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Emulsion-based colloidal nanosystems for oral delivery of doxorubicin: Improved intestinal paracellular absorption and alleviated cardiotoxicity

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ABSTRACT

We have previously reported that the limited intestinal absorption *via* the paracellular pathway may be the primary cause of the low oral bioavailability of doxorubicin (DOX). In this study, we have formulated medium chain glycerides-based colloidal nanosystems to enhance the intestinal paracellular absorption of DOX and reduce its cardiotoxicity. The DOX formulations prepared by the construction of pseudo-ternary phase diagram were characterized in terms of their droplet size distribution, viscosity, drug loading, and drug release. Further evaluation was conducted by an *in vitro* Caco-2 transport study as well as *in situ/in vivo* intestinal absorption, bioavailability and toxicity studies. Compared with DOX solution, these formulations enhanced the absorptive transport of DOX across Caco-2 cell monolayers at least partly due to the paracellular-enhancing effects of their lipidic components. Moreover, the *in situ* intestinal absorption and *in vivo* oral bioavailability of DOX in rats were markedly enhanced. In addition, no discernible damage was observed in the rat jejunum after oral administration of these DOX formulations while the cardiac toxicity was significantly reduced when compared with intravenous DOX solution. Taken together, the medium chain glycerides-based colloidal nanosystems prepared in this study represent a potentially effective oral delivery system for DOX.

1. Introduction

Doxorubicin (DOX) is an anthracycline glycoside antibiotic with a mechanism of impairing DNA synthesis during tumor cell division (Schwarzbach et al., 2002). It is one of the most widely used anticancer drugs for the treatment of lymphoma, osteosarcoma and other sarcomas, carcinomas, and melanoma (Choi et al., 2011). The most common dosing mode of DOX is a single intravenous injection but this may lead to an undesired systemic exposure profile with an excessively high (toxic) level in the initial and subsequent fast decay below the minimum therapeutic level (Bromberg and Alakhov, 2003). It has been generally believed that long-term exposure to drug at modest concentrations would be more beneficial than a pulsed supply of drug at higher concentrations (Kalaria et al., 2009). Thus, much effort has been devoted for achieving prolonged systemic exposure to DOX, and the most successful case has been DOXIL®, a pegylated liposomal DOX.

However, oral chemotherapy would be more advantageous over the current regimens *via* the intravenous route (DeMario and Ratain, 1998; Le Lay et al., 2007). Oral delivery could provide a relatively prolonged systemic exposure profile with less fluctuation leading to lower toxicity and improved efficacy (Zhang and Feng, 2006). Moreover, the oral mode of cancer treatment is non-invasive, cost (time and labor)-saving, and available for outpatient, resulting in a better patient compliance and improved quality of life, particularly for the elderly and for patients with advanced or relapsed cancer (Bromberg, 2008; Dong and Feng, 2005). Thus, oral chemotherapy may be a potential alternative to the current DOX regimen.

Despite many recent studies on the oral delivery of DOX (Benival and Devarajan, 2012; Choi et al., 2011; Jain et al., 2012; Kalaria et al., 2009; Ke et al., 2008), its development still remains challenging due

Abbreviations: DOX, doxorubicin; AUC, total area under the plasma concentration–time curve from time zero to time infinity; C_{max}, peak plasma concentration; T_{max}, time to reach a C_{max}; F_{rel}, extent of relative oral bioavailability.

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to the limited intestinal absorption and low oral bioavailability. In these previous studies, it has been often assumed that the P-gp-mediated efflux and cytochrome P450 (CYP) 3A-mediated first-pass metabolism in the intestine and liver are the main barriers to the oral absorption of DOX. However, our recent work has revealed that the limited and paracellular intestinal absorption of DOX (corresponding to BCS class III) probably due to its physicochemical properties (aqueous solubility = 50 mM; log P = −0.5; apparent Caco-2 permeability = 0.102×10^{-6} cm/s) may be the major factor that is responsible for the low oral bioavailability in contrary to what had been earlier reported (Kim et al., 2013). Therefore, this necessitates a new formulation strategy for developing an effective oral delivery system of DOX, *i.e.*, enhancing the intestinal absorption of paracellularly transported BCS class III drugs.

Over the past decades, lipid-based colloidal systems including microemulsion have been used mainly for the oral delivery of poorly water-soluble drugs (BCS classes II and IV) (Kawakami et al., 2002a,b; Yin et al., 2009). However, recent studies tend to focus on microemulsion as a drug delivery system for enhancing the oral absorption of BCS class III drugs which include fexofenadine, famotidine, calcein, hydroxysafflor yellow A, and earthworm fibrinolytic enzyme (Cheng et al., 2008; Gundogdu et al., 2011; Jha et al., 2011; Koga et al., 2010; Qi et al., 2011). Microemulsions may enhance the oral absorption of paracellularly transported BCS class III drugs because they contain oils and surfactants, some of which have been well recognized as paracellular permeation enhancers. Microemulsion could thus be applied in developing oral delivery systems for DOX.

