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In vivo MR detection of vascular endothelial injury using a new class of MRI contrast agent

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Abstract—A new class of dye-based MRI contrast agents, EB-DTPA-Gd, was designed and synthesized. The contrast agent was found to accumulate at the site of endothelial injury when the reagent was applied to isolated porcine blood vessels or in an ex vivo experiment using rat. In vivo MR detection of vascular endothelial injury was also successful in rat with its common carotid artery injured by balloon treatment. These results indicate that EB-DTPA-Gd is potentially useful for the diagnosis of vascular diseases.

1. Introduction

Magnetic resonance imaging (MRI) has been used as an effective noninvasive diagnostic tool. For more effective diagnosis, various MRI contrast agents have been reported.[1,2] In T1-weighted MR imaging, gadolinium ion-chelate complexes have primarily been applied, as the gadolinium ion interacts with hydrogen in water molecules and enhances the T1-relaxation.[2] As a result, clearer images can be obtained when using these gadolinium ion complexes. In this context, many kinds of site-specific MR-contrast agents have been developed.[3–6] These agents have been designed with the intent of conjugating the targeting unit with the MR detection unit. In the site-specific MRI strategy, blood vessel is one of the most promising targets, as diagnosis of vascular disease in its early stages is essential to a successful treatment intervention. However, the development of such vascular disease-specific MRI has not yet been achieved. Various biomolecules such as antibodies for integrin,[3,4] ICAM-1,[5] or fibrin[6] that interact with proteins related to vascular disease have been used in the design of such MRI reagents. The use of these biomolecules, however, has proved to be impractical in terms of cost and volume of the agents. Another possible approach involves the use of synthesized organic molecules. It has been found that MS-325 (4,4-diphenylcyclohexyl phosphodiester Gd-DTPA derivative) forms a high-molecular weight complex with albumin before its adsorption onto plaque. As such, this molecule has been applied to the imaging of blood vessels in the SLE mouse.[7]

We, however, have focused our attention on endothelium lesions as a target of the MRI contrast agent for the diagnosis of vascular disease. The vascular endothelium plays an important role in the regulation of vascular homeostasis. As such, any damage to the endothelium often leads to a serious vascular disorder such as spasms in myocardial infarction.[8] In these lesional sites, it may be possible that a certain type of molecule could interact with the extracellular matrix or the smooth muscle layer through hydrophobic or electrostatic interactions due to a loss or weakening of endothelial regulation of substances.[9] We therefore

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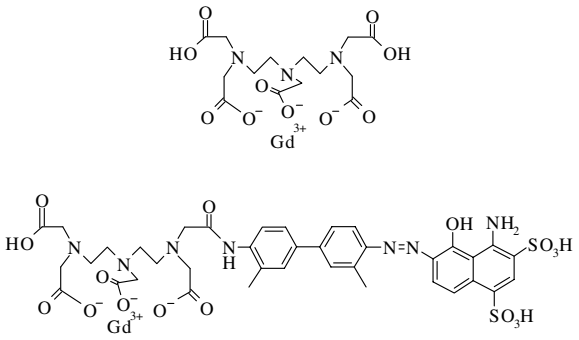

Figure 1. Chemical structure of MRI contrast agents, Gd-DTPA and EB-DTPA-Gd.

screened various organic dyes that have been used in histochemistry and found some azo-dyes that were selectively adsorbed to endothelium-denuded regions in an isolated porcine aorta sample. These dyes can interact with proteins or various tissues probably due to their good hydrophilicity or hydrophobicity balance, although accurate molecular mechanism of the binding has not been elucidated. Of these dyes, we have found Evans Blue capable of most effectively identifying the endothelium-injured region.

We have recently reported the synthesis and basic properties of an endothelium lesion-specific MRI contrast agent, EB-DTPA-Gd (Fig. 1).[10] The reagent was designed using the chemical structure of Evans Blue. This agent selectively accumulates on the endothelium-denuded surface of the porcine aorta section and enhances its signal intensity of the surface, as observed on the T1-weighted MR images.

