JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 1359–1364

Received December 21, 2006
Accepted June 28, 2007
DOI 10.1211/jpp.59.10.0005
ISSN 0022-3573

Stealth and non-stealth nanocapsules containing camptothecin: in-vitro and in-vivo activity on B16-F10 melanoma

Gecioni Loch-Neckel, Daiane Nemen, Ana Cristina Puhl, Daniel Fernandes, Marco Augusto Stimamiglio, Márcio Alvarez Silva, Mirela Hangai, Maria Claúdia Santos Silva and Elenara Lemos-Senna

Abstract

Camptothecin (CPT) is an alkaloid that displays considerable antitumour activity, but clinical use has been limited by its poor water solubility and the instability of the lactone moiety (active form) in physiological media. We have therefore formulated the drug into nanocarrier systems in an attempt to improve its therapeutic properties. This study evaluates the effect of intraperitoneally administered stealth and non-stealth nanocapsules containing CPT on lung metastatic spread in mice inoculated with B16-F10 melanoma cells, and on the cytotoxic activity against B16-F10 melanoma cells in-vitro. Poly (D,L-lactide) PLA (non-stealth) and methoxy polyethylene glycol-(D,L-lactide) (PLA-PEG) (stealth) nanocapsules (49 and 66.6 kDa) were prepared by interfacial deposition of preformed polymer. CPT, as free drug or as drug-loaded nanocapsules, was administrated at a dose of 0.5 mg kg^{-1} at 3-day intervals for 17 days. Free drug and CPT-loaded nanocapsules reduced the number of metastatic nodules by 45.09–91.76% ($P < 0.05$ vs positive control). However, only CPT-loaded PLA-PEG 49 kD nanocapsules significantly decreased the number of lung metastases when compared with free drug ($P < 0.05$). The administration of CPT-loaded nanocapsules and free drug did not result in neutropenia at the administered dose. The improved effectiveness of pegylated nanocapsules was attributed to protection of the drug by nanoencapsulation and to reduced uptake of particles by macrophages located in the lymph nodes. This assumption was supported by the in-vitro study, in which both PLA and 49 kDa PLA-PEG nanocapsules containing CPT were more cytotoxic than the free drug against B16-F10 melanoma cells.

Laboratório de Farmacotécnica, Departamento de Ciências Farmacêuticas, Universidade Federal de Santa Catarina, Brazil

Gecioni Loch-Neckel, Daiane Nemen, Ana Cristina Puhl, Daniel Fernandes, Elenara Lemos-Senna

Laboratório de Neurobiologia e Hematologia Celular e Molecular, Departamento de Biologia Celular, Embriologia e Genética, Universidade Federal de Santa Catarina, Brazil

Marco Augusto Stimamiglio, Márcio Alvarez Silva

Laboratório de Oncologia Experimetal e Hemopatias, Departamento de Análises Clínicas, Universidade Federal de Santa Catarina, Universidade Federal de Santa Catarina, Brazil

Mirela Hangai, Maria Claúdia Santos Silva

Correspondence: Elenara Lemos-Senna, Laboratório de Farmacotécnica, Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal de Santa Catarina, Campus Trindade, Florianópolis, 88040-970, Brazil. E-mail: lemos@ccs.ufsc.br

Acknowledgements: The authors wish to thank Rede Nanobiotec/CNPq for financial support.

Introduction

Camptothecin (CPT) is a cytotoxic alkaloid found in *Camptotheca acuminata*. CPT displays considerable anticancer activity, via a mechanism involving the inhibition of topoisomerase I, an enzyme involved in DNA replication, recombination and transcription. This drug encloses in its structure a highly conjugated pentacyclic ring with an α-hydroxylactone moiety at carbon 12, which is essential for its in-vitro and in-vivo antitumour activity. However, CPT is very poorly water soluble, limiting its administration by intravenous infusion. Furthermore, CPT is unstable at physiological pH, undergoing a fast non-enzymatic hydrolysis of the lactone ring, leading to formation of the less active and more toxic carboxylate form (Wall & Wani 1996; Iyer & Ratain 1998). Reduced cell membrane binding, membrane diffusibility and intrinsic potency against the topoisomerase target may explain the reduction in cytotoxic activity that accompanies opening of the lactone ring of CPT (Hatefi & Amsden 2002).

