Colloids and Surfaces A: Physicochem. Eng. Aspects 444 (2014) 54–62

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Preparation, characterization and evaluation of bufalin liposomes coated with citrus pectin

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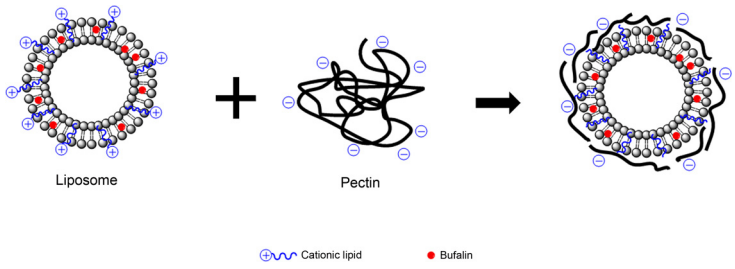
HIGHLIGHTS

- A pectin-coated liposomal formulations was prepared by forming an ion-complex.
- The pectin-coated liposome has an excellent stability and mucoadhesiveness properties.
- The pectin-coated liposome would be a promising drug carrier system for colon cancer.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 June 2013
Received in revised form
25 November 2013
Accepted 7 December 2013
Available online 31 December 2013

Keywords:
Citrus pectin
Liposomes
Bufalin
Colon cancer

ABSTRACT

The aim of this study was to investigate the potential effect of pectin for liposomal drug delivery systems. An orthogonal L9 (3^3) test was designed to optimize the preparation condition of cationic bufalin liposomes coated with commercially available citrus pectin (CPL). The change in particle size, zeta potential, entrapment efficiency, stability, mucoadhesion and anticancer effect were evaluated. The results showed that CPL had an excellent stability and mucoadhesive properties, and the drug release *in vitro* was modest prolonged and sustained. Furthermore, the inhibition effect of liposomes on SW480 colon cancer cells was dramatic enhanced due to a block of cell cycle at G0/G1 phase, and CPL had a higher inhibition rate than bufalin liposomes (BFL) because of the anticancer effect of citrus pectin. It is concluded that CPL is a potentially promising drug carrier system treatment for colon cancer.

1. Introduction

Colon cancer is one of the most prevalent cancers throughout the world, accounting for over 1 million new cases and about half a million deaths every year [1]. Currently, the effective cure rate of colon cancer has not been achieved with surgery, and the risk of recurrence is high. Chemotherapy remains the primary cure or control strategy for patients in colon cancer phase III [2,3]. Therefore, it is important to develop novel potent chemotherapeutic agents for the treatment of colon cancer [4].

One of such chemotherapeutic agent is bufalin, which is purified from Chinese traditional medicine Chan Su [5]. Some recent researches have revealed that bufalin exhibits significant activity against a wide variety of malignancies, including gastric cancer, prostate cancer, ovarian cancer and colon cancer [6]. However, it is still somewhat controversial that the health benefits of oral administration of bufalin are often overshadowed by its drawbacks such as high toxicity [7], poor water solubility [8], short half-life, narrow

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http://dx.doi.org/10.1016/j.colsurfa.2013.12.006

therapeutic window and the little difference between the effective dose and the toxic dose. Therefore, other substitutive agents with the same positive effect and fewer side-effects are needed to explore alternative treatment strategies.

In the past few decades, nano-scale therapeutic systems have become novel therapeutic modalities for combating cancers [9]. Liposomal drugs are agents which are first approved and widely used in the pharmaceutical field since it has many advantages including the affinity to biological membrane and the ability to improve the unfavorable physicochemical properties of drugs [10–12]. However, the disadvantages of liposomal drugs such as the aggregation during storage, fusion of the membranes, loss of encapsulated material, hydrolysis and oxidation of the lipids show a balance number of risks and benefits [13–15]. In recent years, these potential drawbacks have stimulated the research of alternatives to the classical liposomal drugs. More recently, some studies have been reported on stabilize liposomes by using substrates, including silicon particles [16]; gold nanocages [17] and polyelectrolyte-based capsules [18,19]. Moreover, several researches indicated that modifying by attaching polymers on the bilayer surface could improve the stability of liposome as well as avoid the uptake of liposomes by macrophages of the reticuloendothelial system. Many polymers have been employed in the surface coating of liposome, including pectin, amylopectin, dextran and pullulan [20]. As the results, the toxicity of drugs might be diminished whereas the mucoadhesive properties increased [21]. From the aforementioned advantages, pectin can be a good polymer candidate for bonding onto liposomes.

