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Characterization and evaluation of a folic acid receptor-targeted cyclodextrin complex as an anticancer drug delivery system

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ABSTRACT

To improve the water solubility and tumor targeting ability of docetaxel (DTX), and thus enhance the drug's antitumor efficacy and safety, a novel folate receptor (FR)-targeted cyclodextrin drug delivery vehicle (FA-CD) was successfully synthesized. The synthesis of the designed cyclodextrin was confirmed by Fourier transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (1 H NMR), and differential scanning calorimetry (DSC). The in vitro cytotoxicity was investigated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the results showed that no significant differences ($p > 0.05$) appeared in cytotoxicity between the different cyclodextrins in the different cell lines. Besides, the DTX/FA-CD inclusion complex was prepared. The cellular uptake and competition assays were examined using the HepG2, HeLa, and KB cell lines, which have different levels of folate receptor expression. Interestingly, the Cy5.5/FA-CD complex had higher uptake in the HepG2, HeLa, and KB cells, compared with non-targeted Cy5.5/CD complex ($p < 0.001$). The time-dependent drug uptake into KB cells observed by LSCM confirmed the drug delivery via endocytic routes. Data from the competition assays, especially in KB cells, showed that a significant inhibitory effect ($p < 0.001$) was obtained when the concentration of FA was increased, and suggested that the Cy5.5/FA-CD was internalized through a FR-mediated mechanism. Moreover, the in vitro bioactivity assay also demonstrated efficient antitumor activity, and the order of the cell viabilities (% of control) was OB > HepG2 > HeLa > KB for DTX/FA-CD ($p < 0.001$). For DTX/CD, however, it displayed minimum antitumor behavior in all cell types. An apoptosis study by FCM and LSCM also revealed that the FA-modified complexes were more effective in inducing apoptosis in FR-expressing cells. Finally, an in vivo biodistribution study in KB-bearing healthy mice revealed that the DTX/FA-CD complex has enhanced tumor-targeting efficacy and diminished systemic side effects. These results suggest that the novel FR-targeted cyclodextrin complex is a promising alternative as an anticancer drug delivery system.

1. Introduction

Docetaxel (DTX) is a potent anticancer drug that shows a broad spectrum of activity as is approved for the treatment of prostate, breast and lung cancer. Unfortunately the use of the drug is limited by its systemic toxicity and low solubility (Hudachek and Gustafson, 2011). DTX is a very sparingly water-soluble drug (0.0019 mg/mL), which makes it difficult to formulate. It is currently administered by the parenteral route and is commercially formulated as a solution containing polysorbate 80, which can cause severe allergic reactions and peripheral neuropathology in some patients (Baker et al., 2009). In clinical practice, these severe hypersensitivity reactions require the oral administration of dexamethasone and antihistamine before delivery of the DTX infusion. Thus, the search for alternative delivery systems for DTX is of great importance.

DTX has a narrow margin of safety, and is used in combination with other drugs at its maximum tolerated dose to achieve maximum cancer cell killing (Huang et al., 2014). The drug kills tumor cells by cytotoxic mechanisms or activating a host immune response, thereby inhibiting the proliferation processes of tumor cells, and inducing apoptosis (Cotter, 2009). However, most patients do not respond to treatment with DTX and they often experience severe adverse effects such as diarrhea and alopecia. The primary reason for this is because the drug kills both normal and tumor cells as a result of its poor cell selectivity. Drug resistance and dose-limiting toxicities are the major problems for its use in cancer chemotherapy (Hoang et al., 2014).

So for DTX, improving its solubility will solve only one of the drug's problems but it is obviously needed as a starting point for the design

