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Research paper

Anticancer activity of cisplatin-loaded PLGA-mPEG nanoparticles on LNCaP prostate cancer cells

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Abstract

The in vitro anticancer activity of cisplatin-loaded PLGA-mPEG nanoparticles on human prostate cancer LNCaP cells was investigated. The uptake of the PLGA-mPEG nanoparticles by the LNCaP cells was also studied. Blank PLGA-mPEG nanoparticles exhibited low cytotoxicity, which increased with increasing PLGA/PEG ratio in the PLGA-mPEG copolymer used to prepare the nanoparticles, possibly due to the increased cell uptake observed with increasing PLGA/PEG ratio. PLGA-mPEG nanoparticles loaded with cisplatin exerted in vitro anticancer activity against LNCaP cells that was comparable to the activity of free (non-entrapped in nanoparticles) cisplatin. Little differences in the in vitro anticancer activity of the different nanoparticle compositions were found, which may result from the differences observed between the different nanoparticles compositions in the uptake by the LNCaP cells and in the leakage of cisplatin from the nanoparticles during incubation with the cells. Visual evidence of nanoparticles' uptake by the LNCaP cells was obtained with nanoparticles labeled with PLGA(4165)-PyrBu(274) or dextran-rhodamine B isothiocyanate using fluorescence microscopy. Moreover, in some cases fluorescence around or inside cell nuclei was observed, which, if verified by further studies, would indicate that PLGA-PEG nanoparticles might prove to be useful in site-specific delivery of drugs whose site of pharmacological activity is cell nucleus.

Keywords: Poly(lactide-co-glycolide)-methoxy-poly(ethylene glycol); Nanoparticles; PLGA-mPEG; Cisplatin; Cytotoxicity; LNCaP cells; Anticancer activity; PLGA-PEG

1. Introduction

Although cisplatin is one of the most potent anticancer agents available today [1], its use is associated with serious side effects. A more selective administration (targeting) of cisplatin to cancer cells is thus required in order to reduce drug toxicity and enhance its therapeutic potential. Several attempts for a more selective cisplatin administration have been described in the literature, including its systemic administration in the form of soluble drug–polymer conjugates, such as the complexes with polycarboxylates [2], poly(amidoamines) [3], polyamidoamine dendrimers [4] and the complexes with *N*-(2-hydroxypropyl) methacrylamide [5], or in the form of colloidal carriers, such as pegylated liposomes [6], poly(aspartic)acid–poly(ethylene glycol) micelles [7] and poly(caprolactone)–poly(ethylene glycol) or poly(caprolactone)–poly[2-(*N*,*N*-dimethylamino)ethyl methacrylate] micelles [8]. The association of cisplatin with long-circulating carriers alters drug pharmacokinetics and results in increased drug accumulation in tumors, based on the "enhanced permeability and retention" (EPR) effect. The EPR effect is a result of leaky capillaries adjacent to solid tumors and a lack of a lymphatic system for the drainage of drugs back to the systemic circulation [9].

We have considered the feasibility of using long circulating PLGA-mPEG nanoparticles [10] for the passive targeting of cisplatin to tumors after iv administration of the

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cisplatin-loaded nanoparticles. We showed recently that the intravenous administration of PLGA-mPEG nanoparticles loaded with cisplatin to mice resulted in prolonged cisplatin residence in systemic circulation [11]. In this communication, we studied the cytotoxicity of blank PLGA-mPEG nanoparticles and the in vitro anticancer activity of cisplatin-loaded PLGA-mPEG nanoparticles on human prostate LNCaP cells. The uptake of the PLGA-mPEG nanoparticles by the LNCaP cells was also investigated.

2. Materials and methods

2.1. Materials

D,L-Lactide and glycolide were purchased from Boehringer Ingelheim (Germany). They were re-crystallized twice from ethyl acetate and dried under high vacuum at room temperature before use. Monomethoxy-poly(ethylene glycol) (mPEG, molecular weight 5000) was obtained from Sigma (St. Louis, MO) and dried under high vacuum at room temperature before use. Cisplatin (*cis*-platinum diammine dichloride), stannous octoate, sodium cholate and dextran-rhodamine B isothiocyanate (molecular weight 70000) were also purchased from Sigma (St. Louis, MO). The δ-pyrene butanol was from Aldrich. All culture media and supplements were from Biochrom AG (Berlin, Germany).

2.1.1. Cell culture

The human prostate cancer epithelial cell line LNCaP (ATCC) was grown routinely in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamycin and 2.5 μg/ml amphotericin B. Cultures were maintained at 37 °C, 5% CO_2 and 100% humidity.

