Journal of Controlled Release 136 (2009) 187–195

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Encapsulation of the synthetic retinoids Am80 and LE540 into polymeric micelles and the retinoids' release control

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ARTICLE INFO

Article history:
Received 25 December 2008
Accepted 27 February 2009
Available online 14 March 2009

Keywords:
Polymeric micelle
Retinoid
Ion-pairing
Sustained release
Controlled release

ABSTRACT

The objective of this study was to encapsulate two synthetic retinoids Am80 and LE540 into polymeric micelles and to control the retinoids' release rate in vitro. Highly efficient encapsulation yields of these retinoids were obtained for micelles forming from PEG-poly(benzyl aspartate) block copolymers in the wide range of the benzyl substitution degree. The in vitro release examination for LE540 indicated very stable encapsulation of this retinoid owing to its strongly hydrophobic nature. On the other hand, Am80 exhibited a rapid release in Dulbecco's phosphate buffer saline. An addition of a hydrophobic alkyl amine in the Am80-encapsulation process successfully led to significant retardation of the Am80 release rate. A mechanism of the retardation was considered an increase of Am80 hydrophobicity due to an ion-pairing with the alkyl amine. This paper is the first report on release control in the polymeric micelle carrier system through the ion-pairing between an encapsulated drug and an additive.

1. Introduction

Polymeric micelles are self-assembling nanostructures that are typically composed of amphiphilic block copolymers [1]. Micelles have recently received much attention as a promising drug delivery carrier because a large quantity of hydrophobic drugs can be encapsulated into the micelle core in a stable manner. In systemic administration the drug-encapsulating micelles are expected to demonstrate various advantages; e.g., long-circulation in the blood stream owing to the nano-size and the hydrated-surface property of the micelles, and selective accumulation at tumor tissues owing to the EPR effect [2].

For drug targeting with the polymeric micelle carriers, research and development have focused on hydrophobic and cytotoxic anti-cancer drugs such as doxorubicin [3], paclitaxel [4], and camptothecin and its analogues [5,6]. These drugs cause cell mortality by means of strong cellular dysfunction. We would like to explore an application of polymeric micelle delivery using another type of drug that expresses pharmacological activities by regulating cellular functions. We have selected retinoids for this purpose [7,8].

Retinoids were originally defined as vitamin A and its analogues [9]. Compounds in this family modulate specific nuclear receptors called retinoic acid and retinoid X receptors (RARs and RXRs). Each of these receptors includes three subtypes (α, β, and γ). By binding to these receptors retinoids regulate cellular events including differentiation, proliferation, and apoptosis [10,11]. All-trans retinoic acid (ATRA) is clinically approved against acute promyelocytic leukemia (APL). This is the first approval of the differentiation therapy against cancer, indicating retinoids' high potency in clinical applications [12]. In an updated definition, retinoids are expanded to include molecules that bind to RARs and RXRs [13,14], regardless of their similarity in molecular structure to vitamin A. Researchers have reported that synthetic retinoids, such as 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437) [15] and *N*-(4-hydroxyphenyl) retinamide (4-HPR) [16], have induced apoptosis in neoplasma. Although researchers have not yet fully elucidated the mechanism underlying the related actions of these synthetic retinoids, CD437- and 4-HPR-induced apoptosis includes an RAR-independent pathway. Many other synthetic retinoids have been designed to improve pharmacological effects and to decrease adverse effects [17,18].

For the current research project, we selected two synthetic retinoids, Am80 and LE540, as encapsulated drugs (Chart 1). One reason for our decision to select these retinoids is their attractive pharmacological activities. Am80, an RAR-α/β specific agonist, was approved in Japan in 2005 for relapsed or refractory APL [19]. Its antimyeloma [20] and atherosclerosis inhibition effects in vivo have also been reported [21]. Since this synthetic retinoid has little binding affinity to cellular retinoic acid-binding proteins (CRABP),

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doi:10.1016/j.jconrel.2009.02.024

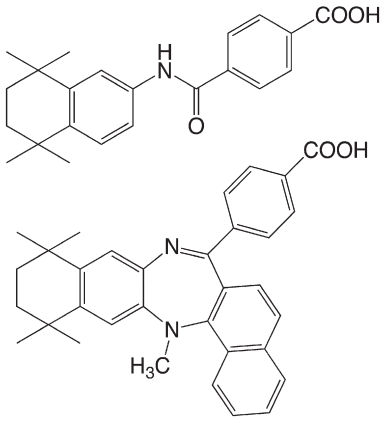

Chart 1. Am80 and LE540 (top and bottom).

CRABP-dependent retinoid-resistance will be avoided. Moreover, Am80 is much more stable than ATRA in the contexts of light, heat, and oxidation. In comparison, LE540 is an RAR antagonist, and its inhibition activity for Am80-induced HL-60 cell differentiation has been reported [22]. Targeting and the controlled release of these retinoids through micelle encapsulation will expand their therapeutic applications, especially when the target is solid tumor tissue for Am80.

Chemical-structure characteristics of Am80 and LE540 constitute the other reason for the selection. We use the hydrophobic frames of these retinoids to encapsulate the compounds into micelles. As compared with Am80, LE540 has bulkier hydrophobic moiety. Therefore, these two retinoids not only possess high therapeutic potential, but also benefit analysis on relations between the chemical structures of drugs and the corresponding encapsulation-release behaviors of the retinoids.