Several hydrophilic derivatives have been developed in order to overcome the low aqueous solubility of CPT. In spite of improvements in water solubility provided by chemical modification, topotecan and irinotecan (CPT-11) – CPT derivatives approved for human use – are also susceptible to inactivation in physiological media (Garcia-Carbonero & Supko 2002; Hatefi & Amsden 2002). In addition, diarrhoea and myelosuppression have been reported as the most important dose-limiting toxicities of CPTs, the severity of which depends on the administration schedule (O'Leary & Muggia 1998; Garcia-Carbonero &

Supko 2002). Thus, in view of the biological relevance of the intact lactone ring, alternative strategies to improve the solubility and therapeutic properties of CPTs have been sought. One way to accomplish these goals has been to formulate CPT and its analogues into nanocarrier delivery systems. In general, drugs incorporated into nanocarrier systems exhibit changed biodistribution and decreased toxicity compared with the free drug, therefore leading to new therapeutic opportunities.

Several studies have demonstrated the advantages of nanoencapsulation as a strategy to delivery CPTs (Tyner et al 2004; Mu et al 2005; Noble et al 2006). Although nanocarrier systems have potential application in cancer therapy, nanocapsules are rapidly removed from the circulation by phagocytosis and preferentially targeted to the liver and spleen, limiting delivery of the drug to other organs. So-called stealth nanocapsules that display hydrophilic surface coating such as polyethylene glycol (PEG) are designed to resist recognition and uptake by phagocytic cells, and exhibit long plasma residence after intravenous administration (Gref et al 1995; Ameller et al 2003). In the case of CPTs, drug delivery into pegylated stealth nanocarriers may provide improved chemical stability of the encapsulated drug and prolonged drug exposure.

Previously, we have demonstrated that CPT-loaded microspheres are able to inhibit the growth and the metastatic spread to the lungs of B16-F10 melanoma cells injected intravenously into mice, in the same way as free drug, but with a significant reduction in drug toxicity (Dora et al 2006).

In this study we evaluate the effect of the administration of stealth and non-stealth CPT-containing nanocapsules on lung metastatic spread in mice injected with B16-F10 cells. In addition, we wanted to verify the cytotoxic activity of the nanosuspensions on B16-F10 cells in-vitro. Haematological toxicity was also evaluated.

Materials and Methods

Materials

CPT was purchased from Sigma-Aldrich (St Louis, MO, USA). Benzyl benzoate and polyoxyethylene sorbitan monooleate (Tween 80) were obtained from Delaware (Porto Alegre, Brazil), and sorbitan monooleate (Span 80) from Beraca (São Paulo, Brazil). Poly(D,L-lactide) (PLA) 16 kDa was obtained from Boehringer Ingelheim (Ingelheim am Rhein, Germany); 49 kDa and 66.6 kDa methoxy polyethylene glycol-(D,L-lactide) (PLA-PEG; 20% PEG) were kindly provided by Alkermes Inc. (Cincinnati, OH, USA). Except for methanol used in HPLC analysis (Tedia, Fairfield, CT, USA), all other reagents and solvents were of analytical grade and were used as received.

Cells

A highly metastatic B16-F10 mouse epithelial-like melanoma cell line was donated by Bio-Rio (Rio de Janeiro, Brazil). Cells were cultured in RPMI-1640 medium (Sigma-Aldrich), buffered with 2 g L^{-1} HEPES and 1.5 g L^{-1} sodium bicarbonate, and supplemented with 4.5 g L^{-1} dextrose, 100 units mL^{-1} penicillin, 100 μmol mL^{-1} streptomycin and 10% fetal bovine serum, in a humidified atmosphere containing 5% CO_2 at 37 °C. Cells were harvested with a trypsin:EDTA (0.05:0.03 w/v) solution, washed and inoculated into mice in phosphate-buffered saline (PBS; pH 7.4), as described below.

Preparation of nanocapsule suspensions

Nanocapsule suspensions were prepared by an interfacial deposition process after a solvent displacement process, as described by Fessi et al (1989). Briefly, 40 mg PLA or PLA-PEG (49 or 66.6 kDa) was dissolved in 10 mL acetone containing 0.12 mL benzyl benzoate, 40 mg Span 80 and 0.30 mg CPT. This organic phase was then poured into an aqueous phase (20 mL) containing 0.15% Tween 80 (w/v) (pH ≤ 5.0) with magnetic stirring. Acetone was eliminated by evaporation under reduced pressure and the final volume of the suspension was adjusted to 10 mL.

