Characterization of Fe(III)-Deferoxamine and Mn(II)-Pectin as Magnetic Resonance Imaging Contrast Agents¹⁾

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To find new contrast agents for magnetic resonance imaging (MRI), the spin-lattice relaxation time (T_1)-reducing activities of metal complexes of EDTA, *N*-hydroxyethylethylenediamine-*N,N',N'*-triacetic acid (HEDTA), diethylenetriamine-*N,N,N',N'',N''*-pentaacetic acid (DTPA), deferoxamine, mugineic acid, and pectin with Fe(III) or Mn(II) were investigated. Strong activity was found in Fe(III)-deferoxamine, Fe(III)-mugineic acid, or Mn(II)-pectin. In the actual MRI tomogram, Fe(III)-deferoxamine exhibited a contrast-enhancing effect comparable with that of Gd(III)-DTPA, and a much stronger effect was observed for Mn(II)-pectin. Fe(III)-deferoxamine and the Mn(II)-pectin appear to be candidates, respectively, as a new intravenous contrast agent and an oral gastrointestinal one.

Key words MRI; contrast agent; manganese; iron; deferoxamine; pectin

Magnetic resonance imaging (MRI) is useful for diagnosing many diseases such as cancer and cerebral apoplexy.^{2–4)} The absence of radiation damage and high resolution in tomography have broadened the scope of applications of this technique. Gd(III)-diethylenetriamine-*N,N,N',N'',N''*-pentaacetic acid (DTPA) has been used clinically until the present as a contrast agent for MRI.^{5–8)} However, Gd is a lanthanide with anticoagulant action, and DTPA is a synthetic chelator not necessarily specific for the Gd(III) ion. DTPA has high affinity for Ca and Mg ions (stability constant $K_{ML}^M = 10^{10}$ [Ca], 10^9 [Mg]). Recently, serious side effects have been reported for Gd-DTPA.^{9–11)} Consequently, safer contrast agents for MRI are being sought. Gd(III) has seven unpaired electrons in the *f* orbitals, and its paramagnetism causes the contrast-enhancement in MRI based on the spin-lattice relaxation time (T_1)-shortening action of Gd(III). There should be considerable contrast-enhancing activity for the complex of Mn(II) or Fe(III), with each possessing five unpaired electrons in the *d* orbitals. In fact, green tea, the water-extract of high manganese-content crude drugs, or blueberry juice have all been previously shown to be satisfactory oral gastrointestinal contrast materials,^{1,12–14)} and their active constituents were identified as manganese(II) complexes with organic compounds or a free manganese(II) ion which reduce the T_1 of water protons.^{13–15)}

In the present study, to find novel contrast agents, T_1 -reducing activity, which is more important than T_2 -reducing activity in the contrast-enhancement, was examined in regard to metal complexes with various synthetic chelators such as EDTA, DTPA, and *N*-hydroxyethylethylenediamine-*N,N',N'*-triacetic acid (HEDTA), or natural chelators such as deferoxamine [DF], mugineic acid [MA], and pectin. Also, the contrast-enhancing actions of selected metal complexes were compared with that of Gd(III)-DTPA.

MATERIALS AND METHODS

Materials Deferoxamine mesylate [DF] was kindly provided by Ciba-Geigy (Japan), Limited. Mugineic acid [MA]

was isolated and purified according to the literature.¹⁶⁾ Pectin (from citrus) was from Nacalai Tesque, Inc. (Japan). All other materials were of the highest purity available.

Measurement of Spin-Lattice Relaxation Time (T_1) The T_1 of HOD protons in sample solution was determined by the inversion-recovery method using a Varian NMR spectrometer (model Gemini 200). The solution for measurement was prepared by adding D₂O (450 μ l) to a sample solution (50 μ l).

