International Journal of Pharmaceutics 408 (2011) 208–212

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Preparation and characterization of polymeric pH-sensitive STEALTH® nanoparticles for tumor delivery of a lipophilic prodrug of paclitaxel

B.B. Lundberg*

Department of Biosciences, Åbo Akademi University, Artillerigatan 6, FI-20520, Åbo, Finland

ARTICLE INFO

Article history:
Received 25 November 2010
Received in revised form 27 January 2011
Accepted 31 January 2011
Available online 4 February 2011

Keywords:
Nanoparticles
pH-sensitive
Poly(ε-amino ester)
Cytotoxicity
Paclitaxel
Paclitaxel oleate
Prodrug

ABSTRACT

Paclitaxel is an effective and widely used anti-cancer agent. However, the drug is difficult to formulate for parenteral administration because of its low water solubility and Cremophor EL, the expient used for its formulation, has been shown to cause serious side effects. The present study reports an alternative administration vehicle involving a lipophilic paclitaxel prodrug, paclitaxel oleate, incorporated in the core of a nanoparticle-based dosage form. A hydrophobic poly (β-amino ester) (PbAE) was used to formulate the nanoparticles, which were stabilized with a mixture of phosphatidylcholine, Synperonic® F 108, and poly(ethylene glycol)-dipalmitoyl phosphatidyl ethanolamine. PbAE undergoes rapid dissolution when the pH of the medium is less than 6.5 and is expected to rapidly release its content within the acidic tumor microenvironment and endo/lysosome compartments of cancer cells. PbAE nanoparticles were prepared by an ultrasonication method and characterized for particle size and physical stability. The nanoparticles obtained had a diameter of about 70 nm and a good physical stability when stored at 4 °C. *In vitro* cellular uptake and release of paclitaxel oleate PbAE nanoparticles were studied in Jurkat acute lymphoblastic leukemia cells. The results were compared with pclitaxel oleate in poly(ε-caprolactone) (PCL) particles, that do not display pH-sensitive release behavior, and paclitaxel in PbAE particles. Both uptake and release of the prodrug were faster when administered in PbAE than in PCL, but much slower than those of the free drug in PbAE. Cytotoxicity assay was performed on the formulations at different doses. Paclitaxel and paclitaxel oleate showed almost identical activity, IC50 123 and 128 nM, respectively, while that of the prodrug in PCL was much lower with IC50 at 2.5 μM. Thus, PbAE nanoparticles with the incorporated paclitaxel prodrug paclitaxel oleate may prove useful for replacement of the toxic Cremophor EL and also by improving the distribution of the drug to the tumor.

1. Introduction

Paclitaxel (Taxol®, Bristol-Meyers Squibb) is a diterpenoid natural product indicated as a first-line therapy for the treatment of advanced carcinoma of the breast and ovaries (Rowinsky and Donehower, 1995). The compound is highly hydrophobic and virtually insoluble in water. Currently paclitaxel is formulated in a mixture of polyoxyethylated castor oil (Cremophor EL) and 49.7% (v/v) dehydrated ethanol, Diluent12, USP. This excipient is associated with several pharmacological and pharmacokinetic problems including serious hypersensitivity reactions (Weiss et al., 1990). Alternative administration vehicles have been tested where paclitaxel has been solubilized in liposomes (Sharma and Sraubinger, 1994), o/w emulsions (Lundberg, 1997), and mixed micelles (Alkan-Onyuksel et al., 1994). However, the solubilizing capacity of these formulations is quite limited and the drug is rapidly released from such preparations. Another approach has been to make a lipophilic prodrug of paclitaxel. The hydroxyl moiety at C-2′ offers a handle for synthesis of prodrugs by esterification with fatty acids. Such prodrugs have been shown to improve the stability of the drug-carrier complex and yield an effective cytotoxic action after hydrolytic cleavage of the compounds (Ahmad et al., 1999; Bradly et al., 2001; Lundberg et al., 2003). *In vivo* these prodrugs show greater AUC, higher C_{max}, lower systemic clearance and improved antitumor effect compared to the drug in Diluent 12 (Lundberg et al., 2003; Sparreboom et al., 2003; Debotton et al., 2008).

A promising strategy for improving cancer therapy is the utilization of drug loaded nanosize colloidal carriers. The nanocarriers are expected to be biocompatible, biodegradable and enhance the circulation time in the blood stream. They can be divided into three categories; lipid-based, polymer-based or combinations of both. The proper design of the nanocarriers is essential for achieving efficient drug delivery to cancer cells. Factors that have to be regarded with special attention are; the clearance of colloidal particles by the reticuloendothelial system (RES), access to the extravascular space from the blood stream and the stability of the drug-carrier complex.

