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A unique highly hydrophobic anticancer prodrug self-assembled nanomedicine for cancer therapy

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Abstract

In contrast with common thought, we generated highly hydrophobic anticancer prodrug self-assembled nanoparticles without the aid of surface active substances, based on the conjugation of docetaxel to D-α-tocopherol succinate. The reduction-sensitive prodrug was synthesized with a disulfide bond inserted into the linker and was compared with a control reduction-insensitive prodrug. The morphology and stability of self-assembled nanoparticles were investigated. Cytotoxicity and apoptosis assays showed that the reduction-sensitive nanoparticles had higher anticancer activity than the reduction-insensitive nanoparticles. The reduction-sensitive nanoparticles exhibited favorable *in vivo* antitumor activity and tolerance compared with docetaxel Tween80-containing formulation and the reduction-insensitive nanoparticles. Taken together, the unique nanomedicine demonstrated a number of advantages: (i) ease and reproducibility of preparation, (ii) high drug payload, (iii) superior stability, (iv) prolonged circulation, and (v) improved therapeutic effect. This highly reproducible molecular assembly strategy should motivate the development of new nanomedicines.

Key words: Docetaxel; Prodrug; Self-assemble; Nanomedicine; Disulfide bond

Cancer is a leading cause of morbidity and mortality worldwide, and recent advancements have resulted in a modest impact on patient survival. Nanomedicine has played an important role in developing anticancer drug delivery system with improved efficacy and/or reduced toxicity.[1] However, few nanotherapeutics have been approved by the Food and Drug Administration for cancer therapy because there is a huge gap between the technical requirements and clinical translation.[2,3] A wide range of nanomaterials have been employed for the development of cancer therapeutics, including lipids,[4,5] inorganics,[6,7] polymers,[8,9] or glycan compounds.[10,11] Furthermore, to improve therapeutic performance, increasingly complicated nanostructures have been fabricated. The added complexity of nanocarriers further deters their translation to clinics.[3] Thus the balance between the medical and societal benefits should be taken into account when developing anticancer nanomedicines. Anticancer nanomedicines with good druggability should: (i) by simply and reproducibly prepared, (ii) have high drug loading efficiency to avoid the use of excessively high amounts of carrier materials, (iii) possess good stability ensuring storage quality, and (iv) achieve favorable therapeutic outcomes with low undesired toxicity. However, the nanomedicines meeting the above criteria are limited.[12]

In most cases, previous carrier-based nanomedicines are inherently multicomponent systems, where in addition to loaded active pharmaceutical ingredients, some organic or inorganic materials serve as a delivery vehicle, and sometimes stealth and/or targeting functional materials are employed. The preparation

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of these nanomedicines is accomplished through a series of physical and/or chemical procedures.[13,14] Although many studies have reported improved *in vivo* efficacy, only a few of have achieved clinical translation because of their unpredictable and inconsistent formulation outcomes.[15] In recent years, the use of drug molecules to directly create well-define nanostructures has gained increasing interest due to their simplified formulation optimization.[15] Through precise design, these drug molecules, including polymer–drug conjugates,[16] polypeptide–drug conjugates,[17,18] and small molecule prodrugs,[19,20] could self-assemble and form one-component nanomedicines with controllable and distinct physicochemical properties. The chemical flexibility of small molecule prodrugs and the simplicity of nano-assembly make it appealing for the development of new nanomedicine.

A commonly used strategy to produce self-assembling prodrugs couples drugs with an auxiliary segment, which endows the conjugate with amphiphilicity to form a nanoassembly.[21] Here, we propose a proof-of-concept study using a highly hydrophobic compound to self-assemble and form stable nanoparticles without any surface active agents. The self-assembled nanomedicine is based on the conjugation of docetaxel (DTX) to D-α-tocopherol succinate (VE) with a disulfide bond (DTX-S-S-VE) or an ester bond (DTX-VE) as linker. The disulfide bond, a reduction-sensitive bond, was selected with the aim of enabling the controlled release of DTX from the prodrug after its passage from blood circulation (1-10 μM glutathione (GSH)) into intracellular compartments (1-10 mM GSH).[22] The ester bond, a reduction-insensitive bond, was chosen as a control. DTX-S-S-VE and DTX-VE both led to the construction of nanoassemblies by a simple solvent-displacement procedure, and were termed DSNPs and DNPs, respectively. Their characterization, stability in various media, DTX release, *in vitro* cell activity, and *in vivo* pharmacokinetic and tissue distribution behavior were investigated. *In vivo* antitumor efficacy and toxicity were evaluated using a murine xenograft model. It was expected that the unique nanomedicine might enhance the antitumor efficacy, and that side effects could be reduced simultaneously.

