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Doxorubicin/heparin composite nanoparticles for caspase-activated prodrug chemotherapy

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ABSTRACT

Caspase-activated prodrug chemotherapy is introduced and demonstrated using the composite nanoparticles (NPs), which deliver doxorubicin (DOX) and DEVD-S-DOX together to the tumor tissue. DEVD-S-DOX, DOX linked to a peptide moiety (DEVD), is a prodrug that is cleaved into free DOX by caspase-3 upon apoptosis. DEVD-S-DOX has no therapeutic efficacy, but it changes into free DOX with the expression of caspase-3. With the accumulation of the composite NPs in the tumor tissue by the enhanced permeation and retention (EPR) effect, a small exposure of DOX in the tumor cells initiated apoptosis in a localized area of the tumor tissue, which induced caspase-3 activation. Cleavage of DEVD-S-DOX into free DOX by caspase-3 continued with repetitive activation of caspase-3 and cleavage of DEVD-S-DOX at the tumor site. The composite NPs were characterized with transmittance electron microscopy (TEM) and particle size analyzer. We then evaluated the nanoparticle drug release, therapeutic efficacy, and in vivo biodistribution for tumor targeting using a non-invasive live animal imaging technology and the quantification of DOX with high performance liquid chromatography. DOX-induced apoptosis-targeted chemotherapy (DIATC) was verified by in vitro/in vivo DEVD-S-DOX response to free DOX and cellular uptake behavior of the composite NPs with flow cytometry analysis. Significant antitumor efficacy with minimal cardiotoxicity was also observed, which supported DIATC for improved chemotherapy.

1. Introduction

A tremendous effort has been made to overcome tumor microenvironments for efficient chemotherapy [1–3]. Various strategies have been designed and characterized to demonstrate an improved therapeutic index of chemotherapy [4–6]. Nanoparticles (NPs) are recognized as multifunctional carrier systems that deliver multiple payloads simultaneously. Various combinations of chemotherapeutic drugs have been delivered to tumor sites using NPs [7,8]. Prodrugs have also shown the possibility of overcoming tumor heterogeneity and evolutionary complexity. Pegylation has been utilized to prepare long circulating chemotherapeutic drugs in systemic circulation through the enhanced permeability and retention (EPR) effect [9–12]. By coupling with antibodies through proper linkers, active targeting of chemotherapeutic drugs has been demonstrated to show improved antitumor efficacy [13,14].

In our previous report, a caspase-3 specific activatable prodrug was designed and characterized to suggest a strategy to simplify a

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heterogeneous tumor microenvironment using DEVD-S-DOX, which contained doxorubicin (DOX) linked to a peptide moiety (DEVD: Aspartic acid-Glutamic acid-Valine-Aspartic acid) cleavable by caspase-3 upon apoptosis [6]. DEVD-S-DOX was administered intravenously using continuous infusion for 3 or 5 days. Activation of caspase-3 was induced by a single exposure of irradiation by gamma knife, which irradiated a localized area of the tumor tissue to induce apoptosis. Caspase-3, which was expressed from apoptotic tumor cells by irradiation, cleaved DEVD-S-DOX to transform into free DOX at the tumor site. Then, free DOX cleaved from DEVD-S-DOX continued to activate remaining DEVD-S-DOX by inducing apoptosis on neighboring tumor tissue and this was maintained with repetitive activation of caspase-3 and cleavage of DEVD-S-DOX at the tumor site. This strategy can be simplified and improved effectively using NPs that deliver DOX and DEVD-S-DOX simultaneously. Although apoptosis was induced by a gamma knife in our previous report [6], a small quantity of DOX could replace gamma radiation to trigger apoptosis in a small area of tumor with caspase-3 activation. It was also reported that caspase-3 activation from apoptosis cells significantly sensitized the supportive cancer cells for DOX to induce apoptosis effectively [15]. Based on this, we expect that a small exposure of DOX in tumor cells for caspase-3 activation and subsequent cleavage of DEVD-S-DOX by caspase-3 will be accomplished with co-delivery of DOX and DEVD-S-DOX using a nanoparticle system.

In this study, we designed and characterized the composite NPs containing DOX and DEVD-S-DOX as a carrier system for DOX-induced apoptosis-targeted chemotherapy (DIATC) as presented in Fig. 1. To immobilize DOX and DEVD-S-DOX in the same frame, heparin was utilized to form a heparin/DOX/DEVD-S-DOX complex through an ionic interaction. Subsequently, the heparin/DOX/DEVD-S-DOX composite was stabilized with Pluronic F-68 to form the composite NPs.

