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Studies on PEG-modified SLNs loading vinorelbine bitartrate (I): Preparation and evaluation in vitro

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ABSTRACT

In this study, the conjugate of PEG_{2000}–stearic acid (PEG_{2000}–SA) was used to prepare PEGylated solid lipid nanoparticles loading vinorelbine bitartrate (VB-pSLNs) by cold homogenization technique. The particle size and zeta potential of resulted VB-pSLNs ranged 180–250 nm and 0–10 mV, which were determined using a Zetasizer, respectively. Although the drug entrapment efficiency (EE) slightly decreased after the PEG modification of VB-SLNs, above 60 % EE could be reached. The drug release tests *in vitro* indicated the faster drug release from VB-pSLNs than that from VB-SLNs without PEG modification. To investigate the cellular uptake of VB-pSLNs, the chemical conjugate of octadecylamine-fluorescein isothiocynate (FITC-ODA) was synthesized, and was used as a fluorescence marker to incorporate into nanoparticles. The results from cellular uptake indicated that the phagocytosis of VB-pSLNs by RAW264.7 cells was inhibited effectively by the PEG modification of SLNs, while the uptake by cancer cells (MCF-7 and A549) could be improved significantly. The assay of anticancer activity *in vitro* demonstrated that the anticancer activity of VB was significantly enhanced by the encapsulation of SLNs and pSLNs due to the increased cellular internalization of drug. The results suggested that SLNs and pSLNs could be excellent carrier candidates to entrap VB for tumor chemotherapeutics.

1. Introduction

The concept of drug delivery system (DDS) has been introduced to pharmaceutical science for long time. Various kinds of drug carriers, including liposomes, polymer nanoparticles, polymer micelles, nanoemulsions and solid lipid nanoparticles (SLNs), have been developed to overcome the problems of insufficient drug concentration and serious side effects due to extensive *in vivo* biodistribution of drug, respectively. In vivo fate of the drug, which is entrapped into DDS, is no longer determined by its physicochemical properties. Furthermore, using the carrier system, the drug can obtain a controlled and localized release according to the specific needs of the therapy (Mehnert and Mader, 2001). SLNs attract an increasing attention for their low toxicity (Mosmann, 1983; Müller et al., 1996a), excellent biocompatibility, ability to carry hydrophilic or lipophilic drugs, and controlled or localized release of the active drugs (Müller and Runge, 1998). However, in vitro and in vivo experiments indicated that SLNs also existed some drawbacks as a promoting drug carrier, such as the rather low entrapment efficiency for hydrophilic drug, the comparative low cellular uptake efficiency in vitro and clearance rapidly in vivo by mononuclear phagocyte system (MPS) (Müller et al., 1996b, 1997; Illum et al., 1987; Rudt and Müller, 1993; Bocca et al., 1998), which resulted in the restricted use of SLNs in the field of pharmaceutical science.

Vinorelbine bitartrate (VB) is a semi-synthetic vinca alkaloid that indicates a significant anti-tumor activity through disrupting microtubule. VB has been shown activity in many tumor types and is currently registered for the treatment of advanced breast cancer (ABC) and non-small cell lung cancer (NSCLC) in most countries. VB is easy to be degraded on condition of high temperature and possesses excellent hydrophilicity. In our previous researches (You et al., 2007), SLNs loading VB have been prepared by cold homogenization technique. The results indicated that VB, as a hydrophilic drug, was successfully encapsulated into SLNs with high efficiency about 80%. The drug release behavior in vitro and drug entrapment efficiency could be adjusted through altering the amount of lecithin and oleic acid (OA) in SLN formulations. OA played an important role in drug release behavior and drug entrapment efficiency.

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Many reports have indicated that the PEG modification of drug carriers can inhibit effectively the clearance in vivo by mononuclear phagocyte system and reach a longer plasma half-life (Kajiwara et al., 2007; Han et al., 2007). Joanna et al. (2007) indicated that the PEG-modified emulsion could enhance the accumulation of lipophilic anticancer drugs in tumor tissues and increase the therapeutic index for non-hepatotoxic drugs. Shiokawa et al. (2005) also reported that PEG modification of emulsion enhanced the drug cytotoxicity. Other groups indicated that modifying the colloid surface with a hydrophilic molecular could prolong circulation time *in vivo* (Takino et al., 1994; Redgrave et al., 1992). Bocca et al. (1998) prepared the stealth SLNs using PEG to reduce the uptake in murine macrophages significantly, and improve the physical stability. However, the PEG-modified drug carrier, especially liposomes, frequently presented a decreased uptake of targeting cell, comparing with the non-PEG modified, which was contributed to the increased hydrophilicity of liposome surface after PEG modification (Goren et al., 2000; Pan et al., 2002).

