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Drug resistance reversal activity of anticancer drug loaded solid lipid nanoparticles in multi-drug resistant cancer cells

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ABSTRACT

The aim of our study was to enhance the cytotoxicity of anticancer drugs by reversing the resistance of multi-drug resistant cancer cells. The cytotoxicities of paclitaxel (PTX) and doxorubicin (DOX), either as single agents or loaded in solid lipid nanoparticles (SLN) by a solvent diffusion method, were examined using drug sensitive cancer cells and drug resistant cells by measuring the drug concentration required for 50% growth inhibition (IC_{50}). Compared to Taxol and DOX·HCl solution, both PTX and DOX loaded in SLN exhibited higher cytotoxicities in human breast tumor drug sensitive MCF-7 and drug resistant MCF-7/ADR cells. The ability of PTX loaded SLN and DOX loaded SLN to reverse the drug resistance of MCF-7 cells compared to MCF-7/ADR cells was 31.0 and 4.3 fold, respectively. Both PTX and DOX loaded SLN showed the same trends of enhanced cytotoxicity against a second wild type/drug resistant human ovarian cancer cell pair SKOV3 and SKOV3-TR30 cells. The reversal powers were 3.8 and 1.9 fold for PTX loaded SLN and DOX loaded SLN, respectively.

1. Introduction

Paclitaxel (PTX) and doxorubicin (DOX) are typical and commonly used drugs against a wide spectrum of solid tumors in the clinic. However, similar to other anticancer drugs, even when they are located in the tumor interstitium they can have limited efficacy against numerous solid tumor types, because cancer cells are able to develop mechanisms of resistance to drugs and evade chemotherapy [1]. The multi-drug resistance (MDR) phenotype, mainly due to expression of the MDR gene family encoding for the P-glycoprotein (P-gp) membrane proteins [2], represents an important problem in chemotherapy. The P-gp proteins are capable of extruding various generally positively charged xenobiotics, including some anticancer drugs, out of the cell via an ATP-dependent mechanism leading to intracellular reduction in the concentration of drug [3].

Nanoparticle (NP) delivery systems are known to carry the incorporated PTX or DOX into cells and improve the intracellular concentration of the drug [4]. Studies have shown that intracellular entry of drug loaded NPs delivery systems can be via endocytosis, followed by release of entrapped agent in cytoplasm. This is an alternative route of drug entry that enables bypassing or inhibiting the P-gp-mediated efflux [5,6].

One type of NP delivery system is solid lipid nanoparticles (SLN) developed as a colloidal carrier based on solid lipid materials. The SLN incorporate drugs into the lipid matrix resulting in the advantages of controlled release [7], high bioavailability by nonparenteral administration [8] and better tolerability [9]. In our previous studies [10–12], hydrophobic drugs were encapsulated in SLN with a controlled in vitro release rate. Moreover, higher cellular uptake of incorporated drugs in SLN was also observed. Therefore, SLN loaded anticancer drugs could enter the cells by an endocytotic pathway, thus bypassing the P-gp-dependent efflux, leading to an increased intracellular drug concentration and drug cytotoxicity, and reversing MDR activity in MDR cancer cells.

In this study, PTX and DOX were chosen as hydrophobic cytotoxic drugs. The cytotoxicities of drug loaded SLN prepared by a solvent-dispersion method were investigated in two human cancer cells (human breast cancer MCR-7 cells and human ovarian cancer SKOV3 cells) and their multi-drug resistant variants. Our aim was to evaluate and analyze the drug resistance reversal activity of drug loaded SLN in MDR cells (PTX and DOX resistant cells) compared to free drug solutions.

2. Materials and methods

2.1. Materials

Doxorubicin hydrochloride (DOX·HCl) was a gift from Hisun Pharm. (Zhejiang, China). Paclitaxel was purchased from

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Zhanwang Biochemical (Huzhou, China). Glyceryl monostearate (Monostearin, $C_{21}H_{42}O_4$) (Shanghai Chemical Reagent Co., China) was used as a solid lipid material for nanoparticles and α-hydro-ω-hydroxypoly(oxyethylene)$_{80}$poly(oxypropylene)$_{27}$poly (oxyethylene)$_{80}$ block copolymer (poloaxmer 188, HO$(C_2H_4O)_{80}$ $(C_3H_6O)_{27}(C_2H_4O)_{80}$H) (Shenyang Pharmaceutical university Jiqi Co. Ltd., China) was used as the surfactant. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO, USA). Trypsin and RPMI 1640 Medium were purchased from Gibco BRL (Gaithersberg, MD, USA). Fetal bovine serum (FBS) was purchased from Sijiqing Biologic (Hanghzou, China). All other chemicals were analytical or chromatographic grade.

2.2. Preparation of SLN

Before loading into the SLN, doxorubicin hydrochloride (DOX·HCl) was stirred with twice the number of mole of triethylamine (TEA) in DMSO overnight to obtain the DOX base [13].

The preparation method of drug loaded SLN was developed in our previous studies [10–12]. Briefly, 3 mg hydrophobic drug (PTX or DOX) and 60 mg monostearin were dissolved in 6 ml warm ethanol. The resultant organic solution was quickly dispersed into distilled water (with volume ration 1:10) under mechanical stirring (DC-40, Hangzhou Electrical Engineering Instruments, China) at 400 rpm in water bath of 70 °C for 5 min. The obtained pre-emulsion (melted lipid droplets) was then cooled to room temperature till drug loaded SLN formation was obtained. Blank SLN were also prepared as described above but omitting drug in the organic solution.

