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Improved delivery of the natural anticancer drug tetrandrine

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ABSTRACT

The study aims at designing a nanoparticle-based delivery system to improve the efficacy of the natural compound tetrandrine against lung cancer. Nanoparticles from poly(lactic-*co*-glycolic acid) (PLGA) were prepared by the emulsion solvent diffusion method and characterized for their physicochemical properties and drug-loading efficiency. Furthermore, the cellular uptake and the anti-cancerous activity was studied on A549 cell line. To investigate the surface properties and uptake, three different stabilizers were used to analyze the effect on size and zeta potential of nanoparticles as well as the effect on the cellular uptake. Nanoparticles in the size range of 180–200 nm with spherical shape were obtained with polyvinyl alcohol (PVA), Pluronic-F127 (PF127) and didodecyldimethylammonium bromide (DMAB), 2%, 1% and 0.1%, respectively. An entrapment efficiency of 50–60% with a loading of 1.5–2% was observed. In vitro release profile at pH 7.4 PBS solution showed a consistent release over 168 h. All particle systems showed an improved performance over the pure drug at the same drug concentration. DMAB stabilized particles demonstrated the most pronounced effect against A549 cells compared to pure drug while PVA stabilized particles were least effective in terms of antitumor activity.

1. Introduction

Lung cancer is the major cause of cancer-related mortality throughout the world (Edwards et al., 2014). Among all lung cancers types, over 75% belong to the non-small cell lung cancer (NSCLC). The surgery and chemotherapeutic options seem to be inadequate in curing NSCLC and the overall 5-year survival rate of all stages of NSCLC could only remain approximately 10–15% (Zochbauer-Muller et al., 2002). Unsatisfactory therapeutic effect is mainly due to the narrow therapeutic window of anticancer drugs, the occurrence of side effects (Simon, 2008), and chemotherapy agents not reaching effectively the target tissue and cells.

Tetrandrine (Tet), a bisbenzylisoquinoline alkaloid is the main active ingredient of Stephania tetrandra S. Moore, extracted from the root tuber. Recent pharmacodynamics studies have shown considerable activity against lung cancer cells: for example, Cho and colleagues reported Tet could selectively inhibit the proliferation of lung cancer cells (Cho et al., 2009). Liou et al. studied the molecular mechanism of growth inhibiting and apoptosis inducing when Tet treated lung cancer cell line A549 and showed the anticancer activity of Tet is due to an up-regulation of the cyclin-dependent kinase inhibitor p21, an activation of the apoptosis mediator caspase-3, and a down-regulation of cyclin D1 (Liou et al., 2008). Furthermore, Liu et al. tested Tet in combination with gemcitabine treatment for 240 patients with advanced NSCLC and found Tet may improve short-term efficacy, survival, and mitigate adverse reactions to chemotherapy for patients with NSCLC (Liu et al., 2012). However, poor water-solubility (Tet $c_{saturation}$ = 0.015 mg/mL in pH 7.4 phosphate buffer solution (PBS)) and toxicities such as localized ulcer are some of the limitations of Tet application (Li et al., 2009). Furthermore, free agents fail to achieve long retention in tumor tissue. Therefore, alternative strategies are needed to improve Tet delivery to target the site of action effectively.

Drug loaded nanoparticles (NPs) are promising for intracellular delivery and offer the possibility to be used for local pulmonary delivery of therapeutics for treating lung diseases (Hein et al., 2009; Nafee et al., 2012, 2014). Delivery of therapeutic agents to the site of action for lung diseases may allow for efficient treatment of lung cancers, lung infections and some other respiratory pathologies (Gelperina et al., 2005).

Besides the common benefits obtained from the administration of drug-loaded nanoparticles like improved therapeutic efficiency, reduced side effects and toxicity (Moghimi et al., 2005; Wang et al.,

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2008), drug loaded nanoparticles have a greater chance to escape from the clearance mechanisms of the lung defense systems, compared to microparticles (Chono et al., 2006; Schürch et al., 1990).

Even though not approved nanocarriers from biocompatible and biodegradable materials such as poly(lactic-co-glycolic acid) (PLGA) offer good prerequisites for pulmonary application (De Souza Carvalho et al., 2014). Particle size or better mean mass aerodynamic diameter are key factors for deposition (De Souza Carvalho et al., 2014). An optimal size range is defined in the micrometer range (Chow et al., 2007) but nanoparticulate carriers are also expected to be efficiently inhaled and deposited (Henning et al., 2010) or applied as structured microparticles from nanoparticles. The size also impacts on the clearance mechanisms and the fate of the particles in the deep lung. Diameters lower than 260 nm will only be weakly taken up by macrophages (Mura et al., 2011; Shoyele and Cawthorne, 2006). Moreover, small particles are more efficiently internalized into cancer cells than larger particles (Panyam and Labhasetwar, 2003). Tahara et al. (2009) reported that A549 cellular uptake of PLGA NP increased, when the particle size was controlled to below the sub- micron region (<400 nm).

Independent of size, charges and the surface properties of the nanoparticles play a dominant role when the particles interact with cells or tissues. At physiological conditions, charged nanoparticles have higher deposition efficiencies as compared to neutrally charged nanoparticles: positively charged NPs exhibited more cellular uptake than negatively charged NPs due to the distinct cell surface properties (Lutsiak et al., 2002; Nafee et al., 2012; Qaddoumi et al., 2004).

