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Controlled release and reversal of multidrug resistance by co-encapsulation of paclitaxel and verapamil in solid lipid nanoparticles

Jong-Suep Baek, Cheong-Weon Cho *

College of Pharmacy and Institute of Drug Research and Development, Chungnam National University, Daejeon 305-764, South Korea

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ABSTRACT

Paclitaxel (PTX) has been used in the treatment of wide range of cancers but its entry into cancer cell is restricted by p-glycoprotein (p-gp). Also, it was reported that verapamil (VP) could inhibit p-gp efflux. Hence, three kinds of solid lipid nanoparticles (SLN) such as PVS (PTX and VP co-loaded SLN), PSV (PTX loaded SLN, later added VP) and PVSV (PTX and VP co-loaded SLN, later added VP) were prepared to overcome MDR by combination of PTX and VP. PVS was the SLN loaded with both PTX and VP at the same time. PSV was the SLN loaded with PTX and then modified with VP – complexed hydroxypropyl-β-cylcodextrin (HPCD). Finally, PVSV was the SLN loaded with PTX and half of VP at the same time subsequently, modified with half of VP - complexed HPCD. The physicochemical characterizations of PVS, PSV or PVSV such as particle size, zeta potential, encapsulation efficiency or in vitro PTX release were examined. PVSV showed that release of VP was higher than PTX solution in first 15 h and sustained release of both VP and PTX. PVSV showed significantly higher cytotoxicity and cellular uptake than that of the PTX solution in MCF-7/ADR resistant cells. Furthermore, PVSV significantly down regulated the expression of p-gp than the PTX solution in MCF-7/ADR resistant cells. Based on these findings, this study indicated that the PVSV exhibited great potential for breast cancer therapy

1. Introduction

Paclitaxel (PTX) is an anticancer agent used in the treatment of a wide range of tumors including breast, lung, prostate, ovarian and pancreatic cancers (Shenoy et al., 2009). However, because of low therapeutic index and the poor aqueous solubility of 1 μg/ml of PTX, the available commercial formulation of PTX is Taxol®, a concentrated solution containing 6 mg/ml of PTX in cremophor EL (polyoxyl 35 castor oil) and dehydrated alcohol (1:1, v/v), which is diluted 5–20-fold in normal saline or dextrose solution before administration (Kim et al., 2001). Also, Abraxane®, which is an albumin-bound form and the first solvent-free nanoparticle of PTX with a mean particle size of about 130 nm, was commercialized. Still, serious side effects of PTX such as hypersensitivity, neurotoxicity, nephrotoxicity and vasodilatation attributable to intravenous administration of the cremophor EL-based formulation have been reported (Weiss et al., 1990). For this reason, the extensive clinical application of PTX is extremely limited (Singla et al., 2002) and there is a need for the development of alternate formulation of PTX having better aqueous solubility and reducing side effects. Accordingly, a number of alternative formulations were investigated, which included nanoparticles, liposome, microspheres, PTX–polymer conjugates, dendritic polymers, implants or water-soluble prodrugs (Singla et al., 2002; Lee et al., 2003).

Another major problem in the clinical treatment of cancer with PTX is multidrug resistance (MDR), which is a frequent phenomenon whereby cancer cells become resistant to the cytotoxic effects of various structurally and mechanistically unrelated chemotherapeutic agents (Ho et al., 2007). Once the MDR occurred, the intracellular drug accumulation was reduced and the sensitivity of tumor cells to drugs was significantly decreased. Therefore, development of MDR is a major obstacle to the success of cancer chemotherapy. In past years, various mechanisms of MDR have been proposed, including the overexpression of multidrug efflux pumps like p-glycoprotein (p-gp) (Krishnamachary and Center, 1993), reduced in topoisomerase activity (Deffie et al., 1989), modifications in glutathione metabolism (Zhang et al., 1998) or altered expression of apoptosis-associated protein Bcl-2 (Kirkin et al., 2004) and tumor suppressor protein p53 (Viktorsson et al., 2005). Among these mechanisms, overexpression of p-gp encoded by the MDR1 is the most commonly encountered in successful cancer therapy with PTX (Mechetner et al., 1998). P-gp, a drug

* Corresponding author. Tel.: +82 42 821 5934; fax: +82 42 823 6566.
E-mail address: chocw@cnu.ac.kr (C.-W. Cho).

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efflux transporter, can result in an increased efflux of the cytotoxic drugs from the cancer cells, thus lowering their intracellular concentrations. In many cancer types, nearly 40–50% of the patients was diagnosed with cancer overexpressing p-gp up to 100-fold in the malignant tissue (Rowinsky and Donehower, 1995; Thomas and Coley, 2003). Even though PTX is the drug of choice for the treatment of breast cancer, its use in breast cancer is limited due to the restricted uptake of PTX by p-gp.

On the other hand, verapamil (VP), a first generation p-gp inhibitor, has been reported to be able to reverse completely the resistance caused by p-gp in vitro (Huang et al., 1999). It was reported that PTX is more effective in inhibiting the growth of cancer cells or enhancing PTX concentration in blood when it is co-administered with VP (Choi et al., 2005; Wang et al., 2011).

