Biochemical and Biophysical Research Communications xxx (2017) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Intracellular delivery and passive tumor targeting of a self-assembled nanogel containing carborane clusters for boron neutron capture therapy

Riku Kawasaki [a, b], Yoshihiro Sasaki [a], Kazunari Akiyoshi [a, b, *]

[a] Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan
[b] JST-ERATO, Japan Science and Technology Agency (JST), The Exploratory Research for Advanced Technology (ERATO), Bio-nanotransporter Project, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan

ARTICLE INFO

Article history:
Received 20 December 2016
Accepted 26 December 2016
Available online xxx

Keywords:
Self-assembled nanogel
Carborane
Polysaccharides
Drug delivery system
Boron neutron capture therapy

ABSTRACT

Boron neutron capture therapy, based on the release of thermal neutron irradiation from boron, is a targeted radiation therapy for cancer. Targeted and sufficient accumulation of boron in tumor cells to achieve cytotoxic efficacy and reduce off-target effects remains a challenge. Carborane has been investigated for use as a delivery agent in boron neutron capture therapy because of its high boron content and chemical stability; however, it is cytotoxic, making safe delivery difficult. The aim of this study was to investigate the potential of carborane-bearing pullulan nanogels to safely and effectively deliver boron to tumor cells *in vitro* and *in vivo* and, consequently, assess their potential as a boron neutron capture therapeutic. Murine fibrosarcoma cells (CMS5a) were used for *in vitro* investigations of nanogel cytotoxicity, cell uptake. A mouse fibrosarcoma xenograft model was used to investigate the bio-distribution of nanogels after intravenous administration. The nanogels produced no apparent cytotoxicity and underwent cell uptake in CMS5a cells after a 24 h incubation at up to 2000 μg/mL and 400 μg/mL, respectively. The internalized nanogels were localized around the nuclear membrane. The nanogels were administered intravenously to mice bearing fibrosarcoma xenografts. Nanogel tumor localization likely occurred through the enhanced permeation and retention effect. The nanogels successfully reduced the cytotoxicity of carborane, were internalized into tumor cells, acted as a dual-delivery therapeutic and accumulated in tumors *in vivo*. Consequently, they demonstrate significant potential as a boron neutron capture therapeutic.

1. Introduction

With properties such as a high molecular boron content, and thermal and chemical stability, carborane has been investigated for use in boron neutron capture therapy (BNCT) [1,2]. BNCT is a cell- or tissue-selective radiation therapy for cancer [2]. The therapy is based on the creation of alpha particles and lithium nuclei, which are generated by the release of low-energy thermal neutron irradiation from boron *via* a boron neutron capture reaction. The energy emitted from the nuclei can radiate out up to 10 μm and can cause cell destruction [1]. The destructive range of this technique corresponds to the size of a single cell, suggesting that BNCT could be used to selectively kill specific cells at the irradiated site without injuring normal tissues, if boron could be selectively delivered to tumor cells. Consequently, BNCT has been applied for the treatment of malignant tumors such as melanoma [3] and glioblastoma [4], which require minimally invasive therapy. The success of BNCT is dependent on the effective delivery of a highly concentrated ^{10}B compound to the targeted site.

As a result, various boron carrier systems have been developed to increase the therapeutic value of BNCT [5]. For instance, low molecular weight boron derivatives such as L-boronophenylalanine (L-BPA) [6,7] or water soluble sodium boronocaptate (BSH) [8] have been used in BNCT as boron delivery agents. L-BPA undergoes active uptake in tumor cells *via* amino acid transporters because it is an essential amino acid [7], and hydrophilic BSH accumulates in tumor tissues because of its long retention time in the blood stream [8]. Sufficient accumulation of ^{10}B at the tumor is generally required to

* Corresponding author. Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan.
E-mail address: akiyoshi@bio.polym.kyoto-u.ac.jp (K. Akiyoshi).

http://dx.doi.org/10.1016/j.bbrc.2016.12.176
0006-291X/

Abbreviations

BNCT	boron neutron capture therapy
L-BPA	L-boronophenylalanine
BSH	sodium boronocaptate
PEG	polyethylene glycol
CAP	carborane bearing pullulan
EPR effect	enhanced permeability and retention effect
POA	acryloyl group bearing pullulan
RITC	rhodamine B isothiocyanate
Carborane-SH	1-mercapto carborane

achieve effective BNCT, meaning that high doses are necessary [2,9]. To address this issue, polymeric drugs such as BSH-bearing poly-L-lysine and BSH-bearing polyethylene glycol (PEG) have been developed [9]. These polymer-based boron delivery agents have been shown to enhance the accumulation of ^{10}B at the tumor *via* the enhanced permeation and retention (EPR) effect [10] and therapeutic efficacy is improved. Recently, self-assembled boron agents such as boronated liposomes [11] containing boronated cholesterol and lipids, boronated polymersome using carborane bearing PEG [12], and polymer micelles using BSH bearing PEG and cationic group bearing PEG [13] have been developed with the aim of achieving effective BNCT. The bottom-up method of self-assembly is a powerful technique to construct complicated nano-structures using relatively simple ingredients. Previously, we have designed and synthesized carborane-bearing pullulan (CAP) using carborane as a hydrophobic core to prepare physically cross-linked nanogels. The resulting amphiphilic polysaccharide formed stable self-assembled hybrid nanogels through hydrophobic interactions, and resulted in the formation of highly integrated boron clusters in the nanogel. Here, we evaluate the potential of the hybrid CAP nanogels, which utilize boron clusters as self-association units, to function as a novel boron carrier *in vitro* and *in vivo* (Fig. 1).

