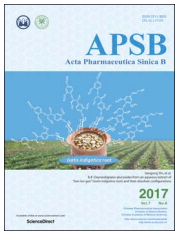
Acta Pharmaceutica Sinica B ▮▮▮▮;▮(▮):▮▮▮–▮▮▮

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

# Development of polyvinylpyrrolidone/paclitaxel self-assemblies for breast cancer

**Pallabita Chowdhury, Prashanth K.B. Nagesh, Sheema Khan, Bilal B. Hafeez, Subhash C. Chauhan, Meena Jaggi, Murali M. Yallapu***

*Department of Pharmaceutical Sciences and Center for Cancer Research, University of Tennessee Health Science Center, Memphis, TN 38163, USA*

Received 11 August 2017; revised 13 October 2017; accepted 20 October 2017

**KEY WORDS**

Drug delivery;
Nanoparticles;
Self-assemblies;
Polymer;
Cancer therapeutics;
Breast cancer

**Abstract** The goal of this investigation was to develop and demonstrate a polymer/paclitaxel self-assembly (PTX-SA) formulation. Polymer/PTX-SAs were screened based on smaller size of formulation using dynamic light scattering analysis. Additionally, fluorescence microscopy and flow cytometry studies exhibited that polyvinylpyrrolidone (PVP)-based PTX-SAs (PVP/PTX-SAs) had superior cellular internalization capability in MCF7 and MDA-MB-231 breast cancer cells. The optimized PVP/PTX-SAs exhibited less toxicity to human red blood cells indicating a suitable formulation for reducing systemic toxicity. The formation of PVP and PTX self-assemblies was confirmed using fluorescence quenching and transmission electron microscopy which indicated that the PVP/PTX-SAs were spherical in shape with an average size range of 53.81 nm as detected by transmission electron microscopy (TEM). FTIR spectral analysis demonstrates incorporation of polymer and paclitaxel functional groups in PVP/PTX-SAs. Both proliferation (MTS) and clonogenic (colony formation) assays were used to validate superior anticancer activity of PVP/PTX-SAs in breast cancer cells over paclitaxel. Such superior anticancer activity was also demonstrated by downregulation of the expression of pro-survival protein (Bcl-xL), upregulation of apoptosis-associated proteins (Bid, Bax, cleaved caspase 7, and cleaved PARP) and $\beta$-tubulin stabilization. These results support the hypothesis that PVP/PTX-SAs improved paclitaxel delivery to cancer cells.



*Corresponding author. Tel.: +901 448 1536; fax: +1 901 448 4731.
E-mail address: myallapu@uthsc.edu (Murali M. Yallapu).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

https://doi.org/10.1016/j.apsb.2017.10.004



Please cite this article as: Chowdhury Pallabita, et al. Development of polyvinylpyrrolidone/paclitaxel self-assemblies for breast cancer. *Acta Pharmaceutica Sinica B* (2017), https://doi.org/10.1016/j.apsb.2017.10.004

## 1. Introduction

Breast cancer (BC) remains the most commonly diagnosed cancer among women in the United States[1]. The most recent data on cancer incidence, mortality, and survival by the American Cancer Society estimated that there will be 63,410 *in situ* cases and 252,710 invasive cases with 40,610 deaths occurring in the US in 2017[1]. Paclitaxel (PTX), is a natural compound derived from the bark of *Taxus Brevifolia*, antimicrotubule and well-established chemotherapeutic agent, which exhibits a broad spectrum of anticancer activity against breast cancer, prostate cancer, leukemia, non-small cell lung cancer, and ovarian cancer[2,3]. Due to its hydrophobicity, it is often formulated with Cremophor EL in ethanol solution, *i.e.*, Taxol®, which limits clinical use due to its adverse side effects, such as increased risk for fatal hypersensitivity reactions[4], non-linear pharmacokinetics[5], prolonged peripheral neuropathy[6], and myelosuppression[7], leading to less bioavailability at the tumor site and therefore reduced efficacy for its action against tumor cells[6,8,9]. Other common side effects of PTX treatment include vomiting, nausea, loss of appetite, and joint pain.

