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Research Paper

Phospholipid-stabilized mesoporous carbon nanospheres as versatile carriers for systemic delivery of amphiphobic SNX-2112 (a Hsp90 inhibitor) with enhanced antitumor effect

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ABSTRACT

Systemic delivery of amphiphobic drugs (insoluble in both water and oil) represents a formidable challenge in drug delivery. This work aimed to engineer a functional mesoporous carbon material to efficiently load SNX-2112, an amphiphobic anticancer agent, and to evaluate its performance in tumor-targeting delivery. Hydrothermal reaction combined with high-temperature activation was used to fabricate glucose-based mesoporous carbon nanospheres (MCNs). SNX-2112-loaded MCNs stabilized by phospholipid (SN-PMCNs) were prepared by the absorption/solvent diffusion/high-pressure homogenization method. The obtained SN-PMCNs were 180 nm around in particle size, showing a high drug load (42.7%) and acceptable physical stability. SN-PMCNs demonstrated an enhanced *in vitro* antitumor effect and increased uptake into cancer cells in comparison with the formulation of SNX-2112 solution (SN-Sol). The *in vivo* antitumor effect and biodistribution in 4T1 xenograft tumor mice, a breast cancer model, were also significantly improved through SN-PMCNs. It was shown that specific clathrin-dependent and non-specific caveolae-dependent endocytosis were involved in the cellular trafficking of SN-PMCNs. Glucose transporter-mediated transport, prolonged body residence time and improved biodistribution via EPR effect were the main mechanisms of enhanced antitumor effect. SN-PMCNs have presented excellent tumor targeting properties and should be a promising carrier to address the systemic delivery of SNX-2112.

1. Introduction

Advances in chemistry, drug design and bioactivity screening have significantly accelerated the drug discovery, resulting in numerous highly active entities. However, the majority of drug candidates are plagued with poor solubility and/or permeability. In fact, over 70% of drugs on the market or under development are identified as BCS II or IV ones [1], many of which are simultaneously insoluble in water and oil (i.e. amphiphobic). Typical amphiphobic drugs include anticancer agents, such as raloxifene [2], paclitaxel [3], and Z-GP-Dox (a doxorubicin derivative) [4]. Systemic delivery of amphiphobic drugs is of high challenge due to poor solubility and incompatibility with excipients.

To achieve systemic delivery of insoluble drugs, a variety of nanoparticle-based formulations have been explored, including liposomes [5], polymeric micelles [6], polymeric nanoparticles [7], lipid nanocarriers [8], and nanocrystals [9]. Although these systems have demonstrated potential in solubilization and targeted delivery of poorly water-soluble drugs, they are less effective in drug loading in the case of amphiphobic drugs due to significant drug precipitation from the carriers. Hence, it is imperative to develop novel nanocarriers for an improved drug load and more effective drug delivery.

Abbreviations: CNs, carbon nanospheres; MCNs, mesoporous carbon nanospheres; SN-PMCNs, SNX-2112-loaded MCNs stabilized by phospholipid; SN-Sol, SNX-2112 solution; EPR effect, enhanced permeability and retention effect; BCS, biopharmaceutics classification system; BCA, bicinchoninic acid; SEM, scanning electron microscope; TEM, transmission electron microscope; DL, drug load; MWCO, molecular weight cut-off; PBS, phosphate buffered saline; FTIR, fourier transform infrared spectroscopy; PDI, polydispersity index; IC_{50}, half maximal inhibitory concentration; RES, reticuloendothelial system.

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In recent years, mesoporous materials have received growing interests in interfacial catalysis, energy reserve, and drug delivery [10]. Mesoporous nanomaterials as drug carriers possess several superior characteristics, such as tunable drug release, modifiable targetability and high drug load [11]. Owing to large pore volume and high specific surface area, mesoporous materials can achieve a high entrapment rate toward amphiphobic drugs by adsorption. Saha et al. synthesized mesoporous carbons using a soft-template technique to orally deliver three model drugs (captopril, furosemide and ranitidine), and showed that the material had excellent potential in the release control and drug loading [12]. In another study, they demonstrated the newer biomaterial of low toxicity and good biocompatibility by the cytotoxicity test and protein adsorption experiment [13]. Hydrophilic mesoporous carbon nanoparticles as carriers of camptothecin have also been reported. The carrier exhibited a good water dispersibility and sustained drug release, and could be internalized into cells to effectively inhibit their growth [14]. Although carbon-based materials are readily available and low-toxic, their merits in drug delivery of amphiphobic drugs have not been fully established. Mesoporous carbon nanospheres (MCNs) can be readily obtained by hydrothermal synthesis [15]. Various substances can be used as carbon sources to fabricate MCNs, such as phenolic resol, sucrose and glucose. It was shown that glucose uptake and glycolytic metabolism were highly active in cancer cells than normal cells [16]. Overexpression of glucose transporter has been verified in a variety of cancer cells, including breast cancer cell [17]. MCNs that use glucose as carbon source are incompletely carbonized, on which glucose residues are retained. Thus, MCNs based on glucose may be a promising carrier for targeted delivery of amphiphobic drug.

