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In vivo Magnetic Resonance Imaging of Atherosclerotic Lesions with a Newly Developed Evans Blue-DTPA-Gadolinium Contrast Medium in Apolipoprotein-E-Deficient Mice

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Key Words
Magnetic resonance imaging · Atherosclerosis · Contrast agent

Abstract
Background: Magnetic resonance imaging (MRI) contrast agents that specifically detect atherosclerotic plaque may be useful for the noninvasive detection of the plaque. We have recently developed a new contrast agent, Evans blue-DTPA-gadolinium (EB-DTPA-Gd), which selectively accumulates vascular lesions with endothelial removal. In this study, we examined whether EB-DTPA-Gd is also useful for in vivo imaging of atherosclerotic plaques. ***Methods:*** We used male apolipoprotein-E-deficient (ApoE–/–) mice of different ages (3, 6 and 12 months old) and age-matched male wild-type mice. After a single intravenous administration of EB-DTPA-Gd (160 μM/kg body weight), MRI T_1 signal was obtained in vivo. ***Results:*** Increased signal intensity in the aortic wall was noted within 10–20 min after intravenous injection of EB-DTPA-Gd and was maintained for 30 min. The MRI enhancement in the aorta of ApoE–/– mice was increased in accordance with age, whereas no such enhancement was noted in wild-type mice. Histological examination demonstrated that there was a topological correlation between the site of MRI enhancement and that of atherosclerotic plaque. ***Conclusions:*** These results indicate that EB-DTPA-Gd is a useful MRI contrast medium for the in vivo detection of atherosclerotic plaques.

Introduction

The endothelium plays an important role in the maintenance of vascular homeostasis, and its dysfunction leads to initiation and progression of atherosclerosis [1, 2]. It is also known that impaired re-endothelialization is

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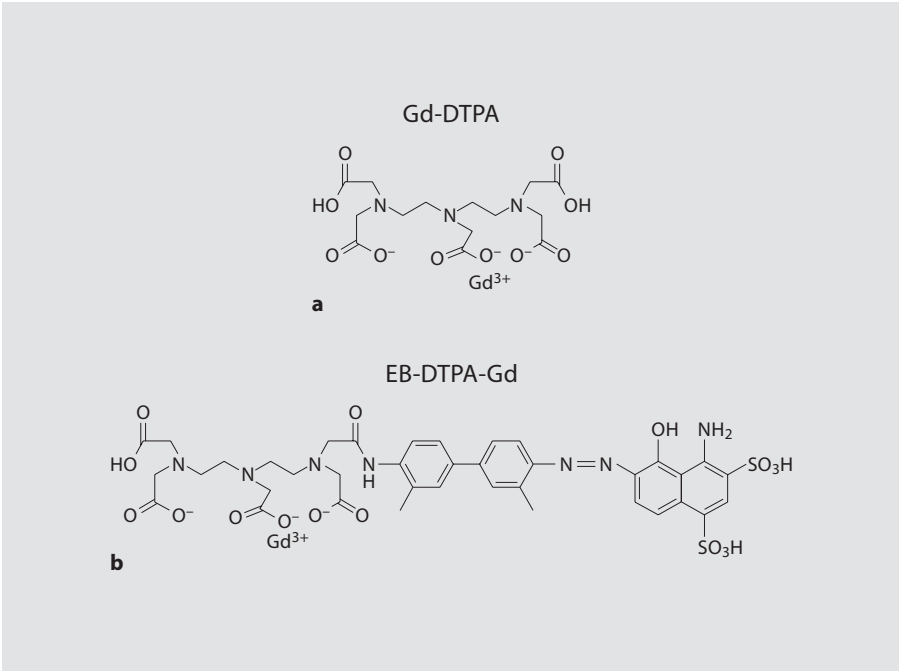

Fig. 1. Chemical structure of Gd-DTPA (**a**) and EB-DTPA-Gd (**b**).

closely related to delayed thrombosis following drug-eluting stent implantation [3, 4]. Imaging methods to detect endothelial dysfunction and the consequent progression of atherosclerotic plaque may aid early intervention in the treatment of vascular diseases [5]. Among them, magnetic resonance imaging (MRI) is emerging as a non-invasive technique with high special resolution and 3-dimensional capacity [6]. Contrast agents that target specific cells or molecules should improve the specificity of MRI and detect pathophysiological processes [7–9].

