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Cationic triblock copolymer micelles enhance antioxidant activity, intracellular uptake and cytotoxicity of curcumin

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ABSTRACT

The aim of the present study was to develop curcumin loaded cationic polymeric micelles and to evaluate their loading, preservation of curcumin antioxidant activity and intracellular uptake ability. The micelles were prepared from a triblock copolymer consisting of poly(ε-caprolactone) and very short poly(2-(dimethylamino) ethyl methacrylate) segments ($PDMAEMA_9$–PCL_{70}–$PDMAEMA_9$). The micelles showed monomodal size distribution, mean diameter of 145 nm, positive charge (+72 mV), critical micellar concentration around 0.05 g/l and encapsulation efficiency of 87%. The ability of the micellar curcumin to scavenge the ABTS radical and hypochlorite ions was higher than that of the free curcumin. Confocal microscopy revealed that the uptake of curcumin by chronic myeloid leukemia derived K-562 cells and human multiple myeloma cells U-266 was more intensive when curcumin was loaded into the micelles. These results correlated with the higher cytotoxicity of the micellar curcumin compared to free curcumin. Intraperitoneal treatment of Wistar rats indicated that PDMAEMA–PCL–PDMAEMA copolymer, comprising very short cationic chains, did not change the levels of malondialdehyde and glutathione in livers indicating an absence of oxidative stress. Thus, PDMAEMA–PCL–PDMAEMA triblock micelles could be considered efficient and safe platform for curcumin delivery.

1. Introduction

Polymer-based nanocarriers show enormous potential, especially for cancer treatment, which relies on their remarkable properties including small size, excellent biocompatibility and biodegradability, prolonged circulation time in the bloodstream, enhanced drug loading capacity, and easy chemical modification or surface functionalization (Gao et al., 2014). In particular, amphiphilic block copolymers can form micelles with core–shell structures in aqueous solution, where the hydrophobic blocks form an inner core surrounded by an outer shell made of hydrophilic blocks (Riess, 2003). Thus, the block copolymer micelles have the ability to solubilize hydrophobic therapeutic drugs and imaging agents by encapsulation within their cores (Nishiyama and Kataoka, 2006; Jia et al., 2013; Lu and Park, 2013). Block copolymers suitable for such applications are composed of biocompatible and biodegradable blocks. The most popular hydrophilic block used in constructing polymer micelles is poly (ethylene glycol) (PEG), which provides the micelles with good biocompatibility and reduced toxicity for cells (Kabanov et al., 2002). As alternates to PEG, poly(*N*-vinyl-2-pyrrolidone) and poly (vinyl alcohol) have also been used to improve biocompatibility or enhance transcutaneous permeation (Jia et al., 2013). The micellar core could be based on hydrophobic blocks such as ε-caprolactone, D,L-lactic acid, butyl acrylate etc. (Colombani et al., 2007; Yang et al., 2007; Mespouille et al., 2008; Zhan et al., 2010; Petrov et al., 2013). Concerning biomedical applications, poly(ε-caprolactone) (PCL), is one of the most attractive and promising polymers owing to its good compatibility, degradability, low immunogenicity and FDA approval (Albertsson and Varma, 2003; Shi et al., 2005; Qiu

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and Bae, 2007; Danhier et al., 2009). For drug release applications, the advantages of PCL include its favorable permeability to drugs and less acidic degradation products as compared to polylactide and polyglycolide (Sinha et al., 2004).

Despite of the potential cytotoxicity, water-soluble polycations have been described for various biomedical applications (Zhang et al., 2008). Among numerous examples, poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA) is widely studied for preparation of gene and drug delivery systems (Verbaan et al., 2005; Karanikolopoulos et al., 2010). PDMAEMA is a biocompatible pH and temperature responsive polymer having a pK_a value around 7.4–7.5, depending on the molecular weight (van de Wetering et al., 1998). At room temperature, PDMAEMA is water-soluble over a wide pH range. It can be absorbed by endocytosis, and can be used as a nonviral DNA vector (van Steenis et al., 2003). The great efficiency of PDMAEMA compared with other methacrylate polymers is due to its ability to destabilize endosomes owing to its tertiary amines acting as a proton sponge and the easy dissociation of the polyplex once present in the nucleus (Arigita et al., 1999). Zhu et al. (2010) have studied different cationic micelles from PDMAEMA–PCL–PDMAEMA triblock copolymers for the co-delivery of siRNA and paclitaxel into cancer cells. They demonstrated that such cationic micelles mediate efficient *in vitro* siRNA transfection and, at the same time, are capable of efficiently delivering paclitaxel into cancer cells, resulting in an enhanced drug efficacy as compared to free paclitaxel. Importantly, it is pointed out that the carriers from copolymers comprising relatively low molar mass of PDMAEMA blocks are easy to prepare with controlled molecular characteristics and have low cytotoxicity.

