# Diagnostic Technique of Rheumatoid Arthritis using Angiogenesis Specific MR Contrast Agents

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## Introduction

To diagnose disease progression in rheumatoid arthritis (RA), imaging techniques are playing an important role for many years. X-ray imaging techniques have been used to diagnose RA, but X-ray images can visualize relatively late stage such as joint space narrowing and bone erosion. On the other hand, magnetic resonance imaging (MRI) can directly show the bone and soft tissues and measure potential inflammatory activity and joint destruction. During early progression, angiogenesis is known to play a significant role in RA. In the current study, we investigated the inflammatory angiogenesis in RA using angiogenesis specific MR contrast agent, Gd-DOTA-RGD. Arginine-glycine-aspartic (RGD) peptide is well known to have a high specific affinity for αvβ3-integrin, which is over-expressed in endothelial cells during angiogenesis. Further, to confirm integrin specific contrast enhancement of Gd-DOTA-RGD, the blocking control experiments were performed

## Material and Methods

To obtain dynamic contrast enhanced (DCE) MRI, used T1-weighted CA which is Gd-DOTA-RGD. It was synthesized using the method described by park, et al [1], and Figure 1 shows the structure of Gd-DOTA-RGD. Two groups which are normal mice (N=6) and RA model mice (N=6) were used for *in vivo* test. RA model which is a murine collagen-induced arthritis (CIA) model was made using the previously described method [2]. The average body weight of DBA/1J mouse was approximately 25 g. A 1.5 Tesla (T) MR unit (GE Healthcare, Milwaukee, WI, USA) was used to obtain MR images with home-made small animal RF coil which is the receive-only, 1-channel, band-pass birdcage type and the inner diameter of the coil was 50 mm. The imaging parameters for the T1-weighted SE sequence were as follows: echo time (TE) = min, repetition time (TR) = 300 ms, receive bandwidth (BW) = 15.63 kHz, field of view (FOV) = 7 cm, slice thickness = 1 mm, number of excitation (NEX) = 4, spacing = 0, matrix = 192 x 128, phase FOV = 0.5, and scan time = 1′ 20″. DCE MR images were dynamically obtained during approximately 2 hours after Gd-DOTA-RGD injection. In no blocking αvβ3-integrin experiment, MR images were obtained pre- and post- Gd-DOTA-RGD (0.1 mmol Gd/kg) injection by tail vein, also blocking αvβ3-integrin experiment almost same with no blocking αvβ3-integrin experiment except for one thing that c(RGDYK) (0.1 mmol/kg) was injection on 30 min before Gd-DOTA-RGD injection. To show difference of contrast enhances, SI Images was converted to SNR images, and then made the Peak value image by using peak value from each pixel of same coordinate from SNR images. Then SNR difference image was obtained from difference between reference image and Peak value image. Figure 2 show image processing concept. Matlab was used to make image processing program for making difference map.

## Results and Discussion

Figure 3 show the induced rheumatoid arthritis at knee joint tissue of RA model by staining CD31. Figure 4a demonstrates that the knee joints in RA model show higher SNR difference (red to yellow) between reference image and DCE-MR images than the knee joints (blue to green) in normal model. In Figure 4b, RA group was enhanced approximately two times more than normal group and RA group slowly reached a peak point than normal group. When αvβ3-integrin receptors were initially blocked by c(RGDYK) and subsequently injected with Gd-DOTA-RGD, there were no differences in enhancement pattern and maximum SNR difference between RA group and normal group. These results from targeting and blocking experiments clearly indicate that Gd-DOTA-RGD is capable of targeting αvβ3-integrin receptors in the inflammatory angiogenesis. Compared to previous tumor angiogenesis study [1], it is also interest to note that the concentration of αvβ3-integrin receptor seems much lower in the RA reflecting inflammatory angiogenesis. Thus, in order to induce an observable contrast enhancement, a relatively large concentration of Gd needed to be loaded per integrin receptor, and the concentration of Gd-DOTA-RGD used in the current study seems to be sufficient to achieve that purpose. Further, Gd-DOTA-RGD enhanced MR images showed kidney enhancement suggesting that elimination of Gd-DOTA-RGD takes place mainly through glomerular filtration. In summary, the current study demonstrates the successful application of Gd-DOTA-RGD as a potential molecular MR agent for inflammatory disease such as RA with specificity to αvβ3-integrin receptor in the inflammatory angiogenesis.

## Reference

1. Park, J.-A. et al. Chembiochem : 9, 2811-3 (2008).
2. Liu, Z. et al. Journal of Clinical Investigation 112, (2003).

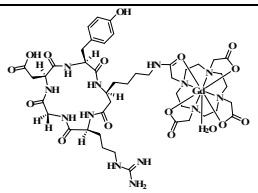


Figure 1. The structure of RGD-DOTA-Gd.

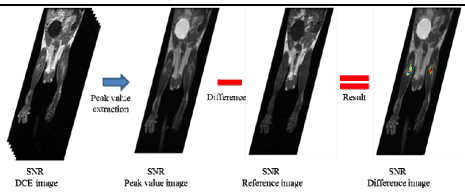
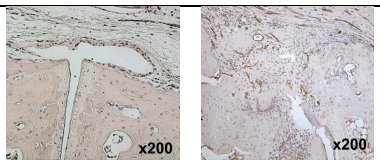
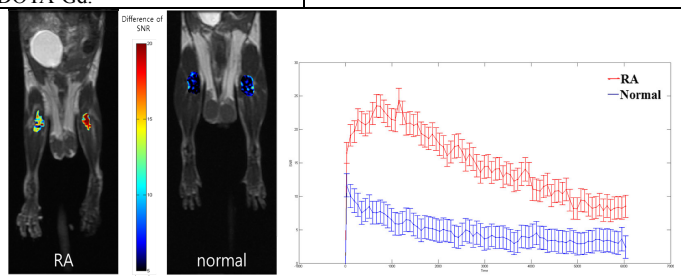



Figure 2. Image process concept for SNR difference image.



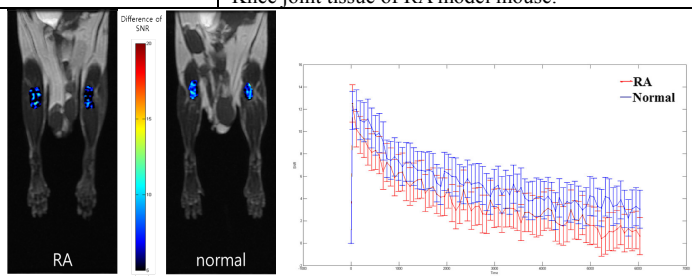

(a) (b)

Figure 3. (a) Knee joint tissue of normal mouse (b) Knee joint tissue of RA model mouse.




(a) (b)

Figure 4. (a)Difference map of RGD-DOTA-Gd. (b)SNR graph which shift to zero point at the knee joint after RGD-DOTA-Gd injection.




(a) (b)

Figure 5. (a) Difference map of RGD-DOTA-Gd after c(RGDYK) injection for blocking αvβ3-integrin. (b)SNR graph which shift to zero point at the knee joint after RGD-DOTA-Gd injection.