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Poly(ethylene oxide)-modified poly(ε-caprolactone) nanoparticles for targeted delivery of tamoxifen in breast cancer

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Abstract

This study was carried out to evaluate and compare the biodistribution profile of tamoxifen when administered intravenously (i.v.) as a simple solution or when encapsulated in polymeric nanoparticulate formulations, with or without surface-stabilizing agents. Tamoxifen-loaded, poly(ethylene oxide)-modified poly(ε-caprolactone) (PEO-PCL) nanoparticles were prepared by solvent displacement process that allowed in situ surface modification via physical adsorption of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO) triblock polymeric stabilizer (Pluronic®). The nanoparticles were characterized for particle size and surface charge. Presence of PEO chains on nanoparticle surface was ascertained by electron spectroscopy for chemical analysis (ESCA). In vivo biodistribution studies were carried out in Nu/Nu athymic mice bearing a human breast carcinoma xenograft, MDA-MB-231 using tritiated [^{3}H]-tamoxifen as radio-marker for quantification. PEO-PCL nanoparticles with an average diameter of 150–250 nm, having a smooth spherical shape, and a positive surface charge were obtained with the formulation procedure. About 90% drug encapsulation efficiency was achieved when tamoxifen was loaded at 10% by weight of the polymer. Aqueous wettability, suspendability, and ESCA results showed surface hydrophilization of the PCL nanoparticles by the Pluronics®. The primary site of accumulation for the drug-loaded nanoparticles after i.v. administration was the liver, though up to 26% of the total activity could be recovered in tumor at 6 h post-injection for PEO-modified nanoparticles. PEO-PCL nanoparticles exhibited significantly increased level of accumulation of the drug within tumor with time as well as extended their presence in the systemic circulation than the controls (unmodified nanoparticles or the solution form). Pluronic® surfactants (F-68 and F-108) presented simple means for efficient surface modification and stabilization of PCL nanoparticles to achieve preferential tumor-targeting and a circulating drug reservoir for tamoxifen.

Keywords: Poly(ethylene oxide)-modified poly(ε-caprolactone) nanoparticles; Targeted delivery; Tamoxifen; Breast cancer

1. Introduction

Tumor-selective delivery of anti-cancer agents at the site of action is desirable for two reasons: to maximize

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cell-kill effect during the tumor growth phase during which majority of the cells remain sensitive to pharmacotherapy and to protect the surrounding healthy cells from exposure to the cytotoxic agent. It is also desirable to maintain a steady infusion of the drug into the tumor interstitium to accomplish continuous extermination of the dividing cells that eventually results in tumor regression. Advances in nanobiotechnology have resulted in evolution of several novel colloidal carrier systems such as liposomes, polymeric micelles, nanoparticles, and nanoemulsions to achieve these multiple objectives (Barratt, 2003; Kataoka et al., 2001; Kim and Lim, 2002; Matteucci and Thrall, 2000; Speiser, 1991; Ulrich, 2002; Vemuri and Rhodes, 1995; Wu et al., 2001; Zasadzinski et al., 2001). Polymeric nanoparticles made from natural or synthetic polymers have drawn major attention due to higher stability, maneuverability for industrial manufacture, and opportunity for further surface nanoengineering (Alonso, 2001; Kreuter, 2001; Moghimi et al., 2001; Muller et al., 2000; Panyam and Labhasetwar, 2003; Rao et al., 2004; Soppimath et al., 2001). They can be tailor-made to achieve both controlled drug release and tumor-targeting by tuning the polymer characteristics and surface chemistry (Gref et al., 2000; Kreuter, 1994; Moghimi et al., 2001; Otsuka et al., 2003). It has been established that nanocarriers can get concentrated preferentially in the tumor mass by virtue of the enhanced permeation and retention (EPR) mechanism and once accumulated, they can act as local drug depot depending upon the make-up of the carrier, thus providing a source for continuous supply of encapsulated therapeutic compound into tumor mass (Brigger et al., 2002; Feng et al., 2004; Kreuter, 1994).