Evans blue (EB) dye that has a high protein affinity interacts with extracellular matrix and vascular smooth muscle cells when vascular endothelial cells are injured [10, 11]. We have recently developed a new EB-DTPA-gadolinium MRI contrast medium (EB-DTPA-Gd) [10] with the chemical structure of EB dye for the detection of vascular lesions associated with endothelial injury (fig. 1) [11]. An in vitro study using an isolated porcine aorta demonstrated that EB-DTPA-Gd effectively stained the denuded area and enhanced T_1-weighed MRI signals [10]. Furthermore, in a rat model of in vivo balloon endothelium removal, enhanced T_1-weighed signals were successfully detected at the injured site of the carotid artery [11]. In the present study, we further examined whether EB-DTPA-Gd enables us to detect atherosclerotic plaques in apolipoprotein-E-deficient (ApoE–/–) mice in vivo.

Methods

Synthesis of EB-EDTA-Gd

The method for the synthesis of EB-DTPA-Gd has been reported previously [10, 12]. Briefly, EB-DTPA was dissolved in deionized water to a concentration of 10 mM, and 1 M aqueous gadolinium chloride solution containing equimolar gadolinium ion was added to EB-DTPA. After adjustment to pH 7, the solution was lyophilized to obtain the desired MRI contrast agent solid, EB-DTPA-Gd. Its chemical structure is shown in figure 1. In the present study, solid EB-DTPA-Gd was dissolved in saline to a final concentration of 20 mM.

Experimental Design

All experimental procedures were performed in accordance with the protocol approved by the Institutional Animal Care and Research Advisory Committee. We used male ApoE–/– mice and male wild-type mice with a C56BL genetic background as non-atherosclerotic controls [13]. After a single intravenous administration of EB-DTPA-Gd (160 μM/kg body weight) through the tail vein, all animals were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg), and then, the MRI T_1 signal was obtained in vivo (1.5 T Magnetom Vision system, Siemens, Germany; T_1-weighted spin-echo, TR/TE 400/14 ms, 1 mm slice thickness, field of view 50 mm, and dot matrix 128 × 256). We reconstructed the coronal images of the aorta and then set the sample area between the ascending (at the level of the aortic valve) and the descending (at the level of the iliac bifurcation) aorta. Pixel intensity was analyzed using Image J (National Institutes of Health, Bethesda, Md., USA).

We performed the following protocols. First, to evaluate the time course of contrast accumulation in the aorta, MRI T_1 signal

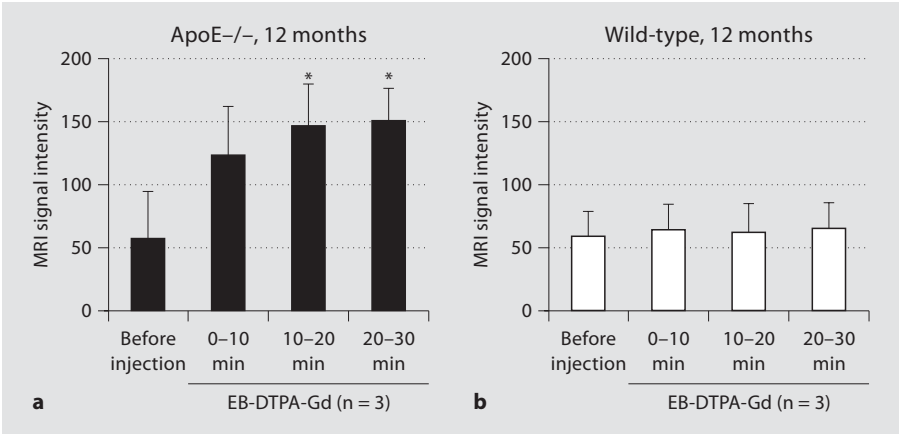

Fig. 2. Time course of MRI signal intensity in the aorta of 12-month-old ApoE–/– mice (**a**) and age-matched wild-type mice (**b**). Increased signal intensity in the aortic wall was noted within 10–20 min after an intravenous injection of EB-DTPA-Gd and was maintained for 30 min. * $p < 0.05$ versus before injection (by ANOVA).