DOX, a model cancer drug, has been proven to be highly effective in cancer therapy, but serious side effects such as, cardiotoxicity and drug resistance, have limited the extended use of DOX [16–18]. Therefore, we utilized DOX and DEVD-S-DOX loaded in the composite NPs to accomplish effective apoptosis-targeted chemotherapy with minimized DOX-associated side effects.

In constructing the NPs suitable for DIATC, low molecular weight heparin and Pluronic F-68 were utilized as building blocks for the NPs.

Heparin is anionic polysaccharide composed of highly sulfated repeating units. It regulates a number of cellular events such as anticoagulation of blood and inhibition of angiogenesis [4,19]. Heparin-induced cancer cell death has also been reported and the heparin-poly(β-amino ester) complex might be the effective agent that induces cancer cell death [20]. Although heparin-associated antitumor efficacy was expected, the main role of heparin in this study was to provide the binding site for DOX and DEVD-S-DOX

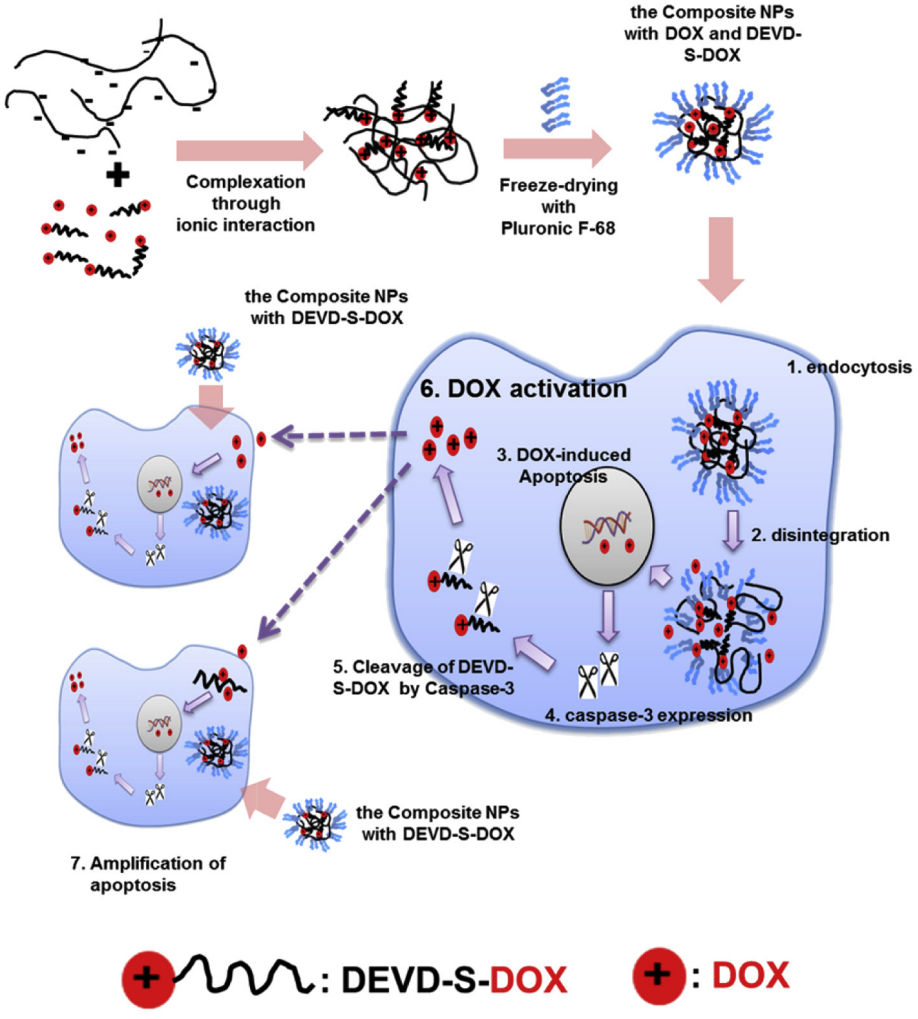

Fig. 1. Schematic description of DOX-induced apoptosis-targeted chemotherapy (DIATC) in tumor cells.