We have reported earlier the formulation development aspects of tamoxifen-containing biodegradable poly(ε-caprolactone) nanoparticulate system (Chawla and Amiji, 2002) and proven the efficient in vitro uptake of these nanoparticles in MCF-7 human breast cancer cells (Chawla and Amiji, 2003). In the present study, we have examined the issues related the surface modification of the nanoparticles using PEO–PPO–PEO triblock surfactants having two different copolymer compositions and ability of the modified nanoparticles to function as long-circulating drug reservoir, unloading a fraction of cargo into liver and tumor with each systemic circulatory cycle.

Among United States Food and Drug Administration-approved polyesters, PCL possesses unique properties such as higher hydrophobicity and neutral biodegradation end products, which do not disturb the pH balance of the degradation medium (Pitt et al., 1981a; Woodward et al., 1985). Over the years, an array of drug delivery systems has been developed using PCL as polymeric material (Pitt et al., 1981b; Sinha et al., 2004; Sinha and Khosla, 1998). Higher hydrophobicity of PCL plays a key role in efficient surface modification strategy that is purely dependent on hydrophobic interactions between the center block (PPO) of the stabilizing surfactant and polymeric core (PCL nanoparticle) (Tan et al., 1993). In the present study, we have used Pluronic® F-68 having 30 residues of propylene oxide (PO) and 76 residues of ethylene oxide (EO) and Pluronic® F-108 having 56 PO residues and 122 EO residues. This mode of adsorption leaves the hydrophilic PEO side arms in a mobile state as they extend outwards from the particle surface and provide stability to the particle suspension by a repulsion effect through a steric mechanism of stabilization involving both enthalpic and entropic contributions (Moghimi et al., 1993). The end result of such an assembly is a stable, slow-eroding colloidal system that is less phagocyte-prone (hence long circulating). Once accumulated within the tumor interstitium by exploiting vascular abnormalities that allows freer access to the tumor mass, the PCL nanoparticle system would increase the drug concentration inside the tumor cells as a result of non-specific endocytic process, followed by gradual release of the drug (Chawla and Amiji, 2003). Such nanoparticle-mediated intracellular delivery is particularly beneficial for tamoxifen therapy as the estrogen receptors are known to be localized in the cytosol and nucleus.

2. Materials and methods

2.1. Materials

PCL with a number average molecular weight of 14,800 Da (as verified by gel-permeation chromatography), was purchased from Polysciences Inc. (Warrington, PA). Pluronic® copolymer surfactants (F-68 NF and F-108 NF) were kindly provided by the Performance Chemical Division of BASF Corporation

(Parsinpanny, NJ). Tamoxifen (free base) was purchased from Sigma Chemical Co. (St. Louis, MO) and tritiated [^{3}H]-tamoxifen with an activity of 85 Ci/mmol was purchased from Amersham Biosciences (Piscataway, NJ). Human breast cancer xenograft cells (MDA-MB-231) were procured from American Type Culture Collection (Rockville, MD). Deionized distilled water (NanoPure II, Bubuque, IA) was used for all aqueous preparations.

2.2. Preparation of PEO-modified PCL nanoparticles

The nanoparticles were prepared by solvent displacement method as reported earlier (Chawla and Amiji, 2002). Briefly, a solution of PCL was prepared in acetone and was introduced into an aqueous solution containing known concentrations of Pluronic® (F-68 or F-108) under magnetic stirring. The rate of addition of organic phase to aqueous phase, volume ratios, and the stirring speed were optimized to ensure batch-to-batch reproducibility. Typically, a 1% (w/v) solution of PCL in 50 ml of acetone was introduced into an aqueous solution of Pluronic®. The rate of addition was controlled through a burette at about 1 ml/min and stirring was maintained at a speed that facilitated formation of a vortex in the solution. The nanoparticles were freeze-dried as such (with surfactant remaining in the bulk) or after removal of excess (unadsorbed) stabilizer either by centrifugation (10,000 rpm for 20 min followed by washing twice with 50 ml of water) and/or equilibrium dialysis (against water using dialysis membrane having 15,000 Da cut-off—from Spectrum Labs, USA which results in selective leaching of free Pluronics in solution through the membrane).

