 (Malvern Inst., Worcestershire, UK). Briefly, 5 mg of sample was suspended in 4 mL of phosphate-buffered saline (PBS, pH 7.4, 10 mM). An electric field of 150 mV was applied to observe the electrophoretic velocity of the microassemblies. All measurements were recorded at 25 °C in triplicate.

2.4.2. Shape characterization of gelatin microassemblies

The shape and surface morphology of microassemblies (GMS and SSGMS) were examined using scanning electron microscopy (SEM; JSM-6100, JEOL, Tokyo, Japan). Microassemblies were fixed on supports with carbon-glue, and coated with gold using a gold sputter module (JFC-1100) in a high vacuum evaporator. The images were captured by SEM at 15 kV.

2.4.3. Quantitative analysis of noscapine in gelatin microassemblies

Different batches (GMS–GMS7 and SSGMS; Table 1) of microassemblies (50 mg) were suspended in 50 mL of 0.02 N hydrochloric acid. Suspensions were heated briefly and set aside for 48 h. Microassemblies were then centrifuged ($8000 \times g$) to remove traces of gelatin and supernatant was filtered with 0.22 μm membrane filters (Millipore). An aliquot of the filtrate was diluted appropriately with 0.02 N hydrochloric acid and analyzed at 311.2 nm in UV/vis spectrophotometer (1800 T-800, Shimadzu, Kyoto, Japan) to determine the encapsulation efficiency of noscapine in microassemblies [19].

2.4.4. Fourier transform-infrared spectroscopy (FTIR)

The spectrum of noscapine, blank GMS, physical mixture of noscapine and blank GMS as well as GMS were scanned using infrared spectrophotometer (Perkin Elmer). Samples were prepared in KBr disc (2 mg sample/200 mg KBr) with a hydrostatic press at a force of 40 psi for 4 min. The scanning range used was 400–4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.4.5. Differential scanning calorimetry (DSC)

Thermal behavior of noscapine, blank GMS, physical mixture of noscapine and blank GMS and GMS was examined using a DSC (DSC7, Perkin-Elmer, Waltham, MA) thermal analyzer. Argon was employed as carrier gas. Analysis was performed at a heating rate of 10 °C/min using an argon flow rate of 35 mL/min. Sample size was 10 mg and curves were recorded at 60–300 °C.