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Influence of formulation variables on the biodistribution of multifunctional block copolymer micelles

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ABSTRACT

The physico-chemical characteristics and composition of block copolymer micelles (BCMs) may influence the pharmacokinetics and consequently, the desired delivery characteristics. In this study the influence of formulation variables such as size, density of targeting ligand [i.e. epidermal growth factor (hEGF)] and the bifunctional chelator (BFC) used for labelling the BCMs with ^{111}In, on the pharmacokinetics and biodistribution in mice were evaluated. BCMs were prepared from Me-PEG$_x$-*b*-PCL$_y$ ($x = 2.5$ k, $y = 1.2$ k for 15 nm BCMs and $x = 5$ k, $y = 5$ k for 60 nm BCMs) with (targeted, 1 or 5 mol% hEGF) or without (non-targeted) hEGF-PEG$_x$-*b*-PCL$_y$. To investigate the effect of the BFC on the pharmacokinetics, the BCMs were labelled with ^{111}In using *p*-SCN-Bn-DOTA (Bn-DOTA-PEG$_x$-*b*-PCL$_y$), H_2N-DOTA (DOTA-PEG$_x$-*b*-PCL$_y$), DTPA anhydride (DTPA-PEG$_x$-*b*-PCL$_y$) or *p*-SCN-Bn-DTPA (Bn-DTPA-PEG$_x$-*b*-PCL$_y$). The resulting 15 nm or 60 nm non-targeted or targeted (1 or 5 mol% hEGF) were injected via a tail vein to mice bearing MDA-MB-468 human breast cancer xenograft that overexpress EGFR, followed by pharmacokinetics and biodistribution studies. Pharmacokinetic parameters were determined by fitting the blood concentration vs time data using a two compartment model with i.v. bolus input. Pharmacokinetic parameters were found to depend on BCM size, the BFC used as well as the density of hEGF on the surface of the BCMs. BCMs labelled with *p*-SCN-Bn-DTPA (^{111}In-Bn-BCMs) showed improved pharmacokinetics (i.e. extended circulation lifetime) and tumor uptake compared to those labelled with DOTA-PEG$_x$-*b*-PCL$_y$, *p*-SCN-Bn-DOTA or DTPA dianhydride. Formulations with a high density of hEGF (5 mol% hEGF) had short circulation half-lives. BCMs labelled with ^{111}In via *p*-SCN-Bn-DTPA showed highest accumulation in the liver and spleen and slower whole body elimination. Smaller sized BCMs were rapidly cleared from the circulation. Increasing the density of hEGF on the surface did not improve tumor uptake due to faster clearance from the circulation. To achieve improved pharmacokinetics and in turn effective exploitation of the EPR effect, *p*-SCN-Bn-DTPA emerged as the optimal BFC for radiolabelling BCMs while a lower density of hEGF gave more favourable organ distribution.

1. Introduction

In recent years, drugs relying on formulations in nanotechnologies such as Doxil®/Myocet® and Fungisome/AmBisome have been approved for clinical use [1–3], while many others have reached late stage clinical development [4–7]. The promise of nanotechnologies in drug delivery relies on their ability to significantly increase the payload of therapeutic agents that reach a diseased site while limiting systemic toxicity [8–10]. Additionally, these nanoparticles when labelled with radionuclides [11,12] and/or contrast agents can be used for imaging, contrast enhancement [13,14] and/or as radiotherapeutics.

Efficient targeting using nano-delivery systems relies largely on passive targeting which is achieved via exploitation of the enhanced permeation and retention (EPR) effect. The size of nanoparticles and presence of steric stabilizing polymers such as poly(ethylene glycol) (PEG) at their surface facilitates retention within the circulation for extended periods following intravenous (i.v.) administration. This prolonged circulation lifetime for the nanoparticles is essential for passive targeting to tumors which have leaky vasculature, via the EPR effect. In addition to the physico-chemical characteristics of nanoparticles (i.e. size, surface properties), the tumor microenvironment is also responsible for determining the extent of extravasation of these nanoparticles into tumors [15].