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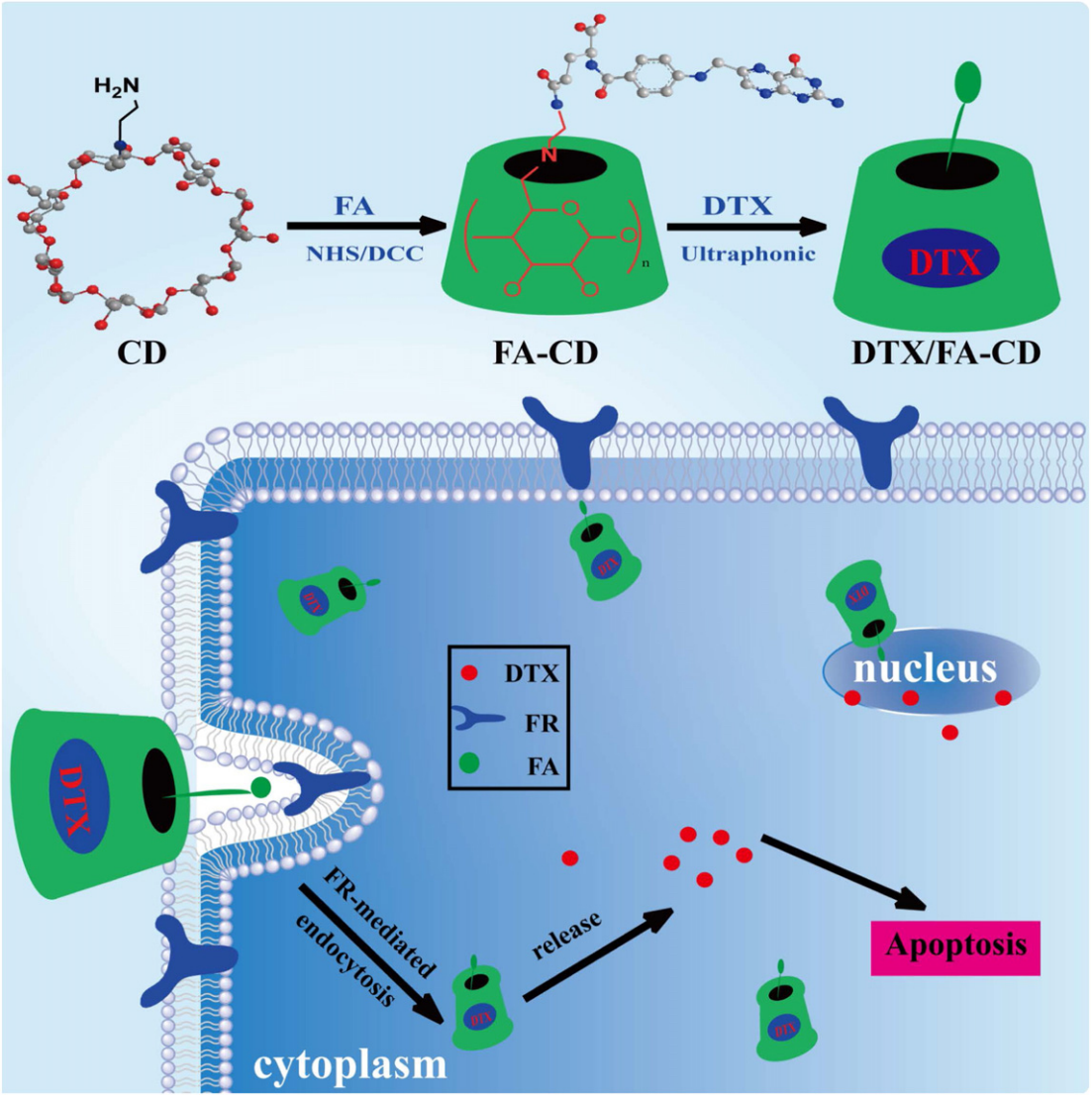
of improved DTX pharmaceutical formulations. To this end, one strategy to improve DTX solubility is the use of cyclodextrin (CDs) and its derivatives as a drug delivery vehicle. Complexation of poorly water-soluble drugs with chemically modified CDs represents a promising strategy for increasing their water solubility, and offers further possibilities through the design of nanoscaled, targeted drug delivery systems.

Folate receptors (FR) are frequently over-expressed on the surface of cancer cells, making the receptor a potential target for a variety of ligand and antibody-directed cancer-targeted therapeutics (Yin et al., 2015; Zhao et al., 2014). Folate binding to different non-viral systems for drug and gene delivery has been studied by some researchers (Ditto et al., 2012; Dou et al., 2014; Wang et al., 2010; Xu et al., 2012). In 2014, Su et al. enhanced the antitumor drug delivery by inserting the folate into the cavity of carboxymethyl-β-cyclodextrin, which was covalently bonded on the surface of nanoparticles (Su et al., 2014). In the same year, Zhang's group constructed folate-conjugated amphiphilic copolymer, folate-poly(ethylene glycol)-poly(D,L-lactide)-β-cyclodextrin (FA-PEL-CD), and used as a novel micellar vector, the in vitro and in vivo experiment all showed improved doxorubicin antitumor efficacy and reduced toxicity (Zhang et al., 2014). We have now explored an alternative strategy that solves the two key problems of DTX: its poor water-solubility and its poor cancer cell selectivity with folic acid functionalized cyclodextrin (FA-CD, Scheme 1).

2. Materials and methods

2.1. Materials

6-Deoxy-6-[(2-aminoethyl) amino]-β-cyclodextrin (CDEn) was purchased from Shandong Binzhou Zhiyuan Biotechnology Co., Ltd. (Shandong, China), *N*-Hydroxysuccinimide (NHS) was obtained from Sigma-Aldrich (St Louis, MO, USA). Folic acid, Dicyclohexyl carbodiimide (DCC), acetone, ether, methanol, ethanol were obtained commercially and used without further purification. Pyridine and DMSO were dehydrated before use. Beyotime Institute of Biotechnology (Zhejiang, China) supplied the cytotoxicity assay kit, Annexin V-FITC apoptosis detection Kit and all cell culture reagents. Cy5.5 NHS ester (Cy5.5, water-insoluble red fluorescent dye) were purchased from Shanghai XiBao Biological Technology Co., Ltd. (Shanghai China). Methanol (HPLC grade) was purchased from Fisher Chemical (Suzhou, China). Other reagents and chemicals were of analytical reagent grade, unless indicated otherwise.



Scheme 1. Schematic diagram of the synthesis of FA-CD and internalization via FR-mediated endocytosis.