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Enhanced anticancer effect of *Brucea javanica* oil by solidified self-microemulsifying drug delivery system

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ABSTRACT

Brucea javanica oil (BJO) is a traditional herbal medicine in China for the effective treatment of various diseases due to multifaceted activities. As the most common formulation, BJO emulsion (BJOE) has been employed to treat carcinomas in clinical practice for many years. Herein, we fabricated a solid self-microemulsifying drug delivery system (S-SMEDDS) of BJO and assessed its antitumor effects on cancer cell lines A549 and DU145. The shape of BJO-loaded S-SMEDDS (BJOS) was approximately spherical and the nanosuspension was stable. Since the proliferation of A549 and DU145 cells was significantly inhibited by BJOS dose-dependently compared to that by BJOE, BJOS had enhanced anticancer effects. Given a lower IC_{50}, DU145 cells were more sensitive to BJOS than A549 cells. Besides, flow cytometry showed that A549 and DU145 cell cycles were arrested in the G0/G1 phase, and the transwell assay demonstrated that their invasion ability decreased apparently after 24 h of exposure to BJOS. In conclusion, BJOS was a promising formulation for improving the treatment of lung cancer and prostate cancer.

1. Introduction

As a fatty acid extracted from the ripe fruit of *Brucea javanica* (L.) Merr. (Simaroubaceae) [1], *Brucea javanica* oil (BJO) is a traditional herbal medicine in China for the treatment of various diseases, such as malaria, amoebic dysentery, lung cancer, gastrointestinal cancer and prostate cancer [2–7]. BJO consists of 85% triglycerides together with other saturated and unsaturated fatty acids. Oleic acid and linoleic acid, which are unsaturated fatty acids, can bind cell membranes as well as inhibit the synthesis of DNA, RNA and proteins in tumor cells [8]. Although BJO has potential pharmacological activities, its clinical applications are limited owing to low oral and systemic bioavailabilities induced by low water solubility. In general, BJO emulsion (BJOE) is the most frequently used formulation for oral and intravenous administrations, which can directly destroy biological membrane systems and induce the degeneration or necrosis of cancer cells [9].

Lung cancer is one of the most malignant tumors worldwide, with 80% of cases being non-small lung cancer (NSCLC). NSCLC is mainly treated by surgery, radiotherapy, chemotherapy and molecular targeted therapy. Most NSCLC patients have entered the locally advanced or late stage at first visit, and the 5-year survival rates are lower than 10% and 5%, respectively [10]. Prostate cancer, which is one of the most common male malignant tumors worldwide, is the second leading cause for deaths. Currently, prostate cancer is primarily treated by surgery, chemotherapy and radiotherapy [11]. Patient's life is greatly threatened by this highly invasive cancer upon metastasis. Therefore, it is necessary to clarify the pathogenic and development mechanisms of lung cancer and prostate cancer, and to control their metastasis [12].

Solid self-microemulsifying drug delivery system (S-SMEDDS) is well-documented to effectively deliver drugs with low aqueous solubilities. Comprising oil, surfactant and cosurfactant, S-SMEDDS can form fine o/w (oil in water) microemulsion droplets under mild stirring followed by dilution in aqueous media [13]. This system is fabricated by incorporating liquid excipients into powders through solidification, which combines the advantages of liquid SMEDDS with solid dosage forms, i.e. high solubility, bioavailability and stability with various dosage form options [14,15]. S-SMEDDS is promising for stabilizing SMEDDS without requiring complicated processing steps associated with liquid formulations [16].

Herein, we engineered a BJO-loaded S-SMEDDS (BJOS) to boost anticancer effects. According to the cytotoxicity assay, DU145 cells were more sensitive to BJOS than A549 cells. The cell cycles of both

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tumor cells were arrested in the G0/G1 phase. The transwell assay demonstrated that the motilities and invasion abilities of A549 and DU145 cells decreased after treatment with BJOS in concentration-dependent manners. In conclusion, this study provides valuable information for improving the treatment of lung cancer and prostate cancer by designing BJOS.

2. Materials and methods

2.1. Materials

BJO that contained 71.72% oleic acid was bought from Jishuizhongnan Natural Refinery Factory (Jiangxi Province, China). Linoleic acid and oleic acid standards were purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd. (China). Phenyl benzoate was obtained from Alfa Aesar Chemical Co., Ltd. (Beijing, China). Gelucire 44/14 (lauroyl polyoxylglycerides) and Transcutol P were bought from Gattefosse Corp. (Brittany, France). Caprylic/capric triglyceride was provided by Crode (Cowick Hall, UK). Polyoxyl 40 hydrogenated castor oil 40 (Cremophor RH 40) and polyethylene glycol (PEG) 6000 were obtained from Damao Chemical Reagents Factory (Tianjin, China). All other reagents and chemicals were analytically pure.

Human lung cancer cells A549 and human prostate cancer cells DU145 were provided by the American Type Culture Collection (CCL-185, HTB-81).

2.2. Detection of BJO components by gas chromatography (GC)

GC was employed to detect BJO components, using linoleic acid and oleic acid as references. High sensitivity was obtained by derivatization based on high boiling points [17]. Sample was prepared according to the method previously reported by our group [18]. In brief, the sample was saponified with KOH-methanol solution in a water bath at 60 °C until all oil droplets dissolved. Boron trifluoride in methanol was added and esterification was carried out at 60 °C. Then the solution was added with 2 mL of n-hexane, vortexed, mixed with 1 mL of saturated NaCl solution, vortexed again and left still. The upper n-hexane solution was collected and vortexed after an equal amount of internal standard benzoic acid/benzoate was added. The resulting mixture was filtered through a 0.22 μm filter membrane and injected for GC.

