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Enhanced doxorubicin delivery and cytotoxicity in multidrug resistant cancer cells using multifunctional magnetic nanoparticles

Chalermchai Pilapong[a,*], Yanee Keereeta[b], Samlee Munkhetkorn[a], Somchai Thongtem[b], Titipun Thongtem[c]

[a] Center of Excellence for Molecular Imaging (CEMI), Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand
[b] Department of Physics and Material Science, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[c] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

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ABSTRACT

Carboxymethyl modified magnetic nanoparticles (CMC-MNPs) have been designed as a vehicle for drug delivery in both drug-sensitive and drug-resistant cancer cells. We have demonstrated that the CMC-MNPs were able to load doxorubicin (DOX) with a high loading efficiency while also maintaining a good colloidal stability in an aqueous solution. According to a drug release study, DOX-loaded CMC-MNPs showed that the pH-dependent drug release property had a much higher release rate in acidic pH. Compared to free DOX, the DOX-loaded CMC-MNPs showed higher DOX accumulation in drug-sensitive cancer cells and much higher accumulation in drug-resistant cancer cells. These results indicate that our nanoplatform is highly efficient as a drug delivery system in both normal cancer cells and MDR cancer cells. In addition, the DOX-loaded CMC-MNPs can also enhance cytotoxicity against drug-resistant cancer cells in comparison to free DOX. The results obtained in this research demonstrate that our nanoplatform may be a promising approach in cancer chemotherapy and for overcoming multidrug-resistant cancer cells.

1. Introduction

Cancer is a leading cause of death worldwide [1], with an estimated 13.1 million deaths in 2030 [2]. Approximately 50% of human cancer treatment is based on chemotherapy. However, the major limitation of this method is multidrug resistance (MDR) [3,4]. Generally, MDR reflects an overexpression of ATP-binding cassette (ABC) transporters on cellular membrane, for example, P-glycoprotein (P-gp), multidrug resistant protein-1 (MRP1), and breast cancer resistant protein (BCRP), which are capable of causing various anticancer drugs such as doxorubicine (DOX), pirarubicin, and paclitaxel to efflux [5–8]. Subsequently, overcoming of MDR in human cancer has become a hot topic in anticancer research. There are several early strategies to overcome MDR in cancer, including a modification of the chemotherapy regime, inactivation of the MDR-associated genes, development of new anticancer drugs, and the use of ABC transporter inhibitors [9,10]. Among these, the development of the ABC transporter inhibitors has received much attention. However, several limitations, including unacceptable cytotoxicity and unwanted pharmacokinetic interaction between the inhibitors and the chemotherapeutics, have prevented their successful translation from research to clinical usage [11–15]. Recently, nanotechnology-based formulations, or nanomedicine, have shown promise as a strategy for cancer treatment because this approach not only provides a new opportunity to overcome MDR cancer cells [9,10,16] but also shows multifunctional platforms for cancer treatment such as theranostics by encapsulating, attaching, and conjugating drugs or specific biomolecules to the nanocarriers[17–21]. Various types of nanocarriers have been developed as multifunctional nanoplatforms for overcoming the MDR cells, such as lipid, polymer, silica, and magnetic nanoparticles [22–26]. These can confirm that encapsulating drugs in nanocarriers has a potential for overcoming MDR. On the other hand, it is well known that pH values vary significantly in different tissue and cellular compartments [27]. The healthy tissues have a normal physiological pH of ~7.4, whereas the extracellular environment of a tumor exhibits a lower pH (~6.8) and even lower in intracellular endosomal/lysosomal compartments (~4.5–5.5). Therefore, pH can be utilized as a useful strategy for cancer targeting [28].

Presently, we have developed a multifunctional nanocarrier which is not only used to overcome MDR but also has pH-responsive drug release behavior. The nanocarrier is composed of magnetic iron oxide nanoparticles (MNPs) coated with carboxymethyl cellulose (CMC). The MNPs were employed as vehicles for drug delivery

* Corresponding author. Tel.: +66858661303.
E-mail address: chalermchai.pilapong@cmu.ac.th (C. Pilapong).

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because their surface can be easily modified with various kinds of drug-carrying molecules and they are also useful for monitoring the therapeutic response via magnetic resonance imaging [17,29,30]. CMC is a cellulose derivative having carboxyl groups bonded on the cellulose back bone. It is water soluble, nontoxic, and biocompatible [31–33].Consequently, negatively charged CMC-coated MNPs (CMC-MNPs) can be easily conjugated with positively charged DOX via electrostatic interaction, leading to highly efficient drug loading.

2. Materials and methods

2.1. Materials

Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) and Iron(II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$) were purchased from Fisher Scientific (USA) and Merck (Germany), respectively. Gibco® RPMI 1640 was purchased from GIBCO™ Invitrogen (USA). Doxorubicin hydrochloride and carboxymethyl cellulose (CMC) sodium salt were purchased from FLUKA (Switzerland). Ammonia solution (30%) was purchased from Mallinckrodt Baker (USA).

2.2. Synthesis of CMC-modified magnetic nanoparticles (CMC-MNPs)

5 mmol of $FeCl_2{\cdot}4H_2O$ and 10 mmol of $FeCl_3{\cdot}6H_2O$ were dissolved in 100 mL of deoxygenated water by continuous stirring. Then, 5 mL of 30% NH_3 solution was added drop-wise into the above solution. The mixture was heated at 90 °C under vigorous stirring for 2 h. The nanoparticle dispersion was then stirred for 1 h upon the adding of 5 mL of CMC solution (16 mg/mL). The precipitate was separated by centrifugation and washed several times with deionized water. Finally, the CMC-MNPs were redispersed in deionized water and kept at 4 °C for further use.

