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Design and characterization of paclitaxel-verapamil co-encapsulated PLGA nanoparticles: Potential system for overcoming P-glycoprotein mediated MDR

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ABSTRACT

Paclitaxel (PTX) is one of the most effective drugs for treating a variety of solid tumors. Due to its poor aqueous solubility, Cremophor EL is used as solvent for its marketed formulation which may cause severe side effects in patients. Another problem in PTX delivery is over expression of P-glycoprotein which contributes to the development of multidrug resistance in cancer cells. Considering Verapamil (VER) calcium channels and P-gp pumps inhibiting effects, it seems co-administration of PTX and VER may increase accumulation of PTX inside cancer cells. Polymeric nanoparticles seem to be one of the best carriers for PTX in order to eliminate Cremophor EL in addition to co-encapsulating ability. The aim of this study is to develop and optimize PLGA nanoparticles for co-encapsulation of PTX and VER.

Coencapsulated PLGA nanoparticles were evaluated for particles size, zeta potential (ZP), drug loading %, encapsulation efficiency% and in vitro release of drugs. Cell cytotoxicity studies were also performed on MCF-7 cell line. PLGA NPs with 470 nm diameter with acceptable PTX and VER loading and release% presented higher cytotoxicity in some concentrations compared with free-PTX after 72 h. Results suggesting PLGA NPs may potentially be useful drug carrier for co-encapsulation of PTX and VER.

1. Introduction

Cancer is a group of diseases involving abnormal cell growth with the potential to metastasize to other parts of the body [1]. Unfortunately, cancers are capable of developing resistance to traditional therapies, and the increasing prevalence of these drug resistant cancers entails further research and development for treatment [2]. The mechanisms of resistance to drugs include epigenetic changes, over expression of drug efflux pumps such as P-glycoprotein (P-gp), drug inactivation, drug target alteration, DNA damage repair, cell death inhibition, and Epithelial-Mesenchymal Transition (EMT). These mechanisms are the most common alterations that can enable or promote direct or indirect drug resistance in human cancer cells [3,4].

A single mechanism or combination of such mechanisms in cancer cells can cause resistance to one or more chemotherapeutics. This is known as multidrug resistance (MDR), which decreases sensitivity to cancer drugs and hinders chemotherapy efficacy [5,6]. Currently, nanoassemblies such as nanoparticles (NPs), quantum dots, dendrimers, liposomes, and micelles have emerged as promising platforms for treatment of drug resistant cancer cells. Nanocarriers have the potential to improve drugs' therapeutic index, divert drug efflux mechanisms, and selectively target tumor cells or cancer microenvironments [7,8].

Nanoparticle platforms have the potential to improve the treatment of cancer, especially their capability of delivering combination chemotherapeutics, which in turn can significantly improve therapeutic efficacy [9].

Polymeric NPs are versatile platforms for controlled and targeted delivery of anticancer drugs and macromolecules such as genes and proteins [10]. Poly lactic-*co*-glycolic acid (PLGA) is one of the most successful polymers used in nanosystems for drug delivery and biomaterial applications because it is degradable in the body and produces the nontoxic metabolite monomers lactic acid and glycolic acid [11,12]. PLGA-based NPs have adaptable

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characteristics for loading various types of drugs (e.g. hydrophilic or hydrophobic small molecules or macromolecules). These NPs protect drugs from degradation, sustain drug release, provide better interaction with biological materials, and possibly target drugs to specific organs or cells. These are some attractive properties of PLGA-based NPs that can provide better drug delivery systems in various biomedical applications such as vaccination, cancer, inflammation and other diseases. In addition, the Food and Drug Administration (FDA) and European Medicine Agency approved PLGA to be used in therapeutic devices for drug delivery in humans [13–15]. Previous studies have been successfully formulated several chemotherapeutics using PLGA-based NPs. These include but are not limited to PTX, doxorubicin, 5-fluorouracil and dexamethasone [16].

