CHINESE JOURNAL OF ANALYTICAL CHEMISTRY
Volume 42, Issue 10, October 2014
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

Cite this article as: Chin J Anal Chem, 2014, 42(10), 1421–1426.

RESEARCH PAPER

Preparation of Novel Biocompatible Macromolecular Magnetic Resonance Imaging Contrast Agent

XIAO Yan[1,2], XUE Rong[1], ZHAN You-Yang[1,2], QI Chen-Li[1], YOU Tian-Yan[1], LI Xiao-Jing[1,*], PEI Feng-Kui[2]

[1] Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China
[2] University of Chinese Academy of Sciences, Beijing 100049, China

Abstract: Poly (aspartic acid-co-leucine) (PL) synthesized with aspartic acid and leucine was conjugated to 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) by ethylenediamine. Then the compound obtained was chelated with gadolinium (III) to form PL-A_2-DOTA-Gd. The structure of PL-A_2-DOTA-Gd was characterized by nuclear magnetic resonance and gel chromatography and its performance was evaluated by cytotoxicity test, heamolysis test, *in vitro* relaxivity determination and animal *in vivo* magnetic resonance characterization. PL-A_2-DOTA-Gd exhibited much lower cytotoxicity than Gd-DOTA. The T1-relaxivity of PL-A_2-DOTA-Gd (15.3 mM^{-1} s^{-1}) was 2.6 times than that of Gd-DOTA (5.8 mM^{-1} s^{-1}) in D_2O. The results of magnetic resonance imaging (MRI) experiments showed a significant enhancement in the rat liver imaging after the intravenous administration of PL-A_2-DOTA-Gd. In addition, the imaging time persisted by using PL-A_2-DOTA-Gd than that of Gd-DOTA and the imaging effect of liver tissue was enhanced by an average of 65.1% ± 5.2% and 21.3% ± 4.9% for PL-A_2-DOTA-Gd and Gd-DOTA, respectively.

Key Words: Magnetic resonance imaging; Poly-(amine acid); Biocompatibility; Liver-specificity

1 Introduction

Magnetic resonance imaging (MRI) has become one of the most important diagnostic tools in the clinic since MRI technology was put forward by Lauterbur[1–3]. In order to enhance the imaging contrast between the normal and the diseased tissues, about 30% of MR imaging require the use of the MRI contrast agents. Up to now, four commercial MRI contrast agents, Gd-DOTA (Dotarem), Gd-DTPA (Magnetvist), Gd-DTPA-BMA and Gd-DO3A are successfully used in the clinical diagnoses[2,3]. However, all these commercial MRI contrast agents have several drawbacks such as low molecular weight (LMW) and low relaxivity and non-targeting[4]. Conjugating these LMW agents to the biomacromolecule could enhance the performance of these MRI contrast agent[4], for instance, (Gd-DOTA)-BSA[5] and (Gd-DTPA)-Dextran[6]. These macromolecular MRI contrast agents, however, have the problems of non-biodegradability, low-specificity *in vivo*[7]. Poly (aspartic acid) is an ideal carrier for small MRI agents due to its excellent water solubility, biocompatibility and biodegradability[8]. It was reported that the introduction of lipophilic group into the backbone of the contrast agent could enhance the liver-selectivity of the agent[9]. Leucine, a natural amino acid which contains the lipophilic group, is believed to be able to enhance the liver-specificity when introducing into Poly (aspartic acid)[10].

In this study, poly (aspartic acid-co-leucine) was used as the carrier to obtain a biocompatible MRI contrast agent with satisfactory liver-imaging. Poly (aspartic acid-co-leucine) was conjugated with LMW contrast agent, gadolinium-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (Gd-DOTA)

Received 26 May 2014; accepted 22 July 2014
*Corresponding author. Email: xjli@ciac.ac.cn
This work was supported by the National Natural Science Foundation of China (Nos. 20975097, 21305134) and the Open Project of State Key Laboratory of Supramolecular Structure and Materials of Jilin University, China (No. 201427).

DOI: 10.1016/S1872-2040(14)60773-8

by ethylenediamine, and the derivative (PL-A_2-DOTA-Gd) was characterized by conventional spectroscopic methods. Compared with Gd-DOTA, PL-A_2-DOTA-Gd exhibited high relaxivity, as well as improved specific liver-imaging time, which provided enough time to optimize the imaging window and obtain the desired imaging, thus could well meet the requirements in the clinical diagnose. PL-A_2-DOTA-Gd is a potential clinical liver contrast agent for MRI due to its good biocompatibility and liver-imaging.

2 Experimental

2.1 Instruments and reagents

Vertex 70 IR spectrophotometer and av-400 NMR spectrometer were purchased from Bruker (USA). TJA POEMS ICP mass spectrometer was from Thermal (USA). Waters 1515 gel chromatograph (Waters, USA), DG-5033A microplate reader (Shanghai Precision Instrument, China), HT-PNMR12-9 1.5T NMR spectrometer and HT-MRSI60-25 1.2T MRI instrument (Shanghai Huantong, China) were also used in the experiment.

