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A novel glyceryl monoolein-bearing cubosomes for gambogenic acid: Preparation, cytotoxicity and intracellular uptake

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ABSTRACT

Lyotropic cubic liquid crystalline nanoparticles, also known as 'cubosomes', have been tested as effective carriers for a variety of drugs due to their ability to enhance delivery efficiency and reduced drug side effects. Cubosomes are colloidal carriers composed of biodegradable Glyceryl monooleate and F127. Being composed of well tolerable and physiological materials, these carriers are well tolerated, compatible and non-toxic. In this study, therefore, we developed a novel, water-soluble, glyceryl monooleate and F127 based multiblock copolymer for Gambogenic acid (GNA) by emulsion-evaporation and low temperature-solidification technique. Physicochemical properties, *in vitro* cytotoxicity, cellular uptake and *in vivo* pharmacokinetic of GNA-loaded cubosomes (GNA-Cubs) were investigated. The results revealed that GNA-Cubs were spherical or ellipsoidal monocellular by dynamic light scattering, meanwhile, 150–250 nm in mean size with narrow polydispersity indexas determined by transmission electron microscopy. Small angle X-ray scattering indicated that GNA-Cubs retain the Pn3m cubic symmetry. Compared with GNA solution, GNA-Cubs exhibited markedly prolonged inhibitory activity in SMMC-7721 cells, as well as time-dependent increases in intra-cellular uptake. Furthermore, *in vivo* pharmacokinetic study showed that the C_{max} values and the AUC of GNA-Cubs were higher than GNA solution approximately 1.2-fold and 9.1-fold, respectively. In conclusion, the results showed that the cubic liquid crystalline nanoparticles could be a potentially nanocarrier in the delivery of GNA for cancer therapy.

1. Introduction

Current cancer therapy seems far away from successful clinical application largely due to renal toxicity, organ failure and photosensitivity. However, there is increasing evidence that traditional medicines have attracted wide interest for the treatment of various cancers (Fu et al., 2012). Gambogenic acid (GNA, $C_{38}H_{47}O_8$, Fig. 1) is an active pharmaceutical ingredient from Gamboge, which is a popular Chinese traditional medicine (Han et al., 2009). Previous reports indicated that GNA possesses diverse anti-tumor activities both *in vitro* and *in vivo*, which is mainly attributed to arresting the cancerous cells to G_0/G_1 via regulating expression of cyclin D1 and cyclo-oxygenase(COX)-2 (Yan et al., 2011). However, clinical therapeutic potential of GNA have some practical disadvantages mainly due to (a) poor aqueous solubility, (b) short biological half-life, (c) excessive irritation to blood vessel and (d) sensitive to light, temperature, which hampered its successful application in clinic. The last but not the least, nanotechnology is a rapidly progressing field and now being applied in the treatment of various cancer therapy (Xia et al., 2013; Zhang et al., 2013). From this point of view, in our previous researches we have successfully prepared GNA PEGylated Niosomes (PEG-NISVs), solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) (Lin et al., 2013; Huang et al., 2013) which effectively reduced toxicity and improved the physical stability of GNA. Nevertheless, these nanocarriers still bear the problems such as low drug encapsulation efficiency, drug loading and residual organic solvent, which were not as economically as possible in industrial production (Sun et al., 2013). Based on above observations, design of other advanced delivery systems is the next challenge for GNA into clinical practice. In recently years, cubosomes (Cubs) as a novel member entered into the nanocarriers, which can be regarded as the non-lamellar analogue of liposome (Mulet et al., 2013). Like liposome, Cubs are lipid bilayer-based particulates based on self-assembled systems in an aqueous

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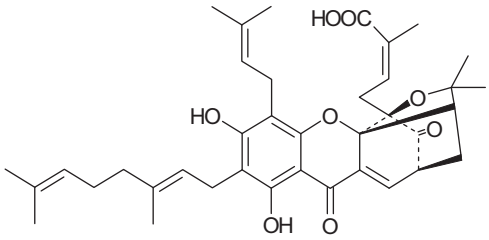

Fig 1. The structure of GNA.

