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Double targeting, controlled release and reversible delivery of daunorubicin to cancer cells by polyvalent aptamers-modified gold nanoparticles

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ABSTRACT

Clinical use of daunorubicin (Dau) in treatment of leukemia has been restricted because of its cardiotoxicity. Targeted delivery of anticancer drugs could decrease their off-target effects and enhance their efficacy.
In this study a modified polyvalent aptamers (PA)-Daunorubicin (Dau)-Gold nanoparticles (AuNPs) complex was designed and its efficacy was assessed in Molt-4 cells (human acute lymphoblastic leukemia T-cell, target). Dau was efficiently loaded (10.5 μM) onto 1 mL of PA-modified AuNPs. Dau was released from the PA-Dau-AuNPs complex in a pH-sensitive manner (faster release at pH 5.5). The results of flow cytometry analysis indicated that the PA-Dau-AuNPs complex was efficiently internalized into target cells, but not into nontarget cells. The results of MTT assay were consistent with the internalization data. PA-Dau-AuNPs complex had less cytotoxicity in U266 cells compared to Dau alone and even Apt-Dau-AuNPs complex. The PA-Dau-AuNPs complex had more cytotoxicity in Molt-4 cells compared to Dau alone and even Apt-Dau-AuNPs complex. Cytotoxicity of PA-Dau-AuNPs complex was effectively antagonized using antisense of polyvalent aptamers.
In conclusion, the designed drug delivery system inherited the properties of efficient drug loading, tumor targeting, pH-dependent drug release and controllable delivery of Dau to tumor cells.

1. Introduction

Leukemia is a type of cancer that usually involves bone marrow and blood cells, resulting to death in many cases [1,2]. Acute lymphoblastic leukemia (ALL) is the most common type of cancer in children [3,4].

Chemotherapy is one of the main treatments of ALL in children. Daunorubicin (Dau), an anthracycline antibiotic, is one of the most common chemotherapy drugs for treatment of acute leukemia and childhood cancer [5,6]. Clinical administration of Dau has been limited, owing to its cumulative cardiotoxicity mainly in children [7,8].

Targeted delivery of anticancer drugs using nanocarriers could increase delivery of anticancer drugs at the tumor site and decrease their off-target effects [9,10].

In recent years, aptamers have been widely used as smart ligands for targeted delivery of chemotherapy drugs. Aptamers are short single-stranded DNA or RNA sequences or short oligopeptides, selected by an in vitro process named SELEX (systematic evolution of ligands by exponential enrichment) [11]. Aptamers bind to a broad variety of targets ranging from small molecules to whole cells with high affinity and specificity [12,13]. They offer unique advantages over antibodies, including low cost, excellent thermal stability, ease of synthesis and modifications and lack of immunogenicity and toxicity [11,14–16]. Because of these unique properties, aptamers have obtained high potential applications in medicine such as therapeutic and diagnostic applications.

Sgc8c aptamer (Apt) tightly binds to protein tyrosine kinase-7 (PTK7, K_d = 1 nM), an important biomarker for T cells acute lymphoblastic leukemia [17,18].

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Recently, gold nanoparticles (AuNPs) have broadly been applied for delivery of therapeutic molecules, because of their unique characteristic, such as convenient synthesis, large surface to volume ratio, ease of functionalization with targeting agents, inert and biocompatible properties and very low toxicity in vivo [19–21].

In this study, a modified polyvalent aptamers (PA)-AuNPs complex was designed for targeted delivery of Dau to Molt-4 cells (T cell line, ALL).

Recently a polyvalent aptamer system was used for targeted delivery of doxorubicin to cancer cells [22]. Unlike current targeted drug delivery systems which usually have monovalent molecular recognition, this polyvalent aptamer system had multivalent molecular recognition (poly aptamers) which could significantly enhance the therapeutic efficacy of anticancer drug. Unlike the mentioned study which had used only one kind of aptamer, in our work the polyvalent aptamer system contains two kinds of aptamers, sgc8c and AS1411 aptamers. AS1411 aptamer specifically binds to nucleolin. Nucleolin is a nuclear protein which could be overexpressed on plasma membrane of certain cancer cells such as lung and pancreatic cancers [23–25]. So, after the internalization of PA-Dau-AuNPs complex into Molt-4 cells by sgc8c aptamer, the complex could be internalized into the nuclei of cancer cells through AS1411 aptamer [26], leading to enhancement of the therapeutic efficacy of Dau. Also, in this study AuNPs were used as carriers for delivery of Dau and polyvalent aptamers to increase the therapeutic efficacy of the targeted drug delivery system because of their high capacity for loading of drug and polyvalent aptamers.

