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Synthesis and evaluation of Gd-DTPA-labeled arabinogalactans as potential MRI contrast agents

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Abstract—Arabinogalactan derivatives conjugated with gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA) by ethylenediamine (Gd-DTPA-CMAG-A_2) or hexylamine (Gd-DTPA-CMAG-A_6) have been synthesized and characterized by means of Fourier transform infrared spectra (FTIR), ^{13}C nuclear magnetic resonance (^{13}C NMR), size exclusion chromatography (SEC), and inductively coupled plasma atomic emission spectrometry (ICP-AES). Relaxivity studies showed that arabinogalactan-bound complexes possessed higher relaxation effectiveness compared with the clinically used Gd-DTPA, and the influence of the spacer arm lengths on the T_1 relaxivities was studied. Their stability was investigated by competition study with Ca^{2+}, EDTA, and DTPA. MR imaging of Wistar rats showed remarkable enhancement in rat liver and kidney after i.v. injection of Gd-DTPA-CMAG-A_2 (0.079 ± 0.002 mmol/kg Gd^{3+}): The mean percentage enhancement of the liver parenchyma and kidney was $38.7 \pm 6.4\%$ and $69.4 \pm 4.4\%$ at 10–30 min. Our preliminary in vivo and in vitro study indicates that the arabinogalactan-bound complexes are potential liver-specific contrast agents for MRI.

Keywords: Magnetic resonance imaging; Contrast agent; Liver-specific; Arabinogalactan; Relaxivity

1. Introduction

Magnetic resonance imaging (MRI) is one of the most useful diagnostic techniques in clinical medicine because it allows researchers and doctors to image the body in a noninvasive manner.[1,2] Image quality can be enhanced by the administration of contrast agents, which enhance various portions of the MR image by changing, usually decreasing, the relaxation time of the tissue water and, thus, allowing the area of interest to be much more conspicuous than the surrounding tissues. Currently, more than 35% of all MRI examinations are accompanied by administration of contrast agents.[3] Up to now, four kinds of gadolinium complexes (Gd-DTPA, Gd-DTPA-BMA, Gd-DOTA, and Gd-HPDO3A) have been used worldwide for intravenous administration. These comparatively small molecular agents have been used successfully to enhance the imaging of brain and central nervous system. However, these small hydrophilic complexes are nonspecific extracellular contrast agents and are excreted rapidly through the kidneys, which may limit their use in other parts of the body. Therefore, there is a significant need for agents that target specific organs, regions of the body, or diseased tissue to gain the greatest diagnostic value.[4–7] In recent years, the research and development for liver-specific contrast agents with high relaxivities and kinetic stability have been quite active.[8–11] Some groups became

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interested in contrast agents which enter hepatocytes through hepatic asialoglycoprotein receptor (ASGP-R), which is an organ-specific lectin, not found anywhere in the body except on the surface of hepatocytes.[12–14] Arabinogalactan, a polysaccharide isolated from plants, is known to be specifically absorbed by hepatocytes via the ASGP-R. The presence of numerous terminal galactose residues and the high degree of branching of arabinogalactan are responsible for its bindings to the ASGP-R.[15] The polysaccharide and its derivatives have been used as carriers to deliver drugs to hepatocytes via this receptor.[16,17] A spin-labelled arabinogalactan,[12] as well as an arabinogalactan-stabilized ultrasmall superparamagnetic iron oxide (AG-USPIO)[13] has been reported as liver-specific contrast agents for MRI by targeting hepatocyte ASGP-R.

We had previously described Gd-DTPA directly linked to natural polysaccharides via ester bonds as contrast agents for MRI.[18,19] As a continuation, the aims of this study were to synthesize arabinogalactan-linked Gd-DTPA (Gd-DTPA-CMAG-A_n; $n = 2$ or $n = 6$) connected via amide groups. The influence of the spacer arm lengths and Ca^{2+}, EDTA, or DTPA on the r_1 relaxivity were investigated. Furthermore, we evaluated the potential application of Gd-DTPA-CMAG-A_2 as a liver-specific contrast agent by in vivo experiments.