Herein, we further improved the SLN formulation loading VB by PEG modification in order to overcome the drawbacks of the comparative low cellular uptake ability and rapid clearance in vivo by MPS. PEG-modified SLNs (pSLNs) were prepared using PEG_{2000}–SA by cold homogenization technique (Bocca et al., 1998). The conjugate of octadecylamine-fluorescein isothiocynate (ODA-FITC) was synthesized, and encapsulated into SLN formulation as a fluorescence probe to investigate the cellular uptake of SLNs and pSLNs. In our previous researches (Yuan et al., 2007), ODA-FITC and lipid matrix precented high incorporation efficiency (above 97%), and the leakage of ODA-FITC from lipid nanoparticles was lower than 7% during in vivo transport. In present studies, SLNs without PEG modification (SLNs), SLNs modified by 5% (w/w) PEG_{2000}–SA (5%pSLNs), SLNs modified by 10% (w/w) PEG_{2000}–SA (10%pSLNs) and SLNs modified by 15% (w/w) PEG_{2000}–SA (15%pSLNs) were prepared to investigate the influence of the incorporating PEG amount on the cellular uptake. RAW264.7 (a mouse macrophage cell line) was employed to investigate the phagocytosis to pSLNs by MPS. MCF-7 (human breast adenocarcinoma cell line) and A549 (human alveolar basal epithelial cell line) were employed to study the uptake to SLNs by cancer cells. Furthermore, the effect of incorporating PEG_{2000}–SA into lipid matrix on drug entrapment efficiency, in vitro release behavior and the cytotoxicity were also investigated in details.

2. Materials and methods

2.1. Materials

Glyceryl monostearate (GMS) was provided by Shanghai Chemical Reagent Co. Ltd. (China); lecithin E_{80} and oleic acid were purchased from Lipoid (Germany); vinorelbine bitartrate was kindly donated from Hangzhou Huadong Pharmaceutical Co. Ltd. (China); BCA Protein Assay Kit and PEG_{2000}–SA was purchased from Beyotime Institute of Biotechnology and Tokyo Kasei Kogyo Co. Ltd., respectively. Poloxamer 188 (F_{68}) was purchased from BASF (Germany). Methylthiazolyldiphenyl-tetrazolium bromide (MTT), RPMI-1640 medium, fetal bovine serum, penicillin–streptomycin solution, phosphate buffered saline (PBS, pH 7.4), trypsin–EDTA solution were purchased from Sigma (USA). Dimethyl sulfoxide (DMSO) and ethanol were analytical reagent grade. De-ionized (DI) water (Millipore) was used throughout the experiments.

2.2. Preparation of nanoparticles

SLNs and pSLNs with or without VB were prepared by cold homogenization technique reported in our previous study (You et al., 2007). Briefly, GMS and PEG_{2000}–SA were melted with water bath (60 °C). Lecithin, oleic acid, ODA-TITC (only for blank SLNs) and VB (no VB for blank SLNs) were dissolved in 1 ml ethanol, and the organic solution was then added into melted GMS and PEG_{2000}–SA by drop-by-drop under 60 °C water bath. After removing ethanol, the mixture was cooled by pouring the mixture in liquid nitrogen. The obtained solid dispersion was ground to form microparticles under the ice-cold condition. The microparticles were suspended in aqueous phase containing 1% F_{68} and 20% sugar, and homogenized at $18{,}000 \times g$ for 0.5 h by a homogenizer (Polytron PT4000, Switzerland), followed by homogenization at 20,000 psi using a high pressure homogenizer (Emulsiflex C-5, AVESTIN, Canada) under an ice-bath condition.

2.3. Measurement of particle size and zeta potential

Particle size and zeta potential of the nanoparticles were measured by photon correlation spectroscopy using Zetasizer (PCS3000, Malvern). Samples were diluted appropriately with aqueous solution containing 1% F_{68} and 20% sugar.

2.4. Determination of drug entrapment efficiency

The VB contents were measured by high performance liquid chromatograph (HPLC). The chromatographic system consisted of a Waters 515 HPLC pump equipped with a 20 μl loop. Waters Symmetry C_{18} (5 μm, 3.9 mm × 150 mm) analytical column was used with Waters 2487 UV-Dural λ Absorbance detector. The wavelength was 267 nm. Mobile phase was methanol/0.05 M KH_2PO_4 solution (pH 4.5) (60:40), and flow rate was kept at 0.8 ml/min.

Drug entrapment efficiency was determined by ultracentrifugation. The drug entrapment efficiency was calculated from the ratio of the drug amount incorporated into SLNs to the total charged drug amount. Ultracentrifugation was carried out using Centrisart, which consist of filter membrane (molecular weight cutoff 10,000 Da) at the base of the sample recovery chamber. About 0.5 ml of SLNs dispersion containing about 0.2 mg VB was placed in the outer chamber and the sample recovery chamber was placed on the top of the sample. The unit was centrifuged at $3000 \times g$ for 15 min. SLNs along with encapsulated drug remained in the outer chamber and dispersion medium moved to the sample recovery chamber through filter membrane. The amount of the drugs in the dispersion medium was estimated by HPLC analysis.