Drug-loaded nanoparticles were prepared by a similar procedure. A known quantity of tamoxifen was dissolved in acetone along with the PCL before introduction into aqueous medium which resulted in entrapment of the drug within polymeric matrix that was formed due to its limited solubility in the aqueous phase.

2.3. Characterization of nanoparticles

2.3.1. Particle size

The lyophilized nanoparticles were resuspended and diluted suitably in deionized distilled water and particle size was determined by Coulter® N4-Plus submicron particle sizer (Coulter Corporation, FL) at multiple scattering angle detection.

2.3.2. Measurement of surface charge

A suitably diluted aqueous suspension of nanoparticles was mounted in a 90 Plus particle sizer/zetasizer (Brookhaven Instruments, NY) and mean zeta potential was computed based on Smoluchowski equation (Smoluchowski, 1917).

2.3.3. Electron spectroscopy for chemical analysis (ESCA) studies

Freeze-dried PCL nanoparticles that were obtained by different ways of removal of free Pluronic® from bulk (centrifugation, dialysis) were subjected for ESCA at the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO) at the University of Washington (Seattle, WA). The spectra of the freeze-dried samples were recorded on Surface Sciences Instrument X-probe spectrophotometer with a monochromatized A1 X-ray source. A 5.0 eV flood gun was used to neutralize the surface charge. The surface elemental composition was determined using standard Scofield photoemission cross sections. Identification of chemical functional groups was obtained from the high-resolution peak analysis of the carbon 1s (C 1s) envelopes.

2.4. Biodistribution study

2.4.1. Development of tumor model

The experimental protocol involving usage of radioactive material in animals was approved by the Institutional Animal Care and Use Committee and the Office of Environmental Health and Safety at Northeastern University.

The tumor cells MDA-MB-231 were grown in culture flasks containing Dulbecco's Minimum Essential Medium modified with fetal bovine serum, sodium pyruvate, buffer and antibiotics. They were harvested and resuspended in serum-free medium (SFM) before injecting into nude mice. Female athymic mice (Nu/Nu, Charles River Laboratory, MA), 4–6 weeks old, weighing about 20–25 g were housed in polycarbonate cages having free access to sterilized rodent pellet diet and water. One million tumor cells suspended in 100 μl

of SFM were injected subcutaneously into the mammary pad of mice under light anesthesia. Solid tumors developed within 2 weeks post-tumor inoculation and once the tumor volume reached approximately 200 mm^3, the animals were chosen for experimental treatment.

2.4.2. Drug treatment

Tritiated [^{3}H]-tamoxifen-loaded PCL nanoparticles (we added 10 μCi of hot tamoxifen to 50 mg of cold tamoxifen) were suspended in saline so as to contain approximately 1 μCi dose per 100 μl volume. Animals were divided into groups of 4 each (per time-point of 1 and 6 h post-injection) for control (saline), plain injection (tamoxifen citrate), control nanoparticle (PCL nanoparticles prepared without any Pluronic®), PCL nanoparticles with Pluronic® F-68 and PCL nanoparticles with Pluronic® F-108. All Pluronic® containing formulations used 0.1% (w/v) of surfactant during preparation and free surfactant from bulk was eliminated by a single centrifugation/washing cycle. All injections were given via the tail vein using 24-guage needle.