imaging was obtained using 12-month-old ApoE–/– mice at 0, 10, 20 and 30 min following an intravenous administration of EB-DTPA-Gd (n = 3). We also compared the enhancement of the aorta by EB-DTPA-Gd between 12-month-old ApoE–/– mice and age-matched wild-type C57BL mice (n = 3 each). Second, because endothelial dysfunction and atherosclerosis are developed in accordance with age, we examined MRI signal intensity in 3-, 6- and 12-month-old ApoE–/– mice (n = 3, n = 4, and n = 4, respectively). Third, we performed an ex vivo vascular analysis using 12-month-old mice. Following the in vivo acquisition of MRI, the aorta was carefully isolated in full length and then longitudinally opened. Localization of atherosclerotic plaques was assessed using Sudan Red dye.

Statistics

Results are shown as means ± SD. Comparisons between 2 groups were made by unpaired Student's t test. Comparisons among 3 or more groups were carried out by a one-way analysis of variance (ANOVA). $p < 0.05$ was considered statistically significant.

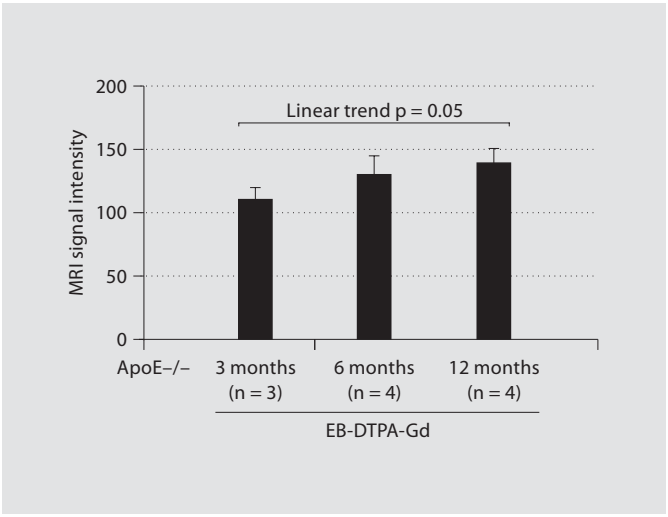

Fig. 3. Comparison of signal intensity among differently aged ApoE–/– mice.

Results

In ApoE–/– mice, increased signal intensity in the aortic wall was noted within 10–20 min after an intravenous injection of EB-DTPA-Gd and was maintained for 30 min with 1.4% of the coefficient of variation, whereas it was not evident in wild-type mice (fig. 2). Thus, the signal intensity measured at 10–20 min was significantly different between the 2 groups (age-matched at 12 months; ApoE–/– 146 ± 33 vs. wild-type 62 ± 23; $p < 0.05$). Figure 3 shows comparisons of signal intensity among differently aged ApoE–/– mice. There was a tendency of age-dependent increment of intensity in the aortic wall of ApoE–/– mice ($p = 0.05$).

We then examined the topological correlation between the site with MRI T_1 signal enhancement and that of atherosclerotic plaque identified macroscopically. Figures 4 and 5 show representatives of C57BL and ApoE–/– mice, respectively. In wild-type mice (fig. 4), no significant MRI T_1 signal enhancement was noted in the aorta with minimal staining by Sudan Red. In contrast, in ApoE–/– mice (fig. 5), the coexistence of the T_1 signal and the lesions stained by Sudan Red dye were noted all over in the atherosclerotic aorta. The MRI T_1 signal was particularly enhanced in advanced atherosclerotic plaques. These findings indicate the high affinity and accumulation of EB-DTPA-Gd to atherosclerotic plaques in vivo.

