Journal of Controlled Release 259 (2017) 203–211

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Andrographolide-loaded polymerized phenylboronic acid nanoconstruct for stimuli-responsive chemotherapy

Jinhwan Kim [a,1], Junseok Lee [b,1], Yeong Mi Lee [b], Swapan Pramanick [a], Sooseok Im [c], Won Jong Kim [a,b,c,*]

[a] Department of Chemistry, Pohang University of Science and Technology (POSTECH), Pohang 37673, Republic of Korea
[b] Center for Self-assembly and Complexity, Institute of Basic Science (IBS), Pohang 37673, Republic of Korea
[c] School of Interdisciplinary Bioscience and Bioengineering, Pohang University of Science and Technology (POSTECH), Pohang 37673, Republic of Korea

ARTICLE INFO

Article history:
Received 14 August 2016
Received in revised form 5 October 2016
Accepted 26 October 2016
Available online 28 October 2016

Keywords:
Andrographolide
Phenylboronic acid
Boronic ester chemistry
Drug delivery
Tumor targeting

ABSTRACT

Along with the successful discovery of paclitaxel as an anticancer drug, natural products have drawn great attention in drug discovery. Recently, andrographolide (AND) from *Andrographis paniculata* was reported to provide several benefits, including an anticancer effect. However, the extremely low solubility of the compound in an aqueous medium was an obstacle to overcome for the systemic administration and clinical application of AND. Based on our previous report, we formulated a water-soluble nanoconstruct by forming a boronic ester between the *cis*-1,3-diol of AND with hydrophilically polymerized phenylboronic acid (pPBA). The release of loaded AND was controlled by intracellular conditions, specifically, by low pH and high ATP concentrations, due to the pH- and diol-dependent affinity of the boronic ester. Because of the intrinsic property of the PBA moiety, the pPBA-AND nanoconstruct exhibited an excellent tumor targeting ability both *in vitro* and *in vivo*. Finally, a significant inhibition of tumor growth was observed *in vivo*. Taken together, our strategy, which is based on the formulation of a soluble nanoconstruct using hydrophilically polymerized PBA and a *cis*-diol, is plausible and provides a delivery system for a wide variety of chemotherapeutics. This strategy has applications not only in cancer therapy but also broader fields such as anti-inflammation or immunotherapy.

1. Introduction

For several decades, secondary metabolites derived from natural organisms such as plants or microbes have been regarded as "natural products", and they have attracted an enormous interest for various clinical applications [1–3]. To date, many kinds of natural products have been discovered and their biological activities have been evaluated [4,5]. In particular, paclitaxel from the pacific yew, salicylic acid from willow, and penicillin G from *Penicillium citrinum* are good examples of successfully commercialized natural products. Recently, an active compound called andrographolide (AND) was discovered from the extract of *Andrographis paniculata*, an herbaceous plant known as "king of bitter" [6,7]. Several studies have found that AND, along with its derivatives, modulates cellular signaling pathways. Moreover, they exhibit various beneficial effects such as antioxidant defense, anti-inflammatory effect, immunomodulatory activity, hepatoprotective activity, antiviral effect, and anticancer effect [8–11]. Especially, many reports have demonstrated that anticancer activity of AND is exerted by affecting various cancer-related factors, thus inhibits the proliferation, metastasis, and angiogenesis of cancer cells. In spite of these studies suggesting AND as a promising candidate for anticancer chemotherapeutics, an *in vivo* evaluation of its effect as an anticancer drug has been barely reported due to the extremely low solubility of AND in an aqueous medium [12]. Similar to other hydrophobic anticancer drugs, including paclitaxel and camptothecin, addition of a surfactant or organic solvent such as dimethyl sulfoxide (DMSO) or alcohol could be an alternative to solubilize AND in an aqueous medium [13–15]. However, these solubilizing agents may induce acute hemolysis leading to fatal systemic damage; additionally, the concern of cryptotoxicity still exists [16–18].