2.2. Methods

2.2.1. Synthesis and characterization of PLGA-mPEG copolymers

Poly(lactide-co-glycolide)-monomethoxy (polyethylene glycol) (PLGA-PEG) copolymers were synthesized by a melt polymerization process under nitrogen, using stannous octoate as catalyst [12]. They were characterized with regard to composition by ^{1}H NMR and molecular weight and molecular weight distribution (P.I. $= M_w/M_n$, polydispersity index) by gel permeation chromatography (GPC) [12]. The following copolymers were synthesized: (1) PLGA(7)mPEG(5) with composition LA:GA:EO = 0.6:0.3:1.0, $M_w = 15 \times 10^3$ and P.I. = 1.6, (2) PLGA(31)mPEG(5) with composition LA:GA:EO = 2.6:1.5:1.0, $M_w = 39 \times 10^3$ and P.I. = 2.3 and (3) PLGA(57)mPEG(5) with composition LA:GA:EO = 4.3:2.8:1.0, $M_w = 57 \times 10^3$ and P.I. = 3.6. The number in parentheses following each block in the word PLGA-PEG designates the molecular weight in kilodaltons of the respective block. LA, GA, and EO stand for lactic acid, glycolic acid and ethylene oxide components, respectively. The molecular weight and the polydispersity index of the mPEG used were measured by GPC to be $M_w = 5200$ and P.I. = 1.1, respectively.

2.2.2. Synthesis and characterization of PLGA-pyrene butanol copolymer

A copolymer of PLGA with the fluorescent pyrene-butanol (PLGA-PyrBu) was also synthesized. Briefly, lactide, glycolide and pyrene-butanol, in due proportions, were dissolved in toluene (pre-dried with molecular sieves) in a three-necked polymerization flask. The set-up allowed for solvent refluxing and the continuous flow of dried nitrogen over toluene. The temperature was set at 110 °C. Stannous octoate (0.05% w/w, dissolved in *n*-heptane) was added in the flask and the polymerization was allowed to proceed for 3 h. Then, toluene was evaporated off under a nitrogen stream and the solid residue was dissolved in dichloromethane. This solution was transferred to an excess volume of diethyl ether (dropwise) and the purified copolymer was dried under vacuum at room temperature.

2.2.3. Preparation and characterization of PLGA-mPEG nanoparticles

PLGA-mPEG nanoparticles loaded with cisplatin were prepared by a modified double emulsion method. Cisplatin (2.5 mg) was dissolved in a mixture of water with dimethylformamide (350 μl water and 250 μl dimethylformamide), and the solution was emulsified in a PLGA-mPEG solution in dichloromethane (typically 50 mg polymer in 2 ml solvent) using probe sonication (Bioblock Scientific, model 75038) at 15 W for 1 min. This emulsion was transferred to an aqueous solution of sodium cholate (6 ml, 12 mM) and the mixture was probe sonicated at 15 W for 2 min. The w/o/w emulsion formed was gently stirred at room temperature in a fume hood until the evaporation of the organic solvent was complete. The nanoparticles were purified by centrifugation and reconstitution of the precipitate in fresh water and filtered through a 1.2 μ filter (Millex AP, Millipore). Blank (without drug) nanoparticles were also prepared with the same method. For the preparation of fluorescent nanoparticles, dextran-rhodamine B isothiocyanate or PLGA-pyrene butanol (typically 10 mg) was co-dissolved with PLGA-mPEG in the organic phase of the emulsion.

The size and ζ (zeta) potential of the nanoparticles were determined using photon correlation spectroscopy (pcs) and microelectrophoresis, respectively, in a Malvern Z-sizer 5000 instrument (five runs per sample). The ζ potential of the nanoparticles was measured in phosphate-buffered saline (0.01 M, pH 7.4).

The drug content of the nanoparticles was determined using a direct procedure. Lyophilized samples of the nanoparticles (1 ml) were dissolved in dimethylformamide (DMF) and the solutions were assayed for drug content by measuring their absorbance at 307.5 nm. The following equation was applied:

$$\% \text{ loading} = W_d/W_{np} \times 100 \quad (1)$$

where W_d is the amount of drug (mg) found in the sample (lyophilized nanoparticles) and W_{np} is the amount (mg) of the sample (lyophilized nanoparticles).