Herein, we report on medium chain glycerides-based colloidal nanosystem, based on water-in-oil (W/O) microemulsion, for the oral delivery of DOX, with the expectation to enhance the intestinal permeation of DOX *via* the paracellular pathway. To date, very few attempts have been made to develop an emulsion-based colloidal nanosystem for the oral delivery of paracellularly transported BCS class III drugs including DOX. Therefore, this study could provide new findings regarding the application of microemulsion in these oral drug delivery systems.

2. Materials and methods

2.1. Materials

A human colonic epithelial cell line, Caco-2 cells, was obtained from the American Type Culture Collection (Rockville, MD). DOX (hydrochloride salt) was purchased from Boryung Pharmaceutical Co. (Gunpo, South Korea). [^{14}C] Mannitol (51 mCi/mmoL) was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, penicillin–streptomycin, and trypsin–EDTA were purchased from Gibco Laboratories (Grand Island, NY, USA). Captex and Capmul MCM were kindly donated by Abitec Co. (Columbus, OH, USA). PEG-8 caprylic/capric glycerides (labrasol) were kindly donated by Gattefossé Co. (Saint Priest, Cedex, France). Propranolol hydrochloride (an internal standard for the high-performance liquid chromatographic (HPLC) analysis of DOX), non-essential amino acid solution, Hank's balanced salt solution (HBSS), N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), D-glucose, and DMSO were purchased from Sigma–Aldrich Co. (St. Louis, MO). Other chemicals were of reagent grade or HPLC grade.

2.2. Construction of pseudo-ternary phase diagrams

The pseudo-ternary phase diagram was constructed to determine the components and contents for the formation of W/O microemulsions (Constantinides et al., 1996). The surfactant mixtures (S_{mix}) were prepared by blending Span 80 and Tween 80 (F1) or Capmul MCM and labrasol (F2) in a fixed weight ratio of 2:1. Then, the oil phase (Captex 355) and S_{mix} were mixed, where the ratios of oil to S_{mix} were varied from 9:1 to 1:9 (w/w). Each clear mixture was titrated with distilled water (DW), while stirring the mixture at room temperature to allow equilibrium. Following the addition of aliquot of water phase, the mixture was visually examined for transparency. The points from clear to turbid and turbid to clear were designated as emulsion and microemulsion, respectively. Based on the results of the pseudo ternary phase diagrams, two microemulsion formulations (F1 and F2) were selected for further experiments: 50% Captex 355, 40% Span 80/Tween 80 mixture, and 10% aqueous phase for F1; 55% Captex 355, 35% Capmul MCM/labrasol mixture, and 10% aqueous phase for F2.

2.3. Determination of maximum loading content and preparation of DOX formulations

In order to determine the maximum loading content of DOX in microemulsion formulations, excess amount of DOX was first dissolved into water followed by mixing in a shaking incubator (Jeio-Tech, Seoul, Korea) at 100 rpm for 48 h at 25 °C. Then, excess DOX was removed by centrifugation at 16,000 × g for 5 min at 25 °C. The supernatant (saturated DOX aqueous solution) was taken as an aqueous phase to prepare the microemulsions following the above mentioned compositions. They were further mixed in a shaking incubator at 100 rpm for 48 h at 25 °C. Excess DOX, if any, was removed by centrifugation at 16,000 × g for 5 min at 25 °C. The content of DOX in the formulation was measured by HPLC assay after an appropriate dilution with methanol. Based on these results of the maximum loading content of DOX in microemulsions, 20 mg/mL of DOX aqueous solution was used to prepare F1 and F2 formulations containing 2 mg/mL of DOX for further studies.

2.4. Characterization of DOX formulations

2.4.1. Mean droplet size and distribution

The droplet size and distribution of DOX formulations (F1 and F2) were measured by an electrophoretic light-scattering spectrophotometer (ELS-8000, OTSUKA Electronics Co. Ltd., Japan). The DOX formulations were transferred to a standard quartz cuvette, and their droplet size and polydispersity index were determined *via* dynamic He–Ne laser (10 mW) light-scattering at an angle of 90° at 25 °C. Data analysis was conducted using a software package (ELS-8000 software) supplied by the manufacturer.

2.4.2. Viscosity

The viscosity of F1 and F2 was measured by DV-E Viscometer (BROOKFIELD, USA) using a #16 spindle at a speed of 100 rpm at room temperature.

2.4.3. Transmission electron microscopy

The morphology of F1 and F2 was examined by an energy-filtering transmission electron microscopy (TEM) (LIBRA120, Carl Zeiss, Germany) with a 80 kV accelerating voltage. The DOX formulations were negatively stained by 2% sodium phosphotungstate (pH 7) and placed on carbon-coated 400 mesh copper grids followed by drying at room temperature before measurements.

2.4.4. Changes of DOX formulations after dilution

To evaluate the changes in the droplet size of DOX formulations after dilution, each formulation (F1 and F2) containing 2 mg/mL DOX was 10-fold diluted with normal saline at 37 °C, and then, the droplet size of the diluted formulations was measured as mentioned above. To evaluate the changes in the formulations' colloidal