Methods

Synthesis and purification of DTX-VE and DTX-S-S-VE

For DTX-VE, DTX was reacted with vitamin E (VE) succinate directly. For DTX-S-S-VE, VE was firstly reacted with dithiodiglycolic acid anhydride, then, DTX was added to the intermediate product. Both prodrugs were purified by column chromatography. Detailed procedures are shown in Supplementary Material.

Preparation and characterization of nanoassemblies

Briefly, a solution of prodrug in ethanol (10 mg/mL) was added slowly to water under stirring (800 rpm) at room temperature. Ethanol was completely evaporated under a vacuum by a rotary evaporator. The aqueous suspension of the self-assembled nanoparticles (NPs) was obtained with light blue opalescence, and stored at 4 °C. PEGylated NPs (DSNPs and DNPs) were achieved just by mixing NPs with DSPE-PEG2000 solution.

The particle size, zeta potential and polydispersity index (PDI) of the resulting NPs were evaluated using a Zetasizer (Nano ZS, Malvern, UK). The measurements were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). The morphology and size of samples were characterized by transmission electron microscopy (TEM) (Hitachi, Japan). A drop (10 μL) of NPs solution was deposited on a carbon-coated copper grid. After 5 min, the excess solution was removed by filter paper. Grids were then stained with 1% uranyl acetate (5 μL) for 15 s and air dried. The samples were imaged using a Hitachi H600 TEM operated at 100 KeV.

In vitro release study of DSNPs

The *in vitro* release of DSNPs was carried out by dialysis method.[23] Briefly, 200 μL DSNPs (2 mg/mL) were transferred into a dialysis bag. The sealed bags were immediately put into conical flasks containing phosphate buffer saline (PBS) at pH 7.4 with different concentrations of GSH: (i) PBS with 10 mM GSH, (ii) PBS with 0.1 mM GSH, (iii) PBS with 0.01 mM GSH, (iv) PBS with 0.002 mM GSH, and (v) pH 7.4 PBS, which were placed in conical flasks containing 20% ethanol as solubilizer in order to improve the solubility of DTX. The flasks were placed in an air bath with shaking at 100 rpm at 37 °C. At predesigned time intervals, 100 μL sample was withdrawn and analyzed by ultra-high performance liquid chromatography (Waters ACQUITY UPLC) on a reverse ODS Cosmosil-C18 column (150 mm × 4.6 mm, 5 μm) with acetonitrile/water (55:45, v/v) as eluting solution. The samples were detected using an ultraviolet–visible detector at 230 nm wavelength, and delivered at a flow rate of 0.8 mL/min.

In vitro conversion in rat plasma

The solutions of DSNPs and DNPs were incubated in rat plasma at 37 °C under the shaking of 100 rpm for 24 h. Then, 50 μL samples were taken for analysis at 0, 1, 2, 3, 5 and 24 h. The plasma samples were processed according to the operation steps of the pharmacokinetic study, and the DTX content was measured by UPLC–MS/MS.

Physical stability

DSNPs were incubated in distilled water, RPMI 1640 medium, PBS at pH 7.4, and different percentages of bovine serum albumin (BSA) at 37 ± 2 °C (100 rpm) for 24 h. Samples were taken for analysis at pre-determined intervals. Changes in particle size and PDI were chosen to evaluate the stability of DSNPs.

In vitro anticancer activity studies

Human lung adenocarcinoma cells (A549) and human prostate cancer cells (PC-3) were cultured in DMEM and RPMI-1640 medium respectively, supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 μg/mL streptomycin and 100 U/mL penicillin. Cells were grown in a humidified atmosphere with 5% CO_2 at 37 °C. The cells were subcultured until 80% confluence by treatment with trypsin–EDTA.