Journal of Drug Delivery Science and Technology 35 (2016) 24–29

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Preparation and characterization of polymeric micelles loaded with a potential anticancer prodrug

Lisa Marinelli [a], Ivana Cacciatore [a], Erika Fornasari [a], Carla Gasbarri [a], Guido Angelini [a], Agostino Marrazzo [b], Assunta Pandolfi [c], Domitilla Mandatori [c], Yang Shi [d], Cornelus F. van Nostrum [d], Wim E. Hennink [d], Antonio Di Stefano [a, *]

[a] Department of Pharmacy, University G. d'Annunzio, Via dei Vestini, 66100, Chieti, Italy
[b] Department of Drug Science, University of Catania, Viale Andrea Doria 6, 95125, Catania, Italy
[c] Department of Experimental and Clinical Sciences, School of Medicine and Health Sciences, University Chieti-Pescara, Chieti, Italy
[d] Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Universiteitsweg 99, P.O. Box 80082, 3508 TB, Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 6 May 2016
Received in revised form
13 June 2016
Accepted 15 June 2016
Available online 17 June 2016

Keywords:
Amphiphilic block copolymers
Drug delivery system
HPMA
R-(+)-MRJF4
Polymeric micelles

ABSTRACT

Polymeric micelles based on HPMA [*N*-(2-hydroxypropyl) methacrylamide] polymers were recently evaluated as drug delivery systems of several anticancer drugs. The development of polymeric micelles to solubilize R-(+)-MRJF4, a potential anticancer prodrug, is reported in this paper. Two different amphiphilic block copolymers based on PEG-HPMA [(ω-methoxypoly (ethylene glycol)-*b*-(*N*-(2-benzoyloxypropyl) methacrylamide)-*co*-(*N*-(2-lactoyloxypropyl) methacrylamide) (PEG-HPMA-Bz-L) and (ω-methoxy poly (ethylene glycol)-*b*-(*N*-(2-benzoyloxypropyl) methacrylamide (PEG-HPMA-Bz)] were synthesized and investigated for this purpose. Results showed that both polymers were able to efficiently solubilize the drug at concentrations of 2 and 4 mg/mL and polymer concentration of 9 mg/mL yielding polymeric micelles with a size of 53–83 nm. Release studies showed that the formulation obtained using PEG-HPMA-Bz-L slowly released R-(+)-MRJF4 for 7–8 days. Moreover, cytotoxicity studies performed on C6 glioma cells revealed that, after 48 h, R-(+)-MRJF4-loaded PEG-HPMA-Bz and PEG-HPMA-Bz-L micelles possessed a higher antiproliferative activity when compared to free R-(+)-MRJF4, implying that the formulations could be internalized by the cells. Taken together, our results suggest that PEG-HPMA-Bz-L polymeric micelles are interesting to optimize the therapeutic efficacy of R-(+)-MRJF4.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Amphiphilic block copolymers have been extensively studied for biomedical applications due to their capability to associate and organize, in aqueous media, in core-shell structures above a certain concentration [1–4]. These nanometer systems have favorable features, such as prolonged circulation time, increased drug availability, and controlled drug release resulting in improved therapeutic effectiveness [5–10]. Such systems are characterized by a peculiar structure composed of a hydrophobic core surrounded by a hydrophilic shell.

The micellar core, due to its chemical nature, has a good capability to solubilize significant amounts of poorly water-soluble chemotherapeutics [11–14], ameliorate their unfavorable pharmacokinetics, as well as their stability.

The hydrophilic corona, most frequently composed of PEG, is responsible for their colloidal stability and protection against protein absorption and opsonization in the circulation, thus resulting in a prolonged circulation time [15–17].

After systemic administration, polymeric micelles, typically ranging between 10 and 100 nm, can accumulate in tumor and diseased tissues through the EPR effect [18,19] avoiding fast removal by macrophages particularly present in liver and spleen. Block copolymers with a molecular weight below 50000 g/mol can be excreted via renal elimination [20], thus they are preferred for the design of polymeric micelles for drug delivery purposes.

