Paclitaxel-loaded PEGylated PLGA-based nanoparticles: In vitro and in vivo evaluation

Fabienne Danhiera, Nathalie Lecouturier, Benoît Vromana, Christine Jérômee, Jacqueline Marchand-Brynaertc, Olivier Feron, Véronique Préata

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The purpose of this study was to develop Cremophor® EL-free nanoparticles loaded with Paclitaxel (PTX), intended to be intravenously administered, able to improve the therapeutic index of the drug and devoid of the adverse effects of Cremophor® EL. PTX-loaded PEGylated PLGA-based were prepared by simple emulsion and nanoprecipitation. The incorporation efficiency of PTX was higher with the nanoprecipitation technique. The release behavior of PTX exhibited a biphasic pattern characterized by an initial burst release followed by a slower and continuous release. The in vitro anti-tumoral activity was assessed using the Human Cervix Carcinoma cells (HeLa) by the MTT test and was compared to the commercial formulation Taxol® and to Cremophor® EL. When exposed to 25 µg/ml of PTX, the cell viability was lower for PTX-loaded nanoparticles than for Taxol® (IC50 5.5 vs 15.5 µg/ml). Flow cytometry studies showed that the cellular uptake of PTX-loaded nanoparticles was concentration and time dependent. Exposure of HeLa cells to Taxol® and PTX-loaded nanoparticles induced the same percentage of apoptotic cells. PTX-loaded nanoparticles showed greater tumor growth inhibition effect in vivo on TLT tumor, compared with Taxol®. Therefore, PTX-loaded nanoparticles may be considered as an effective anticancer drug delivery system for cancer chemotherapy.

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1. Introduction

Paclitaxel (PTX), a major anticancer drug isolated from the bark of Taxus brevifolia, has anti-neoplastic activity particularly against various types of solid tumors. PTX is approved in many countries for its use as second line treatment of ovarian and breast cancers [12]. PTX has a unique mechanism of action. It disrupts the dynamic equilibrium within the microtubule system and blocks cells in the late G2 phase and M phase of the cell cycle, thereby inhibiting cell replication [3]. It has been reported that Taxol® induces bcl2 phosphorylation followed by apoptosis [4]. PTX is poorly soluble in water [5]. To enhance its solubility and allow parenteral administration, PTX is currently formulated (Taxol®) at 6 mg/ml in a vehicle composed of 1:1 blend of Cremophor® EL and ethanol. However, Cremophor® EL causes side effects e.g. hypersensitivity, nephrotoxicity and neurotoxicity as well as effects on endothelial and vascular muscles causing vasodilatation, labored breathing, lethargy and hypotension [6].

Numerous investigations have shown that both tissue and cell distribution profiles of anticancer drugs can be controlled by their entrapment in submicronic colloidal systems. Accordingly, a number of alternative formulations were investigated for the solubilization of PTX, including liposomes, microspheres, nanoparticles and polymeric micelles [7–10]. The rationale behind this approach is to increase antitumor efficacy while reducing systemic side effects [11]. Nanoparticulate drug delivery systems have been studied for several decades now, and many of the features that make them attractive drug carriers are well known. Moreover, nanoparticles can escape from the vasculature through the leaky endothelial tissue that surrounds the tumor and then accumulate in certain solid tumors by the so-called Enhanced Permeation and Retention (EPR) effect [12]. A Cremophor®-free formulation of PTX nanoparticles, Abraxane®, has recently been approved by the FDA for recurrent metastatic breast cancer [13].

The aim of this study was to develop a polymeric drug delivery system for PTX, Cremophor® EL-free, intended to be intravenously administered. To achieve this aim, PTX-loaded PEGylated PLGA-based nanoparticles were prepared by nanoprecipitation method [14]. Poly

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(lactide-co-glycolide) (PLGA) was chosen for its biodegradability properties, its biocompatibility and its approval by FDA. Poly(ε-caprolactone-co-ethylene glycol) (PCL–PEG), an amphiphilic copolymer, was added to take advantage of PEG repulsive properties [15,16] and to provide a higher stability of nanoparticles in biological fluids [17]. The nanoparticles were characterized in terms of size and charge. In vitro drug release was evaluated. In vitro anti-tumoral activity of PTX-loaded nanoparticles was performed using Human Cervix Carcinoma cells (HeLa). In vitro cellular uptake and apoptosis were also studied. Finally, in vivo tumor growth inhibition of PTX-loaded nanoparticles was also investigated in TLT-tumor bearing mice.

