Title
Pharmacokinetics and antitumor efficacy of paclitaxel-cyclodextrin complexes loaded in mucus-penetrating nanoparticles for oral administration

Author(s) names & affiliations
Patricia Calleja\textsuperscript{a}, Socorro Espuelas\textsuperscript{a}, Leticia Corrales\textsuperscript{b}, Ruben Pio\textsuperscript{h,c}, Juan M. Irache\textsuperscript{a}
\textsuperscript{a}Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Navarra, Pamplona, Spain
\textsuperscript{b}Division of Oncology, Center for Applied Medical Research (CIMA), Pamplona, Spain
\textsuperscript{c}Department of Biochemistry, School of Sciences, University of Navarra, Pamplona, Spain

Correspondence:
Prof. Juan M. Irache
Dep. Pharmacy and Pharmaceutical Technology
University of Navarra
C/ Irunlarrea, 1
31080 – Pamplona
Spain
Phone: +34948425600
Fax : +34948425619

E-mail: jmirache@unav.es
Abstract

Aims: Authors report a novel approach for enhancing the oral absorption of paclitaxel by encapsulation in poly(anhydride) nanoparticles containing cyclodextrins and poly(ethylene glycol). Materials & Methods: Formulations were prepared by the solvent displacement method. Subsequently, pharmacokinetics and organ distribution assays were evaluated after oral administration to C57BL/6J mice. Additionally, antitumor efficacy studies were performed in a subcutaneous tumor model of Lewis lung carcinoma. Results: Paclitaxel loaded nanoparticles displayed sizes between 190-300 nm. Oral nanoparticles achieved drug plasma levels for at least 24h, with an oral bioavailability of 55-80%. Organ distribution studies revealed that paclitaxel orally administered in nanoparticles underwent a similar distribution to intravenous Taxol®. For in vivo antitumor assays, oral strategy maintained a slower tumor growth than intravenous Taxol®. Conclusion: Paclitaxel orally administered in poly(anhydride) nanoparticles, combined with cyclodextrins and poly(ethylene glycol), displayed sustained plasma levels and significant antitumor effect in a syngenic tumor model of carcinoma in mice.

Keywords

Oral chemotherapy, pharmacokinetics, antitumor efficacy, paclitaxel, poly(anhydride) nanoparticles, cyclodextrins, poly(ethylene glycol) 2000.
Introduction

Paclitaxel (PTX) is a widely used anticancer agent in patients with advanced breast, ovarian and non-small cell lung cancers [1], among other uses. It works by promoting the stabilization of the microtubules during cell replication stopping cell cycle and therefore, inducing apoptosis [2]. However, PTX presents a very low water solubility (<0.3 mg/ml) so it is formulated using a mixture of ethanol and Cremophor® EL (1:1, v/v) for intravenous (i.v.) administration.

Cremophor® EL has been proven to be responsible for the severe hypersensitivity reactions observed after administration [3, 4]. To minimize or avoid these reactions, patients are pretreated with antihistamines and corticosteroids. In addition, the i.v. route presents certain risks requiring specially qualified staff. In addition, patients have to undergo long infusion times (at least 3 hours for PTX), hospitalization and repeated cycles every 3 weeks until disease regression or no response [5]. In this context, the oral alternative to the traditional intravenous chemotherapy is a new trend gaining interest. Aside from a better patient compliance and a possible lower cost [5], the oral administration of anticancer medication would imply an increase in the patient’s quality of life since patients would be less dependent on hospital care, gaining in autonomy [5, 6].

Nevertheless, PTX has a low oral bioavailability since it is metabolized by the cytochrome P450 and it is substrate of the P-glycoprotein (Pgp). Both mechanisms are highly expressed in the gastrointestinal (GI) tract limiting the permeation of the drug through the intestinal membrane [7]. For PTX, plenty of works have arisen lately to attempt its oral administration. Alternatives vary from the co-administration of Taxol® with selective Pgp inhibitors such as cyclosporin A [8], to its encapsulation in drug delivery systems, such as micelles [9, 10], self-microemulsifying formulations [11], lipid nanoparticles [12, 13] or biodegradable polymeric nanoparticle [14-17]. Biodegradable nanoparticles are promising carriers to improve the oral administration of drugs (namely anticancer agents), vaccines and therapeutic proteins [18]. Nanoparticles help to enhance the oral bioavailability of poorly water soluble drugs protecting from degradation and
promoting the absorption at intestinal level. In such a way, poly(anhydride) nanoparticles prepared from Gantrez® AN polymer have been described as interesting carriers for oral delivery [19]. Gantrez® AN has been reported to present bioadhesive properties within the GI mucosa and a low oral toxicity [20]. Besides, the surface of these polymeric nanoparticles can be easily modified. Poly(anhydride) nanoparticles have been reported to encapsulate PTX [14, 17, 21] combined with cyclodextrins and poly(ethylene glycol) (PEG), which have additionally been identified as Pgp inhibitors [22-24]. In these previous works, an enhancement of the oral bioavailability of PTX in rats and an increase in the residence time in the GI tract was described.

So, the major objective of this work was to assess the antitumor efficacy of orally administered PTX encapsulated in poly(anhydride) nanoparticles combined with cyclodextrins and/or PEG for the first time in C57BL/6J mice. Firstly, pharmacokinetic and tissue distribution studies were developed and finally, the antitumor activity in Lewis lung carcinoma (3LL)-bearing mice was evaluated. Additionally, a novel strategy combining both cyclodextrins and PEG with poly(anhydride) nanoparticles is also here presented to attempt the oral administration of PTX.

**Materials & methods**

1. **Materials**

PTX (USP XXVI, grade>99.5%) and docetaxel (DCX) (grade>99.0%) were purchased from 21CECpharm (UK). Poly(methyl vinyl ether-co-maleic anhydride) (PMV/MA) or poly(anhydride) [Gantrez® AN 119; MW 200,000] was purchased from ISP/Ashland Inc (Spain). Taxol® was provided by Bristol-Myers-Squibb (USA). Phosphate buffered saline (PBS), glycine, β-cyclodextrin (CD) and 2-hydroxylpropyl-β-cyclodextrin (HPCD) were obtained from Sigma Aldrich (Germany) and disodium edetate (EDTA) and poly(ethylene glycol) 2000 (PEG2000) were provided by Fluka (Switzerland). All reagents and chemicals used were of analytical grade.
Lewis lung carcinoma cell line was obtained from the American Type Culture Collection (USA). Cells were cultured in RPMI 1640 medium (GibCo, Life Technologies, UK) supplemented with L-glutamine, 10% Fetalclone® (Thermo Fisher Scientific, USA), streptomycin (100 µg/ml) and penicillin (100 U/ml) (Invitrogen, USA) and passaged by trypsinization. For in vivo assays, C57BL/6J mice (20-22 g) were purchased from Harlan (Spain) and kept in standard animal facilities with free access to food and drinking water. Animal experiments were approved by the Ethical Committee for Animal Experimentation at University of Navarra (protocols 147-11 and 129-11).

