European Journal of Pharmaceutics and Biopharmaceutics 73 (2009) 230–238

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Novel self-assembling PEG-p-(CL-*co*-TMC) polymeric micelles as safe and effective delivery system for Paclitaxel

Fabienne Danhier [a], Nicolas Magotteaux [a], Bernard Ucakar [a], Nathalie Lecouturier [a], Marcus Brewster [b], Véronique Préat [a,*]

[a] Université Catholique de Louvain, Unité de Pharmacie Galénique, Brussels, Belgium
[b] Johnson and Johnson, Pharmaceutical Research and Development, Division of Janssen Pharmaceutica, Beerse, Belgium

ARTICLE INFO

Article history:
Received 20 April 2009
Accepted in revised form 29 June 2009
Available online 3 July 2009

Keywords:
Polymeric micelle
Chemotherapy
Self-assembly
Nanomedicine
Anti-tumor efficacy

ABSTRACT

Paclitaxel (PTX) is an effective anti-cancer drug currently used to treat a wide variety of cancers. Unfortunately, nonaqueous vehicle containing Cremophor® EL is associated with serious clinical side effects. This work aimed to evaluate the ability of polymeric micelles to (i) solubilize PTX without Cremophor® EL and to be used as a (ii) safe and (iii) effective delivery system for PTX. Hence, we developed novel self-assembling poly(ethyleneglycol)$_{750}$-block-poly(ε-caprolactone-*co*-trimethylenecarbonate) (PEG-p-(CL-*co*-TMC)) polymeric micelles which form micelles spontaneously in aqueous solution. The solubility of PTX increased up to three orders of magnitude. The PTX-loaded micelles showed a slow release of PTX with no burst effect. The HeLa cells viability assessed by the MTT test was lower for PTX-loaded micelles than for Taxol® (IC_{50} 10.6 vs. 17.6 μg/ml). When solubilized in micelles, PTX induced apoptosis comparable with Taxol®. The maximum tolerated doses (MTD) of PTX-loaded micelles and Taxol® in mice were 80 mg/kg and 13.5 mg/kg, respectively, after intraperitoneal administration; and 45 mg/kg and 13.5 mg/kg, respectively, after intravenous administration. Similar anti-tumor efficacy of PTX-loaded micelles and Taxol® was observed at the dose of 13.5 mg/kg on TLT-tumor-bearing mice, while the body weight loss was only observed in Taxol® group. However, as higher dose was tolerated (80 mg/kg – IP), a higher growth delay was induced with PTX-loaded micelles. These results demonstrated that PTX-loaded self-assembling micelles present a similar anti-tumor efficacy as Taxol®, but significantly reduced the toxicity allowing the increase in the dose for better therapeutic response.

1. Introduction

Polymeric micelles have been described as promising nanocarriers. Their hydrophobic core increases the solubility of poorly water-soluble drugs, whereas the hydrophilic corona allows a long circulation of the drug by preventing the interactions between the core and the blood components [1,2]. Due to their prolonged circulation time, polymeric micelles are able to accumulate at certain biological sites characterized by vascular abnormalities, such as tumors, through the enhanced permeation and retention (EPR) effect. Endothelial cells of tumors are poorly aligned and present wide fenestrations. Polymeric micelles, which generally have a diameter less than 100 nm, are able to penetrate inside the tumors (enhanced permeation). Typically, this phenomenon is coupled with defective lymphatic drainage which allows the polymeric micelles to remain in the tumor (retention) [3,4].

Recently, novel self-assembling diblock copolymers made up of ε-caprolactone (CL), trimethylene carbonate (TMC) and mmePEG$_{750}$ (mmePEG$_{750}$-p-(CL-*co*-TMC)) have been shown to form micelles spontaneously upon gentle mixing with water. Neither organic solvent nor the dialysis or evaporation step is required for the entrapment of the drug. PEG-p-(CL-*co*-TMC) micelles show a high kinetic and thermodynamic stability (CMC = 20 μg/ml). These copolymers are also biocompatible, noncytotoxic and nonhemolytic (up to 10% w/v). They increase the solubility of poorly soluble-water drugs by one to four orders of magnitude [5–7].

Paclitaxel (PTX) is known as one of the most effective anti-cancer drugs marketed today. Significant anti-tumor activity has been demonstrated in clinical trials against a wide variety of tumors, including ovarian carcinoma, metastatic breast cancer, head and neck cancers and nonsmall lung cancer [8–10]. PTX has a low therapeutic index and a very low aqueous solubility [11]. PTX is currently formulated (Taxol®) at the concentration of 6 mg/ml dissolved in mixture of Cremophor® EL (polyethoxylated caster oil) and ethanol (50:50, v/v). However, Cremophor® EL induces significant toxicity, including hypersensitivity, nephrotoxicity and neurotoxicity. Therefore, the current challenge is to formulate

* Corresponding author. Université Catholique de Louvain, Unité de Pharmacie Galénique, Avenue Mounier, 73-20, B-1200 Brussels, Belgium. Tel.: +32 2 7647320; fax: +32 2 7647398.
E-mail address: veronique.preat@uclouvain.be (V. Préat).

0939-6411/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2009.06.015

PTX in a nontoxic vehicle. Accordingly, a number of alternative formulations were investigated for the solubilization of PTX, including liposomes, microspheres, nanoparticles (e.g. Abraxane®) and polymeric micelles [12–15].

The aim of this work was to investigate whether the self-assembling PEG-p-(CL-*co*-TMC) system can be used both to solubilize PTX avoiding the use of Cremophor® EL and to constitute a safe and effective delivery system for PTX. Therefore, the solubility of PTX in the PEG-p-(CL-*co*-TMC) micellar solutions was determined. The *in vitro* release of PTX from the micelles was assessed. The *in vitro* anti-tumoral activity and the induction of apoptosis of PTX-loaded micelles were performed using Human Cervix Epithelial Carcinoma cells (HeLa). Biodistribution studies were performed on TLT-tumor-bearing mice with C^{14}-labelled polymers. The safety of PTX-loaded micelles was evaluated *in vivo*. Finally, *in vivo* anti-tumor efficacy was also investigated on TLT-tumor-bearing mice.

2. Materials and methods

2.1. Materials

PTX was purchased from Calbiochem (Darmstadt, Germany). Taxol® was obtained from Brystol-Myers Squibb. The mmePEG$_{750}$-p(CL-*co*-TMC) (50:50 M ratio) diblock copolymers were synthesized by the Johnson and Johnson Center for Biomaterials and Advanced technologies (Somerville, NJ, USA). Monomethyl ether polyethylene glycol with a chain length of 750 g/mol (mme PEG$_{750}$) was purchased from Fluka (Milwaukee, WI, USA). ε-Caprolactone (CL) was purchased from Union Carbide (Danbury, CT, USA), and trimethylene carbonate (TMC) from Boehringer Ingelheim (Petersburg, VA, USA). [^{14}C]-PEG-p(CL-*co*-TMC) (50:50 M ratio) polymer was synthesized by Perkin Elmer employing ε-[caprolactone-2,6-^{14}C]. HeLa (Human Cervix epithelial Carcinoma) cells were acquired from ATCC (American Type Culture Collection, Manassas, VA, USA). Cremophor® EL, 4,6-diamidino-2-phenylindole (DAPI) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, trypsin–EDTA and penicillin–streptomycin mixtures were from Gibco® BRL (Carlsbad, CA, USA). Ultra-purified water was used throughout, and all other chemicals were of analytical grade. Male NMRI mice were purchased from Janvier, Genest-St. Isle, France.

2.2. Micelles formulation

2.2.1. Polymer synthesis and characterization

The copolymer was synthesized by ring-opening polymerization as described previously [5] (Fig. 1.). Briefly, the reaction was initiated by PEG monomethylether of 750 Da (mmePEG$_{750}$) at a molar monomer/initiator ratio of 13.3:1. ε-Caprolactone or [^{14}C]ε-caprolactone and trimethylene carbonate were added at 1:1 molar ratio. The reaction was catalyzed by stannous octoate and was allowed to run for 24 h at 160 °C. The polymer was then devolatilized under vacuum at 90 °C for 48–72 h. The molecular weight and the polydispersity of the diblock polymer were determined by gel permeation chromatography and the monomer ratio in the polymer was determined by NMR spectroscopy.

