Colloids and Surfaces B: Biointerfaces 135 (2015) 726–734

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Effects of doxorubicin mediated by gold nanoparticles and resveratrol in two human cervical tumor cell lines

Gheorghe Tomoaia[a], Ossi Horovitz[b], Aurora Mocanu[b], Andreea Nita[b], Alexandra Avram[b], Csaba Pal Racz[b], Olga Soritau[c], Mihai Cenariu[d], Maria Tomoaia-Cotisel[b,*]

[a] Iuliu Hatieganu University of Medicine and Pharmacy, Orthopedics and Traumatology Department, 47 Traian Mosoiu Str., 400132 Cluj-Napoca, Romania
[b] Babes-Bolyai University of Cluj-Napoca, Chemical Engineering Department, 11 Arany J. Str., 400028 Cluj-Napoca, Romania
[c] Ion Chiricuta Oncologic Institute of Cluj-Napoca, Cancer Cells Investigation Section, 34-36 Republicii Str., 400015 Cluj-Napoca, Romania
[d] University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Department of Veterinary Reproduction, Obstetrics and Gynecology, 3-5Calea Manastur, 400372 Cluj-Napoca, Romania

ARTICLE INFO

Article history:
Received 20 March 2015
Received in revised form 16 July 2015
Accepted 20 August 2015
Available online 28 August 2015

Keywords:
Doxorubicin
Gold nanoparticles
Resveratrol
Doxorubicin nanocomplexes
HeLa cells
CaSki cells

ABSTRACT

Green synthesis of gold nanoparticles capped with resveratrol (GNPs) and their physical and chemical characterization by UV–vis spectra, FTIR, DLS, XRD, TEM and AFM are reported. The GNPs are highly stable, with average diameter of about 20 nm. Then, supramolecular nanoassemblies of GNPs and doxorubicin (Dox), Dox-GNPs complexes, were prepared and morphologically characterized. The stability of these Dox nanocomplexes is high in phosphate buffer saline as estimated by UV–vis spectra, TEM and AFM analysis. Effects of resveratrol (Resv), Resv-Dox mixtures, GNPs and Dox-GNPs complexes on HeLa and CaSki cells, after 24 h drug incubation, were assessed using MTT cell viability assay. Results showed strong anticancer activity for Resv-Dox mixtures and Dox-GNPs complexes in the two human cervical carcinoma cell lines. Clearly, both Resv and GNPs can mediate the anticancer activity of Dox at its very low concentration of 0.1 μg/mL, reaching the cytotoxicity of Dox alone, at its concentration up to 20 times higher. Cytotoxic effects of Resv-Dox mixtures and Dox-GNPs complexes have been found for the first time in HeLa and CaSki cells. Furthermore, the apoptosis induction in HeLa and CaSki cells was evidenced for Resv-Dox mixtures and Dox-GNPs complexes by flow cytometry using Annexin V-FITC/propidium iodide cellular staining. For CaSki cells, the apoptosis was also demonstrated, mainly for the treatment with Dox-GNPs complexes, by MTT formazan cellular staining visualized in phase contrast microscopy. Our results provide strong evidence that novel drug delivery vehicles developed on Dox-GNPs nanocomplexes and Resv could have wide applications in cancer diagnosis and treatment.

1. Introduction

There is an increasing interest in using gold nanoparticles for medical applications, for instance as vectors for the transport of drugs [1,2]. Along with the classical methods of obtaining gold nanoparticles by reduction of $HAuCl_4$ with sodium citrate [3,4], or with sodium borohydride [5], green syntheses are preferred [6,7], in order to avoid the use of toxic chemicals.

It is already recognized that gold nanoparticles offer a particularly unique set of physical, chemical and photonic properties. Consequently, they were applied in cancer therapy as carriers for different anticancer drugs, like doxorubicin, or as agents that, after penetration in the tumor cells, can generate by absorption of external radiations a heat shock [8–10]. Undoubtedly, the unique interactions between the nanoscale materials and cells can promote the development of new and potentially more efficacious treatment approaches decreasing the drug dose and improving the response of tumor cells [11].