2.2.4. In vitro cytotoxicity study

The toxicity of blank PLGA-mPEG nanoparticles, cisplatin-loaded PLGA-mPEG nanoparticles and free cisplatin (control to the cisplatin-loaded nanoparticles) against LNCaP prostate cancer cells was investigated by the MTT assay [13]. LNCaP cells were seeded in 24-well plates at a density of 10,000 cells per well in 500 μl RPMI-1640 supplemented with 10% fetal bovine serum. Twenty-four hours after plating, different amounts of a cisplatin solution in water or blank nanoparticles or cisplatin-loaded nanoparticles (suspended in water) were added in the wells. After predefined time periods (24–72 h) of incubation at 37 °C, 50 μl of MTT solution (5 mg/ml in PBS, pH 7.4) was added into each well and plates were incubated at 37 °C for 2 h. The medium was withdrawn and 200 μl acidified isopropanol (0.33 ml HCl in 100 ml isopropanol) was added in each well and agitated thoroughly to dissolve the formazan crystals. The solution was transferred to 96-well plates and immediately read on a microplate reader (Bio-Rad, Hercules, CA, USA), at a wavelength of 490 nm. The experiments were performed in triplicate and repeated three times. Cytotoxicity was expressed as % reduction in cell viability, which was calculated from the ratio between the number of cells treated with the different cisplatin formulations and that of non-treated cells (control).

2.2.5. Study of the uptake of nanoparticles by LNCaP cells

LNCaP cells were seeded in 24-well plates at a density of 10,000 cells per well and cultured in 500 μl RPMI-1640. Fifty microliters (300 μg) of PLGA-mPEG nanoparticles labeled with 20% w/w PLGA(4165)-PyrBu(274) was added in the wells. At predefined time periods of incubation at 37 °C, the supernatants were removed, cells were washed thrice with PBS, dissolved in DMF and the amount of label associated with the cells was assayed by fluorescence measurements (λ excitation: 375 nm, λ emission: 402 nm). The number of cells involved in the uptake was quantified by measuring the total protein content of the cells following the same as above procedure (in control wells) but, instead of dissolving the cells with DMF, the cells were dissolved in 5% SDS in 1 N NaOH and the protein content of the solutions was measured by the BCA method [14]. The uptake of nanoparticles was calculated from the amount of label that was incorporated in the cells and was expressed as mg of nanoparticles per mg of protein and as % w/w of nanoparticles taken by the cells.

The uptake of nanoparticles by the LNCaP cells was also examined with fluorescence microscopy. The cells were cultured in 4-well tissue culture slides (Lab-Tek chamber slides, Nunc). Fifty microliters (300 μg) of PLGA-mPEG nanoparticles labeled with PLGA(4165)-PyrBu(274) (20% w/w) or dextran-rhodamine B isothiocyanate (20% w/w) was added in the wells. After incubation for several time points at 37 °C, cells were washed three times with ice-cold PBS, pH 7.4, fixed with 3% paraformaldehyde, washed five times with ice-cold PBS, mounted by using 4,6-diamidino-2-phenylindole (DAPI)-containing Vectashield mounting fluid (Vector Laboratories) and viewed and photographed using a digital camera equipped Zeiss fluorescence microscope equipped with appropriate filters for pyrene-butanol (excitation in the range 350–375 nm with an emission of 402 nm), rhodamine (excitation in the range 510–560 nm with an emission of 590 nm) and DAPI (targeting DNA in the cell nucleus; excitation at 358 nm and emission at 461 nm). The uptake of free (non-entrapped in nanoparticles) labels by the cells was also studied by adding in the cell incubation medium 5 μl of PLGA(4165)-PyrBu(274) solution in DMSO or 5 μl of dextran-rhodamine B isothiocyanate solution in water. Free labels were added at amounts generating the same label concentrations in the cell incubation medium with that generated when nanoparticle-entrapped labels were added in the cell incubation medium.

3. Results

3.1. Nanoparticle characteristics

The basic characteristics of the three different nanoparticle types involved in this study are presented in Table 1. The average size of the different types of nanoparticles ranged between 130 and 160 nm and their loading with cisplatin between 1.99% and 2.96%. All nanoparticles exhibited low negative ζ potential values, consistent with the presence of the electro-neutral PEG corona around the PLGA core of the nanoparticles.