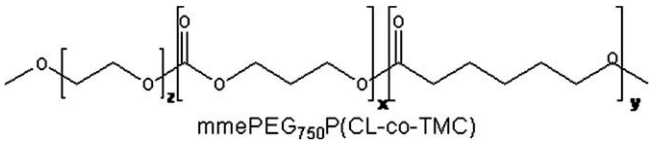

Fig. 1. Representation of the structure of mmePEG$_{750}$-p(CL-*co*-TMC) copolymers.

Radiolabelling of mmePEG$_{750}$-p(CL-*co*-TMC) was generated by replacing unlabelled ε-caprolactone by ε-[caprolactone-2,6-^{14}C] during the polymerization. The radiochemical purity of [^{14}C]-mmePEG$_{750}$-p(CL-*co*-TMC) was checked by a HPLC system equipped with a radioactivity detector as described earlier (>97%) [16,17].

2.2.2. Preparation of PTX-loaded micelles: solubility studies

The solubility of PTX in 1%, 5% and 10% (w/v) of PEG-p(CL-*co*-TMC) in water was determined. A stock solution of PTX (2.5 mg/ml) in acetonitrile was evaporated. The liquid polymer was added to the vial and mixed overnight at 37 °C. Water was then added drop wise to form the micelles. The excess drug was removed by filtration through 0.45 μm PVDF filters [5,6,18–19].

The amount of PTX solubilized was determined in triplicate by HPLC (Agilent 1100 series, Agilent Technologies, Diegem, BE). The mobile phase consisted of acetonitrile/water (70:30 v/v). The reverse phase column was a CC 125/4 Nucleod UR 100-5 C18. The column temperature was maintained at 30 °C. The flow rate was set at 1.0 ml/min, and the detection wavelength was 227 nm. Sample of 50 μl solution was injected. The HPLC was calibrated with standard solutions of 5–100 μg/ml of PTX dissolved in acetonitrile (correlation coefficient of R^2 = 0.9965, LOD = 1.6 μg/ml, LOQ = 5 μg/ml, coefficients of variation <4.3%). Micelles were dissolved in acetonitrile [19,20].

2.2.3. Physicochemical characterization of PTX-loaded micelles

The particle size and ζ potential of the micelles were determined, respectively, by photon correlation spectroscopy (PCS) and laser Doppler velocimetry, combined with phase analysis light scattering (PALS) using a Zetasizer® Nano ZS (Malvern Instruments, UK). The measurements were performed in triplicate in PBS (phosphate buffer solution, pH 7.4).

2.3. In vitro evaluation

2.3.1. Release of PTX from PTX-loaded micelles

The *in vitro* release of PTX from PEG-p(CL-*co*-TMC) micelles and Taxol® was evaluated by a dialysis method. PTX-loaded micelles (PTX = 1.8 mg/ml, 10% copolymers) were prepared in PBS, and Taxol® was diluted in PBS (PTX = 1.8 mg/ml). A volume of 1.5 ml of these solutions were placed into dialysis membrane tubing (Spectra/Por 7, regenerated cellulose, Spectrum Laboratories) with a 2000 Da molecular weight cut-off and immersed into 50 ml PBS at 37 °C with stirring. Dialysis was performed for 24 h and the amount of PTX released into medium was measured after 10 min, 1, 2, 4, 19 and 24 h. The PTX concentration in the different samples was determined by HPLC as described in Section 2.2.

2.3.2. Anti-tumoral activity

HeLa cells were grown in DMEM with 10% (v/v) fetal bovine serum, 100 IU/ml of penicillin G sodium and 100 μg/ml of streptomycin sulfate. The cells were maintained in an incubator supplied with 5% CO_2 at 37 °C.

In vitro cytotoxicity of Taxol®, PTX-loaded micelles, Cremophor® EL and drug-free micelles was determined by the MTT assay. HeLa cells were seeded in 96-well plates at the density of 2500 viable cells per well and incubated for 24 h to allow cell attachment. Cells were then incubated with the different formulations (PTX concentrations of 0.025, 0.25, 2.5, 12.5 and 25 μg/ml; Cremophor® EL concentrations corresponding to Taxol® formulation: 0.22–2.2 mg/ml and 10% PEG-p(CL-*co*-TMC) copolymers) for 24, 48 and 72 h. The supernatant of each well was removed. Wells were then washed twice by PBS and incubated with DMEM containing MTT (5 mg/ml) for an additional 4 h. MTT was aspirated off and DMSO was added to dissolve the formazan crystals [21]. Absorbance was

measured at 570 nm using a BioRad microplate reader. Untreated cells were taken as control with 100% viability, and cells without addition of MTT were used as blank to calibrate the spectrophotometer to zero absorbance. Triton X-100 1% was used as a positive control of cytotoxicity [19,22]. The results were expressed as mean values ± standard deviation of 5 measurements.

2.3.3. Apoptosis induced by PTX-loaded micelles

Apoptosis was identified morphologically by 4,6-diamidino-2-phenylindole (DAPI) staining. HeLa cells were seeded in 6-well plates containing a coverslip with 10^5 cells per well and cultured at 37 °C for 24 h. Cells were then incubated for 4 h with Taxol®, PTX-loaded micelles (PTX concentration of 25 μg/ml) or culture medium as control. Samples were then fixed with 4% paraformaldehyde in PBS at room temperature for 15 min and stained with 0.2 μg/ml DAPI in PBS at room temperature for 15 min, then washed twice with PBS and once with water. Coverslips were mounted onto glass slides and the slides were examined using a fluorescent microscope with a 340/380 nm excitation filter and LP 430 nm barrier filter. Enumeration of apoptotic nuclei (about 200 cells were counted) were made on slides picked up at random by two independent experimenters. Clusters of apoptotic bodies were given as a single count [19].

2.4. In vivo evaluation

2.4.1. Tumor model

All experiments were performed in compliance with guidelines set by national regulations and were approved by the ethical committee for animal care of the faculty of medicine of the Université Catholique de Louvain. Transplantable liver tumors (TLTs) [23] were implanted in the gastrocnemius muscle in the rear leg of 8 week-old male NMRI mice. The treatments were applied when the tumor reached 8.0 ± 0.5 mm in diameter (approximately 7 days). This tumor model was chosen because it is a fast growing model which presents intrinsic resistance. Indeed, when tumor reaches 9–10 mm, tumor vasculature is totally changed and necrosis appears. The resultant high tumor interstitial fluid pressure is described to constitute a barrier for efficient anti-cancer drug delivery. It is a major cause of the unsuccessfulness of chemotherapy. It is so a particularly interesting model to study the efficacy of nanocarriers which are known to be less affected by this enhanced IFP in tumors [24].

2.4.2. Biodistribution of PTX-loaded PEG-p(^{14}C-CL-co-TMC) micelles

PTX-loaded [^{14}C]-micelles with a volume of 200 μl was injected through the tail vein of mice (1.5 mg/ml PTX and 100 mg/ml PEG-p(CL-*co*-TMC)). Blood samples were taken via retro-orbital venous plexus puncture from mice at 5, 20 min, 1, 4, 24 and 48 h after i.v. administration of micelles. Three mice were used for each interval time. The terminal half-life ($t_{1/2\beta}$) was determined from the slope of the terminal linear portion of the log blood concentration–time profiles. Five mice per group were sacrificed at 24 and 48 h after i.v. administration of PTX-loaded micelles. The organs and tissues (heart, lung, liver, spleen, kidneys, tumor and contro-lateral muscle) were removed and weighed. Tissue Solubilizer Biolute-S (Serva, Germany) with a volume of 100 μl and 100 μl of hydrogen peroxide (35%) were added to 50 μl of the blood samples or to the tissues or organs, and these mixtures were incubated at room temperature overnight. [16].

