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A TPGS-incorporating nanoemulsion of paclitaxel circumvents drug resistance in breast cancer

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ABSTRACT

Paclitaxel resistance is usually developed in clinical chemotherapy, which remains a major obstacle for successful cancer treatment. Herein, we attempted to develop a TPGS incorporating nanoemulsion of paclitaxel (NE-PTX) to circumvent the drug resistance in breast cancer. NE-PTX was prepared by a self-assembly technique and the physicochemical properties were characterized. The efficacy of NE-PTX on overcoming paclitaxel resistance was measured by in vitro and in vivo evaluation. The measured results indicated that NE-PTX was nanometer-sized droplets with the mean diameter of 24.93 ± 3.45 nm. The IC_{50} value of paclitaxel in resistant MCF-7/ADR cells was greatly reduced from 101.45 μg/mL to 5.39 μg/mL, which indicated that the paclitaxel resistance was effectively reduced by NE-PTX. The reversal of paclitaxel resistance could mainly ascribe to the significant inhibition of P-gp activity and enhancement of anti-cancer activity. Moreover, the tumor volume in resistant tumor xenograft model treated with NE-PTX was only 10.06% of that of paclitaxel solution group, and the tumor inhibitory rate of NE-PTX reached 93.84%, which effectively verified the efficacy of NE-PTX on treating paclitaxel resistance. Thereby, NE-PTX could provide an effective strategy for circumventing paclitaxel resistance in breast cancer.

1. Introduction

Paclitaxel (PTX), one of the most effective chemotherapeutic agents, presents great potency in treatment of a broad spectrum of solid tumors, such as breast cancer, refractory ovarian cancer and non-small cell lung carcinoma (Goldspiel, 1997; Howat et al., 2014; Megerdichian et al., 2014). However, multidrug resistance is usually developed in clinical trials using PTX alone or in combination with other antineoplastic agents, which presents one of the major obstacles for cancer chemotherapy (Murray et al., 2012; Szakacs et al., 2006). The PTX resistance can result from a variety of mechanisms such as overexpression of P-glycoprotein (P-gp), alteration of microtubule regulatory proteins, deregulation of apoptotic signaling pathway, mutation of tubulins and over-expression of copper transporters (Le and Bast, 2011; Murray et al., 2012; Yusuf et al., 2003). Even worse, PTX resistance occurred in more than 70% of patients at the time of initial diagnosis and almost in all patients upon relapse (Yusuf et al., 2003). Therefore, there is a great necessity to find a new approach to circumvent PTX resistance for clinical chemotherapy.

Nano-based drug delivery systems have demonstrated great potential to bypass the resistance mechanisms that have evolved in conventional chemotherapy (Ji et al., 2012; Patel et al., 2013). Therein, nanoemulsions are usually oil in water droplets with the mean droplet size ranging from 20 nm to 200 nm (Abouzeid et al., 2014; Ganta and Amiji, 2009; Gao et al., 2011; Talekar et al., 2012). Nanoemulsions are usually formulated with a variety of pharmaceutical acceptable excipients, such as oils, surfactants and functional materials, which can offer a promising vehicle for enhancing the aqueous solubility and oral bioavailability of hydrophobic drugs, targeted drug delivery, diagnosis, and cancer therapy (Choudhury et al., 2014; Ganta and Amiji, 2009; Gianella et al., 2011; Ragelle et al., 2012; Zhang et al., 2013). However, to date, few reports of nanoemulsion based drug delivery system have been mentioned for circumventing multidrug resistance in chemotherapy.

Tocopheryl polyethylene glycol 1000 succinate (TPGS), a derivative of vitamin E succinate, has attracted increasing attention for treatment of drug resistance in chemotherapy (Guo et al., 2014; Kutty and Feng, 2013; Saadati and Dadashzadeh, 2014; Tan et al., 2014; Wang et al., 2013; Zhang et al., 2014). Herein, we developed a self-assembled TPGS incorporating nanoemulsion of PTX (NE-PTX) to overcome the drug resistance

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in breast cancer. The in vitro efficacy of NE-PTX on reversal of PTX resistance was firstly evaluated in sensitive MCF-7 and resistant MCF-7/ADR cells. Then, the possible mechanism of NE-PTX on reversal of PTX resistance was elucidated. Moreover, the in vivo behavior of NE-PTX and its anti-cancer activity in resistant MCF-7/ADR cell induced tumor xenograft were investigated to evaluate the effectiveness on circumventing PTX resistance in chemotherapy of breast cancer.

2. Experimental

2.1. Materials

Paclitaxel (PTX, >99%) was purchased from Shanghai Sunve Pharmaceutical Co., Ltd. (Shanghai, China). D-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS), Tween 80, coumarin-6 and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were supplied by Sigma–Aldrich (St. Louis, USA). Nile red and rhodamine 123 was obtained from J&K scientific Co., Ltd. (Shanghai, China). Medium chain triglyceride (MCT) was purchased from Tieling Beiya Pharmaceutical Oil Co., Ltd. (Liaoning, China). All other chemicals and solvents were of analytical or high performance liquid chromatography (HPLC) grade.

