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Gadolinium-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid conjugate of arabinogalactan as a potential liver-targeting magnetic resonance imaging contrast agent

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ABSTRACT

A novel biocompatible macromolecule (AG-CM-EDA-DOTA-Gd) was synthesized as a liver magnetic resonance imaging (MRI) contrast agent. AG-CM-EDA-DOTA-Gd consisted of a carboxymethyl-arabinogalactan unit conjugated with gadolinium-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (Gd-DOTA) via ethylenediamine, and was specifically designed to bind to hepatocyte asialoglycoprotein in vivo, in an effort to develop a potential new tool for the diagnosis of liver diseases. The T_1-relaxivity (8.87 $mmol^{-1}\,L\,s^{-1}$) of AG-CM-EDA-DOTA-Gd was 1.86 times than that of Gd-DOTA (4.76 $mmol^{-1}\,L\,s^{-1}$) in D_2O at 9.4 T and 25 °C. MRI experiments showed significant enhancement in rat liver following the intravenous administration of AG-CM-EDA-DOTA-Gd (0.094 mmol Gd^{3+}/kg body weight), which persisted for longer than Gd-DOTA (0.098 mmol Gd^{3+}/kg body weight). The mean percentage enhancements in the liver parenchyma were 85.2 ± 6.5% and 19.3 ± 3.3% for AG-CM-EDA-DOTA-Gd and Gd-DOTA, respectively. The results of this study therefore indicate that AG-CM-EDA-DOTA-Gd could be used as a potential liver-targeting contrast agent for MRI.

1. Introduction

Magnetic resonance imaging (MRI) is one of the most important imaging modalities in clinical diagnosis.[1,2] Despite the importance of MRI, its application to the diagnosis of specific diseases in clinical practice has been limited by its inability to provide effective contrast imaging between the soft tissues because of the relaxation time of water protons. To enhance the quality of the contrast imaging in MRI, about one-third of MRI diagnoses are achieved with the use of contrast agents.[3,4] MRI contrast agents can enhance the relaxation rates of water molecules, and can therefore be used to provide additional information in the image relative to the areas that have not been treated with the agent.[4] Several MRI contrast agents, including Dotarem (Gd-DOTA), ProHance (Gd-HP-DO3A), and Gadovist (Gd-DO3A-burol), have been used successfully in the clinical practice for the diagnosis of specific diseases.[5,6] These clinical MRI contrast agents, however, possess several undesirable properties, including small sizes, low relaxation rates, poor tissue-targeting abilities, and rapid clearances.[5–7] Most of the issues associated with these agents could be overcome by using macromolecules, such as albumin,[8,9] polysaccharides,[10] poly (L-lysine),[11] poly (glutamic acid),[12] PEG[13], polyamidoamine dendrimer,[14] and liposome,[15] which contain low-relaxivity contrast agents. These macromolecular conjugates generally exhibit longer blood elimination half-lives and higher water solubility properties, as well as showing much lower levels of toxicity, higher thermodynamic stability, and lower immunogenicity.[16,17] Arabinogalactan (AG), a high water-soluble polysaccharide, satisfies the aforementioned strict requirements. Larch AG can be readily extracted from the Larch tree with high purity, reproducible molecular weight, and physicochemical properties.[18] The natural polysaccharide Larch AG consists of L-arabinose and D-galactose residues. Numerous D-galactose residues in AG allow AG to bind to the asialoglycoprotein receptor (ASGP-R). ASGP-R is only located on the surface of hepatocytes.[19] Previous work has shown that 30% of the dose of AG was found in the liver following the intravenous injection of AG to rats. The good water-solubility, biodegradability, and biocompatibility properties of AG, as well as its ability to target the hepatocytes, make AG and its derivatives particularly useful liver-targeting carriers.[18–21]