Ultima Gold® scintillation cocktail of 10 ml was added to these samples before counting the radioactivity in a scintillation counter (Packard Tricarb 2900 TR). The percent of administered dose and the percent of administered dose per gram of wet tissue values were calculated using the following equations:

$$\%\ \text{Administered dose} = \frac{\text{Bq in tissue sample}}{\text{Bq of injected micelles}} \times 100$$

$$\%\ \text{Administered dose/g tissue} = \frac{\%\ \text{Admnistered dose}}{\text{Weight of wet tissue in g}}$$

2.4.3. Toxicity of PTX-loaded micelles

To determine the toxicity of PTX-loaded micelles, the maximum tolerated dose (MTD) of PTX-loaded micelles and Taxol® administered intraperitonealy or intravenously was investigated in mice. To evaluate the intraperitoneal route, mice were injected once with PTX-loaded micelles at the doses of 13.5, 30, 50, 80 and 120 mg/kg. Taxol® was also injected intraperitonealy to mice at doses of 13.5, 30, 50 and 80 mg/kg. The intravenous route was also investigated injecting PTX-loaded micelles at doses of 13.5, 22.5, 45 and 67 mg/kg (due to the volume needed to be injected, doses were divided in three injections for 3 consecutive days). Taxol® was also injected once intravenously to mice at doses of 13.5, 30 and 50 mg/kg. Mice were observed for 2 weeks in all groups, and the number of surviving mice was recorded. The MTD was defined as the allowance of a median body weight loss of 15% of the control and causes neither death due to toxic effects nor remarkable changes in general signs within 1 week after administration.

2.4.4. Anti-tumor efficacy of PTX-loaded micelles

The effect of PTX-loaded micelles on TLT growth was assessed by daily measurement of the diameter of the tumors with an electronic caliper. When TLT tumors (implantation described above) reached 8.0 ± 0.5 mm in diameter, the mice were randomly assigned to a treatment group. Four groups of mice (six mice per group) were treated: group 1, PBS injection; group 2, nonloaded micelles; group 3, Taxol® (PTX concentration of 13.5 mg/kg; diluted in PBS); group 4, PTX-loaded micelles (PTX dose of 13.5 mg/kg). The treatments were injected through the tail vein. After treatment, tumors were measured daily until they reached a diameter of 18 mm, at which time the mice were sacrificed. Body weight was also monitored every day.

TLT-tumor-bearing mice were also treated with PTX-loaded micelles at PTX doses of 30, 50 and 80 mg/kg, administered intraperitonealy. Tumor diameters and body weights were also recorded daily.

2.5. Statistics

All results are expressed as mean ± standard deviation. Two-way ANOVA and Bonferroni post test were performed to demonstrate statistical differences ($p < 0.05$); IC_{50} values were calculated from a dose–response graph (not shown) with sigmoidal function with variable Hill slope, using the software GraphPad Prism 5 for Windows.

3. Results

3.1. Micelles formulations

3.1.1. Polymer and characterization

The chemical composition of the polymers (Fig. 1) determined by proton NMR was in good agreement with the ratio of the charged monomers: the CL/TMC molar ratio for mmePEG$_{750}$-p-(CL-*co*-TMC) was 50:50 mol/mole. The weight-averaged molecular weights (Mws) of mmePEG$_{750}$-p-(CL-*co*-TMC) and [^{14}C]-mmePEG$_{750}$-p-(CL-*co*-TMC) were 5242 Da (PD = 1.9) and 5188 Da (PD = 1.6), respectively. The polymer critical micellar concentration was in the range of 20 μg/ml (0.002%; w/v) [16]. The polymers were liquid at room temperature and formed micelles spontaneously in the presence of aqueous media.

3.1.2. Preparation and physicochemical characterization of PTX-loaded micelles

The influence of the percentage of PEG-p(CL-*co*-TMC) on the solubility of PTX is illustrated on Fig. 2. PEG-p(CL-*co*-TMC) increased the solubility of PTX by three orders of magnitude. In the formulations containing 10% of PEG-p(CL-*co*-TMC), the solubility of PTX increased from the aqueous solubility of 1 μg/ml [11] to 1.82 ± 0.11 mg/ml. The higher the polymer concentration, the higher the solubility. Micelles containing 10% of PEG-p(CL-*co*-TMC) were chosen for the future experiments because this formulation was able to solubilize therapeutic concentrations of PTX.

PEG-p(CL-*co*-TMC) block copolymers spontaneously formed micelles [5,6]. Consistent with previous data [16], the size and ζ potential of micelles containing 10% of PEG-p(CL-*co*-TMC) were 23.9 ± 0.6 nm (PDI: 0.08) and −3.1 ± 0.4 mV (width: 3.4), respectively. The percentage of the polymer in the formulations and the solubilization of PTX did not influence the size and ζ potential of micelles (data not shown).

3.2. In vitro evaluation

3.2.1. Release of PTX from PTX-loaded micelles

The result of the cumulative PTX release profile from PEG-p(CL-*co*-TMC) micelles at 37 °C in PBS (pH 7.4) is shown in Fig. 3. PTX released from Taxol® was also investigated as control. Only 42% PTX was released from the micelles in 4 h while 88% PTX was released from Taxol® during the same period. The PEG-p(CL-*co*-TMC) micelles released 76% PTX during 24 h. No significant burst release was observed as compared to Taxol®, as described by other groups [15,25,26].

3.2.2. Anti-tumoral activity

In vitro anti-cancer activity of PTX-loaded micelles was performed on HeLa cells. Cell viability was measured by the MTT assay 24, 48 and 72 h post treatment by Taxol®, Cremophor® EL, PTX-loaded micelles and 10% PEG-p(CL-*co*-TMC) copolymers (Fig. 4). The range of concentrations of PTX (0.025–25 μg/ml) corresponds to plasma levels of the drug achievable in humans [27]. The range of concentrations of Cremophor® EL (0.022–2.2 mg/ml) corresponds to different concentrations of Taxol® tested and their relative concentrations in solution. After 24 h, the cell viability was more inhibited by PTX-loaded micelles than by Taxol® at 12.5 μg/ml of PTX ($p < 0.001$) (Fig. 4A). The IC_{50} at 24 h of Taxol® and PTX-loaded micelles treatments were 17.6 and 10.6 μg/ml, respectively.

As reported previously [19,28], Cremophor® EL (2.2 and 1.1 mg/ml) reduced cell viability to 59% and 54%, respectively, after 24 h of incubation. In contrast, PEG-p(CL-*co*-TMC) (100 mg/ml) was not cytotoxic (82% of cell viability).

After 48 h and 72 h of incubations, all concentrations of PTX completely inhibited cell growth. This decrease in cell viability measured by the MTT test could result from an inhibition of cell growth or from cytotoxicity. Only the lowest concentration of Cremophor® EL was not cytotoxic after 48 and 72 h of incubations, whereas PEG-p(CL-*co*-TMC) copolymers did not decrease the cell viability. For the lowest concentrations of PTX (0.025–0.25 μg/ml), no significant difference between PTX-loaded micelles and Taxol® was observed (data not shown).

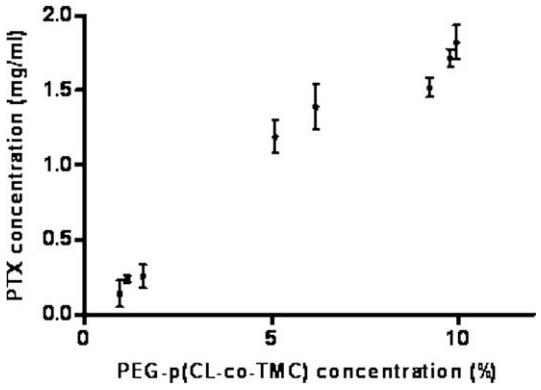

Fig. 2. Solubility (mg/ml) of PTX in PEG-p(CL-*co*-TMC) copolymers. Each data represents the mean ± standard deviation ($n = 3$).