* Corresponding author. Tel.: +358 2 215 4273; fax: +358 2 2410014.
E-mail address: bolundbe@hotmail.com

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.01.061

Coating of carriers with a hydrophilic polymer like poly (ethylene glycol) modified phosphatidyl ethanolamine (PEG-PE) results in slow clearance (STEALTH® properties) and a major improvement of the utility of carriers (Lundberg et al., 1996; Charrois and Allen, 2003). The extravasation problem can be overcome by the use of particles with small size. *In vivo* experiments with small, long-circulating liposomes have shown that a particle size smaller than 100 nm is favorable for tumor uptake and antitumor activity (Mayhew et al., 1992). Fast leakage of the drug out of the carrier is also a major problem with nanocarriers. The drug can be anchored in the surface layer or in the core of the particle. A high lipophilicity of the drug improves the retention through hydrophobic interaction with the carrier. The drug delivery can also be improved by use of a carrier that responds to intracellular stimuli such as pH, whereby the encapsulated drug can be released at the desired site (Potineni et al., 2003; Shenoy et al., 2005).

The aim of this study was to design a combined lipid–polymer nanoparticle with pH-sensitive and stealth properties for the administration of the prodrug paclitaxel oleate and to find a simple and reliable method for the preparation of such drug-carrier. The ultimate goal is to find a drug-carrier system well suited for active targeting drug delivery, regarding both cellular delivery and pharmacological action. Systems with targeted immunoliposomes and immunoemulsions have shown extensive cellular uptake, but combined with relatively small gain in *in vitro* cytotoxic action (Lopes de Menezes et al., 1999; Lundberg et al., 2004). One possible explanation for this contradictory action is that the drug-carrier complex is immobilized in intracellular compartments or exocytosed. A drug-carrier from which the drug can be released into the cytosol could help to solve this problem.

2. Materials and methods

2.1. Materials

L-α-phosphatidylcholine, from egg yolk (EPC), dipalmitoyl phosphatidyl ethanolamine, poly(ethylene glycol) 2000, Synperonic® F 108, (3-[4,5-dimethylthiazol-2-yl]-2-5-diphenyltetrazolium bromide) (MTT), carbonyldiimidazole, poly(ethylene glycol) methyl ether-block-poly(ε-caprolactone) (PCL) were from Sigma–Aldrich (St. Louis, MO). Paclitaxel was obtained from Alexis (Läufelfingen, Switzerland) and [^{3}H]paclitaxel from Moravek Biochemicals (Brea, CA). Paclitaxel oleate and [^{3}H]paclitaxel oleate were synthesized and characterized by the methods used by Mayhew et al. (1996). In short, equimolar amount of oleoyl chloride was added to paclitaxel in chloroform and incubated at room temperature for 6 h. The esterification takes place preferably at the 2′-hydroxyl of paclitaxel because that group is more reactive than the 7-hydroxyl. After addition of water the reaction mixture was extracted with chloroform. The final product was purified by preparative TLC on silica plates and the purity was checked with analytical silica gel TLC. The hydrophobic poly (β-aminoester) PbAE (MW ∼10,000) was synthesized and purified as described by Potineni et al. (2003) by reaction of 4,4′-trimethyldipeperidine with 1,4-butanediol diacrylate in dimethylformamide for 48 h at 50 °C and purified by precipitation in ethyl ether, washed, and vacuum dried. PEG-PE was synthesized by a method based on the reaction of PEG 2000 with carbonyldiimidazole, followed by addition of DPPE (Allen et al., 1991). The compound was purified by adding a small amount of water to the evaporated reaction residue and the resulting PEG-PE micelles were dialyzed against water using a Spectra/Por® CE 300,000 MWCO dialysis bag (Spectrum Medical Industries, Inc., Houston, TX) for 1 week and then lyophilized (Mauruyama et al., 1992) The purity of the product was checked by silica gel TLC.

2.2. Preparation and characterization of nanoparticles

PbAE and PCL drug-loaded nanoparticles were prepared by an ultrasonication procedure. The surface components EPC (2 mg), Synperonic®F 108 (1 mg), and PEG-PE (0.2 mg) and the core components PbAE (2 mg) or PCL (2 mg) were dispersed into a conic test tube together with the drug (100 nmol) from stock solutions, the solvent was evaporated under a stream of nitrogen and the samples lyophilized overnight. Then 2 ml of phosphate-buffered saline (PBS) was added to the mixture, the sample heated to 60 °C, vortexed for 30 s and then sonicated for 5 min with a Branson sonifier, using continuous mode and an output of 1.5. The sonicated preparations were filtered through a sterile 0.4 μm filter and stored at 4 °C. The loading capacity and efficiency of paclitaxel oleate were determined from radioactivity measurements. The particle size of the nanocarriers was measured by quasielastic laser light scattering using a Malvern Zetasizer Nano. The physical stability of the nanoparticles during storage was determined by repeated measurements of particle size by the particle sizer and by centrifugation for 30 min at 10,000 × *g* and quantization of the dispersed phase by scintillation counting.