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The ultrasmall superparamagnetic iron (USPIO) particles coated with arabinogalactan and their derivatives have been used extensively as contrast agents in liver-targeting MRI experiments.[22,23] These arabinogalactan-USIPO contrast agents provide hypointensity in the liver. However, these negative contrast agents are inherently polydisperse and ill characterized. Previous study in this area has shown that liver-selective positive contrast agents are superior to negative contrast agents in terms of their ability to detect the hepatocellular carcinoma (HCC).[24]

Herein, we report the development of AG-CM-EDA-DOTA-Gd as a novel conjugate for use as a potential positive MRI contrast agent with liver-selectivity. The conjugate was synthesized and characterized. The evaluation of the conjugate as an MRI contrast was conducted using T_1 relaxivity measurement and in vivo MRI studies in rats.

2. Result and discussion

2.1. Characterization of AG-CM-EDA-DOTA-Gd

The FTIR spectra of AG (a), AG-CM (b), AG-CM-EDA (c) and AG-CM-EDA-DOTA (d) are shown in Figure 1. Compared with the spectrum of AG (a), the band at 1600 cm^{-1} in the spectrum of AG-CM (b) showed that the carboxylate groups had been incorporated into AG. The new band at 1660 cm^{-1} in the spectrum of AG-CM-EDA (c) was assigned to the NHCO group, which indicated that the acylamino groups had combined with AG-CM-EDA.[25,26] The band at 1645 cm^{-1} in the spectrum of AG-CM-EDA-DOTA (d) indicated that DOTA had been successfully linked to AG-CM-EDA through the carboxylate.[27]

The structures of AG-CM, AG-CM-EDA, and AG-CM-EDA-DOTA were further characterized by the ^{13}C NMR spectroscopy (Fig. 2). Compared with the ^{13}C NMR spectrum of AG (a), the spectrum of AG-CM (b) contained two new peaks at 178.1–177.2 and 68.3 ppm, which were attributed to the carbon atoms of COONa and CH_2 moieties of the carboxymethyl ester, respectively.[28] The ^{13}C NMR spectrum of AG-CM-EDA (c) contained peaks at 171.2, 40.1, and 39.3 ppm, which were assigned to the carbon atoms of CONH, $NHCH_2$, and CH_2NH_2 groups, respectively.[29] The C^{13} NMR spectrum of AG-CM-EDA-DOTA (d) contained peaks at 172.3 and 162.6 ppm, which were assigned to the C-7 (C-7′, C-7″) and C-1 of DOTA carboxyl, as well as peaks at 56.3, 54.2, 51.6, 48.1, and 43.7 ppm, which were attributed to the C-2, C-5 (C-5′, C-5″), C-6 (C-6′, C-6″, C-6‴), C-3 (C-3′), and C-4 (C-4′) of DOTA CH_2 carbons (Scheme 1).[29,31] The disappearance of the peak attributed to the carbon atom of the CH_2NH_2 moiety indicated that all of these α-amino groups had been replaced by DOTA. These results therefore confirmed that the DOTA had been successfully linked to AG-CM-EDA.

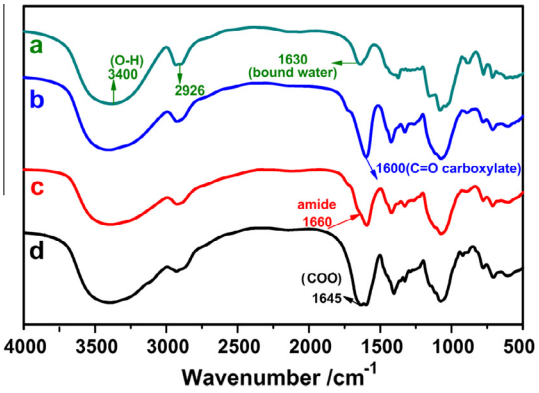

Figure 1. The FTIR spectra of AG (a), AG-CM (b), AG-CM-EDA (c), AG-CM-EDA-DOTA (d).

The molecular weight of AG-CM-EDA-DOTA was 12437 g/mol. The mean number of bound Gd-DOTA residues per AG-CM-EDA-DOTA-Gd molecule was found to be in the range of 7∼8, which was obtained using the reverse complexometric method as well as ICP-AES.