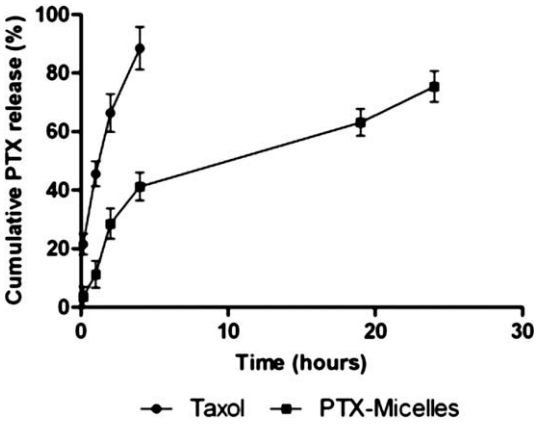

Fig. 3. Cumulative PTX release from PTX-loaded micelles and Taxol®. The results were expressed as mean values ± standard deviation of 3 measurements.

3.2.3. Apoptosis induced by PTX-loaded micelles

DAPI staining of DNA was performed to evaluate if the solubilization of PTX into polymeric micelles could maintain the apoptosis induced by PTX. The nuclei of untreated HeLa cells showed homogenous fluorescence with no evidence of segmentation and fragmentation after DAPI staining. Exposure of the cells to Taxol® and PTX-loaded micelles (25 μg/ml of PTX) for 4 h at 37 °C led to segregation of the cell nuclei into segments, indicating breakdown of the chromatin followed by DNA condensation. The percentage of apoptotic cells induced by Taxol® (17 ± 4%) and PTX-loaded micelles (15 ± 2%) was not significantly different ($p > 0.05$) (Fig. 5). Similar results were observed for other PTX concentrations (2.5 and 12.5 μg/ml) and incubation time (8 h) (data not shown).

3.3. In vivo evaluation

3.3.1. Biodistribution of PTX-loaded PEG-p(^{14}C-CL-co-TMC) micelles

The pharmacokinetics and the tissue distribution profiles of PEG-p(^{14}C-CL-*co*-TMC) micelles after intravenous administration were evaluated in TLT-tumor-bearing mice to check if the PEG-p(^{14}C-CL-*co*-TMC) micelles could accumulate in tumors by an EPR effect. The total radioactivity decreased in the blood with a $t_{1/2\beta}$ of 13 ± 0.6 h. 3.6% of administered dose still remained in the blood 48 h post-injection (Fig. 6A). Blood concentrations remained at least 48 h above the CMC.

After 24 h, PEG-p(^{14}C-CL-*co*-TMC) micelles mainly accumulated in lung, liver and spleen (8.9%, 7% and 6.6% administered dose/g, respectively), followed by the heart and kidneys (4% and 1.2% administered dose/g, respectively). More importantly, a significant tumor accumulation (localized in the gastrocnemius muscle) was observed after 24 h compared to the contro-lateral muscle (6.6% vs. 3.4% administered dose/g) ($p < 0.001$). After 48 h, the accumulation of PEG-p(^{14}C-CL-*co*-TMC) micelles decreased in all organs and reached 1.9% administered dose/g in the tumor (Fig. 6B).

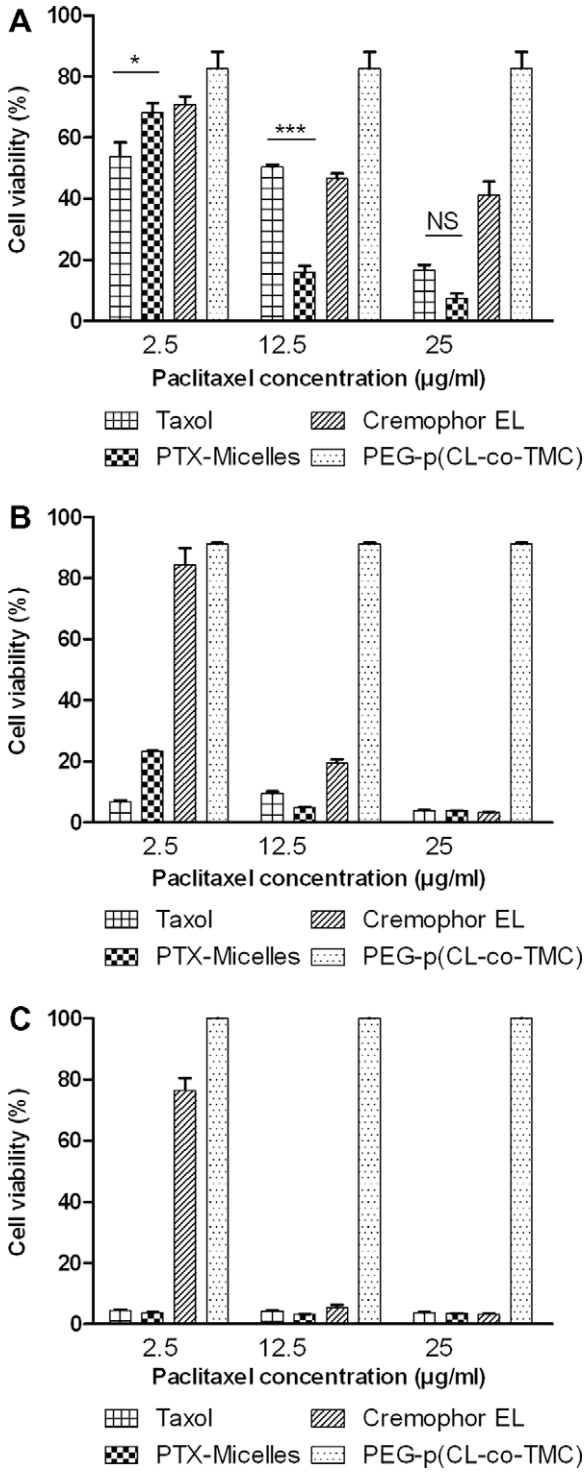

Fig. 4. Viability of HeLa cells incubated with Taxol®, PTX-loaded micelles (PTX concentrations 2.5, 12.5 and 25 µg/ml), Cremophor® EL (concentrations corresponding to Taxol® formulation: 0.22, 1.1 and 2.2 mg/ml), and drug-free micelles (10% copolymers) at day 1 (A), day 2 (B) and day 3 (C). Cell viability was determined by the MTT assay as described in Section 2.5. Untreated cells were taken as a negative control and Triton X-100 1% was used as a positive control. The results were expressed as mean values ± standard deviation of 5 measurements of two independent experiments. $^{*}p < 0.05$, $^{***}p < 0.001$.

3.3.2. Toxicity of PTX-loaded micelles

To examine the toxicity, mice were injected both intraperitonealy (IP) and intravenously (IV) with various doses of PTX-loaded micelles and Taxol®. Firstly, after intraperitoneal injections, the toxic response such as severe prostration, apathy and respiratory distress were observed directly after injection for the highest dose of Taxol® (80 mg/kg – IP); the death of mice was observed within 2 h after injection. Body weight loss, disheveled hair and apathy were begun to be observed 1 and 2 days after injection for mice treated by Taxol® at the doses of 30 and 50 mg/kg – IP, respectively. For mice treated with PTX-loaded micelles, these symptoms were only observed 4 days after injection for the highest dose tested (120 mg/kg – IP). For the lowest doses of PTX-loaded micelles, no change in body weight was observed. The dose–toxicity relationship observed on day 6 is shown in Fig. 7. The increase in the Taxol® doses induced a rapid decrease in the survival rate. All mice died within 2 h when treated with 80 mg/kg – IP of Taxol® while the survival rate fell to 0% for mice treated with PTX-loaded micelles at the dose of 120 mg/kg – IP after 4 days. Interestingly, at 80 mg/kg – IP PTX-loaded micelles, the survival rate was 100%. After intravenous injections, the death of mice was observed within 2 h when treated with Taxol® (30 mg/kg – IV) while at the dose of 13.5 mg/kg – IV, no adverse event and no body weight loss was observed. For mice treated with PTX-loaded micelles, the death of mice occurred from the dose of 67.5 mg/kg – IV after 3 days. Below this concentration, no side effect and no body weight loss was observed.

On the basis of these results, the intraperitoneal MTDs of 80 mg/kg and 13.5 mg/kg were chosen for PTX-loaded micelles and Taxol®, respectively, and the intravenous MTDs of 45 mg/kg and 13.5 mg/kg were chosen for PTX-loaded micelles and Taxol®, respectively. Interestingly, the MTD of Taxol® was the same for the intraperitoneal or the intravenous route.