In the present study, we applied the vascular endothelial lesion-specific contrast agent, EB-DTPA-Gd, to living rat for both ex vivo and in vivo MR imaging. First, the isolated porcine blood vessels, the endothelium of which is partially removed by scalpels, was treated with EB-DTPA-Gd in the presence of serum proteins. We next attempted ex vivo and in vivo MR imaging of the rat vascular injury at the common carotid artery, which was injured with a balloon catheter.

2. Experimental

2.1. In vitro evaluation of an isolated porcine aorta treated with EB-DTPA-Gd in the presence and absence of serum protein

A vascular endothelial lesion-specific MRI contrast agent module, EB-DTPA, was successfully synthesized, as described previously.[10,11] Complexation of EB-DTPA with gadolinium ion was achieved as follows; EB-DTPA was dissolved at 10 mM in water containing a ca. 1.5 M excess of Na_2CO_3 and equimolar gadolinium chloride was added. After adjustment to pH 7, the solution was lyophilized to obtain the desired MRI contrast agent solid, EB-DTPA-Gd. To evaluate the effects of serum proteins, EB-DTPA-Gd was dissolved in porcine serum[12] or pure water to a final concentration of 10 mM.

The specimens of porcine aorta were extracted and opened to a rectangular shape. The endothelium of the left half of the aorta was then carefully removed by scalpel (Fig. 2a). The blood vessel section was stained with each MRI contrast agent solution for 10 s and washed with saline. The aorta section was then evaluated by MR imaging (1.5 T MAGNETOM VISION system (SIEMENS, Germany), T1-weighted spin-echo, TR/TE = 400/14 m s, 3 mm slice thickness, field-of-view 50 mm, and dot matrix 128*256). The obtained MRI image was analyzed using NIH Image software.

2.2. Ex vivo or in vivo evaluation of EB-DTPA-Gd using the rat

A solid EB-DTPA-Gd was dissolved in saline to a final concentration of 24 mM. Rats (about 300 g) were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg), and then a balloon injury of the left carotid artery was made as previously described.[13] The MRI contrast agent solution was injected to the rat via the jugular vein at 160 μmol/kg. In the ex vivo experiment, the right and left common carotid arteries were extracted after 10, 30, or 120 min of reagent injection. The arteries were then opened and fixed on glass plate, and evaluated T1-weighted MR images using the same parameters as those for the in vitro experiment as described earlier.

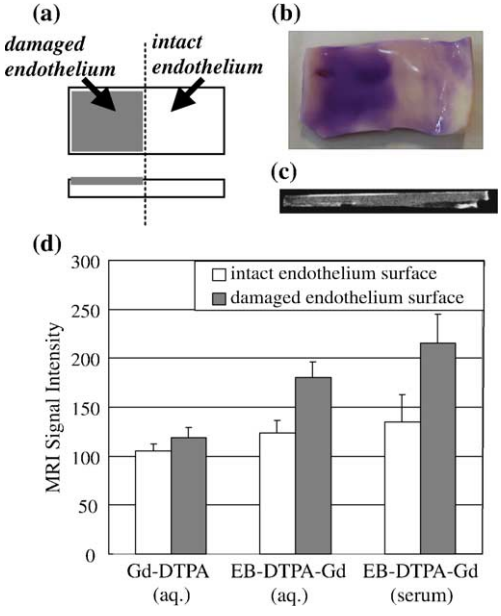

Figure 2. (a) Schematic illustration of the porcine aorta section. The left-half endothelium was removed, and the right half was intact. (b and c) The photograph and T1-weighted MR image (side view) of porcine aorta section stained with EB-DTPA-Gd in the presence of serum proteins. (d) Comparison of the surface MRI signal intensities of the porcine aorta section. The error bars represent the standard deviation ($N = 3$).