3.2. Cytotoxicity of blank PLGA-mPEG nanoparticles

All three nanoparticle types exhibited low toxicity against LNCaP cells, with appreciable cytotoxicity (higher than 20% reduction of cell viability) being observed only after exposing the cells at high nanoparticle concentrations (Fig. 1). At 24 h for example, more than 600 μg nanoparticles per ml were required in order to cause cell growth inhibition higher than 20%. As expected, the cytotoxicity increased with increasing nanoparticle concentration and incubation time. The composition of the nanoparticles appeared to have a significant effect on nanoparticle

Table 1
Nanoparticle characteristics

Type	Size (nm)	P.I.	ζ potential	Loading (% w/w)
PLGA(7)mPEG(5)	134.3 ± 5.2	0.189	−5.7	1.99
PLGA(31)mPEG(5)	151.1 ± 6.6	0.254	−7.4	2.77
PLGA(57)mPEG(5)	159.8 ± 6.2	0.209	−9.3	2.96

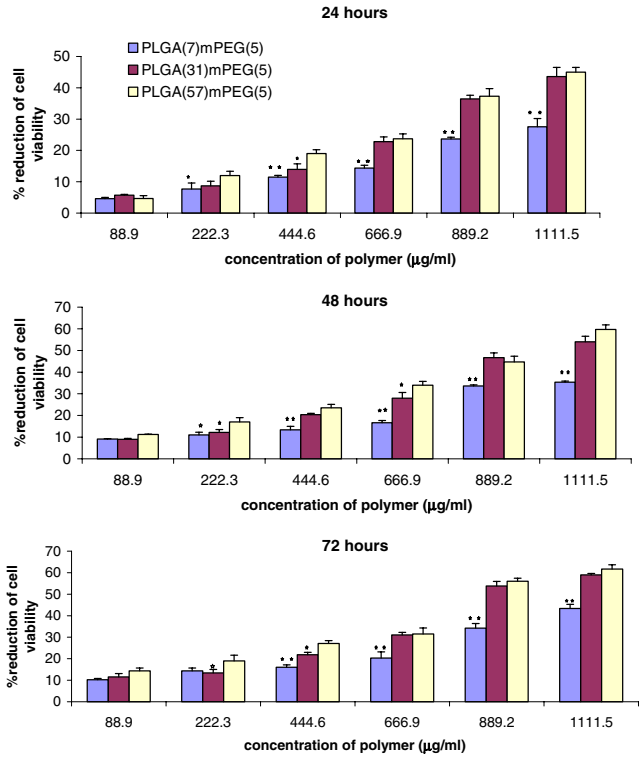

Fig. 1. Cytotoxicity of blank PLGA-mPEG nanoparticles on LNCaP cells. Statistical significance of differences between the data of PLGA(57)mPEG(5) nanoparticles and the data of the other two nanoparticle compositions are reported as: $^{*}p < 0.05$ and $^{**}p < 0.01$.

cytotoxicity: increasing the PEG content of nanoparticles (i.e. decreasing PLGA/mPEG ratio) resulted in decreased cytotoxicity. In most nanoparticle concentrations, the effect of composition on cytotoxicity was statistically significant ($p < 0.05$ or $p < 0.01$) (Fig. 1).

3.3. In vitro anticancer activity of PLGA-mPEG nanoparticles loaded with cisplatin

The in vitro anticancer cytotoxic activity of free cisplatin and cisplatin-loaded PLGA-mPEG nanoparticles on LNCaP cells, expressed as % reduction of cell viability, is shown in Fig. 2. The 50% growth inhibitory concentration (IC_{50}) values for free cisplatin and cisplatin-loaded nanoparticles were estimated from the available cytotoxicity data and are given in Table 2. The nanoparticles loaded with cisplatin exhibited in vitro anticancer activity comparable to that of free cisplatin (Fig. 2 and Table 2). The activity of both free and nanoparticle-entrapped cisplatin increased with increasing cisplatin concentration and incubation time. The differences in anticancer activity between the different nanoparticle types of cisplatin were low. Thus, when added at quantities generating the same cisplatin level in cell incubation medium, the PLGA(31)mPEG(5)/cisplatin nanoparticles appeared to be slightly more cytotoxic than the PLGA(7)mPEG(5)/cisplatin nanoparticles and these slightly more cytotoxic than the PLGA(57)mPEG(5)/cisplatin nanoparticles (Fig. 2 and Table 2).