2. Materials and methods

2.1. Materials

PCL–PEG, PLGA–PEG and PLGA–FITC polymers were synthesized by ring opening polymerization [16]. Molecular weights were determined by size exclusion chromatography (SEC) and NMR as described previously (Table 1).

PTX was purchased from Calbiochem (Darmstadt, Germany). Taxol® (Brystol-Myers Squibb) was obtained from Cliniques Universitaires Saint-Luc, Belgium. Taxol® contains 6 mg/ml of PTX and 528 mg/ml of Cremophor® EL.

HeLa (Human Cervix 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). Dubelcco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS), 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.

NMRI mice were purchased from Janvier, Genest St Isle, France.

2.2. Preparation of Paclitaxel-loaded nanoparticles

First, nanoparticles were prepared by simple emulsion technique [18]. Briefly, 7.5 mg of PLGA–PEG in 500 µl of dichloromethane and 7.5 mg of PCL–PEG in 500 µl of dichloromethane were added to 35 mg of PLGA. 2 mg of PTX was mixed with the polymer solution. 2 ml of sodium cholate 1% was added before a sonication of 15 s (70 W). The emulsion formed was added drop-wise on 100 ml of sodium cholate 0.3% under magnetic stirring. To evaporate dichloromethane, the solution was stirred 45 min at 37 °C.

Nanoparticles were also prepared by the interfacial deposition method (nanoprecipitation) [14]. Briefly, an organic solution of PLGA (70 mg), PLGA–PEG (15 mg), PCL–PEG (15 mg) and PTX (1 mg) in 10 ml acetone was added to 20 ml of water under magnetic stirring at room temperature overnight to allow the evaporation of acetone.

To remove the non-encapsulated drug, the suspensions were filtered (1.2 µm) and ultra-centrifuged at 22,000 g for 1 h at 4 °C. The pellets were suspended in 10 ml of ultra-purified water.

2.3. Determination of Paclitaxel content in nanoparticles

The drug loading efficiency was determined in triplicate by HPLC (Agilent 1100 series, Agilent Technologies, Diegem, BE). The mobile phase consisted in 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 solution was injected at a volume of 50 µl. The HPLC was calibrated with standard solutions of 5 to 100 µg/ml of PTX dissolved in acetonitrile (correlation coefficient of R2 = 0.9965). The limit of quantification was 0.6 ng/ml. The coefficients of variation (CV) were all within 4.3%. Nanoparticles were dissolved in acetonitrile and vigorously vortexed to get a clear solution. The encapsulation efficiency was defined by the ratio of measured and initial amount of PTX encapsulated in nanoparticles [19].

\[
\text{Encapsulation efficiency} (\%) = \frac{\text{Amount of PTX in nanoparticles}}{\text{Initial amount of PTX}} \times 100
\]

The recovery corresponds to the ratio of the amount of PTX in the supernatant and in the pellets to the initial amount of PTX.

\[
\text{Recovery} (\%) = \frac{\text{Amount of PTX supernatant} \times \text{amount of PTX in NP}}{\text{Initial amount of PTX}} \times 100
\]

2.4. Physicochemical characterization of Paclitaxel-loaded nanoparticles

The average particle size and size polydispersity of the nanoparticles prepared in water were determined by photon correlation spectroscopy in water using a Malvern Nano ZS (Malvern Instruments, UK). Potential of nanoparticles was measured in KCl 1 mM with a Malvern Nano ZS (Malvern Instruments, UK) at 25 °C. The instrument was calibrated with standard latex nanoparticles (Malvern Instruments, UK). Experimental values were the average of 3 different formulations.