Paclitaxel (PTX) is an antineoplastic agent with unique mechanism of action used in the treatment of a wide range of tumors including lung, breast, ovarian, prostate, and pancreatic cancers [17]. It disrupts the dynamic equilibrium within the microtubules that are involved in mitosis, cell shape, cell motility, and transport within the cell, thereby inducing cell death [18,19]. Due to a low therapeutic index and the poor aqueous solubility of 1 μg/ml, PTX is formulated in a 1:1 combination of the solubilizing agent Cremophor EL (polyethoxylated castor oil) and dehydrated ethanol for I.V. injection [20,21] However, clinical applications of this combination often causes a variety of side effects such as allergic reactions, neurotoxicity, and nephrotoxicity resulting from the Cremophor EL as well as precipitation of PTX after dilution in the infusion solution. PTX is a substrate of P-gp which contributes to MDR in various types of cancer cells. Therefore, PTX-MDR is a remarkable problem in clinical treatment with PTX [17]. Overexpression of P-gp is often associated with adverse prognosis leading to decline in the therapeutic efficacy of chemotherapy in cancer patients. In many cancer types almost half of the patients were diagnosed with cancer overexpressing P-gp up to 100-fold in the malignant tissue [22]. Different inhibitors of P-gp have been utilized in the treatment of different cancers to overcome this major obstacle [23,24]. Verapamil (VER) is a potent vasodilator that blocks calcium channels and is considered to be a P-gp inhibitor that can reverse P-gp-based MDR. Hence, co-administration of a P-gp inhibitor VER along with an anticancer drug PTX can be a practical attempt to conquer MDR in cancer patients [25,26]. The usual reported resistance modulator verapamil dose is higher (5 times) than its antiarrhythmic dose (0.4–1.2 μM). Previous reports indicating a synergic effect for inhibiting resistant Hela cell line in after administration of free verapamil with doxorubicin loaded liposomes [27]. Another study reported higher MDR-3T3 cell cytotoxicity induction with a halved IC50 when free verapamil added to PTX loaded liposomes [28]. There is also a report of PTX-VER co-encapsulated SLN with higher cellular uptake and cell cytotoxicity in MCF-7/ADR cell lines [17].

PLGA (poly-lactid-*co*-glycolide acid) is commonly used as nontoxic biodegradable and biocompatible drug delivery carrier [29]. Considering PLGA suitable properties such as higher lipophilic drug entrapment efficiency and controllable release manner in drug delivery, we hypothesized that co-encapsulation of PTX with VER for reversal of MDR seems to be effective. To our knowledge this is the first study to deliver PTX and VER concurrently in a single drug delivery system based on PLGA nanoparticles. So the main objective of this study was to develop and characterize PLGA-based nanoparticles for co-delivery of PTX and VER.

2. Materials and methods

2.1. Materials

Paclitaxel was purchased from Samyang Biopharm (Korea). Verapamil was purchased from Sobhan Darou (Iran). PLGA (50:50) was purchased from Hangzhou starshine Pharmaceutical (China). Polyvinyl alcohol was purchased from Fluka (Germany). Acetonitrile (ACN) and dichloromethane (DCM) were purchased from Samchun (Korea). All other chemicals and reagents including Acetic acid, Mannitol, Methanol (MeOH), Potassium phosphate dibasic, and Sodium Hydroxide were purchased from Merck (Germany).

2.2. The concurrent analysis of PTX and VER

PTX and VER concentrations were simultaneously measured by HPLC system (CECIL, Adopt CE 4200, UK). A C18 column (250 mm × 4.6 mm × 5 mm) was used at room temperature. A mixture of ACN and water (60:40, v/v) adjusted at pH 3 was used as mobile phase at a flow rate of 1.5 ml/min. The detection wavelength was set at 227 nm.

2.3. PTX and VER standard curve validation

A primary stock solution was used to make standard solutions with different concentrations. Specifically, PTX and VER were dissolved in acetonitrile and methanol, respectively. Different PTX (12.5, 25, 50, 100 and 200 μg/ml) and VER (1.25, 2.5, 5, 10 and 20 μg/ml) concentrations were obtained by serial dilution. To attain PTX and VER standard curves, equal volumes of the two solutions were mixed and each concentration was injected to the HPLC. The area under the curve (AUC) for each peak was analyzed and the AUC was plotted as a function of the solution concentration. Samples ($n = 5$) were tested three times a day (Intraday) for three days (Interday). Calibration curve was validated for specificity, linearity, precision and accuracy.

2.4. Preparation of PTX-VER loaded PLGA NPs

A modified emulsification/solvent evaporation technique was used to encapsulate the drugs within the NPs [30]. Eight different formulations were prepared applying 2-level full factorial experimental design using Design expert 10® software (Table 1). According to Table 2, known amounts of PLGA and PTX were added into DCM to prepare an organic phase. Then, a known amount of solution of VER was added drop wise to the organic phase and sonicated simultaneously for 1 min. Then the formed emulsion was gently added into PVA aqueous solution. This emulsion was gently stirred at room temperature for several hours to evaporate the organic solvent. The resulting suspension was collected by centrifuge (15,000 rpm, 30 min) and was freeze-dried in mannitol solution 2%. Eight different formulations (F1–F8) were constructed (Table 2) to determine suitable parameters for developing an optimized PLGA-based PTX-VER NPs.

2.5. PTX-VER loaded PLGA NPs characterization

2.5.1. Particle size and zeta potential

The particle size and zeta potential of the PTX-VER loaded NPs were measured using particle size analyzer (NANO-flex®, USA).

Table 1
Factors, factor levels and responses used in 2-level full factorial experimental design.

Factors	Type of factors	Factors level		Response	
X1	PLGA%	2	5	Y1	Particle Size (nm)
X2	PVA%	0.5	1	Y2	Zeta potential
X3	Drug amount (mg)	2	5	Y3	Drug loading%
				Y4	Encapsulation efficiency%