Recently, variety of well-defined amphiphilic copolymers consisting of poly(ε-caprolactone) and poly(2-(dimethylamino) ethyl methacrylate) segments arranged in diblock (Mespouille et al., 2007, 2008), triblock (Motala-Timol and Jhurry, 2007; San Miguel et al., 2008; Zhu et al., 2010), graft (Han et al., 2014), and star-shaped (Zhou et al., 2010) architectures have been synthesized and their potential as drug carriers has been studied. Although, the results concerning the drug loading/release of paclitaxel (Zhu et al., 2010), chlorambucil (San Miguel et al., 2008) and aspirin (Zhou et al., 2010) from the different systems are intriguing, it seems that the preparation of micellar carrier based on linear block copolymers is easier and more preferred.

In the present work we report on the evaluation of cationic micelles based on well defined PDMAEMA–PCL–PDMAEMA triblock copolymer for encapsulation of the hydrophobic drug curcumin and its cellular uptake. Curcumin is a highly hydrophobic molecule possessing antioxidant, anti-inflammatory, anticancer effects etc. (Kuo et al., 1996). However, the low water solubility and hydrolytic instability influenced its oral absorption. The present study aims to develop micellar curcumin formulation able to preserve antioxidant activity of curcumin and to improve its intracellular transport. The efficient curcumin loading is considered taking in account the hydrophobicity of PCL core. On the other hand, the positive charge of PDMAEMA micellar corona could influence the interaction between the micelles and cell membranes. As far as we know this is the first report on the cellular uptake of curumin encapsulated in PDMAEMA–PCL–PDMAEMA triblock copolymer micelles. Finally, the safety of the cationic micelles was studied by evaluation of glutathione (GSH) and malondialdehyde (MDA) as markers of antioxidant defense system and lipid peroxidation in liver of rats.

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) diol (HO—PCL_{70}—OH, CAPA™ 2803, molar mass 8000 g/mol, kindly donated by Solvay Chemicals) was precipitated in cold methanol (−40 °C), filtered and dried under vacuum at 40 °C overnight. DMAEMA (Aldrich) was stirred overnight over calcium hydride (Merck) and distilled under reduced pressure. 2-Bromoisobutyryl bromide (Aldrich), triethylamine (Fluka), bipyridine (BiPy; Aldrich), CuCl (Aldrich), methanol (Merck), tetrahydrofuran (THF; Merck), 1,4-dioxane (Merck), and SiO_2 (63–200 μm) were used as received. Dichloromethane (DCM; Aldrich) was stirred overnight over calcium hydride and distilled. K_2HPO_4, KH_2PO_4, KCl, 2,2′-dinitro-5,5′-dithiodibenzoic acid (DTNB), Thiobarbituric acid were supplied by Merck. Curcumin was purchased from Sigma–Aldrich (Germany). Chronic myeloid leukemia derived cells (K-562) and human multiple myeloma cell line (U266) were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ GmbH, Braunschweig, Germany).

2.2. Synthesis of the macroinitiator

HO—PCL_{70}—OH (7 g, 0.875 mmol) was reacted with 2-bromoisobutyryl bromide (0.32 ml, 2.625 mmol) in toluene (80 ml) in the presence of TEA (0.365 ml, 2.625 mmol) for 24 h at 20 °C. The reaction mixture was then filtered to remove the insoluble hydrobromide salt and then toluene was evaporated. The product was dissolved in 30 ml THF; the macroinitiator was precipitated in 300 ml cold methanol (−40 °C) and recovered by filtration. Finally, Br—PCL_{70}—Br was dissolved in 30 ml 1,4-dioxane and freeze-dried.

2.3. Synthesis of the triblock copolymer

Br—PCL_{70}—Br (1 g, 0.1227 mmol), BiPy (0.048 g, 0.307 mmol) and CuCl (0.0243 g, 0.245 mmol) were placed in a round bottom flask, degassed by three times repeated vacuum/argon cycles, dissolved in methanol (2 ml) and purged with dry argon under stirring for 60 min. Then, 0.83 ml freshly distilled and degassed DMAEMA (4.91 mmol) was added, followed by polymerization at 60 °C for 7 h. Purification was achieved by precipitation of the reaction mixture in cold methanol (−40 °C) and filtration. The copolymer was re-dissolved in DCM and passed through a silica column to remove the Cu(II) catalyst. Finally, DCM was evaporated under vacuum; the copolymer was dissolved in 1,4-dioxane and freeze dried.

2.4. Block copolymer characterization

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy was performed using a 250 MHz Bruker AC-spectrometer in $CDCl_3$.

Size exclusion chromatography (SEC) analysis were performed with four PL-Gel columns (length 30 cm, diameter 0.8 cm, 5 μm, pore sizes 100, 10^3, 10^4, 10^5 Å) with refractive index detector using THF with 0.25 wt.% tetrabutylammonium bromide as eluent at a flow rate of 0.5 ml/min at room temperature. The molar mass and polydispersity index (PDI) were determined using narrow polystyrene standards

2.5. Preparation of drug loaded micelles

Curcumin loaded PDMAEMA–PCL–PDMAEMA micelles were prepared by dialysis method. Curcumin (0.2 mg/ml) and the copolymer (2 mg/ml) were dissolved in 5 ml of dioxane and the solution was stirred (700 rpm) for 30 min. After that, approximately 2 ml purified water was added dropwise to the solution. The resulted micellar dispersion was transferred into dialysis membrane (MWCO 6000–8000 g/mol, Spectrum Labs) and dialyzed against water during 4 h with a refreshment of the outer water every 1 h.