In order to circumvent these side effects, various alternative nanoparticle-based PTX formulations were developed. One of the most widely used formulations is Abraxane®, which is a Cremophor-free and albumin-bound paclitaxel nanoparticle formulation (diameter ~120 nm), developed as an alternative formulation to PTX (Taxol®)[2]. This serum albumin bound PTX nanoformulation is approved by the US Food and Drug Administration for the treatment of breast, lung and pancreatic cancer. This formulation facilitates crossing across endothelial layers of cells and achieves 33% higher PTX concentration at the tumor site[10]. Additionally, Abraxane® was able to increase the maximum tolerated dose (MTD) by 70–80% compared to PTX. Another PTX nanoformulation, Genexol PM® was approved in South Korea for first line treatment for metastatic or recurrent breast cancer, non-small cell lung cancer, and also used in combination with carboplatin for ovarian cancer. It is a polymeric micellar formulation of polyethylene glycol (PEG), poly(D,L-lactide), and PTX. NK-105 (block copolymer of PEG and polyaspartate modified with 4-phenyl-1-butanol with PTX) is another nanoparticular micellar formulation that has entered phase III clinical trial for metastatic and recurrent breast cancer. NK-105 exhibits prolonged circulation, enhanced area under the curve (AUC) by 20 times in contrast to PTX and also had higher antitumor activity[11]. Other clinically used (or under clinical development) PTX formulations/nanoformulations include Paclical® (paclitaxel combined with Oasmia's excipient technology XR17)[12], Lipusu® (paclitaxel liposome)[13], paclitaxel injection concentrate for nanodispersion (PICN)[14], SB05 (positively charged liposome embedded with paclitaxel)[15], LEP-ETU (liposome-entrapped paclitaxel)[16], and Triolimus (micelle containing paclitaxel, rapamycin and 17-AAG)[17]. Self-assembly and solid dispersion techniques were a common approach to generate these clinically relevant PTX nanoformulations.

The goal of this study was to develop a PTX nanoformulation using a polymer-based self-assembly technique, using a polymer excipient already in use in the pharmaceutical industry. The self-assembly process is a well-established, simple, and rapid fabrication method to generate nanosized architecture materials for drug delivery applications[18–20]. We selected 8 biocompatible polymers for generating self-assembled polymer/PTX nanoformulations. Table 1 provides structures of polymers used in this study. Among those, Poloxamer 188 [a nonionic copolymer containing hydrophobic poly (propylene oxide) (PPO) and two hydrophilic poly(ethylene oxide) (PEO) units] is widely used for generating micelles by the self-assembling technique where the PEO units align at the outer lining and PPO falls in the inner core leading to the formation of micelle above the critical micellar concentration. We expect polymeric micelles will be formed using Poloxamer 188[21]. Whereas, the other seven polymers were commonly used as drug delivery carriers for generating solid dispersions or polymeric nanoparticles[22–24]. The motive of this study is to minimize any other external agents being added for the formulation development. Self-assembly is a process where the various components are held together by inter-particulate assembly[25]. There are reports stating that synergistic interactions between self-organizing particles and a self-assembling matrix material can lead to hierarchically ordered structures[26]. Accordingly, we developed nanostructured formulations of hierarchical order using PTX and various polymers without any external reagents or binders. Such self-assembly formulations may have the potential to circumvent the shortcomings of PTX and provide advantages such as: ease of preparation; smaller particle size; an efficient binding ability to PTX; and enhanced particle uptake in cancer cells. In this investigation, we report an optimized PVP/PTX-SA formulation with enhanced cellular uptake and superior *in vitro* cytotoxicity in breast cancer cells, compared to PTX.

## 2. Materials and methods

### 2.1. Materials

All laboratory reagents, solvents, and chemicals were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) or Fisher Scientific (Pittsburgh, PA, USA) unless otherwise mentioned. Cell culture plastics were purchased from Sarstedt, Inc. (Newton, NC, USA). All chemicals were used as received without any further purification.

### 2.2. Cell culture, growth conditions and treatment

Breast cancer cell lines (MCF7 and MDA-MB-231) were obtained through American Type Culture Collection (Manassas, VA, USA) and stored as low passage frozen aliquots upon cell culture expansion. These cell lines were thawed and cultured under sterile conditions for all experiments (<3–4 months after thawing). Both cell lines were cultured in Dulbecco's modified Eagle's medium-high glucose (DMEM-Hi) medium containing 4.5 g/L of glucose, 10 nmol/L of nonessential amino acids, 100 nmol/L of sodium pyruvate, 1× antibiotic/antimycotic (Gibco, Thermo Fisher Scientific, Grand Island, NY, USA) and 10% heat-inactivated fetal bovine serum (FBS, Atlanta Biologicals, Lawrenceville, GA, USA) at 37 °C in a humidified atmosphere (5% $CO_2$ and 95% air condition, ThermoScientific, Waltham, USA). In all cell culture experiments, monodispersed cell lines after trypsinization were plated on either 6-, 12-, or 96-well plates, and were allowed to adhere overnight to the plate before implementing treatments.

### 2.3. Preparation of polymer-PTX-SAs (Poly/PTX-SAs)

Eight polymer-based drug delivery vehicles, such as polyvinyl alcohol (PVA, MW 31,000–50,000), polyvinylpyrrolidone (PVP, MW 40,000), polyethyleneimine (PEI, MW 25,000), poly(methyl vinyl ether-alt-maleic hydrochloride) (PMEAVH, MW 216,000),

Please cite this article as: Chowdhury Pallabita, et al. Development of polyvinylpyrrolidone/paclitaxel self-assemblies for breast cancer. *Acta Pharmaceutica Sinica B* (2017), https://doi.org/10.1016/j.apsb.2017.10.004