In this study, we perform encapsulation of Am80 and LE540 into micelles forming from PEG-poly(benzyl aspartate) block copolymers and analyze the retinoids' encapsulation behaviors and release rates. Am80 and LE540 are sufficiently hydrophobic in water for easy and efficient encapsulation into a polymeric micelle. However, Am80 is soluble in Dulbecco's phosphate buffered saline (D-PBS) and is rapidly released from a polymeric micelle. Therefore we have attempted to control the release rates by means of an addition of hydrophobic compounds interacting with Am80. This is a novel methodology for the control of drug-release rates from polymeric micelle carriers. Even though addition of oppositely charged hydrophobic compounds have been frequently used in traditional drug carriers such as microparticles, the first time application of this method for the polymeric micelle carriers is very important. The reason for the importance is that extremely stable drug encapsulation (e.g., 10^{-19} cm^2/s diffusion coefficient [23]) is required due to a very small micellar inner core such as 10 nm in diameter as a drug container.

2. Materials and methods

2.1. Materials and equipment

For the present study, α-methoxy-ω-amino poly(ethylene glycol) (MeO-PEG-NH_2) (Mw 5-kDa) was purchased from NOF Corp. (Tokyo, Japan). And β-benzyl L-aspartate was purchased from Kokusan Chemical (Tokyo, Japan). Triphosgene was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and used as received. β-Benzyl L-aspartate *N*-carboxyanhydride (BLA-NCA) was prepared from β-benzyl L-aspartate and triphosgene according to the conventional method [24]. DMF was distilled at a reduced pressure before use. All other reagents used were of reagent grade. ^{1}H-NMR measurements were carried out with a Varian Unity Inova NMR spectrometer (Varian Technologies Japan Ltd., Tokyo, Japan) at 400 MHz. A Spectra/Por 6 dialysis membrane (Spectrum Laboratories Inc., CA, USA) (1-kDa cut-off) was used for dialysis. An HPLC analysis was carried out with an HPLC system (PU-2080 plus pump; MX-2080-32 dynamic mixer; UV-2070 plus UV detector; and RI-2031 plus RI detector, Jasco Corp., Tokyo, Japan) equipped with a TSK-gel ODS-80Ts reverse-phased column (150×4.6 mm i.d., Tosoh Corp., Tokyo, Japan). Dynamic light scattering (DLS) measurements were performed in 1.0% (w/w) aqueous solutions at 25 °C with a DLS-7000 (Otsuka Electronic Co. Ltd., Osaka, Japan). Particle size distribution in terms of weight fractions was calculated using a non-negative least squares (NNLS) algorithm.

2.2. Synthesis of amphiphilic diblock copolymers

Polymers used for encapsulation of retinoids are composed of a PEG-poly(aspartic acid) (PEG-P(Asp)) main chain and pendant benzyl groups (Table 1). PEG-poly(benzyl L-aspartate) (PEG-PBLA) polymers **1** and **2** were prepared by ring-opening polymerization of BLA-NCA from a primary amino terminal of MeO-PEG-NH_2 [25]. The PEG-P(Asp(Bzl)$_x$) polymers **3**–**5** were obtained as follows: (1) complete removal of benzyl groups from PEG-PBLA [26], and (2) partial esterification of PEG-P(Asp) with benzyl bromide (BzlBr) [27].

2.2.1. Synthesis of PEG-b-poly(β-benzyl L-aspartate) (PEG-PBLA)

PEG-b-poly(β-benzyl L-aspartate) (PEG-PBLA) block copolymers were synthesized according to literature [25]. Amino-terminated poly (ethylene glycol) MeO-PEG-NH_2 as a macroinitiator was mixed with BLA-NCA in a dichloromethane–DMF mixed solvent (9:1 v/v), and this mixture was stirred at 35 °C for 17 h under a nitrogen atmosphere. The reaction mixture was dropwisely added into diethyl ether cooled in ice. The resulting precipitate was collected by filtration, washed with diethyl ether, and dried under a reduced pressure. The degree of polymerization (DP, i.e., the average unit number of polymer chain) of the PBLA block was determined by ^{1}H-NMR spectroscopy in chloroform-*d*. The determination was based on an integration ratio between the proton assigned to the methylene of PEG (3.8–3.4 ppm) and the benzyl methylene of PBLA (5.2–4.9 ppm). The polymers **1** and **2** were estimated to be 24 and 28, respectively.

Table 1
Synthesis of PEG-P(Asp (Bzl)$_x$) block copolymers.

Polymer	Source						Product	
	PEG-P(Asp)[a]	BzlBr		DBU		BnBr/DBU	Yield	D.s. Bzl[b]
	g (Asp mmol)	g (mmol)	mol.eq./Asp	g (mmol)	mol.eq./Asp		(mg)	(%)
3	0.501 (1.51)	0.261 (1.52)	1.01	0.200 (1.31)	0.87	1.16	0.597	80
4	0.501 (1.51)	0.172 (1.00)	0.66	0.141 (0.93)	0.61	1.08	0.531	53
5	0.501 (1.51)	0.115 (0.67)	0.44	0.093 (0.61)	0.40	1.10	0.516	33

[a] PEG-P(Asp) 5-24 was prepared by hydrolysis of PEG-PBLA 2.
[b] Degree of benzyl substitution.