2.3. Cell line

Jurkat acute lymphoblastic leukemia T-cells were purchased from American Type Culture Collection (Rockville, MD). The cells were grown in RPMI 1640 medium (Gibco™). The media were supplemented with 2 mM L-glutamine, 10 μg mL^{-1} streptomycin, 10 μg mL^{-1} and 10% heat-inactivated fetal calf serum. Cells were maintained at 37 °C and gassed with 5% CO_2 in air.

2.4. Nanoparticle drug uptake and release by cells

For quantitative studies of cellular uptake of drug, nanocarriers were loaded with [^{3}H]-paclitaxel oleate or [^{3}H]-paclitaxel. The experiments were performed in growth media with 5×10^6 cells, which were seeded into Ø10 cm Petri dishes. For continuous exposure studies, the dishes were incubated at 37 °C and at periodic time intervals the cells were thoroughly washed with cold PBS and harvested for scintillation counting. For drug release studies the cells were allowed to accumulate drugs for 4 h, then the cells were washed with sterile PBS and new media added. The incubation was continued and again the cells were washed and counted at periodic time intervals.

2.5. Cytotoxicity assays

Jurkat cells (10^6 cells per well) were seeded into 24-well plates and the *in vitro* cytotoxicity of the PbAE and PCL nanocarriers loaded with paclitaxel oleate or paclitaxel was measured with a proliferation assay utilizing tetrazolium dye, MTT (Mosmann, 1983). Control experiments included drug-free preparations. The cells were incubated for 24 h at 37 °C in growth medium. At the end of the incubation time, tetrazolium dye in PBS was added, the formed reduction product was spun down, dissolved in EtOH:DMSO 1:1 and read at 570 nm.

3. Results and discussion

3.1. Nanoparticle preparation and characterization

Therapeutics like paclitaxel, which require intracellular administration and subsequent trafficking to the nucleus, benefit by a drug carrier that respond to intacellular stimuli such as pH (Soppimath et al., 2001; Lee et al., 2008). PbAE used in this study as core material is insoluble at physiological pH (7.4), but become soluble in aqueous media at pH below 6.5 (Lynn et al., 2001; Akinc and Langer,

2002) and is thus suitable as a drug delivery system for intracellular release of the drug upon exposure to acidic endosomal vesicles. An additional favorable factor is the acidic tumor microclimate, with pH around 6.0, common in solid tumors.

The standard method for preparation of polymer nanoparticles is the solvent displacement method which is quite laborious involving organic solvents, centrifugation and washing (Shenoy et al., 2005; Pulkkinen et al., 2008). In this study a sonication method was chosen because it has proven to be convenient and effective for producing nanosize lipid emulsions (Lundberg, 1997). In a series of initial experiments the composition as well as the sonication conditions, time, output and temperature, were varied to obtain a small size, good stability and effective drug loading. The efficiency of the emulsification process was recorded by size and stability measurements. A combined polymer–lipid composition was chosen because experiences from lipid emulsion preparation have shown that a combination of phosphatidylcholine and a non-ionogenic surfactant as stabilizing components yield small particles with high stability combined with good biocompatibility (Lundberg, 1997; Lundberg et al., 2003). In this study EPC and the non-ionic surfactant Synperonic F® 108 proved to be a good combination as stabilizing components. As expected the weight ratio between the polymeric core component, PbAE or PCL, and the surface active components was critical for good result. After the initial experiments a standard composition of polymer:EPC:Synperonic® F 108:PEG-PE 1:1:0.5:0.1 was chosen. Paclitaxel oleate and paclitaxel loading capacity and efficiency were evaluated by adding a known amount of the drugs per weight of polymer. The loading capacity increased linearly with drug concentration up to 3% (w/w) and with an efficiency of around 93%.

The size of the drug carriers is a very important factor for efficient drug delivery. When large colloidal particles are administered intravenously they are rapidly taken up by RES (Poste et al., 1982), while small particles (Litzinger et al., 1994) and those with a hydrophilic surface (Chow et al., 2009) show slow clearance rate. In this study the hydrophilic surface was provided by PEG-PE, which has been shown to give long-circulating nanoparticles (Lundberg et al., 1996). A problem with particulate drug carriers is how they might leave the vascular space and reach their site of action. The size of the largest pores of an intact endothelium is around 100 nm (Mayhew et al., 1992). However, experiments with long-circulating liposomes have shown that the pore size of most solid human tumors range from 200 to 600 nm (Monsky et al., 1999). Due to the porosity of the tumor vasculature particulate drug-carriers are preferentially distributed in the tumor due to the enhanced permeability and retention (EPR) effect and resulting in much higher values than after administration of the free drug (Maeda et al., 2000). The sonication procedure used in this study produced very small PbAE nanoparticles with a *Z*-average diameter of about 70 nm. The size distribution of a representative preparation is shown in Fig. 1. The PCL nanoparticles were somewhat larger with a *Z*-average diameter of about 85 nm. The nanoparticles thus have proper physical properties for gaining access to the tumor tissue.