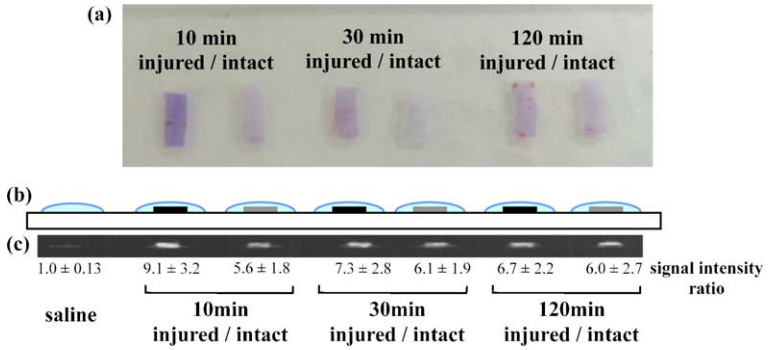

Figure 3. Ex vivo results for the opened common carotid arteries of the rat after the EB-DTPA-Gd injection via jugular vein. (a) Photograph of the extracted rat common carotid arteries at the elapsed times (10–120 min) after the injection of EB-DTPA-Gd. (b and c) Schematic illustration and MR image of both arteries dipped into saline (7 μL). The signal intensity ratio was calculated by comparing the MRI signal intensities of each artery in the saline drop with that of the saline drop alone.

The similar procedures were repeated for in vivo MR imaging of another rat. The animal was anesthetized and kept alive after its left carotid artery being injured by balloon catheter method as described.[13] After the injection of the MRI contrast agents, serial T1-weighted spin-echo MR images of the left carotid artery through transaxial plane were obtained. MR equipment used for this in vivo experiment was a 0.2 T unit (MRP-20, Hitachi, Japan). The parameters for the sequence were as follows; TR/TE = 500/25 m s, 2.5 mm slice thickness, field-of-view 200 mm, and dot matrix 256*192. As a control, 24 mM Gd-DTPA saline solution was used in a similar manner.

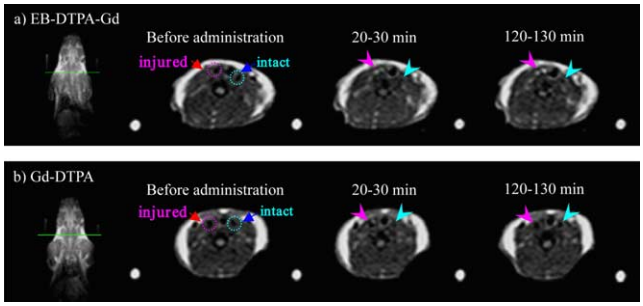

Figure 4. In vivo MR images of the rat with administration of (a) EB-DTPA-Gd and (b) Gd-DTPA via left femoral vein. The left common carotid artery was injured, and the right one was intact. The arrows in the figure indicate each artery.

3. Results and discussion

Various dyes, including Evans Blue and Congo Red, have been reported to bind to serum proteins.[14] It therefore seemed likely that EB-DTPA-Gd would also bind to serum proteins. We therefore investigated whether the presence of the serum proteins affect the characteristics of EB-DTPA-Gd, namely ability to accumulate at the sites of endothelial injury. The endothelium condition is schematically illustrated in Figure 2a. In the presence of serum proteins, EB-DTPA-Gd selectively accumulated on the endothelium injured surface (Fig. 2b). T1-weighted MR images revealed significant signal enhancement only on the endothelium-removed surface of the specimens, treated both with porcine serum and pure water solution of EB-DTPA-Gd (Fig. 2c and d). This result indicates that the serum does not affect the endothelium lesion-specific binding of EB-DTPA-Gd. The nonspecific binding of EB-DTPA-Gd slightly increased if compared with the result in the absence of serum proteins. This increase may be due to formation of the high-molecular weight complex of EB-DTPA-Gd with serum proteins.

