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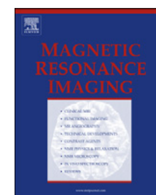

A new biodegradable and biocompatible gadolinium (III) -polymer for liver magnetic resonance imaging contrast agent

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ABSTRACT

A new biodegradable and biocompatible gadolinium (III) -copolymer (ACL-A_2-DOTA-Gd) has been developed as a potential liver magnetic resonance imaging (MRI) contrast agent. ACL-A_2-DOTA-Gd consisted of a poly (aspartic acid-co-leucine) unit bound with 1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid-gadolinium (Gd-DOTA) via the linkage of ethylenediamine. In vitro, the biodegradable experiment and cytotoxicity assay showed the biodegradability and biocompatibility of this gadolinium-polymer. ACL-A_2-DOTA-Gd presented an increase in relaxivity of 2.4 times than the clinical Gd-DOTA. In vivo, gadolinium (III)-copolymer was mainly accumulated in the liver, and it could be excreted via the renal and hepatobiliary mechanism. The average enhancement of ACL-A_2-DOTA-Gd (60.71 ± 5.93%, 50–80 min) in liver was 2.62-fold greater than that of Gd-DOTA (23.16 ± 3.55%, 10–30 min). ACL-A_2-DOTA-Gd could be as a potential liver MRI contrast agent with a long time-window.

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1. Introduction

Magnetic resonance imaging (MRI) is one of the most widely used diagnostic techniques in the clinic [1–3]. Various small MRI contrast agents have been developed, and successfully used for improving the diagnostic actuary and the imaging contrast of MRI in the clinical practice, such as Dotarem (Gd-DOTA), Gadovist (Gd-DO3A-burol), and Magnetvist (Gd-DTPA) [4,5]. These commercial gadolinium-agents, however, possess several undesirable properties, including low-relaxivities, non-distribution and rapid clearances, resulting in a heavy dose to obtain the desirable MR imaging, which are harmful for the patients with weak organs [5]. Loading a low-molecular weight drug into a macromolecule would alter the body distribution, always leading to a delayed the release of small drugs into the body fluids and prolong the time of the pharmaceutical efficacy [6]. Covalent linkages of these small contrast agents to the macromolecules [7] also cause specific-distribution, long half-life period and low-dosage. These macromolecular contrast agents [7] could therefore solve these mentioned above problems of small agents, such as Gd-DOTA-PAMAM [8], Gd-DOTA-dextran [9], Gd-DOTA-liposome [10], and Gd-DOTA-PEG [11]. Despite the advantages of these macromolecular MRI contrast agents, their applications to the diagnosis of diseases in the clinic are limited by their non-biodegradability, low organ specificity, extreme excretion and immunogenicity [7,12]. The desirable carriers for MRI contrast agents, therefore, possess highly specific distribution, are appropriately biodegradable, and have low immunogenic properties.

Poly (aspartic acid) (PASP), a water-soluble polymer, satisfies these aforementioned stringent requirements, and it has been used widely as the pharmaceutical carrier [13–15]. PASP can be readily synthesized with physicochemical properties [16]. Due to the protein-like structure of PASP, it is superior to the mentioned above macromolecules to be the carrier for the small agents [16,17]. Hence, PASP can also be used as the carrier for small MRI contrast agents. Previous studies have shown that the efficiency of an MRI contrast agent in the liver imaging is related to its lipotropy, and modifying the backbone or side-chain of MRI agents via the lipids can enhance the imaging contrast in the liver [4,7,18]. L-leucine, as an essential amino acid, has isopropyl with lipophilic property [19], and proper incorporation of L-leucine into the PASP can therefore lead to increase the lipophilicity of this copolymer. Therefore, we chose this copolymer (poly-(aspartic acid-co-leucine)) as the carrier for the commercial MRI contrast agent (Gd-DOTA).

Herein, a new biodegradable and biocompatible gadolinium-copolymer (ACL-A_2-DOTA-Gd) was developed for a potential liver MRI contrast agent. ACL-A_2-DOTA-Gd was synthesized and characterized using conventional spectroscopic techniques. The evaluation of ACL-A_2-DOTA-Gd for liver contrast agent was conducted by in vitro cytotoxicity assay, hemolytic test, biodegradable experiment, T_1 relaxivity measurement, and in vivo MRI study and distribution in rats.