An important factor from a practical point of view is the physical stability of the nanoparticles. The stability was recorded by the light scattering method and by centrifugation at 10,000 × *g* for 30 min. The physical stability proved to be very good and no significant change in particle size or loss of material was noted up to 3 months of storage at 4 °C. However, it should be noted that the storage of the preparations at low temperature is essential because PbAE is subject to hydrolytic surface degradation in PBS (pH 7.4) at 37 °C (Potineni et al., 2003). The sonication method thus proved to be effective for small scale production of polymer–lipid nanoparticles but for large scale production high pressure homogenization might be more convenient.

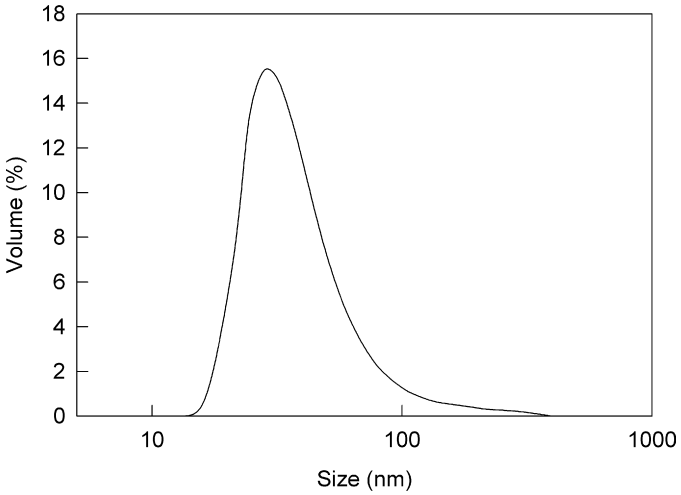

Fig. 1. Size distribution by volume of a typical PbAE nanoparticle preparation measured by laser light scattering.

3.2. Drug uptake and release

The amount of drug in nanoparticles that is concentrated within the cells varied according to the exposed dose. The concentration-dependent uptake of paclitaxel oleate and paclitaxel by Jurkat cells during 4 h is presented in Fig. 2. The accumulation of paclitaxel from PbAE nanoparticles was much larger than that of paclitaxel oleate from PbAE and PCL nanoparticles. Paclitaxel and paclitaxel oleate in PbAE reached saturation at about 1.5 μM. Paclitaxel oleate in PCL did not reach saturation within the concentration range studied and the intracellular amount of drug approached that of the drug in PbAE at the highest concentrations studied.

Time course for the uptake of drugs in nanoparticles is shown in Fig. 3. Paclitaxel in PbAE is accumulated very fast in the cells and a steady state is reached at 1 h. A slight efflux of the drug is in fact noted after that time point. This behavior indicates that a major portion of the uptake process is governed by surface transfer. Such a transfer process is thought to involve desorption into and diffusion trough the aqueous interface of the exchanging molecules (Phillips et al., 1987). In fact, it has been shown that the *in vitro* release of paclitaxel from polymeric nanoparticles is almost complete after 1 h (Pulkkinen et al., 2008) and that about 65% of the

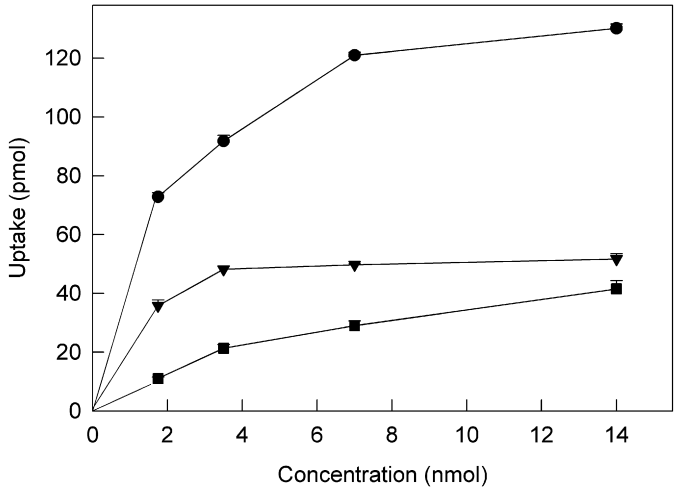

Fig. 2. Concentration-dependent cellular uptake of paclitaxel (●) and paclitaxel oleate (▼) in PbAE nanoparticles and of paclitaxel oleate (■) in PCL nanoparticles during a 4 h incubation at 37 °C in growth media (mean ± S.E., $n = 4$).