The anti-tumor activity of the natural polyphenol, *trans*-resveratrol (3,4',5-trihydroxy- *trans* -stilbene), was reported in a large number of papers [12–15], but its mechanism of action remains unidentified. *In vitro* and *in vivo* studies recommended the use of resveratrol especially in human cancer chemoprevention and in combination with chemotherapeutic drugs, because of its good tolerability, anti-inflammatory and anti-aging properties [14–18]. Apparently, it possesses a unique antitumor function and is capable of reversing multidrug resistance of doxorubicin resistant cells in human breast cancer [19], and of inhibition of tumor invasion *in vitro* against ovarian cancer cells [20].

* Corresponding author. Fax: + 40 264590818.
E-mail addresses: mcotisel@gmail.com, mtcotisel.ubbcluj@yahoo.ro (M. Tomoaia-Cotisel).

http://dx.doi.org/10.1016/j.colsurfb.2015.08.036
0927-7765/

Cervical cancer is the second most common malignancy in relative young women worldwide and one of the most aggressive gynecological diseases [21]. It remains a current leading cause of cancer-related death for women in developing countries. The persistent infection with high risk human papillomavirus (HPV types 16 and 18) is the most important risk factor in this oncological localization, and was detected in over 70% of cases of cervical cancer [22,23]. In addition, HPV positive cervical cancer cells have an inherent resistance to chemotherapy induced apoptosis [24]. Thus, new advanced chemotherapeutical approaches are needed in cervical cancer. Single-agent cisplatin is the standard therapy for advanced cases. A combination therapy of cisplatin with topotecan was reported to improve the patients' response rates [25]. Pegylated liposomal doxorubicin was used as salvage chemotherapy in recurrent cervical cancer, but the patients' response was low [26]. On the other hand, the effectiveness of chemotherapy has been limited by doxorubicin side effects and/or its toxicity. It was also found that resveratrol can reduce doxorubicin induced side effects [27–29]. Alternatively, resveratrol was found to enhance the effects of doxorubicin in the treatment of breast cancer, and thus can be an effective adjuvant in breast cancer therapy [30]. Nevertheless, further research is necessary to improve drug internalization into tumor cells [31], without an increased toxicity for normal tissues, and to foster the development of more efficient approaches using very low (therapeutic) drug dose.

The present paper aims to investigate the effects of gold nanoparticles, obtained by $HAuCl_4$ reduction with *trans* - resveratrol, alone or in association with doxorubicin, on the two human cervical cancer (HeLa, HPV-18 positive, and CaSki, HPV-16 positive) cell lines. This study also presents the effects of doxorubicin, alone or in a mixture with resveratrol against the two tumor cell lines. We demonstrate for the first time the combined effects of doxorubicin, gold nanoparticles and resveratrol on these cervical cancer cells, at doxorubicin very low dose.

2. Materials and methods

All chemicals were of analytical grade or of the highest purity available and were used as received without further purification. Aqueous solutions were prepared with double distilled water, which was further deionized (18 Mohm·cm resistivity) in Elgastat water purification system.

Tetrachloroauric(III) acid trihydrate ($HAuCl_4 \cdot 3H_2O$) 99.5% was purchased from Merck (Darmstadt, Germany), *trans*-resveratrol (Resv) 98% from Antai Bio-tech. Co., Ltd. (Shanghai, China), doxorubicin hydrochloride (doxorubicin, Dox, 98%) was obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany), NaOH was purchased from Merck.

Dulbecco's phosphate buffered saline (PBS), Sigma–Aldrich, without calcium chloride and magnesium chloride, pH 7.4, was used for the preparation of all solutions further utilized in cell cultures.

