Active Drug Targeting of a Folate-Based Cyclodextrin–Doxorubicin Conjugate and the Cytotoxic Effect on Drug-Resistant Mammary Tumor Cells *In Vitro*

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ABSTRACT: Active drug targeting is an effective therapeutic approach for the treatment of malignant cancers and novel types of drug carriers have been developed. In this study, we developed a cyclodextrin (CD)-based novel carrier–drug conjugate, called per-FOL-β-CD-ss-DOX, which has folic acid (FA) molecules at the end of primary hydroxyl groups of β-CD and a pH-cleavable spacer with an anticancer drug, doxorubicin (DOX), at the end of secondary hydroxyl groups. This per-FOL-β-CD-ss-DOX exhibited a significant cellular uptake as compared with the free DOX solution by EMT6/P cells, which activate the expression of folate receptor (FR). Cellular uptake of per-FOL-β-CD-ss-DOX was significantly inhibited in the presence of FA and was also inhibited at 4°C. The conjugate exhibited remarkable cytotoxic effects in EMT6/AR1 cells, which are resistant to DOX, whereas free DOX solution did not exhibit this effect. These results suggest that per-FOL-β-CD-ss-DOX can be taken up into cells via FR-related endocytosis and the cleaved DOX derived from it in endosomes could escape the efflux caused by P-glycoprotein, resulting in the cytotoxic effect. Therefore, the drug delivery by per-FOL-β-CD-ss-DOX may be a useful approach for drug delivery to FR-expressing cells such as drug-resistant malignant cancers. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2934–2940, 2015

Keywords: cyclodextrin; drug resistance; folate; per-FOL-β-CD; receptor-mediated endocytosis; drug delivery; cancer; supramolecular chemistry

INTRODUCTION

Drug delivery systems are newly designed for the optimization of medical therapies such as those using anticancer drugs. Particularly, the concept of drug targeting is an efficient and important approach for the targeted pathological sites such as cancer cells.[1–3] Drug targeting can prevent nonspecific distribution to other organs, resulting in the reduction of side effects. Recently, carrier-based drug targeting by liposomes, micelles, albumin, emulsions, metal-based particles, and cyclodextrin (CD) has been extensively developing. In addition, specific ligands, antibodies, and antigen-binding fragments have been conjugated with carriers to home in on target sites. Active drug targeting by drug carriers is a promising strategy for maximizing therapeutic effects.

The use of receptor-mediated cellular uptake has received attention as a method to overcome drug resistance, which is a challenging issue in the treatment of cancer.[4–6] The overexpression of drug efflux transporters such as P-glycoprotein on cell membranes is known as drug resistance.[7] Therefore, the therapeutic effect of anticancer drugs is attenuated by the reduction of drug concentration in cells by the transporters. Several articles have reported drug nanocarriers to improve drug resistance. For example, Kobayashi et al.,[8] reported that transferrin-conjugated liposomes containing doxorubicin (DOX) were taken up by transferrin receptor-overexpressed drug-resistant cancer cells by receptor-mediated endocytosis and exhibited a therapeutic effect. The mechanism that exhibits a therapeutic effect on drug-resistant cancer cells involves the release of drug from the endosome after receptor-mediated endocytosis that efficiently reaches the nuclei where DOX can intercalate the DNA and induce apoptosis. Therefore, active drug targeting by carriers is an effective method to overcome drug resistance.

Cyclodextrins, which are cyclic oligosaccharides, are promising drug carriers and have been extensively used for various purposes such as for improving drug solubility, drug stability, and bioavailability as well as taste masking.[9,10] Our group previously developed a novel CD that has a high density of folate molecules (seven folate molecules per CD), called per-FOL-β-CD. Folate is a known target ligand in the treatment of malignant cancers that overexpress folate receptor (FR),[11–17] and the use of folate-conjugated drug carriers is an effective strategy for overcoming drug-resistant tumors. Dr. Arima's group reported the investigations using similar CD compounds containing anticancer drugs that exhibited antitumor effects.[18] In the present study, to extend our previous research, we developed DOX-conjugated per-FOL-β-CD, called per-FOL-β-CD-ss-DOX (Fig. 1a). The per-FOL-β-CD-ss-DOX contains a disulfide bond, which is expected to be cleaved under acidic conditions such as those present in endosomes,[19,20] and releases DOX. DOX has a narrow window for cancer therapy; thus, active targeting of DOX is an efficient approach for drug delivery. We used a drug-resistant cell line to investigate the cytotoxic effect of per-FOL-β-CD-ss-DOX on resistance to DOX.