2.5. In vitro drug release studies

The drug release behavior from VB-pSLNs was performed by the dialysis bag method. Phosphate buffer (PBS, pH 7.4) was used as dissolution medium. The dialysis bag (molecular weight cutoff 7000 Da) could retain nanoparticles and allow the diffusion of free drug into dissolution media. The bags were soaked in DI water for 12 h before use. 1 ml of VB loaded SLNs dispersion was poured into the bag with the two ends fixed by clamps. The bags were placed in a conical flask and 10 ml dissolution media was added. The conical flasks were placed into a thermostatic shaker (HZQ-C; Haerbin Dongming Medical Instrument Factory, Haerbin, China) at 37 °C at a rate of 60 times per min. At 0.5, 1, 2, 4, 8, 12, 24, 36 and 48 h after test, the medium in the conical flask was completely removed for analysis and fresh dialysis medium was then added to maintain sink conditions. The drug contents in samples were analyzed by the HPLC method. All the operations were carried out in triplicate.

2.6. Cellular uptake

In a 24-well plate, MCF-7, A549 and RAW264.7 cells were seeded at a density of 1×10^5 cells/ml in 1 ml of growth medium and incubated for 24 h to attach. Cells were then incubated with Fluorescent SLNs (ODA-FITC labeled SLNs) suspension in growth medium for different time. After the incubation, the cells were washed twice with ice-cold PBS (pH 7.4) and directly observed under a fluorescence microscope (Leica DM4000 B, Leica, Germany).

To assay cellular uptake quantitatively, after washing the cells twice with ice-cold PBS (pH 7.4), 100 μl trypsin PBS solution (2.5 mg/ml) was added. After incubated for 10 min, the cells were harvested by adding 1 ml PBS, and then treated by probe-type ultrasonication for 5 times (4 °C, 200 W, active every 5 s for a 60 s duration) to obtain the cell lysate. Finally, the cell lysate was centrifuged at 10,000 rpm for 10 min, and the supernatant was supplied to fluorescence assay by using fluorometer (F-4000, HITACHI Co., Japan) (excitation: 495 nm; emission: 517 nm). To eliminate the error due to the difference of cell density, the cellular uptake percentage of fluorescent SLNs was calibrated by the amount of protein. The amount of protein was determined by BCA protein Assay Kit (Beyotime biotechnology, China) (Liu et al., 2004; Zheng et al., 2004). The cellular uptake percentage of fluorescent SLN was calculated from the following equation.

$$\text{Cellular uptake percentage} = \frac{(\text{Fluorescene value of sample}/\text{Protein amount of sample}) \times \text{Protein amount of controlled group}}{\text{Fluorescene value of total SLNs}} \times 100\%$$

2.7. Cytotoxicity

Cytotoxicity of various kinds of SLNs was assayed against MCF-7 and A549 cells by the MTT method. Control experiments were carried out using the complete growth culture medium only (serving as non-toxic control). MCF-7 and A549 cells (1 ml) at a density of 1×10^5 cells/ml were seeded in a 24-well plate in the complete growth culture medium. After culturing for 24 h, the media were exchanged with 1 ml culture medium containing a specified concentration of free VB, VB-SLNs, 5%VB-pSLNs, 10%VB-pSLNs and 15%VB-pSLNs. After specified intervals (48 h), the culture medium from each well was removed and then 1 ml of the complete growth culture medium and 60 μl MTT solution (5 mg/ml in PBS) were then added to each well. The medium was removed after incubating for 4 h. Then, the formazan crystals were solubilized with 1 ml DMSO. The amount of formazan was then determined from the optical density at 570 nm by a microplate reader (BioRad, Model 680, USA). The results were expressed as percentages relative to the result obtained with the non-toxic control. The cytotoxic effect of blank SLNs was also evaluated by the same method. All the experiments were performed in triplicate.

3. Results and discussion

3.1. Preparation of SLNs

In present study, pSLNs was prepared by mixing the PEG_{2000}–SA into lipid matrix. There were two methods to modify the particles using PEG. One is to graft PEG by chemical reaction on the surface of particles. The other is to prepare PEG-modified particles by mixing PEG into materials. In previous researches, we have once tried to modify SLNs by chemical grafting method. But, because of low melting point of SLNs and intensive condition for chemical graft, pSLNs loading VB with low drug entrapment efficiency were obtained (data not shown). Meanwhile, drug degradation was also accelerated during such process. As a result, mixing the PEG_{2000}–SA into the lipid matrix was chosen for preparing pSLNs as reported by Bocca et al. (1998).

Fig. 1 demonstrated the process of preparation of pSLNs by cold homogenization technique. Firstly, the PEG_{2000}–SA molecules dispersed into lipid matrix uniformly, and then, the hydrophilic PEG groups could expose to the surface of particles due to sharply increasing specific surface area under the condition of powerful mechanical force and shearing force. It is supposed that the probability of PEG group exposing into the aqueous medium on the particle surface increases on increasing the amount of PEG_{2000}–SA incorporating into lipid matrix.

