

A novel blood-pooling MR contrast agent: Carboxymethyl-diethylaminoethyl dextran magnetite

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Abstract. Gadofosveset trisodium is available as a prolonged pooling vascular contrast agent for magnetic resonance imaging. As gadolinium (Gd)-based agents may increase the risk for nephrogenic systemic fibrosis in patients with severe renal insufficiency, the present study synthesized carboxymethyl-diethylaminoethyl dextran magnetite (CMEADM) particles as a blood-pooling, non-Gd-based contrast agent. CMEADM particles carry a negative or positive charge due to the binding of amino and carboxyl groups to the hydroxyl group of dextran. The present study evaluated whether the degree of charge alters the blood-pooling time. The evaluation was performed by injecting four groups of three Japanese white rabbits each with CMEADM⁻, CMEADM²⁻, CMEADM⁺ (surface charges: -10.4, -41.0 and +9.6 mV, respectively) or with ultrasmall superparamagnetic iron oxide (USPIO; -11.5 mV). The relative signal intensity (SI_{rel}) of each was calculated using the following formula: SI_{rel} = (SI post-contrast - SI pre-contrast / SI pre-contrast) x 100. Following injection with the CMEADMs, but not with USPIO, the *in vivo* pooling time was prolonged to >300 min. No significant differences were attributable to the electric charge among the CMEADM⁻, CMEADM²⁻ or and CMEADM⁺ particles when analyzed with analysis of variance and Tukey's HSD test. Taken together, all three differently-charged CMEADM²⁻ particles exhibited prolonged vascular enhancing effects, compared with the USPIO. The degree of charge of the contrast agents used in the present study did not result in alteration of the prolonged blood pooling time.

Introduction

Ultrasmall superparamagnetic iron-oxide nanoparticles (USPIOs) have been used as negative contrast agents. They exert T2- and T1-shortening effects, and their potential as

an angiographic contrast agent has been investigated (1,2). Its long blood half-life makes it possible to use USPIO as a blood-pooling agent during the early phase of magnetic resonance angiography (MRA), however, USPIO is not suitable for the late phase of MRA, due to the presence of phagocytic Kupffer cells (3). Gadofosveset, a gadolinium (Gd)-based blood-pooling agent, has been approved by the U.S. Food and Drug Administration for aortoiliac MRA in certain patients (4). A single dose can be injected for first-pass imaging; a post-injection interval of 10 to 20 min has been suggested for optimal steady-state imaging (5). However, the use of gadofosveset trisodium may increase the risk for nephrogenic systemic fibrosis (NSF) in patients with renal impairment (6).

Xiao *et al* (7) and Nitta *et al* (8-10) reported a novel, long-circulating blood-pooling contrast agent, which involved iron-based substances. Nitta *et al* synthesized carboxymethyl-diethylaminoethyl dextran magnetite (CMEADM) particles (8-10) and used this Gd-free contrast agent in experimental magnetic resonance imaging (MRI) studies (8,9). As with superparamagnetic iron-oxide nanoparticles (SPIOs) and USPIOs, CMEADM is based on iron-oxide. As CMEADM is coated with polymers, including dextran, to prevent aggregation, its pooling time in blood vessels is longer, compared with USPIO (11-13), and its surface carries a negative or a positive charge due to the binding of amino and carboxyl groups to the hydroxyl group of dextran (Fig. 1). The present study evaluated whether the degree of charge on CMEADM altered the blood pooling time in rabbits.

Materials and methods

Animals. All experiments were approved in advance by the ethical animal experiments committee of Shiga University of Medical Science (Otsu, Japan), and performed according to the Animal Care Guidelines of Shiga University of Medical Science. Female Japanese white rabbits (3.0 kg) were purchased from Japan SLC, Inc. (Tokyo, Japan). Prior to each MRI session, the rabbits were anesthetized with intramuscular injections of a mixture of ketaminehydrochloride (25 mg/kg Ketalar 50; Sankyo Yell Yakuhin Co., Ltd., Tokyo, Japan) and medetomidine hydrochloride (0.1 mg/kg; Domitor; Meiji Seika Co., Ltd., Tokyo, Japan). All animals were housed in a temperature-controlled room (24±1°C) on a 12-h light/12-h dark cycle. They had access to standard laboratory chow *ad libitum*.