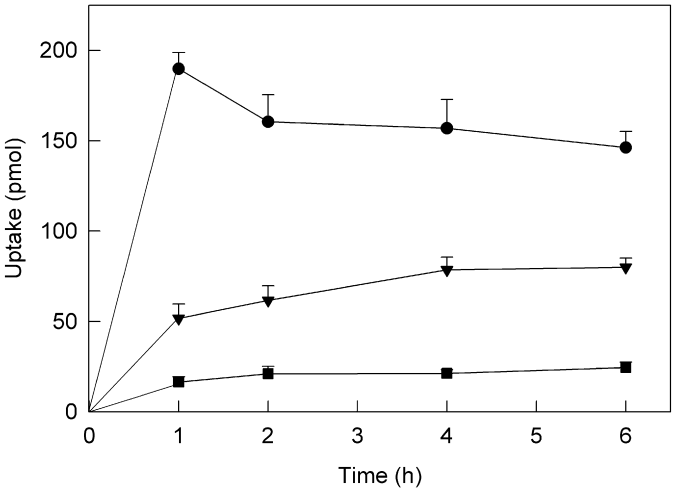

Fig. 3. Time course of cellular uptake obtained with a concentration of 0.7 μM of paclitaxel (●) and paclitaxel oleate (▼) in PbAE nanoparticles and of paclitaxel oleate (■) in PCL nanoparticles incubated in growth media at 37 °C (mean ± S.E., $n = 4$).

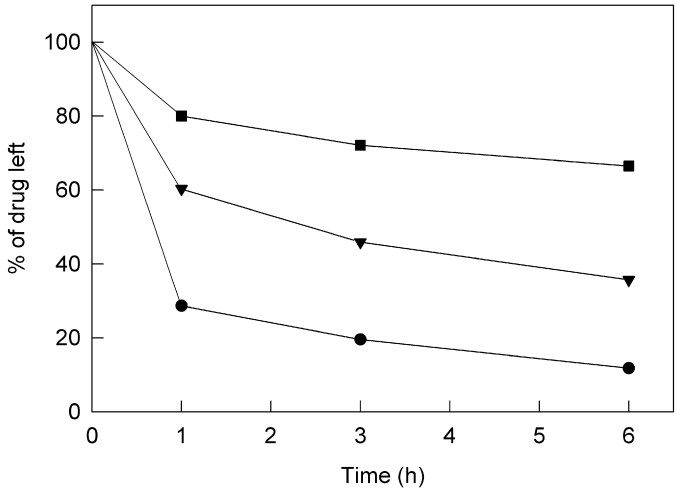

Fig. 4. Time course of cellular release of paclitaxel (●) and paclitaxel oleate (▼) accumulated by PbAE nanoparticles and paclitaxel oleate (■) accumulated by PCL nanoparticles (mean ± S.E., $n = 4$).

drug accumulation by cells from lipid emulsion particles is due to surface transfer (Lundberg, 1997). On the other hand, the results indicate a different mechanism for the accumulation of the prodrug paclitaxel oleate. Paclitaxel has a water solubility of 12.8 μM at 20 °C and a $K_{O/W}$ of 311 (Lundberg, 1997), while paclitaxel oleate is much more lipophilic with a water solubility of 34 nM and a $K_{O/W}$ of 8074 (Lundberg et al., 2003). In fact the $K_{O/W}$ value of the prodrug are in the same range as that of the non-exchangeable lipid cholesteryl oleoyl ether at 10,755. The uptake of nanoparticles is predominantly governed by nonspecific endocytosis (Panyam and Labhasetwar, 2004). The low water solubility and high $K_{O/W}$ value of the prodrug result in a slow leakage from the drug-carrier as shown for lipid nanosize emulsions (Lundberg et al., 2003). It can thus be deduced that the prodrug is taken up mainly by nonspecific endocytosis together with the nanoparticle and this constitutes the first limiting step. The second step, the accumulation of free drug, is governed by the fate of the nanoparticles within the intracellular compartments. The PCL nanoparticles are highly stable at the intracellular conditions and the influx will continue at an extent and rate governed by the endocytotic process. It is likely that the drug together with the carrier will stay for a relatively long time in the endo-lysosomal compartments and the equilibrium process will be slow (Figs. 2 and 3). The scenario for the prodrug in PbAE seems to be different due to the pH-triggered dissolution behavior. The drug together with the nanoparticle is taken up by endocytosis, but once within the cell the nanoparticles quickly disintegrate in acidic endo-lysosomal compartments releasing the free drug within the intracellular matrix. Thus the drug is spread widely in the cell and the accumulation of drug will proceed faster than for drug administered in PCL.

The drug release studies are represented in Fig. 4. For paclitaxel in PbAE a fast drop in intracellular concentration down to a baseline value was noted, after which the leakage was slow. The PCL-paclitaxel oleate nanoparticles, when accumulated in cells during the incubation period, undergo slow degradation resulting in near-steady concentration of drug. The PbAE-paclitaxel oleate nanoparticles, on the other hand, are rapidly dissolved within the cells and free drug is released, after which there is diffusion of the drug out of the cells.