Measurement of encapsulation efficiency and drug content

Drug loading of nanocapsules was determined by reverse-phase HPLC. Analysis was carried out using a Supelcosil LC-18 column (15 cm × 4.6 mm ID, 5 μm; Supelco, St Louis, MO, USA). The mobile phase was 50:50 (v/v) methanol:10 mM KH_2PO_4, adjusted to pH 2.8 with phosphoric acid, delivered at a flow rate of 1.0 mL min^{-1}. The injection volume was 20 μL and CPT was detected by UV absorption at 254 nm. Samples were injected in triplicate and the CPT concentration was determined by comparing the peak area corresponding to the drug with that obtained with a standard CPT solution. Encapsulation efficiency (%) was estimated as the difference between the total concentration of CPT found in the nanocapsule suspensions after complete dissolution in methanol and the concentration of drug in the supernatant obtained by a suspension ultrafiltration/centrifugation procedure using Ultrafree-MC membranes (100 000 nominal molecular weight limit; Millipore, Billerica, MA, USA). The CPT content was expressed in μg mL^{-1} suspension.

Particle size and zeta potential

Mean particle diameter and zeta potential were determined by photon correlation spectroscopy and laser-Doppler anemometry, respectively, using a Zetasizer 3000HS (Malvern Instruments, Malvern, Worcestershire, UK). For all measurements, each sample was diluted to the appropriate concentration with filtered distilled water. Each size analysis lasted 120 s and was performed at 25°C with an angle detection of 90°. For measurements of zeta potential, samples of nanocapsules were placed in the electrophoretic cell, where a potential of ±150 mV was established. The ζ potential values were calculated from mean electrophoretic mobility values using Smoluchowski's equation.

Evaluation of the antimetastatic activity of CPT-loaded nanocapsules

Sixty-day-old Swiss female mice were used. The animals were housed at 23 ± 2°C and 60 ± 10% humidity in a 12-hour light–dark cycle. Food and water were given ad libitum. In-vivo assays were approved by our university's Ethics Committee for Animal Use and were based on the principles of animal care.

Nine groups of eight mice were used (see Table 2) in the evaluation of the antimetastatic activity of the nanocapsules. Mice were inoculated with 3×10^4 B16-F10 cells suspended in 100 μL PBS pH 7.4 via the intraorbital vein. Starting on the second day after inoculation of B16-F10 cells, free CPT, or unloaded or CPT-loaded nanocapsules were administered intraperitoneally at a dose of 0.5 mg kg^{-1} at intervals of 3 days (i.e. on days 2, 5, 8, 11 and 14), corresponding to a total administered dose of 2.5 mg kg^{-1}. In order to improve the dispersion of CPT in the vehicle, CPT was suspended in PBS pH 7.4 containing 0.3% (w/v) sodium carboxymethylcellulose and 0.2% (w/v) Tween 80. Negative control mice received only the vehicle; positive controls received both the cells and the vehicle. Animals were killed by asphyxiation in a carbon dioxide chamber after 17 days. The lungs were excised and fixed with a 10% formaldehyde solution and metastatic colonies were counted using a dissection microscope. The number of pulmonary metastases observed in the mice treated with free drug, vehicle, unloaded nanocapsules and CPT-loaded nanocapules were compared statistically.

Haematological toxicity

The toxicity of free drug and CPT-loaded nanocapsules was evaluated in blood collected by cardiac puncture and stored in heparinized propylene tubes. Haemograms were obtained by flow cytometry using a haematological counter (Serono Baker System 9000 counter coupled with a haematology analyser; Allentown, PA, USA).

Evaluation of in-vitro cytotoxicity

In-vitro cytotoxicity was evaluated using the 3-(4,5-dimethiazol-zyl)-2-5-diphenyltetrazolium bromide (MTT) assay (Van de Loosdrecht et al 1991). Free CPT, unloaded or CPT-loaded nanocapsule suspensions were added to B16-F10 cells in different concentrations in a maximum volume of 20 μL. Cells were incubated at 37°C for 24 h. After incubation, MTT was added to each well, followed by a 3 h incubation. Cells were centrifuged, the supernatant discarded and the formazan precipitated was dissolved with 100 μL 0.04 N isopropyl alcohol/HCl solution. The absorbance was determined at 540 nm using a microplate reader. Control groups were plated with only MTT reagent. All assays were performed in triplicate.