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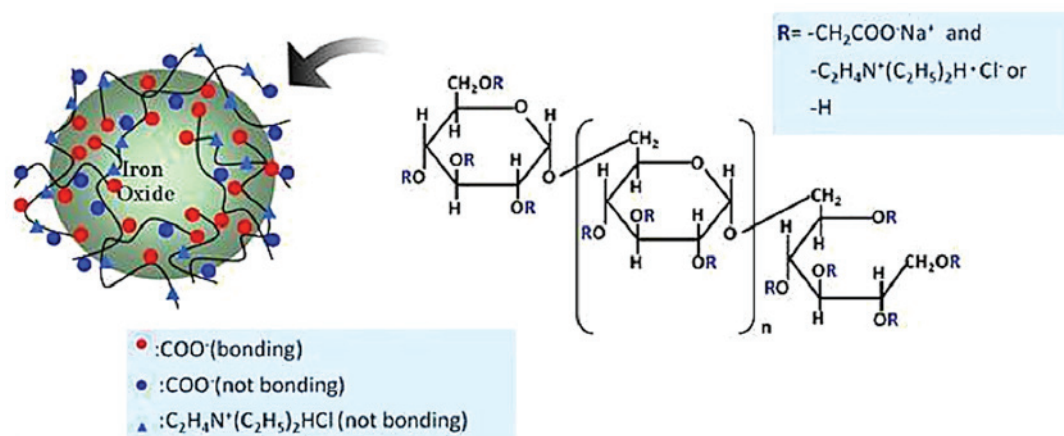


Figure 1. Surface of carboxymethyl-diethylaminoethyl dextran magnetite particles carries either a negative or positive charge due to the binding of amino and carboxyl groups to the hydroxyl group of dextran.

A total of 12 Japanese white rabbits were randomly divided into four groups of three rabbits. The animals received an intravenous injection of 40 $\mu\text{mol Fe/kg}$ of differently-charged CMEADM or USPIO (Table I).

Following pre-scanning, one of four types of iron nanoparticles, CMEADM⁻, CMEADM²⁻ and CMEADM⁺ with surface charges of -10.4, -41.0, and +9.6 mV, respectively, or USPIO (-11.5 mV), was injected intravenously into the auricular vein. All contrast agents were obtained from Meito Sangyo Co., Ltd. (Tokyo, Japan). MRA images were obtained immediately following injection, and at 30, 60, 180 and 300 min post-injection.

MRI. MRI scanning was performed on a 1.5T MRI instrument (SIGNA Excite HDx; GE Healthcare Life Sciences, Shanghai, China) with a circularly polarized head coil and the following scanning parameters: 3D fast-spoiled gradient echo; repetition time, 9.0 ms; echo time, 1.4 ms; flip angle, 25°C; field-of-view, 320x240 mm; slice thickness, 1 mm; matrix, 256x256.

The relative signal enhancement, or relative signal intensity (SI_{rel}), was measured at the abdominal aorta and the inferior vena cava (IVC) on 1-mm-thick coronal images. SI_{rel} was calculated using the following formula:

$$SI_{\text{rel}} = (SI_{\text{post-contrast}} - SI_{\text{pre-contrast}} / SI_{\text{pre-contrast}}) \times 100$$

These values were calculated by dividing the signal of the aorta or IVC by the background SI observed over time. Subsequent to the experiments, rabbits were sacrificed by injecting the heart with an overdose of pentobarbital (Dainippon Sumitomo Pharma Co., Ltd., Tokyo, Japan).

