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Dual anticancer drug/superparamagnetic iron oxide-loaded PLGA-based nanoparticles for cancer therapy and magnetic resonance imaging

N. Schleich[a], P. Sibret[b], P. Danhier[c], B. Ucakar[a], S. Laurent[d], R.N. Muller[d,e], C. Jérôme[b], B. Gallez[c], V. Préat[a,*,1], F. Danhier[a,1]

[a] Université Catholique de Louvain, Louvain Drug Research Institute, Pharmaceutics and Drug Delivery, Avenue Mounier, B1 73.12, 1200 Brussels, Belgium
[b] Université de Liège, Center for Education and Research on Macromolecules, Sart Tilman B6, 4000 Liège, Belgium
[c] Université Catholique de Louvain, Louvain Drug Research Institute, Laboratory of Biomedical Magnetic Resonance, Avenue Mounier, B1 73.08, 1200 Brussels, Belgium
[d] Université de Mons, Department of General, Organic, and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, 7000 Mons, Belgium
[e] CMMI – Center for Microscopy and Molecular Imaging, Rue Adrienne Bolland, 8, B-6041 Gosselies, Belgium

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ABSTRACT

We developed dual paclitaxel (PTX)/superparamagnetic iron oxide (SPIO)-loaded PLGA-based nanoparticles for a theranostic purpose. Nanoparticles presented a spherical morphology and a size of 240 nm. The PTX and iron loading were 1.84 ± 0.4 and 10.4 ± 1.93 mg/100 mg respectively. Relaxometry studies and phantom MRI demonstrated their efficacy as T_2 contrast agent. Significant cellular uptake by CT26 cells of nanoparticles was shown by Prussian blue staining and fluorescent microscopy. While SPIO did not show any toxicity in CT-26 cells, PTX-loaded nanoparticles had a cytotoxic activity. PTX-loaded nanoparticle (5 mg/kg) with or without co-encapulated SPIO induced *in vivo* a regrowth delay of CT26 tumors. Together these multifunctional nanoparticles may be considered as future nanomedicine for simultaneous molecular imaging, drug delivery and real-time monitoring of therapeutic response.

1. Introduction

Theranostics is a newly emerging concept which involves simultaneous execution of therapeutic and diagnostic approaches for personalized medicine. Nanoparticles (NP) can be designed to encapsulate a wide variety of chemotherapeutic and diagnostic agents for the delivery of these agents to tumor cells (Lammers et al., 2010). Nanoparticles can target tumors by a passive process. Passive targeting implies that nanoparticles are smaller than the fenestrations of endothelial cells and can therefore enter the interstitium to be finally entrapped in the tumor. The combination of leaky vasculature and poor lymphatic drainage results in the well-known enhanced permeability and retention (EPR) effect (Maeda et al., 2000; Danhier et al., 2010).

Paclitaxel (PTX), a major anti-cancer drug has anti-neoplasic activity particularly against various types of solid tumors (Singla et al., 2002). PTX disrupts the dynamic equilibrium within the microtubule system and blocks cells in the late G_2 phase and M phase of the cell cycle, thereby inhibiting cell replication (Schiff et al., 1979). PTX is poorly soluble in water. To enhance its solubility and allow its parenteral administration, PTX is currently formulated at 6 mg/ml in a vehicle composed of a mixture of Cremophor® EL and ethanol (1:1) (Taxol®) (Weiss et al., 1990).

Magnetic resonance imaging (MRI) is a non-invasive imaging technique, presenting a high spatial resolution which is suitable for cancer detection and therapeutic response assessment (Brindle, 2008). However, its low sensitivity represents a major limitation. Superparamagnetic iron oxide (SPIO) as MRI contrast agent addresses this limitation. SPIO can produce predominant T_2 relaxation effect, resulting in a signal reduction on T_2-weighted images. The magnetic field heterogeneity around the particles, through which water molecules diffuse, induces the dephasing of the proton magnetic moments. Consequently, a T_2 effective transverse relaxation is shortened (Bjornerud and Johansson, 2004; Ling et al., 2011).