The pH value of the obtained SLN dispersion above was adjusted to 1.20, to allow SLN aggregation, by adding 0.1 M of hydrochloric acid. Then the SLN precipitate was harvested by centrifugation at 46,282 × g for 15 min (3K30, Sigma, Germany). The precipitate of SLN or NLC was collected for drug entrapment efficiency and drug loading determination.

The SLN precipitate was re-dispersed in the aqueous solution containing 0.1% poloxamer 188 (w/v) by probe-type ultrasonic treatment with 20 sonic bursts (200 W, active every 2 s for a 3 s duration) (JY92-II, Scientz Biotechnology Co. Ltd., China).

Then the resultant dispersion was fast frozen under −64 °C in a deep-freezer (Sanyo Ultra Low Temperature Freezer MDF-192, Japan) for 5 h and then the sample was moved to the freeze-drier (Freezone 2.5L, LABCONCO, USA). The drying time was controlled in 72 h and then the SLN powders were collected for in vitro release study.

2.3. Particle size and zeta potential measurement

The blank or drug loaded SLN in dispersion after sonication were diluted 20 times with distilled water, of which the volume average diameter and zeta potential were determined with Zetasizer (3000HS, Malvern Instruments, UK).

2.4. Drug entrapment efficiency and drug loading determination

The collected SLN precipitate was re-dispersed in phosphate buffer solution (PBS, pH 7.2, μ = 0.1 M) medium and subjected to vortex mixing (XW-80A, Instruments factory of Shanghai Medical University, China) at 2800 rpm for 3 min to dissolve the surface attached drugs, and treated with centrifugation at 46,282 × g for 15 min. Drug content in the supernatant was measured as follows.

For PTX, the supernatents were diluted in PBS medium containing 2 M sodium salicylate, and quantified by HPLC (Agilent 1100 series, USA), using C18 column (Diamohsil^{TM} 250 mm × 4.6 mm, 5 μm) in 35 °C, a UV detector (Agilent, USA) at a set wavelength of 227 nm. The mobile phase was a mixture of acetonitrile and water (50:50, v/v) with flow rate of 1.0 ml/min. Injected volume of the sample was 20 μl. The calibration curve of peak area against concentration of paclitaxel was $y = 32.4x - 16.3$ under the concentration of paclitaxel 0.5–120 μg/ml (R^2 = 0.9994, where y = peak area and x = paclitaxel concentration), the limit of detection was 0.01 μg/ml.

For DOX, the PBS medium contained 0.1% (w/v) sodium lauryl sulfate, and DOX concentration was determined by use of a fluorescence spectrophotometer (F-2500, Hitachi, Japan), excitation at 505 nm and emittion at 560 nm. The calibration curve of fluorescent intensity against concentration of doxorubicin was $y = 244x + 11.399$ under the concentration of doxorubicin 0.2–10 μg/ml (R^2 = 0.9993, where y = fluorescent intensity and x = doxorubicin concentration), the limit of detection was 0.05 μg/ml.

The drug entrapment efficiency (EE) and drug loading (DL) of SLN were calculated from Eqs. (1) and (2):

$$\mathrm{EE}(\%) = \frac{W_a - W_{s_1} - W_{s_2}}{W_a} \times 100 \quad (1)$$

$$\mathrm{DL}(\%) = \frac{W_a - W_{s_1} - W_{s_2}}{W_a - W_{s_1} - W_{s_2} + W_L} \times 100 \quad (2)$$

where W_a was the amount of drug added in system, W_{s_1} was the analyzed amount of drug in supernatant after the first centrifugation, W_{s_2} was the analyzed amount of drug in supernatant after the second centrifugation. W_L was the weight of lipid added in system.

2.5. In vitro release study

To investigate the release kinetics of drugs from SLN, separated SLN precipitate containing PTX or DOX was re-dispersed in respective release medium (sodium salicylate and sodium lauryl sulfate for PTX and DOX particles respectively as described above in Section 2.4) and mixed by vortexing at 2800 rpm for 3 min, and then shaken horizontally (Shellab1227-2E, Shellab, USA) at 37 °C and 60 strokes/min for 24 h. One ml of the dispersion was withdrawn from the system at various time intervals and centrifuged. The drug concentration in release medium was measured as described in Section 2.4. The amount of the released drug at each time was then calculated. Each formulation was investigated independently three times.

2.6. Cell culture

MCF-7 (human breast cancer cells) and MCF-7/ADR (multi-drug resistant variant) were donated from the first Affiliated Hospital, College of Medicine, Zhejiang University (Hangzhou, China). SKOV3 (human ovarian cancer cells) and SKOV3-TR30 (multi-drug resistant variant) were obtained from Women's Hospital, College of Medicine, Zhejiang University (Hangzhou, China). Cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2 in RPMI 1640 medium supplemented with 10% FBS, 100 units/ml penicillin, and 100 units/ml streptomycin.

2.7. Cytotoxicity assay and reversal power calculation

Using PTX solution (Taxol^{TM}, a 50:50 mixture of Cremophor EL and ethanol) and DOX·HCl solution as control, the cytotoxicities of PTX loaded SLN and DOX loaded SLN were performed against MCF-7, MCF-7/Adr, SKOV3 and SKOV3-TR30 cells. Briefly, cells were seeded in a 96-well plate at a seeding density of 10,000 cells per well in 0.2 ml of growth medium consisting of RPMI 1640 with 10% FBS and antibiotics. After cells were cultured at 37 °C for 24 h, the growth medium was removed and growth medium containing the different amount of drug, in solution or loaded in SLN,