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Docetaxel (DTX)-loaded polydopamine-modified TPGS-PLA nanoparticles as a targeted drug delivery system for the treatment of liver cancer

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ABSTRACT

Polydopamine-based surface modification is a simple way to functionalize polymeric nanoparticle (NP) surfaces with ligands and/or additional polymeric layers. In this work, we developed DTX-loaded formulations using polydopamine-modified NPs synthesized using D-α-tocopherol polyethylene glycol 1000 succinate-poly(lactide) (pD-TPGS-PLA/NPs). To target liver cancer cells, galactosamine was conjugated on the prepared NPs (Gal-pD-TPGS-PLA/NPs) to enhance the delivery of DTX via ligand-mediated endocytosis. The size and morphology of pD-TPGS-PLA/NPs and Gal-pD-TPGS-PLA/NPs changed obviously compared with TPGS-PLA/NPs. *In vitro* studies showed that TPGS-PLA/NPs, pD-TPGS-PLA/NPs and Gal-pD-TPGS-PLA/NPs had similar release profiles of DTX. Both confocal laser scanning microscopy and flow cytometric results showed that coumarin 6-loaded Gal-pD-TPGS-PLA/NPs had the highest cellular uptake efficiency in liver cancer cell line HepG2. Moreover, DTX-loaded Gal-pD-TPGS-PLA/NPs inhibited the growth of HepG2 cells more potently than TPGS-PLA/NPs, pD-TPGS-PLA/NPs, and a clinically available DTX formulation (Taxotere®). The *in vivo* biodistribution experiments show that the Gal-pD-TPGS-PLA/NPs are specifically targeted to the tumor. Furthermore, the *in vivo* anti-tumor effects study showed that injecting DTX-loaded Gal-pD-TPGS-PLA/NPs reduced the tumor size most significantly on hepatoma-bearing nude mice. These results suggest that Gal-pD-TPGS-PLA/NPs prepared in the study specifically interacted with the hepatocellular carcinoma cells through ligand–receptor recognition and they may be used as a potentially eligible drug delivery system targeting liver cancers.

Statement of Significance

Polydopamine-based surface modification is a simple way to functionalize polymeric nanoparticle surfaces with ligands and/or additional polymeric layers. In this work, we developed docetaxel (DTX)-loaded formulations using polydopamine-modified NPs synthesized from D-α-tocopherol polyethylene glycol 1000 succinate-poly(lactide) (pD-TPGS-PLA/NPs). To target liver cancer cells, galactosamine was conjugated on the prepared NPs (Gal-pD-TPGS-PLA/NPs) to enhance the delivery of DTX via ligand-mediated endocytosis. Both confocal laser scanning microscopy and flow cytometric results showed that coumarin 6-loaded Gal-pD-TPGS-PLA/NPs had the highest cellular uptake efficiency for liver cancer cell line HepG2. The *in vivo* biodistribution experiments show that the Gal-pD-TPGS-PLA/NPs are specifically targeted to the tumor. Furthermore, the *in vivo* anti-tumor effects study showed that injecting DTX-loaded Gal-pD-TPGS-PLA/NPs reduced the tumor size most significantly on hepatoma-bearing nude mice. These results suggest that Gal-pD-TPGS-PLA/NPs prepared in the study specifically interacted with the hepatocellular carcinoma cells through ligand–receptor recognition and they could be used as a potentially eligible drug delivery system targeting liver cancers.

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1. Introduction

Liver cancer is one of the most prevalent cancers and has become the 3rd leading cause of cancer death after lung cancer and stomach cancer around the world [1]. Up to now, liver cancer is mainly clinically treated by chemotherapy besides surgery. However, most anticancer drugs have high toxicity and low specificity, leading to systemic toxicity and severe side effects. Polymeric nanoparticles (NPs) have been employed as promising carriers of anticancer drugs, functioning as sustained, controlled and targeted drug delivery systems to improve the therapeutic effects and to reduce the side effects on normal organs [2–6]. In the existing systems, short interfering RNAs (siRNAs) delivered by liposomes has get promising results and has been the first successful clinical trial against liver cancer [7,8]. Poly(lactide) (PLA) is one of the most often used FDA-approved biodegradable polymers in drug delivery [9,10]. TPGS is a water-soluble derivative of natural vitamin E, which is generally recognized as an excellent emulsifier due to its bulky structure and large surface area. Moreover, co-administration of TPGS can enhance cytotoxicity, suppress P-glycoprotein-mediated multi-drug resistance, and increase the oral bioavailability of anticancer drugs [11,12]. TPGS-PLA copolymers can greatly improve drug encapsulation efficiency (EE), accelerate drug release, and enhance the cellular uptake of drug-loaded NPs to meet therapeutic needs [13,14]. The preparation techniques of polymeric nanoparticles include nanoprecipitation, dialysis, solvent evaporation and multiple emulsions [14,15].