The preparation formulas and results of fluorescent SLNs (containing FITC-ODA) and VB loaded SLNs are listed in Tables 1 and 2, respectively. The particle sizes were controlled about 200 nm in order to eliminate the influence of different particle size on cellular uptake. From Table 2, it was observed that the absolute value of zeta potential of SLNs decreased from about 10 to 0 mV by increasing the amount of PEG_{2000}–SA, which could contribute to the improvement of surface hydrophilicity with the incorporating the PEG_{2000}–SA into the lipid matrix (Zimmermann and Müller, 2001; Gasco et al., 1997). For the same reason, the zeta potential of VB loaded SLNs declined from 16 mV to 4 mV with increasing the amount of PEG_{2000}–SA.

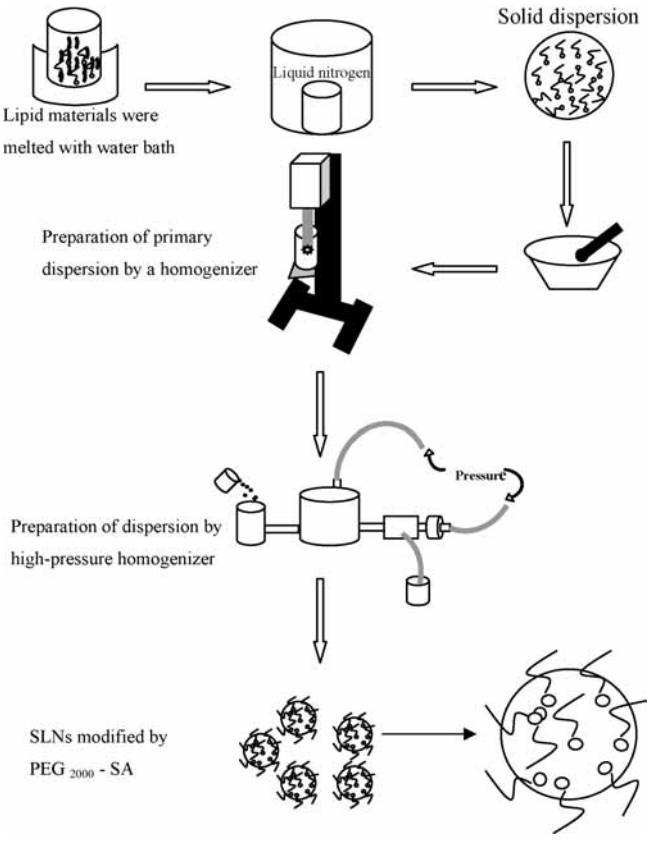

Fig. 1. Schematic of preparation process of SLNs by cold homogenization technique. (○) means SA, (≀) means PEG group.

Table 1
Formulas for the preparation of fluorescent and VB loaded nanoparticles

Carrier	Composition (mg)									
	Fluorescent SLNs					VB loaded SLNs				
	GMS[a]	L[b]	OA[c]	PEG_{2000}–SA[d]	ODA-FITC[e]	GMS	L	OA	PEG_{2000}–SA	VB[f]
SLNs	200	90	14	0	30	200	90	14	0	14
5%pSLNs	184	90	14	16	30	184	90	14	16	14
10%pSLNs	168	90	14	32	30	168	90	14	32	14
15%pSLNs	152	90	14	48	30	152	90	14	48	14

[a] Glyceryl monostearate.
[b] Lecithin E_{80}.
[c] Oleic acid.
[d] The conjugate of PEG_{2000}–stearic acid.
[e] The conjugate of octadecylamine-fluorescein isothiocynate.
[f] Vinorelbine bitartrate.

Table 2
The size, zeta potential and entrapment efficiency of different SLN formulations (mean ± S.D., $n = 3$)

Carriers	Fluorescent SLNs		VB loaded SLNs		
	Size (nm)	Zeta (mV)	Size (nm)	Zeta (mV)	EE (%)
SLNs	243.9 ± 26.8	−10 ± 1.6	210.2 ± 29.7	15.6 ± 2.7	72.7 ± 3.8
5%pSLNs	253.9 ± 36.0	−9.8 ± 1.6	197.8 ± 49.2	8.3 ± 4.8	62.6 ± 1.7
10%pSLNs	233.8 ± 16.5	−0.8 ± 1.6	186.8 ± 48.8	3.7 ± 1.6	62.8 ± 2.4
15%pSLNs	245.2 ± 21.6	4.2 ± 9.4	181.9 ± 37.8	4.0 ± 1.6	60.3 ± 3.1

3.2. Cellular uptake

Firstly, SLNs without PEG modification, with particle sizes of about 200, 300, 500 and 800 nm, were prepared in order to investigate the effect of particle size on cellular uptake. Fig. 2 displays the effect of particle size on cellular uptake of SLNs. It was demonstrated that the smaller particles are easier to be uptake by cells. As also depicted in Fig. 2, the cellular uptake percentage of particles with 200 nm diameter is about 1.5-fold higher than that of particles with 800 nm diameter after 12 h incubation.