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Active targeting may also be pursued as a strategy to improve the efficacy of drug formulations. Active targeting requires the surface conjugation of targeting moieties that are specific for receptors selectively present or overexpressed at a diseased site. Indeed numerous preclinical studies have demonstrated that active targeting of nanoparticle-based formulations can result in improvements in efficacy in comparison to the non-targeted formulations [16–18]. However, the relationship between the therapeutic effect of the actively targeted nanoparticles and distribution at the whole body, tumor and cellular levels is less clear. Overall, it is critical that the addition of the targeting moiety does not result in a reduction in the circulation lifetime of the nanoparticle and in turn a decrease in tumor accumulation via the EPR effect and efficacy [19,20].

The pharmacokinetics of nano-delivery systems depends on their surface properties, size and morphology [21–26]. Based on the present literature it is debateable whether or not the presence (and therefore the density) of targeting moieties at the surface of vehicles influences their pharmacokinetic properties. Some studies have shown that incorporation of targeting moieties leads to a reduction in circulation lifetime while others show no such difference in comparison to the non-targeted delivery systems [16,17,27]. However, in the case of immunoliposomes conjugated using whole IgG there is consensus that the Fc mediated clearance and increased opsonisation of the antibody labelled vehicles leads to poorer pharmacokinetics [27]. While the increased opsonisation has been well documented in some cases for nano-delivery systems conjugated with antibodies or antibody fragments, few studies have examined such effects for small ligands [< 10 kDa, e.g. epidermal growth factor (EGF)]. As well, given the recent interest in image-guided drug delivery and molecular imaging, delivery systems are frequently being labelled with radionuclides via use of a bifunctional chelating (BFC) agent. As has been shown with some proteins (e.g. affibodies conjugated with DOTA or DTPA derivatives) the use of BFC agents can have a profound influence on the pharmacokinetics of biomolecules [28–31]. However, the effects of such BFC agents on the pharmacokinetics and biodistribution of nano-delivery systems have not been studied in detail.

Our group is exploring the use of BCMs for the treatment and diagnosis of EGFR overexpressing cancers [11,12,32]. As shown in Fig. 1, the BCMs consist of a stabilizing methoxypoly(ethylene glycol)-block-polycaprolactone copolymer (MePEG-*b*-PCL), an ^{111}In radionuclide carrying PEG-*b*-PCL copolymer and a targeting component hEGF-PEG-*b*-PCL. In the current study, the influence of several formulation variables (i.e. BCM size, density of EGF and BFC agent selected) on the pharmacokinetics and biodistribution of the actively targeted micelles were examined in order to maximize their delivery to the target site.

2. Materials and methods

2.1. Materials

$MePEG_x$ ($x = 2.5$ k, and $x = 5$ k, polydispersity index (PDI) = 1.06) obtained from Sigma-Aldrich (St. Louis, MO) and NH_2PEG_x ($x = 2.5$ k, and $x = 5$ k, PDI = 1.07) obtained from JenKem Technology (Allen, TX), were dried twice by azeodistillation in toluene. ε-Caprolactone (CL, 99%) was obtained from Sigma-Aldrich and dried with *n*-butyl lithium (at −78 °C), calcium hydride and molecular sieves, prior to use. Tetrahydrofuran (THF, 99.9%) and toluene (99.9%) were obtained from Sigma-Aldrich and were refluxed over a sodium–benzophenone complex and distilled under nitrogen. HCl (1.0 M in diethyl ether), sodium (99.5%), and diethylenetriaminetetraacetic acid dianhydride (DTPA dianhydride) were obtained from Sigma-Aldrich. 2-(4-thiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (*p*-SCN-Bn-DOTA), 1,4,7.10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid mono(1-hydroxysucinimide ester) (DOTA-NHS-ester) and 2-(4-isothiocyanatobenzyl)-diethylenetriaminepentaacetic acid (*p*-SCN-Bn-DTPA) were obtained from Macrocyclics (Dallas TX). hEGF was obtained from Meridian Life Sciences (Memphis, TN). $^{111}InCl_3$ was purchased from Nordion (Kanata, ON).