Statistical analysis. The results were analyzed by performing analysis of variance followed by Tukey's HSD test using IBM SPSS 20 statistical software (IBM SPSS, Armonk, NY, USA) for Windows. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Visibility of vasculature. The vessels were clearly visualized. Compared with the group injected with USPIO, the thoracic

and abdominal aorta, and the IVC manifested significantly higher SI values following the injection of the three types of CMEADM. Even at 300 min-post injection, the thoracic and abdominal aorta, and the IVC were clearly visible (Fig. 2).

Irrespective of their charge, with all three CMEADMs used in the present study, the vascular enhancing effect persisted for up to 300 min on the MRA images. By contrast, with the conventional USPIO, the intravascular SI was markedly decreased and had almost disappeared at 180 min.

Relative signal enhancement. SI_{rel} continued to be present in the abdominal aorta and IVC 30, 60, 180 and 300 min following the delivery of CMEADM⁻, CMEADM²⁻ or CMEADM⁺ (Figs. 3 and 4). At 180 and 300 min, the elevation of SI_{rel} persisted on all the CMEADM⁻ images, but not on the USPIO images. No significant differences were found in the enhancing abilities of CMEADM⁻, CMEADM²⁻ or CMEADM⁺ at 30 min post-injection.

Discussion

The present study compared standard USPIO and CMEADMs for their efficacy as blood-pooling MR contrast agents. All three differently-charged CMEADMs (CMEADMU⁻, CMEADMU²⁻ and CMEADMU⁺) elicited a sufficiently prolonged vascular enhancing effect in the rabbits. Investigations are underway to determine whether these contrast agents qualify as novel blood-pooling MR contrast agents in the clinical setting.

The present study documented that, with the newly synthesized iron nanoparticles, CMEADM, the retention of SI_{rel} in the blood vessels of the rabbits was prolonged. As these agents are Gd-free, they can be used in patients with renal impairment, including stage 4 or 5 chronic kidney disease (glomerular filtration rate $< 30 \text{ ml/min per } 1.73 \text{ m}^2$), who may be at risk for NSF when Gd agents are used. Although the injection of iron oxide nanoparticles may elicit cellular disorders, they may represent an alternative contrast agent for use in patients with renal impairment (14).

Conventional USPIOs have been used as negative contrast agents. However, as documented in the present study, their

Table I. Properties of the injected differently charged CMEADM or USPIO contrast agents.

Contrast agent	Particle size (nm)	Iron concentration (mg/ml)	T2 relaxivity (r2: mM/s)	T1 relaxivity (r1: mM/s)	Surface charge (mV)
CMEADM ⁻	32.0	15.0	114.0	34.0	-10.4
CMEADM ²⁻	29.0	15.0	91.0	34.0	-41.0
CMEADM ⁺	32.0	15.0	106.0	33.0	9.6
USPIO	28.0	15.0	95.0	34.0	-11.5

CMEADM, carboxymethyl-diethylaminoethyl dextran magnetite; USPIO, ultrasmall superparamagnetic iron-oxide nanoparticle.