2.5. In vitro drug release

PTX-loaded nanoparticles (1 mg of PTX) were dispersed in 10.0 ml of PBS (phosphate buffer solution, pH 7.4) and incubated at 37 °C under magnetic stirring. At determined time intervals, the tube was ultra-centrifuged at 22,000 g for 1 h at 4 °C. 1 ml of supernatant mixed with 1 ml of acetonitrile was analyzed by HPLC as described in Section 2.3 [20].

2.6. In vitro anti-tumoral activity

HeLa cells were grown in DMEM supplemented with 10% (v/v) fetal bovine serum and 100 IU/ml of penicillin G sodium and 100 µg/ml of streptomycin sulfate. The cells were maintained in an incubator supplied with 5% CO2/95% air humidified atmosphere at 37 °C.

HeLa cells were seeded in 96-well plates at the density of 2500 viable cells per well and incubated 24 h to allow cell attachment. The cells were then incubated with Taxol®. PTX-loaded nanoparticles (PTX concentrations of 0.025, 0.25, 2.5, 12.5 and 25 µg/ml at a PTX/polymers percentage of 0.7%), Cremophor® EL (0.022 to 2.2 mg/ml) and drug-free nanoparticles (polymers concentration 10 mg/ml) for 24, 48 and 72 h. At determined time, the formulations were replaced with DMEM containing MTT (5 mg/ml) and cells were then incubated for additional 4 h. MTT was aspirated off and DMEM 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 positive control of cytotoxicity [22]. The results were expressed as mean values ± standard deviation of 5 measurements.

| Table 1: Chemical description of the polymers included in the formulations |
|-----------------|-----------------|
| Polymer | Mn (SEC) / g/mol | Mn (NMR) / g/mol |
| PLGA | 22,000 | |
| PLGA–PEG | 23,600 | |
| PLGA–FITC | 29,300 | 16,500–4600 |
| PCL–PEG | 4,200 | |
| PCL–FITC | 15,200–4600 | |

a Poly styrene calibration.

b Determined by NMR by the following formula: \((I_{4.7}/2)/(I_{5.2} + I_{4.7}/2)\)×100, where \(I_{4.7}\) is the signal intensity of the glycolide unit at 4.7 ppm (CH2OC=O) and \(I_{5.2}\) is the signal intensity of the lactide unit at 5.2 ppm (CH(CH3)OC=O).
Table 2

<table>
<thead>
<tr>
<th></th>
<th>Simple emulsion</th>
<th>Nanoprecipitation</th>
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</thead>
<tbody>
<tr>
<td>PTX encapsulation efficiency (%)</td>
<td>37±2.5</td>
<td>70±4</td>
</tr>
<tr>
<td>PTX/polymers (%)</td>
<td>0.4±0.02</td>
<td>0.7±0.04</td>
</tr>
<tr>
<td>PTX recovery (%)</td>
<td>78±3</td>
<td>96.7±1.6</td>
</tr>
<tr>
<td>Size (nm)</td>
<td>190±4.5</td>
<td>112±4</td>
</tr>
<tr>
<td>PDI</td>
<td>0.16±0.002</td>
<td>0.18±0.005</td>
</tr>
<tr>
<td>ζ potential (mV)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−7.7±2.6</td>
<td>−0.55±5.7</td>
</tr>
<tr>
<td>ζ deviation (mV)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.18±0.98</td>
<td>4.6±1.02</td>
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<sup>a</sup> Measured by photon correlation spectroscopy with a Malvern Nano ZS.
<sup>b</sup> Determined with a Malvern Nano ZS.

2.7. In vitro cellular uptake of nanoparticles

Fluorescent nanoparticles were prepared by incorporating PLGA–FITC instead of PLGA in the formulations as described in Section 2.2. Twelve-well plates were seeded with 100 000 HeLa cells per well and the cells were incubated at 37 °C for 24 h to allow cell attachment. After 24 h, the medium was replaced by PTX-loaded nanoparticles (PTX concentrations 2.5, 12.5 and 25 µg/ml) for 30 min, 2 h and 24 h. After incubation, the cells were removed and the wells were washed with ice-cold PBS. The cells were then harvested by trypsinization and centrifuged at 254 g for 5 min at 4 °C. Finally, the cells were resuspended in 500 µl of PBS and stored on ice until analysis. The percentage of fluorescent cells in the population and the fluorescence intensity were measured using a flow cytometer (FACSscan, Becton Dickinson). Data were analyzed using the CellQuest (Becton Dickinson) software. The individual fluorescence of 10 000 cells was collected for each sample [23]. The counting of nanoparticles number was also determined by flow cytometry [16].

