

NANOPARTICLES OF BIODEGRADABLE POLYMERS FOR CANCER CHEMOTHERAPY

Khin Yin Win (1), Li Mu (2), Chi-Hwa Wang (1), Si-Shen Feng (1, 2)

(1) Chemical and Environmental Engineering
Department
National University of Singapore
Singapore

(2) Bioengineering Division
National University of Singapore
Singapore

INTRODUCTION

Paclitaxel, a naturally occurring diterpenoid originally extracted from the Pacific Yew tree, is one of the best antineoplastic drugs, which has been used against a wide spectrum of cancers, including breast cancer, ovarian cancer, lung cancer, head and neck carcinomas, and acute leukemia. However, the success of its clinical application is limited by its low therapeutic index and low solubility in most pharmaceutical solvents. More effective chemotherapy using paclitaxel is relying on development of its new dosage forms, among which nanoparticles of biodegradable polymers and lipid bilayer vesicles (liposome) seem the most prospective.

Currently, the only available dosage form of paclitaxel is Taxol® for intravenous (i.v.) infusion, which is a solution of paclitaxel in an adjuvant called Cremophor EL, which causes serious side effects such as hypersensitivity reactions, nephrotoxicity, neurotoxicity and cardiotoxicity [1-4]. Also, Taxol® infusion is cumbersome for the patients and limits the use of frequent dosing schedule for a prolonged systemic exposure to the drug. Thus, the development of successful paclitaxel delivery system devoid of Cremophor EL is essential for a better clinical administration. Moreover, it would be ideal solution to achieve best therapeutic efficacy and least side effects and to greatly improve the quality of life of the patients if oral paclitaxel could become bioavailable.

Nanoparticles of biodegradable polymers could provide such an ideal solution for intravenous or oral delivery of paclitaxel as well as of other anticancer drugs. Bioadhesive nanoparticles with appropriate coating have been shown to increase oral bioavailability of drugs due to the increased residence time of the nanoparticles within the gastrointestinal (GI) tract and the increased contact time with the intestinal epithelium cells and hence the increased uptake. Moreover, appropriate coating of nanoparticles may provide engineering make-ups to escape from the recognition of P-glycoproteins, which have been found to be responsible for the oral unavailability and the multidrug resistance of paclitaxel.

The objective of this study was to develop a new polymeric delivery system of paclitaxel for clinical administration with higher

therapeutic efficacy and less side effects, and with further development, to promote oral chemotherapy. This system developed can also be applied to other anti-cancer drugs.

EXPERIMENTAL METHODS

Nanoparticle Preparation

Nanoparticles were prepared by the solvent extraction/evaporation method. In brief, an oil phase solution of dichloromethane (DCM) or other organic solvents containing poly (DL-lactide-co-glycolide) (PLGA, 50:50) and paclitaxel was emulsified in an aqueous solution containing PVA or vitamin E TPGS using a microtip probe sonicator. The polymer solution also contained 0.05%(w/v) Coumarin 6 as a fluorescent marker. The resulted emulsion was then stirred to evaporate DCM and particles were collected by centrifugation followed by freeze-drying.

Characterization of Nanoparticles

Nanoparticle size and size distribution were determined by laser light scattering with particle size analyzer (90 Plus, Brookhaven Inst, Huntsville, NY) at a fixed angle of 90° and at a temperature of 25°C. The size distribution was given by the polydispersity index. The morphology of nanoparticles was investigated by scanning electron microscopy (SEM) (Jeol JSM 5600LV). The zeta potential as an indicator of surface charge and particle stability in dispersion was determined by a zeta potential analyzer (Zeta Plus, Brookhaven Instruments, Huntsville, NY). The encapsulation efficiency of paclitaxel in particles was analyzed by HPLC system.

In Vitro Release Studies

The amount of paclitaxel released from particles into phosphate buffered saline (PBS, pH 7.4), under in vitro conditions, was measured by high performance liquid chromatography (HPLC).

In Vitro Cytotoxicity

Human colon adenocarcinoma cell lines, HT-29 cells and Caco-2 cells, were used for cytotoxicity study. The cells were incubated with

paclitaxel-loaded particles or Taxol®, respectively, for 24 hours. Cytotoxicity was determined by MTT assay, in which the optical density at 570 nm was determined by microplate spectrophotometer.

