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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,

were added respectively. Blank SLN at different concentrations in growth medium were also added to cells. All the experiments were performed in triplicate.

The cells were further incubated for 48 h at 37 °C. Then 100 μl of fresh growth medium containing 50 mg MTT was added to each well and cells were incubated for 4 h at 37 °C. After removing the unreduced MTT and medium, each well was washed with 100 μl of PBS and 180 μl of DMSO were then added to each well to dissolve the MTT formazan crystals at room temperature. Finally, the plates were shaken for 20 min at room temperature and the absorbance of formazan product was measured at 570 nm in a microplate reader (BioRad, Model 680, USA). Survival percentage was calculated as compared to mock-treated cells (100% survival).

The reversal power was calculated from Eq. (3):

$$\text{Reversal power} = \frac{R_f/R_N}{S_f/S_N} \quad (3)$$

where R_f was the IC_{50} value of drug solution against drug resistant cells, R_N was the IC_{50} value of drug loaded SLN against drug resistant cells; S_f was the IC_{50} value of drug solution against drug sensitive cells, S_N was the IC_{50} value of drug loaded SLN against drug sensitive cells.

2.8. Analysis of reversal activity

2.8.1. Cellular uptakes of fluorescent SLN

The conjugate of octadecylamine-fluorescein isothiocyanate (ODA-FITC) was synthesized according to our previous researches [12], and encapsulated into SLN as a fluorescence probe to investigate the cellular uptakes. MCF-7, MCF-7/ADR, SKOV3 and SKOV3-TR30 cells were seeded in a 24-well plate at a seeding density of 10,000 cells per well in 1 ml of growth medium and allowed to attach for 24 h. Cells were incubated with FITC-ODA loaded SLN suspension (the concentration were 100 μg/ml) in growth medium for different times. After the incubation, cells were washed thrice with PBS (pH 7.4) to remove the fluorescent SLN adsorbed on the cell membrane, and then directly viewed under a fluorescence microscope (Leica DM4000 B, Leica, Germany).

2.8.2. Cellular uptake of drug

Cells were seeded in a 24-well plate at a seeding density of 10,000 cells per well in 1 ml of growth medium and allowed to attach for 24 h. Then cells were incubated with free drug solution, drug loaded SLN (drug concentration: 2 μg/ml) in growth medium for 1, 2, 4, 12, 24 h. After the cells were washed with PBS thrice, 100 μl trypsin PBS solution (2.5 μg/ml) was added. The cells were further incubated for 5 min. According to the method reported by us previously [12], the cells were then harvested by adding 400 μl methanol, and were subjected to probe-type ultrasonic treatment (400 W, 10 cycles with 2 s active-3 s duration, JY92-II, Scientz Biotechnology Co. Ltd., China) in an ice bath. The obtained cell lysate was centrifuged at 22,360 × g for 10 min. The PTX content in the supernatant after centrifugation was measured by a HPLC method as described in Section 2.4, while the DOX content in the supernatant after centrifugation was determined using a fluorescence spectrophotometer as described in Section 2.4.

The protein content in the cell lysate was measured using the Micro BCA protein assay kit. The drug uptake percentages were calculated from Eq. (4):

$$\text{Drug uptake percentage } (\%) = \frac{C/M}{C_0/M_0} \times 100 \quad (4)$$

where C was intracellular drug concentration at a particular time, M was unit weight (milligram) of cellular protein at a particular time, C_0 was initial drug concentration, M_0 was initial unit weight (mg) of cellular protein.

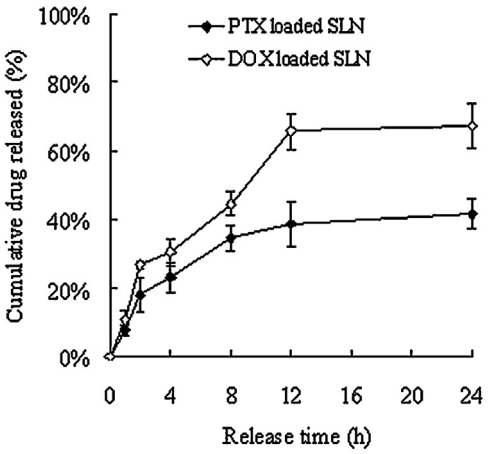

Fig. 1. In vitro release profiles of paclitaxel or doxorubicin loaded SLN. Mean ± SD, $n = 3$.