SNX-2112 is a heat-shock protein 90 (Hsp90) inhibitor that can degrade Hsp90 client proteins to induce cell cycle arrest and apoptosis, thereby killing cancer cells [18]. However, SNX-2112 is almost insoluble in water and oil, and also poorly soluble in other lipophilic excipients. The solubility is just 7.55 μg/mL in water and 90.1 μg/mL in soybean oil. The Log*P* and pK_a are determined to be 1.89 and 11.67, respectively. The insoluble nature significantly limits its development of the compound toward clinical stages. Although there are hydrophilic hydroxyl and carboxyl groups on the surfaces of MCNs, MCNs are hydrodynamically unstable as injectable carriers. In this work, phospholipid-stabilized MCNs (PMCNs) were developed for systemic and specific delivery of SNX-2112 (Scheme 1). Multiple techniques such as loading drug by adsorption, stabilizing carriers by phospholipid coating, and targeting delivery by glucose transporter are integrated into the engineering of SNX-2112-loaded PMCNs (SN-PMCNs). The suitability and antitumor effects of SN-PMCNs were evaluated by a series of *in vitro* experiments and a breast cancer xenograft mouse model.

2. Materials and methods

2.1. Materials

SNX-2112 and AT-533 (internal standard, an analogue of SNX-2112) with a purity of >98.0% were kindly provided by Prof. Yifei Wang (Biomedicine Research and Development Center, Jinan University, Guangzhou, China). Soybean lecithin (S100) was supplied by Lipoid (Ludwigshafen, Germany). Chlorpromazine, simvastatin, Filipin, trypsin and EDTA were obtained from Sigma–Aldrich (Shanghai, China). Glucose, 5-aminofluorescein and sucrose were purchased from Aladdin (Shanghai, China). RPMI 1640, DMEM, fetal bovine serum (FBS) and penicillin–streptomycin were purchased from Gibco BRL (Gaithersburg, MD, USA). BCA protein assay kit, RIPA lysis buffer and phenylmethanesulfonyl fluoride (PMSF) were obtained from Beyotime Institute of Biotechnology (Shanghai, China). Deionized water was prepared by a water purifier (Chengdu, China). All other chemicals and reagents used were of analytical grade.

2.2. Synthesis and characterization of MCNs

Carbon nanospheres (CNs) were synthesized by hydrothermal reaction using glucose as carbon source [19]. Typically, 6 g of glucose was dissolved in 60 mL of deionized water to form a clear solution, and then the solution was placed in a Teflon-lined autoclave and maintained at 200 °C. To obtain suitable CNs, the hydrodynamic size was monitored using a particle size analyzer (Zetasizer Nano ZS, Malvern, Worcestershire, UK) in real time. The reaction was terminated by cooling the system to room temperature. The products were filtrated and washed alternately with ethanol and water. The purified CNs were dried at 70 °C for 8 h followed by impregnation with $ZnCl_2$ solution (0.8 M) for 12 h. The impregnated CNs were harvested by filtration and proceeded to dry at 80 °C for 12 h. An activation procedure was performed on the material at 400 °C under N_2 atmosphere for 3 h. After cooling down, the material was subjected to HCl (0.5 M) disintegration to create porosity. Finally, MCNs were obtained after washing and drying.

MCNs were characterized by BET nitrogen adsorption, scanning and transmission electron microscopies. The surface area and pore size of MCNs were calculated based on the N_2 adsorption/desorption isotherm obtained from a surface area and porosity analyzer (Micrometritics TriStar, Norcross, USA). MCNs were first outgassed at 250 °C for 3 h under vacuum to a final pressure of 0.25 Pa and then the isotherm was measured at 77 K over the relative pressure (P/Po). The specific surface area and adsorbed volume of MCNs were determined using the Brunauer–Emmett–Teller (BET) equation. The surface area was calculated from the BET model. The pore volume versus diameter distribution was calculated by analyzing the adsorption and desorption branches of the isotherm using the Barrett–Joyner–Halenda (BJH) method [20].

To inspect the surface morphology, MCNs were immobilized to supporters by drying them under a lamp. Fixed particles were then coated with platinum/palladium and photographed using a Zeiss XL-30E SEM (Oberkochen, Germany). For insight into the interior morphology, the sample was prepared as above without surface coating and observed with a Philips Tecnai 10 TEM (Amsterdam, Netherlands).

2.3. Adsorption isotherm of MCNs versus SNX-2112

Adsorption isotherm was plotted by accumulative absorption of SNX-2112 against time at 25 °C. In detail, 50 mg of MCNs was added into 50 mL of SNX-2112 solution (dissolved in 80% ethanol, 15 mg/mL) and agitated with a magnetic stirrer. At predetermined intervals, the sample (200 μL) was withdrawn and centrifuged at 6000*g* for 10 min to separate SNX-2112-loaded MCNs from the system. The concentration of SNX-2112 in supernatant (free drug) was determined by HPLC as described below. The accumulative adsorption quantity of SNX-2112 relative to MCNs at various time points (q_t, mg/g) was calculated by the equation: $q_t = (C_0 - C_t) \cdot V/M$, where C_0 and C_t (mg/mL) respectively represent the initial and real-time concentration of SNX-2112, V denotes the volume of system, and M is the weight of MCNs used.

2.4. Preparation of SN-PMCNs

SN-PMCNs were prepared by an absorption/solvent-diffusion/high pressure homogenization technique. Briefly, 30 mg of SNX-2112 was dissolved in 1 mL 80% ethanol, into which 30 mg of MCNs was introduced. After absorption for 6 h, the solvent was removed by evaporation at 45 °C under reduced pressure until

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