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Multifunctional quercetin conjugated chitosan nano-micelles with P-gp inhibition and permeation enhancement of anticancer drug

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ABSTRACT

In this study, quercetin-chitosan conjugate (QT-CS) was synthesized for oral delivery of doxorubicin (DOX) to improve its oral bioavailability by increasing its water solubility, opening tight junction and bypassing the P-glycoprotein (P-gp). The prepared QT-CS self-assembled into micelles which could encapsulate DOX with high encapsulation rate, small particle size (136.9 nm) and strong zeta potential (+16.2 mV). QT-CS-DOX micelles displayed sustained-release profile in gastrointestinal simulation fluid (pH 1.2/pH 7.4). QT-CS micelles could promote cellular uptake of doxorubicin, which was 2.2 folds higher than that of free doxorubicin. The trans epithelial electrical resistance (TEER) value of Caco-2 monolayer cells was significantly reduced (about 57%) by drug loaded QT-CS micelles, leading to a high apparent permeability coefficient (P_{app}) of doxorubicin, which was 10.17 folds higher than that of free doxorubicin. Above results indicate that QT-CS micelles are promising vehicles for the oral delivery of insoluble anticancer drugs.

1. Introduction

Chemotherapy, by way of intravenous injection or infusion in clinical treatment, is one of the most effective treatments for cancer. Such a way causes rapid increase of anti-cancer drugs concentration in the plasma above the maximum tolerable concentration (MTC) and then fast excretion of the drug from the circulation system, resulting in serious side effects and limited therapeutic effects (Mei et al., 2013). The oral route, an alternative administration of chemotherapy, can maintain appropriate blood drug concentration as well as decrease corresponding side effects, thus gives promise therapeutic effect. Moreover, it is considered as painless, convenient, compliant and cost saving (Gaucher, Satturwar, Jones, Furtos, & Leroux, 2010).

Unfortunately, most anticancer drugs are BCS Biopharmaceutical classification system (BCS) class IV, low solubility and low permeability, which is difficult to penetrate across the intestinal epithelium, reach blood circulation (Tian et al., 2017). The elimination by P-glycoprotein (P-gp) based multidrug efflux pumps located on the surface of intestine epithelial cells, leading to low oral bioavailability of anticancer drugs (Bellamy, 1996). Nanomedicines have good application prospects in realizing drug sustained-release, drug targeted delivery, and improving the bioavailability of poorly soluble drugs. Nanomedicines have been approved on the market partially for decades (Table 1). There are more than 51 FDA-approved nanomedicines and 77 products in clinical trials. Therefore, nanomedicines have broad research and application prospects (Bobo, Robinson, Islam, Thurecht, & Corrie, 2016). In past decades, nanoscale carriers, such as liposomes, polymeric micelles or nanoparticles, had been increasingly investigated as promising strategies for oral drug delivery system (Liu et al., 2016; Lu et al., 2017; Lucio et al., 2017; Uhl et al., 2017). It was proven that the nanocarriers were able to improve the solubility, stability and permeability of the drug, thereby promote the oral bioavailability of the drug (Feng et al., 2013; He et al., 2017; Liu et al., 2012; Sonaje et al., 2011). In our previous study, a nanoparticle was developed by the ionic interactions between chitosan (CS) and carboxymethyl chitosan (CMCS) for oral delivery of doxorubicin hydrochloride (DOX·HCl) (Feng et al., 2015). Chitosan can open tight junction between intestinal epithelium and enhance the membrane permeability of DOX. However, we found that P-gp, which is highly expressed in intestinal cells, has a strong efflux effect on DOX, preventing DOX from being absorbed by intracellular pathways. Therefore, on the basis of previous work, we have prepared a new carrier by combining chitosan and quercetin with P-gp

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Table 1
List of some FDA-Approved Nanomedicines.

Name	Material Description	Nanoparticle Advantage	Indication(s)	Year(s) approved
Adagen®/pegademase bovine (Sigma-Tau Pharmaceuticals)	PEGylated adenosine deaminase enzyme	Improve circulation time and decreased immunogenicity	Severe combined immunodeficiency disease (SCID)	1990
Cimzia®/certolizumab pegol (UCB)	PEGylated antibody fragment (Certolizumab)	Improved circulation time and greater stability *in vivo*.	Crohn's disease	2008
			Rheumatoid arthritis	2009
			Psoriatic Arthritis	2013
			Ankylosing Spondylitis	2013
Macugen®/Pegaptanib (Bausch & Lomb)	PEGylated anti-VEGF aptamer	Improved stability of aptamer as a result of PEGylation	Macular degeneration, neovacular age-related	2004
Estrasorb™ (Novavax)	Micellar Estradiol	Controlled delivery of therapeutic	Menopausal therapy	2003

inhibitory effect. This nanocarrier can not only promote DOX cross-cell transport but also promote intracellular transport, thereby further enhance the membrane permeability of DOX

