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Hyaluronic acid-decorated poly(lactic-co-glycolic acid) nanoparticles for combined delivery of docetaxel and tanespimycin

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ABSTRACT

Multiple-drug combination therapy is becoming more common in the treatment of advanced cancers because this approach can decrease side effects and delay or prevent drug resistance. In the present study, we developed hyaluronic acid (HA)-decorated poly(lactic-co-glycolic acid) (PLGA) nanoparticles (HA-PLGA NPs) for co-delivery of docetaxel (DTX) and tanespimycin (17-AAG). DTX and 17-AAG were simultaneously loaded into HA-PLGA NPs using an oil-in-water emulsification/solvent evaporation method. Several formulations were tested. HA-PLGA NPs loaded with DTX and 17-AAG at a molar ratio of 2:1 produced the smallest particle size (173.3 ± 2.2 nm), polydispersity index (0.151 ± 0.026), and zeta potential (−12.4 ± 0.4 mV). Approximately 60% and 40% of DTX and 17-AAG, respectively, were released over 168 h *in vitro*. Cytotoxicity assays performed *in vitro* using MCF-7, MDA-MB-231, and SCC-7 cells showed that dual drug-loaded HA-PLGA NPs at a DTX:17-AAG molar ratio of 2:1 exhibited the highest synergistic effect, with combination index values of 0.051, 0.036, and 0.032, respectively, at the median effective dose. Furthermore, synergistic antitumor activity was demonstrated *in vivo* in a CD44 and RHAMM (CD168) – overexpressing squamous cell carcinoma (SCC-7) xenograft in nude mice. These findings indicated that nanosystem-based co-delivery of DTX and 17-AAG could provide a promising combined therapeutic strategy for enhanced antitumor therapy.

1. Introduction

In recent years, it has become clear that the use of single chemotherapeutic drugs for the treatment of cancer has limitations and may have inadvertently contributed to the development of acquired chemoresistance, owing to neoplastic heterogeneity and rapid mutation. Hence, combined use of two or more chemotherapeutic drugs with different mechanisms of action has been adopted as a standard regimen in clinical practice. This overcomes the limitations of single-drug therapy and confers additional benefits such as dose reduction, amelioration of non-specific side effects and toxicity, eradication of resistant cancer cells, and synergistic effects (Davis, Chen, & Shin, 2008; Jia et al., 2009; Ramasamy, Tran, et al., 2014; Ramasamy, Kim, et al., 2014; Shin, Alani, Rao, Rockich, & Kwon, 2009). Many drug combination regimens have already been studied in clinical trials and approved for use in patients (Lane, 2006). However, chemotherapeutic agents have different pharmacokinetics, systemic distributions, and cellular uptake profiles, which can make dosing and administration of multiple agents to patients very difficult and may even prove counter-productive in the longer term (Kang et al., 2010; Zheng et al., 2013). These issues can be addressed by delivering multiple agents using a single nanocarrier system (Hu, Aryal, & Zhang, 2010; Lee & Nan, 2012). Ideally, a well-designed nanocarrier can provide ratiometric drug loading, targeted delivery, temporal drug release, and consistent industrial scalability. It also helps to circumvent the issues associated with the compatibility, stability, and toxicity of toxic excipients such as cremophor and dimethyl sulfoxide (DMSO) that are used to solubilize chemotherapeutic agents, which are often poorly water soluble (Huang et al., 2014; Xiong, Yanez, Kwon, Davies, & Laird Forrest, 2009).

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Various platforms for nanoparticle (NP)-assisted combination chemotherapy have been developed for concurrent or sequential delivery of multiple agents; these include liposomes, polymeric NPs, and dendrimers (Katragadda, Fan, Wang, Teng, & Tan, 2013; Wang et al., 2011; Wong & Chiu, 2011). Of these, polymeric NPs have been the most widely-researched platform due to their relatively higher stability, physicochemical tunability and tailorability, nanosize and narrow size distribution, higher encapsulation efficiency for drugs with low water solubility, and availability of surface functional groups for ligand conjugation. One of the most extensively-used biodegradable polymers in nanoparticulate anticancer drug delivery is poly(lactic-co-glycolic acid) (PLGA), which exhibits minimal systemic toxicity (Kumari, Yadav, & Yadav, 2010; Mittal, Sahana, Bhardwaj, & Kumar, 2007; Toro, Betti, & Spampinato, 2004). Surface modification of PLGA with certain hydrophilic polymers enables these NPs to increase their selectivity for cellular binding and internalization (Danhier et al., 2012). For example, attachment of hyaluronic acid (HA), a naturally occurring linear polysaccharide, to the surface of PLGA NPs has been reported to facilitate specific binding to receptors for ligands such as CD44 and RHAMM (CD168); these are generally overexpressed by tumor cells and thereby facilitate internalization of the NPs by receptor-mediated endocytosis (Jeong et al., 2012; Qhattal & Liu, 2011).