2.8. Apoptosis induced by PTX-loaded nanoparticles

Apoptosis was identified morphologically by 4,6-diamidino-2-phenylindole (DAPI) staining. HeLa cells were seeded in 6-well plates containing a coverslip with 100 000 cells per well and cultured at 37 °C for 24 h. Cells were then incubated for 4 h with Taxol®, PTX-loaded nanoparticles (PTX concentration of 25 µg/ml) and culture medium as control. Samples were then fixed with 4% paraformaldehyde in PBS at room temperature for 15 min, stained with 0.2 µg/ml DAPI in PBS at room temperature for 15 min and washed twice with PBS and with water. Coverslips were mounted onto glass slides. Slides were then 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) was made on slides picked up at random by two independent experimenters. Clusters of apoptotic bodies were given as a single count [24].

2.9. In vivo tumor growth inhibition study

The transplantable liver tumor TLT was implanted in the gastrocnemius muscle in the posterior leg of 8 weeks old male NMRI mice. All experiments were approved by the ethical committee for animal care of the faculty of medicine of the Université Catholique de Louvain.

The effect of PTX-loaded nanoparticles on TLT growth was assessed by measuring the diameter of the tumors with an electronic caliper. When tumors reached 8.0±0.5 mm in diameter, the mice were randomly assigned to a treatment group. Three groups of mice (6 mice per group) were treated: group 1: PBS injection; group 2: Taxol® (PTX concentration of 1 mg/kg; diluted in PBS); group 3: PTX-loaded nanoparticles (PTX concentration of 1 mg/kg). The treatments were injected through the tail vein. After treatment, tumors were measured every day until they reached a diameter of 18 mm, at which time the mice were killed. Body weight change was also monitored.

2.10. Statistics

All results are expressed as mean±standard deviation. One-way or Two-way ANOVA and Bonferroni post test were performed to demonstrate statistical differences (p<0.05); IC<sub>50</sub> 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. Survival curves were also calculated from the software GraphPad Prism 5 for Windows.

3. Results and discussion

3.1. Physicochemical characterization of Paclitaxel-loaded nanoparticles

Chemical description of polymers used in the formulations is summarized in Table 1. The influence of the preparation method on the incorporation efficiency and on the recovery rate is illustrated in Table 2. Nanoparticles prepared with the nanoprecipitation method could achieve higher incorporation efficiency (70%) than with the simple emulsion technique (37%). The loss of PTX was also higher with the simple emulsion technique. Hence, the nanoprecipitation method was chosen for the formulation of PLGA based PEGylated nanoparticles.

Nanoparticles obtained by simple emulsion and nanoprecipitation were characterized in term of size and ζ potential (Table 2). Nanoprecipitation technique generated nanoparticles smaller than those obtained with the simple emulsion technique (112 vs 190 nm). Both with techniques, nanoparticles exhibited a narrow size distribution (polydispersity index <0.2). Nanoparticles obtained by both techniques exhibited a ζ potential close to neutrality, confirming the presence of PEG chains shielding the negative charges present at the nanoparticle surface.

Consistent with previously published data [16,25], the presence of PTX compared to free-drug nanoparticles did not affect the size and the ζ potential of nanoparticles (data not shown).

3.2. In vitro drug release

The in vitro release profile of PTX-loaded nanoparticles was investigated in PBS at 37 °C. The cumulative percentage release is shown in Fig. 1. After the initial burst release for about 4 h, the release rate of PTX slowed down and became an almost zero-order rate of release (R<sup>2</sup>=0.97). PTX released in the first 4 h was equivalent to 46.9±5.7% of the initial drug load of nanoparticles. After 11 days, the amount of cumulated PTX release was 75.3±2.7%. The burst release of PTX may be due to the dissolution and diffusion of the drug that was poorly entrapped in the polymer matrix, while the slower and continuous release may be
attributed to the diffusion of the drug localized in the PLGA core of the nanoparticles. Similar burst effect was observed previously [18,26].