In this study, we synthesized a polymer-drug conjugate, quercetin-chitosan, based on carbodiimide reaction of water soluble chitosan (WCS) and quercetin. This polymer-drug conjugate consisted of the hydrophobic quercetin (QT), an inhibitor of P-gp drug efflux pumps, and the hydrophilic WCS, which could initiate the cascade of TJs disruption and promote intestinal adsorption. Therefore it was expected the amphiphilic quercetin-chitosan conjugate could self-assemble polymeric micelles in an aqueous solution and encapsulate hydrophobic doxorubicin (DOX) to improve the oral bioavailability of DOX by increasing drug water solubility, mucosal permeability and bypassing the P-gp drug efflux pumps (Fig. 1).

2. Materials and methods

2.1. Materials

Chitosan (deacetylation degree of 84.2%, molecular weight of 157.9 kDa.) was purchased from Laizhou Haili Biotechnology Product Co., Ltd (Shandong, China). Quercetin (QT, ≥ 98%), Dimethyl sulfoxide (DMSO, ≥ 98%), Trimethylamine (≥ 99 %) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC•HCl, ≥98%), N-hydroxy succinimide (NHS, 98%), pyrene (98%) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, 98%) were obtained from Sigma-Aldrich (St. Louis, USA). Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were provided by Hyclone (Logan, UT, USA). DOX was supplied by Zhejiang Hai zheng Co. Ltd. (China). All other chemicals were of analytical grade and were used without further purification.

2.2. Depolymerization of chitosan

The water-soluble chitosan (WCS) was prepared by oxidative degradation with $NaNO_2$ at room temperature (Mao et al., 2004). Briefly, 1 g chitosan was dissolved in 100 mL 6% (v/v) acetic acid solution under magnetic stirring. When chitosan was completely dissolved, 100 mg $NaNO_2$ was added and the reaction was performed at room temperature for 1 h. The reaction mixture was subsequently neutralized with NaOH to pH 8.0. The reaction mixture was dialyzed (molecular weight cutoff 3500) against distilled water for 3 days and the sample was placed in a freezer at −20 °C for 24 h and the sample was completely frozen. The sample was then placed in a lyophilizer and dried in a vacuum environment at −50 °C for 24 h.

Under fixed conditions, there is a corresponding relationship between the molecular weight of chitosan and its intrinsic viscosity. The molecular weight of chitosan can be calculated by measuring the intrinsic viscosity of chitosan samples. The viscosity-average molecular weights of original chitosan and water soluble chitosan were calculated using the classical Mark-Houwink equation (Mao et al., 2004) (Supporting information Fig. S1). The structure and deacetylation degree of original chitosan and water soluble chitosan were determined by ^{1}HNMR. The deacetylation degree was determined from the integral of the Acetyl signal compared with that of C1 signals of glucosamine and N-acetylglucosamine (Varum, Anthonsen, Grasdalen, & Smidsrad, 1991) (Supporting information Fig. S2, Table S1). The experimental details were provided in supporting information.

2.3. Synthesis and Characterization of quercetin-chitosan conjugate (QT-CS)

Quercetin-chitosan conjugate was prepared by carbodiimide reaction of water-soluble chitosan and QT-hemisuccinate. Briefly, succinic

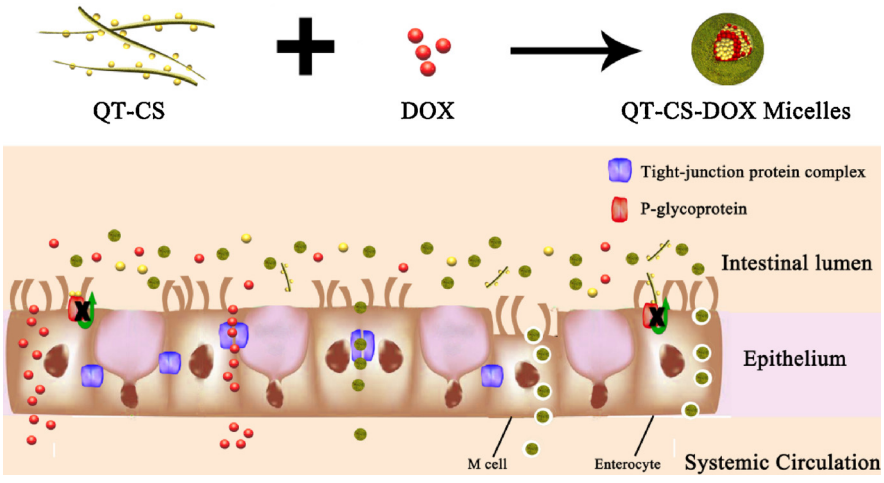

Fig. 1. Scheme of the effect of QT-CS Micelles on improving the oral absorption of DOX.