Targeted drug delivery, which can carry drugs to specific organs or tissues, has been highlighted in cancer nanotechnology [16–19]. Introducing various targeting ligands, such as antibodies, peptides, nucleic acids, and small molecules, has significantly improved the specificity to deliver drugs within the tumor cells via endocytosis mechanisms [20–24]. Targeted drug delivery also significantly decreases toxic side effects compared to traditional chemotherapy. Hepatic carcinoma cells are known to recognize galactose- and N-acetylgalactosamine-terminated glycoproteins through asialoglycoprotein (ASGP) receptors located on their surfaces [25], and ASGP receptor-mediated targeted therapy has been generally recognized as one of the most effective targeted drug delivery systems in treating hepatocellular carcinoma [26–30].

NPs are typically modified with cell-interactive ligands to promote cell binding and uptake abilities. However, due to the lack of reactive functional groups, the NP surface is commonly activated by reactive linkers, coupling agents or prefunctionalization, followed by complicated and inefficient purification processes to remove catalysts and excess reactants [31–34]. Dopamine polymerization has been used to introduce a reactive chemical group on the surface of NPs [35,36]. In weak alkaline conditions (~pH 8–8.5), dopamine catechol is oxidized to quinone which reacts with other catechols and/or quinones to form polymerized dopamine, finally giving a water-insoluble polymer film on solid surfaces. Functional ligands possess nucleophilic functional groups such as amine and thiol that can be incorporated into the surface layer via Michael addition and/or Schiff base reactions [37–39]. Being both simple and versatile, this method has been widely used to functionalize various types of substrates since its discovery in 2007 [35–37].

DTX, as one of the most active anticancer drugs for solid tumors [40,41], is a mitotic inhibitor for cell cycle progression by inducing tubulin polymerization and by arresting cells in the G2-M phase [42]. The aim of the study was to develop DTX-loaded formulations for the treatment of liver cancers. To this end, we conjugated galactosamine as a target moiety on the polydopamine layer introduced to the surface of TPGS-PLA/NPs. The particle size distribution, zeta potential, surface morphology, drug loading content (LC), encapsulation efficiency (EE), drug release profile, cellular uptake efficiency and cytotoxicity against HepG2 cells were investigated *in vitro*. Additionally, the *in vivo* biodistribution and anti-tumor effects of the prepared NPs on hepatoma-bearing nude mice were also evaluated.

2. Materials and methods

2.1. Materials

Dopamine hydrochloride, galactosamine hydrochloride, coumarin 6, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and IR-780 were purchased from Sigma–Aldrich (St. Louis, MO, USA). DTX (purity: 99.9%) was bought from Shanghai Jinhe Bio-Technology Co., Ltd. (Shanghai, China). Copolymer TPGS-PLA was synthesized as described previously [43]. Human hepatoma cell line HepG2 and human nasopharyngeal carcinoma cell line CNE-2 were get from American Type Culture Collection (ATCC).

2.2. Preparation of DTX- or coumarin 6-loaded TPGS-PLA/NPs

DTX-loaded TPGS-PLA NPs were synthesized as described previously [44]. Briefly, 10 mg DTX and 100 mg TPGS-PLA copolymers were dissolved in 8 ml of acetone. Then the solution was dropwise added into 100 ml of 0.03% (w/v) TPGS aqueous solution using a 1 ml injector under stirring. After stirring overnight at room temperature to remove acetone, the particles were collected by being centrifuged at 20,000 rpm for 20 min and then washed three times in 10 ml of deionized water to remove TPGS emulsifier and unencapsulated drug. The precipitated NPs were prepared for polydopamine coating. Fluorescent coumarin 6-loaded NPs were fabricated by the same protocol, only with DTX replaced by 2 mg coumarin 6.

2.3. Prime-coating with polydopamine

Polydopamine-coated NPs (pD-TPGS-PLA) were synthesized by incubating TPGS-PLA NPs in 0.5 mg/mL dopamine hydrochloride dissolved in a 10 mM Tris buffer (pH 8.5) for 3 h at room temperature with stirring. The suspensions turned dark when dopamine hydrochloride was added, indicating dopamine was successfully polymerized. The obtained pD-TPGS-PLA/NPs were centrifuged and lyophilized for galactosamine conjugation.

2.4. Conjugation of galactosamine to pD-TPGS-PLA/NPs

The functional ligand galactosamine was bound to the surface of the pD-TPGS-PLA/NPs via the Michael addition reaction [35]. Briefly, pD-TPGS-PLA/NPs were resuspended in Tris buffer (10 mM, pH 8.5) containing galactosamine. The final concentrations of NPs and ligand were 1 and 2 mg/mL, respectively. After 2 h of incubation at room temperature with stirring, the resulting NPs (designated as Gal-pD-TPGS-PLA/NPs) were centrifuged, washed three times with deionized water and then lyophilized. Dopamine polymerization includes brief incubation of the preformed NPs in a weak alkaline solution of dopamine, followed by secondary incubation with amine- or thiol-terminated functional ligands in aqueous solution via the Michael addition reaction. Using this method, we functionalized pD-TPGS-PLA NPs with galactosamine as the targeting ligand. The surface modification of

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