3. Results

3.1. Characteristic of SLN

Blank and drug loaded SLN were prepared by a solvent diffusion method. The volume average diameters, zeta potential and the polydispersity indexes of the resulting SLN are listed in Table 1. As SLN used in our study were the redispersions in 0.1% poloxamer 188 solution, the results were the data of SLN redispersions. From Table 1, it was also found that the sizes of drug loaded SLN were higher than that of blank SLN due to the incorporation of drug in the SLN matrix which increased the amount of solid phase. All of the absolute values for zeta potential of the SLN were above 30 mV, which demonstrated that the nanoparticles redispersion was a physically stable system. Table 1 also reports the encapsulation efficiency (EE) and drug loading (DL) of drug loaded SLN. About 60 wt% EE and 3 wt% DL could be reached by the present preparation method.

3.2. In vitro release study

Fig. 1 shows the PTX and DOX release profiles from drug loaded SLN formulations within 24 h. The cumulative amount of PTX released over 24 h was 41.6%, while the cumulative amount of DOX released over 24 h was 67.3%. It can be seen from the results that the PTX presented sustained release profile from PTX loaded SLN. As PTX is a more lipophilic drug with better affinity for lipid material compared to DOX, the PTX loaded SLN shown a relatively slower in vitro release profile than DOX loaded SLN.

3.3. The anti-cancer and reversal MDR activities of drug loaded SLN

Blank SLN formulations showed very high value of IC_{50} in both the breast and ovarian cell lines and their multi-drug resistant variants (Table 2), suggesting these lipid materials were safe to prepare SLN as drug carriers.

Table 2 reports IC_{50} values for drug solution and drug loaded SLN against drug sensitive cells and drug resistant cells. Fig. 2 shows cell survival curves after exposuring to drug solution and drug loaded SLN. From Table 2 and Fig. 2, it was found that SLN apparently improved the cytotoxicity of PTX comparing to Taxol in sensitive human breast cancer cells (MCF-7). In the MDR cells (MCF-7/ADR), the IC_{50} value of Taxol was nearly 30-fold higher than sensitive cells, however, PTX loaded in SLN had an even lower IC_{50} value than the sensitive cells, which meant the PTX loaded SLN could totally

Table 1
Properties of blank and drug loaded SLN ($n = 3$).

Formulation	d_v (nm)	PI (–)	ζ (−mV)	EE (%)	DL (%)
Blank SLN	273 ± 44	0.101 ± 0.015	36.4 ± 2.1	–	–
PTX loaded SLN	437 ± 68	0.436 ± 0.068	41.2 ± 5.2	61.8 ± 1.7	2.92 ± 0.15
DOX loaded SLN	481 ± 31	0.300 ± 0.038	46.6 ± 4.3	58.2 ± 2.8	2.83 ± 0.13

d_v, PI, ζ, EE and DL indicate the volume average diameter, polydispersity index, zeta potential, drug entrapment efficiency and drug loading, respectively.

Table 2
Cytotoxicities of blank SLN, drug solution and drug loaded SLN against drug sensitive cells and drug resistant cells ($n = 3$).

