International Journal of Biological Macromolecules 120 (2018) 702–710

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Inducing sustained release and improving oral bioavailability of curcumin *via* chitosan derivatives-coated liposomes

Mei-Ping Tian, Rui-Xi Song, Ting Wang, Meng-Jie Sun, Ya Liu *, Xi-Guang Chen *

College of Marine Life Science, Ocean University of China, 5# Yushan Road, Qingdao 266003, China

ARTICLE INFO

Article history:
Received 5 June 2018
Received in revised form 23 July 2018
Accepted 26 August 2018
Available online 29 August 2018

Keywords:
Liposome
Polymer coating
Oral drug delivery

ABSTRACT

Liposomes (LPs), a delivery vehicle for stabilizing drugs, the characteristics of being easy to aggregate and fuse limit its application. Polymer coating is a promising way to tackle these issues. In this study, the potential of carboxymethyl chitosan (CMCS) and quaternary ammonium chitosan (TMC)-coated liposomes (CMCS/TMC-LPs) for improving the oral delivery capacity of curcumin (CUR) was explored. CMCS/TMC-LPs were prepared by electrostatic adsorption in a layer-by-layer manner. CMCS/TMC-LPs were spherical and had not obvious change in particle size and morphology after storage at 4 °C for 7 and 14 days. CMCS/TMC-LPs possessed favorable gastric acid tolerance (the cumulative drug release rate <10%) due to stable structure. The hemolysis test and Cell Counting Kit-8 (CCK8) assay appeared satisfactory biocompatibility of CMCS/TMC-LPs. The pharmacokinetics exhibited that oral absolute bioavailability of CUR loaded CMCS/TMC-LPs was about 38%, which was around 6 folds and 3 folds higher than CUR loaded LPs and CUR loaded TMC-LPs, respectively. The *in vivo* experiments showed that CMCS/TMC-LPs could prolong the retention time of CUR in systemic circulation and generate high level of CUR in liver, spleen and lung. Thus, CMCS/TMC-LPs may be a promising carrier for improving the efficacy and safety of orally administered drugs.

1. Introduction

Over the past decades, oral administration has gained wide application for tumor-targeted drug delivery in clinic. The oral administration route has clear advantages such as painless administration and improved patient compliance [1]. However, oral delivery of drugs faces several barriers, including pre-systemic degradation, limited mucosal diffusion, and poor intestinal epithelial membrane permeability [2]. In recent years, the combination of drugs *via* nanocarriers has emerged as a promising strategy for oral administration [3]. It has been reported that nanoformulations can protect the drugs from gastro-intestinal degradation, promote drug absorption through the intestinal mucosa and improve the oral bioavailability of drugs [4–6].

Curcumin (CUR) is a polyphenol compound, which was found to be an effective anti-oxidant and anti-inflammatory agent [7,8]. Therapeutic potential of CUR as anti-cancer drug, including the treatment of colon cancer in tumors, was also described [9–11]. However, the therapeutic application of CUR is limited due to its poor aqueous solubility, extensive first-pass metabolism and rapid systemic elimination [12]. Therefore, a carefully designed carrier could significantly facilitate CUR delivery and broaden the range of its possible pharmaceutical applications.

Liposomes (LPs) are widely used as delivery vehicles for stabilizing drugs and overcoming barriers to cellular and tissue uptake [5]. Liposome-drug complex is one of the most popular formulations for delivery system because of theirs good biocompatibility and sustained-releasing potential [13]. Although LPs have various advantages as a carrier, they are easy to result in aggregation, fusion, low resistance to gastric pH and phospholipids hydrolysis, which may lead to leakage of encapsulated drugs [14,15]. To address those problems, different types of biocompatible polymers had been employed to improve the efficiency of liposomal systems [4,16]. However, there are still certain pitfalls in terms of limited stability of the nanoparticles and their transport across mucosal barrier [17]. Chitosan or its derivatives have biocompatibility, biodegradability and immunogenicity, and their coating on LPs can not only maintain the advantages of LPs but also have been found to increase their stability and provide them with mucoadehsive properties [10,18–22]. Chitosan is only soluble in acid solutions, which limits its biomedical application [23]. Quaternary ammonium chitosan (TMC) is derivative of chitosan for reversing or preventing the deficiency of chitosan resulting from its good solubility

* Corresponding authors.
E-mail addresses: yaliu@ouc.edu.cn (Y. Liu), xgchen@ouc.edu.cn (X.-G. Chen).

https://doi.org/10.1016/j.ijbiomac.2018.08.146
0141-8130/

over a wide pH range [24]. However, it was reported that the electrostatic interaction between TMC and negative charged cell membranes could result in the cells rupturing [25]. Carboxymethyl chitosan (CMCS) is synthesized from the carboxymethylation reaction of chitosan and has also received attention because of its improved solubility and biocompatibility [6,26]. In addition to this, the carboxyl group on CMCS makes it obtain high capacity to bind Ca^{2+} [27], which could deprive the divalent ions from extracellular matrix and increase the parallular permeability of the epithelium [28].

