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Enhanced cytotoxic activity of bile acid cisplatin derivatives by conjugation with gold nanoparticles

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ABSTRACT

This article explores the potential cytotoxic activity of bile-acid cisplatin derivatives like bisursodeoxycholate (ethylenediamine)platinum(II), PtU2, when conjugated with gold nanoparticles, being a promising alternative to cisplatin in the treatment of cancer due to their lower toxicity. For our purpose we analyzed the intracellular delivery ability of these compounds after conjugation with 20-nm gold nanoparticles (PtU2-AuNPs) in the MG63 (osteosarcoma) cell line. Same platinum uptake after incubation with PtU2 and PtU2-AuNPs-derivatives is associated with a higher cytotoxic activity in case of the platinum–gold nanoparticle conjugate, the overall IC50 of PtU2 being reduced more than 10 fold for these new conjugates. When conjugated with gold nanoparticles, this bile-acid derivative is more efficient than the platinum compound alone in terms of their cytotoxic activity.

1. Introduction

The platinum compounds have clinical utility as antitumoral drugs because of their cytotoxic activity, cisplatin (CDDP) being one of the most widely used anticancer agents for the treatment of various solid tumors [1]. Its side effects (nephrotoxicity, myelotoxicity, neurotoxicity, nausea, and vomiting) and the development of resistance by targeted cells have been drawbacks which have fostered the development of new cisplatin analogues, including bile acid-cisplatin derivatives [2]. These derivatives have potential cytotoxic activity and reduced toxicity, owing to their lower lability and their amphipathic character, making them a suitable alternative for CDDP, as anti-tumoral drugs. Also the square-planar platinum(II) complex bearing two ursodeoxycholate (UDC) ligands used in this study and synthesized by our group presents intrinsic fluorescence, which may contribute to a better understanding of drug activity, metabolic pathways and transport mechanism [3].

Gold nanoparticles (AuNPs) have recently emerged as an attractive candidate for drug delivery applications [4]. AuNPs dramatically improve therapeutic outcomes of multiple drug-related therapies based on drug delivery, which attract the attention of "NanoMedicine" [5–7]. The gold core is essentially inert, non-toxic (in short- and medium-term direct assays), biocompatible and provides stability, making it an ideal starting point for carrier construction [8]. A wide range of conjugation chemistries (self-assembled monolayers) provide versatility on chemical functional groups that can be used to bound distinct compounds. As a consequence of such versatility, AuNPs offer the diversity to incorporate multiple therapeutic drugs or biomacromolecules by covalent and non-covalent conjugation on the surface of a NP [9]. Such drug delivery systems have proven to improve solubility and stability in complex biological media compared to the corresponding free drugs [10].

In this work, we show an enhanced cytotoxic activity of UDC-cisplatin conjugated to AuNPs in an osteosarcoma cell line (MG63), a model for one of the most untreatable and painful malignant tumors whose standard treatment requires radical surgery and neoadjuvant therapy [11].

2. Material and methods

2.1. Chemicals

All chemical reagents were of analytical grade and used as purchased with no additional purification. Dichloro(ethylenediamine)platinum(II), cisplatin, HUDC (ursodeoxycholic acid) and gold nanoparticles (gold colloidal solution) were purchased from Sigma-Aldrich (St. Louis, MO). Gold nanoparticles were characterized with UV–vis absorption spectroscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM), showing an absorption band at 520 nm and an average size of 20 nm. Annexin V-FITC, propidium iodide staining solution and annexin V binding buffer 10× were purchased from Immunostep (Salamanca, Spain). Penicillin/streptomycin, trypsin, fetal bovine serum, L-glutamine and phosphate buffered saline (PBS) were obtained from GIBCO

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(Carlsbad, CA). Dimethylsulfoxide was obtained from Merck (Darmstadt, Germany).

The bisursodeoxycholate(ethylenediamine)-platinum(II), [Pt(UDC)$_2$(en)], PtU2 compound was synthesized as previously described by Pérez-Andrés et al. [3]. PtU2 solutions were prepared by dissolving the solid compound in ultrapure water. The conjugates of PtU2 with gold nanoparticles (PtU2-AuNP) were prepared by mixing the solid PtU2 compound directly in the colloidal solution of the nanoparticles. All the solutions were vortexed for at least 20 min and then stored at 4 °C until use in cell cultures. The conjugates were characterized by absorption spectroscopy, fluorescence emission spectroscopy, TEM, SEM and dynamic light scattering (DLS).

2.2. Cell line

The MG63 cell line was cultured in RPMI (Invitrogen, CA, USA) supplemented with 10% calf fetal serum, 1% L-glutamine and 2% penicillin/streptomycin, at 37 °C in a 5% CO_2 humid atmosphere, in the presence of different concentrations (0–150 μM) of PtU2, AuNPs and PtU2-AuNPs for the analysis of the cytotoxic activity and the carrier activity. The cells were cultured for 24 and 48 h in the different conditions evaluated. When necessary, the cells were counted using a Neubauer counting chamber. All the experiments were performed on exponentially growing cells, with a doubling time between 24 and 36 h.