Formulation	IC_{50} (μg/ml)		Reversal power	IC_{50} (μg/ml)		Reversal power
	MCF-7	MCF-7/ADR		SKOV3	SKOV3-TR30	
Blank SLN	549 ± 29	564 ± 22	–	544 ± 24	558 ± 24	–
Taxol	0.290 ± 0.011	8.61 ± 0.28	–	0.160 ± 0.003	9.35 ± 0.25	–
PTX loaded SLN	0.092 ± 0.002	0.088 ± 0.003	31.0	0.089 ± 0.008	1.36 ± 0.05	3.8
DOX·HCl solution	0.176 ± 0.005	6.20 ± 0.22	–	0.52 ± 0.08	1.83 ± 0.11	–
DOX loaded SLN	0.120 ± 0.005	0.99 ± 0.09	4.3	0.48 ± 0.07	0.91 ± 0.07	1.9

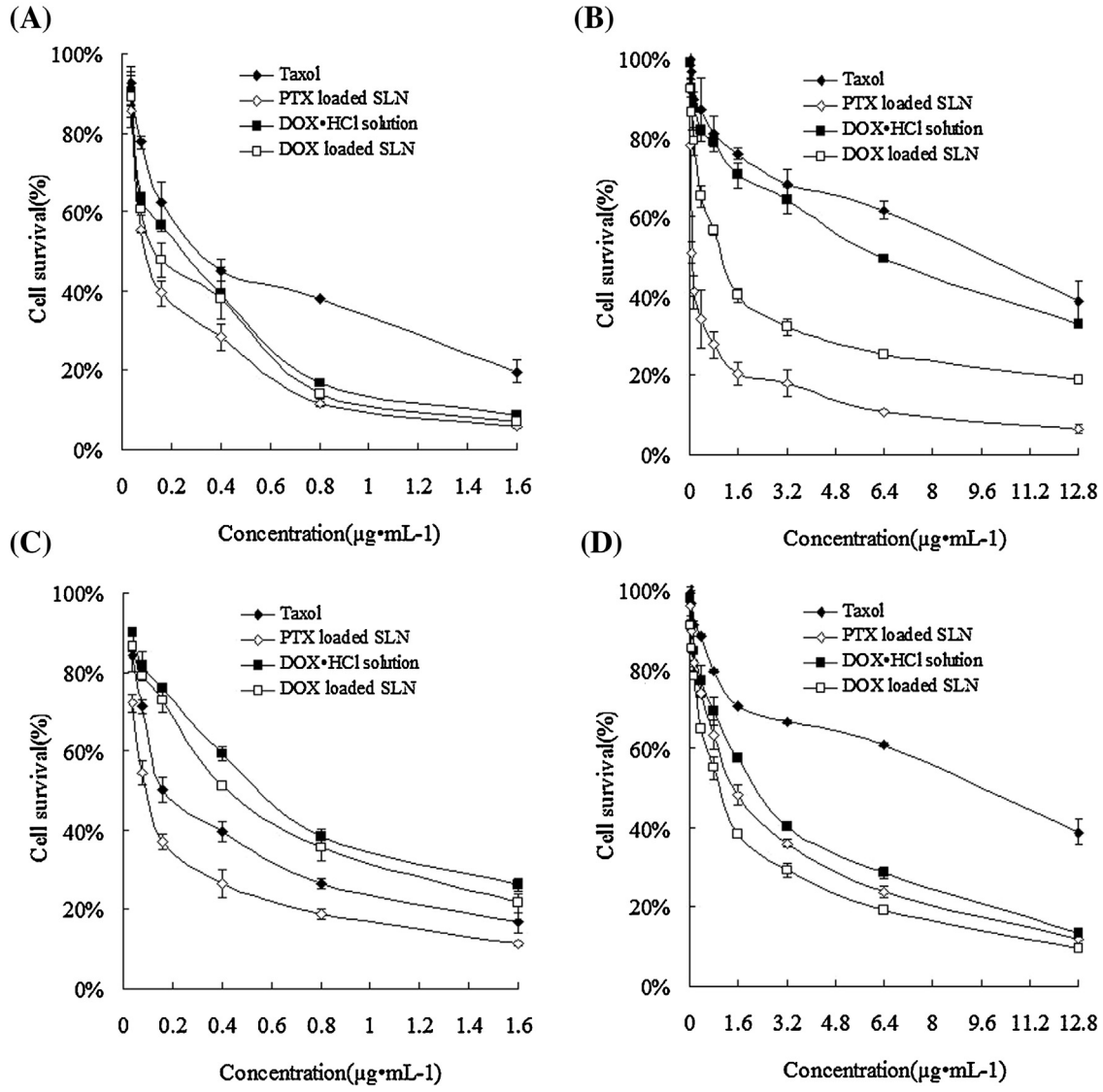

Fig. 2. Cell survival curves against drug concentration after MCF-7 (A), MCF-7/ADR (B), SKOV3 (C) and SKOV3-TR30 (D) were incubation with drug solution and drug loaded SLN. Mean ± SD, $n = 3$.