medium. Whereas, Cubs component lipid bilayers are twisted and contorted, resulting in the formation of three–dimensional structures with continuous hydrophobic and hydrophilic regions (Gordon et al., 2012). Compared to other drug delivery systems, Cubs can offer increased encapsulation due to the high surface area imparted by the greater proportion of lipid comprising the particle. Besides, research manifested that Cubs are able to incorporate large amounts of drugs of varying physicochemical properties and can be administered orally, parenterally, or percutaneously (Murgia et al., 2013). Cubs applications, therefore, have demonstrated great potential for utilization as particulate carriers in the fields of both drug and vaccine delivery. Materials used in Cubs have found in natural lipids, cationic and nonionic surfactants, and polymer systems. The lipid widely used to construct bicontinuous cubic phases is the monoglyceride monoolein spontaneously form bicontinous cubic phases upon the addition of water. In our study, therefore, we have employed glyceryl monooleate (GMO), which has a stiff and gel-like appearance with high biocompatibility, bioadhesivity as well as easy production protocol. Furthermore, GMO is a non-toxic material classified as GRAS (generally recongnized as safe), and the formulations that could meet with the regulatory standards by the Food and Drug Administration (FDA) (Garg et al., 2007). Surfactants, such as Pluronic F127 (F127) and polyvinyl alcohol, are used in the production of Cubs as a stabilizing agent to increase the viscosity of the system and to support the framework network structure of the system (Dong et al., 2006). In this work, henceforth, the efforts have been made to prepare GNA-Cubs comprising GMO and F127 for the encapsulation of GNA by emulsion-evaporation and low temperature-solidification technique. The physicochemical properties of GNA-Cubs, including particle size, Zeta potential, entrapment efficiency, stability and *in vitro* drug release kinetics in phosphate buffer (pH 7.4, 37 °C), were systematically investigated. In addition, *in vivo* pharmacokinetic of GNA-Cubs in rats were investigated. Finally, the *in vitro* antitumor activity and intracellular uptake of GNA-Cubs in the human hepatic carcinoma (SMMC-7721) cells was evaluated in detail. MTT assay was employed to investigate the inhibition effect on cellular viability. Diamidino-2-phenylindole (DAPI) staining was performed to visually examine the fluorescent morphology changes of the cells incubated with GNA-Cubs. Flow cytometry was utilized to determine the influence of GNA-Cubs on apoptosis of SMMC-7721 cells.

2. Materials and methods

2.1. Materials

GNA (purity: 98%) was isolated from gamboges in Prof. Wang Xiaoshan's laboratory. GMO was purchased from Tianjin heowns Biochemical Technology Co., Ltd. (Tianjin, China). F127 was obtained from BASF (Ludwigshafen, Germany). Sephadex gel G-50 was purchased from Beijing Ruida Henghui Science & Technology Development Co., Ltd. (Beijing, China). Cell culture medium (RPMI-1640,Waymouth), 0.25% trypsin-EDTA and heat-inactivated fetal bovine serum (FBS) were obtained from Gibco (Grand Island, USA), while the MTT were purchased from Sigma (Sigma–Aldrich, Germany and USA) and stored at −4 °C in the dark. Dimethyl sulfoxide (DMSO) and streptomycin/penicillin was obtained from Sigma (USA). DAPI and Annexin V-FITC/PI were obtained from Bestbio Biotechnology (Shanghai, China). The human hepatic carcinoma (SMMC-7721) cell line was obtained from Institute of Biochemistry and Cell Biology, Institute for Biological Sciences, Chinese Academy of Science (Shanghai, China). All other chemicals and solvents were analytical purity grade, and water used in the study was bidistilled and deionized.

2.2. Preparation of GNA-Cubs

For preparing lipid-based colloidal nanostructured dispersions there are two common approaches: a bottom–up and a top–down approach, have been recently reviewed in literatures (Pan et al., 2013). While associating drug properties with experimental conditions, we creatively adopt employing emulsion evaporation and low temperature-solidification method to acquire GNA-Cubs (Li et al., 2014; Dai et al., 2010). The procedure was optimized by single factor experiment and the optimal process was described as follow. In briefly, GNA (10.00 mg) and GMO (100.00 mg) were dissolved into ethanol (5.00 mL) absolutely in a water bath at 60 °C to obtain the organic phase. 40 mL aqueous solution with F127 (12.00 mg) was heated to equivalent temperature. Then, the resulting organic solution was rapidly injected into the aqueous solution within isothermal condition under a mechanical stirrer (ETS-D4.IKA, Germany) with 1000 rpm in a water bath for 2 h to get the homogeneous emulsion. Preliminary experiments demonstrated that there was no residual organic solvents in the homogeneous emulsion, then the obtained hot pre-emulsion was rapidly dispersed into 20 mL of distilled water (0∼2 °C) in ice bath with stirring at 1000 rpm for 1 h. The dispersion of GNA-Cubs was achieved by lipid recrystallisation.

2.3. HPLC analysis of GNA-Cubs

The samples were assayed for GNA by using HPLC method reported earlier (Lin et al., 2013). HPLC was equipped with Shimadzu LC-15C, SPD-15CUV-spectrophotometric detector system. The analytical column was a Cosmosil $5C_{18}$-MS-11 column (4.6ID × 250 mm,5 μm), and the column temperature was set at 30 °C. The mobile phase was methanol and 0.1% phosphoric acid solution (90:10, v/v) and was delivered at a flow rate of 1.0 mL/min. The injection volume was 20 μL and detection of GNA was carried out at 360 nm.

2.4. Characterization of GNA-Cubs

The mean particle diameter (MD), polydispersity index (PDI) and Zeta potential of GNA-Cubs were evaluated by dynamic light scattering using a Zetasizer (Malvern Instrume Malvern, UK). Before measurement, all samples were diluted with deionised water (distilled water) to obtain a suitable concentration for measurement and every sample was examined in triplicate.

2.5. Morphology of GNA-Cubs

The morphology of the GNA-Cubs was characterized using a transmission electron microscope (JEM1400, JEOL Ltd., Tokyo, Japan). In general, GNA-Cubs were diluted with water and placed on a membrane coated grid surface with a filter paper, and then negatively stained with 2% phosphotungstic acid (2%, w/v) for 3 min and dried at room temperature before being loaded onto the microscope.