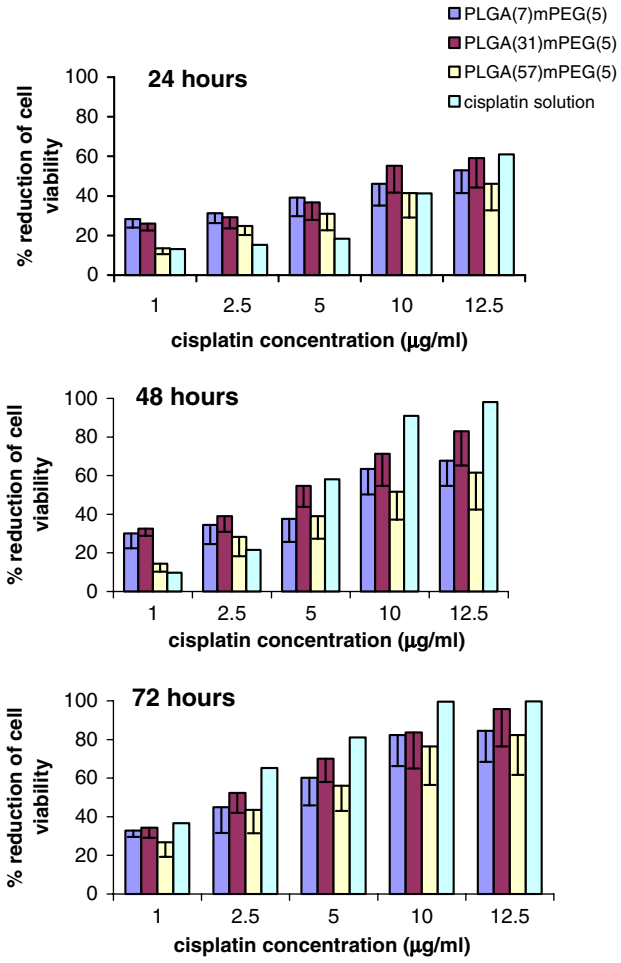

Fig. 2. Cytotoxicity of free cisplatin and cisplatin-loaded PLGA-mPEG nanoparticles on LNCaP cells. Variability of data was small (standard deviations normally less than 10% of respective means) and is not shown. The cytotoxicity of the carriers at the concentrations used to provide for the specific cisplatin concentrations is indicated by the part of the bars defined by the black solid lines originating from the top of the bars.

Table 2
IC_{50} values (μg/ml) of free cisplatin and cisplatin-loaded PLGA-mPEG nanoparticles

Formulation	24 h	48 h	72 h
Free cisplatin	10.3	5.2	1.8
PLGA(7)mPEG(5)/cisplatin	11.2	8.7	4.3
PLGA(31)mPEG(5)/cisplatin	9.3	5.5	2.7
PLGA(57)mPEG(5)/cisplatin	13.9	11.4	4.5

3.4. Uptake of PLGA-mPEG nanoparticles by LNCaP cells

The uptake of PLGA-mPEG nanoparticles by LNCaP cells in vitro, expressed as mg of nanoparticles per mg of protein and as % w/w of nanoparticles internalized by the cells, is shown in Fig. 3. The uptake of nanoparticles increased with incubation time. The composition of the nanoparticles had a significant effect on nanoparticles' uptake: increasing the PEG content of nanoparticles (i.e.

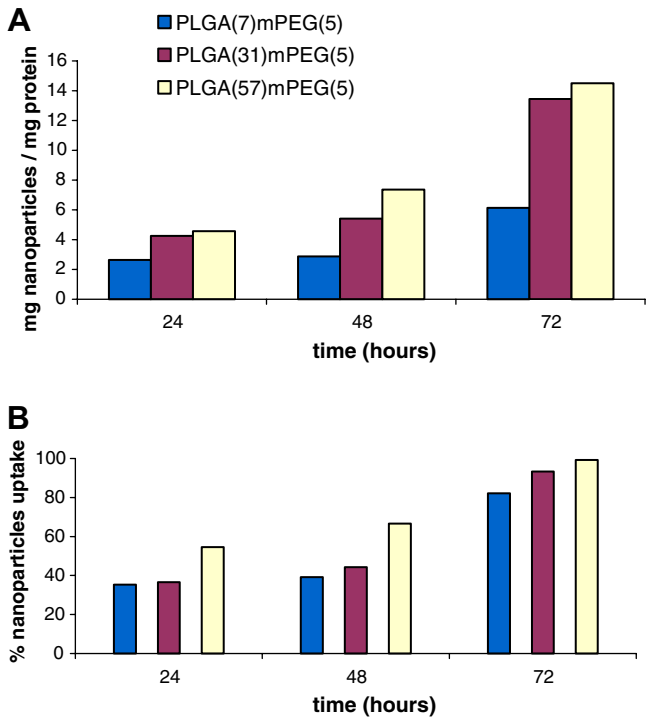

Fig. 3. Uptake of PLGA-mPEG nanoparticles by LNCaP cells in vitro expressed as (A) mg nanoparticles/mg protein and (B) % nanoparticles uptake.

decreasing PLGA/mPEG ratio) resulted in decreased uptake. After 72 h, the uptake of PLGA(57)mPEG(5) nanoparticles was complete (99.2%) as compared to only 70.1% of the least efficiently internalized PLGA(7)mPEG(5) nanoparticles (Fig. 3B). The uptake of PLGA(31)mPEG(5) at the same time was 93.2%.

