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Paclitaxel-loaded micelles enhance transvascular permeability and retention of nanomedicines in tumors

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ABSTRACT

Paclitaxel (PTX)-loaded polymeric micelles (M-PTX) have been shown to enhance the blood flow and oxygenation of tumors 24 h after treatment. We hypothesized that these changes in the tumor microenvironment could lead to an enhancement of the EPR (enhanced permeability and retention) effect. M-PTX, administered 24 h before analysis, increased the accumulation of macromolecules, nanoparticles and polymeric micelles in tumors. This increased EPR effect could be linked to normalization of the tumor vasculature and decreased interstitial fluid pressure. M-PTX used as a pre-treatment allowed a more effective delivery of three nanomedicines into tumors: polymeric micelles, liposomes and nanoparticles. These experiments demonstrate an enhanced EPR effect after M-PTX treatment, which lead to better availability and enhanced efficacy of a subsequent treatment with nanomedicines.

1. Introduction

One of the major limitations of the efficacy of conventional chemotherapy is poor entry of anti-cancer drugs into tumors (Heldin et al., 2004; Jain and Stylianopoulos, 2010; Minchinton and Tannock, 2006). Tumor targeting by nanomedicine-based therapeutics has emerged as a promising approach to overcome the lack of specificity of conventional chemotherapeutic agents. The design of these agents is based on anatomical and physiological differences between normal and tumoral tissues. Nanocarriers (20–200 nm in diameter) can extravasate and accumulate within the interstitial space of tumors by transiting through endothelial pores 10 and 1000 nm in diameter. The general absence of functional lymphatic vessels in most tumors contributes to nanocarrier entrapment and retention. This passive phenomenon is termed the "enhanced permeability and retention (EPR) effect" (Maeda et al., 2009).

Paclitaxel (PTX) is a potent anti-cancer chemotherapy widely used for treatment of solid tumors, particularly of the breast and ovary (Singla et al., 2002). PTX promotes the polymerization of tubulin causing cellular death by disrupting the normal tubule dynamics required for mitotic division (Adams et al., 1993). PTX also has broad inhibitory effects on angiogenesis, which include decreasing the proliferation, mobility and cord formation of endothelial cells, as well as *in vivo* angiogenesis and VEGF-induced neovascularization (Belotti et al., 1996; Myoung et al., 2001).

Previously, we developed self-assembling diblock copolymers made up of ε-caprolactone (CL), trimethylene carbonate (TMC) and mme-PEG$_{750}$ (mmePEG$_{750}$-p(CL-*co*-TMC)). These polymeric micelles were shown as safe and effective delivery system for PTX. *In vitro*, the IC$_{50}$ (HeLa cells) at 24 h of Taxol® and PTX-loaded micelles were 17.6 and 10.6 μg/ml, respectively. mmePEG$_{750}$-p(CL-*co*-TMC) copolymers were not cytotoxic (100 mg/ml) (Danhier et al., 2009a). Additionally, mmePEG$_{750}$-p(CL-*co*-TMC) copolymers have been shown biocompatible, non-cytotoxic and non-hemolytic (up to 20% w/v) (Vandermeulen et al., 2006). *In vivo*, these PTX-loaded polymeric micelles (M-PTX) enhanced the maximum tolerated dose (MTD) of PTX compared to Taxol® (the current

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commercialized form of PTX which contains Cremophor® EL) from 13.5 mg/kg to 80 mg/kg after intraperitoneal injection. Unloaded-micelles were shown to be not cytotoxic *in vivo*. Polymeric micelles are known to extravasate from blood vessels into tumors through fenestrations present in the tumor endothelium and to be retained within the tumor tissue by the EPR effect (Danhier et al., 2010; Maeda, 2001). M-PTX have been shown to improve tumor oxygenation 24 h after treatment, thereby causing radiosensitization. This increased tumor pO_2 was explained by both an increased blood flow and an inhibition of cellular O_2 consumption resulting from the anti-tumor activity of PTX (Fig. 1) (Danhier et al., 2012).

New vessel formation in tumors is initiated by an imbalance between pro-angiogenic and anti-angiogenic factors. Anti-angiogenic therapies should destroy the tumor vasculature, thereby depriving the tumor of oxygen and nutrients. However, some anti-angiogenic agents are able to transiently "normalize" the abnormal tumor vasculature. This leads to remodeling of the vasculature which presents a structure that is more similar to normal vessels, and reduced interstitial fluid pressure (IFP). This concept of normalized vessels could suggest that these "normal" vessels could avoid the EPR effect and compromise the delivery of drugs to tumors. Jain resolved this paradox by comparing properties of normal, tumor and normalized vessels. It appeared that normalized vessels remain permeable to large molecules (Jain, 2005). Hence the normalization induces a better delivery of the therapeutic entities, by keeping intact the EPR effect and decreasing the IFP (Fig. 1). Although mostly described in mouse tumor models, several clinical trials have provided evidence of a normalized vasculature in cancer patients treated with anti-angiogenic agents (Heldin et al., 2004; Jain and Stylianopoulos, 2010).