2. Preparation of PTX-cyclodextrin complexes

The preparation of inclusion complexes of PTX and cyclodextrins (HPCD or CD) was performed by the method established by Agüeros et al. [21]. Briefly, 10 mg of PTX were dissolved in 2 ml of ethanol and added to 8 ml water containing the oligosaccharide. After a 72 h agitation, ethanol was evaporated under reduced pressure (Büchi R-144, Switzerland) and the resulting suspensions filtered through a 0.45 µm membrane filter. Finally, the obtained solution was evaporated under vacuum in the rotary evaporator in order to obtain a solid dry residue.

3. Preparation of poly(anhydride) nanoparticles

PTX-cyclodextrin inclusion complexes (PTX-HPCD or PTX-CD) were encapsulated in poly(anhydride) nanoparticles by a solvent displacement method as described previously [21]. Pegylated nanoparticles were prepared following the method published by Zabaleta et al. [17] with minor modifications.

3.1. Preparation of poly(anhydride) nanoparticles loaded with PTX-cyclodextrin complexes: PTX-HPCD-NP and PTX-CD-NP
PTX-cyclodextrin complexes were dispersed and incubated under magnetic stirring for 30 minutes in acetone containing 100 mg of the poly(anhydride) polymer previously dissolved. Nanoparticles were formed by the addition of an ethanol/water mixture (1:1, v/v). After elimination of the organic solvents under reduced pressure, the resulting suspensions were purified twice by centrifugation 27,000xg for 20 min. Supernatants were removed and pellets resuspended in water. Finally, the formulations were frozen and lyophilized (Genesis 12EL, Virtis, USA) using sucrose (5% w/v) as cryoprotector.

Formulations were named as follows: PTX-HPCD-NP (nanoparticles containing PTX-HPCD inclusion complex) and PTX-CD-NP (nanoparticles containing PTX-CD inclusion complex).

3.2. Preparation of peglated nanoparticles loaded with PTX-CD (PTX-CD-NP-PEG)

PTX (inclusion complex with CD) was dispersed in acetone containing poly(anhydride) polymer and PEG2000. Afterwards, the nanoparticles were formed by the addition of a mixture of ethanol and water (1:1 volume). Organic solvents were eliminated under reduced pressure and the resulting suspension was purified by centrifugation and, finally, freeze-dried using sucrose (5%) as cryoprotector.

3.3. Preparation of peglated nanoparticles loaded with PTX (PTX-NP-PEG)

Following the method published by Zabaleta and co-workers [17], PTX was incubated with Gantrez® AN and PEG2000 under magnetic stirring for 30 min in acetone. Then, nanoparticles were formed by the addition of an ethanol/water mixture (1:1 by vol.). The organic solvents were eliminated under reduced pressure and the suspension purified twice by ultrafiltration in Vivaspin tubes (300,000 MWCO, Sartorius, Germany) at 3,000 xg for 20 min. Pellets were resuspended in water and finally, the formulations were frozen and freeze-dried using sucrose (5%) as cryoprotector.
4.  Characterization of poly(anhydride) nanoparticles

4.1.  Physicochemical characterization

The mean hydrodynamic diameter of the nanoparticles and the zeta potential were determined by photon correlation spectroscopy (PCS) and electrophoretic laser Doppler anemometry, respectively, using a Zetamaster analyzer system (Malvern Instruments Ltd., UK). The diameter of the nanoparticles was determined after dispersion in ultrapure water (1:10) and measured at 25°C by dynamic light scattering angle of 90°C. The zeta potential was measured in 0.1 mM KCl solution. The yield of the process was calculated by gravimetry as previously published [21].

4.2.  Quantification of the amounts of PEG and CD associated with the nanoparticles

The amounts of PEG and cyclodextrins (either HPCD or CD) bound to the nanoparticles were estimated as published elsewhere [25, 26] by quantification in supernatants collected from the purification steps. The technique assessed was HPLC (Agilent model 1100 series LC, Germany) attached to an Evaporative Light Scattering Detector (ELSD) (Alltech, USA) [25, 26]. Each sample was assayed in triplicate and results were expressed as the amount of PEG or cyclodextrin per mg of nanoparticle.

4.3.  PTX content in nanoparticles

The amount of PTX loaded in the nanoparticles was quantified by HPLC-UV [14]. The equipment was an Agilent model 1100 series LC and a diode-array detector set at 228 nm. The chromatographic system was equipped with a reversed-phase 150 mm x 3 mm C18 Phenomenex Gemini column (particle size 5 µm). The mobile phase consisted of phosphate buffer (0.01 M, pH 2) and acetonitrile (50:50 v/v) eluted at 0.5 ml/min. For analysis, nanoparticles were solubilized with acetonitrile (1:5 v/v) and assayed in triplicate. Results were expressed as the amount of PTX (µg) per mg nanoparticles.
5. **Pharmacokinetic studies**

C57BL/6J mice were randomly divided into treatment groups. The experimental groups were: (a) PTX-HPCD-NP, (b) PTX-CD-NP, (c) PTX-NP-PEG and (d) PTX-CD-NP-PEG. Each animal received PTX orally loaded in poly(anhydride) nanoparticles at a dose of 25 mg/kg body weight (bw). As controls, one group received i.v. Taxol® via tail vein and another group was treated with the same commercial formulation orally; both treatments at a dose of 25 mg/kg bw. Previous to the drug administration, animals were fasted overnight to avoid interference with the absorption, allowing free access to water.

Blood samples (300 µl) were obtained from 3 animals per time point at 0 min, 10 min, 30 min, 1, 3, 6, 8, 24, 48 and 72 hours after administration. EDTA was used as anticoagulant agent. Blood volume was recovered intraperitoneally with an equal volume of normal saline pre-heated at body temperature. Plasma was separated into clean tubes by centrifugation at 2500xg for 10 minutes and kept frozen until analysis.