2.4.3. Estimation of the drug disposition

Animals were sacrificed after fixed time-points by carbon dioxide euthanasia and tumor, liver and blood (by sino-arbital vein puncture) were collected. Blood was used as such and for the tissues (tumor and liver), a 10% (w/v) homogenate was prepared in water and 1.5 ml each was taken in a scintillation vial. All tissues and fluids (blood) were digested with Scintigest® fluid (from Fisher Scientific, USA, 1 ml, incubation for 2 h at 50 °C) and decolorized with hydrogen peroxide (200 μl of 30% solution, incubation for 30 min at 50 °C). Then, scintillation cocktail (ScintiSafe® Econo 1, from Fisher Scientific, USA, 10 ml) was added and the sample was allowed to quench for 2 h in dark before measuring in a liquid scintillation analyzer (TriCarb 1600TR, Packard Instrument Co., CT). The counts-per-minute were converted into μCi using appropriate calibration curves.

3. Results and discussion

In our earlier report, we have described the standardization of preparation procedure, morphological analysis by scanning electron microscopy, drug–polymer interaction study by calorimetry, in vitro PCL nanoparticle biodegradation in the absence and presence of lipase, tamoxifen loading and release study and cellular uptake by the MCF-7 cells in vitro (Chawla and Amiji, 2002). In yet another investigation, we reported further insights into cellular uptake and intracellular concentrations of tamoxifen when presented to the tumor cells in vitro in nanoparticulate formulation (Chawla and Amiji, 2003). The present investigation is the continuing part of the same—providing supplementary evidence for surface modification and in vivo tumor disposition profiles using different types of PEO-containing surface modifying agents.

3.1. Characterization of nanoparticles

Our previous investigation showed scanning electron micrographs of distinct spherical nanoparticles with smooth surface (Chawla and Amiji, 2002). In the present study, we limited the characterization account to effect of stabilizer concentration and copolymer ratio on particle size and zeta potential (see Table 1 for details). There was no significant difference in particle size distribution with the variation in copolymer ratio (PEO:PPO) or concentration of stabilizer used for preparation. Investigations into effect of these variables have indicated a reduction in mean particle size with an increase in hydrophilic PEO groups in the stabilizer (Moghimi et al., 2001). Insignificant differences in particle size results in our case could be due to insufficient density and altered conformation of the hydrophilic portion of the triblock polymer on nanoparticle surface. However, nanoparticles prepared without any surfactant showed marked aggregation, hydrophobicity and increased diameter.

The PCL nanoparticles, with or without adsorbed PEO group showed a negative surface charge. However, drug encapsulation switched the surface charge to positive—predominantly due to presence of surface localized drug (Chawla and Amiji, 2002). Cationic surface charge is desirable as it promotes interaction of the nanoparticles with cells, and hence increases the rate and extent their internalization.

The method of nanoparticle preparation and drug encapsulation is highly favorable for hydrophobic compounds, such as tamoxifen, resulting in excellent entrapment levels (>90%). The system allowed good loading levels (about 20% by weight) for tamoxifen—a

Table 1
Characterization of the control and poly(ethylene oxide)-modified poly(ε-caprolactone) nanoparticles

Formulation	Particle size (nm)	Zeta potential (mV)
Control PCL NP (no stabilizer/drug)	762.9 ± 140.8[a]	−19.2 ± 3.3
PCL NP (0.1% F-68)[b]	206.2 ± 22.5	−27.7 ± 0.5
PCL NP (0.5% F-68)[b]	195.4 ± 18.9	−23.3 ± 0.6
Tamoxifen-loaded PCL NP (0.1% F-68)[b]	210.3 ± 24.8	+22.3 ± 0.9
PCL NP (0.1% F-108)[b]	198.2 ± 16.7	−17.9 ± 0.9
PCL NP (0.5% F-108)[b]	182.5 ± 19.6	−15.8 ± 0.6
Tamoxifen-loaded PCL NP (0.1% F-108)[b]	201.4 ± 20.8	+19.7 ± 0.8

NP = nanoparticles.
[a] Values are mean ± S.D. for triplicates (for particle size) and sextuplicates (for zeta potential).
[b] Terms within parentheses indicate concentration (as percentage w/v) and type of stabilizer used during preparation of nanoparticles.

desirable parameter for increasing efficiency of targeted drug delivery.