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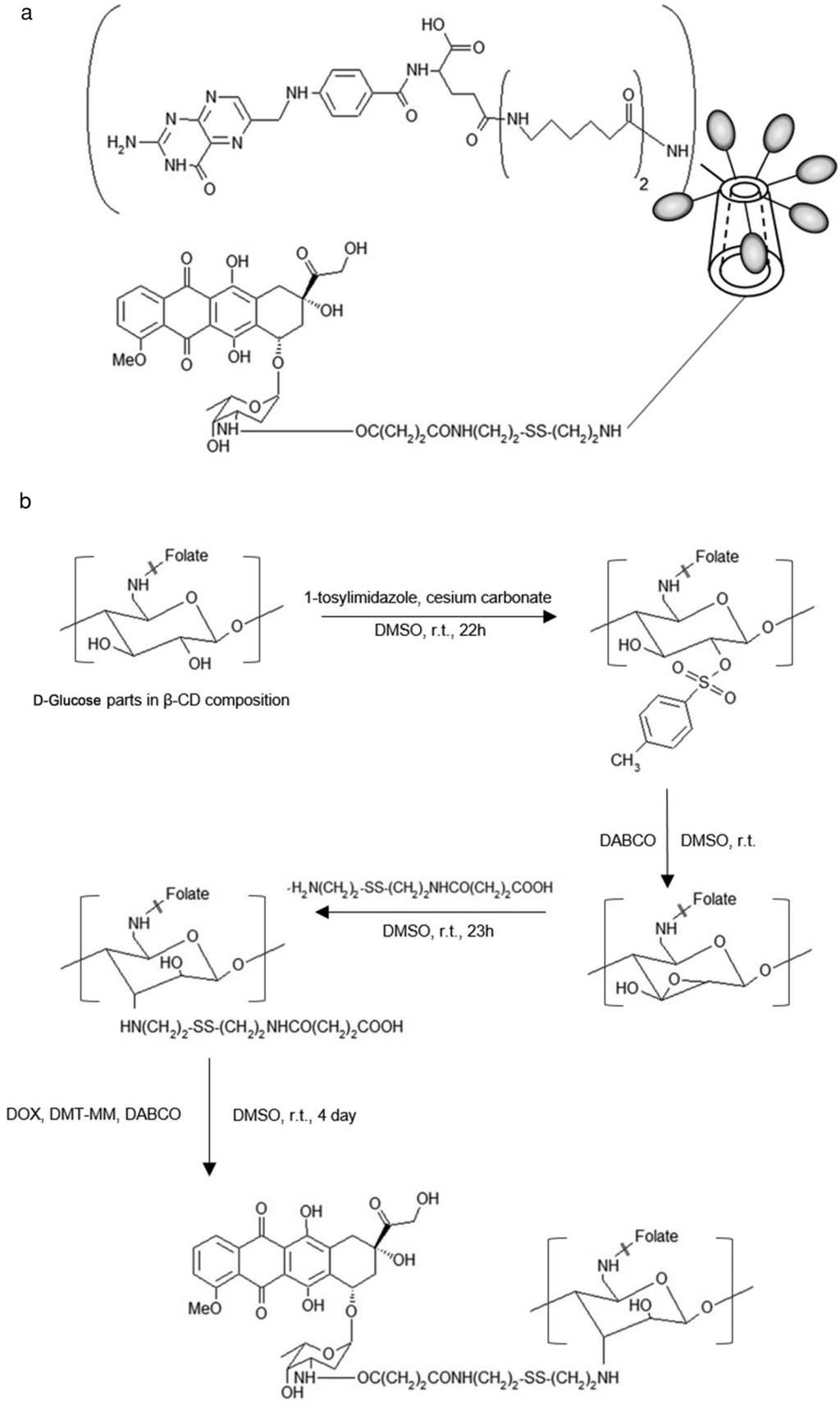

Figure 1. Per-FOL-β-CD-ss-DOX. (A) Structure and (B) Synthetic pathway.