Fig. 3 displayed the cellular uptake images of 0%pSLNs, 5%pSLNs, 10%pSLNs, and 15%pSLNs. It was shown that the fluorescence intensity in RAW264.7 cells became weaker with the treatment from 0%pSLNs to 15%pSLNs, which demonstrated that the phagocytosis ability of RAW264.7 decreased by increasing the amount of PEG fraction on SLNs surface. Fig. 4A indicated the results of quantitative cellular uptake for SLNs and pSLNs in RAW264.7. A sharp decline of cellular uptake form 0%pSLNs to 15%pSLNs in RAW264.7 cells was also found, which suggested that the increase of PEG (hydrophilic group) density on particle surface resulted in the improvement of hydrophilicity of SLNs (Cavalli et al., 2000), and subsequently decrease of phagocytosis ability. As previous report (You et al., 2007), the nanoparticles prepared by cold homogenization technique was owing to the powerful mechanical force and shearing force. Herein, the PEG_{2000}–SA molecules were dispersed into lipid matrix uniformly, the hydrophilic PEG groups could expose on the surface of particles under the condition of powerful mechanical force and shearing force. On the other hand, it was reasonable that the amount of PEG exposed on the surface of nanoparticles could increase with increasing the amount of PEG mixed in lipid matrix.

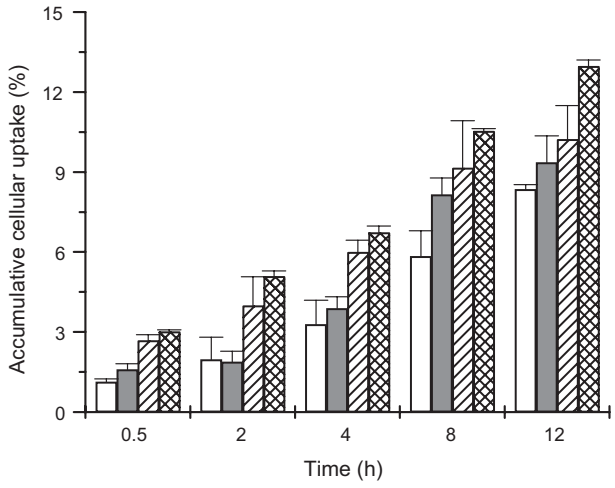

Fig. 2. Effect of particle size on cellular uptake. (□) 800 nm; (■) 500 nm; (▨) 300 nm; (▩) 200 nm.

The cellular uptake images of various SLNs by MCF-7 and A549 cells are also displayed in Fig. 3. Contrasting the results of pSLNs cellular uptake by RAW264.7, the cellular uptake of pSLNs in tumor cells increased with increasing the amounts of PEG_{2000}–SA in pSLNs. Furthermore, the results of quantitative cellular uptake of pSLNs in tumor cells (Fig. 4B and C) also demonstrated that cellular uptake ability enhanced with increasing the amount of PEG_{2000}–SA in pSLNs. Shiokawa et al. (2005) had also reported that the cellular uptake of PEG coated microemulsion enhanced comparing with that of microemulsion without PEG. The PEGylated liposomes loading doxorubicin showed significantly lower cytotoxicity in vitro than the free drug because of the PEG coating. In contrast, the PEGylated microemulsion loading aclacinomycin A (ACM) showed a 1.7-fold higher cytotoxicity (IC_{50}) than free ACM. However, the mechanism of the difference in cellular uptake of PEGylated carriers is not clear. These results seem to imply that the interaction of PEG in various carriers with the cell membrane might be different. The structure of pSLNs is similar with the microemulsion with PEG-coating, and the property difference between two carriers is that pSLNs is solid structure with higher melting point than that of microemulsion. The nanoparticles surface possessing proper balance between hydrophilicity and lipophilicity might be more benefic for the uptake by cancer cells. The cell membrane exists in hydration shell, which could hinder the particles from approaching to the cell membrane if the particle surface is excessively lipophilic. While the excessive hydrophilicity could prevent the internalization of nanoparticles into cells due to the interaction between nanoparticles and the lipophilic components of the cell membrane could be interfered. Moreover, PEG_{2000}–SA possesses the lower melting point (about 50 °C), which leads to the decline in melting point and viscosity of pSLNs. The particles with low viscosity can be easier to inset into cell membrane. Our previous researches had demonstrated that the cellular uptake of lipid