3.2. Surface modification by physical adsorption

Pluronics® (also known as poloxamers) are non-ionic PEO–PPO–PEO triblock polymers with the general formula $HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_aH$. They are commercially available in different grades, which vary from liquids, semi-solids and to solids. In pharmaceutical industry, Pluronics® have been used as emulsifying agents, solubilizing agents, surfactants, and wetting agents. As the "*a*" and "*b*" block numbers change (hence a change in hydrophilic–lipophilic balance), there is a change in physicochemical properties of the triblock copolymer that in turn changes the applicability of the polymer. Pluronic® F-108 NF (poloxamer 338) has a bulkier central block as well as longer side arms ($a = 122$; $b = 56$) as compared to Pluronic® F-68 NF (poloxamer 188, $a = 76$; $b = 30$).

It has been established that PEO/PPO block copolymers adsorb onto surface of oil-in-water emulsions or any hydrophobic nanoparticular systems via their hydrophobic PPO central block (Moghimi et al., 1993, 2001; Monfardini and Veronese, 1998). The strength of the polymer adsorption and the resultant polymer conformation is dependent on the proportion and the size of both PPO and PEO segments as well as the physicochemical properties of the nanoparticle surface (Moghimi and Hunter, 2000; Monfardini and Veronese, 1998). For a particle of a given size, it is the size of the polymer's hydrophobic center block that determines the surface concentration or density rather than its flanking tails (Li et al., 1994). The binding affinity of Pluronic® F-108 to PCL nanoparticles will be higher due to greater number of PPO residues as compared to Pluronic® F-68. While triblock copolymer of similar PPO size showed comparable surface concentration, longer PEO chains were associated with thicker adsorption layers as well as greater chin dynamics (Li et al., 1994). As a result, one can expect thicker adsorbed layer assembly for Pluronic® F-108 as compared to Pluronic® F-68 on PCL nanoparticles.

The solvent displacement technique followed for preparation of PCL nanoparticles facilitates instant adsorption of PPO–PEO groups when an organic solution of polymer–drug solution is introduced into aqueous solution containing the stabilizer (Scholes et al., 1999). A lower surface tension prevails at the newly formed interface that promotes finer dispersion of the incoming hydrophobic polymer and simultaneously assists quick hydrophobic interactions between the PPO and freshly formed PCL nanoparticle surface (Stolnik et al., 2001).

After evaporating the organic solvent by stirring at room temperature for 24 h, majority of the excess (unadsorbed) Pluronic® was eliminated from the bulk by a single centrifugation-washing step or by dialysis across a membrane having 15,000 molecular weight cut-off. Different treatments allowed us to obtain qualitative data on extent of PEO remaining firmly adsorbed on nanoparticle surface and aqueous re-dispersability of the lyophilized nanoparticles.

Qualitative surface analysis of the nanoparticles was done by ESCA. It is a surface-sensitive technique that measures the elemental composition and identifies the chemical functional groups in the 10 nm thick surface layer. Only those electrons that leave the surface without energy loss will contribute to the peak signifying

Table 2
High-resolution C 1s peak analysis of ESCA on surfaces of the control and poly(ethylene oxide)-modified poly(ε-caprolactone) nanoparticles

Formulation	Relative peak area (%)		
	─C─H─ (285.0 eV)	─C─O─ (286.4 eV)	─C═O─ (288.1 eV)
Control PCL NP (no stabilizer)	67	18	13
PCL NP (0.1%, F-68, as is)	34	60	5
PCL NP (0.5%, F-68, as is)	13	84	2
PCL NP (0.5%, F-68, dialyzed)	17	80	2
PCL NP (0.1%, F-68, washed once)	59	27	13
PCL NP (0.1%, F-68, washed twice)	69	18	12
PCL NP (0.1%, F-108, as is)	20	76	3
PCL NP (0.1%, F-108, washed once)	55	33	11
PCL NP (0.1%, F-108, washed twice)	61	24	14

