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Preparation, characterization, biodistribution and antitumor efficacy of hydroxycamptothecin nanosuspensions

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ABSTRACT

Hydroxycamptothecin (HCPT) has shown activity against a broad spectrum of cancers, but its therapeutic efficacy is impaired by its poor solubility and delivery challenges. In this study, HCPT nanosuspensions were prepared with precipitation-combined ultrasonication and characterized by dynamic light scattering (DLS), scanning electron microscopy (SEM) and X-ray powder diffraction (XRD). The HCPT nanosuspensions were spherical with a smooth surface and a small size of 150–200 nm. The lyophilized powders for the HCPT nanosuspensions were amorphous and displayed sustained release in vitro. Compared to commercial HCPT injection, in vivo experiments with HCPT nanosuspensions showed significantly increased HCPT concentrations in the blood and all tissues of the tested as well as improved tumor targetability and liver targetability. Meanwhile, nanosuspensions displayed better anticancer efficacy than injection on H22 bearing mice (81.20% vs. 56.39%, in tumor inhibition rate). Therefore, HCPT nanosuspensions seem very promising for the treatment of hepatic cancer.

1. Introduction

Camptothecin (CPT) is a natural alkaloid that was isolated in the 1960s from the extracts of *Camptotheca acuminate*, a plant native to China (Wall et al., 1966). Due to the potent antitumor activity of CPT, many CPT derivatives have been synthesized and their bioactivity and pharmacodynamics have received increasing attention. Hydroxycamptothecin (HCPT, Fig. 1), a CPT analog with a 10-hydroxy substituent, has shown the best antitumor effects activity (Wall et al., 1966), and lower toxicity in both experimental animals and human trials than CPT (Han, 1994). Therefore, HCPT has been widely used in the treatment of gastric carcinoma, hepatocarcinoma, leukemia and tumors of the head and neck (Pu et al., 2009). However, HCPT can exist in either the carboxylate form or the lactone form, depending on the surrounding pH. Under acidic conditions (pH < 4), HCPT mainly exists primarily as the lactone. At physiological pH (pH 7.4), approximately 90% of the HCPT is in the carboxylate form. It has been proven that the presence of the α-hydroxy- δ-lactone ring of HCPT is crucial for its antitumor activity (Hertzberg et al., 1989) and that the lactone is the more effective inhibitor of topoisomerase I (Slichenmyer et al., 1993). In addition, the carboxylate form has shown several side effects in clinical trials (Wani et al., 1980; Mallery et al., 2001). Unfortunately, the lactone form shows very poor solubility in water and physiological fluids, which greatly limits its pharmaceutical development and clinical utilization. To solve this problem, the development of a safe and high performance delivery system for the insoluble HCPT lactone is necessary.

Recently, the design of special dosage forms and techniques for HCPT have been evaluated to overcome its hydrophobicity and instability. These approaches have included liposomes (Cortesi et al., 1997; Saetern et al., 2004; Zhang et al., 2004; Sadzuka et al., 2005; Zhao et al., 2006), microspheres (Mallery et al., 2001; Zhao et al., 2006; Lu and Zhang, 2006), submicron emulsions (Zhao et al., 2007), chitosan complexation (Zhou et al., 2010), and water- and lipid-soluble prodrugs (Croce et al., 2004). However, there are shortcomings with these formulations, such as low drug loading capacity, low encapsulation, poor physical stability and adverse side effects from the solubilizing or encapsulating excipients.

Nanosuspensions are an intriguing delivery system consisting essentially of pure drug nanoparticles (<1000 nm) and include only a small amount of surfactants and/or polymeric materials for stabilization. In this system, drugs exist in a nano-sized particle. Nano-sized particles have large specific surface areas and thus can increase drug saturation solubility and dissolution velocity, which are both very important parameters for improving the bioavailability of drugs. In addition, because of its nanometer

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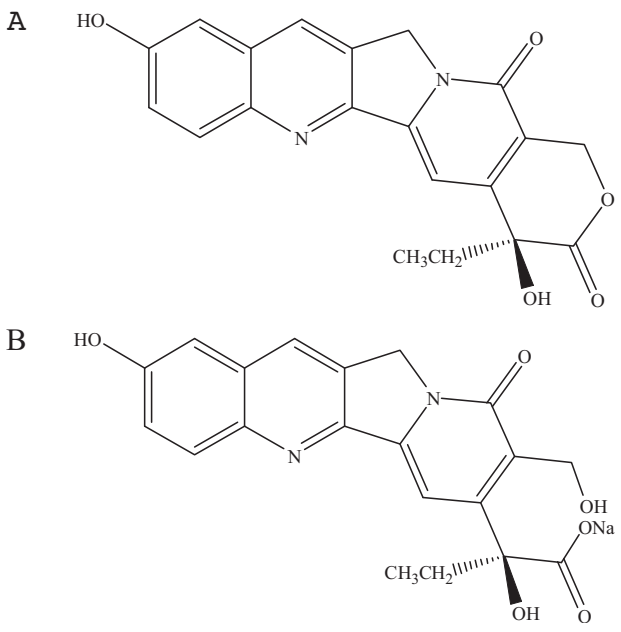

Fig. 1. The chemical structure of hydroxycamptothecin (A – lactone form; B – carboxylate form).

size and safe composition, nanosuspensions can be given by various routes of administration, including the oral (Liversidge and Cundy, 1995), parenteral (Peters et al., 2000), ocular (Pignatello et al., 2002) and pulmonary pathways (Jacobs and Muller, 2002). Nanosuspensions, when intravenously administered, can alter the drug biodistribution and increase drug accumulation in tumors through the enhanced permeability and retention effect (EPR) effect.