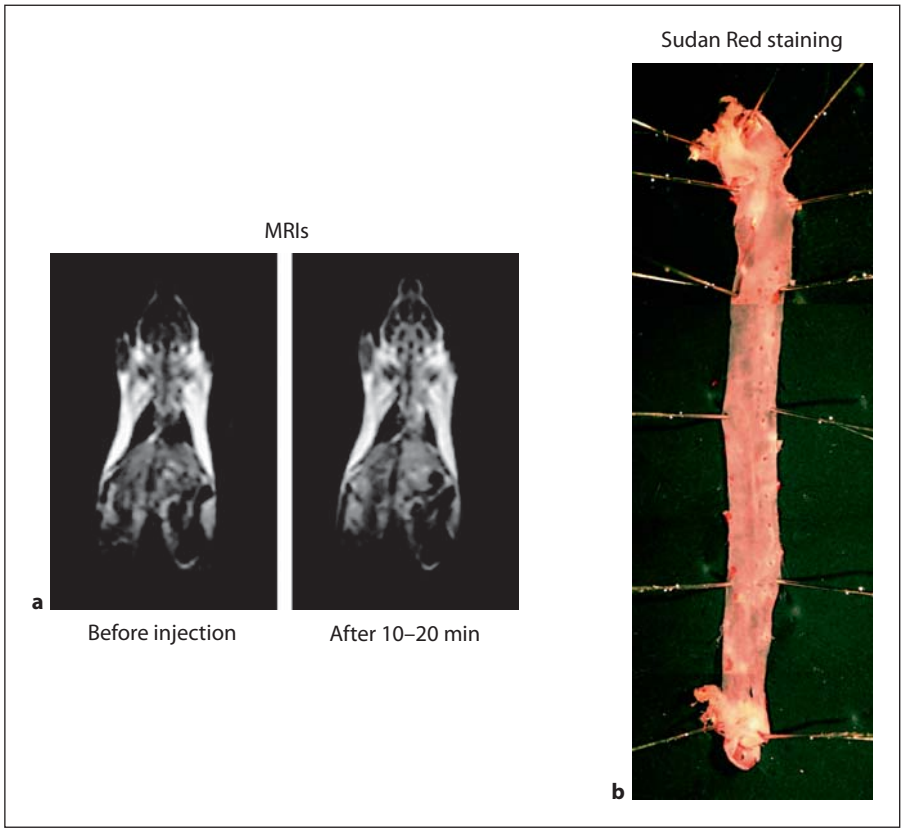

Fig. 4. Representative MRIs (T_1 weighted; **a**) and macroscopic photograph of the aorta stained by Sudan Red dye (**b**) in the same wild-type mouse. No significant MRI T_1 signal enhancement was noted in the aorta with minimal atherosclerotic plaque.

