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A highly stable norcantharidin loaded lipid microspheres: Preparation, biodistribution and targeting evaluation

Jinlong Ma [a], Huan Teng [a], Juan Wang [a,b], Yu Zhang [a,c], Tianyang Ren [a], Xing Tang [a], Cuifang Cai [a,*]

[a] School of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, China
[b] Shenyang Jinchang Pharmaceutical Co., Ltd., China
[c] Jiangsu Kanion Pharmaceutical Co., Ltd., China

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ABSTRACT

The purpose of this study was to prepare norcantharidin (NCTD)-loaded lipid microspheres (LMs) with a high encapsulation efficiency (EE) and stability during sterilization. The NCTD–phospholipid complex (NPC) was produced and characterized to increase the lipophilic properties of NCTD and a novel concentrated homogenization method was applied for the preparation of LMs. The results of the UV, DSC and IR investigations confirmed the formation of NPC. The oil–water partition coefficient (log *P*) of NPC was significantly increased with a value of -1.34 ± 0.06 at pH 7.4, nearly 224 times higher than that of NCTD. A concentrated emulsion was prepared based on a homogenization method and then diluted with water. After optimization of the NPC formation and emulsion preparation process, the EE was dramatically increased from 21.6% to 84.6%, and a highly sterilization stability was achieved with only a minor change in particle size from 168.2 ± 39.4 nm to 173.4 ± 43.5 nm. The tissue distribution of NPCLM was measured after intravenous administration to rats of a dose of 3.9 mg/kg with NCTD injection (NI) as the reference. Considerably increased concentrations of NCTD in the liver, spleen and lung were detected with NPCLM and the values were 1.67, 1.49 and 1.06 times higher than in the NI group, respectively while, in the kidney, the concentration was slightly reduced 0.96-fold. Overall, based on these techniques, this NPCLM with an improved EE and stability offers great promise in clinical applications and industrial-scale production along with a potentially increased targeting effect on the liver and reduced toxicity in the kidney.

1. Introduction

Norcantharidin (NCTD), a demethylated analogue of cantharides isolated from the dried body of the blister beetle, is a potent anti-cancer drug used for the treatment of primary hepatic carcinoma, breast cancer and abdominal cancer (Wang, 1989). Compared with the other anticancer drugs, the distinguishing features of NCTD include its low degree of myelosuppression, induction of leukocytosis and minor effect on normal cells (Yi et al., 1991), (Chen et al., 2012). Therefore, NCTD is more attractive for chemotherapy because of its potent anti-hematoma activity and synergistic therapeutic effect.

However, the significant side-effects of NCTD, including cardiac and renal damage, limit its use in clinical situations (Liu et al., 1995). NCTD is usually administered by means of a high-dose injection which produces intense irritation at the injection site (Wang et al., 2006). When administered by the oral route, NCTD has to be given in a high dose to increase its antitumor efficacy due to its poor intestinal absorption, which can produce severe gastrointestinal and urinary toxic effects. Therefore, it is very important to find a new drug delivery system for NCTD to reduce these side effects.

To obtain a safer and more effective NCTD treatment, many new alternative formulations have been studied to improve the targeted delivery, such as *N*-trimethyl chitosan nanoparticles (Guan et al., 2012), lipid microspheres (Wang et al., 2006), polycaprolactone microspheres (Wang et al., 2008), NCTD-polymer conjugates (Hong et al., 2009), and pH-sensitive liposomes (Qiaoling et al., 2012). However, most of these dosage forms have been found to be clinically unsuccessful because of lower encapsulation efficiency, the presence of an organic solvent and poor physicochemical stability during long-term storage.

* Corresponding author: Tel.: +86 24 23986343; fax: +86 24 23911736.
E-mail address: bbpharmcai@sina.com (C. Cai).

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In recent years, lipid microspheres (LMs) have attracted much attention as an ideal carrier for anticancer drugs. Compared with other drug carriers, LMs are physically stable, biodegradable, biocompatible, and easy to prepare (Lundberg et al., 1996; Fukui et al., 2003). Moreover, sustained drug release can be obtained because the drug is contained in the inner phase and does not come into direct contact with body tissues and fluids (Lu et al., 2005). In addition, it also improves the stability of hydrolyzable materials and reduces the effect of the drug on tissues. Consequently, LM is an ideal carrier for NCTD.

LMs are usually used as parenteral delivery carriers for lipophilic drugs, such as prostaglandin E1, diazepam, and non-steroidal anti-inflammatory drugs (Constantinides, 1995; Singh and Ravin, 1986; Yamaguchi and Mizushima, 1994). To ensure an encapsulation efficiency of more than 80%, the oil–water partition coefficient (log *P*) of the drug must be greater than 3. However, the problem is that all drugs of commercial interest to be formulated as i.v. emulsions do not have a sufficiently high solubility and oil–water partition coefficient in standard LCT and MCT oils, while the development of a new oil phase is too expensive (Akkar and Müller , 2003).

The oil–water partition coefficient (log *P*) of NCTD is approximately −3.69 (pH 7.4) so during the process of preparing a coarse emulsion, NCTD dissolved in the oil phase would diffuse into the water, which would reduce the encapsulation efficiency of the formulation.