2.2. Synthesis and characterization of MePEG-b-PCL, NH_2-PEG-b-PCL and HOOC-PEG-b-PCL

Carboxy-terminated poly(ethylene glycol)-*b*-poly(ε-caprolactone) (HOOC-PEG_x-*b*-PCL_y), methoxy-terminated PEG_x-*b*-PCL_y ($MePEG_x$-*b*-PCL_y) and amino-terminated PEG_x-*b*-PCL_y (H_2N-PEG_x-*b*-PCL_y) copolymers were prepared as previously reported [32–34]. N-hydroxysuccinimidyl PEG_x-*b*-PCL_y activated ester (NHS-PEG_x-*b*-PCL_y) and hEGF-PEG_x-*b*-PCL_y were synthesized as previously described [34]. The conjugation efficiency (and the amount of hEGF conjugated to BCMs) was determined by the μBCA assay (Pierce Scientific) following the manufacturer's guidelines.

DTPA derivatization of H_2N-PEG_x-*b*-PCL_y was accomplished using DTPA bis-anhydride or *p*-SCN-Bn-DTPA and was purified as previously described [11,32]. DOTA-PEG_x-*b*-PCL_y and Bn-DOTA-PEG_x-*b*-PCL_y were obtained following the similar procedure as for the DTPA derivatives.

Block copolymers (in $CDCl_3$) were characterized by ^{1}H NMR using a Gemini 200 spectrometer (200 MHz for ^{1}H). The molecular weights of polymers were determined by analyzing the ratio of the proton intensities in the ^{1}H-NMR spectra. Gel permeation chromatography (GPC) measurements to determine the molecular weight and PDI of the polymers were carried out at 40 °C using a Waters 2695 liquid chromatography system equipped with three Waters Styragel HR 4E columns (10 μm, 7.8 × 300 mm) connected in series (Waters Inc.; Mississauga, ON). The column effluent was connected to a refractive index detector (Waters Inc., Mississauga, ON) which was previously calibrated using polystyrene standards (Polysciences Inc.; Warrington, PA). THF with 1% triethylamine was used as mobile phase at a flow rate of 1.0 mL/min at 40 °C. The data were analyzed using Empower software (Waters Inc.; Mississauga, ON). The critical micelle concentration (CMC) of the copolymer was determined using an established method with 1,6-diphenyl-1,3,5-hexatriene (DPH) as the fluorescent probe [35].

2.3. Preparation of ^{111}In-labelled block copolymers

DTPA/Bn-DTPA-conjugated PEG_x-*b*-PCL_y, or DOTA/Bn-DOTA-conjugated PEG_x-*b*-PCL_y (10–25 μg) were radiolabelled with ^{111}In-acetate ($^{111}InCl_3$/sodium acetate, 37–100 MBq) via incubation at pH 6.0 for 30 min. Purity of the complex was determined on Whatman ITLC-SG strips (Pall Life Sciences; St. Laurent, QC, Canada) developed in 100 mM sodium citrate pH 5.0. ^{111}In-labelled block copolymers remained at the origin ($R_f = 0$) while free ^{111}In-DTPA/^{111}In-acetate migrated with the solvent front ($R_f = 1$). A radiochemical purity of 96–98% was achieved.

2.4. Preparation of ^{111}In-labelled block copolymer micelles

$MePEG_x$-*b*-PCL_y micelles (BCMs) were prepared by the "dry down" method as previously described [11]. Briefly, 60 nm or 15 nm BCMs were prepared by dissolving 50 mg of $MePEG_{5k}$-*b*-PCL_{5k} or $MePEG_{2.5\text{-}k}$-*b*-$PCL_{1.2k}$, respectively, in 1 mL DMF and stirring for 4 h, after which the solution was dried under an atmosphere of N_2 and reduced pressure in a vacuum overnight. The resulting dry film was then hydrated with 1 mL of filtered Milli-Q water at 60 °C with vortexing. The hydrated micelle solution was then stirred at room temperature for 48 h followed by 1 h sonication at room temperature. The hydrodynamic diameters of BCMs were determined by dynamic light scattering (DLS) at an angle of 90° at room temperature (90Plus Particle Size Analyzer; Brookhaven Instruments Corp.; Holtsville, NY). Sample solutions of 1–20 mg/mL BCMs were prepared from the stock and