Optimization of Parameters for Preparation of Docetaxel-loaded PLGA Nanoparticles by Nanoprecipitation Method

Wei SHI (施 伟) 1*, Zhan-jie ZHANG (张占洁) 1†, Yin YUAN (袁 昱) 1†, En-ming XING (邢恩明) 1†, You QIN (秦 奕) 1†, Zhen-jun PENG (彭振军) 2, Zhi-ping ZHANG (张志平) 2†, Kun-yu YANG (杨坤禹) 2†

1 Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China
2 School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Summary: The purpose of this study was to develop docetaxel-poly (lactide-co-glycolide) (PLGA) loaded nanoparticles by using nanoprecipitation method and optimize the relative parameters to obtain nanoparticles with higher encapsulation efficiency and smaller size. The physicochemical characteristics of nanoparticles were studied. The optimized parameters were as follows: the oil phase was mixture of acetone and ethanol, concentration of tocopheryl polyethylene glycol succinate (TPGS) was 0.2%, the ratio of oil phase to water phase was 1:5, and the theoretical drug concentration was 5%. The optimized nanoparticles were spherical with size between 130 and 150 nm. The encapsulation efficiency was (40.83±2.1)%. The in vitro release exhibited biphasic pattern. The results indicate that docetaxel-PLGA nanoparticles were successfully fabricated and may be used as the novel vehicles for docetaxel, which would replace Taxotere® and play great roles in future.

Key words: docetaxel; nanoparticles; optimization; poly (lactide-co-glycolide); cancer

Docetaxel, a member of to xoid family similar to paclitaxel, has been widely used in the treatment of lung cancer, breast carcinoma, ovarian cancer, and head and neck cancers[1-3]. However, the clinical application of docetaxel is impeded by its poor solubility, low bioavailability and high toxicity. Although Tween 80 can enhance the solubility of docetaxel, it also brings some adverse effects such as hypersensitivity and incompatibility with common polyvinyl chloride intravenous administration sets[4, 5]. Meanwhile, chemotherapy with docetaxel causes bone marrow suppression, peripheral neurotoxicity, and fluid retention[6]. Fortunately, several novel carriers including liposomes and nanoparticles are recommended to solve these problems. They increase efficacy of therapeutics by reducing systemic exposure, increasing therapeutic concentrations at the disease site, and modifying exposure time by providing sustained release of therapeutics[7-10].

Among the novel carriers, nanoparticles made by biodegradable polymers are the ideal vehicles for docetaxel to solve the problems of poor solubility and co-solvent. Besides, polymeric nanoparticles may consequently reduce the side effects and toxicity of the drug while increase its therapeutic efficacy. Furthermore, nanoparticles can escape from the leaky vasculature around the tumor and accumulate in the tumor[11]. It was reported recently that nanoparticles can overcome multidrug resistance caused by P-glycoprotein and increase the drug content in the tumor[12]. Other important advantages of the nanoparticles include simple preparation with polymer and the high stability either in fluids or during storage[13].

Poly (lactide-co-glycolide) (PLGA) has been widely used as a drug delivery carrier as it has good biocompatibility and biodegradability[14]. In our study, docetaxel-loaded PLGA nanoparticles were fabricated by classical nanoprecipitation method. D-α tocopheryl polyethylene glycol succinate (vitamin E TPGS or simply, TPGS) has been widely used as solubilizer, emulsifier, absorption enhancer and additive in drug delivery formulations[15, 16]. TPGS can be used as excipient to overcome multidrug resistance (MDR) and inhibit P-glycoprotein (P-gp) to increase the bioavailability of anticancer drugs[17-19].

In our research, TPGS was first used as emulsifier for nanoparticles of docetaxel, which will result in high drug encapsulation efficiency, high cellular uptake in vitro, high therapeutic effects in vivo, longer half life and higher therapeutic effects[20-21]. Docetaxel-loaded PLGA nanoparticles was fabricated by nanoprecipitation method and then characterized by laser light scattering (LLS) for particles size and size distribution, and field emission scanning microscopy for morphology. The drug encapsulation efficiency (EE) and in vitro release kinetics were measured by high-performance liquid chromatography. In our further study, we are going to investigate docetaxel-loaded PLGA nanoparticles as radiosensitizer in vitro and in vivo.

1 MATERIALS AND METHODS

1.1 Materials

Docetaxel of purity 99% was purchased from Jinhe Limited, China. PLGA (Resoner RG503H) was supplied from Boehringer Ingelheim, Germany. Long lipophilic tracer Dil was purchased from Invitrogen, USA. Vitamin E TPGS was purchased from Eastman Chemical, Kingsport, USA, Docetaxel (Taxotere®) was purchased from Sanofi-Aventis (China). Methylene chloride (dichloro-
methanol, DCM), ethanol and phosphate-buffered solution (PBS) were of analytical grade. Methanol and acetonitrile were of HPLC grade. All other chemicals were of analytical grade and used as received. All reagent water used in the laboratory was pretreated with Milli-Q Plus System (Lillipore Corporation, MA). Amicon Ultra-4 centrifugal filter was purchased from Millipore, USA.