3.3. In vitro anti-tumoral activity

The in vitro cytotoxic activity of Taxol®, Cremophor® EL, drug-free nanoparticles and PTX-loaded nanoparticles was evaluated by the MTT assay using the HeLa cell line. The range of concentrations of PTX (0.025 to 25 µg/ml) corresponds to plasma levels of the drug achievable in humans [27]. The range of concentrations of Cremophor® EL (0.022 to 2.2 mg/ml) corresponds to the concentrations present in solution of different concentrations of Taxol® tested.

At the concentration of 25 µg/ml Taxol®, cell viability lower than 8% was achieved after 24 h while lower PTX concentrations achieved the same low cell viability after 48 h. The IC50 at 24 h was 15.5 µg/ml (Fig. 2A).

As reported previously [28,29], Cremophor® EL was cytotoxic at concentration above 1 mg/ml. (Fig. 2B).

When encapsulated in nanoparticles, 25 and 12.5 µg/ml PTX totally inhibited cell viability after 24 h of incubation while a 40% decrease in cell viability was achieved at lower concentrations of PTX (2.5 to 0.025 µg/ml). IC50 value for HeLa cells decreased from 15.5 µg/ml for Taxol® to 5.5 µg/ml for the PTX-loaded nanoparticles after 24 h incubation. At 12.5 µg/ml and 2.5 µg/ml PTX, PTX-loaded nanoparticles contributed to higher reduction in cell viability than Taxol® (p < 0.01 and 0.05 respectively) (Fig 2D). Cell viability was totally suppressed after 48 h of incubation. No cytotoxic activity was observed for the drug-free nanoparticles at 10 mg/ml polymers.

It should be emphasized that in the case of Taxol® a significant effect was attributed to the excipient Cremophor® EL (absent in nanoparticles), whereas in the case of PTX-loaded nanoparticles the cytotoxicity observed was only attributed to PTX (drug-free nanoparticles were non-cytotoxic). This decrease in cell viability, measured by the MTT test can result from an inhibition of cell growth or from cytotoxicity.

Both incubation time and concentration played a major role in the in vitro cytotoxicity of PTX. For longer incubation periods a larger number of cells enter the G2 and M cell cycle phases during which PTX is more active [3]. During the first 24 h of incubation, the significant amount of free PTX released from the nanoparticles could be available to mediate some cytotoxicity. Nevertheless, the cytotoxic effect may be a result of the presence of free PTX, or PTX-loaded nanoparticles or a combination of both.

Consistent with other published data, the incorporation of PTX into nanoparticles enhanced its anti-tumoral activity compared to Taxol® [30], showing smaller IC50 over Taxol® [20].

3.4. In vitro cellular uptake of nanoparticles

To check if the drug-loaded nanoparticles were internalized in tumor cells, the cellular uptake of PTX-loaded nanoparticles was evaluated on HeLa cells at different time intervals and different concentrations of PTX using flow cytometry. The percentage of cells that showed an increase of fluorescence compared to the untreated control cells and the fluorescence intensity are shown in Fig. 3.

As the nanoparticles contained the same ratio of PTX/polymers, the increase of PTX concentrations from 2.5 to 25 µg/ml lead to an increase in