Recently, we synthesized R-(+)-MRJF4, a novel haloperidol metabolite II (HP-mII) prodrug (a sigma-1 antagonist and sigma-2 agonist), obtained through conjugation with 4-phenylbutyric acid

* Corresponding author.
E-mail address: antonio.distefano@unich.it (A. Di Stefano).

http://dx.doi.org/10.1016/j.jddst.2016.06.006
1773-2247/© 2016 Elsevier B.V. All rights reserved.

(PhBA) [a histone deacetylase inhibitor (HDACi)] endowed with anticancer activities (Fig. 1) [21,22]. However, its poor water solubility (1.2 μg/mL) is a main restriction for administration.

To overcome this problem, in the present study we encapsulated R-(+)-MRJF4 into polymeric micelles based on HPMA polymers [23]. pHPMA is a synthetic, water-soluble, biocompatible, non-immunogenic, and highly multifunctional polymer that has been evaluated in clinical trials as doxorubicin-conjugated polymeric prodrug [24–28]. Notably, two chemically modified HPMA polymers, PEG-HPMA-Bz (ω-methoxy poly (ethylene glycol)-*b*-(*N*-(2-benzoyloxypropyl) methacrylamide and PEG-HPMA-Bz-L (ω-methoxypoly (ethylene glycol)-*b*-(*N*-(2-benzoyloxy-propyl) methacrylamide)-*co*-(*N*-(2-lactoyloxypropyl) methacrylamide) were recently investigated to improve the solubility of curcumin [14,26]. The first polymer is not water soluble since it contains only benzoyl groups as side chains that confer it a high grade of hydrophobicity; the second one contains hydrolytically sensitive moieties, such as lactic acid esters side groups, that improve the hydrophilicity of the polymer and thus its water solubility [29]. Moreover, the two polymers have another feature: PEG-HPMA-Bz is not thermosensitive while PEG-HPMA-Bz-L is a thermosensitive polymer that forms micelles above its cloud point [29].

In this study we used the two above mentioned polymers to solubilize the hydrophobic prodrug R-(+)-MRJF4 and improve its potential anticancer activity against C6 glioma cells (the structures of these polymers are shown in Scheme 1). The prodrug-loaded polymeric micelles were subjected to physico-chemical characterization (size, encapsulation efficiency, loading capacity), *in vitro* release studies, and *in vitro* cytotoxic assays against C6 glioma cells to assess their potential as formulations of an anticancer drug.

2. Materials and method

Acetonitrile (ACN), tetrahydrofuran (THF), dichloromethane (DCM), dimethylformamide (DMF), and molecular sieves (0.4 nm) were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). The syntheses of R-(+)-MRJF4 and the polymers PEG-HPMA-Bz and PEG-HPMA-Bz-L were performed as previously reported [22,26,29].

The identity of the synthesized compounds was confirmed by ^{1}H NMR spectroscopy and their purities were evaluated by analytical HPLC. ^{1}H NMR spectra were recorded on a Gemini 300 MHz spectrometer (Varian Associates Inc. NMR Instruments, Palo Alto, CA). The weight average molecular weight (M_w), the number average molecular weight (M_n), and the Polydispersity Index (PDI) of the synthesized polymers were determined by gel permeation chromatography (GPC)[29]HPLC equipment was a Waters 600 HPLC pump (Waters Corporation, Milford, MA, USA), provided with a Waters 2996 photodiode array detector, a 20 μL Rheodyne injector loop and a computer-integrating apparatus.

Size and PDI of the polymeric micelles were analyzed by dynamic light scattering (DLS). DLS analysis was carried out using a Malvern 4700 system (Malvern Ltd., Malvern, U.K.) consisting of an Autosizer 4700 spectrometer, a pump/filter unit, a model 2013 air-cooler argon ion laser (75 mW, 488 nm, equipped with a 2500 remote interface controller, Uniphase) and a water bath, and a computer with DLS software (PCS, version 3.15, Malvern), operating at 25 °C at a fixed angle of 90°.

