Process Biochemistry 50 (2015) 2298–2306

Contents lists available at ScienceDirect

Process Biochemistry

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

Doxorubicin functionalized gold nanoparticles: Characterization and activity against human cancer cell lines

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ARTICLE INFO

Article history:
Received 9 May 2015
Received in revised form
10 September 2015
Accepted 6 October 2015
Available online 20 October 2015

Keywords:
Gold nanoparticles
Green synthesis
Doxorubicin
Cytotoxicity
MTT assay
Cancer cell lines

ABSTRACT

The aim of the study was to synthesize doxorubicin (DOX)-functionalized gold nanoparticles (GNPs) by a green method and to evaluate their anticancer potential against human cancer cell lines. These GNPs were synthesized with a green chemistry method and characterized by ultraviolet (UV) spectrophotometry, Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), and Zetasizer measurements. Surface plasmon resonance studies showed a clear UV-Visible peak at 532 nm, suggesting the formation of GNPs. FT-IR and XRD were used to determine the surface characteristics (presence of phytoconstituents) and crystalline nature of GNPs, respectively. The TEM and Zetasizer studies revealed a particle size of 74.7 ± 2.47 nm with a zeta potential of -19.13 ± 0.2. The synthesized GNPs were loaded with DOX by simple incubation method and evaluated for particle size, zeta potential, FT-IR and XRD to confirm drug loading. An in vitro anticancer assay of DOX-loaded GNPs (D-GNPs) against human cancer cell lines showed variations in responsiveness to D-GNPs, with significant activity against breast, lung, and prostate cancer cell lines. However, no significant difference was found in the percent cell viability of cervical, liver, and pancreatic cancer cell lines between DOX and D-GNPs. The results of the in vitro anticancer assay of D-GNPs against human cancer cell lines supports their potential for in vivo applications in cancer treatments.

1. Introduction

According to the World Health Organization (WHO), cancer is one of the leading causes of morbidity and mortality worldwide, with approximately 8.2 million cancer related deaths reported in 2012 [1]. Although novel anticancer agents are being synthesized and evaluated continuously [2–4], physicians have very limited choices for treatment due to the side effects associated with cancer chemotherapy. Thus, this warrants research into modifying drug formulations with the aim of reducing side effects by specifically targeting cancer cells. One such approach is the use of nanotechnology, with different nano-formulations such as solid lipid nanoparticles [5], nanoliposomes [6], polymeric nanoparticles [7], and self-emulsifying drug delivery systems [8] being used to modify the existing dosage form of anticancer drugs so as to target cancer cells. Recently, the synthesis and applications of gold nanoparticles (GNPs) have been increasingly studied for their inert and stable nature [9].

GNPs exhibit unique chemical and physical properties that facilitate loading and their role as a carrier for transporting the drug to the targeted sites. As GNPs are chemically inert, nontoxic in nature, and easily synthesized, with a functional or charged surface, they find many biomedical applications such as tissue or tumor imaging, photothermal therapy, biosensing, and optics. Moreover, their biocompatibility and high affinity for large biomolecules such as drugs, proteins, enzymes, DNA, and amino acids further aid drug targeting and delivery [10,11]. The literature reveals that GNPs can be synthesized using chemical reducing agents [12–14] or green chemistry methods [15–21]. GNPs synthesized by chemical reducing agents are expensive, as toxic chemicals that may pose potential biological and environmental risks in the end products are used. In comparison, the green chemistry method, which uses plant extracts as the source of reducing agent, is an economical and environment-friendly method that yields nontoxic and biocompatible GNPs with enhanced stability [15–21]. *Pterocarpus marsupium*, commonly known as the Indian kino tree, is well known for its antioxidant phytochemicals such as flavonoids, tannins, carbohydrates, polyphenols, and proteins [22], which can be used

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http://dx.doi.org/10.1016/j.procbio.2015.10.007
1359-5113/

as a green source in the large-scale, simple, and eco-friendly production of GNPs.

Doxorubicin hydrochloride (DOX) is a drug used to treat various malignant cancers such as solid tumors and soft tissue cancers. However, its use is limited due to serious side effects such as cardiac myopathy leading to congestive heart failure [23]. GNPs can act as inert carriers for anticancer drugs such as DOX due to their inherent responsiveness to cancer cells. Thus, the desired therapeutic index can be attained with fewer doses, consequently reducing the associated side effects.

Hence, the present study investigates the green synthesis of GNPs using plant extracts of *Pterocarpus marsupium* and its characterization. The study was extended to evaluate the biomedical applicability of DOX-functionalized GNPs for their in vitro anticancer activity against human cancer cell lines.

2. Materials and methods

The heartwood of *Pterocarpus marsupium* was collected in September 2013 from Jamboti, which is located in the Western Ghats around 20 km southwest of Belgaum near Khanapur in Belgaum District, Karnataka, India. This sample was authenticated by the Regional Medical Research Centre, Indian Council of Medical Research, Belgaum, Karnataka, India (specimen number: RMRC-922). DOX was obtained as free sample from RPG Life Sciences Ltd., Mumbai, India. Gold (III) chloride hydrate (99.99% metal basis) was purchased from Sigma–Aldrich, Pune, India. All human cancer cell lines and normal mouse fibroblasts used in this study were procured from the National Centre of Cell Science, Pune, India. Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), and Pen Strep (a mixture of penicillin and streptomycin) was procured from Gibco Life Technologies (Auckland, New Zealand); gentamycin (4 mg/ml) and amphotericin (5 mg/ml) were purchased from Himedia Pvt. Ltd. (Mumbai, India).