2.2. Cell culture and animals

Human breast cancer cell line (MCF-7) was obtained from Shanghai Cell Resource Center of Shanghai Institute for Biological Sciences, Chinese Academy of Sciences (CAS). Doxorubicin-resistant derivative cell line (MCF-7/ADR) was supplied by KeyGEN Biotech (Nanjing, China). Cells were grown in RPMI 1640 culture media (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Biochrom, Germany), 100 units/mL penicillin G sodium and 100 μg/mL streptomycin sulfate. Cells were kept at 37 °C in a humidified and 5% CO_2 incubator. To maintain the resistant phenotype of MCF-7/ADR cells, cells were incubated in RPMI 1640 culture media with 1.0 μg/mL of doxorubicin.

BALB/c nude mice (18–22 g, ♀) were provided by Shanghai Experimental Animal Center, CAS. Animals were kept under the animal care facility and acclimatized for 5 days prior to the experiment. The experiments were performed according to the protocols approved by the Institutional Animal Care and Use Committee of Shanghai Institute of Materia Medica, CAS.

2.3. Preparation and characterization of NE-PTX

NE-PTX was prepared by a self-assembly technique as previously described method (Zhang et al., 2013). Briefly, PTX, TPGS, MCT and Tween 80 (1:20:25:40, w/w) were dissolved in anhydrous alcohol and evaporated to dryness under reduced pressure (Heidolph Laborata 4000, Germany) to form a thin film. Then, the film was dispersed into double-distilled water at 37 °C with gentle shaking to form NE-PTX spontaneously. As control, blank self-assembled nanoemulsion (blank SN) composed with equivalent TPGS, MCT and Tween 80 was prepared in the same procedure.

The morphology of NE-PTX was measured under a transmission electron microscope (TEM, JEM-1230, Japan) after negative staining with 1% (w/v) uranyl acetate. Meanwhile, the size distribution of NE-PTX was determined by dynamic light scattering (DLS) on an instrument of Nano ZS 90 (Malvern, UK).

To determine the entrapment efficiency (EE) and drug loading (DL) of PTX in NE-PTX, free PTX was separated from NE-PTX by ultrafiltration method (10,000 Da, Millipore) with centrifugation at 3000 × *g* for 5 min. The drug amount in the filtrate and NE-PTX was quantified by HPLC analysis. The measurement was performed on HPLC system (Agilent 1100, USA) with the Sunfire™ C_{18} column (250 mm × 4.6 mm, I.D. 5 μm, Waters, USA); column temperature, 30 °C; mobile phase, acetonitrile:water (60:40, v/v); flow rate, 1.0 mL/min; detection wavelength, 227 nm. The EE and DL were calculated as the following:

$$EE = \frac{(W_t - W_f)}{W_t} \times 100\%$$

$$DL = \frac{(W_t - W_f)}{W_c} \times 100\%$$

therein, W_t referred to the total amount of PTX in NE-PTX, W_f presented as the amount of free PTX, and W_c was that of total ingredients in NE-PTX.

The in vitro release profiles of PTX from NE-PTX were measured at 37 °C in phosphate buffered solution (PBS) at different pH values (5.0, 6.0, 7.4 and 7.4 with 10% serum). Briefly, 0.5 mL of freshly prepared NE-PTX was sealed into dialysis bags with the molecular weight cut-off of 7000 Da, immersed into 20.0 mL of various release media and shaken at 100 rpm (HZ-9611k thermostatic oscillator, Hualida, China). The release media was replaced with fresh pre-warmed solution at predetermined time intervals. Samples were collected for quantification by the aforementioned HPLC analysis.

2.4. In vitro evaluation of NE-PTX on reversal of PTX resistance

The in vitro cytotoxicity of NE-PTX was measured in MCF-7 and MCF-7/ADR cells. Briefly, cells were seeded into 96-well plate at 8×10^3 cells/well and cultured overnight. NE-PTX or PTX solution (DMSO solution) was added to each well at a certain concentration and incubated for further 48 h. Afterwards, the cytotoxicity of NE-PTX was measured by MTT assay (Bio-Rad model 550, USA). The cell viability was defined as the ratio between the absorbance value of samples and that of negative control. The half-maximal inhibitory concentration (IC_{50}) of each group was calculated.

2.5. Cell cycle analysis

MCF-7/ADR cells were seeded into 6-well plates at 3×10^5 cells/well and cultured overnight prior to the analysis. NE-PTX or PTX solution was added to each well at 2.0 μg/mL of PTX and incubated for further 48 h. By contrast, blank SN was incubated with cells at an equivalent concentration of NE-PTX. Then, cells were collected and stained with 10 μg/mL of propidium iodide (PI, Sigma) for the measurement. Thereafter, cells were analyzed by the FACSCalibur flow cytometry system (BD, USA). The cell percentage in each phase of cell cycles was calculated by the ModFit software (Verity Software House, ME).

2.6. P-gp expression in MCF-7/ADR cells

MCF-7/ADR cells were seeded into 12-well plates at 5×10^4 cells/well and cultured for 24 h for the attachment. NE-PTX, PTX solution (2.0 μg/mL of PTX) or equivalent blank SN was added to each well and incubated for further 24 h. Then, cells were collected, rinsed and resuspended in PBS (pH 7.4). The P-gp was labeled with Phycoerythrin (PE)-anti-human MDR1 (CD243, P-gp, ABCB1, eBioscience, USA) according to the manufacturer's protocols. As control, the nonspecific labeling was corrected by its isotype control (PE-Mouse IgG2a, eBioscience, USA). The fluorescent intensity was determined by FACSCalibur system (BD, USA) and analyzed with the CellQuest software. By contrast, the P-gp expression in sensitive MCF-7 cells and resistant MCF-7/ADR cells was performed as control.