Preparation of Sample Solution for T_1 Measurement 20 μ l of each stock solution (metal concentration: 1000 ppm) for atomic absorption spectrometry was added to 40 μ l of each chelator solution (20 mM), followed by a 940 μ l of each buffer solution (0.1 M phosphate buffer pH 7, 0.025 M borate buffer pH 7, and 0.025 M acetate buffer pH 5). The resulting solution contains metal (20 ppm [*ca.* 0.36 mM for Fe, Mn and 0.14 mM for Gd] and a chelator (0.8 mM).

Measurement of Contrast-Enhancing Activity in Magnetic Resonance Imaging (MRI) NMR images were obtained using a General Electric Medical Systems Signa Advantage 5 instrument with a 1.5 T superconducting magnet by spin echo (SE; repetition time [TR], 500 ms; echo time [TE], 13 ms; flip angle [FA], 90°) or 3-dimensional spoiled gradient echo (3D-SPGR; TR, 11.3 ms; TE, 2.1 ms; FA, 20°) methods.

Preparation of Metal Complexes A 1:1 Fe(III)-DF complex was easily prepared by mixing DF (0.08 mmol) and Fe(III) ion (as Fe(ClO₄)₃·8H₂O) in aqueous solution (pH adjusted to 7.0 with *d*-NH₄OH and NH₄HCO₃ reagents). The complex solution was applied to a mini-column (Sep-Pak C₁₈, Waters) in 0.05 M NH₄HCO₃ buffer (pH 7.0) and then eluted with MeOH. The reddish fractions were pooled, concentrated, and freeze-dried overnight.

The 1:1 Fe(III)-MA complex was prepared in the same manner as the Fe(III)-DF complex. However, the complex could not be purified by the mini-column because it does not adsorb on the column. So, the complex was purified by washing the residue obtained by evaporating water from the reaction solution using acetone to remove NH₄ClO₄, followed by

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Fig. 1. Chemical Structures of Various Chelators

DF, Siderophore from *Streptomyces pilosus*; MA, phytosiderophore from barley.

freeze-drying overnight.

The Mn(II)-pectin complex was prepared by mixing 0.8% pectin aqueous solution (50 ml) and Mn(II) ion (0.05 mmol as MnCl_2), then adjusting its pH to 5.0 with 1 M NaOH. The solution was concentrated to ca. 17 ml by ultrafiltration (YM05 membrane, Amicon). The concentrated solution was diluted to 50 ml with water, and concentrated again by the same method. After a total of three times concentration by ultrafiltration to remove low-molecular weight substances, Mn(II)-pectin was obtained by freeze-drying the viscous solution.

RESULTS AND DISCUSSION

Figure 1 shows the chemical structures of chelators (ligands) examined here and Fig. 2 the relationship between the T_1 and pH of the metal (Fe^{3+} , Mn^{2+} , and Gd^{3+}) solution without a ligand. T_1 of Mn^{2+} solution was about 2.5 s at a pH range of 1 to 7. T_1 of Gd^{3+} solution increased from 3 s at pH 5 to 7 s at pH 7, though there was no change from pH 1 to 5. T_1 (1–2 s) of the acidic Fe^{3+} solution was markedly different from T_1 (10–12 s) of the neutral Fe^{3+} solution. T_1 thus clearly depends on pH.

At a physiological pH (pH 7), the T_1 of each metal complex solution was compared with that of Gd(III)-DTPA commercially available (Fig. 3). For Mn(II), T_1 was only 3 s for the metal solution, but it increased in the case of complexation with EDTA, HEDTA, DTPA, and MA. Significant T_1 -reducing activity was observed for Mn(II)-pectin. Gd complexation with EDTA or DTPA caused no significant change in T_1 . Fe complexation with the chelators, except pectin, reduced T_1 . Reduction was greatest for EDTA and DF, whose complexation with Fe(III) caused T_1 -reducing activity essentially the same as that of Gd(III)-DTPA. EDTA has a strong affinity not only for Fe(III) but also essential metals such as Ca ($K_{\text{ML}}^{\text{M}}=10^{11}$) and Mg (10^9). Fe(III)-EDTA would thus not be suitable as an intravenous contrast agent. On the other hand, DF is a Fe(III)-specific chelator ($K_{\text{ML}}^{\text{M}}=10^{31}$), a

