Colloids and Surfaces B: Biointerfaces 160 (2017) 254–264

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Full length article

Gold nanoconjugates reinforce the potency of conjugated cisplatin and doxorubicin

Sana Iram[a], Manaal Zahera[a], Salman Khan[a], Imran Khan[a], Asad Syed[b], Abu Ayoobul Ansary[c], Fuad Ameen[b], Omar H.M. Shair[b], Mohd Sajid Khan[a,*]

[a] Nanomedicine & Nanobiotechnology Lab, Department of Biosciences, Integral University, Lucknow, 226026, India
[b] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, 11451, Saudi Arabia
[c] Biochemical Sciences Division, CSIR-National Chemical Laboratory, Pune, 411008, India

ARTICLE INFO

Article history:
Received 5 February 2017
Received in revised form 21 August 2017
Accepted 6 September 2017
Available online 7 September 2017

Keywords:
B-AuNPs
B-AuNPs conjugated CIS and DOX
Cisplatin
Doxorubicin
Cytotoxicity
Osteosarcoma

ABSTRACT

Osteosarcoma or osteogenic sarcoma is the most common and prevalent cancerous tumor of bone and occurs especially in children and teens. Recent treatment strategy includes a combination of both chemotherapy and surgeries. Although, the use of single drug-based chemotherapy treatment remains unsatisfactory. Therefore, combinatorial therapy has emerged as a potential strategy for treatment with limited side- effects. Here, we evaluated the combinatorial anticancerous effect of cisplatin (CIS) and doxorubicin (DOX) bioconjugated bromelain encapsulated gold nanoparticles (B-AuNPs conjugated CIS and DOX) in the treatment of osteosarcoma. The synthesized B-AuNPs conjugated CIS and DOX were characterized by various characterization techniques like UV–vis spectroscopy, TEM, DLS and zeta potential to ensure the synthesis, size, shape, size distribution and stability. Drug loading efficiency bioconjugation of CIS and DOX was ensured by UV–vis spectroscopy. Bioconjugation of CIS and DOX was further confirmed using UV–vis spectroscopy, TEM, DLS, Zeta potential and FT-IR analysis. The combinatorial effect of CIS and DOX in B-AuNPs conjugated CIS and DOX showed highly improved potency against MG-63 and Saos-2 cells at a very low concentration where primary osteoblasts didn't show any cytotoxic effect. The apoptotic effect of B-AuNPs conjugated CIS and DOX on osteosarcoma and primary osteoblasts cells were analyzed by increased permeability of the cell membrane, condensed chromatin and deep blue fluorescent condensed nucleus. The results clearly showed that B-AuNPs conjugated CIS and DOX significantly improved the potency of both the chemotherapeutic drugs by delivering them specifically into the nucleus of cancer cells through caveolae-dependent endocytosis. Thus, the greater inhibitory effect of combinatorial drugs (B-AuNPs conjugated CIS and DOX) over single drug based chemotherapy would be of great advantage during osteosarcoma treatment.

1. Introduction

Osteosarcoma/osteogenic sarcoma is the most prevalent malignant and aggressive tumor of bone that occurs primarily in the long bones, particularly the proximal tibia and distal femur and especially occurs in adolescents and children aged between 10 to 24 years [1,2]. Although various conventional therapies such as radiotherapy, chemotherapy and surgical resection have evolved and made significant progress in osteosarcoma treatment (5-year survival rate to 65%), they are always associated with severe side effects. The prognosis of patients with osteosarcoma is still poor and up to 20% patients are diagnosed only at metastatic stage which is the biggest cause of concern [3]. Besides significant progress in osteosarcoma treatment, still, there is no substantial improvement occurring in osteosarcoma treatment and remains elusive [4]. The mainstay osteosarcoma treatment is the single drug based chemotherapy which leads to drug resistance [5].

Therefore, combinatorial therapy has been considered as a promising treatment method to minimize the side-effects and improve the therapeutic efficiency of drugs [6]. In combination therapy, anticancerous drugs believed to act synergistically towards suppression of cancer cells. The adaptations of cancer cells can be delayed by administration of chemotherapeutic drugs with different molecular pathways and further reduce the mutations in cancer cells. Anticancerous drugs with similar molecular pathways could act synergistically towards higher target selectivity and bet-

* Corresponding author.
E-mail address: research.sajid@gmail.com (M.S. Khan).

http://dx.doi.org/10.1016/j.colsurfb.2017.09.017
0927-7765/

ter therapeutic efficiency [7]. In this study, a unique combination of CIS and DOX was selected to improve their effect in the treatment of osteosarcoma. CIS is one the most potent anticancer drug against osteosarcoma and lung cancer. Its acts on DNA and eventually triggers several signal transduction pathways which include p53, p73, MAPK and ATR and finally lead to activation of apoptosis [8]. Nephropathy and drug resistance are two major side effects of CIS. DOX (an anthracycline), a very potent anticancer drug, works by generating free radicals which damage DNA, protein and cell membrane [9]. It also interrupts topoisomerase II- mediated DNA repair and intercalates into DNA [10]. Its side effects include cardiomyopathy, secondary malignancies extravasations and tissue necrosis, etc.