2. Materials and methods

2.1. Materials

Pullulan (Mw, 100,000) was purchased from Hayashibara (Okayama, Japan). Carborane, sulfur, di-methoxy ethylene, dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), ethanol, hexyl amine, Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) and diethylether were purchased from Wako (Japan). *n*-butyl lithium hexane solution, dibutyltin dilaurate, and rhodamine B isocyanate were purchased from Sigma Aldrich (St. Louis, MO, USA), and 2-acryloyl-oxyethyl isocyanate was purchased from Showa Denko (Tokyo, Japan). The cell counting kit-8 was purchased from Dojindo (Kumamoto, Japan). Acryloyl group bearing pullulan (POA) was synthesized using a procedure similar to one reported previously [14]. Rhodamine B labeled POA (substitution degree, 20 per 100 glucose units), was synthesized using isothiocyanate chemistry [14]. Briefly, POA (5 mg/mL), Dibutyltin dilaurate (DBTDL) (10 mM), and rhodamine B isothiocyanate (RITC, 300 μM) were dissolved in DMSO and stirred for 24 h at 45 °C in the dark condition. The resulting solution was precipitated twice into a mixture of diethylether and ethanol (8:2, v:v). The obtained solid material was resuspended in DMSO and dialyzed against deionized water using a Spectra/Por 7 dialysis membrane with a molecular weight cut-off of 3.5 kDa. After dialysis and filtration using a polyvinylidene difluoride (PVDF) membrane, the solution was lyophilized to obtain rhodamine B labeled POA (POARh). The degree of substitution of rhodamine B was determined to be 0.2 per 100 glucose units by measuring the absorbance at 580 nm. Rhodamine B labeled CAP was synthesized using thiol-ene click chemistry [15], from Rhodamine B labeled POA and 1-mercapto carborane (carborane-SH) [16]. POARh (1 mg/mL), carborane-SH (3 mM), TCEP (9 mM), and hexyl amine (3 mM) were dissolved in DMF (30 mL) and stirred for 7 days. The resulting solution was precipitated twice into a

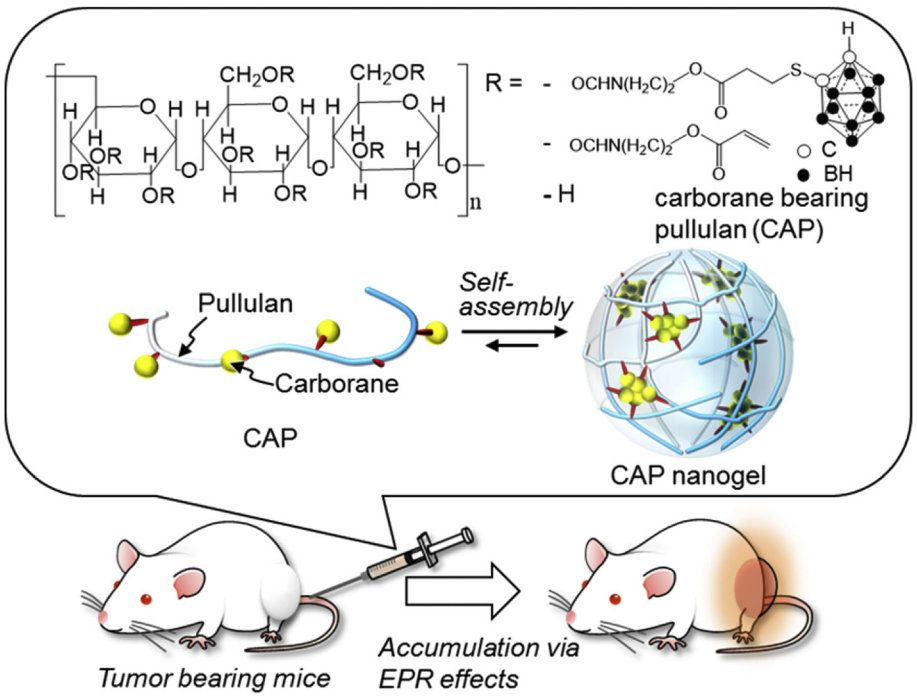

Fig. 1. Chemical structure of carborane bearing pullulan (CAP) for boron neutron capture therapy (BNCT).

Please cite this article in press as: R. Kawasaki, et al., Intracellular delivery and passive tumor targeting of a self-assembled nanogel containing carborane clusters for boron neutron capture therapy, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2016.12.176