A conclusion deduced from the fast leakage of paclitaxel from cells as well as from nanoparticles is that this type of preparations might be used as sustained-release vehicles, but not as a stable system for active targeting of drug. On the other hand, the prodrug paclitaxel oleate in PbAE is more stable and the residue time in cells is longer, rendering it better suited for targeting purposes.

3.3. *In vitro* cytotoxic activity

The *in vitro* cytotoxic activity of paclitaxel oleate in PbAE and PCL and paclitaxel in PbAE nanoparticles was tested on Jurkat acute lymphoblast leukaemia T-cells during 24 h incubation in growth media. The cell viability was checked by a proliferation assay utilizing MTT. The drug-free nanoparticles had no significant cytotoxic activity. Dose–response curves for the preparations are shown in Fig. 5. It can be noted that the cytotoxic activities of paclitaxel oleate and paclitaxel in PbAE were almost identical. Notable is that intact prodrug has no cytotoxic activity until metabolized to the active molecule paclitaxel (Bradly et al., 2001). Apparently hydrolytic cleavage of the ester linkage at the C-2′-hydroxyl is fast and the active free drug can perform its action. On the other hand paclitaxel oleate in PCL showed much lower activity with an IC50 value of 2.5 μM compared to 128 and 123 nM for paclitaxel oleate and paclitaxel in PbAE, respectively. This behavior can be correlated to the much lower drug accumulation by PCL particles (Figs. 2 and 3)

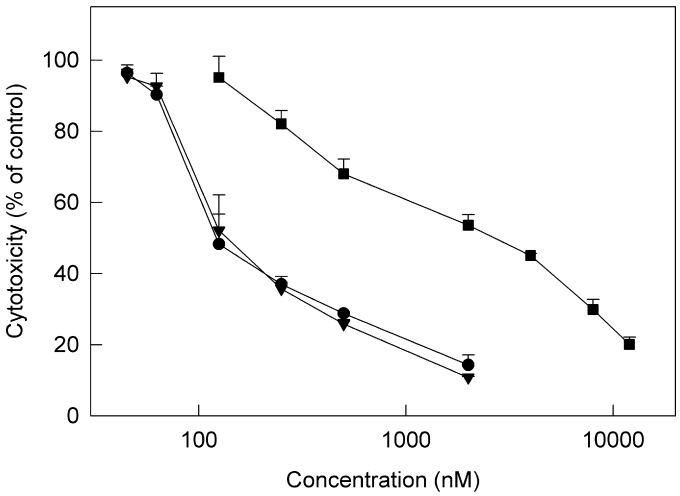

Fig. 5. Percentage cell viability as a function of concentration for paclitaxel (●) and paclitaxel oleate (▼) in PbAE nanoparticles and paclitaxel oleate (■) in PCL nanoparticles (mean ± S.E., $n = 4$).

and be explained on the basis of polymer characteristics. The drug encapsulated in PCL nanoparticles is retained within the particles upon reaching the intracellular matrix, while for the drug in PbAE the payload is rapidly dissolved into the cytosol after ingestion of the particles, resulting in efficient cell kill. The cytotoxicity values obtained in this study can be compared with those obtained by Shenoy et al. (2005) for paclitaxel in PbAE and PCL. They noted a relatively slight preference for paclitaxel in PbAE, which might be explained by the difference in retention of paclitaxel oleate and paclitaxel in the nanoparticles. The leakage of the free drug from the nanoparticles is much faster than that of the prodrug (Lundberg et al., 2003; Pulkkinen et al., 2008). That means that paclitaxel in PCL, well inside the cell will leak out into the cytosol while paclitaxel oleate will be retained inside the PCL particle for a longer time. In contrast, the drug-PbAE complex will disintegrate well inside the cell and both paclitaxel oleate and paclitaxel will be able to exert their action within the same timeframe.

It can be concluded that PbAE loaded with the paclitaxel fatty acid ester prodrugs seems to be a promising delivery system for paclitaxel. This statement is also supported by *in vivo* studies showing greater AUC, higher C_{max} and lower systemic clearance (Wolff et al., 2003; Lundberg et al., 2003; Debotton et al., 2008), combined with better *in vivo* antitumor activity (Bradly et al., 2001; Mayhew et al., 2001) compared to paclitaxel in Cremophor EL/ethanol. In addition to solving the solubility issue, improving the pharmacokinetic properties and pharmacologic action these prodrugs offer the possibility of active targeted drug delivery that may improve the therapeutic index (Huang and Oliff, 2001; Debotton et al., 2008).

In the past decade, a number of nanoparticle-based drug delivery systems have been developed for therapeutic applications (Lee et al., 2008). Among these systems *nab*-Paclitaxel (Abraxane®), an albumin-bound nanoparticle formulation of paclitaxel, that takes advantage of the increased delivery of albumin to tumor through receptor-mediated transport, has been clinically approved (Petrelli et al., 2010). The aim of the present study was to form a platform for pH-sensitive selective targeting by use of surface-functionalized ligands in line with previous studies with lipid immuno-nanoparticles (Lundberg et al., 2004).