Citrus pectin (CP), as a promising polymer, is non-toxic, biodegradable and biocompatible material, in addition to the capability to form gels. It is a natural occurring polysaccharide, including mainly of D-galacturonic acid units with varying degrees of methylesterified carboxyl groups [22]. CP has a great potential to be a colonic drug delivery carrier due to its prolonged retention in gastro-intestinal tract and nearly complete degradation by the bacteria living in colon [23]. More importantly, the role of CP as an inducer of malignant cell apoptosis cannot be ignored. The study of Bergman et al. showed that CP exhibited a dose-dependent anti-proliferation effect, which was compatible with the results of Olano-Martin et al. [24–26].

In this study, we aim to design the citrus pectin-liposome nanocomplexes for improving the stability and mucoadhesiveness of liposome, meanwhile enhance the anti-colon cancer effectiveness.

2. Materials and methods

2.1. Materials

L-α-Phosphatidylcholine (Egg PC), cholesterol, citrus pectin, mucin (extracted from porcine stomach, type II) and MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) from porcine stomach (type II) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The cationic lipid dipalmitoyl trimethylammoniumpropane (DPTAP) and rhodamine-phosphoethanolamine (Rb-PE) was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Bufalin was purchased from ChenGuang Technology Development Co. Ltd. (BaoJi, China).

2.2. Optimization of BFL preparation conditions by orthogonal design method

2.2.1. Preparation of BFLs

BFLs were prepared according to the ethanol injection method and their lipid compositions were as follows: EPC, cholesterol and bufalin = 20:4:4.8 (mass ratio, total 14.4 mg total lipid/mL ethanol), and 5.0 mol% cationic lipid DPTAP was added to prepare cationic liposomes. Briefly, the lipids with the above compositions were dissolved in 2 mL of ethanol, and rapidly injected into 20 mL of phosphate buffer saline (PBS, pH 7.4), magnetic stirred at 60 °C for 1 h. The characteristic opalescence of colloidal dispersions was occurred as soon as ethanolic solution was in contact with the aqueous phase. Liposomes formed spontaneously after further volatilizing the residual ethanol. The liposomes suspension was immediately extrusion with a Lipex extruder (LipoFast-Pneumatic, Avestin, Canada) five times using polycarbonate membranes (0.2 μm pore size, Whatman, UK) to achieve the desired size.

2.2.2. Single-factor test of liposomes preparation

Liposomes preparation was dependent on numerous factors, and effects of four single factors (ratio of bufalin to lipid, ratio of cholesterol to EPC, hydration media and volume of media) on the encapsulation efficiency rate of liposomes were observed.

2.2.3. Orthogonal test of preparation of liposomes

Based on our previous research and the single-factor experiments, the three factors, ratio of drug to lipid (w/w) (A), ratio of cholesterol to EPC (w/w) (B), and the volume of media (C) were mainly effective factors on encapsulation efficiency rate of liposomes. Therefore, an orthogonal L9 (3^3) test design was used for optimizing the preparation condition of liposomes and the encapsulation efficiency was chosen as determination index. Three levels per factor were used and nine reacting conditions were designed according to orthogonal test as L9 (3^3). These three factors at three levels were as follows: the ratio of drug to lipid (1:5, 1:10 and 1:15, w/w), the ratio of cholesterol to EPC (1:3, 1:4 and 1:5, w/w), the volume of media (10, 20 and 30 mL).

In addition, several verification experiments were carried out according to the optimal condition, and the encapsulation efficiency was test in order to investigate whether the experimental results were consistent with regression model.

2.3. Preparation of CPLs

2.3.1. Purification of CP

Purity of the commercially available CP was needed before use. A stock solution of the CP was freshly prepared by dissolved in PBS to a concentration of 2.0% (w/v), and then centrifuged for 30 min at 4000 rpm. The CP solution was further diluted with PBS to achieve the final concentration of 0.4% (w/w) for each experiment. The solution was filtered through a 2 μm polycarbonate membrane filter and stored in 4 °C.

2.3.2. Coating of the liposomes with pectin

For the preparation of CPL, an equal volume of the cationic liposomal suspension was added dropwise into the pectin solution and mixed under continuous magnetic stirring for 0.5 h.

2.4. Size, charge, morphology and entrapment efficiency of liposomes

The average particle size and charge of the liposomes were analyzed using a zeta potential analyzer (Malvern Zetasizer Nano ZS90, Malvern, UK). The sample was diluted with deionized water prior to the determination of surface properties. Mean size and polydispersity index were obtained from 70 times measurements. The morphologies of liposomes were observed by transmission electron microscope (TEM) (Hitachi H-7650, Tokyo, Japan). For TEM studies, carbon coated samples were placed over a copper grid and samples were negatively stained with 1% phosphotungstic acidshape.