Fig. 2. Viability of HeLa cells with (A) Taxol®, (B) Cremophor® EL and (C) PTX-loaded nanoparticles. Cells were incubated with different concentrations for 1, 2 and 3 days as described in Section 2.6. (D) Viability of HeLa cells incubated for 24 h with Taxol® (PTX concentrations: 25, 12.5 and 2.5 µg/ml), Cremophor® EL (concentrations corresponding to Taxol® formulation: 2.2, 1.1 and 0.22 mg/ml), PTX-loaded nanoparticles (PTX concentrations: 25, 12.5 and 2.5 µg/ml) and drug-free nanoparticles (polymers concentration 10 mg/ml). Cell viability was determined by the MTT assay. Untreated cells were taken as negative control and Triton X-100 1% was used as positive control. The results are expressed as mean values±standard deviation of 5 measurements of two independent experiments. *p<0.05, **p<0.01.
polymer concentration and nanoparticles number from approximately $10^8$/ml to $10^9$/ml. The uptake of the nanoparticles loaded with 25 µg/ml of PTX was very fast; 98% of the cells were fluorescent after 30 min. After 2 and 24 h, the percentage of fluorescent cells was the same, 99% respectively ($p < 0.05$). The fluorescence intensity increased with the incubation time ($p < 0.05$). The cellular uptake of nanoparticles loaded with 12.5 µg/ml of PTX was the same than for nanoparticles loaded with 25 µg/ml of PTX. For nanoparticles loaded with 2.5 µg/ml of PTX, the percentage of fluorescent cells increased with the incubation time ($p < 0.05$). The cellular uptake of PTX-loaded nanoparticles was time-dependent. The percentage of fluorescent cells and the fluorescence intensity increased with the concentration of the nanoparticles ($p < 0.05$). The cellular uptake of PTX-loaded nanoparticles was thus also concentration-dependent.

These results are in accordance with those of anti-tumoral activity studies: almost total inhibition of cell viability and high cellular uptake were achieved after 24 h incubation. Generally, nanoparticles are non-specifically internalized into cells via endocytosis or phagocytosis [31,32]. Nevertheless, it is possible that the FITC-labeled nanoparticles were not internalized in cells but adsorbed non-specifically to the surface of the cells. Other experiments should be carried out in order to distinguish these two mechanisms.

3.5. Apoptosis induced by PTX-loaded nanoparticles

To examine if the encapsulation of PTX in nanoparticles modify the apoptosis induced by PTX, DAPI staining of DNA was performed [33]. The nuclei of untreated HeLa cells showed homogenous fluorescence with no evidence of segmentation and fragmentation after DAPI staining. Exposure of the cells for 4 h at 37 °C to Taxol® and PTX-loaded nanoparticles (25 µg/ml of PTX) led to segregation of the cell nuclei into segments, indicating breakdown in the chromatin followed by DNA condensation. The percentage of apoptotic cells induced by Taxol® and
PTX-loaded nanoparticles was not significantly different indicating that nanoparticles did not affect the mechanism of action of PTX compared to Taxol® (Fig. 4). PTX encapsulated in the nanoparticles or released in the extracellular medium was able to induce apoptosis. Similar results were observed for other PTX concentration (12.5 and 2.5 µg/ml) and incubation time (8 h) [data not shown].

3.6. In vivo tumor growth inhibition study

The in vivo anti-tumor efficacy of PTX-loaded nanoparticles and Taxol® was evaluated in TLT tumor-bearing mice. The PTX-loaded nanoparticles inhibited tumor growth most efficiently, followed by Taxol® (p < 0.05) [Fig. 5A]. The study on the control (PBS) group ended on the 7th day because the tumor volume was excessively enlarged (about 18 mm), while other groups lasted until the 11th day for Taxol® and the 14th day for PTX-loaded nanoparticles (p < 0.001) [Fig. 5B]. Body weight measurements showed no significant differences between the groups throughout the study. There was a slight increase in body mass as a result of natural animal growth (data not shown).

When encapsulated into nanoparticles, PTX could attain tumor site through EPR effect and maintain the effective therapeutic concentra-

tion for a long period of time. The nanoparticle formulations allowed to suppress the use of Cremophor® EL which causes serious adverse effects.

4. Conclusion

The aim of the study was to design polymeric nanoparticles loaded with the anticancer drug PTX. PTX-loaded in the nanoparticles were more cytotoxic on Hela cells than Taxol®. However in contrast to Cremophor® EL, no cytotoxicity of the polymers was observed. Apoptosis induced by the PTX-loaded nanoparticles and Taxol® was similar. Cellular uptake of PTX-loaded nanoparticles was concentration and time dependent. In mice, PTX-loaded nanoparticles showed noticeable anti-tumor efficacy and enhanced survival rates, compared to Taxol®. Based on these results, it can be concluded that the formulations developed in this work may be considered as an effective anticancer drug delivery for cancer chemotherapy.

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