As is: the bulk nanoparticle suspension was lyophilized as such without any post-preparation treatment (washing or dialysis). Terms within parentheses indicate concentration (as percentage w/v), type of stabilizer used during preparation of nanoparticles and post-preparation treatment before lyophilization for ESCA.

that element and electron that originate from far below the surface (i.e., >10 nm distance) suffer energy loss through collisions and are unable to register their signal (Potineni et al., 2003). Table 2 summarizes the results obtained thereof showing high-resolution –C–H– (hydrocarbon), –C–O– (ether) and –C=O– (carbonyl) peaks in the C 1s envelope of the control and PEO-modified nanoparticles at their characteristic binding energies. The surface presence of the PEO chains was confirmed by an increase in the ether (–C–O–) signature of the spectra, which is indicative of the presence of ethylene oxide residues (Potineni et al., 2003). The nanoparticle samples that were lyophilized with Pluronic® remaining as such in bulk (samples 2, 3 and 7) show marked presence of PEO residues. Repeated washings with water caused gradual stripping of the hydrophilic coating resulting in exposure of the underlying PCL substrate (samples 6 and 7). However, the washing process did leave behind significant amount of PEO groups on PCL surface, when the anchoring central block was bulkier—as with Pluronic® F-108 (samples 8 and 9). The process of removal of triblock surfactant from the bulk by dialysis (up to 72 h) resulted only in partial success. We used nanoparticles that were washed once for all further experiments as presence of free (unadsorbed) Pluronic® in the bulk (especially in smaller amounts like in samples 2 and 7) was not expected to cause major complications in vivo due to safety profile of the copolymer. Under the light of earlier investigations (Moghimi, 1997; Moghimi and Hunter, 2000), free Pluronic® may instead be beneficial as the nanoparticles can acquire in situ coating of Pluronic® or Pluronic®–protein complex within the systemic circulation when they are introduced 1–3 h after the i.v. injection of free Pluronic®.

Additionally, another simple way of perceiving surface modification is the visible hydrophilicity of the nanoparticle, which otherwise show marked hydrophobic behavior. Hence, all samples that showed surface presence of PEO residues by ESCA were easily redispersible in aqueous media and showed good wettability behavior compared to those which showed no evidence of surface-modification (samples 1 and 6).

3.3. Biodistribution study

We carried out biodistribution study with respect to three principal sites of nanoparticle disposition—liver, tumor and blood. When we computed the recovered radioactivity, we could account for more than 90% of the injected dose in all cases. We chose the widely accepted in vivo breast tumor model—based on MDA-MB-231 xenograft—as it is a simpler model and is well characterized as compared to MCF-7, which requires estrogen priming for growth (Oka et al., 1996). In principle, upon introduction into systemic circulation via tail vein, a fraction of the nanoparticle population would find its place in the liver due to prompt recognition and uptake by the macrophages. The fraction that evades this process (by virtue of PEO adsorbed layer) will remain in the circulation and gets targeted into the solid tumor via the capillaries with open fenestrations (EPR phenomenon). It is known that in a transplantable animal tumor model, the ma-

jority of the tumor vasculature is usually in a pro-angiogenic state (Griffioen and Molema, 2000) and hence presence of capillaries that promote EPR effect. The PEO–PPO–PEO triblock copolymers render nanoparticles anti-adhesive—by virtue of the extended PEO configuration on the particle surface which acts as steric barrier reducing the extent of clearance by circulating macrophages of the liver and promoting the possibility of undergoing EPR process.