The administration of anticancerous drugs in combination did not result in improved therapeutic efficacy, despite the significance of combination therapy [11]. The diversity in biodistribution, pharmacokinetics and membrane transport mechanism of two anticancer drugs would result in extreme difficulty in dosing and poor accumulation in cancerous cells [12]. Therefore, it is necessary to entrap/conjugate multiple anticancerous drugs to a single nanocarrier so that it can help in the release of drug molecules in a predetermined manner. In this regard, nanocarrier used should have high loading capacity for both anticancerous drug and remain stable for a longer period in the bloodstream to avail enhanced retention (EPR) effect and permeability [13]. There have been various nanoparticles- based delivery strategies have been developed such as PEGylated PLGA NP-encapsulated paclitaxel and etoposide used against osteosarcoma [14], camptothecin-containing nanoparticle drug conjugated with bevacizumab against renal cell carcinoma [15], Doxorubicin conjugated bisphosphonate nanoparticles against osteosarcoma [16], methoxy-poly (ethylene glycol) aldehyde conjugated with doxorubicin and curcumin against Hep G-2 cancer cells [17] and doxorubicin-carbonane conjugated polymeric nanoparticles used for cancer therapy [18]. Nanoparticles with size (<200 nm) can abscond from reticuloendothelial system (RES) and circulate in the blood for prolonged time periods and through enhanced permeation and retention effect (EPR) nanoparticles can accumulate at the tumor site [19] as compared to conventional drugs. Among various nanoparticles, gold nanoparticles were selected because of their perceived non toxic nature, biocompatibility, tunable surface chemistry due to which additional functional groups can be conjugated [20]. Gold nanoparticles have the property of a large surface to volume ratio, which gives an ample number of anticancerous drug molecules being delivered to cancer cells.

Entry of nanoparticles into the cell is a very dynamic process and almost all endocytic pathways are energy dependent processes [21]. It depends upon physical and interfacial characteristics of NPs such as surface charge, size, shape and hydrophobicity. It also depends on cell type and properties of plasma membranes [22] such as receptor density, receptor type, the recycling rate of receptors and membrane fluidity [23]. Interactions of NPs and cell membranes with the biological environment are also crucial for the process [24]. A number of pathways may be used simultaneously for the uptake of nanoparticles, although with varying efficiency. Nanoparticles which are recognized by opsonins, such as blood serum proteins, complement component (C3, C4, and C5) and immunoglobulin (IgG and IgM), take phagocytosis route of cell entry after binding with cell surface receptors. Eventually, the cargo fuse with lysosomes and [25] destroyed by acidification and enzymolysis in the lysosomes. Therefore, to produce desired effects, nanomedicines must by pass this route to avoid degradation. Cell uptake studies revealed that the surface charge has a remarkable role in the uptake of nanoparticles.

Since, targeted drug delivery to cancerous cells is a highly appealing application of nanotechnology. Hence, tumor targeting by using nanosystems has come up with a variety of advantages, including reduced side effects, improved cancer bearing survival rate enhanced tumor targeting and elevated drug uptake [26]. Certain nanomedicines (or nanosystems) with FDA approval have been emerged and shown better clinical performance than traditional drugs [27]. Further, it has been proved that stable gold bioconjugates work exceptionally better than the pure drugs at a much lower concentration with substantially reduced side effects [28]. Since, CIS and DOX are highly effective, but with severe side effects and follow the almost same mode of action and form DNA adducts. Hence, a noble delivery system (B-AuNPs conjugated CIS and DOX) has been developed to deliver these drugs specifically into the nucleus of the cancer cells.

The present study investigates the enhanced, and the synergistic effect of the uniquely combined CIS and DOX conjugated bromelain (cysteine protease) encapsulated gold nanoparticles (B-AuNPs conjugated CIS and DOX). In this regard, we hypothesized those two anticancerous drugs (CIS and DOX), when administered together would significantly increase therapeutic efficiency in cancer treatment. The characterization of B-AuNPs conjugated CIS and DOX and bioconjugation of CIS and DOX was confirmed by UV–vis spectroscopy, DLS, Zeta potential, TEM, and FTIR analyses. The drug loading efficiency was calculated by UV–vis spectroscopy method. The cytotoxic effect of B-AuNPs, B-AuNPs conjugated CIS and DOX, and individual drugs evaluated on osteosarcoma cells (MG-63and Saos-2) and primary osteoblasts cells using MTT assay. Apoptotic effect of B-AuNPs conjugated CIS and DOX further confirmed by DAPI staining.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and reagents

Tetrachloroauric [III] acid ($HAuCl_4$), cisplatin, doxorubicin and bromelain were bought from Sigma-Aldrich. Unless stated otherwise, all chemicals and solvents were of analytical grade and were utilized as received.

2.2. In vitro *synthesis of B-AuNPs by using bromelain as reducing and capping agent*

In vitro synthesis of B-AuNPs was done by taking reaction mixtures of 3 ml, each containing 1 mM [$HAuCl_4$] [prepared in Phosphate buffer (50 mM)] and 1 mg/ml freshly prepared bromelain. The reaction mixture was incubated at 40 °C for 48 h. For control, a separate reaction completed in the absence of bromelain. The reaction mixture was removed at regular intervals of time and analysis was done by UV–vis spectroscopy to confirm the synthesis of nanoparticles. After the reaction completion, 50% v/v of ethanol treatment was used to remove unbound bromelain, and further by centrifugation (30,000*g*, 30 min) nanoparticles were collected, reaction mixture washed twice using Milli-Q water and used for further characterization.

2.3. *Bioconjugation of B-AuNPs with a combination of cisplatin and doxorubicin*

In vitro, synthesized gold nanoparticles were bioconjugated to the mixture of anticancerous drugs cisplatin and doxorubicin. The activator 1-Ethyl-3-(3-dimethyl) carbodiimide (EDC) used to bind the free amino group(s) of all the anticancerous drugs with the carboxylate group(s) present on bromelain [29]. The 5 ml reaction mixture containing 50 mM HEPES buffer, 250 μg drugs (125 μg CIS + 125 μg DOX), and 250 μg of B-AuNPs was used for coupling