5.1. **Determination of PTX plasma concentration by HPLC-UV**

The amount of PTX was determined in plasma by HPLC-UV following the method described by Agüeros et al. [14] An aliquot (100 µl) of plasma was mixed with 25 µl of internal standard solution (DCX, 4 µg/ml in ethanol). A liquid–liquid extraction was accomplished by adding 4 ml of tert-buthylmethyleneether. The organic layer was transferred to a clean tube and evaporated until dry (Savant, Spain). Finally, the residue was dissolved in 125 µl of reconstitution solution (acetonitrile–phosphate buffer 0.01 M pH 2; 50:50 v/v) and placed in the HPLC. The UV detection of PTX was performed at 228 nm.

5.2. **Calculation of pharmacokinetic parameters**
The pharmacokinetic analysis was performed based on a non-compartmental model using WinNonlin 5.2 software (Pharsight Corporation, USA). The following parameters were estimated: area under the curve (AUC), half-life of the terminal phase (t₁/₂), mean residence time (MRT), peak plasma concentration (Cₘₐₓ) and time to reach the peak plasma concentration (Tₘₐₓ). In addition, the relative oral bioavailability (F %) of PTX was calculated using the ratio of dose-normalized AUC values following oral and i.v. administrations:

\[ F \% = \frac{AUC_{oral}}{AUC_{i.v.}} \times 100 \text{ [eq. 1]} \]

where \( AUC_{oral} \) and \( AUC_{i.v.} \) correspond to the areas under the plasmatic curve for the oral and i.v. administrations, respectively.

6. Organ distribution of PTX

To study the amount of drug in organs, animals received PTX loaded in the poly(anhydride) nanoparticles orally at a dose of 25 mg/kg bw. Treatment groups were: PTX-HPCD-NP, PTX-CD-NP, PTX-CD-NP-PEG and PTX-NP-PEG. In addition, a group of mice was treated with i.v. Taxol® at the same dose (25 mg/kg bw) as control.

After administration, mice were sacrificed at different time points by cervical dislocation under isoflurane anesthesia and the following organs were harvested: liver, lung, spleen, kidneys, ovaries, stomach and intestine. In the group receiving Taxol®, animals were sacrificed at 30 min, 3h, 8h and 24 hours post-administration. On the other hand, the animals receiving pegylated nanoparticles (PTX-NP-PEG) were sacrificed at 3h, 8h, 24h and 72 hours. Finally, for the other treatment groups (PTX-HPCD-NP, PTX-CD-NP and PTX-CD-NP-PEG), the animals were killed at 8 hours exclusively. Time points were selected based on the plasmatic curves obtained for the different formulations in mice.

Before quantifying the amount of PTX in the different tissues, organs were individually weighed and homogenized in 1 ml of PBS pH 7.4 using a Mini-bead Beater (BioSpect Products
Inc, USA). Later, the homogenized organs were centrifuged at 10,000 xg for 10 minutes and the supernatants collected and stored at -80°C until analysis.

6.1. Measurement of PTX levels in tissue samples by HPLC-UV

For the determination of PTX in the different tissues, a liquid-liquid extraction method followed by reverse-phase HPLC analysis was performed. The extraction method was adapted from Agüeros et al [14]. Standardized calibration curves were used for each organ. DCX was used as internal standard and the conversion of the PTX/DCX chromatographic areas to concentration was performed. Aliquots (200 µl) of the tissues were mixed with 25 µl of the DCX solution (5 µg/ml in ethanol). After mixing, a liquid-liquid extraction was accomplished by adding 3 ml of t-buthylmethylether. Next, the mixture was centrifuged at 3000 xg for 5 min and then, the clear organic layer was transferred to clean tubes and evaporated until complete dryness. Finally, the residue was dissolved in 125 µl of acetonitrile-phosphate buffer (0.01 M pH2, 50:50 v/v), and quantified by HPLC-UV at a wavelength of 228 nm. A new calibration curve was done for every set of samples for each tissue studied with a established detection limit of 200 ng/ml.

7. Antitumor efficacy studies

7.1. Animal model

In vivo antitumor efficacy was evaluated with a tumor model set up by inoculation of the Lewis Lung Carcinoma cells to mice. Before the implantation, 3LL cells were maintained at 37°C and 5% CO₂ in RPMI supplemented with 10% Fetalclone® and antibiotics. Prior to the inoculation of cells to animals, a mycoplasma assay was performed to ensure the absence of contaminants in the cell culture samples.
On the day of the experiments, 3LL cells (1 × 10^5) were mixed with Growth Factor Reduced Matrigel Matrix (BD Biosciences, USA) (1:1, v/v), and injected subcutaneously on the right flank of mice under isoflurane anesthesia.

7.2. Antitumor study

Treatments were started on day 8 after inoculation when tumors were palpable and reached approximately 100 mm³. On that day (considered day 1 of treatment), mice were randomly distributed into the following groups: control group, commercial Taxol® treatment group (administered i.v.) and nanoparticle treatment groups (PTX-HPCD-NP, PTX-CD-NP, PTX-NP-PEG and PTX-NP-PEG administered orally). Each group consisted of 8 tumor-bearing animals.

Taxol® (dose 10 mg/kg bw) was diluted with sterile normal saline (0.9%) to facilitate injection and administered daily via the tail vein. The lyophilized nanoparticle formulations were resuspended in water (300 µl) prior to the oral administration at a dose of 25 mg/kg bw of PTX in nanoparticles. The treatment schedule for the nanoparticles was: pegylated nanoparticles, PTX-NP-PEG, every 3 days and cyclodextrin containing formulations, PTX-HPCD-NP, PTX-CD-NP and PTX-CD-NP-PEG, daily. As established in the approved protocol, when tumor volumes reached 2000 mm³, animals were sacrificed considering tumors life-threatening.

Throughout the study, tumors were measured with a caliper every two days. Tumor volumes were calculated according to the following formula:

\[
Tumor \text{ Volume (mm}^3) = \frac{L \times W^2}{2} \quad [eq. 2]
\]

in which the L corresponded to the largest diameter and W to the shortest diameter of the tumor, perpendicular to length.

In addition, tumor growth delay (TGD) and tumor doubling time (DT) were determined as pharmacodynamic parameters [27, 28].

DT was calculated using the following equation:
\[ DT = (t_f - t_0) \times \frac{\log 2}{\log V_f - \log V_0} \quad [eq. 3] \]

in which \( t_f - t_0 \) correspond to the difference between two measurements and \( V_f \) and \( V_0 \) indicate the tumor volumes at two points of measurements. TGD was calculated as the time in days required for tumors to reach a mean volume of 500 mm\(^3\).