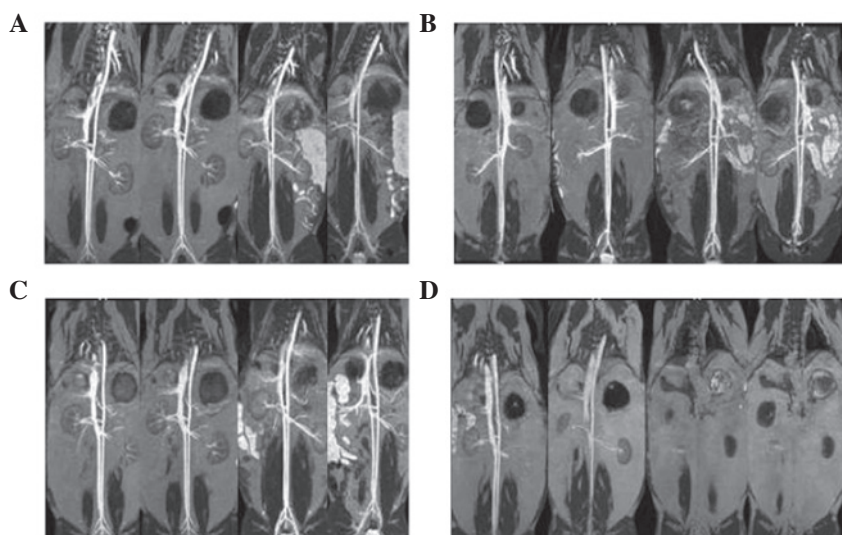


Figure 2. Vascular enhancement. On the magnetic resonance angiography images, the vascular enhancing effect persisted for up to 300 min with injections of (A) CMEADM⁻, (B) CMEADM²⁻ and (C) CMEADM⁺. By contrast, with (D) conventional USPIO, intravascular signal intensity was markedly decreased and almost absent at 180 min. CMEADM, carboxymethyl-diethylaminoethyl dextran magnetite; USPIO, ultrasmall superparamagnetic iron-oxide nanoparticle.

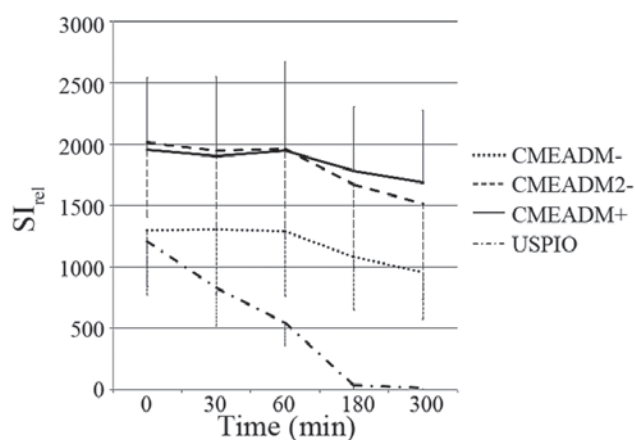


Figure 3. SI_{rel} patterns in the aorta. In the aorta, the SI_{rel} elicited by the three different CMEADMs remained high up to 60 min and then decreased. At 180 and 300 min, SI_{rel} values were significantly higher in the CMEADM²⁻ and CMEADM⁺ groups, compared with the USPIO group ($P < 0.05$). Data are presented as the mean of the three rabbits in each group. SI_{rel} , relative signal intensity; CMEADM, carboxymethyl-diethylaminoethyl dextran magnetite; USPIO, ultrasmall superparamagnetic iron-oxide nanoparticle.

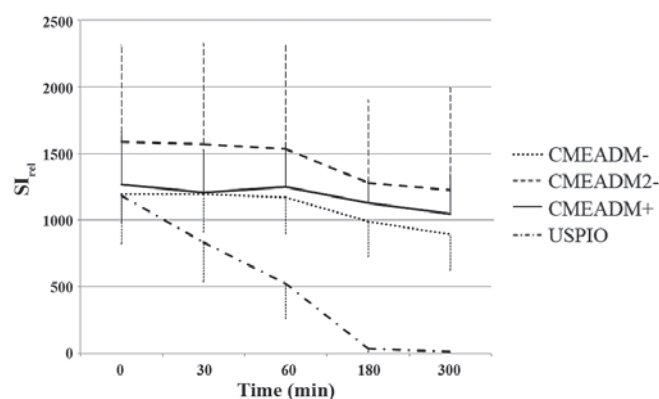


Figure 4. SI_{rel} patterns in the IVC. SI_{rel} patterns in the IVC were similar to those in the aorta. At 180 min, signal enhancement in the IVC was significantly higher in all charged CMEADMs groups, compared with the USPIO group ($P < 0.05$). At 300 min, SI_{rel} was significantly higher for CMEADM²⁻, compared with USPIO ($P < 0.05$). Data are presented as the mean of the three rabbits in each group. SI_{rel} , relative signal intensity; IVC, inferior vena cava; CMEADM, carboxymethyl-diethylaminoethyl dextran magnetite; USPIO, ultrasmall superparamagnetic iron-oxide nanoparticle.