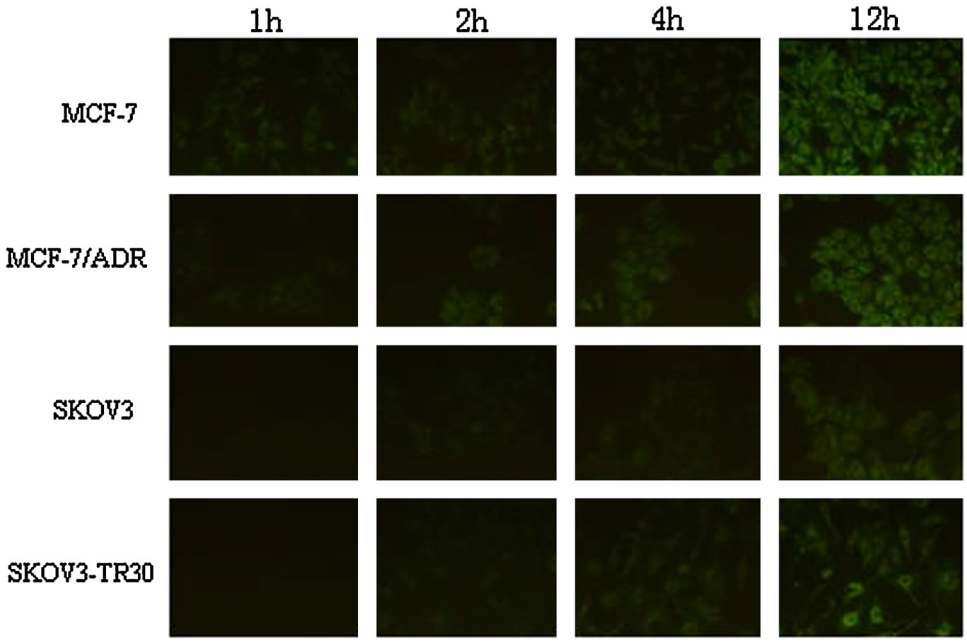

Fig. 3. Fluorescence images of the MCF-7, SKOV3 cells and their multi-drug resistant variants when incubated with fluorescent SLN (the concentrations of the SLN were 100 μg/ml). Original magnification 40×.

reverse the PTX-resistant activity in MCF-7/ADR cells. As a result, the reversal fold of PTX in SLN against MCR-7/ADR was 31.0, which could be caused by the enhanced endocytosis of SLN delivered drug bypassing or "inhibiting" the pump efflux by P-gp.

In MCF-7 cells, SLN could not apparently improve the cytotoxicity of DOX over DOX·HCl solution much. This result may be due to the burst drug release behavior of DOX loaded SLN at the initial stage, which led to a lower DOX concentration internalized into cells. However, in MCF-7/ADR cells, after loading into SLN, the cytotoxicity of DOX was improved about 6-fold, and the reversal power of SLN was 4.3.

In SKOV3 cells, PTX loaded SLN did show an apparent cytotoxicity improvement in both sensitive and resistant cells, with reversal power of 3.8 (Table 2). Also in SKOV3 cells, DOX loaded SLN showed a poorer improvement of cytotoxicity in both sensitive and resistant cells, as a result, the reversal power of DOX loaded SLN was below 2.

3.4. Analysis of reversal activity

3.4.1. Cellular uptakes of fluorescent SLN

Fig. 3 shows the fluorescence images when MCF-7, MCF-7/ADR, SKOV3 and SKOV3-TR30 cells were incubated with fluorescent SLN for different incubation time.