7.3. **Measurement of vascular endothelial growth factor (VEGF)**

Blood samples were obtained to evaluate vascular endothelial growth factor (VEGF) levels as angiogenesis marker. For this purpose, a commercial kit (Mouse VEGF Immunoassay Quantikine® ELISA kit, R&D Systems, USA) was used. Blood samples were obtained at the beginning of the study (basal levels of VEGF in plasma) and every 2 days from 3 animals in each group randomly. Blood (300 µl) was extracted from mice and plasma was recovered by centrifugation at 2500 xg for 10 min and frozen at -80°C until analysis. Samples were processed as specified in the commercial kit and the plate was read at 450 and 540 nm using a microtite plate reader (Labsystems, Finland).

8. **Statistical analysis**

Data are expressed as the mean ± standard deviation (S.D.) of at least three experiments. One-way ANOVA with Bonferroni post-test, Mann-Whitney U-test or Kruskall-Wallis tests were used to investigate statistical differences. In all cases, \( p < 0.05 \) was considered to be statistically significant. All data processing was performed using GraphPad Prism 4.0 statistical software (GraphPad Software, USA).

**Results**

1. **Preparation and characterization of poly(anhydride) nanoparticles**
Poly(anhydride) nanoparticles were successfully prepared by the solvent displacement method. The main physicochemical characteristics of the different poly(anhydride) nanoparticles loaded with PTX are summarized in Table 1. In the first place, nanoparticles containing the drug-cyclodextrin complexes displayed bigger sizes (250-300nm) than the nanoparticles including PEG (190-200 nm). It is interesting to note that the addition of PEG to the formulation containing PTX-CD complexes, PTX-CD-NP-PEG, decreased the mean size of the resulting nanoparticles.

Regarding the zeta potential, interestingly nanoparticles containing PEG (PTX-CD-NP-PEG and PTX-NP-PEG) presented a slightly more negative surface charge, around -55 mV. The nanoparticles formulated with just cyclodextrins displayed a surface charge around -46 mV. Furthermore, the yield of the process was calculated to be between 60% and 73% for PEG and CD formulations, respectively.

The amounts of excipients (cyclodextrins and PEG) associated with the different formulations were estimated by HPLC-ELSD. On one hand, for cyclodextrin containing nanoparticles, the amount of oligosaccharide depended on the type of cyclodextrin used. Thus, HPCD showed a higher ability to associate with the poly(anhydride) polymer than β-cyclodextrin correlating with the previous results by Agüeros et al [14]. The amount of cyclodextrin for the 2 formulations containing β-CD was similar (PTX-CD-NP and PTX-CD-NP-PEG: 80-88 µg/mg NP approximately), lower than PTX-HPCD-NP. On the other hand, when PEG was added to the formulation containing PTX-CD complex, the amount of PEG was decreased (42 µg/mg NP) compared to the nanoparticles with no cyclodextrin at all, PTX-NP-PEG (55 µg/mg NP).

Focusing on the amount of PTX loaded in the nanoparticles, differences were obtained between the formulations. For the nanoparticles formulated with HPCD, PTX content was estimated in 149 µg PTX/mg NP. However, for the nanoparticles containing β-cyclodextrin (PTX-CD-NP), the amount of PTX was significantly lower (3 times lower), around 50 µg/mg NP. Besides, when PEG was added to the formulation containing PTX-CD complexes, almost a 40%
increase in the drug loading was observed, from 50 to 70 µg/mg NP, enhancing the encapsulation of the anticancer drug. Finally, for PTX-NP-PEG, the amount of PTX was calculated to be around 110 µg/mg NP.

2. Pharmacokinetic study

The plasma concentration-time curve after a single i.v. administration of Taxol® at 25 mg/kg is shown in figure 1. The i.v. plasma profile of Taxol® presented a nonlinear profile, with detectable plasma levels of PTX until 12 hours post-administration.

Figure 2 shows the plasma concentration profiles of PTX after a single oral dose of 25 mg/kg to mice when administered as commercial Taxol® or encapsulated in poly(anhydride) nanoparticles. When commercial Taxol® was administered orally, PTX plasma levels were detected at low concentrations and decreased rapidly, displaying no detectable levels after 8 hours. Thus, the plasma levels for oral Taxol® were low, close to the quantification limit of the HPLC technique (80 ng/ml).

On the contrary, PTX plasma levels found after the oral administration of the poly(anhydride) nanoparticles were significantly higher than oral Taxol®, 10-15-fold higher. In all cases, there was an initial rapid rise for the first 2 hours reaching the maximum concentration (C_max) followed by slow decline prolonged for at least 24 hours for PTX-HPCD-NP and PTX-CD-NP and up to 72 hours for the PEG containing formulations (PTX-NP-PEG and PTX-CD-NP-PEG).

Table 2 summarizes the main pharmacokinetic parameters estimated. Firstly, for the i.v. commercial formulation, the AUC was 101 µg h/ml with a maximum concentration (C_max) of 113 µg/ml. The MRT was 3.2 h and the half-life of the terminal phase (t_1/2) was estimated to be 2.5 h.

As seen in table 2, the C_max for the nanoparticles was significantly higher than for oral Taxol®. Within the different poly(anhydride) nanoparticles, the rank order of C_max values obtained was: PTX-NP-PEG > PTX-CD-NP-PEG > PTX-HPCD-NP > PTX-CD-NP. Similarly, the highest AUC value
was obtained for pegylated nanoparticles (PTX-NP-PEG). In addition, this AUC value for PTX-NP-PEG was 38%, 48% and 21.5% higher than for PTX-HPCD-NP, PTX-CD-NP and PTX-CD-NP-PEG, respectively. However, in all cases \( T_{\text{max}} \) appeared to be similar (\( T_{\text{max}}=1-1.5 \) h).

Additionally, MRT of PTX encapsulated in nanoparticles was between 18 to 30 hours, significantly higher than that obtained for Taxol® (1.3 h). Conversely, the values of \( t_{1/2} \) for all the nanoparticles were similar (between 14 to 18h).

Finally, the oral bioavailability of PTX delivered in nanoparticles was calculated to be around 55% and 59% for PTX-CD-NP and PTX-HPCD-NP, and 67% and 81% for PTX-CD-NP-PEG and PTX-NP-PEG, respectively. These values were 33-fold higher (on average) than the bioavailability estimated for oral Taxol® (Fr= 2.3%).

