Gd complexes of diethylenetriaminepentaacetic acid conjugates of low-molecular-weight chitosan oligosaccharide as a new liver-specific MRI contrast agent

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1. Introduction

Magnetic resonance imaging (MRI) is one of the most powerful, noninvasive imaging techniques capable of providing high-resolution visualization of specific tissues or organs in the body and has been extensively used in neurological, cardiovascular, and oncological diagnosis [1,2]. Contrast agents (CAs) are employed in MRI to improve the contrast effect, as they interact with the surrounding water protons in the body and shorten their relaxation time to provide better image contrast [3]. Presently, MRI CAs used in a large number of MRI techniques are usually gadolinium(III) (Gd³⁺) based complexes. Some representative advantages of using the paramagnetic Gd³⁺ ion as metal center result from their unique properties such as (I) high magnetic moment, and (II) suitable electron relaxation time [4]. To date, there are known various types of Gd³⁺-based MRI CAs applied for clinical usage, like Gd-DTPA (DTPA = diethylenetriaminepentaacetic acid), Gd-DOTA (DOTA = 1,4,7,10-tetra-azacyclododecane-1,4,7,10-tetraacetic acid), Gd-DTPA-CSn (n = 6, 8, 11) as a new class of contrast agent as well as its magnetic property in a pilot magnetic resonance imaging. The efficacy of the contrast agent was assessed by measuring the longitudinal relaxivity (r1). FLASH imaging in phantoms in vitro and signal intensity in vivo of the rat abdominal axial imaging. The r1 of Gd-DTPA-CS4,5 was up to 11.65 mM⁻¹·s⁻¹, which was 3 times higher than that of the analogous MRI contrast agent Gd-DTPA in commercial use. In vivo MR images of rat obtained with Gd-DTPA-CS4,5 showed strong signal enhancement in liver and the vessels of the liver parenchyma during the extended period of time. The present study suggests that the new synthesized gadolinium complexes can be used as a new class of practical liver-specific MRI contrast agent because of its superior performance compared with Gd-DTPA.

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Chitosan, as a naturally existing and abundantly available amino-poly saccharide, may be recommended as an ideal structure. Chitosan is the N-deacetylated derivative of chitin, which is the principal component of protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters and cell walls of some fungi [21]. Chitosan has many outstanding properties like biocompatibility, biodegradability, non-toxicity [22,23]. Besides, the low-molecular-weight chitosan oligosaccharides (CS) with narrow polydispersity index (PDI) and suitable degree of polymerization (DP) possess more excellent and attractive bioactivity [24,25].

In this paper, a new kind of MRI CAs Gd-DTPA-CS\(_n\) (\(n\) is DP of CS), modifying Gd-DTPA with different molecular weight chitosan oligosaccharides, was synthesized. The performance of Gd-DTPA-CS\(_n\) as the potential CA is determined by means of in vitro study as well as in vivo experiments.

2. Materials and methods

2.1. Chemicals

Chitosan oligosaccharides with different DP and narrow PDI (\(n\) included 6, 8, 11; PDI = 1.04, 1.06, 1.10, respectively) were presented by Hainan Provincial Key Lab of Fine Chemistry. GdCl\(_3\)·6H\(_2\)O was gained by dissolving Gd\(_2\)O\(_3\) into excessive HCl. Gd-DTPA complex was synthesized according to a routine method [26]. 4-Dimethylaminopyridine was purchased from JingChun reagent Co. Ltd. (Shanghai, China). Sprague–Dawley (SD) rats were purchased from Animal Experimental Center of Hainan Normal University (Hainan, China). All other reagents (A.R.) were used as received without further purification.