In drug delivery system, a nano-sized formulation has been widely attempted because it enables tumor-specific extravasation *via* an enhanced permeation and retention (EPR) effect [19–26]. Thus far, various types of nano-sized delivery systems, including liposomes, polymeric micelles, and inorganic nanoparticles, have been employed [27–31]; of these, liposomes, polymeric micelles, and self-assembled conjugates are the most commonly applied strategies. However, liposomes and polymeric micelles lack *in vivo* stability after administration, leading to nonspecific leakage of the loaded drug [32,33]. In addition, self-assembled conjugates require the chemical modification of drug molecules, which may reduce their therapeutic effect; they also require complicated synthetic procedures, which may hinder highly reproducible results [21,32,33]. Remarkably, the chemical structure of AND suggests an

* Corresponding author at: Department of Chemistry, Pohang University of Science and Technology (POSTECH), Pohang 37673, Republic of Korea.
E-mail address: wjkim@postech.ac.kr (W.J. Kim).
[1] These Authors are contributed equally to this work.

http://dx.doi.org/10.1016/j.jconrel.2016.10.029
0168-3659/

ingenious solution for a delivery system that overcomes aforementioned challenges for a drug delivery carrier when combined with hydrophilic polymers and phenylboronic acid (PBA). It has been widely known that PBA, (chemical formula: $C_6H_5B(OH)_2$), has a unique ability to form a boronic ester bond with a *cis*-1,2- or -1,3-diol, a common moieties of saccharides; thus, it has been incorporated as a ligand in applications for sugar sensors or affinity column of carbohydrates [34–37]. Specifically, PBA seems like a highly appropriate chemical moiety for delivery applications for two reasons. First, the binding affinity of PBA and diol is highly sensitive to external conditions including pH, sugars, and H_2O_2, making PBA moieties the preferred choice in stimuli-responsive drug delivery systems [38–41]. Second, PBA itself can act as a cancer targeting ligand because it exhibits specific binding with sialylated epitopes that are overexpressed on the surface of various types of tumors [42–44]. Using these characteristics, our group has reported a stimuli-responsive cross-linked polyethyleneimine for effective gene delivery based on the interaction between PBA and galactose [45]. Similarly, in the case of AND, which has 5-hydroxymethyl and 6-hydroxyl groups in the *cis* position to form a 1,3-*cis*-diol, it is expected that AND and PBA would form a boronic ester bond, subsequently generating their hydrophobic chemical structure. PBA is conjugated on a hydrophilic polymer; therefore, it is obvious that a self-assembled structure would be formed from the hydrophilic polymer backbone and the hydrophobic PBA-AND complex. The fact that they form a self-assembled structure could provide a great opportunity to expand the accessibility of AND to a drug delivery system.

By employing particular properties of PBA and AND in biomedical applications, we developed a simple and unique polymeric platform for the systemic administration of AND to be used as effective chemotherapy by overcoming limitations of aforementioned previous studies (Scheme 1). The PBA moiety was grafted on poly(methyl vinyl ether *alt* maleic anhydride) (pMAnh) to form poly(phenylboronic acid) (pPBA). It is worth highlighting that the synthetic procedure of pPBA does not require the usage of neither toxic solvents nor catalysts, thus it is free from residual solvent toxicity; additionally, it is highly atom economical and favors green chemistry. Next, a nanoconstruct was formulated by simple mixing pPBA and AND at the desired molar ratio. The pPBA-AND nanoconstruct was preferred because of several advantages: 1) it did not require any complicated synthetic procedure; 2) large amounts of carboxylic acid (derived from hydrolyzed pMAnh) readily solubilized and stabilized AND in an aqueous medium; 3) the residual PBA moiety exhibited a tumor targeting effect; 4) the hydrodynamic size of ~100 nm was suitable for biomedical applications; and 5) loaded AND was released specifically intracellularly. This unique strategy could provide an innovative platform for the delivery of hydrophobic AND and its derivatives, not only for cancer therapy, but also for more broad applications such as anti-inflammatory therapy or immunotherapy.