Recently, we reported the successful preparation of individual DTX- and 17-AAG-loaded PLGA NPs with sustained release profiles and cytotoxic activities against breast cancer cell lines (Pradhan et al., 2013, 2014). However, in this study, we developed an HA-coated PLGA nanocarrier system for the combined delivery of docetaxel (DTX) and tanespimycin (17-AAG) and investigated its synergistic effects on cancer cell lines and tumor-bearing mice. 17-AAG, a geldanamycin analog, was the first heat shock protein 90 (Hsp90) inhibitor to enter clinical trials and it has completed phase II trials for the treatment of a range of cancers (Modi et al., 2011; Pacey et al., 2012; Solit et al., 2008). As a single drug therapy, 17-AAG has demonstrated its efficacy in multiple myeloma and breast cancer (Richardson et al., 2010). It has also exhibited marked synergistic effects in combination with a broad range of anticancer drugs in different tumor cell lines (Zhou, Feng, Mueller, Liu, & Johnson, 2004). Typical examples include paclitaxel/17-AAG, etoposide/17-AAG, and DTX/17-AAG combinations (Ramalingan et al., 2008; Shin et al., 2009). 17-AAG has been shown to potentiate the effects of taxols in various tumor models and is currently an important element in combination chemotherapy for some relapsed cancers (Sain et al., 2006; Sawai et al., 2008).

In the present study, HA-PLGA NPs were prepared using an oil-in-water (o/w) emulsification solvent evaporation technique. The HA-PLGA NPs were characterized with respect to particle size, polydispersity index, morphology, encapsulation efficiency, and *in vitro* release. In addition, we evaluated the efficacy of HA-PLGA NPs by performing *in vitro* cytotoxicity studies in the MCF-7 and MDA-MB-231 human breast cancer cell lines, and the SCC-7 murine squamous cancer cell line. Antitumor efficacy was also assessed *in vivo* using SCC-7 cells in a nude mouse xenograft model.

2. Materials and methods

2.1. Materials

PLGA (50:50 molar ratio of lactide:glycolide, product number: DLG 2A, MW 18,000) with free carboxyl end groups was purchased from Evonik Degussa (Seoul, Korea). DTX was donated by Hanmi Pharm. Co. (Hwaseung, Korea) and 17-AAG was purchased from LC Laboratories (Woburn, Massachusetts, USA). Hyaluronic acid (3 kDa) was supplied by B&K Technology Group Co. Ltd. (China). Hexadecyl trimethyl ammonium bromide (CTAB), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) reagent, 4,6-diamidino-2-phenylindole (DAPI), and coumarin-6 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Cell culture medium (RPMI 1640 and high glucose DMEM), trypsin-EDTA, penicillin, streptomycin, and fetal bovine serum (FBS) were provided from Invitrogen Inc. (Carlsbad, CA, USA). All other chemical reagents were of analytical grade and were used without further purification.

2.2. Preparation of HA-decorated PLGA NPs for co-delivery of DTX and 17-AAG

The dual drug-loaded HA-decorated PLGA NPs were prepared using an o/w emulsification and solvent evaporation technique with a slight modification. Briefly, 10 mg of PLGA, 1.614 mg of DTX, and 1.172 mg of 17-AAG (at molar drug ratio of 1:1) were dissolved in 1 mL of ethyl acetate. This organic solution was then added dropwise under ultrasonication into 10 mL of aqueous solution containing CTAB (0.25–2.0% w/v). The resulting o/w emulsion was evaporated by magnetic stirring under mild heating (25–30 °C) in order to remove the organic solvent, prior to filtration through 0.45 μm syringe filter. HA aqueous solution (0.25–2.0% w/v) was added dropwise into the clear nanoemulsion with mild stirring. The NPs were isolated by low-speed centrifugation (3000 × *g*, 10 min) followed by freeze-drying using a suitable cryoprotectant. Drug-free HA-PLGA NPs were produced in a similar manner. When determining the effect of CTAB and HA concentrations on the physicochemical properties of the resultant NPs, only one variable was altered at a time.

2.3. Characterization of DTX and 17-AAG loaded HA-decorated PLGA NPs

2.3.1. Hydrodynamic diameter and zeta potential measurement

A NanoZS light-scattering particle size analyzer (Malvern Instruments, Malvern, UK) with non-invasive back scatter (NIBS®) technology was used with the manufacturer's software (NanoDTS, version 6.34) employing cumulant analysis to determine the *Z*-averaged hydrodynamic size (nm), polydispersity index (PDI), and zeta potential (mV) of the HA-PLGA NPs. The data were expressed as the mean ± standard deviation (S.D.).

2.3.2. Morphological characterization

Morphological studies of NPs were conducted using transmission electron microscopy (TEM) and atomic force microscopy (AFM). TEM (H-7600, Hitachi, Japan) was performed by staining a small drop of the NP suspension with 2% phosphotungstic acid (PTA) solution. The stained sample was deposited onto a carbon-coated copper grid and dried for 30 min at room temperature. AFM was performed in air using a Multimode NanoScope IV system (Veeco, Santa Barbara, CA, USA) operated in tapping mode. Samples for AFM imaging were prepared by depositing a few μL of the micellar dispersion (*ca.* 0.5 mg/mL) onto a positively charged mica surface for 2 min, followed by air-drying. The spherical particles were found aggregated on the mica surface. The images were measured using the manufacturer's software.

2.4. Encapsulation efficiency and loading capacity

The content of DTX and 17-AAG in HA-PLGA NPs was assayed by high-performance liquid chromatography (HPLC; Hitachi, Japan). For DTX analysis, a reverse-phase Hypersil Gold C_8 column (GL science, 5 μm, 4.6 mm × 250 mm) and a UV–vis detector (L-2420) were used. The mobile phase consisted of acetonitrile:methanol:0.01 M acetate buffer (pH 5.0) (45:15:40, v/v) at a