It was found that the uptake of SLN were without obvious differences between drug sensitive cells and drug resistant cells in both MCF-7 and SKOV3 cells. On the other hand, the uptake of fluorescent SLN increased with incubation time in all four cell lines. The SLN may enter the cells via endocytosis, which is not a P-gp dependent pathway.

3.4.2. Cellular uptake of drug

Fig. 4A shows the cellular uptake percentages of PTX at different incubation time when the MCF-7 and MCF-7/ADR cells were incubated with PTX in different formulations. The cellular uptake percentages of PTX loaded in SLN obviously increased more compared with that of Taxol. The cellular uptake percentages of PTX delivered by SLN in MCF-7/ADR cells were similar or even higher than that in MCF-7 cells at the same incubation time. This result was consistent with the total reversal of PTX-resistant activity in MCF-7/ADR cells, in which the cytotoxicity of PTX loaded SLN in MCF-7/ADR cells was higher than that in MCF-7 cells.

Fig. 4B shows the cellular uptake percentages of DOX at different incubation times when the MCF-7 and MCF-7/ADR cells were incubated with DOX in different formulations. The cellular uptake percentages of DOX loaded in SLN obviously increased compared with that of DOX·HCl solution. However, the cellular uptake percentages of DOX delivered by SLN in MCF-7/ADR cells were still lower than that in MCF-7 cells at the same incubation time. This result was consistent with the partial reversal of DOX-resistant activity in MCF-7/ADR cells, in which the cytotoxicity of DOX loaded SLN in MCF-7/ADR cells was still lower than that in MCF-7 cells.

Fig. 5A shows the cellular uptake percentages of PTX at different incubation time when the SKOV3 and SKOV3-TR30 cells were incubated with PTX of different formulations. The cellular uptake percentages of PTX loaded in SLN obviously increased compared with that of Taxol. However, the cellular uptake percentages of PTX delivered by SLN in SKOV3-TR30 cells were still lower than that in SKOV3 cells at the same incubation time. This result was consistent with the partial reversal of PTX-resistant activity in SKOV3-TR30 cells, in which the cytotoxicity of PTX loaded SLN in SKOV3-TR30 cells was still lower than that in SKOV3 cells.

Fig. 5B shows the cellular uptake percentages of DOX at different incubation time when the SKOV3 and SKOV3-TR30 cells were incubated with DOX of different formulations. The cellular uptake percentages of DOX loaded in SLN obviously increased compared with that of DOX·HCl solution. However, the cellular uptake percentages of DOX delivered by SLN in SKOV3-TR30 cells were still lower than that in SKOV3 cells at the same incubation time. This result was consistent with the partial reversal of DOX-resistant activity in SKOV3-TR30 cells, in which the cytotoxicity of DOX loaded SLN in SKOV3-TR30 cells was still lower than that in SKOV3 cells.

4. Discussion

In this study, we prepared SLN by the method of solvent-dispersion. The EE and DL of obtained SLN was around 60 wt% and