2.2. Relaxivity

2.2.1. The r_1 relaxivity of AG-CM-EDA-DOTA-Gd

To assess the suitability of AG-CM-EDA-DOTA-Gd as a potential MRI contrast agent, the T_1 values of the material were measured at 2.1 and 9.4 T, respectively. Dotarem (Gd-DOTA), which has been used in the clinic for MRI diagnosis, was used as a control.

The ability of the paramagnetic MRI contrast agent to enhance the water relaxation rate is referred to the longitudinal relaxivity (r_1), which is calculated by the Eq. (1)[1]

$$(1/T_1)_{obs} = (1/T_1)_d + r_1[M] \quad (1)$$

where $(1/T_1)_{obs}$ is the observed relaxation rate, $(1/T_1)_d$ is the water proton relaxation rate of the pure water solvent, and $[M]$ is the total concentration of the paramagnetic compounds, respectively.

Table 1 shows the relaxivities of AG-CM-EDA-DOTA-Gd and Gd-DOTA in D_2O, which were obtained by linear regression by Eq. (1). The T_1-relaxivity (8.87 $mmol^{-1}$ L s^{-1}) of AG-CM-EDA-DOTA-Gd was 1.86 times than that of Gd-DOTA (4.76 $mmol^{-1}$ L s^{-1}) in D_2O at 9.4 T and 25 °C. The T_1 relaxivities of contrast agents were also measured at a low frequency. As shown in Table 1, the T_1 relaxivity (10.73 $mmol^{-1}$ L s^{-1}) of AG-CM-EDA-DOTA-Gd was 1.92 times than that of Gd-DOTA (5.59 $mmol^{-1}$ L s^{-1}) in D_2O at 2.1 T. This increase in T_1-relaxivity values of AG-CM-EDA-DOTA-Gd compared with those of Gd-DOTA were attributed to (1) an increase in the size of the contrast agent resulting from the conjugation of Gd-DOTA with AG-CM-EDA, which would have led to the increased rotational correlation time;[30] and (2) the capturing of water molecules within the carrier (AG), which would lead to the increased number of outer sphere-coordinated water molecules.[27,31,32]

2.2.2. Albumin binding study

Serum albumins are the major proteins in the plasma with important physiological properties, and bovine serum albumin (BSA) has been used extensively in studies involving serum albumins, because of its well-characterized molecular structure, low price, and structural similarity to human serum albumin (HSA). There was a marked increase in the T_1-relaxivity (9.22 $mmol^{-1}$ L s^{-1}) of AG-CM-EDA-DOTA-Gd in a solution of BSA (0.725 mmol L^{-1}), which was 1.04 times than that of AG-CM-EDA-DOTA-Gd (8.87 $mmol^{-1}$ L s^{-1}) in D_2O (Table 1). This result was attributed to the presence of other paramagnetic substances in the BSA solution besides free AG-CM-EDA-DOTA-Gd.[33] It is noteworthy that the possibility of AG-CM-EDA-DOTA-Gd being decomposed under these conditions could be ignored because of the weak interaction between Gd^{3+} and the serum album.[33,34] This phenomenon can be explained in terms of part of the AG-CM-EDA-DOTA-Gd being non-covalently bound to BSA. Analysis referring to Ref. [35] showed that approximately 2.1% of the AG-CM-EDA-DOTA-Gd existed as AG-CM-EDA-DOTA-Gd · BSA in a 0.725 mmol L^{-1} BSA solution with an equilibrium constant of 0.031.

2.3. In vitro cytotoxicity assay of AG-CM-EDA-DOTA-Gd

Human liver cells (L02 cells) were used to evaluate the biocompatibility of AG-CM-EDA-DOTA-Gd. The cells were incubated with various concentrations of AG-CM-EDA-DOTA-Gd and Gd-DOTA, respectively. At the same concentration of [Gd], the cytotoxicity