Solid lipid nanoparticles (SLN) are well tolerated in living systems since they are constructed from physiological compounds and easily metabolized. In addition, SLN made from biodegradable solid lipids exist in the submicron size range have attracted increasing attention in recent years. The advantages of SLN are as follows: possibility of controlled drug release and drug targeting, protection of incorporated compound against chemical degradation, no biotoxicity of the carrier and no problems with respect to large scale production (Mehnert and Mader, 2001; Muller et al., 2002). Hydroxypropyl-β-cyclodextrin (HPCD) is more water-soluble than the parent molecule, β-cyclodextrin and has hydroxypropyl-ester groups attached to the hydroxyl groups in position 2 (Patel and Purohit, 2008). In addition, HPCD has known to form inclusion complex with many compounds, which prevents the oxidation of oils and involatile flavors and solubilizes insoluble compounds (Hamada et al., 2006). Our group had published the possibility of modifying SLNs of PTX with HPCD to get higher aqueous solubility (Baek et al., 2012) and cellular uptake of PTX (Baek and Cho, 2013).

Therefore, the aim of this study is to co-encapsulate PTX and VP in SLNs using HPCD to achieve higher cellular uptake by inhibiting p-gp and to evaluate MDR effect of PTX with the combination of VP in SLN.

2. Materials and methods

2.1. Materials

PTX was provided by Samyang Genex (Daejeon, Korea). VP and HPCD were purchased from Sigma–Aldrich (Steinheim, Switzerland). Stearic acid was purchased from Daejung Chemical (Cheongwon, Korea). Poloxamer 188 was obtained from BASF (Ludwigshafen, Germany) and soy lecithin was purchased from Junsei (Tokyo, Japan). All other chemicals and reagents used were of analytical grade.

2.2. Preparation of three different types of SLN

Three different types of SLN were prepared using modified hot sonication method (Table 1). In case of co-loading of PTX and VP in SLN, called as PVS, stearic acid (100 mg) was melted at 80 °C in a water bath. PTX (5 mg) and VP (5 or 10 mg) were dissolved in 0.5 ml of ethanol and then injected into melted stearic acid under sonication. Both lecithin (75 mg) and poloxamer 188 (75 mg) were dispensed into 3 ml of distilled water and sonicated for 10 min at 80 °C in a water bath. They were added into above melted solution under sonication. The solution was then dispersed in 2 ml of distilled water at 4 °C and sonicated for 10 min.

On the other hand, in case of PSV, PTX was loaded for SLN, subsequently VP-complexed HPCD was added. Briefly, stearic acid (100 mg) was melted at 80 °C in a water bath. PTX (5 mg) was dissolved in 0.5 ml of ethanol and then injected into melted stearic acid under sonication. Both lecithin (75 mg) and poloxamer 188 (75 mg) were dispensed into 3 ml of distilled water and sonicated for 10 min at 80 °C in a water bath. They were added into above melted solution under sonication. The solution was then dispersed in 2 ml of distilled water at 4 °C and sonicated for 10 min. And then, HPCD (200 or 400 mg) complexed with VP (5 or 10 mg) was added under shaking for 30 min.

Next, PVSV was prepared by modifying the preparation of PVS and PSV. Briefly, stearic acid (100 mg) was melted at 80 °C in a water bath. Both PTX (5 mg) and VP (2.5 mg or 5 mg) were dissolved in 0.5 ml of ethanol and then injected into melted stearic acid under sonication. Both lecithin (75 mg) and poloxamer 188 (75 mg) were dispensed into 3 ml of distilled water and sonicated for 10 min at 80 °C in a water bath. They were added into above melted solution under sonication. The solution was then dispersed in 2 ml of distilled water at 4 °C and sonicated for 10 min. And then, HPCD (400 mg) complexed with VP (2.5 mg or 5 mg) was added under shaking for 30 min.

As a control, PTX solution was prepared as commercial Taxol® formulation for the study of in vitro release study, in vitro cytotoxicity study, cellular uptake study and western blotting assay.

Table 1
Composition of three different types of SLN.

Formulation unit (mg)	PVS1	PVS2	PSV1	PSV2	PVSV1	PVSV2
PTX	5	5	5	5	5	5
VP	5	10	5	10	5	10
Stearic acid	100	100	100	100	100	100
Poloxamer 188	75	75	75	75	75	75
Lecithin	75	75	75	75	75	75
HPCD	–	–	400	400	400	400

2.3. Analysis of drug loading and encapsulation efficiency of SLN

The SLN solution (0.5 ml) were solubilized with 1 ml of ethanol, heated at 80 °C for 30 min and then cooled down at −20 °C for 30 min. This solution was centrifuged at 3000 rpm for 5 min to precipitate the undissolved solid stearic acid, filtered through a 0.2 μm filter and injected into the HPLC system. An Agilent 1100 liquid chromatography system with an autosampler and UV detector were used. The column used was a C_{18} column (4.0 × 250 mm, 5 μm particle size, Supelco™; MetaChem, USA). The flow rate of the mobile phase was 1 ml/min and the detection wavelength of PTX or VP was set to 227 nm or 283 nm, respectively. The mobile phase of PTX or VP was a mixture of water and acetonitrile (60:40, v/v) or water and acetonitrile (30:70, v/v) adjusted pH 2.5 by formic acid, respectively. Drug loading and encapsulation efficiency (EE) were calculated as follows (Trickler et al., 2008).

$$\text{Drug loading}(\%) = \frac{\text{weight of the drug in particles}}{\text{weight of the particles}} \times 100$$

$$\text{EE}(\%) = \frac{\text{weight of the drug in particles}}{\text{weight of the feeding drugs}} \times 100$$

2.4. Measurements of particle size and polydispersity

The particle size and zeta potential analysis of three different types of SLNs including PVS, PSV or PVSV were performed by laser scattering analyzer (ELS-8000, Otasuka Electronics, Osaka, Japan). The lyophilized SLN was dispersed in water, added to the sample dispersion unit and sonicated in order to minimize the inter-particle interactions. The obscuration range was maintained between 20 and 50%. The instrument was set to measure the