The results of in vivo biodistribution studies have been summarized in Figs. 1 and 2. Fig. 1 depicts the tumor concentrations of different standard (plain injection, unmodified PCL nanoparticles) and test (PCL nanoparticles surface-modified with Pluronics® F-68 and F-108) formulations of tamoxifen. For 1 h time-point, we noticed a higher tumor concentration being reached with Pluronic®-modified nanoparticles, maximum being with F-108. There was no significant difference in concentrations obtained among two standards. However, at 6 h post-injection, the amount retained within tumor was maximal for nanoparticles having Pluronic® F-68 as the adsorbed layer, though no significant difference was seen among the test formulations. A similar trend is observed for blood component for 1 and 6 h time-points (Fig. 2), the test formulations remained in the systemic circulation for longer times

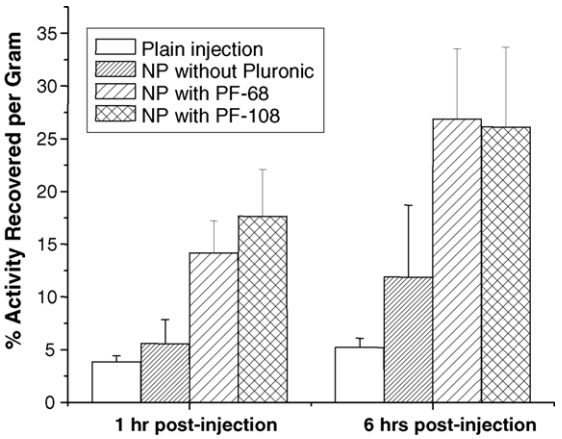

Fig. 1. Tumor concentrations as a function of time of [^{3}H]-tamoxifen administered as controls and in poly(ethylene oxide) (PEO)-modified PCL nanoparticles in Nu/Nu mice bearing human breast carcinoma (MDA-MB-231) xenografts. PEO-modified PCL nanoparticles were prepared by physical adsorption of Pluronic® F-68 (PF-68) and Pluronic® F-108 (PF-108) (NP = nanoparticles). Results are expressed as mean ± S.D. (n = 4).

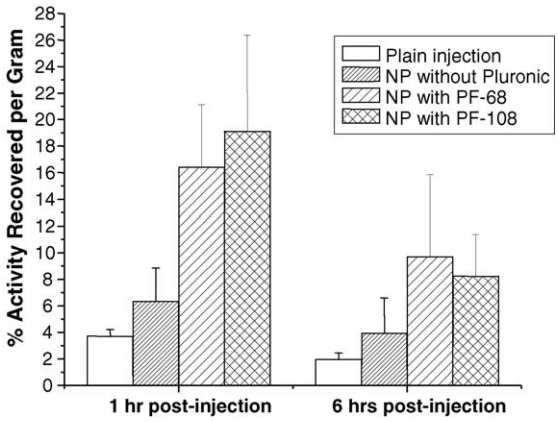

Fig. 2. Plasma concentrations as a function of time of [^{3}H]-tamoxifen administered as controls and in poly(ethylene oxide) (PEO)-modified PCL nanoparticles in Nu/Nu mice bearing human breast carcinoma (MDA-MB-231) xenografts. PEO-modified PCL nanoparticles were prepared by physical adsorption of Pluronic® F-68 (PF-68) and Pluronic® F-108 (PF-108) (NP = nanoparticles). Results are expressed as mean ± S.D. (n = 4).

than the controls. In case of plain injection more than 90% of the injected dose reached the liver within first hour as compared to 83% for unmodified PCL nanoparticles. For the test formulations, the values were 74% (F-68) and 67% (F-108) of the injected dose in the liver at 1 h. After 6 h, the liver concentrations were reduced for all formulation by 2–7% indicating possible degradation/metabolism of the drug. We observed a gradual transfer of the nanoparticle load from the circulatory compartment to the tumor compartment for both surface-modified nanoparticle formulations. Pluronic® F-68 appears to be dominating disposition at first hour and Pluronic® F-108 dominating at the sixth hour, though no significant difference was seen among themselves at both time-points. It is evident that PEO modification did result in significant alteration of drug disposition when introduced as nanoparticle formulation—the surface-modified versions inhibiting macrophage recognition and favoring tumor uptake.