Visual evidence of nanoparticles' uptake by the LNCaP cells at different incubation times (0.5–24 h) was obtained with fluorescence microscopy using nanoparticles labeled with PLGA(4165)-PyrBu(274) or dextran-rhodamine B isothiocyanate (Fig. 4). With all nanoparticle compositions, intense fluorescence was observed inside the cells at incubation time periods of 3 h or longer. In control experiments with non-labeled nanoparticles no fluorescence was observed within the cells (data not shown). Administration of the pure (non-entrapped in nanoparticles) labels resulted in extremely low fluorescence intensity within the cells (Fig. 4A and C). As shown in Fig. 4B, D–F, all nanoparticle types tested entered the cells. In all cases fluorescence was located in the cytoplasm, whereas in some cases fluorescence appeared to be located also around or inside nuclei.

4. Discussion

We have considered the feasibility of using long circulating PLGA-mPEG nanoparticles as carriers for the passive targeting of cisplatin to tumors. We have shown that the intravenous administration of PLGA-mPEG nanoparticles loaded with cisplatin to mice resulted in prolonged cisplatin residence in systemic circulation [11]. In this work, the in vitro anticancer activity of cisplatin-loaded PLGA-mPEG nanoparticles on prostate cancer LNCaP cells was investigated.

In the present study, the nanoparticles were prepared by a modified double emulsion process, which we have found that results in higher loading (% w/w) of nanoparticles with cisplatin [15] compared with the double emulsion method used previously [11]. Loading efficiency tends to be a little higher as the PLGA/PEG ratio of the copolymer used to prepare the nanoparticles increases (Table 1). This may be due to the fact that an increase of the PEG proportion renders the nanoparticles more hydrophilic, which would facilitate water (of external aqueous phase) penetration, drug dissolution in the water and drug leakage from the nanoparticles during nanoparticle preparation.

According to the data obtained (Fig. 1) even after prolonged contact with the cells (72 h), the blank PLGA-mPEG nanoparticles exhibited low cytotoxicity. Low cytotoxicity for PLGA(45)-PEG(5) nanospheres against human breast cancer MCF-7 cells was reported recently [16], in agreement with our results. The cytotoxicity of the blank nanoparticles was dependent on the composition of the PLGA-mPEG copolymer: an increase of the PEG content of the nanoparticles (i.e. a decrease of the PLGA/mPEG ratio in the copolymer by increasing the molecular weight of the PLGA block) caused a decrease in nanoparticle cytotoxicity. The cytotoxicity of PLGA(7)mPEG(5) nanoparticles, which were made by a copolymer with a much lower PLGA/PEG ratio than the other two types of nanoparticles, exhibited significantly lower toxicity than the other types of nanoparticles. The difference in cytotoxicity between PLGA(31)mPEG(5) and PLGA(57)mPEG(5) nanoparticles was relatively low. Since no or little degradation of the PLGA-mPEG nanoparticles in the neutral (buffered) cell incubation medium is expected to occur within the incubation times used [11], no significant cytotoxicity could have arisen from polymer degradation products. The cytotoxicity of the nanoparticles is, then, probably related to the efficiency of nanoparticles' uptake by the cells and to the inherent toxicity of the PLGA-mPEG copolymer following nanoparticles' uptake. The uptake of PLGA(7)mPEG(5) nanoparticles by LNCaP cells was much lower than that of the other two types of nanoparticles, whereas the uptake of PLGA(31)mPEG(5) nanoparticles was a little lower than that of PLGA(57)mPEG(5) nanoparticles (Fig. 3). The reason why the uptake of nanoparticles by the cells was decreased with decreasing PLGA/PEG ratio in the copolymer used to make the nanoparticles is that decreasing PLGA/PEG ratio increases the PEG content of the nanoparticles. An increase of the PEG content has been found to decrease phagocytic uptake of pegylated nanospheres [17]. Increasing PEG content causes an increase of the PEG density on nanoparticle surface [18], which