Based on these results, *in vivo* anti-tumor efficacy studies of PTX-loaded micelles compared to Taxol® will be performed by the intravenous route at the PTX dose where all mice survive (13.5 mg/kg). Other *in vivo* anti-tumor efficacy studies will be assessed to evaluate intraperitoneal injections of PTX-loaded micelles at doses until 80 mg/kg.

3.3.3. Anti-tumor efficacy

The *in vivo* anti-tumor efficacy of PTX-loaded micelles and Taxol® was evaluated in TLT-tumor-bearing mice (Fig. 8). All treatments were statistically different to the PBS control and nonloaded micelles ($p < 0.001$). Taxol® displayed the similar tumor inhibition rates as PTX-loaded micelles at the dose of 13.5 mg/kg. The time to reach 18 mm (time where mice needed to be sacrificed) was 13 days for PTX-loaded micelles and 14 days for Taxol® ($p < 0.05$) (Fig. 8A). However, the toxic effects (body weight loss) were only observed in Taxol® group but not in PTX-loaded micelles group (Fig. 8B).

Finally, we checked the hypothesis that PTX-loaded micelles allow to administer higher doses of PTX than when formulated in Cremophor® EL, to increase its efficacy. Hence, the *in vivo* anti-tumor efficacy of PTX-loaded micelles at higher doses of PTX (30, 50 and 80 mg/kg) was evaluated in TLT-tumor-bearing mice (Fig. 9). Due to the volume needed to be administered, treatments were injected intraperitonealy. All treatments were statistically different to the PBS control ($p < 0.001$). For mice treated with PTX-loaded micelles (30, 50 and 80 mg/kg), the time to reach 18 mm was 11, 14 and 17 days ($p < 0.001$) as compared to 7 days for nontreated mice ($p < 0.001$). No change in body weight was observed for mice treated with PTX-loaded micelles for all doses tested (data not shown).

4. Discussion

Polymeric micelles have widely been studied as drug carriers due to their distinctive characteristics including the solubilization

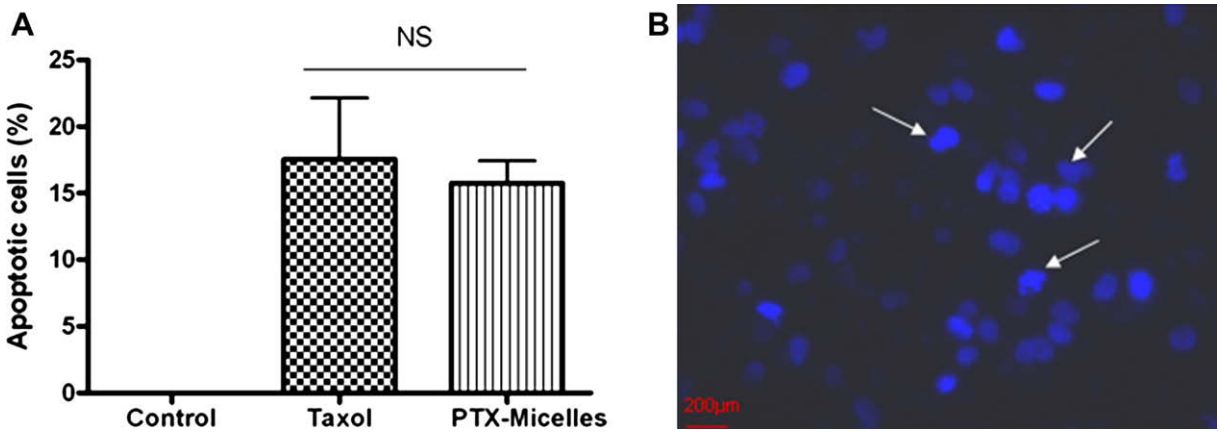

Fig. 5. DAPI staining of HeLa cells after incubation for 4 h of culture medium as control, Taxol® and PTX-loaded micelles at PTX concentration of 25 µg/ml and PEG-p(CL-*co*-TMC) concentration of 100 mg/ml. (A) Percentage of apoptotic cells induced by Taxol® and PTX-loaded micelles. (B) Fluorescent microscopy of apoptotic cells induced by PTX-loaded micelles.

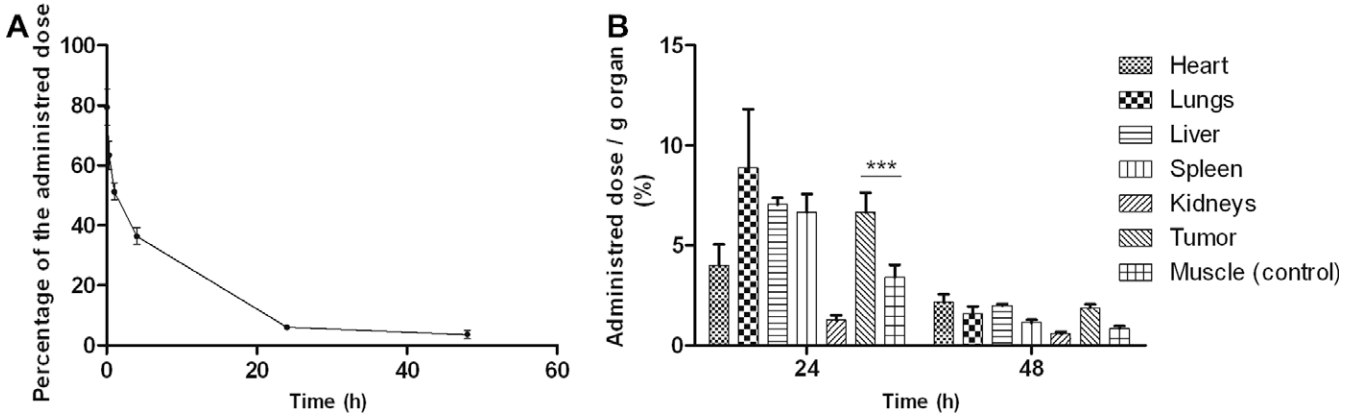

Fig. 6. (A) Plasma concentrations of PEG-p(^{14}C-CL-*co*-TMC) micelles-related radioactivity after i.v. administration of 200 µl PTX solubilized in 10% PEG-p(CL-*co*-TMC) solution (1.5 mg/ml PTX and 100 mg/ml PEG-p(^{14}C-CL-*co*-TMC)) evaluated in TLT-tumor-bearing mice. Each point represents the mean ± SD (n = 3). (B) Biodistribution profiles after 24 and 48 h of PEG-p(^{14}C-CL-*co*-TMC) micelles: percentage of the administered dose per gram of tissue. Each column represents the mean ± SD (n = 5).

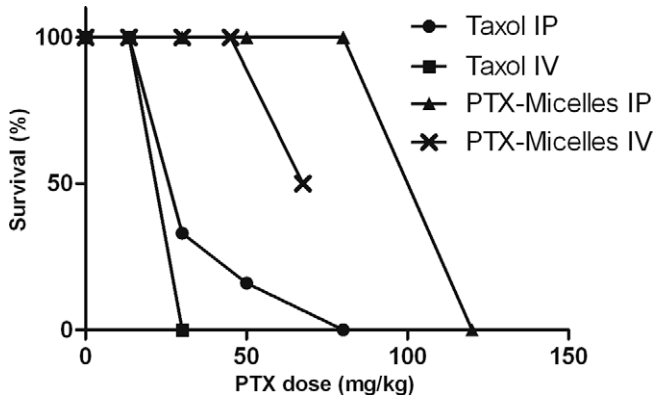

Fig. 7. Toxicity of PTX-loaded micelles and Taxol® injected intraperitonealy or intravenously to NMRI mice (n = 6). The survival percentage at day 6 is presented.

of poorly water-soluble drugs, thermodynamic stability, long-circulation potential and passive targeting to tumors by EPR effect [3].