2.1. Synthesis of GNPs

The plant extract was prepared by boiling 10 g of air-dried plant material (heartwood) with 100 ml of deionized water for 10 min. This was then filtered with Whatman filter paper #41 and used in the synthesis of GNPs. GNPs are prepared by adding the aqueous plant extract to 0.5 mM gold chloride solution. A visual color change followed by UV-Visible spectroscopy was used to confirm the formation of GNPs. The synthesized GNPs were retrieved from the mixture using a high-speed refrigerated centrifuge (Kubota 6500, Osaka, Japan) with a rotor model AG-506R at a relative centrifugal force (RCF) of 36,873 maintained at 4 °C for 20 min.

2.2. Characterization of GNPs

A Malvern Zetasizer (Nano ZS, Malvern, UK) was used to study the particle size, the particle size distribution, the polydispersity index (PDI), and the zeta potential of GNPs. The Fourier transform infrared (FT-IR) spectrum of lyophilized GNPs was recorded using the KBr pellet method with an FT-IR Shimadzu spectrophotometer. The X-ray diffraction (XRD) data of GNPs were obtained using a Philips PRO expert diffractometer at room temperature with nickel-filtered Cu Kα radiations, operating at a 40-kV voltage, 30-mA current, and 7–70° (2θ) range. The morphology of the GNPs was confirmed with transmission electron microscopy (TEM) using a 120-kV Hitachi (H-7500) instrument equipped with a charge-coupled device (CCD) camera. The colloidal dispersion of GNPs was deposited on a carbon-coated copper grid and scanned to obtain images.

2.3. Preparation and characterization of DOX-loaded GNPs

Varying volume ratios of GNPs to DOX (Table 1) were incubated at room temperature for 24 h. DOX-loaded GNPs (D-GNPs) were analyzed for loading efficiency, particle size, zeta potential, PDI, XRD, and drug release. For determining the loading efficiency of GNPs, the D-GNPs were centrifuged and the supernatant was analyzed for the unloaded drug. The loading efficiency was calculated by the following formula:

$$\text{Percent loading efficiency} = \frac{\text{Total doxorubicin - doxorubicin in supernatant}}{\text{Total doxorubicin}} \times 100 \quad (1)$$

The drug release of D-GNPs was studied under simulated physiological conditions using phosphate buffer with a pH of 7.4 at 60 rpm and 37 °C. The particle size, PDI, zeta potential, and XRD were measured according to the standard procedure [24].

2.4. Cytotoxicity studies

Human breast (MDA-MB-231), cervical (HeLa), liver (Hep G2), lung (A549), pancreatic (MIA-Pa-Ca-2), and prostate (LN-CAP-FCG) cancer cell lines and normal mouse fibroblasts (L929) were seeded in 96-well flat-bottomed tissue culture plates at densities of 1×10^4 cells/well per 0.1 ml of the medium (DMEM, FBS, penicillin, streptomycin, gentamycin, and amphotericin). These cells were allowed to adhere for a period of 24 h in a CO_2 incubator maintained at 37 °C and 5% CO_2 with 95% humidity until the completion of experiments. The cell count was determined using a hemocytometer before the experiment. The medium was discarded and replaced with fresh medium (0.1 ml) containing different concentrations of DOX (25 μg/ml), GNPs, and D-GNPs equivalent to 25, 20, and 15 μg/ml of DOX, followed by incubation at 37 °C. At the end of 24 h, the medium in each well was discarded and 50 μl of the MTT solution (5 mg/ml in phosphate-buffered saline) was added to each well, and the plate was incubated at 37 °C. After 4 h, 100 μl of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formed formazan crystals, and the absorbance was recorded with a 492-nm filter using an enzyme-linked immunosorbent assay (ELISA) plate reader. The relative cell viability (%) was expressed as a percentage proportional to the untreated control cells and calculated as follows [25]:

$$\text{Percent cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of untreated cells}} \times 100 \quad (2)$$

2.5. Cell uptake studies

Human breast (MDA-MB-231), cervical (HeLa), liver (Hep G2), lung (A549), pancreatic (MIA-Pa-Ca-2), and prostate (LN-CAP-FCG) cancer cell lines were seeded in 96-well flat-bottomed tissue culture plates at densities of 2×10^4 cells/well per 0.2 ml of the medium (DMEM, FBS, penicillin, streptomycin, gentamycin, and amphotericin). The cells were allowed to adhere for a period of 24 h in a CO_2 incubator maintained at 37 °C and 5% CO_2 with 95% humidity until the completion of experiments. The medium was discarded and replaced with varying concentrations (0.2 ml) of DOX (25 μg/ml), GNPs, and D-GNPs equivalent to 25, 20, and 15 μg/ml of DOX prepared in medium containing 10% fetal calf serum, followed by incubation at 37 °C. After 2 h, the supernatant was collected and the cells were lysed in the well with 0.5% w/v sodium lauryl sulfate solution (200 μl) to determine the DOX content in the cell lysate and supernatant.