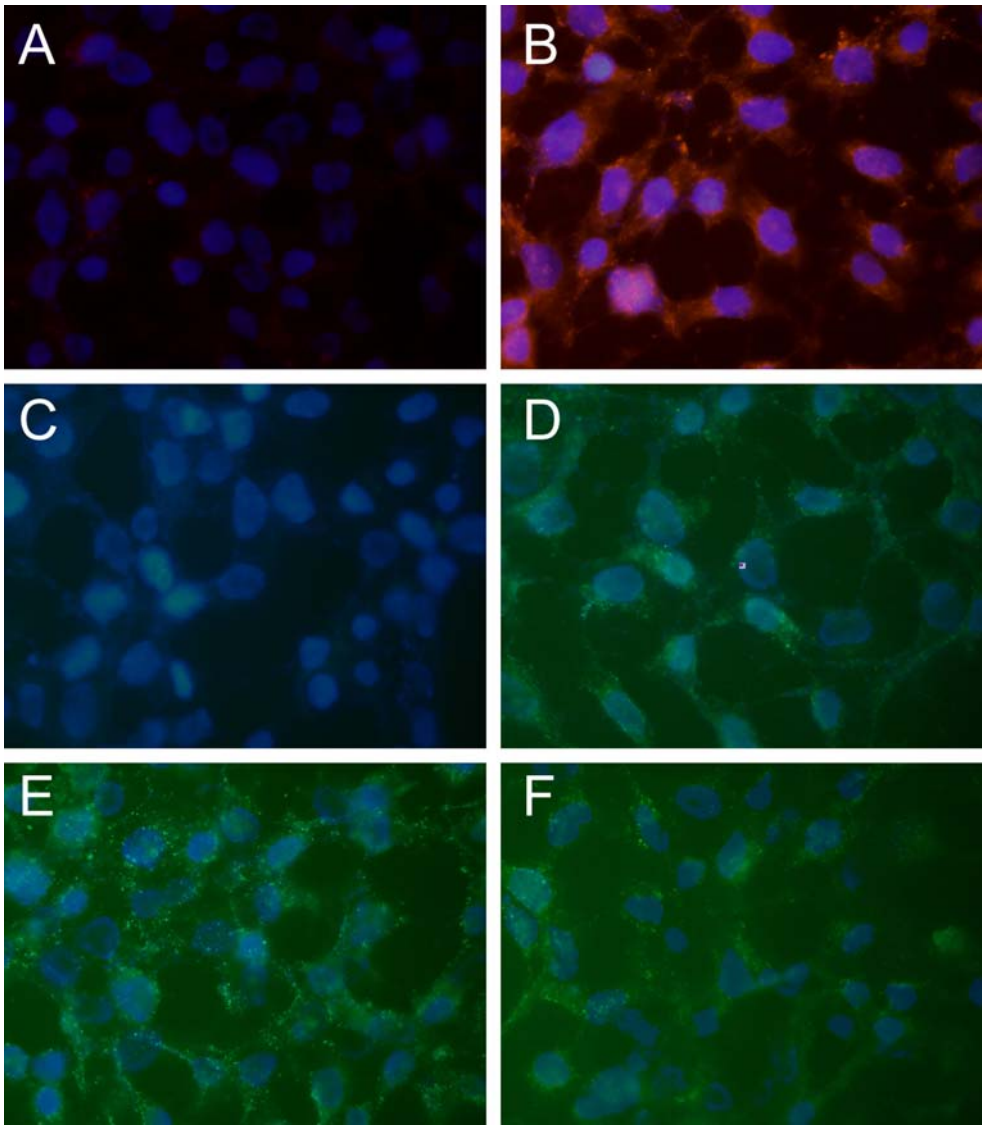

Fig. 4. Fluorescence microscopy images of the uptake of PLGA-mPEG nanoparticles by LNCaP cells. Cells were incubated for 12 h with (A) rhodamine B isothiocyanate, (B) dextran-rhodamine B isothiocyanate-labeled PLGA(31)mPEG(5) nanoparticles, (C) pure (nonentrapped in nanoparticles) PLGA(4165)-PyrBu(274), (D) PLGA(4165)-PyrBu(274)-labeled PLGA(31)mPEG(5) nanoparticles, (E) PLGA(4165)-PyrBu(274)-labeled PLGA(7)mPEG(5) nanoparticles and (F) PLGA(4165)-PyrBu(274)-labeled PLGA(57)mPEG(5) nanoparticles. DAPI was used to visualize nuclei (blue) and overlay images are shown in all cases (magnification 60×). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

probably results in a more effective steric barrier to nanoparticles' uptake by cells. The differences in the efficiency of uptake by the cells between the different types of nanoparticles may explain their differences in cytotoxicity. Thus, the PLGA(7)mPEG(5) nanoparticles, which exhibited significantly lower uptake by the cells than the other two types of nanoparticles, also exerted significantly lower cytotoxicity than the other types of nanoparticles. Additionally, the PLGA(31)mPEG(5) nanoparticles, which exhibited lower uptake than the PLGA(57)mPEG(5) nanoparticles, also caused slightly lower cytotoxicity than the PLGA(57)mPEG(5) nanoparticles.