References

Ahmad, I., Masters, M.R., Schupsky, J.J., Nguyen, J., Ali, S., Janoff, A.S., Mayhew, E., 1999. Growth inhibition of a human ovarian tumor by a novel paclitaxel derivative in SCID mice. Oncol. Res. 11, 273–280.

Akinc, A., Langer, R., 2002. Measuring the pH environment of DNA delivered using nonverbal vectors: implications for lysosomal traffickin. Biotechnol. Bioeng. 78, 503–508.

Alkan-Onyuksel, H., Ramakrishnan, S., Chai, H.B., Pezzuto, J.M., 1994. A mixed micellar formulation suitable for potential administration of taxol. Pharm. Res. 11, 206–212.

Allen, T.M., Hansen, C., Martin, F., Redemann, C., Yau-Young, A., 1991. Liposomes containing synthetic lipid derivatives of poly(ethylene glycol) show prolonged circulation half-lives in vivo. Biochim. Biophys. Acta. 1066, 29–36.

Bradly, M.O., Webb, N.L., Anthony, F.H., Devanesan, P., Witman, P.A., Hemamalini, S., Chander, M.C., Baker, S.D., He, L., Horwitz, S.B., Swindell, C.S., 2001. Tumor targeting by covalent cojugation of a natural fatty acid to paclitaxel. Clin. Cancer Res. 7, 3229–3238.

Charrois, G.J., Allen, T.M., 2003. Multiple injections of pegylated liposomal Doxorubicin; pharmacokinetics and therapeutic activity. J. Pharmacol. Exp. Ther. 306, 1058–1067.

Chow, T.H., Lin, Y.Y., Hwang, J.J., Wang, H.E., Wang, S.J., Liu, R.S., Lin, W.J., Yang, C.S., Ting, G., 2009. Improvement of misdistribution and therapeutic index via increase of polyethylene glycol on drug-carrying liposomes in an HT-29/luc engrafted mouse model. Anticancer Res. 29, 2111–2120.

Debotton, N., Parnes, M., Kadouche, J., Benita, S., 2008. Overcoming the formulation obstacles towards targeted chemotherapy: *in vitro* and *in vivo* evaluation of catatonic drug loaded immunonanoparticles. J. Control. Release 127, 219–230.

Huang, P.S., Oliff, A., 2001. Drug-targeting strategies in cancer therapy. Curr. Opin. Genet. Dev. 11, 104–110.

Lee, E.S., Gao, Z., Bae, Y.H., 2008. Recent progress in tumor pH targeting nanotechnology. J. Control. Release 132, 164–170.

Litzinger, D.C., Builting, A.M., Van Rooijen, N., Huang, L., 1994. Effect of liposome size on the circulation time and intraorgan distribution of amphipathic poly(ethylene glycol)-containing liposomes. Biochim. Biophys. Acta 1190, 99–107.

Lopes de Menezes, D.E., Kirchmeier, M.J., Gagne, J.-F., Pilarski, L.M., Allen, T.M., 1999. Cellular trafficking and cytotoxicity of anti-CD19-targeted liposomal doxorubicin in B lymphoma cells. J. Liposome Res. 9, 199–228.

Lundberg, B.B., Mortimer, B.C., Redgrave, T.G., 1996. Submicron lipid emulsions containing amphipathic polyethylene glycol for use as drug-carriers with prolonged circulation time. Int. J. Pharm. 134, 119–127.

Lundberg, B.B., 1997. A submicron lipid emulsion coated with amphipathic polyethylene glycol for potential administration of Paclitaxel (taxol). J. Pharm. Pharmacol. 49, 16–21.

Lundberg, B.B., Risovic, V., Ramaswamy, M., Wasan, K.M., 2003. A lipophilic paclitaxel derivative incorporated in a lipid emulsion for parental administration. J. Control. Release 86, 93–100.

Lundberg, B.B., Griffiths, G., Hansen, H.J., 2004. Cellular association and cytotoxicity of anti-CD74-targeted lipid drug-carriers in B lymphoma cells. J. Control. Release 94, 155–161.

Lynn, D.M., Amiji, M.M., Langer, R., 2001. pH-responsive polymer micro spheres: rapid release of encapsulated material within the range of intracellular pH. Angew. Chem. Int. Ed. Engl. 40, 1707–1710.

Maeda, H., Wu, J., Sawa, T., Matsumura, Y., Hori, K., 2000. Tumor vascular permeability and the EPR effect in macromolecular therapeutics; a review. J. Control. Release 65 (1–2), 271–284.

Mauruyama, K., Yuda, T., Okamoto, A., Kojima, S., Suginaka, A., Iwatsuru, M., 1992. Prolonged circulation time in vivo of large unilamellar liposomes composed of distearoyl phosphatidylcholine and cholesterol containing amphipathic poly(ethylene glycol). Biochim. Biophys. Acta. 1128, 44–49.