2.1. Preparation of gold nanoparticles (GNPs) and Dox-GNPs nanocomplexes

Gold nanoparticles were obtained by reduction of $HAuCl_4$ with Resv in basic solution, by a modified procedure [7]. Briefly, 100 mL 10^{-3} M aqueous $HAuCl_4$ solution was mixed under continuous stirring, at room temperature, with 10 mL freshly prepared Resv (25 mg) solution in 0.02 M NaOH, so that the final resveratrol concentration after mixing is 10^{-3} M. The obtained nanogold colloidal solution is stable for more than one year.

Supramolecular nanoassemblies of biogenic gold nanoparticles capped with resveratrol (GNPs) with different Dox content were prepared by mixing the colloidal solution of nanogold (Au content, 179 mg/L) with aqueous Dox (42 mg/L doxorubicin hydrochloride) solution by self-assembly in aqueous medium, as well as in presence of PBS.

2.2. Characterization of the colloidal GNPs solutions

UV–Vis spectra were obtained using a Jasco UV–vis V-650 spectrophotometer with 10 mm path length quartz cuvettes in the 190–900 nm wavelengths range.

TEM images were obtained with a transmission electron microscope JEOL – JEM 1010. The GNPs colloidal solutions (approximately 7 μL for each sample) were deposited on 300 mesh electrolytic copper grids, coated with a carbon layer, and adsorbed for 1 min. The excess solution was removed with filter paper and the samples were air dried. The images have been recorded with JEOL standard software.

Atomic force microscopy, AFM, was performed on the nanostructured film of nanoparticles obtained by adsorption from colloidal solutions on glass for 10 s, washing with water and natural drying. AFM imaging was obtained using the AFM JEOL 4210 equipment, operated in tapping mode [32,33], using standard cantilevers with silicon nitride tips (resonant frequency in the range of 200–300 kHz, spring constant 17.5 N/m). Different areas from 10 μm × 10 μm to 0.5 μm × 0.5 μm were scanned on the same film. The images (2D- and 3D- topographies, phase and amplitude images, and cross-section profiles in the film along a selected direction) were processed by the standard procedures.

Zeta potential and dynamic light scattering (DLS) measurements were performed using the Malvern Zetasizer Nano-ZS90, on the colloidal gold solution.

X-Ray Diffraction (XRD) investigations were carried out using a Bruker D8 Advance diffractometer, in Bragg–Brentano geometry, equipped with a X-ray tube with copper target (Kα line, wavelength 1.541974 Å), a Ge (111) monochromator and an EyeLynx position detector.

FTIR spectra were recorded using KBr pellets, containing the sample powders (obtained by evaporation of the GNPs colloidal solution) with a spectrometer JASCO 6100 in the 4000–400 cm^{-1} range of wave numbers, with a 2 cm^{-1} resolution.

2.3. Biological assays

2.3.1. Cell culture

Human epitheloid cervical carcinoma cells lines, HeLa (HPV-18 positive) and CaSki (HPV-16 positive), were purchased from ECACC (European Collection of Cell Cultures, Public Health England, London). HeLa cells were cultured in DMEM, Dulbecco's Modified Eagle Medium, low glucose with 10% FCS (fetal calf serum), 2 mM L-glutamine, 100 U/mL penicillin, 100 U/mL streptomycin, and 1% NEA (nonessential aminoacids), at 37 °C in 5% carbon dioxide humidified air. For culturing CaSki cells we used RPMI-1640 medium with 10% FCS, 1% antibiotics, 2 mM L-glutamine. All cell culture reagents were purchased from Sigma–Aldrich Chemie GmbH (Germany).

2.3.2. MTT viability test

MTT test is used as a cellular viability assay to monitor drug toxicity [34]. Cells were cultivated in Cole flasks until reaching the confluence. After detachment of cells with 0.25% trypsin-EDTA and centrifugation, HeLa and CaSki cells were seeded in 96 wells plates at a cell density of 15×10^3 cells/well in 200 μL complete medium. After a cultivation period of 24 h for accommodation, 10 μL/well from each treatment was administrated in culture microplates. The effects on cell viability of different Dox concentrations (2.10, 6.25 and 12.5 μg/mL), Resv solution (0.75 μg/mL),