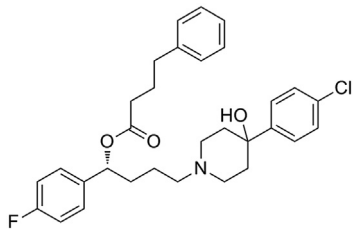


Fig. 1. Structure of R-(+)-MRJF4.

3. Experimental

3.1. Synthesis and characterization of block copolymers

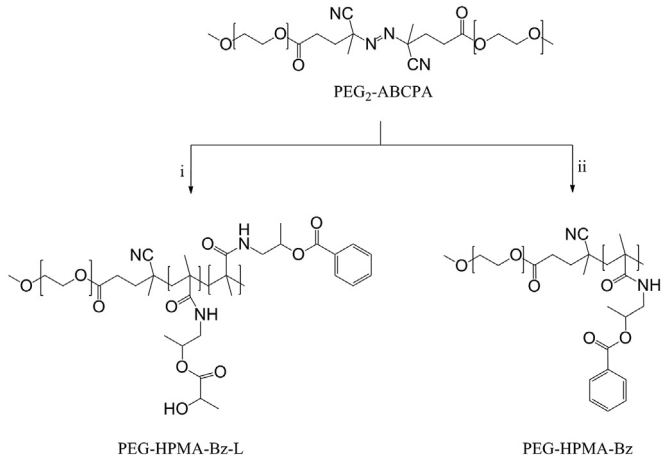
Block copolymers were prepared following free radical polymerization using different monomers (HPMAm-L, HPMAm-Bz $mPEG_2$-ABCPA (M_n of mPEG = 5000 g mol^{-1}) as macroinitiator [30]. The monomer/initiator ratio was 150:1 mol/mol. The monomers and the macroinitiator, at a concentration of 300 mg/mL, were dissolved in ACN. The resulting solution was flushed with N_2 for at least 20 min, heated at 70 °C and stirred for 24 h. Next, the polymers were precipitated by dropwise addition of the mixture to an excess of diethyl ether. After centrifugation, different procedures were applied to obtain the final polymers: the thermosensitive block copolymer (PEG-HPMA-Bz-L) was dissolved in water and dialyzed for two days at 4 °C, using membranes with a cut-off of 12–14 kDa while the non-thermosensitive copolymer (PEG-HPMA-Bz) was recovered without purification.

The polymers were fully characterized by ^{1}H NMR spectroscopy and GPC measurements. The NMR signals were assigned as previously reported [29].

GPC was performed using samples at the concentration of 5 mg/mL using a PL gel 5 μm polystyrene packed MIXED-D (Polymer Laboratories) column characterized by 5 μm particle size, 300 mm × 8 mm i.d. using DMF containing 10 mM of LiCl as eluent with a flow of 0.7 mL/min and the temperature was set at 40 °C [30]. PEG of defined molecular weights, ranging from 238 to 1015000 Da, were used for calibration.

3.2. Critical micelle concentration (CMC)

The CMC of the block copolymers was determined using pyrene as a fluorescent probe. Fluorescence excitation spectra of pyrene were obtained as a function of the polymer concentration using Jasco FP-6500 spectrofluorimeter. The excitation spectra were recorded at 37 °C from 300 to 360 nm with the emission



Scheme 1. Synthesis of PEG-HPMA-Bz-L and PEG-HPMA-Bz: (i) HPMAm-Bz/HPMAm-Lac_1 25/75; (ii) HPMAm-Bz by free radical polymerization (ACN, 70 °C) using PEG_2-ABCPA as macroinitiator.

Download English Version:

https://daneshyari.com/en/article/2482992

Download Persian Version:

https://daneshyari.com/article/2482992

Daneshyari.com