Hence, in the present paper, CMCS and TMC were used to form protective shell on the liposomal surface in a lay-by lay manner through electrostatic adsorption effect (Scheme 1). TMC was deposited to form the cationic polymeric layer onto negatively charged liposomal surface (TMC-LPs) and CMCS was further coated on the surface of TMC-LPs (CMCS/TMC-LPs) [29]. TMC could adsorb on the outer surface of LPs due to its fixed positive charge and prevent interactions between particles through steric stabilization to improve their stability [30,31]. Negatively charged CMCS was coated on the surface of TMC-LPs, which could further improve the stability and biocompatibility of CMCS/TMC-LPs [32]. CMCS/TMC-LPs were characterized for particle shape, size and zeta potential. The stability of nanoparticles was evaluated by transmission electron microscope (TEM) and dynamic light scattering (DLS) after storage at 4 °C for 7 and 14 days. The release profiles of CUR from nanoparticles were evaluated in simulated gastric and intestinal fluid. Cytotoxicity assay of CMCS/TMC-LPs was conducted through mouse fibroblast cells (L929) and human colorectal adenocarcinoma cells (Caco-2). Bioavailability and tissue distribution were also studied to assess efficiency of CMCS/TMC-LPs as oral drug delivery system.

2. Materials and methods

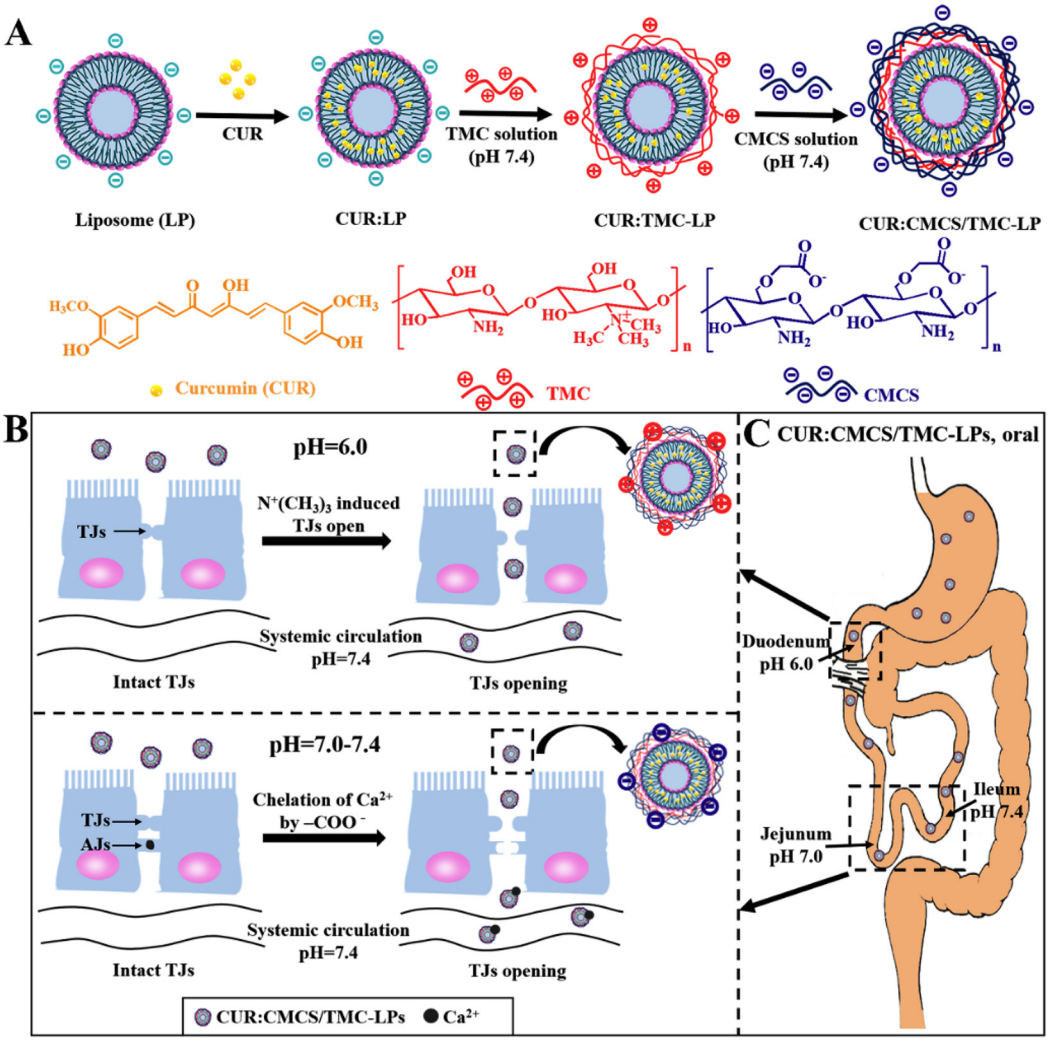
2.1. Materials

Chitosan (molecular weight 10 kDa, 90% degree of deacetylation) was purchased from Zhejiang Aoxing Biotechnology (Taizhou, China). Curcumin (CUR) was purchased from L-α-Phosphatidylcholine from Dalian Mellon Biotechnology Co. Ltd. (China). Egg yolk, Cell Counting Kit-8 (CCK-8) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Sigma-Aldrich (St. Louis, USA). Other reagent chemicals were all of analytical grade and used without further purification.

2.2. Preparation of LPs, TMC-LPs and CMCS/TMC-LPs

2.2.1. LPs preparation

LPs were prepared according to the thin-film dispersion method [33]. In brief, lecithin (SPC, 70 mg) was dissolved in chloroform/ethanol



Scheme 1. (A) Preparation of CUR:CMCS/TMC-LPs; (B) Schematic diagrams displaying the transport mechanisms of CUR: CMCS/TMC-LPs in small intestine (AJs: adherens junctions, TJs: tight junctions); (C) Oral delivery of CUR:CMCS/TMC-LPs in the gastrointestinal tract.