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Carborane confined nanoparticles for boron neutron capture therapy: Improved stability, blood circulation time and tumor accumulation

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ABSTRACT

Carborane confined nanoparticles based on the core cross-linked and boron-containing micelles (CL micelles) were prepared using the radical polymerization of poly(ethylene glycol)-*block*-poly(lactide) copolymer (PEG-*b*-PLA), which contained an acetal group at the PEG end and a methacryloyl group at the PLA end (acetal-PEG-*b*-PLA-MA), with polymerizable carborane (VB-carborane) as a cross-linker. No leakage of VB-carborane from the CL micelles was observed in PBS even in the presence of 10% fetal bovine serum (FBS) at 37 °C, whereas significant leakage (80%) of VB-carborane was observed for the non-cross-linked (NCL) micelles under the same conditions. To clarify the biodistribution of both types of micelles, ^{125}I (RI: radioisotope)-labeled CL and NCL micelles were injected into tumor-bearing BALB/c mice via the tail vein. The ^{125}I-labeled CL micelles showed a remarkably prolonged blood circulation time (7.9%ID/g) and high tumor accumulation (2.9%ID/g) compared with the ^{125}I-labeled NCL micelles (blood: 3.1%ID/g, tumor: 1.8%ID/g) at 24 h after injection. Moreover, the biodistribution of the VB-carborane (boron species) was also evaluated using ICP-MS at 24 h after intravenous injection of the CL and NCL micelles. The boron concentrations in blood and tumor after injection of the CL micelles (blood: 13.5%ID/g, tumor: 5.4%ID/g) were also significantly higher than the concentrations after the injection of the NCL micelles (blood: 1.8%ID/g, tumor: 1.4%ID/g). Note that the biodistribution of the boron species in the CL micelles was similar to that of the ^{125}I-labeled CL micelles, whereas the boron concentrations (%ID/g) in blood and tumor after injection of the NCL micelles were lower than those expected with the ^{125}I concentrations (%ID/g) in blood and tumor. Thus, the boron concentration ratios of the CL micelles to the NCL micelles (CL_{ICP}/NCL_{ICP}) in blood (15.8) and tumor (3.8) were much higher than the ^{125}I concentration ratios of CL_{RI}/NCL_{RI} in blood (2.5) and tumor (1.6). These differences might be caused by the suppression of the leakage of the VB-carborane from the CL micelles in the blood stream due to the existence of the cross-linking bonds between the VB-carborane and the PLA core. Based on these results, the CL micelles composed of PEG-*b*-PLA copolymer cross-linked by carborane represent a promising approach to the creation of a novel boron carrier for boron neutron capture therapy (BNCT).

1. Introduction

Boron neutron capture therapy (BNCT) has attracted much attention as a selective and noninvasive cancer therapy using boron-10 (^{10}B) compounds, which efficiently generate cytotoxic α-particles and ^{7}Li nuclei within the cell diameter (5–9 μm) through the nuclear reaction of the ^{10}B atom with low-energy thermal neutrons [1,2]. Two types of the ^{10}B-compounds, sodium borocaptate (BSH) and L-4-dihydroxyboronylphenylalanine (BPA), have been approved for clinical trials so far. Due to the rapid clearance of these compounds from the blood stream (blood circulation time of BSH: $t_{1/2} < 1$ h) [3] a high dose of the ^{10}B-compound is generally required to allow a sufficient concentration of the ^{10}B-compound to accumulate in the tumor tissue (20–30 μg of ^{10}B atoms per gram of tumor tissue). Additionally, the non-specific distribution of these compounds also reduces the efficiency of their accumulation in the tumor tissue. To improve the effect of BNCT, the effective accumulation of boron compounds in the area of the tumor tissue is one of the key issues.

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A promising strategy in this regard is the concept of drug delivery systems (DDS). Matsumura and Maeda et al. have reported that a DDS based on nanomaterials, including proteins, drug-conjugated polymers and nano-sized particles, enabled the accumulation of drug in the tumor tissue due to the so-called "enhanced permeability and retention" (EPR) effect [4,5]. Indeed, PEG-modified liposomes have been reported to be effective nano-sized carriers of hydrophilic and negatively charged BSH, thus enhancing biocompatibility, blood circulation time and accumulation in the tumor tissue. Although the PEG-modified liposomes encapsulating the BSH showed higher accumulation in the tumor tissues compared to the free BSH due to the EPR effect [6–8], the therapeutic efficacy of the PEG-modified liposomes encapsulating the BSH is still controversial due to the leakage of the encapsulated BSH from the liposome into the blood stream caused by the high ion osmotic pressure of the BSH-loaded interior of the liposome [9]. An alternative approach is the ^{10}B-compound-conjugated liposomes fabricated by covalently linking a lipid (hydrophobic group) with a ^{10}B-compound (hydrophilic group) to suppress the leakage of the ^{10}B-compound into the blood stream [10–12]. The ^{10}B-compound-conjugated liposome constructed from *nido*-carborane afforded a sufficiently high ^{10}B concentration in the tumor tissue, without leakage of the ^{10}B compound into the blood stream. However, the synthesis of *nido*-carborane required complicated preparation steps. Additionally, serious acute toxicity was observed *in vivo* because of the cytotoxicity of *nido*-carborane [11]. Thus, the development of a ^{10}B-compound confined delivery system, which does not allow leakage of the ^{10}B-compound and is not toxic is a promising approach to cancer BNCT.

