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Annonaceous acetogenins (ACGs) nanosuspensions based on a self-assembly stabilizer and the significantly improved anti-tumor efficacy

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ABSTRACT

Annonaceous acetogenins (ACGs) have exhibited antitumor activity against various cancers. However, these substances' poor solubility has limited clinical applications. In this study, hydroxypropyl-beta-cyclodextrin (HP-β-CD) and soybean lecithin (SPC) were self-assembled into an amphiphilic complex. ACGs nanosuspensions (ACGs-NSps) were prepared with a mean particle size of 144.4 nm, a zeta potential of −22.9 mV and a high drug payload of 46.17% using this complex as stabilizer. The ACGs-NSps demonstrated sustained release *in vitro* and good stability in plasma as well as simulated gastrointestinal fluid, and met the demand of both intravenous injection and oral administration. The ACGs-NSps demonstrated significantly increased cytotoxicity against Hela and HepG2 cancer cell lines compared to ACGs in solution (*in vitro* cytotoxicity assay). An *in vivo* study with H22-tumor bearing mice demonstrated that nanosuspensions significantly improved ACGs' antitumor activity. When orally administered, ACGs-NSps achieved a similar tumor inhibition rate at 1/10th the dose of ACGs in an oil solution (47.94% *vs.* 49.74%, $p > 0.05$). Improved therapeutic efficacy was further achieved when the ACGs-NSps were intravenously injected into mice (70.31%). With the help of nanosuspension technology, ACGs may be an effective antitumor drug for clinic use.

1. Introduction

Annonaceous acetogenins (ACGs) are a series of natural substances isolated exclusively from species of the *Annonaceae* family [1]. These substances' chemical structures typically contains a long aliphatic chain with 35–37 carbons, an α, β-unsaturated γ-lactone ring and 0–3 tetrahydrofuran ring(s) [2–4]. Annonaceous acetogenins (ACGs) and single compound purified from ACGs demonstrate antitumor activity against various cancer cells [5–11]. Bullatacin, one of the most active ACGs compounds, was 10^4–10^5 times more effective than doxorubin against both A549 and MCF-7 [12]. It was 300 times as active as taxol in L1210 murine leukemia bearing mice [13]. The 50% inhibitive concentration (IC_{50}) of three bis-tetrahydrofuran compounds from Annona squamosa seeds were 1/10th–1/100th of fluorouracil against Hela, HepG2, SMMC, MKN-45 cell lines [14]. ACGs induce cytotoxicity by inhibiting the mitochondrial complex I (NADH: ubiquinone oxidoreductase) of the electron transport chain [15,16]. This mechanism is different from the majority of anti-tumor drugs. Due to this reason, ACGs may reverse the multiple drug resistance (MDR) of many tumor cell lines because MDR is an energy-requiring and ATP-consuming process [17–21]. Apoptosis induction also played an important role in the antitumor mechanism of ACGs.

However, the poor solubility (less than 1 μg/mL) and a complicated composition restrict the *in vivo* anti-tumor studies of ACGs. Drug delivery technology focuses on improving the solubility. Furthermore, ACGs are a mixture of naturally resourced effective components. This mixture complicates dosage preparation and the corresponding characterization. The current researches

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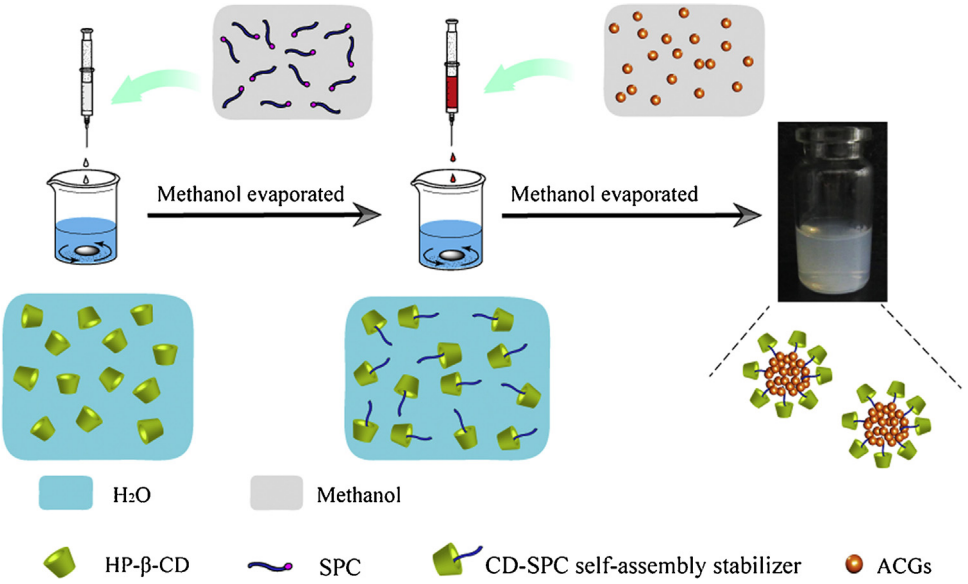

