Visualization of therapeutic angiogenesis by a polymer-based magnetic resonance contrast agent

Jun-ichiro Jo[1,2], Ichio Aoki[1], Tsuneo Saga[1], and Yasuhiko Tabata[2]

[1]*Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, Chiba, Japan,* [2]*Department of Biomaterials, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Kyoto, Japan*

INTRODUCTION

With the recent progress of tissue engineering technology, the effects of regeneration-induction therapy of various tissues have been experimentally demonstrated in animal models. Furthermore, some clinical trials have also been successfully performed. Under these circumstances, it is strongly required to develop a non-invasive imaging method that can accurately evaluate the process of tissue regeneration and healing. Among various imaging modalities, magnetic resonance imaging (MRI), having a high spatial resolution and tissue contrast, is expected to be an optimal modality for this purpose. For the application of MRI for the monitoring of tissue regeneration, effective delivery of MRI contrast agents to the region being regenerated or repaired is essential. In this study, a new polymer-based MRI contrast agent has been designed to evaluate the therapeutic angiogenesis.

METHODS

Diethylenetriaminepentaacetic acid (DTPA) residue of a chelator was chemically introduced to dextran with the molecular weight of 74,000 (dextran-DTPA). Then, gadolinium ions (Gd) and cyclic peptides containing an arginine-glycine-aspartic acid (RGD) sequence (cRGD) with an inherent affinity for the $\alpha_v\beta_3$ integrin expressed on activated endothelial cells during angiogenesis were modified to dextran-DTPA (cRGD-dextran-DTPA-Gd, **Figure 1**). The longitudinal relaxation time (T_1)-weighted images and longitudinal relaxivities of DTPA-Gd, dextran-DTPA or cRGD-dextran-DTPA-Gd were obtained by two-dimensional multi-slice spin echo and saturation-recovery MRI, respectively.

Right femoral, external iliac, and deep femoral and circumflex arteries and veins were surgically ligated to prepare a mouse model of hindlimb ischemia. A laser Doppler analysis and histological evaluation revealed that the hindlimb ischemia was naturally healed accompanied with angiogenesis and the $\alpha_v\beta_3$ integrin was expressed in the ischemic region without any treatments. In this study, mice 7 days after the vascular ligation were used as an angiogenesis model. The T_1-weighted image was obtained after intravenous injection of dextran-DTPA or cRGD-dextran-DTPA-Gd into the mice (n = 5).

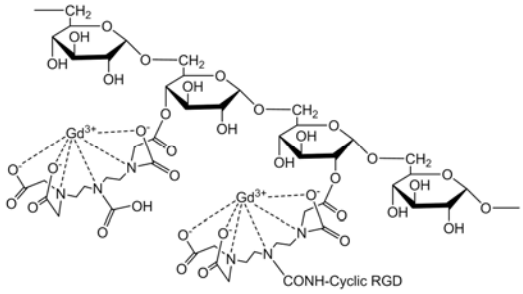

Figure 1. Chemical structure of polymer-based MRI contrast agent designed in this study

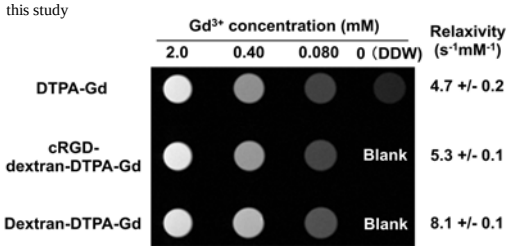

Figure 2. T_1-weighted images and relaxivities of various contrast agents with different concentrations. The T_1-weighted images were obtained by the following parameters: pulse repetition time (TR) = 400 msec; echo time (TE) = 9.57 msec; matrix size = 256 x 256; field of view (FOV) = 4.8 x 4.8 mm; slice thickness (ST) = 2.0 mm; and number of acquisitions (NA) = 1. For T_1 map calculation, the following parameters were used: TR = 500, 750, 1000, 1500, 3000, and 5000 msec, TE = 2.2 msec, matrix size = 256 x 256, FOV = 4.8 x 4.8 mm^2, ST = 2.0 mm, and NA = 1. The longitudinal relaxivity was calculated by the following formula; $R_1 = (1/T_1 - 1/T_0) / C$, where T_0 is the longitudinal time of DDW and C is the corresponding Gd^{3+} concentration.

RESULTS AND DISCUSSION

The longitudinal relaxivities of dextran-DTPA-Gd and cRGD-dextran-Gd were higher than that of low-molecular-weight DTPA-Gd (**Figure 2**). The ischemic-angiogenic region could be clearly detected with MRI by the intravenous injection of cRGD-dextran-DTPA-Gd (**Figure 3**). It was found by the immunohistochemical staining that the $\alpha_v\beta_3$ integrin was expressed in the ischemic-angiogenic region of mice and that the fluorescein-labeled cRGD-dextran-DTPA was co-localized in the integrin $\alpha_v\beta_3$-positive region after the intravenous injection (data not shown). Taken together, it is clearly demonstrated that the affinity of cRGD for the integrin $\alpha_v\beta_3$ enabled the cRGD-dextran-DTPA-Gd to selectively deliver to the ischemic-angiogenic region of mice with hindlimb ischemia.

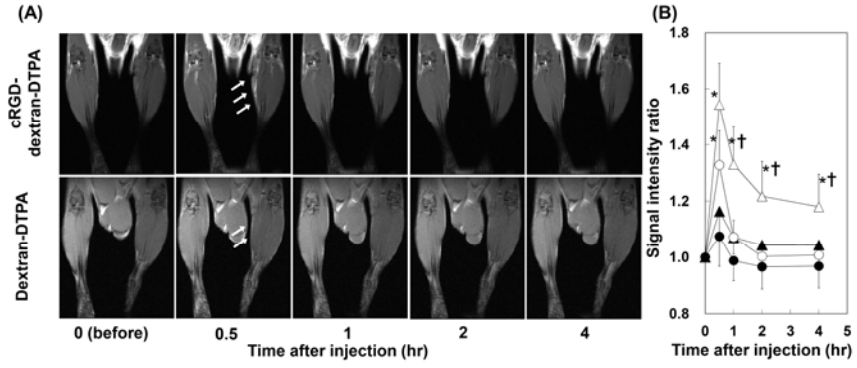

Figure 3. (A) Representative MR images of mice hindlimb region before or after injection of dextran-DTPA-Gd or cRGD-dextran-DTPA-Gd. The mice were placed in the supine position. The right side in the picture is ischemic hindlimb. Two dimensional T_1-weighted multi-slice spin echo MRI with fat suppression was performed in the following parameters: TR/TE = 9.57/250 msec, matrix size = 256 x 256, FOV = 3.2 x 3.2 mm^2, ST = 1.0 mm, and NA =16. Arrows indicate the ischemic-angiogenic region. (B) Time profiles of T_1 signal intensity ratio in the mice hindlimb after injection of dextran-DTPA-Gd (○, ●) or cRGD-dextran-DTPA-Gd (△, ▲). The signals (5 points each) were acquired in the normal (solid) and ischemic-angiogenic region (open). The hindlimb ischemia (5 mice each) was created 7 days before injection. The Gd^{3+} concentration of contrast agent is 5.0 mM. *, $p < 0.05$; significant against the normalized signal ratio in the normal region after the injection of corresponding agent at the corresponding time. †, $p < 0.05$; significant against the normalized signal ratio in the ischemic-angiogenic region after the injection of dextran-DTPA-Gd at the corresponding time.

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