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Synthesis and evaluation of oxidation-responsive alginate-deferoxamine conjugates with increased stability and low toxicity

Meng Tian[a,1], Xi Chen[a,b,1], Zhipeng Gu[a], Hao Li[a], Lu Ma[a], Xin Qi[a], Hong Tan[c], Chao You[a,*]

[a] Department of Neurosurgery, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, PR China
[b] State Key Laboratory of Biotherapy/Collaborative Innovation Center of Biotherapy, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, PR China
[c] College of Polymer Science and Engineering, State Key Laboratory of Polymer Materials Engineering, Sichuan University, Chengdu, Sichuan 610065, PR China

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ABSTRACT

Deferoxamine is commonly used for iron-overload related diseases, its drawbacks such as instability and toxicity, however, significantly limited its utility in clinic. To address these issues, oxidation-responsive alginate-deferoxamine (Alg-DFO) conjugates were synthesized and their structure was characterized. The metabolism studies shown the conjugation of alginate significantly increased the stability of the DFO, with half-life more than 10 times longer than that of the free DFO. Moreover, the conjugates could not only quickly respond to oxidative stimuli and degradation, suggesting their potential to be cleared from the body by responding to iron-overload associated oxidative environment to avoid its accumulation and safety concern, but also protect iron binding capacity of the attached DFO from oxidation. The degradation mechanism for oxidative-response was proposed. In addition, the conjugates shown lower cytotoxicity compared to the free DFO. Taken together, the Alg-DFO conjugates synthesized in this work has promise for treating iron-overload related conditions.

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1. Introduction

Iron overload is clinically associated with numerous diseases, including thalassemia, stroke, as well as neurodegenerative disorders such as Alzheimer's and Parkinson's disease. To treat these conditions, iron chelation therapy is often employed, in which the most commonly used drug in clinic is deferoxamine (DFO) (Selim et al., 2011). However, the use of DFO was significantly hindered by its drawbacks during the past decades. For example, it has a very short plasma half-life within 30 min in vivo, resulting from its rapid metabolism by the globulin in the blood, thus requiring administration by intravenous or subcutaneous infusion over 8–12 h per day, 5–7 days per week which results in very poor patient compliance. Moreover, some complications such as growth retardation, endocrine dysfunction, cardiomyopathy, and peripheral neuropathies are usually associated with the toxicity of DFO.

One solution to this problem involves the use of drug delivery systems. In this regard, the approach using polymer-drug conjugate has shown promise as it effectively prolongs the drug's circulation half-life as well as it enables the drugs to be administered at lower dosages and with less frequency so as to relieve the toxicity (Xiao et al., 2014). For instance, the conjugation of poly (ethylene glycol) (PEG) to drugs such as polypeptides has been widely used to increase circulation half-life and decrease the toxicity of a wide range of drugs. For DFO, there are also some reports that have observed a prolonged half-life when conjugation to polymer carriers, including dextran, PEG copolymer, and hyperbranched polyglycerol (Hallaway, Eaton, Panter, & Hedlund, 1989; Rossi, Zou, Scott, & Kizhakkedathu, 2008; Ul-haq et al., 2013). Besides the circulation half-life, another important consideration for the design of polymer-drug conjugate is that the polymer carrier should be ideally degraded and cleared from the body after fulfillment of its role to avoid its accumulation in tissues resulting in a safety concern. To achieve this goal, recent research has focused on the designing of conjugates with stimuli responsive properties where the carriers on one hand shield the drug during circulation, and on the other hand degrade at the target upon being triggered by some stimuli

* Corresponding author.
E-mail addresses: tianmong007@gmail.com (M. Tian), Youchao@vip.126.com (C. You).
[1] These authors contributed equally to this work.

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present at the local environment, e.g. pH, temperature, oxidative, or redox potential (Wu et al., 2015; Xu, Wang, Li, & Wang, 2014). Iron overload is closely associated with an oxidative environment since iron plays an important role in catalyzing oxidative reactions that yield highly reactive toxic hydroxyl radicals leading to oxidative stress and cell death, activating lipid peroxidation, and exacerbating excitotoxicity. As a result, carriers with oxidative-response may be a potential candidate for DFO conjugation under the condition of iron overload. Alginate is a naturally occurring polysaccharide composed of α-L-guluronate (G unit) and β-D-mannuronate (M unit) arranged as linear homopolymeric and heteropolymeric blocks and it has been widely applied in drug, gene, and cell delivery systems due to its biocompatibility and non-immunogenicity (Biswas, Chattopadhyay, Sen, & Saha, 2015; Martín et al., 2015; Tian, Han, Tan, & You, 2014). In the case of drug carriers, it has been reported that alginate conjugation prolonged the half-life and improved the solubility of the drugs (Brudno et al., 2014; Imai, Fujii, Shiraishi, & Otagiri, 1997; Sreenivasan, 2014). Moreover, alginate is readily degraded under oxidation conditions such as in the aqueous solutions of sodium periodate and peroxide hydrogen (Li et al., 2010; Yang, Li, & Guan, 2004), suggesting its possibility as an oxidation-responsive drug carrier. However, up to now, there is no report on the design and synthesis of oxidation-responsive drug conjugates using alginate as a polymer carrier.