2.3. Carrier activity measurements

In order to study the carrier activity of the conjugates, the medium and the cells were collected separately, and metal content (platinum and gold) was determined in each fraction by inductively coupled plasma mass spectrometry (ICP-MS) at the central facility of Chemical Analysis ("Servicio General de Análisis Químico") of the University of Salamanca (Salamanca, Spain) with an Inductively Coupled Plasma-Mass Spectrometer Elan 6000 model from Perkin-Elmer (Waltham, MA) calibrated from 10 to 1000 ppb with solutions of platinum and gold standard (Panreac, Barcelona, Spain). In all the assays, the sensitivity detection limit was of <0.3 ppb and of <5 ppb for the Pt and Au metals, respectively. Platinum and gold containing samples were mixed with 5 mL of concentrated HNO_3, added with 20 ppb of holmium. After treatment in microwave oven for 15 min, clear solutions of the samples were analyzed for total platinum and gold. Concentration values (ppb) were corrected with respect to holmium signal.

2.4. Cytotoxic activity measurements

The cytotoxic activity of the PtU2 and the PtU2-AuNPs conjugates was evaluated by flow cytometry (FACSCalibur flow cytometer, Becton/Dickinson Biosciences, San Jose, CA, USA) after a 24 and 48 h incubation under the above described culture conditions. The harvested cells were centrifuged (5 min. at 540 g) and re-suspended at a concentration of 10^5 cells were washed once in a PBS solution. Afterward, the cells were stained with the Annexin V-FITC Apoptosis detection kit (Immunostep SL, Salamanca, Spain) according to the manufacturer's instructions. A minimum of 5×10^4 cells were acquired within 1 h. The percentage of death cells were analyzed using Infinicyte software 1.5 (Cytognos, Spain). All the experiments were repeated, at least three times. The Mann–Whitney *U* test was used to estimate the statistical significance of the differences observed between groups of experiments. In all cases, p values ≤ 0.05 were considered to be associated with statistical significance.

3. Results and discussion

AuNPs-citrate nanostructures were coated with the PtU2 compound through non-covalent interactions between the citrate molecules and moieties of the bile-acid cisplatin derivatives. The formation of the conjugates in a non-covalent way is more convenient than a covalent conjugation mainly because of easier drug release as intracellular processing of the prodrug is not required [12,13], together with an easier production and a controllable loading efficiency. The ^{1}H-NMR spectrum in D_2O of the PtU2 compound shows three signals (s, singlet) at 0.6550 ppm, 0.8507 ppm and 0.9230 ppm, which are attributed to the methyl groups C18, C19 and C21, respectively (Fig. 1A). After the formation of the adducts, the corresponding signals slightly shifted to 0.5500 ppm, 0.7878 ppm and 0.8530 ppm, respectively.

The morphological characterization of the conjugates was performed by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) (Fig. 1B–E). Based on TEM images, at first glance, it is possible to distinguish a clear zone surrounding the core of AuNPs, which corresponds to an element with a lower electron density, in our case the platinum compound (Fig. 1D). In turn, the SEM images (Fig. 1E) showed the surface of AuNPs with a rough appearance. The bile-acid platinum compounds tested in this study have an amphipathic micelle-forming character, as was previously described by Criado et al. [2]. Thus, it could be assumed that an interaction between the micelles and the hydrophobic surface of the AuNPs existed.

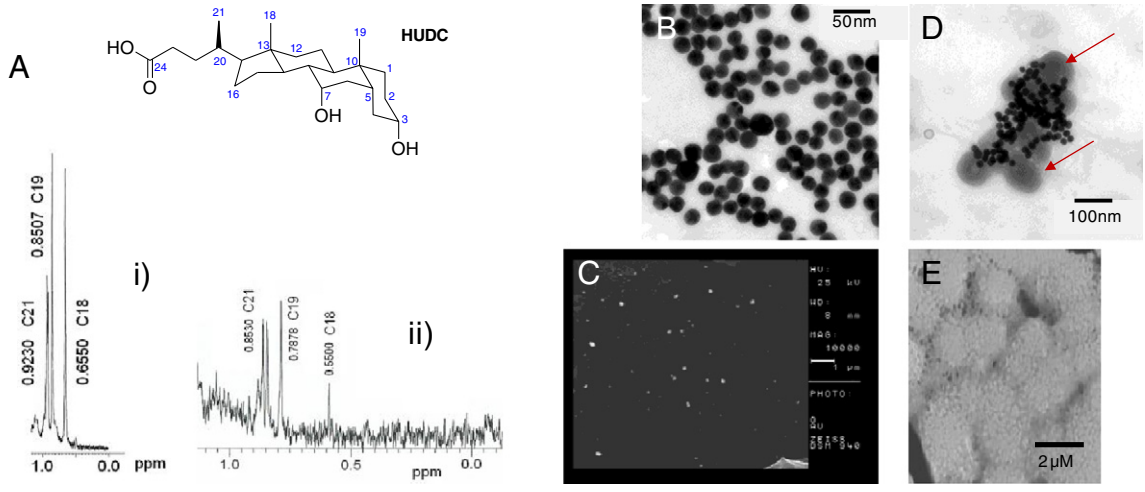

Fig. 1. (A) ^{1}H-NMR spectra of the platinum compound (i) and its incubation adduct with gold nanoparticles (ii). HUDC = ursodeoxycholic acid. TEM (B, D) and SEM (C, E) images of the AuNPs (B, C) and the PtU2-AuNPs conjugate drug complex (D, E) in water.