2. Results and discussion

2.1. Characterization of DTPA-CMAG-A_n

There are several structural types of arabinogalactans.[20,21] Generally, type I and type II of AG are distinguished. The ^{13}C NMR spectrum (Fig. 1) of the AG used in this work is similar as those reported from Larch species (type II).[22,23] The AG has a framework of β-(1→3) D-galactopyranose residues branched at C-6. The side chains consist of single β-D-Gal*p* or dimer structures such as β-Gal*p*-(1→6)-β-Gal*p*, β-Ara*p*-(1→3)-Ara*f*, and β-Ara*p*-(1→3)-Ara*f*.[20]

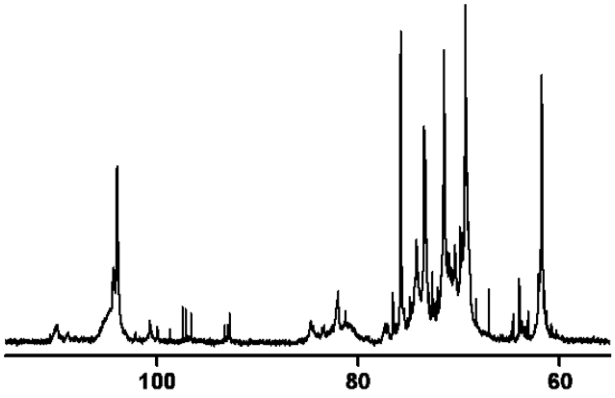

Figure 1. ^{13}C NMR spectrum of AG in 4:1 water–D_2O.

The synthetic route to arabinogalactan-linked Gd-DTPA as MRI contrast agents is depicted in Scheme 1. The DTPA-CMAG-A_2 was characterized by size exclusion chromatography as shown in Figure 2. DTPA-CMAG-A_2 eluted as a single peak with a retention time of 16.5 min, compared to Dextran T-10 (molecular weight: 10 kD) and Dextran T-40 (molecular weight: 40 kD) standards (16.7 and 15.4 min, respectively). The starting arabinogalactan eluted at 16.1 min (spectrum not shown) and a small amount of a low molecular weight arabinogalactan was removed by dialysis. DTPA-CMAG-A_6 eluted at 16.4 min (spectrum not shown) and exhibited an elution profile similar to DTPA-CMAG-A_2.

The FTIR spectra of AG and its derivatives (CMAG, CMAG-A_2, and DTPA-CMAG-A_2) are shown in Figure 3. Arrows point out to some characteristic vibrations.[24,25] The successful incorporation of carboxylate groups onto AG block was verified by the appearance of a peak at 1604 cm^{-1} in the spectrum of CMAG (b). The spectrum of CMAG-A_2 (c) presents a peak at 1650 cm^{-1}, attributed to the –NHCO (amide I), which indicates that the acylamino bond in CMAG-A_2 was formed. After CMAG-A_2 linking to DTPA, the peak appeared at 1738 cm^{-1}, corresponding to the carboxylate in DTPA (d).

The structure of the synthesized polymers was further supported by the ^{13}C NMR spectra. Compared to AG alone, two additional peaks of CMAG at 178.6–177.7 and 68.7 ppm were assigned to COONa and CH_2 carbon atoms of a carboxymethyl ester.[26] The ^{13}C NMR spectrum of CMAG-A_2 exhibited three new peaks at 173.7, 40.7, and 39.9 ppm, corresponding to the carbonyl CONH, the amido α-carbon $NHCH_2$, and the amino α-carbon CH_2NH_2,[27] respectively. The spectrum still contained peaks at 178.6–177.7 ppm, consistent with residual unsubstituted COONa. In the case of DTPA-CMAG-A_2, the peak corresponding to the amino α-carbon disappeared, an indication that all of the CMAG-A_2 amino groups were substituted by DTPA. Some new peaks were observed with the following possible assignments (see Scheme 1):[28,29] 179.2 and 171.2 ppm, the C-1 and C-7 (C-7′, C-7″) of DTPA carboxyls; 60.9, 57.7, 56.8, 53.6, and 49.0 ppm, the C-5, C-6 (C-6′, C-6″), C-2, C-3 (C-3′), and C-4 (C-4′) of DTPA CH_2 carbons.

The IR and ^{13}C NMR results indicated that DTPA was linked to aminated arabinogalactan by an amide function.

The contents of AG derivatives were determined by quantitative assays. The synthesized CMAG sample contained 2.0 mmol of COONa/g. Samples CMAG-A_2 (CMAG-A_6) contained 0.51 (0.50) mmol of NH_2/g and so the residual unsubstituted COONa groups were