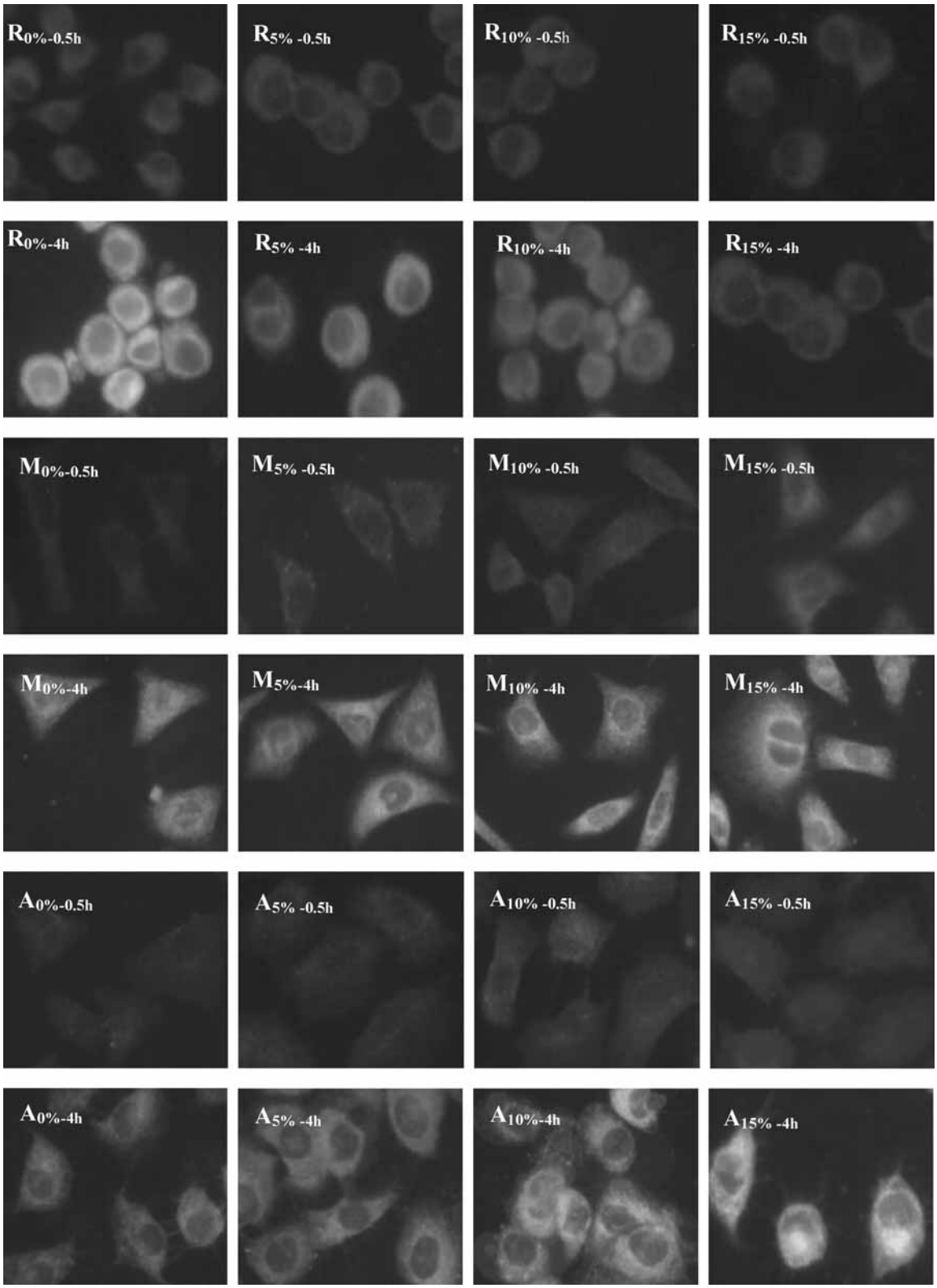

Fig. 3. Fluorescent pictures of cell uptake for SLNs with 0%, 5%, 10% and 15%PEG_{2000}–SA content in RAW264.7 cell line (R), MCF-7 cell line (M) and A549 cell line (A) after incubation for 0.5 and 4 h.

nanoparticles could be improved by incorporating liquid lipid (oleic acid) into solid lipid matrix to decrease the viscosity (unpublished data). Furthermore, Wong et al. (2006a) and Wong et al. (2006b) reported that polymer–lipid hybrid nanoparticles could increase the cellular uptake and retention of drug (doxorubicin hydrochloride). The pSLNs prepared here with PEG_{2000}–SA could possess the similar structure of polymer–lipid hybrid nanoparticles as reported by Wong et al.