Recently, a new class of drug delivery system has emerged, based on nano-sized polymeric micelles composed of PEG-*block*-poly(D,L-lactide) (PLA) copolymers (PEG-*b*-PLA) because the PEG-*b*-PLA micelles showed high biocompatibility, nontoxicity, long blood circulation time and high biodegradability [13–16]. The hydrophobic PLA core is able to accommodate hydrophobic drugs, and the brush-like hydrophilic PEG shell prevents protein adsorption and subsequent non-specific uptake by the reticuloendothelial system (RES) after intravenous injection. However, Chen et al. reported that hydrophobic fluorescent compounds incorporated into the core of the PEG-*b*-PLA micelle showed a loss of micelle integrity within the blood stream due to the interaction between the micelles and some blood components, leading to the rapid leakage of the hydrophobic fluorescent compounds within 15 min after intravenous injection [17]. To solve this problem, many studies have focused on the stabilization of micelles, introducing chemical linkages into either the shell or the core segment to avoid the loss of micelle integrity [18–20]. We have also previously reported the preparation of core-polymerized PEG-*b*-PLA micelles possessing a methacryloyl group at the PLA chain end. Through the radical polymerization [21,22], the core-polymerized micelles eventually showed high dispersibility even after lyophilization as well as high stability against dilution, temperature changes and sodium dodecyl sulfate solution. Indeed, these above-mentioned approaches (polymerized or cross-linked micelles) are critical to the formulation of micelles that are resistant to the loss of micelle integrity. However, even though the polymerized or cross-linked micelles are employed, the leakage of the drug from the polymerized or cross-linked micelles is not completely suppressed due to the diffusion of the drug from the core to the blood stream when the drug is physically entrapped inside the core by hydrophobic interactions [23,24].

In this study, we designed and prepared a core cross-linked and boron-containing micelles (CL micelles) using the radical copolymerization of PEG-*b*-PLA, which contains an acetal group at the PEG end and a methacryloyl group at the PLA end (acetal-PEG-*b*-PLA-MA), with hydrophobic 1,2-bis(4-vinylbenzyl)-*closo*-carborane (model for ^{10}B-compound) as a cross-linker (Fig. 1). Note that the CL micelles suppress the leakage of carborane and the loss of micelle integrity in the blood stream, leading to a prolonged blood circulation time and enhanced accumulation in tumor tissues. The cross-linked core of the micelle is anticipated to gradually collapse through the biodegradation of the PLA segment after the therapy. Moreover, an acetal group at the tethered PEG chain end can be easily converted to an aldehyde group by hydrolysis with acid. The aldehyde group is known to easily react with hydrazide compounds, facilitating the installation of tumor-specific ligand molecules and radioactive tags for monitoring the biodistribution. We believe that the use of the CL micelles composed of acetal-PEG-*b*-PLA-MA and 1,2-bis(4-vinylbenzyl)-*closo*-carborane represents a promising strategy for the fabrication of drug delivery systems, which deploy a sufficient quantity of ^{10}B to tumor tissues.

2. Experimental section

2.1. Materials and methods

Azobisisobutyronitrile (AIBN; Wako Pure Chemical Industries, Ltd., Osaka, Japan) was purified by recrystallization from methanol and dried *in vacuo*. *N*,*N*-Dimethylacetamide (DMAc; Kanto Chemicals Co., Ltd., Tokyo, Japan), L-tyrosine hydrazide (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), sodium cyanoborohydride (Aldrich Chemical Co., Ltd., Milwaukee, WI, USA), *p*-toluenesulfonchloramide sodium salt (chloramine T; Kanto Chemicals Co., Ltd.) and sodium peroxodisulfate (Kanto Chemicals Co. Ltd.) were used as received. Water was purified using the Milli-Q system (Millipore). Dynamic light scattering measurements were conducted in phosphate-buffered saline (PBS) at 37 °C using a Zetasizer Nano-ZS instrument (Malvern, UK) equipped with a 4.0 mW He–Ne laser (633 nm). Zeta potential measurement of the micelles was performed at 37 °C in 5 mM phosphate buffer solution at pH 7.4 using a Zetasizer Nano ZS. ^{1}H NMR spectra were obtained in chloroform-*d* at 25 °C with a JEOL EX270 spectrometer (JEOL, Japan). Chemical shifts were reported in ppm relative to $CDCl_3$ (δ 7.26 ppm). Fluorescence spectra were recorded on an F-7000 spectrometer (Hitachi, Japan). The concentration of boron atoms was determined with ICP-AES using an ICAP-575 emission spectrometer (Nippon Jarrell-Ash, Japan) and ICP-MS using an ELAN DRC II (PerkinElmer,

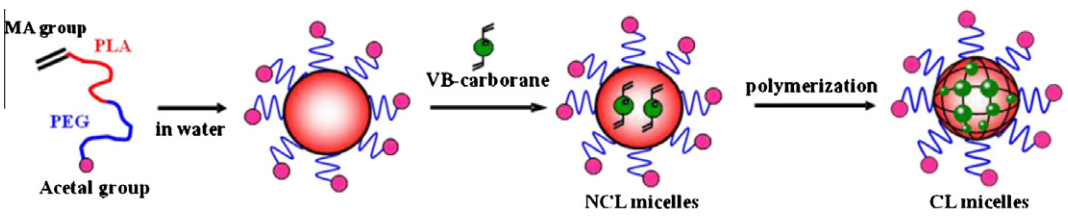

Fig. 1. Schematic illustration of the preparation of NCL and CL micelles composed of acetal-PEG-*b*-PLA-MA and polymerizable carborane (VB-carborane).