Mayhew, E., Lasic, D., Babbar, S., Martin, F.J., 1992. Pharmacokinetics and ant tumor activity of epirubicin encapsulated in long-circulating liposomes incorporating a polyethylene glycol-derivatized phospholipid. Int. J. Cancer 51, 302–309.

Mayhew, E., Franklin, J.C., Bhatia, S., Harmon, P.A., Janoff, A.S., 1996. Hydrophobic taxane derivatives. US Patent 5,580,899.

Mayhew, E., Franklin, J.C., Bhatia, S., Harmon, P.A., Janoff, A.S., 2001. Hydrophobic taxane derivatives. U.S. Patent 6,291,690.

Monsky, W.L., Fukumura, D., Gohongi, T., Angukiewcz, M., Weich, H.A., Torchilin, V.P., Yuan, F., Jain, R.K., 1999. Augmentation of transvascular transport of macromolecules and nanoparticles in tumors using vascular endothelial growth factor. Cancer Res. 59 (16), 4129–4135.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival; application to proliferation and cytotoxicity assay. J. Immunol. Methods 65, 55–63.

Panyam, J., Labhasetwar, V., 2004. Sustained cytoplasm delivery of drugs with intracellular receptors using biodegradable nanoparticles. Mol. Pharm. 1, 77–84.

Petrelli, F., Borgonovo, K., Barni, S., 2010. Targeted delivery for breast cancer therapy: the history of nanoparticle-albumin-bound paclitaxel. Expert Opin. Pharmacother. 11, 1413–1432.

Phillips, M.C., Johnson, W.J., Rothblat, G.H., 1987. Mechanism and consequences of cellular cholesterol exchange and transfer. Biochim. Biophys. Acta. 906, 223–276.

Poste, G., Bucana, C., Raz, A., Bugelski, P., Kirsh, R., Fidler, I.J., 1982. Analysis of the fate of systemically administered liposomes and implications for the use in drug delivery. Cancer Res. 42, 1412–1422.

Pulkkinen, M., Pikkarainen, J., Wirth, T., Tarvainen, T., Haapa-aho, V., Korhonen, H., Seppälä, J., Järvinen, K., 2008. Three-step tumor targeting of Paclitaxel using biotinylated PLA-PEG nanoparticles and avidin–biotin technology: formulation development and *in vitro* anticancer activity. Eur. J. Pharm. Biopharm. 70, 66–74.

Potineni, A., Lynn, D.M., Langer, R., Amiji, M.M., 2003. Poly(ethylene oxide)-modified poly(β-amino ester) nanoparticles as a pH-sensitive biodegradable system for Paclitaxel delivery. J. Control. Release 86, 223–234.

Rowinsky, E.K., Donehower, R.C., 1995. Paclitaxel (Taxol). N. Engl. J. Med. 332, 1004–1014.

Sharma, A., Sraubinger, R.M., 1994. Novel taxol formulations: preparation and characterization of taxol-containing liposomes. Pharm. Res. 11, 889–896.

Shenoy, S., Little, S., Langer, R., Amiji, M., 2005. Poly(ethylene oxide)-modified poly (β-amino ester) nanoparticles as a ph-sensitive system for tumor-targeted delivery of hydrophobic drugs. Part I. *In vitro* evaluations. Mol. Pharm. 2, 357–366.

Soppimath, K.S., Aminabhavi, T.M., Kulkarni, A.R., Rudzinski, W.E., 2001. Biodegradable polymeric nanoparticles as drug delivery devices. J. Control. Release 70, 1–20.

Sparreboom, A., Wolff, A.C., Verweij, J., Zabelina, Y., van Zomeren, D.M., McIntire, G.L., Swindell, C.S., Donehower, R.C., Baker, S.D., 2003. Disposition of docosahexaenoic acid-paclitaxel, a novel taxane, in blood: *in vitro* and clinical pharmacokinetic studies. Clin. Cancer Res. 9, 151–159.

Weiss, R.B., Donehower, R.C., Wiernik, P.H., Ohnuma, T., Gralla, R.J., Trump, D.L., Baker, J.R., Van Echo, D.A., Von Hoff, D.D., Leyland-Jones, B., 1990. Hypersensitivity reactions from taxol. J. Clin. Oncol. 8, 1263–1268.

Wolff, A.C., Donehower, R.C., Carducci, M.K., Carducci, M.A., Brahmer, J.R., Zabelina, Y., Bradley, M.O., Anthony, F.H., Swindell, C.S., Witman, P.A., Webb, N.L., Baker, S.D., 2003. Phase I study of docosahexaenoic acid-paclitaxel: a taxane-fatty acid conjugate with unique pharmacology and toxicity profile. Clin. Cancer Res. 9, 3589–3597.