Fig. 1. Schematic illustration of the formation of ACGs-loaded nanosuspensions.

that focused on ACGs drug delivery systems (intravenous lipid emulsions [22], dropping pills [23] and solid dispersions [24]) have limitations such as low drug loading capacity, poor physical stability and the absence of the *in vivo* research. We hypothesize that encapsulating hydrophobic ACGs into the inner core of nanoparticles for *in vitro* and *in vivo* drug delivery, will improve administration and therapeutic efficacy. Nanosuspensions (NSps) are a promising technology for the delivery of poorly soluble drugs [25,26]. The average sizes of drug NSps are in the nanometer range (10–1000 nm) with a high drug loading capacity and large specific surface [27]. Nanosuspensions may be a good drug delivery system for ACGs.

Stabilizers are amphiphilic molecules or polymers and play a vital role in the preparation of nanosuspensions. Cyclodextrins (CDs) have various applications in drug delivery. They posses a hydrophilic exterior surface and a non-polar interior cavity [28]. This architecture allows CDs to form partial or total inclusion complexes with lipophilic substances in an aqueous environment. Dong et al. has reported a novel approach for the construction of supramolecular polymer micelles (SMPMs) using alpha-cyclodextrins (α-CD)and polycaprolactone (PCL) as building blocks [29]. Inspired by the above, we developed a self-assembly amphiphilic complex of soybean lecithin(SPC) in the hydrophobic cavity of hydroxypropyl-beta-cyclodextrin (HP-β-CD), which could be applied as a stabilizer to prepare ACGs nanosuspensions (ACGs-NSps). The *in vitro* properties, cytotoxicity and *in vivo* anti-tumor effect of the nanosuspensions were investigated.

2. Materials and methods

2.1. Materials

Annonaceous acetogenins (ACGs) were provided by Prof. Wenhua Huang from the Institute of Medicinal Plant Development (ACGs batch number: 091, the composition was seen in Table S1); Hydroxypropyl-beta-cyclodextrin (HP-β-CD) was purchased from Zhiyuan Science and Technology Ltd., Shandong, China. Soybean lecithin (SPC) was obtained from Guangzhou Hanfang Pharmaceutical Company Ltd. Hydroxycamptothecin (HCPT) injections were supplied by Shenghe Pharm. Ltd., Szechwan, China. Acetonitrile (HPLC grade) was purchased from Fisher Scientific (Pittsburgh, PA, USA). All other organic solvents and chemicals were of the highest commercially grade available. The water used in the experiments was deionized.

2.2. Animals and cell lines

Male ICR mice (6–8 weeks old, 20 ± 2 g) were purchased from the Vital River Laboratory Animal Technology Co. Ltd. All animal experiments were performed in accordance with the Guidelines for Ethical and Regulatory for Animal Experiments as defined by the Institute of Medicinal Plant Development (IMPLAD), China. Hela (human cervix carcinoma), HepG2 (human hepatocellular carcinoma), LO2 (human normal liver cell line) and H22 (murine hepatocarcinoma) cell lines were provided by the Cell Culture Center, Institute of Basic Medical Sciences (Beijing, China). Hela and HepG2 were cultured with RPMI 1640 medium (GIBCO, USA) and LO2 was cultured with DMEM medium (GIBCO, USA) containing 10% fetal calf serum (GIBCO, USA), penicillin (100 U/mL) and streptomycin (100 U/mL) at 37 °C and 5% CO_2.

2.3. Chromatographic conditions

The content of the ACGs was determined by HPLC using bullatacin (a major component of ACGs). The HPLC system (DIONEX Ultimate 3000, USA) was equipped with an autosampler and the chromatographic separation was perfomed using a Symmetry C18 column (4.6 mm × 250 mm, 5 μm, Waters, USA) at 30 °C.

The mobile phase was composed of acetonitrile and water (70/30, v/v) and run at a flow rate of 1 mL/min. The detection wavelength was set at 210 nm (UV detector, DIONEX).

2.4. Preparation of ACGs-NSps

ACGs-NSps were prepared using nanoprecipitation. Briefly, SPC was dissolved in methanol, forming a 10 mg/mL solution. 250 μL of the SPC solution was dropped into 5 mL of the aqueous solution containing 0.1% HP-β-CD(w/v)with 500 rpm stirring (EURO-STD S25, IKA, Germany) for 15 min to obtain the amphiphilic stabilizer (defined as HP-β-CD-SPC). The solution was then evaporated under a vacuum to remove methanol. The ACGs were then dissolved in methanol (25 mg/mL). This solution (0.4 mL) was dropped into the resultant HP-β-CD-SPC solution under continuous stir-