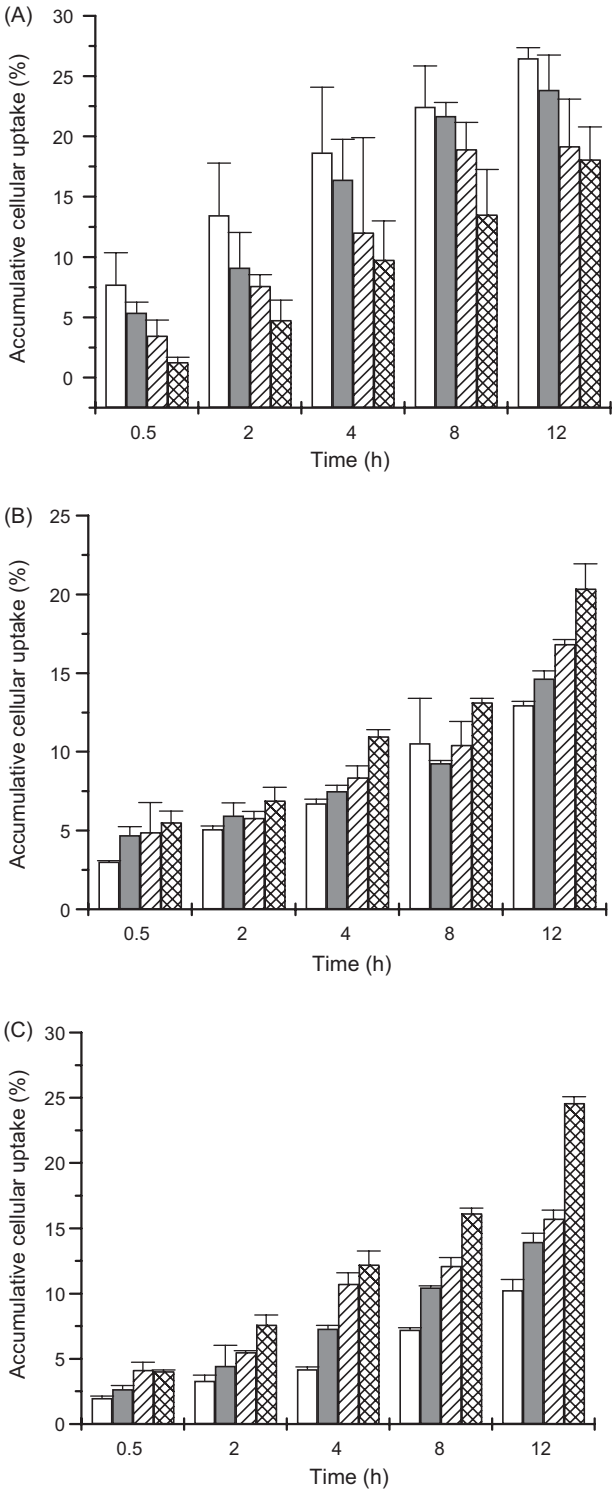

Fig. 4. Quantitative assay of cell uptake of SLNs and pSLNs on RAW264.7 (A), MCF-7 (B) and A549 (C) cells. (□) SLNs; (■) 5% pSLNs; (▨) 10% pSLNs; (▩) 15% pSLNs. All plots were average values ($n = 3$).

3.3. Drug entrapment efficiency

In our previous studies, it was found that the lecithin and oleic acid (OA) in nanoparticles played important roles to improve the drug entrapment. The vinorelbine was amphoteric molecular, which can associate with phospholipid by hydrophobic and hydrophilic interactions between them. The incorporation of OA into lipid matrix could reduce the viscosity of lipid matrix, and consequently improve the combination between drug and lecithin. Otherwise, the drug (VB) molecules dispersed into lipid matrix had the less chance to contact with aqueous medium in preparing process by cold homogenization technique. Hence, the drug entrapment efficiency (EE) of the SLNs could reach up to 80%. After incorporating PEG_{2000}–SA into lipid matrix, the EE of PEG-modified SLNs declined slightly (see Table 2), which could contribute to the increased hydrophilicity of pSLNs. The drug was easily distributed into aqueous medium in the preparing process.

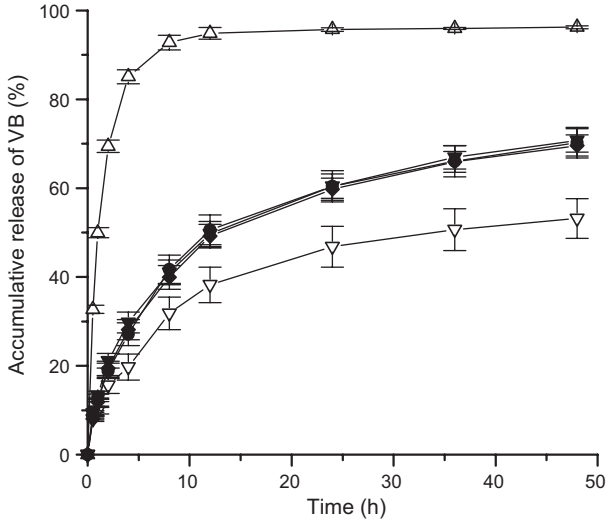

Fig. 5. I*n vitro* drug release profiles of different formulations. (△) free drug; (▽) VB-SLNs; (♦) 5%VB-pSLNs; (●) 10%VB-pSLNs; (▼) 15%VB-pSLNs. All plots were average values ($n = 3$).