Statistical analysis

Statistical analysis of antimetastatic activity was performed using one-way analysis of variance followed by the Newman–Keuls multiple comparison test. A two-way analysis of variance followed by Newman–Keuls multiple comparison test was used to compare the in-vitro cytotoxicity of unloaded and CPT-loaded nanocapsule suspensions. All statistical analyses were performed using the Graph-Pad Prism software (San Diego, CA, USA).

Results and Discussion

Characterization of the nanocapsule suspensions

A summary of the physicochemical properties of the nanocapsule suspensions is presented in Table 1. Monodispersed distributions of particles with mean diameters of 148–177.3 nm were obtained using the nanoprecipitation method. Zeta potential values ranged from −25 mV (when PLA nanocapsules were used) to −17.9 mV (when 66.6 kDa PLA-PEG nanocapules were used). The reduction of the zeta potential can be attributed to the presence of the PEG chains on the surface of the particles, which cover the carboxyl end groups of the PLA located near the surface, masking the negative charge. The gradual modification of the zeta potential can be ascribed to an increase in the thickness of the PEG coating layer with the chain length of PEG (Gref et al 1995).

Encapsulation efficiency was above 80% for all formulations. These high values can be explained by maintenance of the less-water-soluble lactone form of the CPT during the nanoencapsulation process, which was provided by adjusting the pH of the aqueous phase to below 5.0. Drug loading ranged from 8.49 to 26.56 μg mL^{-1} and was highest when 49 kDa PLA-PEG was used to prepare the nanocapsule suspensions.

In-vivo antimetastatic effect

The ability of malignant neoplasms to produce secondary growths (metastases) in organs distant from the primary tumour is the lethal event in the clinical course of most neoplasic diseases. While primary cancers can be resected surgically or irradiated locally, it is usually difficult to use these

Table 1 Encapsulation efficiency, drug content, particle size and zeta potential obtained from camptothecin-loaded nanocapsule suspensions. Data are mean ± s.d. and for encapsulation efficiency and drug content are from nanocapsule suspensions prepared in triplicate

Polymer	Encapsulation efficiency (%)	Drug content (μg mL^{-1})	Mean diameter (nm) (polydispersity index)	Zeta potential (mV)
PLA	93.71 ± 1.71	15.01 ± 0.99	177.3 (0.12)	−25.0 ± 1.0
49 kDa PLA-PEG	97.35 ± 0.43	26.56 ±1.01	148.0 (0.12)	−19.6 ± 0.8
66.6 kDa PLA-PEG	83.90 ± 1.74	8.49 ± 0.33	159.0 (0.17)	−17.9 ± 1.5

therapeutic modalities against a disseminated disease. The lungs and the liver are the earliest sites colonized by the most metastatic tumours (Fidler 1989).

The B16-F10 melanoma cell line is characterized by its ability to produce lung metastases at a rate of 5×10^{-5} per cell per generation (Khanna & Hunter 2005). In this study, stealth and non-stealth nanocapsules were tested for their ability to inhibit the number of lung colonies. An important aspect of the experimental protocol is that the volume of nanocapsules administered was adjusted according to the drug concentration of the suspensions (i.e. the drug loading). Given that intravenous administration was initially intended, nanocapsule suspensions were concentrated 25-fold, with the objective of maximizing the drug concentration and allowing administration of CPT at 1.5–2.0 mg kg^{-1}. However, preliminary assays of spontaneous lung metastases revealed high toxicity of nanocapsule suspensions prepared from PLA, even those prepared without CPT: almost all the animals died before the end of the experiments and showed marked clinical signs (dyspnoea, reduced motor activity) before death. In fact, a previous study had reported that administration of massive doses of PLA nanoparticles leads to the saturation of the mononuclear phagocytic system, prolonging the exposure of the particles to plasma proteins such as the coagulation components, and causing a disseminated intravascular coagulation and associated events. Clinical signs were absent after administration of nanocapsules prepared from PLA-PEG, reported to be the result of stearic repulsion stemming from the high density of PEG chains on the nanoparticle surface, which prevents induction of the coagulation cascade, according to the same mechanism involved in the complement activation (Plard & Bazile 1999). For this reason, the nanocapule suspensions were concentrated only three times and were administered by intraperitoneal injection at a dose of 0.5 mg kg^{-1}.