L-Aspartic acid, *L*-leucine, phosphoric acid, diethyl ether, ethanol, *N*,*N*-dimethyl formamide (DMF) and ethylenediamine were purchased from Sinopharm Chemical Reagent (China). 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), DMEM were purchased from Sigma (USA). Liver cells (L02) were purchased from XinRan Biological (Shanghai, China). Kunming mouse were obtained from College of Basic Medical Sciences, Jilin University.

2.2 Synthesis of PL-A_2-DOTA-Gd

2.2.1 Poly (aspartic acid-co-leucine) (PL)

Poly (aspartic acid-co-leucine) was synthesized according to the reported method[11]. Briefly, aspartic acid (5.99 g, 45 mmol), leucine (3.94 g, 30 mmol) and 85% phosphoric acid (5 mL) were mixed and reacted at 165 °C under 3.19 kPa for 5 h. Then the product was dissolved with 50 mL DMF, and the obtained solution was added to 250 mL deionized water to obtain the crude product. The crude product was washed by water and dried under the vacuum to obtain the final product (PL).

The ^{13}C NMR (DMSO-d_6, δ/ppm) were as follows: 172.9 (–CH_2**C**ONH–, succinimide), 171.7 (–CH**C**ONH–, succinimide), 169.8 (–**C**OCH–, leucine), 49.3 (–CO**C**HNH–, leucine), 46.1 (–**C**HCO–, succinimide), 36.1 (–CH**C**H_2CH–, leucine), 31.7 (–**C**H_2CO–, succinimide), 22.7–19.4 (–CHCH_2CH(**C**H_3)–, leucine).

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

Approximately 2.0 g of PL was dissolved in 24 mL DMF. Then 24 mL of ethylenediamine was added dropwise into the PL solution and reacted at 0 °C for 2.5 h. The reacted solution was then incubated at 25 °C for 2 h. The resulting solution was added to 240 mL ethanol-diethyl ether (2:1, *V*/*V*) to obtain the precipitate. The precipitate was dissolved in 50 mL NaOH solution (0.1 M) and the obtained mixture was then concentrated and dialyzed (MWCO 3500) against deionized water for three days. The residual solution was lyophilized to get the final product (PL-A_2).

The ^{13}C NMR (DMSO-d_6, δ/ppm) were as follows: 172.7–171.8 (–CH_2**C**OONa, –CH**C**OONa, the unit of aspartic acid), 170.8–170.1 (–CH_2**C**ONH–, –CH**C**ONH–, the unit of aspartic acid), 168.3 (–**C**OCH–, leucine), 49.3 (–CO**C**HNH–, leucine), 46.1 (–**C**HCO–, aspartic acid), 40.1 (–CONH**C**H_2CH_2NH_2), 37.2 (–CONHCH_2**C**H_2NH_2), 36.1–35.3 (–CH**C**H_2CH–, the unit of leucine, –**C**H_2CO–, the unit of aspartic acid), 22.7–19.4 (–CHCH_2**C**H(**C**H_3)–, leucine).

2.2.3 Conjugation of DOTA to PL-A_2 (PL-A_2-DOTA)

Approximately 2.0 g of aqueous solution of PL-A_2 (20 mL) was added to the active aster of DOTA solution synthesized according to the method described in the reference[12] at room temperature. After reacted for 24 h under stirring, the obtained product was dialyzed against deionized water for three days, and the residual solution was lyophilized to obtain the final product (PL-A_2-DOTA).

The ^{13}C NMR (DMSO-d_6, δ/ppm) were as follows: 176.9–176.1 (C-7, C-7', C-7" of DOTA), 172.7–171.8 (–CH_2**C**OONa, –CH**C**OONa, the unit of aspartic acid), 170.8–170.1 (–CH_2**C**ONH–, –CH**C**ONH–, the unit of aspartic acid), 168.3 (–**C**OCH–, leucine), 159.1–157.3 (C-1 of DOTA), 65.4–62.3 (C-2 of DOTA), 55.8 (C-5, C-5', C-5" of DOTA), 55.5-53.7 (C-6, C-6', C-6", C-6"' of DOTA), 49.7 (C-4, C-4' of DOTA), 49.3 (–CO**C**HNH–, leucine), 46.1 (–**C**HCO–, aspartic acid), 40.1 (–CONH**C**H_2**C**H_2NH–), 36.1–35.3 (–CH**C**H_2CH–, the unit of leucine, –**C**H_2CO–, the unit of aspartic acid), 22.7–19.4 (–CHCH_2CH(**C**H_3) –, leucine).

2.2.4 Preparation of gadolinium-conjugate (PL-A_2-DOTA-Gd)

The solutions of $GdCl_3$ and PL-A_2-DOTA (with the molar ratios of Gd^{3+} to DOTA of 1.2:1.0) were mixed and stirred overnight at room temperature. Then proper amount of EDTA solution was added to the resulting solution to conjugate the free Gd^{3+}. The mixed solution was concentrated and dialyzed (MWCO 3500) against deionized water for three days. The residual solution was lyophilized to give the final product (PL-A_2-DOTA-Gd). The gadolinium content of PL-A_2-DOTA-Gd was determined to be 8.16% by ICP-OES.