In order to improve the lipophilicity of NCTD, phospholipid complexes of NCTD were developed. Phospholipid complexes are currently used for two groups of drugs for different purposes. One is to reduce the gastrointestinal toxicity caused by non-steroidal anti-inflammatory drugs (NSAIDs), while the other is to increase the solubility and the bioavailability of highly lipophilic and poorly absorbed drugs. (Hüsch et al., 2011). In the case of phytopharmaceuticals, Indena S.p.A. introduced a new product called Phytosom® (Semalty et al., 2010). According to the structure of NCTD, it is prone to hydrolysis and forms a dicarboxylic acid structure which can interact with the amino end of phospholipids, forming hydrogen bonds or exhibiting charge effects. Also, NCTD is a low molecular weight drug and, therefore, the formation of NPC is a suitable way of increasing the lipophilicity of NCTD. In addition, to further increase the EE and reduce the exposure of the drug to water, a concentrated homogenization method was used (initial preparation of a 20% o/w emulsion which was then diluted to 10%). The resulting emulsion had a high encapsulation efficiency, excellent stability, and efficient tissue targeting.

This paper describes the preparation of a new kind of NCTD o/w lipid microspheres loaded with an NCTD–phospholipid complex (NPCLM). The preparation process was systemically investigated and optimized. As a result, we developed a novel method which is suitable for the drug NCTD (log $P \approx -3.69$, pH 7.4) and provides an encapsulation efficiency greater than 80%. All the experimental results prove that NPCLM can withstand thermal sterilization and it has good physical stability and high encapsulation efficiency. Moreover, it is suitable for industrial-scale production.

2. Material and methods

2.1. Materials

The following materials were purchased from the sources in brackets: NCTD (Surui Medicine and Chemical Industry Ltd. Co., Suzhou, China), PL-100 M (Advanced Vehicle Technology Ltd. Co., Shanghai China), Lipoid E80®, oleic acid, cholesterol and medium-chain triglyceride (MCT) (Lipoid KG, Ludwigshafen, Germany), long-chain triglyceride (LCT) (Tieling Beiya Pharmaceutical Co., Tieling, China), Poloxamer 188 (Pluronic F68®) was purchased from BASF AG (Ludwigshafen, Germany), EDTA (Toshihito Pharmaceutical Co., Ltd. in Hangzhou, China) and glycerol (Zhejiang Suichang Glycerol Plant, Zhejiang, China), All other chemicals and reagents were obtained from Tianjin Concord Technology Ltd. Co., Tianjin, China, and were of analytical or chromatographic grade.

All the animals used in this study were obtained from the Experimental Animal Center (Shenyang Pharmaceutical University, Shenyang, China). The experimental protocol were evaluated and approved by the University Ethics Committee for the use of experimental animals and conformed to the Guide for Care and Use of Laboratory Animals.

2.2. Preparation of the NCTD–phospholipid complex (NPC)

The NPC was prepared with phospholipids (E80), cholesterol and NCTD in a suitable ratio. The required amounts of the above materials were dissolved in 10 ml anhydrous alcohol then the mixture was allowed to react at a temperature of 40 °C for 4 h with constant stirring at 100 rpm. Then, the ethanol was completely evaporated in a rotary evaporator and the dried NPC was collected for further processing.

2.3. Characterization of NPC

2.3.1. UV absorption spectra

Appropriate amounts of NCTD and NPC were dissolved in methanol and their UV absorption was scanned from 200–400 nm. Differences in UV absorption characteristics between the test samples were compared.

2.3.2. Fourier transforms infrared spectroscopy (FT-IR)

Fourier transform infrared spectrophotometry (FT-IR Spectrometer, BRUKER IF S-55, Switzerland) was used to study the interaction between NCTD and phospholipid. The IR spectra of NCTD, phospholipid, a physical mixture and NPC were obtained by the KBr method.

2.3.3. Differential scanning calorimetry (DSC)

Samples were sealed in aluminum crimped cells and heated at a rate of 10 °C min^{-1} from 30 °C to 150 °C in a nitrogen atmosphere (DSC-60, Shimadzu, Japan). The peak transition maximum temperatures of NCTD, phospholipid, physical mixture and NPC were determined and compared using a Thermal Analyzer (TA-60 WS, Shimadzu, Japan).

2.4. Solubility studies

2.4.1. Water and oil solubility

In brief, an excess of NCTD was placed in vials, then phosphate buffer solution (PBS) with different pH values was added followed by distilled water, MCT and LCT. The oversaturated solutions were placed in a shaking air bath (HZQ-C, Dongming Medical Instrument Co., Harbin, China) operated at 100 rpm and 25 °C for 72 h to achieve a solubility equilibrium. The resultant suspensions were passed through a 0.45 μm microporous filter then the filtrate was diluted for HPLC analysis. All solubility samples were processed in triplicate.

2.4.2. Oil/water apparent partition coefficient (log P) of NCTD and NPC

Briefly, an excess of NCTD and NPC were added to a solution containing 5 ml double -distilled water (pre-saturated with *n*-octanol) and 5 ml *n*-octanol (pre-saturated with double -distilled water) in sealed glass containers at 25 °C. Each experiment was performed in triplicate. The liquids were agitated at 100 rpm for 72 h, and then centrifuged to remove excess NCTD or NPC (10 min, 8000 rpm). The supernatants were then passed through a 0.45 μm