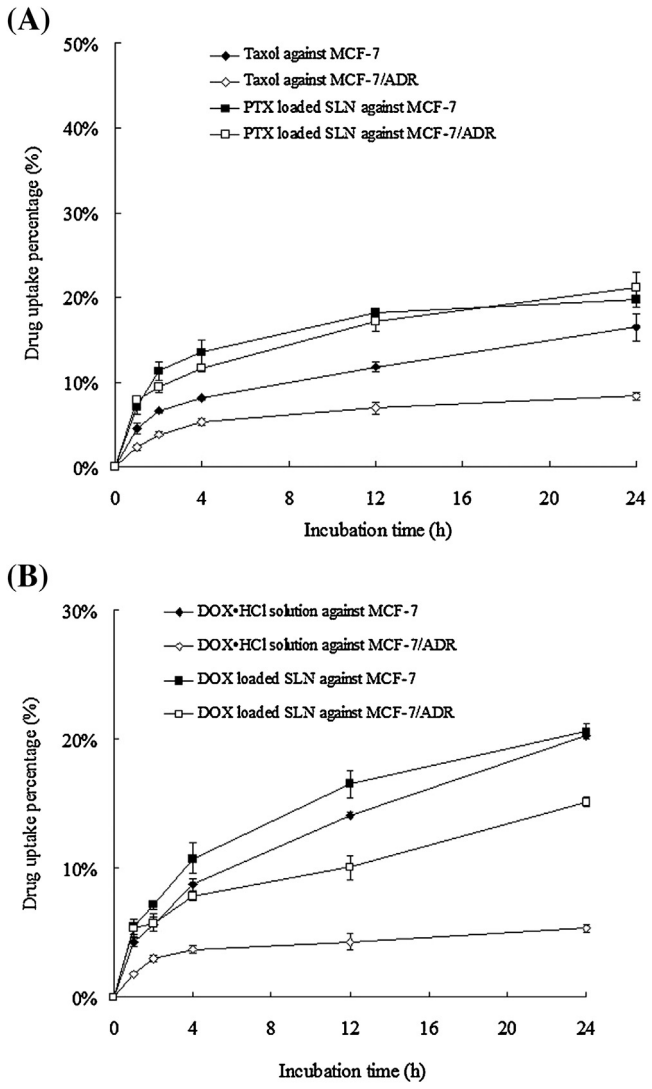

Fig. 4. PTX (A) or DOX (B) uptake percentage against incubation time after MCF-7 and MCF-7/ADR cells were incubated with Taxol and PTX loaded SLN. Mean ± SD, $n = 3$.

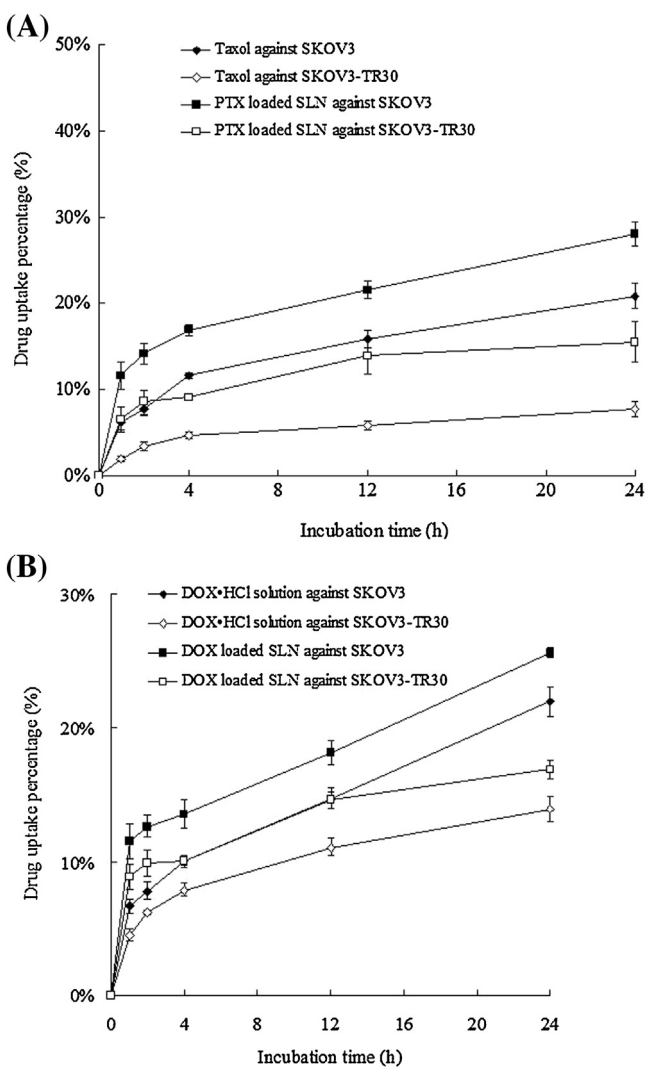

Fig. 5. PTX (A) or DOX (B) uptake percentage against incubation time after SKOV3 and SKOV3-TR30 cells were incubated with DOX·HCl solution and DOX loaded SLN. Mean ± SD, $n = 3$.