In the literature, several studies describe the use of PEG-containing polyesters as micellar carriers of drugs. Mainly biodegradable polyesters consisting of lactide (L or DL), ε-caprolactone, glycolide or amino acids are described [26,29–33]. Compared to most of the polyester-based polymeric micelles described in literature [34,35], the major advantage of our PEG-p(CL-*co*-TMC) micelles is that they are self-assembling upon gentle mixing with water and that not organic solvent neither dialysis or evaporation step is required for the encapsulation of the drug. The solubilization of drugs into micellar carriers described in the literature depended largely on the manufacture method used. Moreover, the toxicity of 10% polymer formulation was better than that of a cyclodextrin formulation and of poly(ethylene oxide)-block-poly(b-benzyl-L-aspartate) micellar system [36,37]. PEG-p(CL-*co*-TMC) copolymers have shown to be biocompatible (slug test – data not shown), noncytotoxic and nonhemolytic (up to 10% w/v) [5,6].

The aim of this work was to investigate if the self-assembling PEG-p-(CL-*co*-TMC) could be used both to solubilize PTX and to administer PTX efficiently without excipient-related toxicity. We demonstrated that PEG-p(CL-*co*-TMC) micelles strongly increased the solubility of PTX, leading to a formulation with potentially therapeutic concentrations of PTX. We have previously shown that PEG-p(CL-*co*-TMC) micelles can solubilize poorly water-soluble drugs, owing to the high hydrophobic nature of the PCL/TMC moiety [5,7,17].

The release of PTX from PEG-p(CL-*co*-TMC) micelles was relatively slow, and no significant burst release was observed. The release of PTX from micelles upon dilution is probably controlled by diffusion as a result of portioning between the

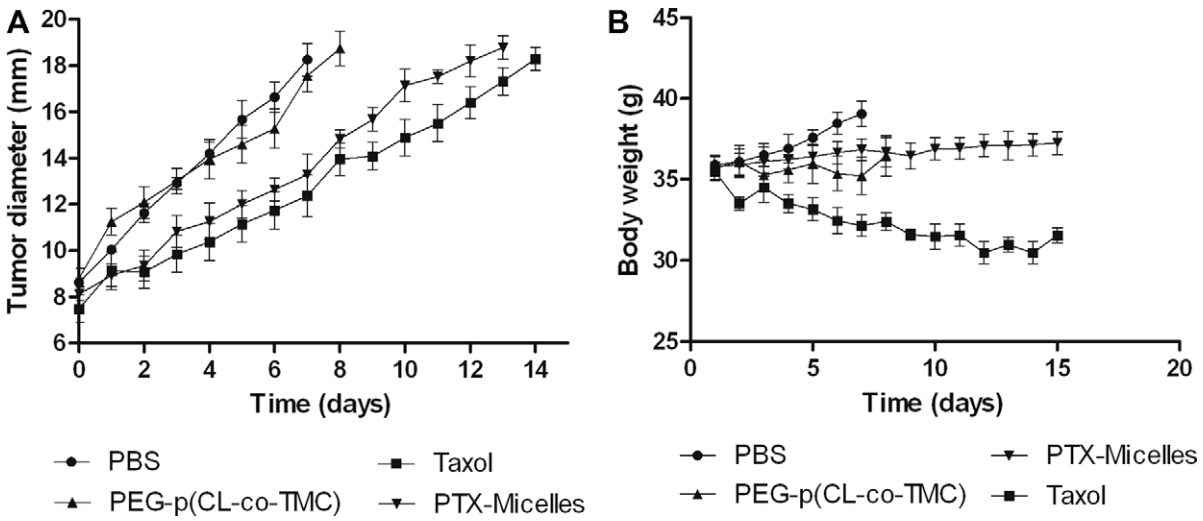

Fig. 8. (A) Anti-tumor effect of PTX-loaded micelles and Taxol® on TLT-tumor-bearing mice. One week after intramuscular TLT-tumor implantation, treatments were injected intravenously. The untreated control received PBS injection and nonloaded micelles; the treatment groups consisted of PTX-loaded PEG-p(CL-*co*-TMC) micelles and Taxol® (PTX concentration 13.5 mg/kg). Each point represents the mean of tumor size ± SD (n = 6). (B) Body weight changes of the mice treated with PBS, nonloaded micelles, PTX-loaded micelles and Taxol® (PTX concentration 13.5 mg/kg).

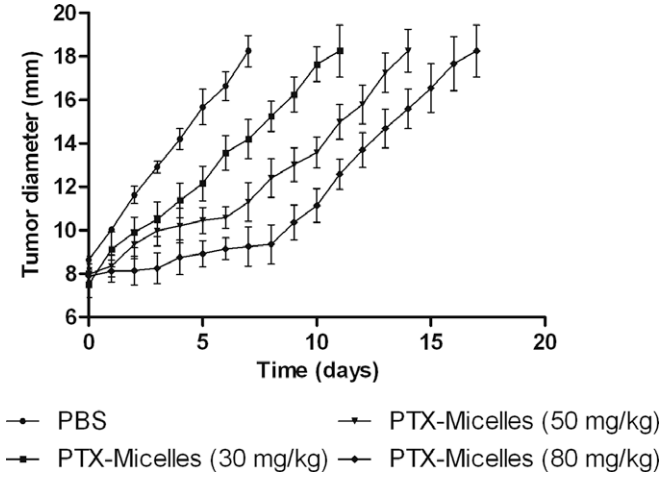

Fig. 9. Anti-tumor effect of PTX-loaded micelles on TLT-tumor-bearing mice. One week after intramuscular TLT-tumor implantation, treatments were injected intraperitonealy. The untreated control received PBS injection; the treatment groups consisted of PTX-loaded micelles (PTX concentration 30, 50 and 80 mg/kg). Each point represents the mean of tumor size ± SD (n = 6).

hydrophobic core and the aqueous phase. This slow release of PTX suggests that micelles could lead PTX into the tumors. Similar PTX release profiles have been described in the literature [15,25,26]. The solubilization of PTX into micelles enhanced its *in vitro* anti-tumoral activity compared to Taxol®, showing a smaller IC_{50} than Taxol®. PTX entrapped in PEG-p(CL-*co*-TMC) micelles were readily available to interact with cancer cells, retaining its cytotoxic activity. In the case of Taxol®, a significant effect may be attributed to the vehicle Cremophor® EL, whereas in the case of PTX-loaded micelles the cytotoxicity observed was only caused by PTX. Indeed, PEG-p(CL-*co*-TMC) micelles were not cytotoxic [6]. The anti-tumor activity of PTX encapsulated in polymeric micelles is consistent with other published data [38,39]. PTX entrapped in micelles was also able to induce apoptosis as reported for Taxol® [40,41]. The internalization of polymeric micelles into cells was previously reported [17,42,43]. The cellular uptake of the radiolabelled PEG-p(CL-*co*-TMC) copolymers was concentration dependent (data not shown). Mathot et al. showed previously that unimers passively diffused across enterocytes while PEG-p(CL-*co*-TMC) micelles utilized fluid-phase pinocytosis for translocation [17]. Similar concentration and time dependence was observed previously [44]. Corresponding with the anti-tumoral activity studies, HeLa cells viability was almost completely inhibited after 24 h while a high cellular uptake was achieved.

After intravenous injection, the blood clearance of radiolabelled PEG-p(^{14}C-CL-*co*-TMC) micelles ($t_{1/2\beta}$) was 13 h, consistent with their $t_{1/2\beta}$ in rats [16] but slightly higher than most polymeric micelles described in literature [34,35]. It was shown previously that PEG-p(^{14}C-CL-*co*-TMC) micelles were not extensively taken up by the RES as compared to nanoparticles or other polymeric micelles. PEG chains diminish opsonization of proteins, which activate clearance by the RES by steric hindrance and other mechanisms [45,46]. PEG-p(^{14}C-CL-*co*-TMC) micelles extravasated from the circulation and were largely retained into the tumor tissue, probably as a result of their small size, suggesting that PTX accumulate in the tumor via the EPR effect. The predominant factor for effective tumor accumulation is the small size of polymeric micelles [47]. PTX-loaded PEG-p(CL-*co*-TMC) micelles could constitute a long delivery system that facilitates accumulation of the drug in tumors by the enhanced permeation and retention (EPR) effect.