In Vitro Particle Uptake Studies

For the cell uptake experiment, the cells were seeded on the coverglass in a chamber system and incubated with Coumarin-loaded particle suspension (100 µg/ml to 250 µg/ml in HBSS, pH 7.4). The qualitative cell uptake study was performed by confocal laser scanning microscope (CLSM) (Zeiss LSM 410) and Fluoview FV300 software, while the quantitative cell uptake study was carried out by microplate reader.

RESULTS AND DISCUSSION

The particle size and the polydispersity index, zeta potential, and drug encapsulation efficiency of the prepared drug-loaded nanoparticles were presented in Table 1. The zeta potential was found to be strongly influenced by the emulsifier applied in fabrication of the nanoparticles. The particles are nanometric and exhibited spherical shape, as shown in Figure 1.

Table 1. Characteristics of paclitaxel-loaded particles

Properties of samples	Sample P1	Sample T1
Emulsifier (w/v)	2% PVA	0.03% vitamin E TPGS
Size ± SD (nm)	293.6 ± 4.8	375.1 ± 14.8
Polydispersity	0.110	0.222
Zeta Potential (mV)	-18.89	-23.98
Encapsulation Efficiency (%)	92.80	55.68

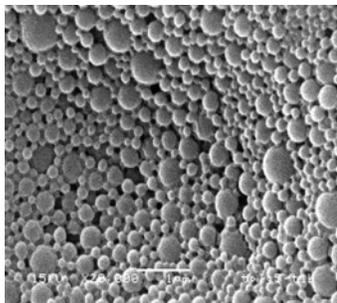


Figure 1. Scanning electron micrograph showing the surface morphology of paclitaxel-loaded PLGA particles prepared using vitamin E TPGS.

The effect of different coating of particles on the in vitro release behavior is presented in Figure 2. The release profile can be controlled by varying polymer type, its molecular weight and copolymer blend ratio and the process parameters. TPGS was chosen as coating material since it acts to solubilize lipophilic compounds. We hypothesized that TPGS could be absorbed intact readily in the GI tract and could inhibit P-gp to enhance nanoparticle absorption to the cells [5-6].

Nanoparticles for oral chemotherapy need to be absorbable to GI tract with a sufficiently high rate and extent. Both HT-29 cells and Caco-2 cells were found to uptake the nanoparticles. Size and surface characteristic dependency of nanoparticle uptake was investigated by the confocal microscopy of the cell monolayers. The effect of coating materials on nanoparticle uptake was evidenced by Figure 3, which shows that nanoparticles coated by TPGS were accumulated in the

intracellular space to a better extent compared with PVA coated nanoparticles.

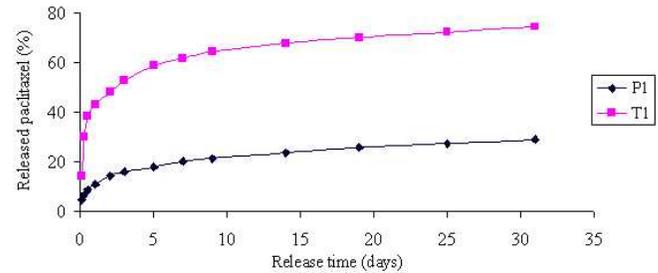


Figure 2. In vitro release profile of paclitaxel-loaded PLGA nanoparticles prepared using PVA and vitamin E TPGS.

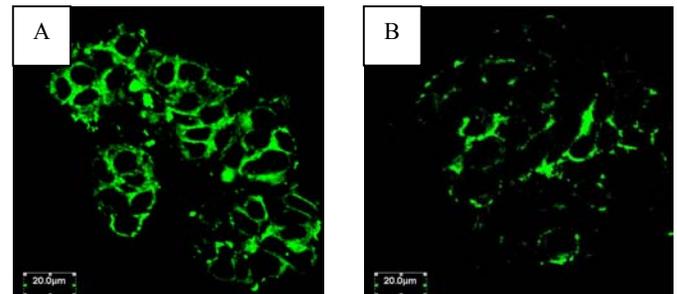


Figure 3. Confocal images of HT-29 cells incubated with Coumarin-loaded particles incorporating: A) vitamin E TPGS, B) PVA. The horizontal bar indicates 20 µm.

CONCLUSION

It is feasible for nanoparticles of biodegradable polymers to be used for clinical administration of paclitaxel to avoid the toxic adjuvant Cremophor EL and to promote oral chemotherapy.

The delivery system developed in this study imposed a great potential.

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