3.4. In vitro release behavior

The *in vitro* drug release profiles of VB loaded SLNs with different formulations are shown in Fig. 5. The drug release rate could be adjusted by using OA and lecithin. Especially, OA played an important role in controlling drug release by improving the compatibility of drug (VB) with lipid materials (You et al., 2007). The drug release mechanism might be the corrosion of lipid materials. Herein, the release behavior between VB loaded SLNs (VB-SLNs) and VB loaded PEG-modified SLNs (VB-pSLNs) were

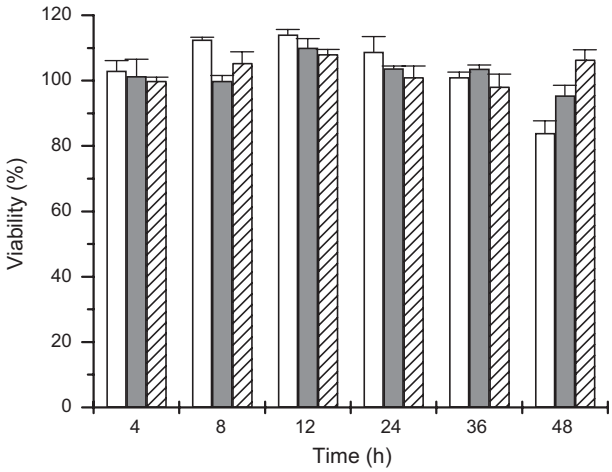

Fig. 6. The assay of biocompatibility of PEG-modified SLNs at the concentration of 250 μg/ml. (□) 5%pSLNs; (■) 10%pSLNs; (▨)15%pSLNs. All plots were average values ($n = 3$).

Table 3
IC_{50} value of free VB, SLNs and pSLNs loading VB against MCF-7 and A549 cells (mean ± S.D., $n = 3$)

Samples	Cell Lines			
	MCF-7		A549	
	IC_{50} ($\mu g\,ml^{-1}$)	Fold	IC_{50} ($\mu g\,ml^{-1}$)	Fold
Free VB	7.64 ± 0.98	1	8.28 ± 1.02	1
VB-SLNs	1.18 ± 0.13	6.5	2.68 ± 0.23	3.1
5%VB-pSLNs	0.716 ± 0.08	10.7	0.962 ± 0.10	8.6
10%VB-pSLNs	0.322 ± 0.07	23.7	0.580 ± 0.05	14.3
15%VB-pSLNs	0.276 ± 0.02	27.7	0.331 ± 0.01	25.0

compared. The drug release rate of VB-pSLNs was faster than that of VB-SLNs, while the drug release rate of VB-pSLNs with different charged amounts of PEG_{2000}–SA were similar. The faster drug release of VB-pSLNs could be contributed to accelerating corrosion of PEG mixed lipid materials. As reported in earlier paper (You et al., 2007), the size of VB-SLNs after release experiments decreased from 225.3 to 173.4 nm, while the size of VB-pSLNs after release experiments decreased from about 200 nm to about 100 nm. The higher decrease extent of particle size after the drug release demonstrated that corrosion of lipid materials could be accelerated by incorporating PEG_{2000}–SA into lipid matrix.

3.5. *In vitro anticancer activity*

The biocompatibility of SLNs was investigated in our previous studies. Herein, the biocompatibility of pSLNs was further investigated. The results are displayed in Fig. 6. The cells could keep above 90% viability after 250 μg/ml pSLNs was incubated with cells for 48 h, which suggested that pSLNs was a safe drug carrier. IC_{50} value of various SLNs loading VB was then investigated to assay the anticancer activity in vitro (Table 3). Comparing with free VB, the anticancer activity in vitro of VB on MCF-7 and A549 cells could be enhanced about 6.5 and 3.1-fold, respectively, by entrapping VB into SLNs. The enhanced fold was enlarged further after entrapping VB into pSLNs. The results could contribute to the increased intracellular drug concentration via the transport of SLNs or pSLNs as the reported by Chawla and Amiji (2003). The higher anticancer activities of VB-pSLNs were due to the enhanced cellular uptake of pSLNs than that of SLNs.

4. Conclusion

PEG-modified SLNs loading vinorelbine bitartrate (VB-pSLNs) was prepared successfully, and pSLNs was demonstrated to be a safe carrier with good biocompatibility. The phagocytosis of pSLNs by RAW264.7 cells was inhibited effectively due to PEG modification, and meanwhile the ability of cancer cell uptake was improved significantly on MCF-7 and A549. Comparing with SLNs loading VB, the drug entrapment efficiency of VB-pSLNs had a slight decline. Otherwise, the drug release rate was accelerated and the drug released more completely in 48 h. The assay of anticancer activity *in vitro* indicated that the anticancer activity of VB was enhanced more sharply by entrapping VB into pSLNs compared with that in SLNs.

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