In the present study, HCPT nanosuspensions were prepared with precipitation-combined ultrasonication, and the formulation was optimized for lyophilization. The percentage of the lactone form in the resultant HCPT nanosuspensions could be increased to over 95%, and the apparent HCPT concentration reached 100 mg/ml, effectively reducing the dosing volume.

The results from in vivo antitumor and tissue distribution tests were studied in parallel with studies of H22-tumor bearing mice after intravenous injection. These tests showed that HCPT nanosuspensions exhibited superior in vivo antitumor effects and a remarkably different biodistribution than the commercially available HCPT injection.

2. Materials and methods

2.1. Materials

Hydroxycamptothecin with greater than 99% purity was kindly provided by Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Dimethylsulfoxide (DMSO, ACS grade) was purchased from Sigma–Aldrich Chemicals, Germany. Fetal calf serum was obtained from Yuanhengjinma Bio-technology Development Co., Ltd. (Beijing, China). HCPT injection (used as the sodium carboxylate in China) was obtained from Shenghe Pharmaceutical Ltd. (Sichuan, China). The water used in the experiments was deionized, and all other organic solvents were of the highest commercially available grade.

2.2. Animals

Female ICR mice (20 ± 2 g) were supplied by Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All of the animals were acclimatized at a temperature of 25 ± 2 °C and a relative humidity of 70 ± 5% under natural light/dark conditions for 1 week and provided food and water ad libitum. All experimental procedures 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.

2.3. Preparation of HCPT nanosuspensions

HCPT nanosuspensions were prepared by the precipitation–ultrasonication method. Briefly, the HCPT bulk powder was dissolved in DMSO (50 °C) to form an organic solution containing 50 mg/ml of drug. The organic solution was then allowed to cool to room temperature. Fetal calf serum was used as an anti-solvent. Both the organic solution and the fetal calf serum were passed through a 0.45 μm filter (Xinya Purification Device Factory, China). Then, 1 ml of the organic solution was slowly introduced into 9 ml of anti-solvent at 20 ± 2 °C under rapid stirring and intense 250 W sonication (Ultrasonic cleaner, Kun Shan Ultrasonic Instruments Co., Ltd., China). The resultant suspensions were centrifuged at $20{,}000 \times g$ for 10 min using a high-speed centrifuge (Sigma–Aldrich Co., Ltd., Germany), and the sediment was redispersed into fresh fetal calf serum using a vortex until no visible clumps were observed. The obtained HCPT nanosuspensions were lyophilized (see Section 2.4.2) to provide the final product for use.

2.4. Physicochemical characterizations of the nanosuspensions

2.4.1. Particle size measurement

The mean particle size (z-average) and the polydispersity index (PDI) of the prepared nanosuspensions were determined by dynamic light scattering (DLS) (Zetasizer nano ZS, Malvern Instruments, UK). All of the measurements were performed with a laser wavelength of 633 nm at 25 °C. Each sample (5 mg/ml) was measured in triplicate with 12 runs in each measurement and a duration of 10 s for each run.

2.4.2. Lyophilization of HCPT nanosuspensions

To enhance the chemical and physical stability, the HCPT nanosuspensions were lyophilized before storage and subsequent use. Briefly, the HCPT nanosuspensions were rapidly cooled to −80 °C for 12 h and then freeze-dried in an LGJ-10B freeze-dryer (Sihuan Laboratory Instruments Co., Ltd., China) under vacuum (pressure < 10 pa) for 24 h. No cryoprotectant was needed for this procedure.

2.4.3. Surface morphology of HCPT nanosuspensions

The morphological evaluations of the HCPT bulk powder and HCPT nanosuspensions prepared by the precipitation method were conducted using scanning electron microscope (SEM, JSM-6360LV, Japan). SEM samples were glued and mounted on metal sample plates. The samples were gold coated with a sputter coater using an electrical potential of 2.0 kV at 30 mA for 240 s. The surface morphology of the HCPT bulk powder and HCPT nanosuspensions was examined operating the SEM at 20 kV.

2.4.4. X-ray powder diffraction measurements

X-ray powder diffraction (XRD) was performed on a Bruker D8 advance instrument controlled by Diffrac plus XRD commander software. The HCPT bulk powder, lyophilized fetal bovine serum and lyophilized HCPT nanosuspensions were scanned over an angular range of 3–80° of 2θ, with a step size of 0.02° and a count time of 3 s per step. Samples were rotated at 30 rpm during the analyses. The generator was set at 40 kV and 100 mA.