The effect of free drug, unloaded and CPT-loaded nanocapsules on lung metastatic spread after inoculation of 3×10^4 B16-F10 melanoma cells is summarized in Table 2. Administration of free drug, CPT-loaded PLA nanocapsules and CPT-loaded 49 kDa and 66.6 kDa PLA-PEG nanocapsules reduced the number of metastatic nodules compared with the positive control (vehicle-treated inoculated mice) by 45.09% ($P < 0.05$), 60.53%, 91.76% and 89.01% (all $P < 0.001$), respectively. CPT-loaded 49 kDa PLA-PEG nanocapsules reduced the number of metastatic nodules compared with the administration of free drug ($P < 0.05$).

It could be argued that nanoencapsulation protected the drug against hydrolysis in physiological medium, thus serving as a repository of the active form of CPT, which in turn may be absorbed from the peritoneal cavity into the circulation. Furthermore, nanoparticles administered by the intraperitoneal route can be absorbed via lymphatic capillaries and can reach the lymph nodes. Since the chemical composition of lymph is similar to that of plasma, containing all the plasma proteins but at lower concentration, particle uptake by fixed macrophages of lymph nodes may occur following opsonisation (Hawley et al 1995; Nishioka & Yoshino 2001). As the interaction with the proteins is dramatically reduced by the presence of hydrophilic coating, the improved effectiveness of the pegylated nanocapsules in reducing metastatic spread to the lungs may to some extent be connected to their ability to escape macrophage recognition.

Toxicity studies

In order to evaluate the toxicity associated with CPT administration, haematological parameters were measured on the 17th day, and compared with values obtained from normal control mice. The red blood cell count, haemoglobin content and hematocrit values were within the normal range for all treated groups. In contrast, an increase in platelet count (thrombocytosis) rather than thrombocytopenia was observed after administration of the unloaded and CPT-loaded nanocapsules, suggesting the presence of an inflammatory process caused by intraperitoneal administration of the particles (results not shown). Myelosupression caused by severe neutropenia is the main dose-limiting toxic effect of CPT (Garcia-Carbonero & Supko 2002). Total white blood cell count and neutrophil count are summarized in Table 2. The lower

Table 2 Effect of free drug and camptothecin (CPT)-loaded nanocapsules on pulmonary metastasis, total white blood cell count and neutrophil count in mice inoculated with 3×10^4 B16-F10 melanoma cells. Free drug or nanocapsules were administered at a CPT dose of 0.5 mg kg^{-1} at 3-day intervals (total dose 2.5 mg kg^{-1}) and the mice were killed on day 17. Positive controls were inoculated mice treated with vehicle. Negative controls received vehicle but were not inoculated. Data are mean ± s.d.

	Number of metastases[a] (n = 8)	Total WBC (per mm^3)[b]	Neutrophil count (% WBC)[b]
Negative control	0	6580 ± 1273	25.80 ± 29.88
Positive control	14.57 ± 2.23	6750 ± 2379	9.00 ± 4.30
Unloaded PLA nanocapsules	7.40 ± 7.40	5520 ± 1596	42.2 ± 28.77
Unloaded 49 kDa PLA-PEG nanocapsules	9.00 ± 6.56	7460 ± 1316	25.40 ± 3.93
Unloaded 66.6 kDa PLA-PEG nanocapsules	9.67 ± 5.61	4825 ± 1035	18.75 ± 2.28
Free CPT	8.00 ± 1.41*	10120 ± 331	32.00 ± 29.91
CPT-loaded PLA nanocapsules	5.75 ± 2.12†	7940 ± 3047	16.20 ± 4.12
CPT-loaded 49 kDa PLA-PEG nanocapsules	1.20 ± 1.64†,‡	6540 ± 902	20.60 ± 7.68
CPT-loaded 66.6 kDa PLA-PEG nanocapsules	1.60 ± 1.52†	6750 ± 2225	23.50 ± 6.34

[a]Statistical significance was evaluated using one-way analysis of variance followed by the Newman–Keuls test. *$P < 0.05$, $^{\dagger}P < 0.001$ vs positive control; $^{\ddagger}P < 0.05$ vs free drug.