Recently, numerous publications have reported various polymer-based nanocarriers (poly(lactide-co-glycolide) (PLGA), poly (L-lactic acid) PLLA, N-(2-hydroxypropyl)methacrylamide (HPMA), poly(e-caprolactone)) for magnetic-imaging (Hamoudeh et al., 2007; Ling et al., 2011; Lu et al., 2009; Talelli et al., 2009). These theranostics successfully allowed the non-invasive assessment of the biodistribution, the visualization of drug distribution, the optimization of strategies, the prediction and real-time

* Corresponding author. Tel.: +32 2 7647320; fax: +32 2 7647398.
E-mail address: veronique.preat@uclouvain.be (V. Préat).
[1] These authors contributed the same way.

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monitoring of therapeutic responses (Lammers et al., 2010). However, these SPIO-loaded nanocarriers are generally limited because of (i) their lack of functional groups on their surface for covalent modification, (ii) their low capacity of drug loading and (iii) the fact that most of polymers used are not approved by the FDA, limiting their potential translation to the clinic.

Previously, we developed PTX-loaded PEGylated PLGA-based nanoparticles showing a lower IC_{50} *in vitro* and improved *in vivo* anti-tumor efficacy when compared to Taxol®, due to the EPR effect (Danhier et al., 2009a). (PLGA) was chosen for its biodegradability properties, its biocompatibility and its approval by the FDA (Danhier et al., 2012a). Poly(ε-caprolactone-b-ethylene glycol) (PCL-b-PEG), an amphiphilic copolymer, was added to take advantage of the repulsive properties of PEG, to provide a higher stability of nanoparticles in biological fluids and to allow the grafting of a targeting ligand (Fievez et al., 2009; Garinot et al., 2007; Pourcelle et al., 2007). We also previously developed RGD-grafted PTX-loaded PEGylated PLGA-based nanoparticles showing an effective $\alpha_v\beta_3$ targeting of the tumor endothelium (Danhier et al., 2009b).

In this study, we aimed at developing these previously described PTX-loaded PEGylated PLGA-based nanoparticles as an effective nanocarrier for dual encapsulation of anti-cancer drug (PTX) and SPIO for a theranostic purpose. Hence, SPIO were prepared by the co-precipitation technique and were encapsulated in PLGA-based nanoparticles. The physico-chemical properties of nanoparticles were characterized by different techniques such as transmission electron microscopy (TEM), dynamic light scattering (DLS) method, electron paramagnetic resonance (EPR) spectroscopy or inductively coupled plasma mass spectroscopy (ICP-MS). Their magnetic properties were evaluated using relaxometry and MRI. Furthermore, we investigated the *in vitro* cellular uptake and cytotoxicity of nanoparticles. Finally, the *in vivo* anti-tumor efficacy was assessed on CT-26 tumor-bearing mice.

2. Materials and methods

2.1. Materials

Iron(II) chloride, iron(III) chloride, oleic acid, sodium hydroxide, chlorhydric acid, 4,6-diamidino-2-phenylindole (DAPI), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT), polyvinylalcohol (PVA, MW = 30–70 kDa), poly(lactic-co-glycolic acid) (PLGA, lactide/glycolide molar ratio of 50:50 MW = 7000–17 000), paclitaxel (PTX) and doxorubicin (DOX) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Perls' Prussian blue kit and commercial SPIO Molday Ion™ were purchased from BioPAL (Worcester, UK). PLGA-*b*-PEG (MW = 10 040–4600), PCL-*b*-PEG (MW = 13 100–5000) and FITC-PLGA were synthesized as previously described (Danhier et al., 2009a,b; Freichels et al., 2011; Garinot et al., 2007). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin–EDTA and penicillin–streptomycin mixtures were from Gibco® BRL (Carlsbad, CA, USA). Murine colon carcinoma CT26 cells were kindly given by Goldyne Savad Institute of Gene Therapy, Hadassah-Hebrew University Hospital (Jerusalem, Israël). Water used in all experiment was prepared by Aqualab purification system.