3. Organ distribution of PTX

Organ distribution of PTX after the administration of i.v. Taxol® and oral nanoparticles were compared in C57BL/6J mice.

Figures 3A and 3B represent the amount of PTX found in different organs (liver, kidneys, spleen, ovaries, lung, stomach and intestine) at different times after the administration of i.v. Taxol® or oral PTX-NP-PEG, respectively. As it can be seen, PTX underwent a rapid and wide distribution in the organs evaluated. Following the i.v. administration, at the shortest time (30 minutes), the highest concentration of PTX was found in liver (30 \( \mu g \) /g tissue), followed by kidney (25 \( \mu g \) /g tissue), spleen (15 \( \mu g/g \) tissue) and lung (10 \( \mu g/g \) tissue). However, after 24 hours, the drug amount in the mentioned organs was remarkably decreased as expected, and a higher concentration was found in the intestine (PTX amount 24 hours post-administration=20 \( \mu g/g \) tissue). Thus, when PTX was orally administered loaded in pegylated nanoparticles, the drug amounts in organs followed a similar trend to that obtained for Taxol®, although with a certain delay in time with respect to i.v. administration. The main distribution at 3 hours for PTX-NP-PEG was in liver (33 \( \mu g/g \) tissue), kidneys (30 \( \mu g/g \) tissue), lung (35 \( \mu g/g \)
tissue) and ovaries (15 µg/g tissue). As time increased, the drug in tissues decreased except in the case of the intestine, with higher levels (25 µg PTX/g tissue approximately) at the longest times evaluated, 72 hours, same trend than for the commercial formulation.

Figure 4 shows the comparative amount of PTX in organs 8 hours post-administration after oral or i.v. administration of the nanoparticles and Taxol® at a dose of 25 mg/kg. In general, the amounts of anticancer drug found at 8 hours were significantly higher (p< 0.05) than for Taxol®. Thus, the largest amounts of drug were found in all cases in liver, kidneys, ovaries and intestine. In these organs, the amount of PTX found was at least 7-fold, 11.5-fold, 4.5-fold and 5.5-fold higher than that of the commercial formulation, respectively. In contrast, slight differences were observed amongst nanoparticles mainly in liver. In liver, pegylated nanoparticles (PTX-NP-PEG and PTX-CD-NP-PEG) displayed slightly higher drug levels than PTX-HPCD-NP and PTX-CD-NP (1.5 times).

Regarding the levels in lung, only the PEG containing formulations (PTX-CD-NP-PEG and PTX-NP-PEG) presented statistically significant differences compared to Taxol®. Furthermore, in lung, for these pegylated formulations (PTX-NP-PEG and PTX-CD-NP-PEG) the amounts of drug were almost 3 times higher than for PTX-HPCD-NP and PTX-CD-NP. In the other studied organs (spleen, ovaries and stomach), the drug amounts were similar to those observed for Taxol® with no statistical differences.

4. Antitumor activity

Figure 5 represents the mean tumor volumes (in mm$^3$) throughout the treatment. As observed, tumors were similar on day 1 in all groups with a mean volume of 100 mm$^3$, approximately. No size regression was observed in the control group, as expected and tumors grew exponentially reaching volumes larger than 2000 mm$^3$ at the end of the study. On the other hand, the daily i.v. administration of Taxol® was capable of maintaining constant volumes up to day 5. After, tumors grew rapidly, presenting on day 10 the biggest volumes of all the treatment groups.
(volume> 1000 mm³). Yet, for the poly(anhydride) nanoparticle groups, tumors grew at a slower rate. Nonetheless, differences were observed between formulations. From day 7 onwards, in the group treated with the β-cyclodextrin formulation (PTX-CD-NP), tumors grew at a faster rate and at the end of the study, mice presented tumor volumes of 700-800 mm³, approximately. The evolution of the tumors for PTX-CD-NP-PEG and PTX-HPCD-NP was similar, with a more sustained growth and a final volume of 575 and 650 mm³, respectively. In contrast, the treatment with PTX-NP-PEG displayed the smallest tumor sizes (volume< 500 mm³) throughout the whole study.

PTX-NP-PEG administered every 3 days and PTX-CD-NP-PEG administered daily were capable of reducing significantly (p<0.01) tumors in mice compared to i.v. Taxol®, administered daily. The pharmacodynamic variables of antitumor response were determined by growth delay and doubling times. As shown in Table 3, the untreated group, Taxol® group and PTX-CD-NP group presented similar doubling times, 1.9, 1.6 and 1.7 days, respectively. Mice treated with nanoparticles containing HPCD had an increase in the doubling times compared to Taxol® (2.3 days vs. 1.6 days). Additionally, the groups receiving pegylated nanoparticles (PTX-CD-NP-PEG and PTX-NP-PEG) had similar DT values (2.5 and 2.6 days, respectively).

Regarding growth delay, animals treated with i.v. Taxol® presented a delay of 7.5 days (2.5 days higher than for the untreated animals). In contrast, the formulation of PTX in nanoparticles clearly inhibited the tumor growth achieving values from 9 to 11 days in growth delay with no substantial differences between treatments.

Figure 6 shows the VEGF profiles throughout the study for the different groups. The concentrations of VEGF at the beginning of the experiment were the basal levels (15 pg/ml) in mice. As observed in figure 6, in general, despite the administration of the different treatments, there was an increase in the VEGF plasma concentration for all the groups. On the initial days of treatment, levels of the angiogenesis marker were maintained similar. However, at the end of the study differences were observed between treatments. On day 11, for the
Taxol® group, the increase was of 10-fold compared to the basal levels whereas for the nanoparticle groups this rise was not as pronounced (5-7-fold).

Discussion

Recently, the combination between poly(anhydride) nanoparticles and either cyclodextrins or PEG has been reported as an interesting approach for the oral administration of PTX [14, 17]. These nanoparticles were able to increase the relative oral bioavailability of the drug in rat up to 70-80%, approximately [14, 17]. Nevertheless, the use of β-cyclodextrin resulted in a quite low drug loading (about 4-5%) compared to the use of HPCD or PEG (around 15-16%). In addition, when cyclodextrins were used, the plasma levels of PTX in rat were maintained for 24 h, whereas pegylated nanoparticles offered sustained plasma levels of the anticancer drug for up 3 days.