Fig. 2. Relationship between T_1 Values of Proton Signals (Water) in Metal Ion Solutions and pH

siderophore from *Streptomyces pilosus*, and is clinically used for hemochromatosis.¹⁷⁾ Considering that iron is an essential metal and that DTPA is not a specific chelator for Gd(III), Fe(III)-DF should be safer and more useful than Gd(III)-DTPA as an intravenous contrast material. Neither Fe(III)-pectin nor FAC (ferric ammonium citrate, a commercially available oral gastrointestinal contrast agent¹⁸⁾) showed strong T_1 -reducing activity, whereas Mn(II)-pectin reduced T_1 strongly and thus may possibly serve as an oral gastrointestinal contrast agent. The pectin-like polysaccharide complex with Mn(II) was previously shown to be responsible for the contrast effect of green tea in MRI.¹³⁾ Mn(II)-pectin, similarly to the active component of green tea, should be quite safe. Table 1 summarizes the T_1 data for water proton signals in various metal-complex solutions (metal: 20 ppm). The Fe(III) complex (Fe: 40 ppm) with MA (a typical phytosiderophore) has activity comparable to that of Gd(III)-

Fig. 3. T_1 Values of Proton Signals (Water) in Metal-Complex Solutions (pH 7)

FAC, Ferric ammonium citrate.

Table 1. T_1 Values (s) of Proton Signals from Water in Various Metal-Complex Solutions (Metal: 20 ppm)

	Mn	Fe	Gd
Metal only	2.5 (0.4)	2.5 (0.2)	3.0
	2.7 (0.3) [pH 1]	1.5 [pH 1]	3.1 [pH 1]
	2.7 [pH 5]	12.1 [pH 5]	3.4 [pH 5]
	2.9 [pH 7]	10.2 [pH 7*]	6.3 [pH 7]
EDTA	2.5 [pH 7*]		
	4.4 [pH 7*]	4.7 [pH 7]	4.9 [pH 7]
HEDTA	3.5 [pH 7]	5.5 [pH 7]	
DTPA	4.6 [pH 7]	5.9 [pH 7]	5.3 [pH 7]
MA			5.7 [pH 7*]
	4.1 [pH 7]	6.8 [pH 7]	
DF	2.6 [pH 7*]	5.2 (40 ppm) [pH 7]	
	2.7 [pH 7]	5.2 [pH 7]	
Pectin (0.5%)	5.2 [pH 7*]	5.8 [pH 7*]	
		3.5 (40 ppm) [pH 7]	
Iron(III) ammonium citrate	1.9 [pH 7]	9.1 [pH 7]	
		8.5	
		8.4 [pH 7]	

pH 7, borate buffer (0.025 M); pH 7*, phosphate buffer (0.1 M); pH 5, acetate buffer (0.025 M). S.D. are given in parentheses.

DTPA (Gd: 20 ppm), and Fe(III)-DF (Fe: 40 ppm) exhibited markedly strong T_1 -reducing activity (3.5 s).