### 1.2 Preparation of Docetaxel-loaded PLGA Nanoparticles

The docetaxel-loaded PLGA nanoparticles were fabricated by a classical nanoprecipitation technique [22, 23]. Briefly, a weighed amount of PLGA with designated ratio between PLGA and docetaxel were dissolved in oil phase to reach 10 mg/mL concentration. TPGS was dissolved into aqueous phase at the designated concentration. The organic phase was subsequently added drop-wise into the aqueous phase under magnetic stirring. The organic solvent was then evaporated overnight under vigorous stirring. The suspension was centrifuged at 3500 r/min for 15 min using the filters with a molecular weight cutoff of 20 kD for three times to collect the nanoparticles. For the blank (without drug loaded) nanoparticles, only the polymers were dissolved in oil phase. The fluorescent nanoparticles were prepared in the same way with docetaxel replaced by Dil. The sterilized nanoparticles were made by fabricating nanoparticles in the super-clean bench under sterile conditions.

### 1.3 Characterization of Nanoparticles

#### 1.3.1 Particle Size and Size Distribution of Nanoparticles

The particle size and size distribution of the nanoparticles were measured by the laser light scattering (Brookhaven DB-525 Zeta PALS). The samples were prepared by diluting the nanoparticles in ultrapure water and sonicated before measurement. A typical result was obtained based on the average from six runs. The measurement was performed at 20°C.

#### 1.3.2 Surface Charge

The surface charge of the nanoparticles was determined by laser light scattering at room temperature in ultrapure water. The suspension of nanoparticles was diluted by ultrapure water and sonicated before measurement. A typical result was obtained based on the average from six runs. The measurement was performed at 20°C.

#### 1.3.3 Determination of Drug-loading Parameters

Drug EE of the nanoparticles was measured by high-performance liquid chromatography (HPLC, Hitachi L-2000, Japan). A reverse phase Inertsil® ODS-3 C18 column (150 cm×4.6 mm, pore size 5 μm, Agilent, China) was used. Briefly, 3 mg of the freeze-dried nanoparticles were dissolved in 1 mL DCM to break polymer matrix and then evaporated overnight. After evaporating DCM, 3 mL mobile phase (50% acetonitrile in water in volume ratio) was added to dissolve the extracted drugs. Sample solution was injected three times to detect docetaxel at 227 nm with a UV/VIS detector. The flow rate was 1.0 mL/min. The calibration curve for the quantification of docetaxel was linear over the range of standard concentration between 100 ng and 150 μg/mL with a correlation coefficient of $R^2=0.9996$.

The drug concentration of docetaxel was calculated from standard curves. Drug EE was calculated as follows:

$$EE\% = \frac{Actual\ amount\ of\ drug\ loaded\ in\ nanoparticles}{Theory\ amount\ of\ drug\ loaded\ in\ nanoparticles} \times 100\%$$

#### 1.3.4 Surface Morphology

The shape and surface morphology of the nanoparticles were imaged by a field emission scanning electron microscopy (FESEM) system at an accelerating voltage less than 15 kV. To prepare samples for FESEM, the nanoparticles were fixed on the stub by a double-sided sticky tape and then coated with platinum layer by JFC-1300 automatic fine platinum coater for 50 s.

#### 1.3.5 In Vitro Drug Release

The release rate of docetaxel from nanoparticles was measured three times in phosphate buffer saline (PBS) (pH=7.4). Weighted docetaxel-loaded PLGA nanoparticles were dispersed in 10 mL of PBS with 0.1% Tween 80 in a dialysis bag. The dialysis bags were incubated in 50 mL of PBS with the same pH and gently shaken at 120 r/min in 37°C water bath. At predetermined time points, the aliquots of release medium were removed and the same volume of fresh solution was added. The drug released in the medium was extracted with 4 mL DCM and analyzed by HPLC as described above.

### 1.4 Statistical Analysis

Statistical analysis was performed by using the Student's $t$-test with $P<0.05$ as significant difference. The experimental results were given in the format of $\bar{x}\pm s$ in the tables and figures.

## 2 RESULTS

#### 2.1 Factors Affecting Diameter and EE of Docetaxel-loaded PLGA Nanoparticles

The effect of variables in the preparation steps on the size and EE of the docetaxel-loaded PLGA nanoparticles was investigated. These variables included the concentration of TPGS, choice of organic phase, the volumetric ratio of organic phase to aqueous phase, and the theoretical drug loading. We evaluated these parameters in terms of particle size, size distribution and EE.

---

**Fig. 1** Influence of concentration of TPGS on the mean size and EE of docetaxel-loaded PLGA nanoparticles