[b]Normal total WBC count: 6000–15 000 per mm^3; normal neutrophil count: 10–40% of total WBC count.

limit of the normal neutrophil count in mice represents 10% of the total white blood count. Thus, administration of encapsulated CPT or free drug did not cause neutropenia in the mice, which can be related to the schedule of dose administration. CPT was administered at 0.5 mg kg^{-1} CPT at 3 day intervals. Dora et al (2006) demonstrated that administration of larger doses of CPT to mice not only caused neutropenia but also diarrhoea and weight loss. In our study, administration of low doses of CPT in nanocapsules inhibited the growth and pulmonary metastasis of B16-F10 melanoma but without causing marked toxicity.

Viability assay (MTT assay)

Data obtained in the in-vitro cytotoxic study with B16-F10 melanoma cells are shown in Figure 1. Incubation of B16-F10 melanoma cells with CPT-loaded nanocapsules caused significant cell death, in a concentration-dependent manner. PLA and 49 kDa PLA-PEG nanocapsules containing CPT induced a significantly higher toxic effect than free drug at the concentration of 0.1 $\mu g\,mL^{-1}$, and the percent of viable cells was 26.3 ± 4.4% and 35.4 ± 4.5%, respectively ($P < 0.05$). At 0.01 $\mu g\,mL^{-1}$, free drug and both types of CPT-loaded nanocapsule suspensions reduced the percentage of viable cells by 20–25% ($P < 0.05$ vs control).

These results indicate that nanoencapsulation protects the drug from inactivation in the culture medium, since the cytotoxic activity of the CPT-loaded nanocapsules against B16-F10 melanoma cells was significantly greater than CPT alone at a concentration of 0.1 $\mu g\,mL^{-1}$. CPT-loaded PLA nanocapsules displayed a high cytotoxicity against B16-F10 melanoma cells, which is in contrast to in-vivo results, in which it was demonstrated that PLA nanocapsule suspensions did not significantly reduce the number of lung metastases compared with administration of free drug. This result corroborates the assumed adsorption of the lymph protein onto PLA nanocapsules after intraperitoneal administration of the suspensions, followed by recognition and uptake of the particles by fixed macrophages of lymph nodes.

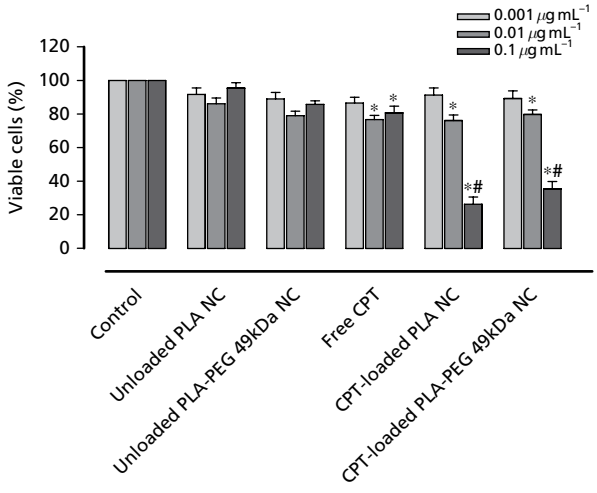

Figure 1 Cytotoxic effect of free camptothecin (CPT), unloaded and CPT-loaded nanocapsules (NC) on B16-F10 melanoma cells. Optical density of control groups was taken as 100% cell viability, as confirmed by the Trypan Blue exclusion method. $^{*}P < 0.05$ compared with control group; $^{\#}P < 0.05$ compared with free CPT at the same concentration (n = 3).

Conclusion

The results obtained in this study suggest that CPT-loaded nanocapsules have antimetastatic and cytotoxic activity. Administration of CPT-loaded nanocapsules or free drug did not result in neutropenia. At the dose administered, all nanocapsule suspensions were effective against the B16-F10 melanoma. However, CPT-loaded 49 kDa PLA-PEG nanocapsule suspensions demonstrated, to some extent, a higher effectiveness against lung metastases than PLA nanocapsules. The benefit provided by pegylated nanocapsules was evidenced not only by the improved stability of CPT in physiological medium, but also by the increased residence time of the particles in the physiological medium and hence of its therapeutic effectiveness.