In this work, our aim was to complete these previous studies in rat and gain insight in the potential use of these nanoparticle vehicles as oral delivery systems for PTX. For this purpose, the pharmacokinetics of PTX as well as its organ distribution and efficacy in an animal tumor model (C57BL/6J mice) were evaluated. In parallel, the effect of pegylation of nanoparticles containing PTX as inclusion complex with CD on their in vivo properties was also investigated. In this work, our aim was to evaluate the oral bioavailability, organ distribution and antitumor efficacy of PTX when loaded in poly(anhydride) nanoparticles in C57BL/6J mice. The strategy here described combined two excipients, PEG and cyclodextrins, with nanoparticles to perform in vivo studies. The poly(anhydride) nanoparticles were obtained by a simple desolvation of the poly(anhydride) polymer in ethanol as described previously by Arbos et al. [29]. The drug content was found to be dependent on the excipients used. Thus, there were differences in the drug loading according to the cyclodextrin selected, as previously stated by Agüeros et al. [14]. On the other hand, when PEG was added to the formulation containing PTX-CD complexes, an increase in the drug loading was evidenced enhancing the drug loading due to
the presence of PEG, which could help solubilize the drug and therefore, enhance the drug encapsulation.

For the pharmacokinetic study, a single dose of 25 mg/kg was selected. I.v.Taxol® presented a characteristic nonlinear pharmacokinetic profile, published previously, associated with the presence of Cremophor® EL in the formula [30]. From the pharmacokinetic analysis, comparable values to the earlier reported by other authors were obtained [31, 32]. However, when commercial Taxol® was administered orally, the plasma concentrations of PTX were low. The calculated oral bioavailability for Taxol® was around 2.5%. This value is in agreement with previously reported values varying from 2 to 10.5% [13, 33, 34]. On the other hand, when PTX loaded in poly(anhydride) nanoparticles was administered orally to mice, the plasma levels of the anticancer drug were higher and more sustained in time. Thus, PTX was found in plasma for at least 24 hours after administration for the case of PTX-HPCD-NP and PTX-CD-NP and up to 72 hours for the formulations containing PEG, PTX-CD-NP-PEG and PTX-NP-PEG. These plasma levels could be considered pharmacologically active since they were above the clinical therapeutic threshold of 0.01mM (equivalent to 85 ng/ml approximately) [33].

Overall, in C57BL/6J mice, the relative bioavailability data obtained for the different poly(anhydride) nanoparticles were high, varying from 55-60% for PTX-CD-NP and PTX-HPCD-NP and from 67 to 81% for the PEG containing nanoparticles, PTX-CD-NP-PEG and PTX-NP-PEG. Interestingly, the combination of PEG with CD enhanced slightly the relative oral bioavailability of PTX.

The results of tissue distribution showed the presence of PTX mainly in liver, kidney and lung at the initial hours after the i.v. Taxol®. At 24 hours post-administration, the highest levels were obtained in intestine, as expected since PTX has been described to suffer elimination by feces [35]. Similarly, the evaluation of the drug levels in organs after the oral administration of the drug loaded in the different poly(anhydride) nanoparticles assessed the highest amounts of anticancer agent in liver, kidneys, intestine, lung and ovaries, such as mentioned for Taxol®.
Previous published results on organ distribution already stated the wide distribution of the anticancer drug, decreasing with time [12, 32]. The presence of the drug in organs clearly correlates with a systemic effect since the drug is firstly absorbed at the GI level and rapidly distributed in plasma reaching the different tissues where it finally would act as anticancer agent. Interestingly, comparing the organ distribution profiles of the oral and i.v. PTX, no differences were observed. In the case of nanoparticles, the drug is clearly absorbed at GI level at a slower rate since it has to undergo a release from the delivery system and therefore, the appearance of the drug in plasma and organs is delayed, compared to i.v. administration.

The antitumor efficacy was studied by measuring the tumor volume every two days after implantation. Using this model, the poly(anhydride) nanoparticles loaded with PTX and orally administered were able to diminish tumor growth compared to i.v. Taxol® as previously observed by Zabaleta et al. [36] Interestingly, the lowest tumor volume was obtained for pegylated nanoparticles, PTX-NP-PEG, administered every 3 days. On the other hand, the effect on tumor growth of PTX-HPCD-NP and PTX-CD-NP-PEG (administered daily) was similar. These results appear to indicate that the presence of sustained and not very high levels of PTX in plasma could be efficient to reduce tumor mass in tumor bearing mice. Furthermore, no signs of toxicity were observed in the nanoparticle receiving animals, indicative of the biocompatibility of the poly(anhydride) nanoparticles in this study.

In addition, VEGF was measured as angiogenesis factor. Taking into account that tumor cells produce VEGF and that PTX induces tumor cell death, the antiangiogenic effect of treatments was evaluated. VEGF did not diminish in blood but there was a slower increase, especially in those animals receiving the nanoparticles treatments. Pegylated nanoparticles (PTX-NP-PEG and PTX-CD-NP-PEG) and PTX-HPCD-NP were able to show a slower rise of VEGF correlating with slower tumor growth.

All in all, the presence of PEG provided a rather higher ability than cyclodextrins to promote the absorption of the drug at the intestinal mucosa. In fact, the combination of the 2
excipients, CD and PEG, apparently favored the interaction of the nanoparticles with the GI mucosa and promoted the absorption of PTX once released displaying higher drug levels in plasma and tissues than for the nanoparticles formulated with CD alone. Herein, the presence of PEG could permit a deeper penetration of the drug delivery system in the mucosa; there, establish a stronger interaction thanks to the hydrophilic nature of PEG and finally, enhance the absorption of the encapsulated drug at the surface of the enterocytes. Since PTX-HPCD-NP and PTX-CD-NP formulations presented bigger sizes and no PEG was attached, the capacity of these carriers to penetrate in the mucus would be more limited remaining instead in more superficial layers. As a result, the plasma and tissue concentrations appeared to be slightly reduced (1.5-3 times lower levels) for poly(anhydride) nanoparticles containing cyclodextrins: HPCD or CD than for the pegylated ones: PTX-CD-NP-PEG and PTX-NP-PEG.

**Conclusions**

In conclusion, the work here presented demonstrated that the combination of poly(anhydride) nanoparticles with cyclodextrins and PEG favored the encapsulation of PTX as inclusion complex with CD increased the loading of the drug enhancing the absorption at the intestinal surface. This is reflected in maintained plasma levels of the drug and the organ distribution achieved after the oral administration of PTX encapsulated in the nanocarriers. In addition, these nanoparticles were able to slow down tumor growth in a murine model using 3LL tumor cell line. So, the combination of PEG and PTX-CD complexes loaded in poly(anhydride) nanoparticles was a successful and novel approach to ameliorate the oral uptake of the anticancer drug and allow sustained plasma levels with a significant tumor activity.