In the present work, the possibility of synthesizing oxidation-responsive alginate-DFO (Alg-DFO) conjugates was explored. We hypothesized that the conjugated alginate on one hand could protect DFO from metabolism by globulin during circulation, thus increasing the stability of DFO and iron chelation, and on the other hand degraded response to oxidative stimuli and cleared from the body (Scheme 1A). To address this possibility, alginate in its partially oxidized form, alginate dialdehyde (ADA), was first synthesized, and then the conjugates were prepared by Schiff-base reaction and followed by reduction (Scheme 1B). The structure of the conjugates was characterized by size-exclusion chromatography (SEC), FTIR, and ^{1}H NMR. The metabolism studies were carried out to evaluate the stability of the conjugates. The oxidative response was determined by the change of the molecular weight (MW) of the conjugates in an aqueous solution of H_2O_2 and the mechanism was proposed. The cytotoxicity of the conjugates was also assessed using endothelial cells and was quantified by MTT assay.

2. Materials and methods

2.1. Materials

Medium viscosity (MV) grade sodium alginate (M/G 1.96, MW 921 kDa), deferoxamine mesylate salt (DFO), and glutaraldehyde and diphenyl tetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Low viscosity (LV) grade sodium alginate (M/G 1.92, MW 365 kDa) was obtained from Qingdao Jingyan Biotechnology Co. LTD (China). Sodium cyanoborohydride ($NaBH_3CN$), and sodium borohydride ($NaBH_4$) were purchased from Aladdin Co. (Shanghai, China). Fetal bovine serum, DMEM, trypsin, penicillin and streptomycin were purchased from Gibco (Grand Island, NY, USA). Sodium periodate, peroxide hydrogen, and other reagents (analytical grade) were obtained from Kelong Co. (Chengdu, China).

2.2. Synthesis of ADA

ADA was synthesized according to previous reports with minor modification (Xu et al., 2013). In brief, 5.0 g of MV or LV alginate sodium (25 mmol of uronate units) was dissolved in 350 ml deionized water and 50 ml ethanol, and then an aqueous solution of sodium periodate (8.3 mmol, periodate/uronic = 0.3) was added under stirring. The reaction solution was stirred for 6 h in the dark at room temperature to oxidize and cleave the C2—C3 bond of the vicinal diol group in the uronate residue, and then 10 ml ethylene glycol was added and the solution was stirred for 2 h to quench the reaction. The synthesized ADA was separated and purified by precipitation with the addition of NaCl (5.00 g) and ethanol (1 l). The precipitant was re-dissolved in about 200 ml deionized water and precipitation with 1000 ml ethanol again, and the process was repeated three times. To further purify the product, the precipitant was re-dissolved in about 200 ml deionized water and then dialyzed using dialysis tube (MWCO, 3500) against deionized water with several changes of water until the dialyzate was periodate free. Finally, the solution was lyophilized to obtain the product. The products synthesized from LV and MV alginate sodium are referred to as LV-ADA and MV-ADA, respectively.

The chemical structure of the ADA was characterized by SEC, FTIR, and ^{1}H NMR. The degree of oxidation (OD) was determined by potentiometric titration aldehyde groups by hydroxylamine hydrochloride/sodium hydroxide method (Xu et al., 2013). The value was calculated by the following Eq. (1).

$$OD = \frac{n(CHO)/2}{W_{ADA}/198.11} \quad (1)$$

where $n(CHO)$ is the molar content of the aldehyde groups; W_{ADA} is the weight of the ADA; 198.11 is the molecular weight of uronate residue.

2.3. Synthesis of Alg-DFO conjugates

0.5 g of ADA was dissolved with deionized water (30 ml) at room temperature under stirring, and then 1.2 g of DFO was added to the solution with stirring, corresponding to a theoretical molar ratio of amino groups in DFO/aldehyde groups in ADA of 1.2. After 2 h, 0.25 g of $NaBH_3CN$, previously dissolved with 2 ml of deionized water, was slowly added to the reaction solution and stirred at room temperature for 4 h, and then 0.5 g of $NaBH_4$ was added to the solution similar to the process of addition of the $NaBH_3CN$. After 24 h, the reaction solution was dialyzed using dialysis tube (MWCO, 3500) against deionized water for 3 days. Finally, the solution was lyophilized to obtain the conjugate. The conjugate synthesized from LV-ADA and MV-ADA were marked as LA-DFO and MA-DFO, respectively.

The chemical structure of the conjugate was characterized by SEC, FTIR, and ^{1}H NMR. The amount of DFO in the conjugate was determined by conversion to the iron-saturated complex, ferrioxamine, using a UV–vis spectroscopy (Analytik jena scandrop 100, Germany) (Hallaway et al., 1989). Briefly, known quantities of conjugates were dissolved and diluted with ferrous sulfate solution and leaving to stand overnight at room temperature. The absorbance at 429 nm was measured and the DFO content was calculated using a standard curve (Supplementary data). The degree of DFO (% DFO) incorporation was calculated and described as moles of DFO attached per uronate residue.

2.4. Characterization

Weight average molecular weight (Mw), number average molecular weight (Mn) and polydispersity index (PDI) of the products were determined by a SEC equipped with a Waters 515HPLC pump, a Waters 2410 refractive index detector and a Waters hydrogel linear column operated at a flow rate of 0.6 ml/min. Each sample was eluted with a solution of 0.2 M $NaNO_3$. Dextranum standards were used for column calibration and as a relative reference for

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