2.2. Preparation of Gd-DTPA-CS\(_n\)

2.2.1. The synthesis of DTPA-CS\(_n\)

The DTPA-bis(anhydride) was accomplished and isolated by the method of Eckelman [27]. The synthetic reaction of Gd-DTPA-CS\(_n\) was modified according to literature [19]. In our process, DTPA bis(anhydride) (5mmol, 1.79g) was dissolved in 50mL anhydrous DMF by heating at 65°C until a clear solution was formed and the solution was then cooled to room temperature. CS\(_{11}\) (5mmol, 9.90g) and a small quantity of 4-dimethylaminopyridine were added to the solution and the resulting mixture was magnetically stirred overnight at ambient temperature. It was then cooled in an ice-water bath after which deionized water (5mmol, 0.09mL) was added in drops. The mixture was stirred continuously for 12h and condensed under reduced pressure. DTPA-CS\(_{11}\) was precipitated and washed three times with anhydrous ethanol and ether respectively to remove the remaining DMF. This product was then purified by

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running over a dextran sephadex G-25 column. A white DTPA-CS$_{11}$ solid (5.2g) was obtained and stored under vacuum for the next step. DTPA-CS$_{6}$ and DTPA-CS$_{8}$ were synthesized and purified in a similar way as explained above.

2.2.2. The synthesis of Gd-DTPA-CS$_{n}$

A slight excess GdCl$_3$·6H$_2$O (2mmol, 0.74g) was added to a solution of DTPA-CS$_{11}$ (1mmol, 1.98g). Then the mixed solution was stirred for 8h. Anhydrous ethanol was added to the solution and the suspension was filtered to leave a white product. The crude product was purified by dialysis against 0.9 % saline for 3days, then against distilled water. The external solution was exchanged 3 times every day until longitudinal relaxation time ($T_1$) of water proton of external solution reached up to 3000ms. Finally, the residual solution was lyophilized to give the desired Gd-DTPA-CS$_{11}$ solid (2.1g, 85% yield) as powder. Gd-DTPA-CS$_{8}$ and Gd-DTPA-CS$_{6}$ were obtained in a similar way. A diagram of the synthesis of Gd-DTPA-CS$_{n}$ is shown in Scheme 1.

2.3. In vitro MRI studies

2.3.1. Determination of $r_1$

Relaxivity is used to compare the efficiency of different contrast agents in relation to their signal enhancement in MRI. The ability of proton relaxation enhancement of a paramagnetic compound is commonly evaluated by the term $r_1$, which is defined as the slope of Eq. (1) and with mM$^{-1}$·s$^{-1}$ as the unit of measurement.

$$\frac{1}{T_1}_{obs} = \frac{1}{T_1}_{d} + r_1[M]$$

Where ($1/T_1$)$_{obs}$ and ($1/T_1$)$_{d}$ are water proton relaxation rates in the presence and absence of the paramagnetic species, and [M] is the concentration of the paramagnetic species. $T_1$ values of Gd-DTPA and Gd-DTPA-CS$_{n}$ ($n=6, 8, 11$) aqueous solution at concentrations ranging from 0.0 to 2.0mM (0.0, 0.4, 0.8, 1.2, 1.6 and 2.0mM) were measured by a standard inversion-recovery sequence on the MicroMR imaging & analyzing system at 32°C and 0.5T (Niumag Technology Co., Ltd., Suzhou, China).

2.3.2. $T_1$-Weight FLASh in vitro

In vitro imaging effect of Gd-DTPA-CS$_{n}$ ($n=6, 8, 11$) is visualized by FLASh images in phantoms. Multislice spin echo (MSE) sequence (TR = 30ms, TE = 0.55ms, NS = 32, Slice thickness = 2.0mm) on the MicroMR imaging & analyzing system was employed for the acquisition of the in vitro imaging. The schematic drawing of a phantom was shown in Fig. 1. The center tube labeled as 0.0mM contained water only. The surrounding five tubes were grouped together with the order of increasing concentrations (0.4, 0.8, 1.2, 1.6 and 2.0mM) counterclockwise. The six-tube phantom was then immersed in a larger tube (o.d. = 5.0mm, i.d. = 3.0mm) filled with 0.2% CuSO$_4$ solution (3.0mL) and sealed airtight without bubbles to keep the magnetic resonance susceptibility artifacts low. FLASh images of phantoms prepared by the samples were analyzed using the Image J software package [29].
In vivo imaging

In this in vivo experiment, twenty SD rats (180–200g) were randomly assigned into two groups: the experimental group (n=10) and the control group (n=10). The rats were anesthetized by intraperitoneal administration of sodium pentobarbital (0.5mL/100g). For comparative analysis, the experimental and the control groups (both 0.080mmol/kg Gd3+) were injected with Gd-DTPA-CS$_{11}$ and Gd-DTPA respectively.