Dose-limiting toxicities by PTX may be related to PTX itself or to the vehicle required to formulate the drug, Cremophor® EL. This excipient is clearly responsible of a lot of serious side effects such as hypersensitivity reaction and neuropathy. Cremophor® EL may positively participate to the cytotoxic effect of PTX. Indeed, it was shown that Cremophor® EL induces a cell cycle block and an efflux pump inhibition independent from PTX. However, it was not demonstrated whether the plasma concentration of Cremophor® EL after Taxol® infusion is relevant in solid tumors [48,49]. Therefore, effective chemotherapy without toxicity using PTX is relying on the development of new delivery systems. Toxicity studies revealed that the highest dose tested administrable intraperitonealy to mice (100% survival rate) was 13.5 mg/kg Taxol®, while this dose was largely enhanced to 80 mg/kg for PTX-loaded micelles. After intravenous injections, the highest dose administrable was the same for Taxol (13.5 mg/kg) and 45 mg/kg for PTX-loaded micelles. The absence of Cremophor® EL and the safety and biocompatibility of PEG-p(CL-*co*-TMC) copolymers in micelles formu-

lations lead to the drastically reduced toxicity associated to PTX-loaded micelles as compared to Taxol. In the literature, comparable MTDs of Taxol® and PTX-loaded micellar formulation have been described [33,34].

Based on MTDs determined from toxicity studies, *in vivo* anti-tumoral activity of PTX-loaded micelles was assessed both by the intravenous and by the intraperitoneal routes on TLT-tumor-bearing mice. After intravenous injections at the same dose of PTX (13.5 mg/kg), PTX-loaded micelles exhibited equal anti-tumor activity on TLT tumor than Taxol®. Nevertheless, mice treated with Taxol® presented a serious body weight loss while no toxic effect was observed in the group of mice treated by PTX-loaded micelles. Interestingly, the tumor growth of mice treated with PBS and non-loaded micelles was similar, confirming that PEG-p(CL-*co*-TMC) copolymers were not cytotoxic *in vivo*. The decreased toxicity of PTX-loaded micelles allows the administration of higher dose of PTX to mice. Hence, mice were treated with PTX-loaded micelles at PTX doses of 30, 50 and 80 mg/kg by intraperitoneal injections. At 80 mg/kg PTX, tumors presented a complete growth inhibition during 8 days (tumor diameter <9 mm). For lower doses tested, only a growth delay was observed. Because of intraperitoneal injections, these results cannot be correlated to those obtained with mice treated with PTX-loaded micelles injected by the intravenous route. Nevertheless, these results indicate that the enhancement of dose induced a better tumor growth inhibition. We clearly demonstrated that PEG-p(CL-*co*-TMC) copolymers, used as vehicle for PTX, combined reduction in toxicity and improvement of anti-tumor efficacy.

5. Conclusions

In conclusion, in comparison to existing diblock copolymers which form polymeric micelles, PEG-p(CL-*co*-TMC) copolymers have the following advantages: (i) they easily form micelles spontaneously in water (no dialysis or organic solvent are needed to produce micelles), (ii) their CMC is very low which allowing them to maintain their micellar form upon dilution in blood, (iii) they enhance the solubility of PTX by three orders of magnitude, (iv) they are not cytotoxic *in vitro* as compared to Cremophor® EL, (v) they present a low blood clearance, a relatively weak capture by the RES, and they accumulate by the EPR effect in tumors, (vi) they allow the administration *in vivo* of PTX without excipient-related toxicity as compared to Taxol®, and (vii) finally, *in vivo*, PTX-loaded micelles allow the enhancement of doses of PTX as compared to Taxol® for better therapeutic efficacy.

Acknowledgements

The authors wish to thank Johnson & Johnson, Pharmaceutical Research and Development, Division of Janssen Pharmaceutica, Belgium for providing Paclitaxel. This work was supported by the FRSM (Belgium).

References

[1] C. Allen, D. Maysinger, A. Eisenberg, Nano-engineering block copolymer aggregates for drug delivery, Colloids Surf. B: Biointer. 16 (1999) 3–27.
[2] G.S. Kwon, Block copolymer micelles as drug delivery systems, Adv. Drug Delivery Rev. 54 (2002) 169–190.
[3] G. Gaucher, M.H. Dufresne, V.P. Sant, N. Kang, D. Maysinger, J.C. Leroux, Block copolymer micelles: preparation, characterization and application in drug delivery, J. Control. Release 109 (2005) 169–188.
[4] K. Greish, J. Fang, T. Inutsuka, A. Nagamitsu, H. Maeda, Macromolecular therapeutics: advantages and prospects with special emphasis on solid tumour targeting, Clin. Pharmacokinet. 42 (2003) 1089–1105.
[5] L. Ould-Ouali, A. Arien, J. Rosenblatt, A. Nathan, P. Twaddle, T. Matalenas, M. Borgia, S. Arnold, D. Leroy, M. Dinguizli, L. Rouxhet, M. Brewster, V. Preat, Biodegradable self-assembling PEG-copolymer as vehicle for poorly water-soluble drugs, Pharm. Res. 21 (2004) 1581–1590.
[6] L. Ould-Ouali, M. Noppe, X. Langlois, B. Willems, P. Te Riele, P. Timmerman, M.E. Brewster, A. Arien, V. Preat, Self-assembling PEG-p(CL-co-TMC) copolymers for oral delivery of poorly water-soluble drugs: a case study with risperidone, J. Control Release 102 (2005) 657–668.
[7] D.J. Latere, L. Rouxhet, M.E. Brewster, V. Preat, A. Arien, Spontaneously self-assembled micelles from poly(ethylene glycol)-b-poly(epsilon-caprolactone-co-trimethylene carbonate) for drug solubilization, Pharmazie 63 (2008) 235–240.
[8] R. Panchagnula, Pharmaceutical aspects of paclitaxel, Int. J. Pharm. 172 (1998) 1–15.
[9] A.K. Singla, A. Garg, D. Aggarwal, Paclitaxel and its formulations, Int. J. Pharm. 235 (2002) 179–192.
[10] C.M. Spencer, D. Faulds, Paclitaxel. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in the treatment of cancer, Drugs 48 (1994) 794–847.
[11] K. Nicolaou, M. Dai, R. Guy, Chemistry and biology of Taxol, Angew. Chem. Int. Edit. Engl. 33 (1994) 15–44.
[12] T. Yang, M.K. Choi, F.D. Cui, J.S. Kim, S.J. Chung, C.K. Shim, D.D. Kim, Preparation and evaluation of paclitaxel-loaded PEGylated immunoliposome, J. Control. Release 120 (2007) 169–177.
[13] R.T. Liggins, H.M. Burt, Paclitaxel-loaded poly(L-lactic acid) microspheres 3: blending low and high molecular weight polymers to control morphology and drug release, Int. J. Pharm. 282 (2004) 61–71.
[14] Z. Zhang, S.S. Feng, The drug encapsulation efficiency, in vitro drug release, cellular uptake and cytotoxicity of paclitaxel-loaded poly(lactide)-tocopheryl polyethylene glycol succinate nanoparticles, Biomaterials 27 (2006) 4025–4033.
[15] K.M. Huh, H.S. Min, S.C. Lee, H.J. Lee, S. Kim, K. Park, A new hydrotropic block copolymer micelle system for aqueous solubilization of paclitaxel, J. Control. Release 126 (2008) 122–129.
[16] F. Mathot, B.L. van Beijsterveldt, V. Preat, M. Brewster, A. Arien, Intestinal uptake and biodistribution of novel polymeric micelles after oral administration, J. Control. Release 111 (2006) 47–55.
[17] F. Mathot, A. des Rieux, A. Arien, Y.J. Schneider, M. Brewster, V. Preat, Transport mechanisms of mmePEG750P(CL-co-TMC) polymeric micelles across the intestinal barrier, J. Control Release 124 (2007) 134–143.
[18] G. Vandermeulen, L. Rouxhet, A. Arien, M.E. Brewster, V. Preat, Encapsulation of amphotericin B in poly(ethylene glycol)-block-poly(epsilon-caprolactone-co-trimethylenecarbonate) polymeric micelles, Int. J. Pharm. 309 (2006) 234–240.
[19] F. Danhier, N. Lecouturier, B. Vroman, C. Jerome, J. Marchand-Brynaert, O. Feron, V. Preat, Paclitaxel-loaded PEGylated PLGA-based nanoparticles: In vitro and in vivo evaluation, J. Control. Release 133 (2009) 11–17.
[20] Y.W. Cho, J. Lee, S.C. Lee, K.M. Huh, K. Park, Hydrotropic agents for study of in vitro paclitaxel release from polymeric micelles, J. Control. Release 97 (2004) 249–257.
[21] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
[22] B. Vroman, M. Mazza, M.R. Fernandez, R. Jerome, V. Preat, Copolymers of epsilon-caprolactone and quaternized epsilon-caprolactone as gene carriers, J. Control. Release 118 (2007) 136–144.
[23] B.F. Jordan, P. Sonveaux, O. Feron, V. Gregoire, N. Beghein, B. Gallez, Nitric oxide-mediated increase in tumor blood flow and oxygenation of tumors implanted in muscles stimulated by electric pulses, Int. J. Radiat. Oncol. Biol. Phys. 55 (2003) 1066–1073.
[24] C.H. Heldin, K. Rubin, K. Pietras, A. Ostman, High interstitial fluid pressure – an obstacle in cancer therapy, Nat. Rev. 4 (2004) 806–813.
[25] L. Han, J. Guo, L. Zhang, Q. Wang, X. Fang, Pharmacokinetics and biodistribution of polymeric micelles of paclitaxel with Pluronic P123, Acta Pharm. Sinica 6 (2006) 747–756.
[26] Y. Wang, L. Tang, T. Sun, C. Li, M. Xiong, J. Wang, Self-assembled micelles of biodegradable triblock copolymers based on poly(ethylene phosphate) and poly(epsilon-caprolactone) as drug carriers, Biomacromolecules 9 (2008) 388–395.
[27] E. Raymond, A. Hanauske, S. Faivre, E. Izbicka, G. Clark, E.K. Rowinsky, D.D. Von Hoff, Effects of prolonged versus short-term exposure paclitaxel (Taxol) on human tumor colony-forming units, Anticancer Drugs 8 (1997) 379–385.
[28] J. Liebmann, J.A. Cook, C. Lipschultz, D. Teague, J. Fisher, J.B. Mitchell, The influence of Cremophor EL on the cell cycle effects of paclitaxel (Taxol) in human tumor cell lines, Cancer Chemother. Pharmacol. 33 (1994) 331–339.
[29] Z. Xie, H. Guan, C. Lu, L. Chen, X. Hu, Q. Shi, X. Jing, A novel polymer–paclitaxel conjugate based on amphiphilic triblock copolymer, J. Control. Release 117 (2007) 210–216.
[30] B. Liu, X. Li, X. Qian, Z. Shen, Y. Ding, L. Yu, Enhanced efficiency of thermally targeted taxanes delivery in a human xenograft model of gastric cancer, J. Pharm. Sci. 97 (2008) 3170–3181.
[31] S. Liu, Y. Tong, Y. Yang, Thermally sensitive micelles self-assembled from poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide)-b-poly(D,L-lactide-co glycolide) for controlled delivery of paclitaxel, Mol. Biosyst. 1 (2005) 158–165.
[32] X. Zhang, J.K. Jackson, H.M. Burt, Development of amphiphilic diblock copolymers as micellar carriers of Taxol, Int. J. Pharm. 132 (1996) 195–206.
[33] C. Zhang, G. Qu, Y. Sun, X. Wu, Z. Yao, Q. Guo, Q. Ding, S. Yuan, Z. Shen, Q. Ping, H. Zhou, Pharmacokinetics, biodistribution, efficacy and safety of N-octyl-O-sulfate chitosan micelles loaded with paclitaxel, Biomaterials 29 (2008) 1233–1241.