2. Materials and methods

2.1. Materials

The aptamer circular template, 5′-GCAGCAGTTAGATAAAAACCACCA CCACCACAACCACCACCACCAAAAATCTAACCGTACAGTATTTTCCCGGC GGC-3′, and aptamer ligation template (primer), 5′-TTATCTAACTGCTG CGCCGCCGGG-3′, and antisense against polyvalent aptamers, 5′-AACC GTACAGTATTTTCCCGGCGGCGCAGCAGTTAGATAA-3′, were purchased from Macrogen (South Korea). Sodium tetrachloroaurate (III) ($HAuCl_4$), daunorubicin (Dau), deoxynucleotides (dNTPs) and Taq DNA polymerase were obtained from Sigma (USA). Trypsin–ethylenediaminetetraacetic acid (EDTA) was purchased from Gibco (USA). DNA T4 ligase was obtained from Thermo Scientific (USA). A Nanosep 30K centrifugal device was purchased from Pall (USA).

2.2. Cell culture

U266 (C151, B lymphocyte, human myeloma) and Molt-4 (C149, T-cell line, human ALL) cells were purchased from Pasteur Institute of Iran and cultured in RPMI 1640 (Gibco) supplemented with 100 units/mL penicillin–streptomycin (Sigma) and 10% fetal bovine serum (FBS, heat inactivated, Gibco).

2.3. Synthesis of AuNPs

AuNPs were prepared by the classical citrate reduction of $HAuCl_4$, according to the previously published protocol [27]. The prepared AuNPs solution was centrifuged at 15,000 *g* for 20 min at 4 °C. The supernatant was eliminated and AuNPs were resuspended in ultrapure water. Concentrations of AuNPs solution was measured based on the Extinction coefficient of $2.7 \times 10^8\ M^{-1}\ cm^{-1}$ at $\lambda =$ 520 nm for 15 nm AuNPs. Size, morphology, and zeta potential of synthesized AuNPs were analyzed by a particle size analyzer (Malvern, UK) and Transmission Electron Microscopy (TEM) (CM120, Philips, Holland).

2.4. Rolling circular amplification

Amplification was performed in a solution (400 μL) containing 1 nmol circular template, 1.2 nmol primer, Taq DNA polymerase (0.25 units/μL), 1.5 mM dNTPs, 100 μL 10X Taq reaction buffer and $MgCl_2$ (4 mM). After incubation for 5 min at 72 °C, formation of the amplified product was investigated by 2.5% agarose gel electrophoresis. The amplified product (polyvalent aptamers, PA) was immediately purified using a Nanosep 30K centrifugal device and quantified using a NanoDrop (NanoDrop 2000, Thermo Scientific, USA).

2.5. Preparation of PA-modified AuNPs

85 μL PA (3 μM) was added to 1 mL AuNPs (6 nM) in ultrapure water and incubated for 1 h. Formation of PA-modified AuNPs was assessed by colorimetric assay. NaCl to final concentration of 50 mM was added to the solutions containing PA-modified AuNPs and the AuNPs alone, and after incubation for 5 min absorbance (A) was recorded using a Synergy H4 microplate reader (BioTeK, USA).

2.6. Dau loading onto the PA

To analyze PA-Dau conjugate formation and estimate the amount of Dau loading onto the PA, increasing concentrations of PA (0–10 μM) were added to a fix concentration of Dau (3 μM) in phosphate buffer saline (PBS, pH 7.4) and fluorescence spectra were recorded.

2.7. Dau loading onto the surface of PA-modified AuNPs

Dau (50 μM) was incubated with 1 mL of PA-modified AuNPs in PBS (pH 7.4) at room temperature for 2 h (Fig. 1). The solution was centrifuged at 15,000 *g* for 20 min to eliminate free Dau. The supernatant was collected and PA-Dau-AuNPs complex was resuspended in the ultrapure water and stored at 4 °C. Dau loading efficiency was determined by measuring A488 of collected supernatant, and using a calibration curve measured under the same conditions.

Furthermore, to assess Dau loading, fluorescence spectra of Dau alone and Dau after incubation with PA-modified AuNPs (10.5 μM final concentration of Dau) were obtained by the microplate reader [$\lambda_{Ex} = 488$ nm, $\lambda_{Em} = 500$–700 nm].

2.8. Synthesis of sgc8c aptamer (Apt)-modified AuNPs and Apt-Dau-AuNPs complex

Apt-modified AuNPs and Apt-Dau-AuNPs complex were prepared like the previous experiments, but here sgc8c aptamer was used instead of polyvalent aptamers (PA).

2.9. Release profile of Dau from the PA-Dau-AuNPs complex

PA-Dau-AuNPs complex was incubated in PBS containing 10% serum at 37 °C with pHs 5.5 and 7.4. At predetermined time intervals, the PA-Dau-AuNPs complex was isolated from the buffer by centrifugation at 15,000 *g* for 20 min and the concentration of released Dau in the supernatant was determined by measuring absorbance at 488 nm.

2.10. Flow cytometry analysis

U266 and Molt-4 cells were seeded in 12-well plates (2×10^5 cells per well). Cells were incubated with 4 μM Dau, Apt-Dau-AuNPs complex and PA-Dau-AuNPs complex (with the same amount of Dau) for 3 h. Cells were centrifuged at 400 *g* for 5 min and incubated with trypsin–EDTA for 5 min, followed by resuspension in PBS for Dau fluorescence analysis on a Partec PAS flow cytometer (Partec