Before and after 5min, 15min, 30min, and 90min intravenous injection (tail vein) of Gd-DTPA-CS$_{11}$ and Gd-DTPA, a series of $T_1$-weighted images of abdominal axial was acquired on a 3.0-T MRI scanner (Signa HDx, GE Co., USA) using fast spin echo (FSE) sequence at room temperature. Enhanced imaging parameters were as follows: repetition time (TR): 500ms; echo time (TE): 15ms; slice thickness (SLT): 2mm; field of view (FOV): 8cm×8cm; and matrix: 256×224.

3. Results and discussion

$T_1$ values for different concentrations of Gd-DTPA-CS$_n$ (n=6, 8, 11) as well as Gd-DTPA in aqueous solution were measured. It is found from Fig. 2, that increased Gd-DTPA-CS$_n$ concentration led to a decrease in $T_1$, that is an increase in longitudinal relaxation rate $R_1$ ($R_1=1/T_1$). Gd-DTPA-CS$_n$ also demonstrated a shorter $T_1$ than Gd-DTPA at the same concentration. Moreover, $R_1$ of each solution was plotted with the Gd$^{3+}$ concentration. The $r_1$ was obtained from the linear regression between $1/T_1$ (s$^{-1}$) and the concentration (mM) according to Eq.(1). It was seen from Table 1, that $r_1$ was 11.65mM$^{-1}$·s$^{-1}$ for Gd-DTPA-CS$_{11}$, 10.36mM$^{-1}$·s$^{-1}$ for Gd-DTPA-CS$_8$ and 7.62mM$^{-1}$·s$^{-1}$ for Gd-DTPA-CS$_6$, which was 3.2, 2.8 and 2.1 times that of Gd-DTPA (3.62mM$^{-1}$·s$^{-1}$), respectively.

Possible explanations for increased relaxivity, relative to commercial contrast agent Gd-DTPA, include: (a) a decrease in rotational correlation rate by virtue of the attachment of the metal chelate to chitosan oligosaccharides with different DP; (b) an increase in the number of inner sphere coordinated water molecules; (c) an increase in the number of outer sphere coordinated water molecules[30–32]. However, compared with $r_1$ of dendrimer-based contrast agents [33,34], the relaxivity of Gd-DTPA-CS$_n$ was lower because the linear characteristic of the chitosan oligosaccharides made the part of Gd-DTPA easier to rotate, which partly reduced the relaxivity resulting from increase of the rotational correlation time of the chelated part of the metal.

The left column of Fig. 3A showed in vitro FLASH images prepared by Gd-DTPA-CS$_n$ (n=6, 8, 11) solutions in phantoms. The intensity in the center tube that contained water only, was used as baseline intensity. The right column of Fig. 3A showed the $T_1$-weighted signal enhancement levels ($\Delta S$%) of FLASH images, which were obtained by deducting the $T_1$-weighted FLASH images from the corresponding baseline intensity. It was seen that proton signal intensity increased with Gd$^{3+}$ complex concentration. $\Delta S$% was defined as follows:

$$\Delta S\% = \frac{\times (\text{pixel signal intensity/averaged signal intensity in the center tube})}{100−100}$$