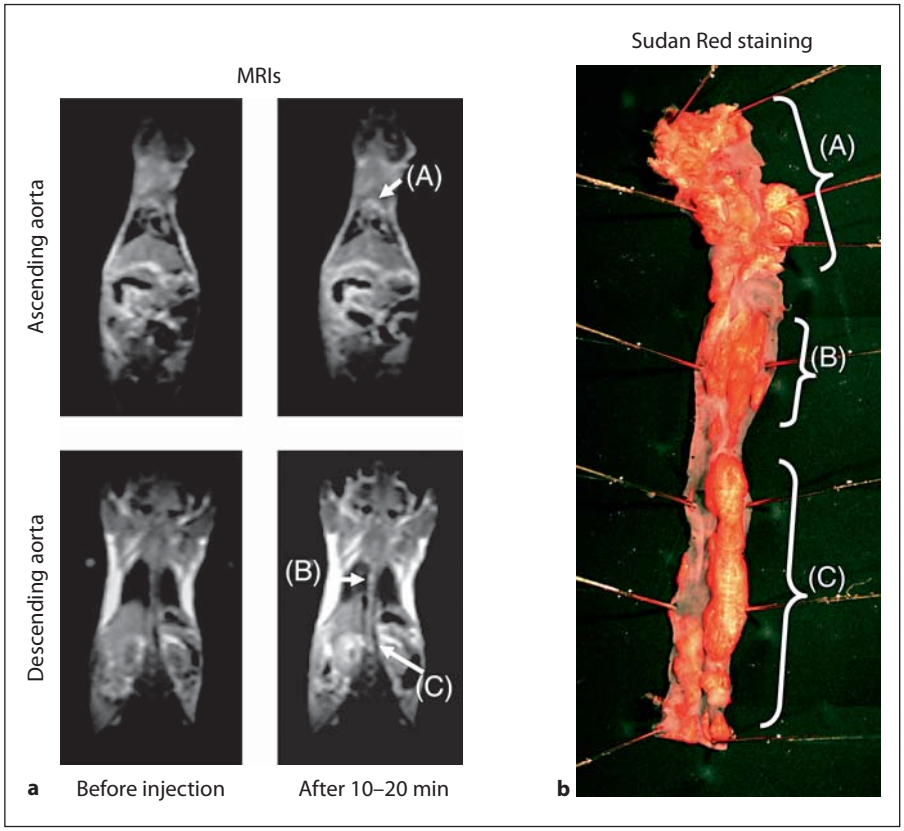

Fig. 5. Representative MRIs (T_1 weighted; **a**) and macroscopic photograph of the aorta stained by Sudan Red dye (**b**) in the same ApoE–/– mouse. There was a topological correlation between the sites of MRI T_1 signal enhancement and those of atherosclerotic plaques (indicated as A, B and C).

Discussion

The major finding of the present study is that EB-DTPA-Gd is useful for in vivo MRI detection of atherosclerotic plaques in ApoE–/– mice. Imaging methods to identify the progression and regression of atherosclerosis should play an important role in the management of patients with atherosclerotic cardiovascular diseases. MRI is widely applied because it enables us to noninvasively and simultaneously evaluate structural and functional changes in the blood vessel [5, 6]. Targeted contrast agents are under development to enhance the capability and specificity of MRI [7–9]. These may aid early intervention for both primary and secondary treatment of cardiovascular diseases.

Our EB-DTPA-Gd is a newly designed MRI contrast agent with EB structure conjugated to the DTPA frame (fig. 1). Originally, EB dye binds to serum protein and stains endothelial-denuded vascular lesions [11, 14]. However, as shown in previous studies [10, 12], the specific binding of EB-DTPA-Gd to the lesion with endothelial damage is independent of serum protein and blood stream. In addition to these unique characteristics, the present study provides an important implication. Our new MRI contrast agent, when intravenously administered, effectively detected atherosclerotic plaques in the aorta in a small animal, such as ApoE–/– mice (fig. 5). Increased accumulation of EB-DTPA-Gd in atherosclerotic plaques is in accordance with the previous study, which demonstrated that in ApoE–/– mice, endothelial dysfunction was strongly associated with plaque formation [15]. Also, in the present study, T_1 signal intensity by EB-DTPA-Gd was increasingly enhanced with age, a consistent finding with the notion that endothelial dysfunction and consequent atherosclerosis are enhanced with age (fig. 3) [1].

Despite the encouraging results of the present study, several limitations should be pointed out. First, only a single dosage of EB-DTPA-Gd was used. Dose dependency, in vivo bioactivity and adverse effects all remain to be examined in future studies. Second, 1.5 T of the magnetic field strength used in the present study was relatively low [16, 17]. Depending on the desired resolution, imaging conditions (e.g., field strength and pulse sequences) should be adjusted.

In conclusion, the present study demonstrates that our new MRI contrast agent, EB-DTPA-Gd, is useful for the in vivo detection of atherosclerotic plaques in mice. The high resolution of MRI and the development of sophisticated contrast agents may offer the promise of detailed in vivo imaging of atherosclerotic plaque and endothelial dysfunction.

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