The purpose of this study was to modulate the surface properties of the formulation parameters for improving the efficacy of the Tet against A549 lung cancer cell. To investigate the surface properties and uptake, three different stabilizers were investigated i.e., the most commonly used hydrophilic polymeric stabilizer polyvinyl alcohol (PVA), Pluronic® F127 (PF127), a non-ionic bifunctional triblock copolymer surfactant (Batrakova and Kabanov, 2008), and a quaternary ammonium salt didodecyldimethylammonium bromide (DMAB), which would render the particles surface positively charged (Bhardwaj et al., 2009). Different nanoparticle formulations were compared in terms of surface charge, drug loading, and release behavior. Furthermore, their cellular uptake and the antitumor effectiveness were assessed in vitro using toxicity testing.

2. Materials and methods

2.1. Materials

Tetrandrine (as a powder with a purity of >98%) was obtained from Wuhan Dinghui Chemical Co., Ltd., (Wuhan, China). PLGA 50:50 (Resomer RG 503, MW: 24,000–38,000 Da) was purchased from Evonik Industries, Germany. Polyvinyl alcohol (PVA) (Mowiol 4-88, Kuraray Specialities Europe GmbH, Frankfurt, Germany), Pluronic® F-127 (PF127), didodecyldimethylammonium bromide (DMAB), fluoresceinamine and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (DMAP) (Sigma–Aldrich, St. Louis, MO, USA) were used as obtained. Dimethylthiazoly-2,5-diphenyltetrazolium bromide (MTT) was obtained from (Promega, Darmstadt, Germany). Millipore water with a resistivity of 18.2 mΩ cm was used throughout the experiment. All other solvents and chemicals were from the highest grade and commercially available.

2.2. Preparation of the fluorescently-labeled PLGA polymer

Fluoresceinamine (FA) bound PLGA (FA–PLGA) was prepared based upon the method introduced by Horisawa et al. (2002) and modified by Weiss et al. (2007). Briefly, PLGA (3.07 g) and FA (0.0583 g) were dissolved entirely in 30 mL of acetonitrile with 0.0408 g of DMAP and incubated at room temperature for 24 h under light protection and gentle stirring. The resultant FA–PLGA was precipitated by the addition of purified water and separated by centrifugation. The polymer was rinsed from excessive reagents (dissolution in acetone and precipitation with ethanol) and then lyophilized (Alpha 2–4 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode Germany).

2.3. Preparation of the tetrandrine-loaded nanoparticles or fluorescently-labeled PLGA nanoparticles with different surfactants

Tetrandrine-loaded PLGA NPs were prepared by the emulsion solvent diffusion evaporation method as previously used (Nafee et al., 2012). Briefly, 20 mg/mL PLGA and Tetrandrine (0.2, 0.3, 0.4, 0.5 mg/mL) were dissolved in ethyl acetate (organic phase). This was then added dropwise into the aqueous phase (containing PVA, PF127 or DMAB), under continuous stirring. After 1 h of stirring the crude emulsion was homogenized using ultrasonifier with 500 J for 30 s (G560E, Scientific Industries, Inc., USA) and then diluted with water to allow ethyl acetate diffusion into the aqueous phase. Subsequently, the suspension was stirred overnight to evaporate ethyl acetate. Nanoparticles were collected using a centrifuge (Rotina420, Hettich, Tuttlingen, Germany) at 14,000 × *g*, for 40 min and redispersed in demineralized water.

The fluorescently-labeled PLGA nanoparticles were prepared by the same method using FA–PLGA instead of normal PLGA.

For purification of Tet-loaded PLGA NPs, centrifugation of the nanoparticles suspension in Vivaspin-20 tubes (Sartorius tubes with a MWCO of 100,000 Da, Goettingen, Germany) at 14,000 × *g*, for 40 min was performed. The NPs on the filter were collected and washed with demineralized water. Then, nanoparticle suspensions were freeze dried and stored until use at room temperature.

2.4. Characterization of nanoparticles

2.4.1. Physicochemical characterization

2.4.1.1. Size, polydispersity and zeta potential determination. The colloidal properties size, size distribution (polydispersity, PDI), and the zeta potential of the NP was measured using a Malvern Zetasizer Nano (Malvern Instruments, Worcestershire, UK) The mean values were calculated from the measurements performed at least in triplicate.

2.4.1.2. Stability evaluation of colloidal properties. Tetrandrine-loaded PLGA NPs were resuspended after centrifugation in pH 7.4 PBS solution and kept at room temperature. Particle sizes were then determined by dynamic light scattering (DLS) every 3 days for 12 days to evaluate colloidal stability.

2.4.2. Morphological characterization

The morphology of NPs was visualized by SEM (SEM EVO HD series, Carl Zeiss, Jena, Germany) using high vacuum, applying an acceleration voltage of 1.5 or 4.0 kV.

For SEM measurement, a drop of the nanoparticles suspension was placed on a silica wafer. The sample was dried under ambient conditions and coated with gold using a Quorum Q 150 ES sputter coater (Quorum Technologies Ltd., Laughton, UK) to render the sample conductive.

2.5. Measurement of drug loading and in vitro release

2.5.1. HPLC analysis for tetrandrine