In the present study, we hypothesized that (i) changes in the tumor microenvironment observed 24 h after treatment with M-PTX enhance the EPR effect, allowing a more effective delivery of anti-cancer nanomedicines into tumors; (ii) the underlying mechanism of enhanced permeability could be related to normalization of the tumor vasculature and finally (iii) M-PTX pre-treatment could be used to enhance the therapeutic response to nanomedicines (Fig. 1).

Two tumor models were used for this study: CT26 colon carcinoma was chosen because of its sensitivity to PTX (Yang et al., 2013) and its angiogenic properties (Lyons et al., 2007). The TLT hepatocarcinoma model was chosen because it is a fast-growing hypoxic model that presents intrinsic radio- and chemo-resistance and it has already been used to exploit vascular "normalization" properties of anti-angiogenic drugs (Segers et al., 2006). Enhanced vascular permeability was first assessed *in vivo* by different methods highlighting intratumoral accumulation of various nano-scaled vectors (FITC-dextran, 35 nm; Evans blue complex, 6 nm; iron oxides, 30 nm and [^{3}H]PTX-loaded-micelles, 23 nm). We also evaluated normalization of the tumor vasculature using whole mount staining (CD-31), immunofluorescence (CD-31 and α-SMA) and the measurement of IFP, using the wick-in-needle technique. Finally, we tested if a second injection of various anti-cancer nanomedicines 24 h after M-PTX could enhance the therapeutic response.

2. Methods

2.1. Polymer synthesis and characterization

The mmePEG$_{750}$-p(CL-*co*-TMC) copolymer (Johnson and Johnson Center for Biomaterials and Advanced Technologies, Somerville, NJ, USA) was synthesized by ring-opening polymerization as described previously. Briefly, the reaction was initiated by PEG monomethylether of 750 Da (mmePEG$_{750}$) at a molar monomer/initiator ratio of 13.3/1. ε-Caprolactone and trimethylene carbonate were added at 1:1 molar ratio. The reaction was catalyzed by stannous octoate and was allowed to run for 24 h at 160 °C. The polymer was then devolatilized under vacuum at 90 °C for 48–72 h. The molecular weight and the polydispersity of the diblock polymer were determined by gel permeation chromatography and the monomer ratio in the polymer by NMR spectroscopy (Ould-Ouali et al., 2004).

2.2. Preparation and characterization of micelles loaded with PTX

PTX-loaded micelles (M-PTX) (PTX, Sigma–Aldrich, USA) were prepared as previously described (Danhier et al., 2009a). For the radiolabeled formulation, PTX was replaced by [2,6-benzamido-^{3}H (N)] paclitaxel (Moravek Biochemicals, USA).

The amount of solubilized PTX in mmePEG$_{750}$-p(CL-*co*-TMC) micelles was determined by HPLC as described previously (Danhier et al., 2009a, 2012). The particle size and ζ potential were determined by photon correlation spectroscopy (PCS) and laser Doppler velocimetry, respectively using a nanosizer ZS (Malvern, UK). Results were analyzed by the CONTIN algorithm and the sizes were calculated based on the volume distributions together with polydispersity indices (PDI). Electrophoretic mobilities were converted to ζ potentials using the Smoluchowski's equation.

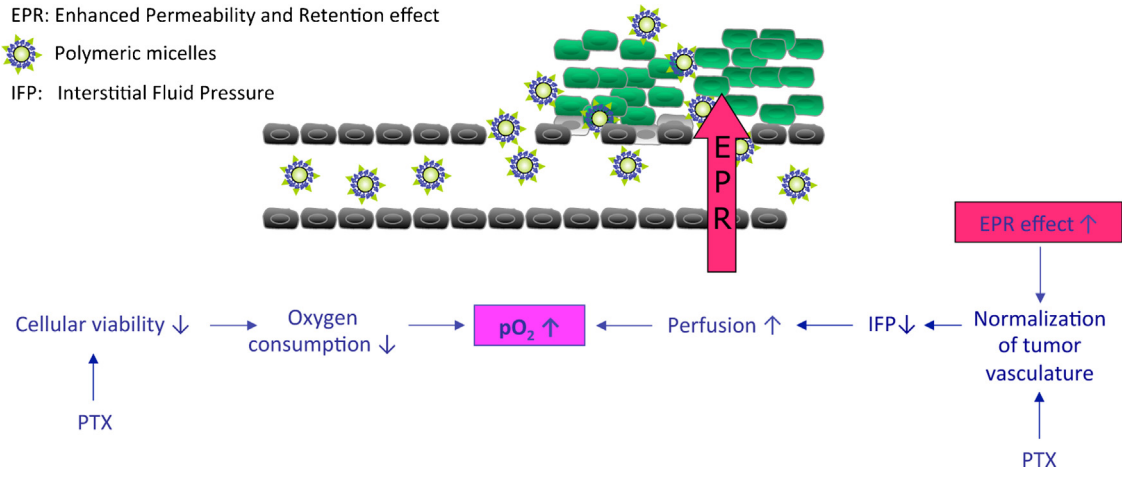

Fig. 1. Schematic representation of the effects of PTX and its influence on the tumoral microenvironment.