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2. Experimental section

2.1. Materials and methods

All reagents in this study were A.R. and used without further purification. L-aspartic acid, L-leucine, 85% phosphoric acid, ethanol, diethyl ether, acetone, ethylenediamine (EDA), dimethylformamide (DMF) and nitric acid were purchased from Sinopharm Chemical Reagent Co., Ltd (SCRC). 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), N-Hydroxysulfosuccinimide sodium salt (Sulfo-NHS), 1-Ethyl-(3-dimethyllaminopropyl) carbodiie hydrochlide (EDC·HCl), gadolinium chloride ($GdCl_3$), bovine serum albumin (BSA), Dulbecco's modified eagle medium (DMEM) and cathepsin B were purchased from Sigma Aldrich, China.

^{13}C NMR spectra were obtained on a Bruker Avance 400 NMR spectrometer. The content of gadolinium (III) was measured on a POMES TJA inductively coupled plasma mass spectroscopy/spectrophotometer (ICP-OES). The molecular weight of the gadolinium–macromolecule was measured on a Waters gel permeation chromatography (GPC) instrument. The average number of bound Gd-DOTA residues per ACL-A_2-DOTA-Gd molecule was obtained by a reverse complexometric titration [9] and ICP-OES. The T_1 water proton relaxation times of gadolinium complexes were measured on a Bruker 90 NMR spectrometer.

2.2. Synthesis of imaging agent

2.2.1. Synthesis of poly-(aspartic acid-co-leucine) (ACL)

L-aspartic acid (7.98 g, 60 mmol), L-leucine (1.98 g, 15 mmol) and 85% phosphoric acid (4.5 ml) were well mixed, and the mixture was added into a round-bottom flask equipped with a rotary evaporator. The mixture was reacted at 160 °C for 5 hours under reduced pressure (24 mm Hg). The resulting mixture was dissolved in 50 ml dimethylformamide (DMF), and the solution was dropwise added into 150 ml deionized water and filtered. The crude product was washed with appropriate deionized water, and the final product (ACL) was acquired via vacuum drying at 55 °C. ^{13}C NMR (DMSO-d_6, δ/ppm): 173.5 (-CH_2**C**ONH-, carbonyl carbon of succinimide unit), 172.3 (-CH**C**ONH-, carbonyl carbon of succinimide unit), 170.1 (-**C**OCH-, carbonyl carbon of leucine unit), 50.8 (-CO**C**HNH-, methylene of leucine unit), 47.4 (-**C**HCO-, methyne carbon of succinimide unit), 36.5 (-CH**C**H_2CH-, leucine unit), 32.6 (-**C**H_2CO-, methylene carbon of succinimide unit), 23.8–20.9 (-CHCH_2**C**H(**C**H)$_3$, methylene carbon of leucine unit).

2.2.2. Synthesis of aminated-poly-(aspartic acid-co-leucine) (ACL-A_2)

ACL (1 g) and EDA (12 ml) were dissolved in DMF (12 ml), respectively. The solution of ACL was dropwise added in to the EDA solution, and the mixture solution was stirred for 6 hours at room temperature. The resulting solution was added into a mixed solution of ethanol and diethyl ether (1:2, 120 ml) and filtered. The crude product was purified by dextran Sephadex G-50. After condensation and lyophilization, aminated-poly-(aspartic acid-co-leucine) (ACL-A_2) was obtained. ^{13}C NMR (DMSO-d_6, δ/ppm): 173.2 ~ 171.7 (-CH_2**C**OONa, -CH**C**OONa, -CH_2**C**ONH-, -CH**C**ONH-, carbonyl carbon of aspartic acid unit; -**C**OCH-, carbonyl carbon of leucine unit), 53.8 (-CO**C**HNH-, methylene of leucine unit), 50.2 (-**C**HCO-, methyne carbon of aspartic acid unit), 38.9 (-CONH**C**$H_2CH_2NH_2$, methylene carbon of aspartic acid unit), 36.7-36.4 (-CONHCH_2**C**H_2NH_2, methylene carbon of aspartic acid unit; -CH**CH₂**CH-, leucine unit; -**C**H_2CO-, methylene carbon of aspartic acid unit), 24.5-20.6 (-CHCH_2**C**H(**C**H)$_3$, methylene carbon of leucine unit).