**Future Perspective**

The oral route is the most commonly accepted route of administration by patients in the clinical settings. The advantages it entails go from a higher patient convenience even to a
higher compliance of the treatment or a decrease in costs since the patients are more involved in the treatment and there is no need of hospital special requirements. However, nowadays only a few anticancer agents can be administered orally. Thus, many factors (low solubility, poor permeability or presystemic metabolism) reduce the oral bioavailability of drugs. In order to overcome these drawbacks and facilitate, or in many cases, achieve the oral administration for cancer treatment, works have focused on developing new drug delivery systems that can permit or enhance the intestinal uptake of the drugs. The development of delivery systems as well as any advances in cancer treatment will entail great advances from a clinical point of view as well as from an economical perspective.

Amongst these delivery systems under investigation, the use of different polymeric nanocarriers has been widely studied and numerous works have been published in this area. The reduction of size and targeting possibilities these systems offer could be a major breakthrough in therapy against cancer. In this context, polymeric nanoparticles and many other nanocarrier systems are currently under research. Although further studies are necessary, the technology and devices capable of offering effective oral delivery of anticancer drugs with poor solubility and permeability is feasible. Further progress in oral chemotherapy is required since there is still no specificity against cancer cells after oral administration. Thus, in future years, more development in this area would be expected and luckily, new oral formulations for anticancer agents will reach clinical development.

**Executive Summary**

- **Pharmacokinetic studies of paclitaxel encapsulated in poly(anhydride) nanoparticles combined with cyclodextrins and/or PEG 2000 in mice**
  - Plasma levels of PTX were significantly higher when orally administered encapsulated in poly(anhydride) nanoparticles than for commercial Taxol®.
The oral bioavailability of PTX encapsulated in poly(anhydride) nanoparticles combined with cyclodextrins and PEG2000 was increased to, at least, 55%.

- **Organ distribution studies in mice**
  - The encapsulation of paclitaxel in poly(anhydride) nanoparticles did not affect the distribution of the drug in the body.
  - Regardless the formulation, paclitaxel was found in liver, spleen, kidney, ovaries and intestine.

- **Antitumor efficacy studies in mice**
  - Paclitaxel encapsulated in poly(anhydride) nanoparticles was able to slow down tumor growth in a murine tumor model.
  - Pegylated nanoparticles administered every 3 days presented smaller tumors compared to cyclodextrin containing nanoparticles administered daily.

- **Combination of β-cyclodextrin and PEG2000 with poly(anhydride) nanoparticles**
  - The association of β-cyclodextrin and PEG2000 to poly(anhydride) nanoparticles permitted an increase in the loading of PTX.
  - The combination of β-cyclodextrin and PEG2000 in poly(anhydride) nanoparticles enhanced the oral absorption of paclitaxel up to 70-80% in C57BL/6J mice.

**References**


** Comprehensive review of benefits and risks of anticancer oral delivery


* Production of paclitaxel-loaded nanoparticles for oral delivery


* One of the first papers related with the inhibitory effect of some excipients on the intestinal P-gp


* One of the first papers related with the inhibitory effect of some excipients on the cytochrome P450 enzymatic complex


* One of the first papers related with the organ distribution of paclitaxel


**Financial & competing interests disclosure**

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**Ethical conduct of research**

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.
Tables

Table 1. Physicochemical characterization of the obtained poly(anhydride) nanoparticles.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Size (nm)</th>
<th>Zeta Potential (mV)</th>
<th>Yield (%)</th>
<th>PDi</th>
<th>PEG content (µg /mg NP)</th>
<th>Cyclodextrin content (µg /mg NP)</th>
<th>PTX Loading (µg PTX/mg NP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTX-HPCD-NP</td>
<td>295 ± 5</td>
<td>-45 ± 3</td>
<td>70 ± 5</td>
<td>0.13</td>
<td>-</td>
<td>98 ± 3.5</td>
<td>149 ± 3.1</td>
</tr>
<tr>
<td>PTX-CD-NP</td>
<td>255 ± 7</td>
<td>-46 ± 6</td>
<td>73 ± 4</td>
<td>0.15</td>
<td>-</td>
<td>80 ± 6.9</td>
<td>49 ± 3.8</td>
</tr>
<tr>
<td>PTX-CD-NP-PEG</td>
<td>220 ± 6</td>
<td>-55 ± 3</td>
<td>65 ± 3</td>
<td>0.15</td>
<td>42 ± 2.4</td>
<td>88 ± 5.8</td>
<td>69 ± 3.1</td>
</tr>
<tr>
<td>PTX-NP-PEG</td>
<td>193 ± 3</td>
<td>-53 ± 2</td>
<td>62 ± 4</td>
<td>0.15</td>
<td>55 ± 2.2</td>
<td>-</td>
<td>112 ± 4.2</td>
</tr>
</tbody>
</table>

Table 2. Pharmacokinetic parameters of PTX obtained after the i.v. and oral administration of the commercial Taxol® and poly(anhydride) nanoparticles encapsulating PTX at a single dose of 25 mg/kg bw to C57BL/6J mice.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Route</th>
<th>AUC (µg h/ml)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>MRT (h)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>Fr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxol®</td>
<td>i.v.</td>
<td>101.2 ± 5.7</td>
<td>112.9 ± 6.7</td>
<td>0.05</td>
<td>3.2 ± 0.8</td>
<td>2.5 ± 0.3</td>
<td>100</td>
</tr>
<tr>
<td>Taxol®</td>
<td>p.o.</td>
<td>2.3 ± 0.9</td>
<td>0.2 ± 0.1</td>
<td>1</td>
<td>1.3 ± 0.8</td>
<td>2.2 ± 0.6</td>
<td>2.3</td>
</tr>
<tr>
<td>PTX-HPCD-NP</td>
<td>p.o.</td>
<td>59.3 ± 5.7</td>
<td>5.2 ± 2.8</td>
<td>1.5</td>
<td>23 ± 2.3</td>
<td>15.3 ± 1.4</td>
<td>58.6</td>
</tr>
<tr>
<td>PTX-CD-NP</td>
<td>p.o.</td>
<td>55.3 ± 5.2</td>
<td>3.6 ± 1.3</td>
<td>1.5</td>
<td>18 ± 1.6</td>
<td>13.6 ± 1.5</td>
<td>54.6</td>
</tr>
<tr>
<td>PTX-CD-NP-PEG</td>
<td>p.o.</td>
<td>67.4 ± 3.8</td>
<td>5.1 ± 2.9</td>
<td>1.5</td>
<td>31 ± 2.8</td>
<td>17.1 ± 1.6</td>
<td>66.6</td>
</tr>
<tr>
<td>PTX-NP-PEG</td>
<td>p.o.</td>
<td>82.0 ± 3.1</td>
<td>5.7 ± 2.6</td>
<td>1.5</td>
<td>29 ± 3.1</td>
<td>18.3 ± 1.2</td>
<td>81.1</td>
</tr>
</tbody>
</table>