The difference in biodistribution pattern would rely on the extent of surface modification (adsorbed layer thickness, packing density and mobility of PEO chains), strength of adherence between the nanoparticle surface and adsorbed layer, and the extent of interactions of the nanoparticles with body fluids and biomacromolecules (like proteins). For a given triblock polymer, it has been found that both surface concen-

trations and adsorbed layer thicknesses are strongly related to the particle size, such that smaller particles take up fewer polymer molecules per unit area than the larger ones (Li et al., 1994). The reduced crowding around each PEO chain results in thinner adsorbed layers and higher chain mobilities (Moghimi et al., 2001). We see in Table 1, the particle size obtained with F-108 are slightly smaller than for F-68, but at the same time, ESCA data represented in Table 2 shows higher degree of accumulation of PEO chains with F-108. The biological results support the ESCA findings—at least for first hour of distribution. After first hour, the PEO-modified nanoparticles appear to undergo differential treatment within systemic circulation with respect to the packing of the adsorbed layer and at the end of 6 h we see F-68 taking the lead. The difference, both in terms of blood concentration or tumor uptake, though not significant, can only be justified by desorption kinetics. All polymer coated nanoparticles are eventually cleared from circulation by macrophages of the RES. The adsorbed stabilizer is either desorbed or eventually displaced by some plasma proteins. Both processes decrease the steric shielding at particular surface sites and make the nanoparticle prone to phagocytosis. Besides, they can also activate complement system resulting in particle fixation by opsonizing complement fragments and stimulation of particle recognition by the activated complement receptors of phagocytic cells. We expect the cascade of events leading to uptake by the RES would take longer than 6 h based on earlier investigations (Chawla and Amiji, 2003).

Once presented in the vicinity of the tumor cells, the PCL nanoparticles will be taken up by the tumor cells by non-specific endocytosis, which rapidly increases the intracellular concentration of the drug resulting in maximal pharmacological effect (Panyam and Labhasetwar, 2003). It has been shown that the nanoparticles are non-toxic to the cells by themselves (Chawla and Amiji, 2003; Woodward et al., 1985). Several speculative mechanisms can induce drug release from extravasated nanoparticles (the population within tumor mass). One possibility is the drug leakage induced by the interstitial fluid surrounding the tumors which contain oxidizing agents, lipases and other hydrolytic enzymes derived from dying tumor cells or the resident inflammatory cells (Moghimi et al., 2001). We have shown in our previous investigations that the biodegradation kinetics of the PCL nanoparticles is much faster in presence of lipases than in buffer alone (Chawla and Amiji, 2002). The tumor mass is rich in lipases and other factors listed above only accelerate the process of degradation and drug release further.

4. Conclusions

Biodegradable polymeric nanoparticles loaded with a hydrophobic anti-cancer drug were successfully surface-modified by physical adsorption of a triblock polymer having central hydrophobic anchoring unit and hydrophilic side chains. These nanoparticles having mean size between 150 and 250 nm showed tumor-selective biodistribution (by virtue of EPR-based passive targeting) with clinically exploitable circulation times. Once accumulated within tumor mass, the nanoparticles are expected to release encapsulated drug by diffusion and concurrent biodegradation. Biodegradation of the PCL polymeric matrix is not expected to cause change in physicochemical environment (like pH, osmolality, chemical make-up, etc.) of the degrading medium—a specific advantage over other polyesters (like polylactides or polyglycolides). Though it is possible to derive at a polymeric nanostructured unit with chemically secured surface modifier (amphiphilic triblock copolymer–poly(ε-caprolactone)–poly(ethylene oxide)–poly(ε-caprolactone)) (Ge et al., 2002), the present study provides evidence for simple means for achieving in situ surface-modification to derive at a tumor-selective nanocarrier.

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