For the next experiment, ex vivo MR imaging of the vascular endothelium injury was performed. In ex vivo study, carotid arteries with and without endothelial injury were clearly distinguished by the presence and absence of EB-DTPA-Gd accumulation, respectively, both by macroscopic observation and by T1-weighted MR images as well (Fig. 3). Figure 3c shows the MR image of the same section of aorta. The MRI signal intensity of the extracted left common carotid artery was largest after 10 min of the EB-DTPA-Gd injection. As time elapsed, the signal intensity of the left carotid artery gradually decreased, and 120 min after injection of EB-DTPA-Gd, it was almost the same as that of the right (intact side) carotid artery. The signal intensity of the right carotid artery slightly increased after EB-DTPA-Gd injection as compared to the baseline level, probably due to nonspecific binding of the contrast agent to the intact endothelium. As a site-specific contrast agent based on small organic molecule, MS-325 has been reported. The agent recognizes a inflammation site in blood vessels of SLE mice.[7] However the mechanism of the specific accumulation at the target site totally depends on the property of albumin, because the agent is carried by albumin. On the other hand, our compound possesses the recognition ability of the endothelial injury by itself.[10] Although the accurate mechanism of the recognition has not been clear.

For the final experiment, in vivo MR detection of the rat vascular injury was performed. The in vivo MRI results are shown in Figure 4. With the injection of EB-DTPA-Gd, the injured left common artery was clearly enhanced compared with the right intact common artery.

Gd-DTPA, however, did not enhance the MRI signal in either injured or intact artery. In contrast to the results of ex vivo study, the largest signal intensity of the endothelial injury was observed 120 min after EB-DTPA-Gd injection. We expected that the difference may be caused by the infiltration of the contrast agent into the tissue through the injured blood vessel surface.

4. Conclusion

Endothelial lesions are essential to the early-stage development of vascular diseases. We designed and synthesized a new dye-based MRI contrast agent, EB-DTPA-Gd, for the detection of such endothelium lesional sites. EB-DTPA-Gd was found to selectively bind to the target regions in extracted porcine aorta or living rat common carotid artery, even in the presence of serum or the blood stream. Finally, we preliminarily succeeded in carrying out noninvasive detection of injured blood vessel regions in living rat using an in vivo MRI technique. The contrast agent and the concept for the design using organic dye will be potentially useful in the development of a reliable diagnostic system that can detect vascular disease in its early stages.

Acknowledgements

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11. Precursor of the EB-DTPA, DMB-DTPA, was synthesized by the coupling of mono-Boc protected dimethylbenzidine and DTPA anhydride and was obtained as a TFA salt. The synthesis of EB-DTPA was performed as described below. All reactions were performed in an ice bath. DMB-DTPA·4TFA (0.53 g, 0.51 mmol) was dissolved in water (15 mL) containing HCl (1.52 mmol). Sodium nitrite (35 mg, 0.51 mmol) was then added in small portions, followed by stirring for 20 min. The diazonium salt solution was added dropwise into 15 mL of an aqueous 1-amino-8-naphthol-2,4-disulfonic acid (0.17 g, 0.51 mmol) solution containing sodium bicarbonate (0.43 g, 4.08 mmol), and then stirred for 3 h. The reaction mixture was lyophilized. The obtained solid was redissolved in water (10 mL), and the desired product was precipitated by concd hydrochloric acid. The precipitate was collected and dried under reduced pressure (0.36 g, 78%). The chemical structure was determined by ^{1}H NMR and elemental analysis.
12. Porcine serum was prepared as follows. Extracted porcine blood was centrifuged at 3000 rpm for 10 min to remove hemocyte. The concentrations of serum proteins obtained as the supernatant were determined to be 55 mg/mL (albumin: 33 mg/mL) by entrusting them to an outsourcer.
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