[34] S.C. Kim, D.W. Kim, Y.H. Shim, J.S. Bang, H.S. Oh, K.S. Wan, M.H. Seo, In vivo evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy, J. Control. Release 72 (2001) 191–202.
[35] D. Le Garec, S. Gori, L. Luo, D. Lessard, D.C. Smith, M.A. Yessine, M. Ranger, J.C. Leroux, Poly(N-vinylpyrrolidone)-block-poly(D,L-lactide) as a new polymeric solubilizer for hydrophobic anticancer drugs: in vitro and in vivo evaluation, J. Control. Release 99 (2004) 83–101.
[36] K. Chakraborty, S. Naik, Therapeutic and hemolytic evaluation of in situ liposomal preparation containing amphotericin-B complexed with different chemically modified b-cyclodextrins, J. Pharm. Pharm. Sci. 6 (2003) 231–237.
[37] B. Yu, T. Okano, K. Sardari, G. Kwon, In vitro dissociation of antifungal efficacy and toxicity for amphotericin-B-loaded poly(ethylene oxide)-block-poly(beta-benzyl-L-aspartate) micelles, J. Control. Release 56 (1998) 285–291.
[38] M.L. Forrest, J.A. Yanez, C.M. Remsberg, Y. Ohgami, G.S. Kwon, N.M. Davies, Paclitaxel prodrugs with sustained release and high solubility in poly(ethylene glycol)-b-poly(epsilon-caprolactone) micelle nanocarriers: pharmacokinetic disposition, tolerability, and cytotoxicity, Pharm. Res. 25 (2008) 194–206.
[39] T. Yang, F.D. Cui, M.K. Choi, J.W. Cho, S.J. Chung, C.K. Shim, D.D. Kim, Enhanced solubility and stability of PEGylated liposomal paclitaxel: in vitro and in vivo evaluation, Int. J. Pharm. 338 (2007) 317–326.
[40] P.B. Schiff, J. Fant, S.B. Horwitz, Promotion of microtubule assembly in vitro by Taxol, Nature 277 (1979) 665–667.
[41] T.K. Yeung, C. Germond, X. Chen, Z. Wang, The mode of action of Taxol: apoptosis at low concentration and necrosis at high concentration, Biochem. Biophys. Res. Commun. 263 (1999) 398–404.
[42] X. Shuai, T. Merdan, A.K. Schaper, F. Xi, T. Kissel, Core-cross-linked polymeric micelles as paclitaxel carriers, Bioconjug. Chem. 15 (2004) 441–448.
[43] N. Rapoport, Combined cancer therapy by micellar-encapsulated drug and ultrasound, Int. J. Pharm. 277 (2004) 155–162.
[44] O. Soga, C.F. van Nostrum, M. Fens, C.J. Rijcken, R.M. Schiffelers, G. Storm, W.E. Hennink, Thermosensitive and biodegradable polymeric micelles for paclitaxel delivery, J. Control. Release 103 (2005) 341–353.
[45] Z. Panagi, A. Beletsi, G. Evangelatos, E. Livaniou, D.S. Ithakissios, K. Avgoustakis, Effect of dose on the biodistribution and pharmacokinetics of PLGA and PLGA-mPEG nanoparticles, Int. J. Pharm. 221 (2001) 143–152.
[46] V.C. Mosqueira, P. Legrand, J.L. Morgat, M. Vert, E. Mysiakine, R. Gref, J.P. Devissaguet, G. Barratt, Biodistribution of long-circulating PEG-grafted nanocapsules in mice: effects of PEG chain length and density, Pharm. Res. 18 (2001) 1411–1419.
[47] R.M. Schiffelers, I.A. Bakker-Woudenberg, G. Storm, Localization of sterically stabilized liposomes in experimental rat *Klebsiella pneumoniae* pneumonia: dependence on circulation kinetics and presence of poly(ethylene)glycol coating, Biochim. Biophys. Acta 1468 (2000) 253–261.
[48] L. Webster, M. Linsenmeyer, M. Millward, C. Morton, J. Bishop, D. Woodcock, Measurement of Cremophor EL following Taxol: plasma levels sufficient to reverse drug exclusion mediated by multidrug resistant phenotype, J. Natl. Cancer Inst. 85 (1993) 1685–1690.
[49] L. van Zuylen, J. Verweij, A. Sparreboom, Role of formulation vehicles in taxane pharmacology, Investig. New Drugs 19 (2001) 125–141.