To confirm the actual contrast-augmentative activity of the selected metal complexes having the strong T_1 -reducing activity in MRI, the activity was examined for Fe(III)-DF, Fe(III)-MA, and Mn(II)-pectin. In Fig. 4, the contrast-augmentative activity of three complexes were compared with those for Gd(III)-DTPA and FAC under the following conditions: metal: 20 ppm, chelator: 0.8 mM, and 0.1 M phosphate buffer (pH 7.0). In the case of the spin echo [SE] mode, Mn(II)-pectin had the strongest activity (signal intensity: ca. 1800). On the other hand, the two Fe(III) complexes showed activity (ca. 800-1000) comparable to Gd(III)-DTPA (ca. 900). In spite of similar activities between Fe(III)-DF and Gd(III)-DTPA, the Fe(III) complex should be preferable to Gd(III)-DTPA as a contrast agent because the combination of the Fe(III)-specific natural chelator and iron, an essential element, is thought to be safer to our bodies than that of the nonspecific synthesized chelator and Gd, a lanthanide. Weak

Fig. 4. Cross-Section Photograph Taken through the Six Bottles Containing Each Metal Complex in MRI Computed Tomography (Spin Echo Method)

A, Photograph; B, signal intensity of each bottle. Metal concentration is 20 ppm. Pectin is 0.5% solution. The other ligands are 0.8 mM. Bottle 1, Gd(III)-DTPA; 2, Fe(III)-DF; 3, Fe(III)-MA; 4, Mn(II)-pectin; 5, FAC; 6, 0.1 M phosphate buffer (pH 7) only.

activity (ca. 600) was observed for FAC.

The isolation of the pure metal complexes is prerequisite for clinical use. Figure 5 shows the contrast-augmentative activity of the isolated metal complexes Fe(III)-DF, Fe(III)-MA, Mn(II)-pectin, and Gd(III)-DTPA at concentrations ranging from 0.046 to 1.25 mM. In the case of Gd(III)-DTPA, Fe(III)-DF, and Fe(III)-MA, the activity was dependent on the complex concentration. The Fe(III) complexes have less activity (ca. one-third) than that of Gd(III)-DTPA in both SE and 3D-SPGR modes, though these three metal complexes (20 ppm) exhibited comparable signal intensities in MRI, as mentioned above (Fig. 4). This could be accounted for by the fact that Gd is three times as high in atomic weight as Fe. In contrast to these complexes, Mn(II)-pectin showed unique behavior in the relationship between the concentration and the activity; it has a distinct maximum at 0.2 and 0.8 mM in the SE and 3D-SPGR modes, respectively. Of special interest is the fact that Mn(II)-pectin exhibited about twice the activity of Gd(III)-DTPA at a concentration of less than 0.2 mM (SE) and 0.4 mM (3D-SPGR) and also markedly stronger activity than that of Mn²⁺ solution. Such a big difference in the

Fig. 5. Contrast-Enhancing Effects for Selected Metal Complexes in MRI Computed Tomography

—◆—, Fe(III)-DF; —■—, Fe(III)-MA; —▲—, Mn(II)-pectin; —×—, Mn^{2+} ; —×—, pectin; —●—, Gd(III)-DTPA, —+—, water. A, Spin echo method; B, 3D-SPGR method.

T_1 -shortening activity between Mn(II)-pectin and Mn^{2+} solution was also observed in our previous report.¹³⁾ To elucidate the reason for this phenomenon, further investigations should be done in the near future.

In conclusion, the Fe(III) complex with the natural iron chelator, DF or MA, especially Fe(III)-DF, has virtually the same contrast-enhancing activity as Gd(III)-DTPA under the same concentration (ppm). In consideration of its safety for our bodies, Fe(III)-DF should serve well as an intravenous contrast agent in MRI. Contrast effects *in vivo* should be confirmed, and the organ-selectivity and the contrast effects against cancer tissues should be examined. Also, Mn(II)-pectin, similar to the active component in green tea, showed much stronger contrast-enhancing activity than FAC or even Gd(III)-DTPA. The significant enhancing effect can be expected for an even lower manganese concentration than that

of green tea and should continue after Mn(II)-pectin passes through, because only 3–4% of orally administered manganese is absorbed through some digestive tracts.^{1,19)} Consequently, Mn(II)-pectin should be considered usable as an oral gastrointestinal contrast agent in MRI, although its usage as an intravenous agent might be difficult because of its high viscosity.

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