Data expressed as mean ± S.D. (n=3). AUC: Area under the concentration-time curve; C<sub>max</sub>: peak plasma concentration; T<sub>max</sub>: time to reach peak plasma concentration; MRT: mean residence time; t<sub>1/2</sub>: half-life of the terminal phase, Fr: relative oral bioavailability. PTX-HPCD-NP: PTX complexed with 2-hydroxyl-propyl-β-cyclodextrin and loaded in poly(anhydride) nanoparticles; PTX-CD-NP: PTX complexed with β-cyclodextrin and loaded in poly(anhydride) nanoparticles; PTX-CD-NP-PEG: PTX complexed with β-cyclodextrin and loaded in poly(anhydride) nanoparticles combined with PEG2000; PTX-NP-PEG: PTX loaded in poly(anhydride) nanoparticles combined with PEG2000. i.v.: intravenous administration, p.o.: oral administration.
Table 3. Pharmacodynamic parameters estimated after PTX treatment either as commercial Taxol® formulation or loaded in poly(anhydride) nanoparticles.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Tumor Volume Doubling Time (DT) (days)</th>
<th>Tumor Growth Delay (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Control</td>
<td>1.9 ± 0.7</td>
<td>5</td>
</tr>
<tr>
<td>(untreated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taxol® i.v.</td>
<td>1.6 ± 0.5</td>
<td>7.5</td>
</tr>
<tr>
<td>PTX-HPCD-NP</td>
<td>2.3 ± 0.3</td>
<td>9.5</td>
</tr>
<tr>
<td>PTX-CD-NP</td>
<td>1.8 ± 0.4</td>
<td>9</td>
</tr>
<tr>
<td>PTX-CD-NP-PEG</td>
<td>2.5 ± 0.3</td>
<td>10.2</td>
</tr>
<tr>
<td>PTX-NP-PEG</td>
<td>2.6 ± 0.3</td>
<td>11</td>
</tr>
</tbody>
</table>

**Figure Captions**

**Figure 1.** PTX plasma concentration-time profile after i.v. administration of Taxol® (dose 25 mg/kg bw). Data are expressed as mean ± S.D., n=3 per time point. Taxol i.v.: intravenous administration of commercial Taxol®.

**Figure 2.** PTX plasma levels after the administration of a single dose of 25 mg/kg bw. Animals received orally commercial Taxol® and PTX loaded nanoparticles: PTX-HPCD-NP, PTX-CD-NP, PTX-CD-NP-PEG and PTX-NP-PEG. Data are expressed as mean ± S.D. (n=3 per time point). PTX-HPCD-NP: PTX complexed with 2-hydroxypropyl-β-cyclodextrin and loaded in poly(anhydride) nanoparticles; PTX-CD-NP: PTX complexed with β-cyclodextrin and loaded in poly(anhydride)nanoparticles; PTX-CD-NP-PEG: PTX complexed with β-cyclodextrin and loaded in poly(anhydride)nanoparticles combined with PEG2000; PTX-NP-PEG: PTX loaded in poly(anhydride) nanoparticles combined with PEG2000.

**Figure 3.** Organ distribution time profiles of PTX in C57BL/6J mice after i.v. administration of Taxol® (A) or oral administration of PTX loaded in pegylated nanoparticles (PTX-NP-PEG) (B); all mice received a single dose of 25 mg/kg bw. Data expressed as mean ± S.D. (n=4 per time point).

**Figure 4.** Comparative organ distribution of PTX following the oral administration of the different poly(anhydride) nanoparticles and i.v. Taxol® (dose=25 mg/kg bw) at 8 hours after administration in C57BL/6J mice. Data expressed as mean ± S.D. (n=4 per time point). *p<0.05 Taxol® vs. nanoparticle formulations: PTX-HPCD-NP, PTX-CD-NP, PTX-CD-NP-PEG and PTX-NP-PEG.

**Figure 5.** Comparative tumor growth inhibition by i.v. Taxol® (dose 10 mg/kg) or oral PTX loaded in poly(anhydride) nanoparticles (dose 25 mg/kg bw) in 3LL tumor-bearing C57BL/6J mice. Results expressed as mean ± S.D. (n=6). *p < 0.05 ANOVA + Bonferroni post-test (PTX-HPCD-NP vs. Taxol® i.v.); **p< 0.01 ANOVA + Bonferroni post-test (PTX-NP-PEG and PTX-CD-NP-PEG vs. Taxol® i.v.)

**Figure 6.** Vascular endothelial growth factor (VEGF) concentration in plasma for the different treatment groups: Taxol® and PTX loaded poly(anhydride) nanoparticles, i.v. and orally administered respectively, on different days throughout the treatment. Data expressed as mean ± S.D. (n=3) *p < 0.05 Mann-Whitney U-test PTX-HPCD-NP vs. Taxol®, PTX-CD-NP-PEG vs. Taxol®, PTX-NP-PEG vs. Taxol®; †p < 0.05 Mann Whitney U-test PTX-CD-NP vs. PTX-HPCD-NP, PTX-CD-NP-PEG and PTX-NP-PEG.
PTX Plasma concentration (ng/ml)

Time (h)

Taxol i.v.
Liver
Spleen
Kidneys
Ovaries
Lung
Stomach
Intestine

3A

3B
TaXo

PTX-HPCD-NP

PTX-CD-NP-PEG

PTX-NP-PEG

Basal levels [15 pg/mL]

* day 1 of treatment
† day 5 of treatment
* day 8 of treatment
* day 11 of treatment

VEGF [pg/mL]

0
40
80
120
160
200

day 11 of treatment
day 8 of treatment
day 1 of treatment
day 5 of treatment