3 wt%, respectively. The low EE and DL might due to the shortage of SLN including limited drug loading capacity, some drug loss into the aqueous system during the formulation, and drug expulsion during storage [14,15]. In addition, the lower EE and DL of DOX loaded in SLN than that of PTX was mainly due to the lower solubility of DOX in water.

The profiles of in vitro release showed the release plateau at 40% for PTX and 65% for DOX over 24 h. After 24 h, the drug was still in SLN, and could be released still PTX or DOX from SLN. These results indicated that PTX and DOX could be released slowly from SLN and be kept at a constant concentration for a relatively long period. Therefore, the frequency of administration might be reduced, which is beneficial for clinical application.

The difference between the release properties of PTX and DOX was attributed to the prolonged release function of SLN. Lipophilic drug was held by the hydrophobic carrier, and the drug was released primarily through dissolution and diffusion. As PTX is a more lipophilic drug with better affinity for lipid material compared to DOX, the PTX loaded SLN shown a relatively slower in vitro release profile than DOX loaded SLN.

In MDR cancer cells, P-gp is an organic cation pump that is capable of transporting a variety of structurally and functionally diverse chemotherapeutic drugs [16]. Owing to the overexpression of P-gp, cancer cells actively efflux the drug, leading to reduced intracellular drug accumulation and decreased therapeutic efficacy.

Based on our previous study, we concluded SLN could be taken up by target cells efficiently due to the membrane affinity of lipid material and nano-scale size of SLN. Herein, SLN loading drugs with controlled release profiles within 24 h, accumulated in cytoplasm, served as a drug-reservoir releasing the drug continuously to the cytoplasm to overcome the drug reduction from P-gp mediated drug efflux. On the other hand, it was reported that the cellular uptake of drug loaded NPs was an ATP-mediated action [3]. Thus, lipid NPs were presumed to act as a competitive inhibitor to P-gp's action, which would be responsible for the drug uptake increase and MDR reversal in multi-drug resistant cells. In this study, compared with free drug solution, the drug loaded in SLN not only improve cytotoxicity against sensitive cancer cells, which were also seen in a previous study in vitro [17] and in vivo [18], but also improve cytotoxicity against resistant cancer cells. As a result, SLN showed MDR reversal activity in both two multi-drug resistant cell lines (MCF-7/ADR and SKOV3-TR30).

In our work, we made drug loaded SLN with considerable anticancer and MDR reversal activity. However, whether and to what

extent SLN could overcome the P-gp's efflux and deliver drug into MDR cells was still uncertain. Thus the cellular uptake of PTX or DOX delivered by SLN was measured to establish a clear relationship between the reversal activity and the drug concentration in cells. Lipid matrix-based SLN can increase the drug transport into cancer cells by efficiently cellular uptake both in sensitive and resistant cancer cells. As low intracellular drug concentration is the universal character in multi-drug resistant cells when the anticancer agent was administrated, the drug concentration increase in the target cells is the key point for reversing the MDR. Thus the cellular drug uptake result of anticancer drug loaded SLN was consistent with its drug resistance reversal activity.

5. Conclusion

Intracellular drug concentration deficiency caused by P-gp-mediated efflux in cancer cells is a key mechanism for the occurrence of MDR. Herein, solid lipid nanoparticles drug delivery system can increase the transport of standard anticancer drugs (PTX or DOX) into cancer cells and enhance the cytotoxicity against sensitive cancer cells and their multi-drug resistant variant cells, compared with free drug solutions. That is to say, lipid matrix based SLN can protect the encapsulated drug from the P-gp efflux mechanism of the cell and overcome multi-drug resistance, and revealing a potential application of this drug resistance reversal mechanism in drug resistant human cancer cells.

Acknowledgments

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