International Journal of Pharmaceutics 511 (2016) 367–379

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Paclitaxel-loaded chitosan oligosaccharide-stabilized gold nanoparticles as novel agents for drug delivery and photoacoustic imaging of cancer cells

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ARTICLE INFO

Article history:
Received 12 May 2016
Received in revised form 29 June 2016
Accepted 12 July 2016
Available online 14 July 2016

Keywords:
Chitosan oligosaccharide
Gold nanoparticles
Paclitaxel
Anticancer
Photoacoustic imaging

ABSTRACT

Polymer nanoparticles have gained significant attention as potential drug carriers for anticancer agents and molecular imaging. Biocompatible gold nanoparticles (AuNPs) were synthesized using chitosan oligosaccharide (COS) as a reducing and stabilizing agent and were subsequently loaded with paclitaxel (PTX) to demonstrate their use in drug delivery and photoacoustic imaging (PAI) of MDA-MB-231 cells. Paclitaxel-loaded chitosan oligosaccharide-stabilized gold nanoparticles (PTX-COS AuNPs) were spherical in shape with an average particle size of 61.86 ± 3.01 nm. PTX-COS AuNPs showed sustained and pH-dependent drug release profiles and exhibited strong cytotoxic effect against MDA-MB-231 cells through the induction of apoptosis with improved reactive oxygen species (ROS) generation and altered mitochondrial membrane potential (MMP) level. The cellular internalization of PTX-COS AuNPs was proven by fluorescence microscopy as well as flow cytometry. PTX-COS AuNPs were also evaluated as a new class of optical contrast agents for photoacoustic imaging (PAI). To the best of our knowledge, this is the first report that describes the use of PTX-COS AuNPs as novel agents for drug delivery and PAI of cancer cells. These results exposed the promising potential of PTX-COS AuNPs in the field of drug delivery, molecular imaging, and cancer therapy in the near future.

1. Introduction

Nanotechnology has immense potential to revolutionize biomedical research with the development of novel and improved nano-products for clinical diagnosis and therapy (Krishnaraj et al., 2014). Polymer nanoparticles have emerged as potential drug carriers for anticancer agents and molecular imaging (Venkatpurwar et al., 2011; Choi et al., 2012). Chitosan (CS), a natural biopolymer, has emerged as an attractive biomaterial for drug-delivery systems because of its biocompatible, biodegradable, and nontoxic nature (Kumar et al., 2004; Xue et al., 2015). However, the main problem of CS is its poor solubility at neutral pH. Chitosan oligosaccharide (COS), a water-soluble hydrophilic backbone (Hu et al., 2008), was developed to overcome these problems and serve as an excellent candidate for drug delivery (Liu et al., 2008). COS has outstanding advantages compared with chitin and CS. COS, a low molecular weight depolymerization product of CS, has recently received considerable attention in biomedical applications because of its low-cost, water-soluble, abundant, biocompatible, biodegradable, and nontoxic nature. In addition, it possesses unique bioactivities, including antimicrobial, antitumor, and antiviral activities (Manivasagan and Oh, 2016). COS is mainly suitable for polymer-drug conjugates because of its accessibility for coupling with the primary amino groups and hydroxyl groups of each polymer subunit and the cationic nature that allows ionic crosslinking (Agnihotri et al., 2004). COS has been improved with hydrophobic residues, including cholesterol, deoxycholic acid,

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http://dx.doi.org/10.1016/j.ijpharm.2016.07.025
0378-5173/

tocopherol, and alkyl groups. This hydrophobically improved COS can form self-assembled nanoparticles, which can be used as carriers for gene and tumor-targeted drug delivery (Chae et al., 2005).

Carbon nanomaterials such as carbon nanotubes and graphene are being constantly researched to explore their applications (De Volder et al., 2013). Carbon nanotubes and graphene are both low-dimensional sp^2 carbon nanomaterials displaying several unique properties that are interesting in a broad range of applications such as drug delivery, imaging, and nanomedicine (Kruss et al., 2013). Since 2004, carbon nanotubes have been used as delivery carriers. *In vivo* cancer treatment with carbon nanotubes has been established in animal experiments by various groups (Liu et al., 2011). Recently, graphene has also shown promise in several biomedical applications. Graphene has been extensively explored as drug delivery and cancer treatment. However, the major drawback of carbon nanomaterials are limited solubility, extremely small, expensive, poor distribution among cells, inability of drugs to cross cellular barriers, (Heister et al., 2009) and particularly a lack of clinical procedures for overcoming multidrug resistant cancer (Jabr-Milane et al., 2008), all limit the clinical administration of chemotherapeutic agents. In recent years, these problems are subject to intensive research worldwide. In this context, a broad range of various types of drug delivery systems have been investigated, including polymers, silica nanoparticles, quantum dots and liposomes. Gold nanoparticles (AuNPs) have emerged as promising agents for drug delivery, therapy, and diagnosis because of their unique physicochemical properties, including surface plasmon resonance (SPR), optical property, and the capability to permit surface modification for further use in biomedical applications (Huang et al., 2007; Manivasagan et al., 2016). Considering the importance of AuNPs, the study focuses on the biosynthesis these nanoparticles in a green way (Singh et al., 2016). Green synthesis does not involve the use of any toxic chemicals, it is environment-friendly, cost-effective, zero energy based and less time-consuming process (Dhand et al., 2016; Manivasagan et al., 2016). Various marine biopolymers like COS is used as sources under 'green synthesis'. Chitosan oligosaccharide-stabilized gold nanoparticles (COS AuNPs) synthesized by the green process and highly biocompatible with pharmaceutical and other biomedical applications (Tagad et al., 2013).