2.2.3. Conjugation of DOTA with aminated-poly-(aspartic acid-co-leucine) (ACL-A_2-DOTA)

A solution of DOTA (1.5 g, 3.7 mmol) in water (20 ml) was activated with sulfo-NHS (0.87 g, 4.0 mmol) and EDC · HCl (1.91 g, 10.0 mmol) at 4 °C for 2.5 hours. A solution of ACL-A_2 (0.5 g, 10 ml) was added into the mixture solution, and the mixture solution stirred for 1 day at room temperature. The resulting solution was purified by dextran Sephadex G-50. After condensation and lyophilization, ACL-A_2-DOTA was obtained. ^{3}C NMR (DMSO-d_6, δ/ppm): 177.6 (C-7, C-7', C-7''/DOTA carboxyl), 172.6 ~ 169.7 (-CH_2**C**OONa, -CH**C**OONa, -CH_2**C**ONH-, -CH**C**ONH-,carbonyl carbon of aspartic acid unit; -**C**OCH-, carbonyl carbon of leucine unit), 160.5 ~ 158.7 (C-1/DOTA **C**ONH carbon),64.1 ~ 62.7 (C-2/DOTA CH_2 carbon), 55.4 (C-5, C-5', C-5''/DOTA carbons),54.3 ~ 52.9 (C-6, C-6', C-6'', C-6'''/DOTA CH_2 carbons; -CO**C**HNH-, methylene of leucine unit), 50.8 (C-3,C-3'/DOTA CH_2 carbons), 50.2 (-**C**HCO-, methyne carbon of aspartic acid unit), 48.7 (C-4, C-4'/DOTA CH_2 carbons), 39.1 (-CONH**C**H_2CH_2NH-, methylene carbon of aspartic acid unit), 36.5–36.2 (-CHCH_2CH-, leucine unit; -**C**H_2CO-, methylene carbon of aspartic acid unit), 24.8–20.3 (-CHCH_2**C**H(**C**H)$_3$, methylene carbon of leucine unit).

2.2.4. Complexation of gadolinium (III) to ACL-A_2-DOTA-Gd

$GdCl_3$ (2.12 mol·L^{-1}, 1.06 ml) was dropwise added into ACL-A_2-DOTA (0.25 g, 15 ml) aqueous solution and reacted for 1 day at room temperature. The indicator (dimethyl phenol orange) of free gadolinium (III) was added into the resulting solution. An appropriate amount of EDTA solution was added into the resulting solution to chelate the free gadolinium (III) until the color of the solution changed from pink to bright yellow. The resulting solution was purified by dextran Sephadex G-50. After condensation and lyophilization, ACL-A_2-DOTA-Gd was obtained.

2.3. In vitro T_1 relaxivity of gadolinium (III)-complexes

The longitudinal relaxation time of ACL-A_2-DOTA-Gd and Gd-DOTA were measured on a 90 M NMR spectrometer using a standard inversion-recovery pulse sequence, at 25 °C. The gadolinium (III)-complexes were dissolved in 25% D_2O aqueous solution and 25% D_2O aqueous solution of BSA (0.725 mmol·L^{-1}) to obtain the gadolinium (III) concentrations at 0, 0.5, 1.0, 2.0, 3.5 and 5.0 mmol·L^{-1}, respectively. The relaxivity (R_1) was defined as the slope of the Eq. (1) [1] in the units of mmol·L·s^{-1};

$$(1/T_1)_{\mathrm{Obs}} = (1/T_1)_{Dia} + R_1 * [Gd] \quad (1)$$

where $(1/T_1)_{\mathrm{Obs}}$, $(1/T_1)_{\mathrm{Dia}}$ and [Gd] were the water proton relaxation rate in the presence of gadolinium (III)-complexes, the water proton relaxation rate in the absence of the gadolinium (III)-complexes, the concentrations of gadolinium (III), respectively.

2.4. In vitro biodegradation of ACL-A_2-DOTA-Gd

ACL-A_2-DOTA-Gd was dissolved in phosphate buffer solution (pH = 5.5) to obtain the concentration of this gadolinium (III)-complexe at 15 mg·ml^{-1}. Cathepsin B was added into the ACL-A_2-DOTA-Gd solution to obtain the concentration of this enzyme at 22 units·ml^{-1}. The mixture solutions were incubated at 37 °C. At the predetermined time intervals, the solutions were collected and analyzed by GPC.

2.5. In vitro cytotoxicity tests of gadolinium (III)-complexes

The healthy liver cells (L-02 cells) were seeded at a density of 4×10^4 cells/well (96 wells) in DMED medium and cultured for 1 day at 37 °C in 5% CO_2 atmosphere. Gadolinium (III)-complexes with various gadolinium (III) concentrations were added into each well and incubated for 3 days at 37 °C in 5% CO_2 atmosphere, respectively. After the incubation, an MTT solution (5 mg/ml, 20 μL/well) was added into each well and incubated for 4 hours. The absorbance of the solutions was measured at 490 nm by a DG-5033A ELISA-Reader

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