pooling time was shorter, compared with the durations observed in the CMEADM groups. Smaller particles are less likely to be phagocytized, compared with large particles (11,13,15),

however, the sizes of CMEADM and USPIO particles are similar. According to Jo *et al* (16), iron oxide nanoparticles with positive surface potential interact ionically with the cell

surface as it is negatively charged. Consequently, the cell internalization of nanoparticles is increased.

The present study hypothesized that the negatively charged surface may affect the pooling time as a result of the decreased likelihood of phagocytosis. The charge of the USPIO in the present study was -11.5 mV. The present study synthesized three types of CMEADM with a similar charge of -10.4 mV (CMEADM), a lower charge of 9.6 mV (CMEADM⁺) and a higher charge of -41.0 mV (CMEADM²⁺), compared with that of USPIO, and calculated their SI_{rel} values. It was found that with all three types of CMEADM, irrespective of their charge, the blood-pooling time was prolonged. This suggested that their surface charge, whether positive or negative, did not affect the pooling time.

Iron nanoparticles, including USPIOs, are phagocytized in the liver and spleen, and metabolized via the same metabolic pathway as hemoglobin iron. However, although CMEADM may be metabolized via a similar pathway, these particles are different from conventional USPIOs, as the iron in CMEADM is covered by an increased quantity of dextran. It may be possible to retard their clearance from vessels by rendering their surface hydrophilic with poloxamers and poloxamines, including dextran (17). Covering CMEADM particles with dextran may reduce their recognition by phagocytes and/or their entrapment by Kupffer cells (8,18). The superior pooling time of CMEADMs in the blood stream may reflect a hydrophilic effect, and investigations are underway to determine whether this is the case.

CMEADM particles are considered to have a longer half-life, compared with USPIOs, and may be more useful for long-term angiographic investigations in humans. Due to the long pooling time of CMEAD in vessels, a single administration of the agent may suffice for the pre- and post-procedure angiographic evaluation of patients undergoing percutaneous transluminal angioplasty of coronary arteries, stent placement (percutaneous coronary intervention) or aortic stent grafting (8). In addition, MRI examinations using CMEADM agents may be used for the diagnosis of patients with intermittent gastrointestinal bleeding. Although Gd contrast agents may elicit complications, including NSF, to the best of our knowledge, there have been no reports of patients with serious adverse effects elicited by the administration of iron nanoparticles to date.

The present study had a number of limitations. First, the number of rabbits included in the investigation was small. However, it was of a sufficient size to enable assessment of statistical significance, and the prolonged enhancement effect of CMEADM 180 min post-injection was clearly demonstrated. Therefore, the number of rabbits used was deemed sufficient to support the conclusions. Second, it was not possible to compare the CMEADM with emerging non-contrast MRA techniques, including quiescent-interval single-shot MRA, with respect to vessel visualization (19). Third, the present study found that visually confirmed vascular enhancement persisted for up to 300 min. This may raise concerns regarding the wash-out kinetics of these agents. Although these agents do not elicit NSF or other renal complications, for example long elimination time, this is a significant limitation. Fourth, CMEADM particles are covered with an increased quantity of dextran, compared with USPIO. The dextran used for particle covering is the same substance used for ferucarbotran. Although the present study suggested that the use of CMEADM is safe, in rare instances, dextran

has been reported to elicit severe anaphylactic reactions (20). Consequently, the safety of CMEADM requires further elucidation. Investigations are underway to determine the elimination routes and kinetics, and to obtain quantitative data.

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