Fig. 3B indicated the good linear relationships between the concentration of Gd-DTPA-CS$_n$ (n=6, 8, 11) and $\Delta S$. The slope of the plot of Gd-DTPA-CS$_{11}$ was the largest and the signal intensity was the strongest at the equivalent concentrations. The results were consistent with the effect of the $T_1$-relaxivity in vitro.
Due to the most excellent properties in vitro, Gd-DTPA-CS$_{11}$ was selected as researchable objective in the in vivo MRI studies with Gd-DTPA as a contrast. The axial T$_1$-weight images of SD rat liver and kidney are shown in Fig. 4, and Fig. 5. It is clear that the rat liver was bright and the signal intensity (SI) increased shortly after administration of Gd-DTPA. Subsequently, the SI in liver decreased slowly after 30 min and the SE in kidney was increased, which indicated Gd-DTPA was removed from the blood and eliminated through the kidney quickly. It indicated undesirable background enhancement due to fast extravasation into the extracellular fluid. Besides, the blood vessels of liver parenchyma of the rat receiving Gd-DTPA always were not observed clearly.

In contrast to the rapid rate of elimination of Gd-DTPA, the experimental group displayed much higher and more prominent enhancement in MR signal intensity in the liver shortly after injection of Gd-DTPA-CS$_{11}$. The blood vessel in liver parenchyma was clearly visible and the SI in the vessel persisted throughout the whole imaging periods. The signal in rat kidney was not detected, since Gd-DTPA-CS$_{11}$ was still not excreted from rat liver into the kidney during this tracking time. The effect of Gd-DTPA-CS$_{11}$ on the liver was more pronounced than Gd-DTPA.

The SI changes ultimately due to distribution (uptake) and elimination (excretion) of the contrast agents, whichever species they were in [35]. It shows that the hepatic uptake rate of Gd-DTPA-CS$_{11}$ is almost equivalent to Gd-DTPA, but the contrast and the intensity in liver were stronger compared to Gd-DTPA. The excretion rate of Gd-DTPA-CS$_{11}$ is much slower than that of Gd-DTPA, which implied that Gd-DTPA-CS$_{11}$ may be utilized as a liver-specific contrast agent to provide prominent imaging effect and stable imaging contrast for a longer time. Gd-DTPA-CS$_{11}$ produced more pronounced effect on liver than Gd-DTPA probably due to a combination of two effects: 1) a prolonged blood circulation time of Gd-DTPA-CS$_{11}$ because of its increased molecular weight; 2) T$_1$ effect in blood gave a shortened T$_1$ of liver since the liver is a well-perfused organ [36]. Therefore, the timing of contrast injection and long data acquisition for Gd-DTPA-CS$_{11}$ become less significant and an optimal imaging window is feasible. The low excretion rate of this agent in kidney is accompanied by the fact that renal MRI intensity enhancement of this contrast agent persisted changeless during the whole imaging period.

4. Conclusions

Gd-DTPA conjugates of low-molecular weight chitosan oligosaccharides with 6, 8, 11 DP and narrow PDI were designed and synthesized. An obtained Gd-DTPA-CS$_{n}$ (n included 6, 8, 11) agents are well soluble in water suitable enough to be injected in an in vivo experiment. Gd-DTPA-CS$_{n}$ displays a higher T$_1$-relaxivity than that of the widely used contrast agent Gd-DTPA, which makes it possible to reduce the risk of the toxicity by lowering the dose of Gd$_{3}^{3+}$ chelates. Gd-DTPA-CS$_{11}$ induces distinct signal increase in liver even in the blood vessels of liver parenchyma during the long-term tracking time. Gd-DTPA-CS$_{n}$ suggests a low toxicity in clinical resulting from the high T$_1$-relaxivity enhancement and the coordination of free Gd$_{3}^{3+}$ with substantial amine groups in nontoxic chitosan oligosaccharides, minimizing Gd$_{3}^{3+}$ release. Besides, Gd-DTPA-CS$_{n}$ shows a low manufactures cost because of the abundant raw material chitosan, simple reaction process, and low energy consumption. It can therefore be concluded that this present synthesized series may put an entry into a new family of practical MRI CAs with more merits than the former Gd-DTPA.

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Appendix A. Supplementary Materials

Supplementary data to this article can be found at http://dx.doi.org/10.1016/j.mri.2012.09.004.

References