2.3. Sample characterization

The size and morphology of the MNPs were observed by using transmission electron microscope (TEM, JEOL JEM-2010, Japan) operated at 200 kV. The surface charge of the MNPs was measured as the zeta potential (ZP) value by using the Malvern system (Zetasizer5, Malvern Instruments, U.K.). The surface chemistry of the MNPs was studied by using the Fourier transform infrared spectrometer (FTIR, Bruker Tensor 27, Germany), with KBr as a diluting agent, and operated in the range of 450–4000 cm^{-1}. Thermal analysis was performed using a thermogravimetric analyzer (TGA, Shimadzu TGA-50 Analyzer, Japan). For the drug conjugation experiment, a UV–visible spectrometer (Agilent 8453, China) was performed.

2.4. DOX entrapment, DOX loading, and DOX release

9 mg of CMC-MNPs was incubated in 1 mL PBS solution (pH 7.4) of DOX for 1 h. After that, the DOX-loaded CMC-MNPs were centrifuged and washed with a PBS buffer. The concentration of doxorubicin in the solutions was determined by measuring UV/Vis absorbance at 480 nm (ε_{480} = 11,500 L/mol cm). The amount of DOX loaded in the CMC-MNPs was determined by comparing the amount in the original and the amount in the supernatant and washing solutions. The DOX entrapping efficiency (%DEE) and the DOX loading efficiency (%DLE) were calculated by using the following equations:

$$\%\text{DOX entrapping efficiency (\%DEE)} = \frac{\text{Weight of DOX in CMC} - \text{MNP} \times 100}{\text{Weight of loaded DOX}}$$

$$\%\text{DOX loading efficiency (\%DLE)} = \frac{\text{Weight of DOX in CMC} - \text{MNPs} \times 100}{\text{Weight of CMC} - \text{MNPs}}$$

To test the pH-dependent DOX release properties, the appropriate amount of the DOX-loaded CMC-MNPs was dispersed into the PBS buffer (pH 7.4) or the acetic buffer (pH 4.5), and then incubated at 37 °C. The supernatants were taken out from the suspension at a given time interval and then were centrifuged again for monitoring the release of DOX via UV–Vis spectrophotometry.

2.5. Intracellular uptake in K562 and K562/ADR cells

Cellular uptakes of free DOX and DOX-loaded CMC-MNPs were studied by using fluorescence microscope. Doxorubicin-sensitive (K562) and doxorubicin-resistant (K562/ADR) human leukemia cell lines were cultured in RPMI 1640 containing 10% FBS, 100 U/mL of penicillin, and 0.1 mg/mL streptomycin in a humidified atmosphere at 37 °C in 5% CO_2 for 24 h. On the day of the experiment, the cells were incubated with free DOX and DOX-loaded CMC-MNPs in culture medium for 5 h. The final concentrations of the cells, and the DOX or DOX-equivalent in the CMC-MNPs were 5×10^4 cell/mL and 1.35 μM, respectively. The cellular uptakes of free DOX and DOX-loaded CMC-MNPs by K562 and K562/ADR were imaged on a fluorescence microscope (Leica, DMI 4000B).

The cellular uptakes of free DOX and DOX-loaded CMC-MNPs were also analyzed by using flow cytometer. The cells were seeded in 24 well plates and cultured in the RPMI 1640 medium overnight. The cells were then treated with free DOX and DOX-loaded CMC-MNPs for 5 h ([DOX] = 1.35 μM). After washing with the PBS buffer, the cells were resuspended in the PBS buffer, and then analyzed by using the flow cytometer.

2.6. Cytotoxicity against K562/ADR

K562/ADR cells were maintained in the RPMI 1640 medium and cultured in a 24-well plate for 24 h at 37 °C in 5% CO_2 for 24 h. Then, various concentrations of free DOX or DOX-loaded CMC-MNPs were added to the designated wells at a density of 5×10^4 cells per well and further incubated for 72 h. Afterward, the cytotoxicity was expressed as the percentage of the cell viability compared to the control.

3. Results and discussion

Carboxymethyl cellulose modified magnetic nanoparticles (CMC-MNPs) were synthesized via an in-situ co-precipitation method. CMC can be coated on MNP by electrostatic interaction of a carboxyl group of CMC with Fe cation on the surface of MNP [33]. This method has various advantages, including simplicity, low cost, and large scale synthesis [34]. The TEM image (Fig. 1a) shows that CMC-MNP is approximately 5 nm in diameter. The Zeta potential (ZP) of the CMC-MNPs and the DOX-loaded CMC-MNPs were measured as -9 ± 0.6 mV and $+33.5 \pm 1.6$ mV, respectively. It can be clearly seen that incorporation of doxorubicin can reduce the negative ZP value of the CMC-MNPs. This may be due to the nature of doxorubicin that it can neutralize carboxylate groups on the surface of the CMC-MNPs. From the colloidal stability point of view, a colloidal suspension is stable if its ZP value is lower than −30 mV or higher than +30 mV [35]. Herein, the ZP value of $+33.5 \pm 1.6$ mV observed in the DOX-loaded CMC-MNPs can hold a great colloidal stability in the aqueous medium. In order to confirm the existence of CMC on the surface of magnetic particles, the FTIR spectrum of the CMC-MNPs was analyzed (Fig. 1b). Vibration peaks at around 1631 cm^{-1} and 1400 cm^{-1} are attributed to the stretching vibration