Using fluorescence microscopy, visual evidence of nanoparticles' uptake by the LNCaP cells was obtained (Fig. 4). The nanoparticles were labeled (separately) with two different fluorescent substances, PLGA(4165)-PyrBu(274) or dextran-rhodamine B isothiocyanate, in order to check if observations were affected by the type of label. Pyrene-butanol was covalently attached to PLGA polymer, which forms the core of the nanoparticles, in order to provide for a stable nanoparticle labeling [19]. With all three nanoparticle types, intense fluorescence was observed inside cells, indicating that the PLGA-mPEG nanoparticles were capable of entering the cells. Moreover, in some cases fluorescence appeared to be located also around or inside cell nuclei, which if verified by further studies, would indicate that PLGA-PEG nanoparticles might prove to be useful in site-specific delivery of drugs whose site of pharmacological activity is cell nucleus.

The PLGA-mPEG nanoparticles loaded with cisplatin exhibited anticancer activity against LNCaP cells comparable to that of free cisplatin (Fig. 2 and Table 2). This indicates that cisplatin remains active after entrapment in the nanoparticles and, providing that the nanoparticles will result in a more selective delivery of cisplatin to tumors in vivo due to the EPR effect, PLGA-mPEG nanoparticles may be expected to be a more potent and safer delivery system for cisplatin than the aqueous solutions used today. Low differences in anticancer cytotoxic activity between the different nanoparticle compositions were observed. The PLGA(31)mPEG(5)/cisplatin nanoparticles were slightly more active than the PLGA(7)mPEG(5)/cisplatin nanoparticles and these slightly more active than the PLGA(57)mPEG(5)/cisplatin nanoparticles (Fig. 2 and Table 2). Although the PLGA(57)mPEG(5) nanoparticles exhibited the most efficient uptake by LNCaP cells (Fig. 3), the PLGA(57)mPEG(5)/cisplatin nanoparticles exhibited lower cytotoxic activity than the other two types of (cisplatin-loaded) nanoparticles. It appears that apart from cell uptake other factors may also influence the cytotoxic activity of the cisplatin-loaded nanoparticles. In our case, this factor cannot be the toxicity of the carrier itself (blank nanoparticles), since at the nanoparticle concentrations applied in this experiment, the carrier did not have significant cytotoxicity. Even at the highest nanoparticle concentrations, which were applied in order to generate the maximum studied cisplatin level (12.5 μg/ml) and which ranged between 365 μg/ml for the most drug-loaded PLGA(57)mPEG(5) nanoparticles and 445 μg/ml for the least drug-loaded PLGA(7)mPEG(5) nanoparticles, the carriers exhibited limited cytotoxicity (Figs. 1 and 2). One possibility could be that the different nanoparticle types exhibited different cisplatin release in the cell incubation medium prior to their uptake by the cells. Released cisplatin would rapidly enter the cells, exerting cytotoxic activity, and differences in the rate of cisplatin release would influence the cytotoxicity results obtained with the different cisplatin-loaded nanoparticle types. Indeed, the different nanoparticle types exhibited different rates of cisplatin release in vitro (release data in reference [11]). The PLGA(7)mPEG(5)/cisplatin nanoparticles were a little more cytotoxic than the PLGA(57)mPEG(5)/cisplatin nanoparticles (Fig. 2 and Table 2), although they exhibited significantly lower cell uptake (Fig. 3), possibly because they released cisplatin in the cell incubation medium much more rapidly than the PLGA(57)mPEG(5)/cisplatin nanoparticles [11]. On the other hand, the PLGA(31)mPEG(5)/cisplatin nanoparticles were a little more active than the other two types of cisplatin-loaded nanoparticles (Fig. 2 and Table 2), possibly because they exhibited both efficient uptake by the cells (Fig. 3) and adequately rapid cisplatin release [11].

5. Conclusions

Blank PLGA-mPEG nanoparticles exhibited low cytotoxicity, which increased with increasing the PLGA/PEG ratio in the PLGA-mPEG copolymer used to prepare the nanoparticles. PLGA-mPEG nanoparticles loaded with cisplatin entered the cells and exerted in vitro anticancer activity against LNCaP human prostate cancer cells that was comparable to the activity of free (non-entrapped in nanoparticles) cisplatin. The results justify further investigation of the anticancer activity of PLGA-mPEG/cisplatin nanoparticles in vivo.

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