2.2. Synthesis of superparamagnetic iron oxides (SPIO) coated with oleic acid

Hydrophobic superparamagnetic iron oxides (SPIO) were synthesized using a classical co-precipitation technique of ferrous and ferric salts in alkaline medium (Massart, 1981). Briefly, 10 mmol iron(III) chloride and 5 mmol iron(II) chloride were mixed together in 12 ml of an hydrochloride aqueous solution (HCl 1 M). This solution was then added dropwise to an aqueous solution of NaOH 1 M containing 3.1 g of oleic acid with stirring on a magnetic stir plate for 20 min under a nitrogen-gas atmosphere at 80 °C. The black precipitate was separated using a magnet, washed three times using absolute ethanol and then dissolved in 50 ml of dichloromethane (DCM). The solution was then placed in an ultrasonic bath for 10 min and centrifuged (4416 rcf, 10 min) to remove the undispersed residue.

Hydrophilic SPIO used as SPIO aqueous solution (SPIO sol) were also synthesized. Briefly 1 ml of DCM dispersion of SPIO coated with oleic acid (40 mg/ml) was added to a suspension of tetramethylammonium 11-aminoundecanoate in DCM (40 mg in 2 ml). After 24 h magnetic stirring, the precipitate was washed three times with DCM and dispersed in water (Yang et al., 2010).

2.3. Preparation of PLGA-based nanoparticles loaded with SPIO and paclitaxel or doxorubicin

Both nanoparticles loaded with SPIO and DOX (SPIO/DOX-NP) and nanoparticles loaded with SPIO and paclitaxel (SPIO/PTX-NP) were prepared by an emulsion–diffusion–evaporation method (Danhier et al., 2009a; Garinot et al., 2007) Briefly, PLGA (14 mg/ml), PLGA-PEG (3 mg/ml) and PCL-PEG (3 mg/ml) were dissolved in 2 ml DCM containing SPIO (Fe concentration: 15 mg/ml). Doxorubicin HCl (0.2 mg/ml) was dissolved in 1 ml DCM containing 0.001 mg/ml triethanolamine beforehand and stirred overnight (Shieh et al., 2011) whereas paclitaxel (3 mg) was added directly. This organic solution was then added to an aqueous solution (4.5 ml) containing 3% (p/v) PVA and emulsified using a vortex for 2 min followed by sonication (2× 30 s, 50 W). The mixture was then added dropwise and under magnetic stirring into an aqueous solution containing 1% PVA and stirred overnight to evaporate the organic solvent. To remove the non-encapsulated drug, the suspension was filtered (1.2 μm) and washed three times with water using ultracentrifugation (11,000 × *g*, 30 min, 4 °C) and suspended in 2 ml water. FITC covalently labeled PLGA was used to prepare fluorescent nanoparticles (Freichels et al., 2011). PTX-loaded nanoparticles (PTX-NP) and SPIO-loaded nanoparticles (SPIO-NP) were also prepared using the same protocol.

2.4. Physico-chemical characterization of nanoparticles loaded with SPIO and paclitaxel or doxorubicin

2.4.1. Thermogravimetric analysis of SPIO

The coating percentage of SPIO with oleic acid was assessed by thermogravimetric analysis (TGA) on a TA Instrument Q500 model, under dry nitrogen flow, with a heating rate of 15 °C/min from RT to 600 °C, in an open platinum pan.

2.4.2. Particles size and morphology determination

The hydrodynamic particle size and size polydispersity of nanoparticles was assessed using a dynamic light scattering method (Nano ZS, Malvern instruments, UK). The Zeta (ζ) potential of the nanoparticles was measured in KCL 1 mM with a Malvern Nano ZS at 25 °C. The morphology of the particles was achieved using transmission electron microscopy (TEM). TEM was carried out with a Philips CM 100 operating at a voltage of 100 kV, equipped with a Megaview G2 camera. Samples for TEM experiments were prepared by spin coating a drop of nanoparticles in DCM on a carbon-coated TEM grid.

2.4.3. Determination of SPIO loading content

The iron content was measured by electron paramagnetic resonance (EPR) using a Bruker EMX EPR spectrometer operating at 9 GHz (Bruker Biospin GmBh, Germany) validated by inductively