References

Ameller, T., Marsaud, V., Legrand, P., Gref, R., Renoir, J. M. (2003) *In vitro* and *in vivo* biologic evaluation of long-circulating biodegradable drug carriers loaded with pure antiestrogen RU. *Int. J. Cancer* **106**: 446–454

Dora, C. L, Alvarez-Silva, M., Trentin, A. G., de Faria, T. J., Fernandes, D., da Costa, R., Stimamiglio, M., Lemos-Senna, E. (2006) Evaluation of antimetastatic activity and systemic toxicity of camptothecin-loaded microspheres in mice injected with B16-F10 melanoma cells. *J. Pharm. Pharmaceut. Sci*. **9**: 22–31

Fessi, H., Puisieux, F., Devissaguet, J. P., Ammoury, N., Benita, S. (1989) Nanocapsule formation by interfacial polymer deposition following solvent displacement. *Int. J. Pharm*. **55**: R1–R4

Fidler, I. J. (1989) Origin and biology of cancer metastasis. *Cytometry* **10**: 673–680

Garcia-Carbonero, R., Supko, J. (2002) Current perspectives on the clinical experiences, pharmacology, and continued development of the camptothecins. *Clin. Cancer Res*. **8**: 641–661

Gref, R., Domb, A., Quellec, P., Blunk, T., Müller, R. H., Verbavatz, J. M., Langer, R. (1995) The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanospheres. *Adv. Drug Del. Rev*. **16**: 215–233

Hatefi, A., Amsden, B. (2002) Camptothecin delivery methods. *Pharm. Res*. **19**: 1389–1399

Hawley, A. E., Davis, S. S., Illum, L. (1995) Targeting of colloids to lymph nodes: influence of lymphatic physiology and colloidal characteristics. *Adv. Drug Del. Rev*. **17**: 129–148

Iyer, L., Ratain, M. J. (1998) Clinical pharmacology of camptothecins. *Cancer Chemother. Pharmacol*. **42**: 31–43

Khanna, C., Hunter, K. (2005) Modeling metastasis *in vivo*. *Carcinogenesis* **26**: 513–523

Mu, L., Elbayoumi, T. A., Torchilin, V. P. (2005) Mixed micelles made of poly(ethylene glycol)-phosphatidylethanolamine conjugate and d-alpha-tocopheryl polyethylene glycol 1000 succinate as pharmaceutical nanocarriers for camptothecin. *Int. J. Pharm*. **306**: 142–149

Nishioka, Y., Yoshino, H. (2001) Lymphatic targeting with nanoparticulate system. *Adv. Drug. Del. Rev*. **47**: 55–64

Noble, C. O., Krauze, M. T., Drummond, D. C., Yamashita, Y., Saito, R., Berger, M. S, Kirpotin, D. B., Bankiewicz, K. S., Park, J. W. (2006) Novel nanoliposomal CPT-11 infused by convection-enhanced delivery in intracranial tumors: pharmacology and efficacy. *Cancer Res*. **66**: 2801–2806

O'Leary, J., Muggia, F. M. (1998) Camptothecins: a review of their development and schedules of administration. *Eur. J. Cancer* **34**: 1491–1548

Plard, J. P., Bazile, D. (1999) Comparison of the safety profiles of PLA_{50} and Me.PEG-PLA_{50} nanoparticles after single dose intravenous administration to rat. *Colloids Surf. B: Biointerf.* **16**: 173–183

Tyner, K. M., Schiffman, S. R., Giannelis, E. P. (2004) Nanohybrids as delivery vehicles for camptothecin. *J. Control. Release*. **95**: 501–514

Van de Loosdrecht, A. A., Nennie, E., Ossenkoppele, G. H., Beelen, R. H. J., Langenhuijsen, M. M. (1991) Cell mediated cytotoxicity against U937 cells by human monocytes and macrophages in a modified colorimetric MTT assay. *J. Immunol. Methods* **141**: 15–22

Wall, M. E., Wani, M. C. (1996) Camptothecin and taxol: from discovery to clinic. *J. Ethnopharmacol*. **51**: 239–254