2. Materials and methods

2.1. Materials

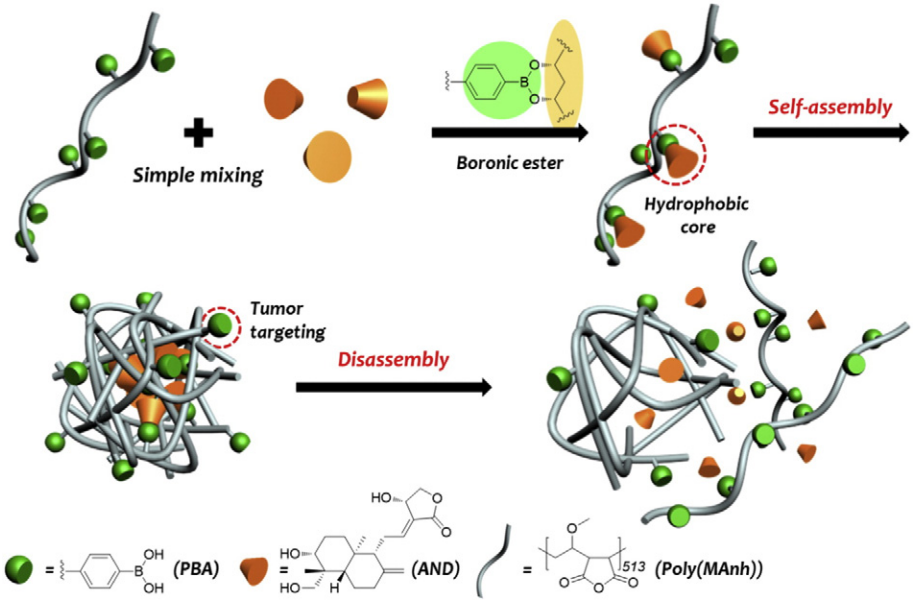
Poly(methyl vinyl ether-*alt*-maleic anhydride) (pMAnh; Mn ~80,000), 3-aminophenylboronic acid monohydrate (PBA-NH_2), adenosine 5′-triphosphate disodium salt hydrate (ATP), thiazolyl blue tetrazolium bromide (MTT) and all other solvents were purchased from Sigma Aldrich Co. (St. Louis, MO). Flamma FCR648-NH_2 was purchased from BioActs (Incheon, Korea). All reagents were used as received without further purification.

2.2. Instrumental methods

TEM image was taken from a transmission electron microscope (JEM-2210, JEOL) and analyzed by Gatan DigitalMicrograph software. UV–vis spectra were measured from a UV–vis spectrometer (UV 2550, Shimadzu) and fluorescence spectra were measured from a spectrofluorophotometer (RF-5301 PC, Shimadzu). Hydrodynamic volume and zeta potential were measured from zetasizer (Nano S90, Nano Z, respectively; Malvern) at 0.1 mM in Dulbecco's phosphate buffered saline (DPBS, pH 7.4) or PBS (pH 5.0). Confocal laser scanning microscope (CLSM) image was obtained from Olympus FV-1000 and analyzed by OLYMPUS FLUOVIEW ver. 1.7a Viewer software.

2.3. Extraction of andrographolide (AND) from A. paniculata

The air-dried powdered leaves of *Andrographis paniculata* (1 kg) were subjected to extraction in a Soxhlet apparatus with light petroleum ether (bp 60–80 °C), $CHCl_3$, and MeOH respectively. The MeOH extract was partitioned between *n*-BuOH and water. The *n*-BuOH extract (15 g), obtained after evaporation of solvent *in vacuo*, was



Scheme 1. Schematic illustration for the formulation and destruction of the pPBA-AND nanoconstruct. The nanoconstruct is formulated by the interaction between PBA (from pPBA) and 1,3-*cis*-diol (from AND), followed by self-assembly based on the hydrophobic interactions of AND and PBA. Furthermore, the nanoconstruct is disassembled, leading to the release of AND, in response to an intracellular stimulus such as acidic pH or increased level of ATP.