Paclitaxel (PTX) is one of the successful anticancer drugs used to treat breast and ovarian cancers but has poor solubility and low therapeutic applications (Lee et al., 2008; Tao et al., 2012). Recently, various drug-delivery vehicles, including polymer nanoparticles (Zhang et al., 2005; Zhang et al., 2008) and liposomes (Xiong et al., 2011) were investigated to increase the solubility of PTX and to avoid the side effects, and among these novel drug-delivery systems, polymer nanoparticles have received a greater interest. Human breast cancer is the second most common cause of cancer deaths in women and is increasingly observed in developing and developed countries (Hsieh et al., 2005; Krishnaraj et al., 2014). The existing cytotoxic compounds used for breast cancer treatment are found to be considerably costly, ineffective and may further induce side effects. To overcome this difficulty, the development of efficient novel drug carriers without any side effects is an urgent need (Nagajyothi et al., 2016). Photoacoustic imaging (PAI) is an emerging technology that allows for the imaging of tissues and cells using contrast agents such as nanoparticles. PTX-COS AuNPs have been used as contrast agents for PAI with photoacoustic tomography (PAT) *in vitro*. The aim of the present study is to evaluate the potential of PTX-COS AuNPs as novel agents for drug delivery and PAI of cancer cells.

2. Materials and methods

2.1. Materials

COS (average molecular weight above 10 kDa) was obtained from Kitto Life Co., Ltd. (Seoul, South Korea). PTX, gold (III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), acridine orange (AO), ethidium bromide (EB), propidium iodide (PI), Hoechst 33342, 2′, 7′-dichlorodihydrofluorescein-diacetate (DCHF-DA), and rhodamine-123 (Rh-123) were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was purchased from Junsei Chemical Co., Ltd (Tokyo, Japan). All chemicals procured from Sigma–Aldrich Co. (St. Louis, MO, USA) were of analytical grade.

2.2. Synthesis of COS AuNPs

COS (0.05 g) was added to 10 mL aqueous solution of 1×10^{-4} M $HAuCl_4{\cdot}3H_2O$, and the solution was kept in magnetic heater stirrer at 80 °C for 1 h, which resulted in a dark ruby red color indicating the formation of AuNPs. The formation of AuNPs was observed with UV–vis spectroscopy using Beckman Coulter DU530 Life Science UV/Vis spectrophotometer (Beckman Coulter, Fullerton, CA, USA). The synthesized AuNPs were collected by centrifugation at 12,000 × *g* for 30 min, washed many times with deionized water by centrifugation, and re-dispersed in water to remove excess gold. The AuNPs dispersion was dialyzed using a dialysis tube with 12,000 Da molecular weight cutoff for 24 h to remove ionic impurities.

2.3. Preparation of PTX-COS AuNPs

For preparing PTX-COS AuNPs, 20 mg of COS AuNPs was dissolved in 10 mL deionized water, added with 1 mg/mL PTX in DMSO, and kept for 24 h at room temperature to allow proper interaction between the drug and COS AuNPs. The resulting solution was sonicated and centrifuged at 12,000 rpm for 30 min and then dialyzed against deionized water for 12 h. The dialysates were kept at −20 °C until required for further experiments.

To determine the drug loading (DL) efficiency and drug contents (DCs), the samples were freeze-dried and then dissolved in DMSO. The amount of PTX was measured by UV spectrophotometry at 490 nm. The DL efficiency and DCs were calculated by Eqs. (1) and (2), respectively:

$$\mathrm{DL}(\%) = \frac{\text{Amount of drug in nanoparticles}}{\text{Amount of drug initially added}} \times 100 \quad (1)$$

$$\mathrm{DC}(\%) = \frac{\text{Amount of drug}}{\text{Amount of drug and polymer}} \times 100 \quad (2)$$

2.4. Characterization of COS AuNPs and PTX-COS AuNPs

The biosynthesized COS AuNPs were subjected to lyophilization and were powdered for X-ray diffraction (XRD) analysis (X'Pert-MPD, Philips, Almelo, Netherlands). The COS, COS AuNPs, PTX, and PTX-COS AuNPs were freeze dried, powdered, and ground with KBr and analyzed using Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer Inc., USA). FTIR spectra of COS, COS AuNPs, PTX, and PTX-COS AuNPs were recorded using spectrum GX spectrometry in diffuse reflectance mode operated at a resolution of 4 cm^{-1} of wavelength from approximately 4000–400 cm^{-1}. The particle size, morphology, and composition of COS AuNPs and PTX-COS AuNPs were analyzed using field emission scanning electron microscopy