Agilent 6820 gas chromatograph (USA) was equipped with Agilent DPFF-AP column (30 m × 0.25 mm × 0.25 μm, USA) for GC. The above solution sample (2 μL) was injected into the GC, and the temperature of injector was kept at 250 °C. The carrier gas was high-purity nitrogen. The temperatures of column oven and hydrogen flame ionization detector were kept at 205 °C and 250 °C respectively.

2.3. Screening of excipients

To find out appropriate surfactants as the compositions of BJOS, the solubilities of BJO in different surfactants, such as Solutol-HS-15, Cremophor RH 40, Transcutol P, F68 and Gelucire 44/14, were measured. BJO (approximately 1 g) was added into 5 mL centrifuge tubes containing 1 g surfactants respectively. Subsequently, the mixtures were shaken by a (37 ± 1)°C water-bath shaker for 48 h until equilibrium was reached. Surfactants with higher solubilities were chosen as the adjuvant candidates for BJOS.

2.4. Preparation of BJOS and BJOE

BJOS was prepared by using BJO as oil, Cremophor RH 40 as surfactant and PEG 6000 as co-surfactant. Briefly, Cremophor RH 40 and PEG 6000 were mixed in a 60 °C water bath, into which BJO with the ratio of 3:5:1 (w/w/w) was added under magnetic stirring at 300 rpm for 10 min. At last, a transparent BJOS formulation formed. The mixture was stored at 4 °C prior to use, and BJOS was prepared by dilution with distilled water for further experiments.

The BJOE was prepared according to the method previously reported by our group [18]. In brief, a mixture of water and soy lecithin (15 g) was quickly added to the preheated BJO, then mixed at high speed and stirred. The crude emulsion was filled to 100 mL with water for injection, and then homogenization to form a final emulsion.

2.5. Characterizations of BJOS

2.5.1. Determination of particle size and zeta potential

BJOS (0.25 mg) was suspended in 8 mL of distilled water and then blended through vortexing for 5 min. The particle size was analyzed with Delsa™ Nano C Particle Size Analyzer (Beckman Coulter Corp., UK) in the range of 2–3000 nm.

2.5.2. Observation of morphological characteristics

Morphology was observed by transmission electron microscopy (TEM) using HITACHI H-7650 transmission electron microscope (Japan). A thin film was created by placing a drop of the prepared solid self-microemulsion on copper grid and covering it by nitrocellulose. The film was thereafter stained negatively by adding a drop of 2% phosphotungstic acid solution. After drying at ambient temperature, the film was finally observed with TEM.

2.5.3. Fourier transformed infrared (FTIR) spectroscopy

BJO, physically mixed BJO and excipients, and BJOS were subjected to FTIR spectroscopy with TENSOR-3 FTIR spectrometer (Bruker Corp., Germany). Ground samples were mixed by potassium bromide (weight ratio: 1%) and compressed at 8 tonnes for 5 min with a hydraulic press. Single beam spectra from 4000 to 400 cm^{-1} were obtained at the resolution of 0.5 cm^{-1} after 20 scans were averaged, and then corrected with the background spectrum of atmosphere. All these spectra were recorded as absorbances and thereafter converted into transmittances. During operation, the instrument was calibrated periodically.

2.5.4. Drug release in vitro

According to the paddle method (Method II, Chinese Pharmacopoeia (2015 Edition)), the release of BJOS was tested in 900 mL of pH 7.4 phosphate buffer solution (PBS) with the paddle speed of 100 rpm at 37 ± 0.5 °C. In brief, BJOS (about 1.62 g) was dropped directly into the medium, and 10 mL aliquots of samples were collected at 5, 10, 15, 20, 30, 45 and 60 min respectively, and then filtered through a 0.45 μm filter membrane. Sampling-induced losses were compensated by adding the same blank medium. Afterwards, 5 mL aliquots of every filtered sample were dried in a water bath at 60 °C and extracted by 1 mL of n-hexane five times. After being collected into 10 mL test tubes, the extractants were dried under nitrogen.

2.6. Cytotoxicity assay

The cytotoxicity of BJOS was tested with the CCK-8 assay. A549 and DU145 cells were cultured with RPMI1640 medium (Gibco) that contained 10% fetal bovine serum (FBS; Gibco BRL) in a 37 °C incubator with an atmosphere of 5% CO_2, and then divided into three groups which were untreated (control group) or treated with BJOS and BJOE respectively.

Subsequently, cells in the exponential growth phase were collected, routinely detached by trypsin and prepared into a suspension in culture medium, with the density adjusted to 1×10^5 mL^{-1} using a cytometer (Beckman Coulter, Danvers, MA, USA). The cell suspension was added into 96-well plates, and then 50, 100, 200, 500 and 1000 μg mL^{-1} BJOS and BJOE were added into each well. The data of 6 different wells in each plate were obtained and averaged. Meanwhile, the cells were incubated under similar conditions throughout this study. CCK-8 (10 μL) was added into each well after 24 h, and the cells were further incubated for 4 h. Then the supernatant was collected, with the