

Endothelial cell-specific MR imaging with gadolinium-based contrast agents

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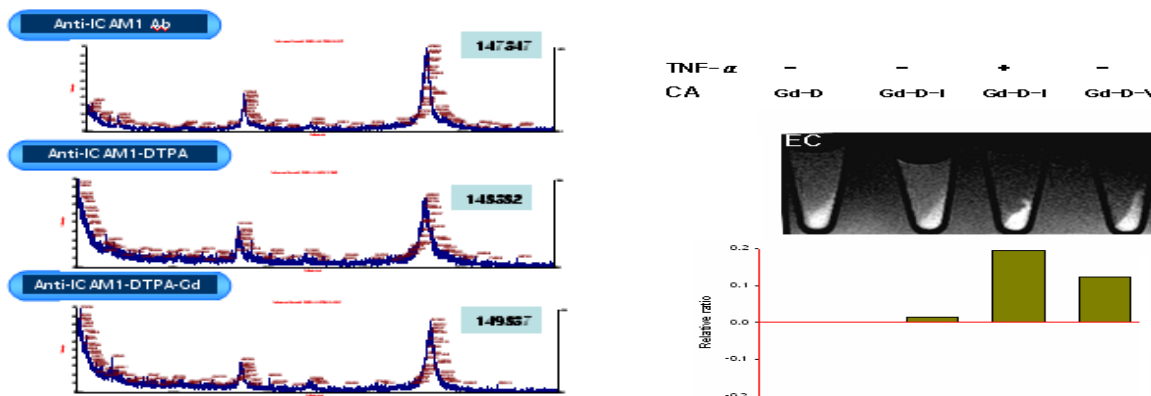
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Abstract Gadolinium chelates are widely used as T1 contrast agents for magnetic resonance imaging (MRI). To noninvasively examine highly vascularized tissues such as hyperangiogenic tumors, we designed blood vessel endothelial cell-targeted MR contrast agents which were prepared with bioconjugation of gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) and anti-VEGFR2 or ICAM-1 antibody. Gd-DTPA-anti-ICAM-1 antibody complex exhibited predominant binding to the endothelial cells which were stimulated with 2 nM TNF- α , a potent inducer of ICAM-1 on endothelial cells.

Introduction The advantage of the small molecule-Gd chelates is possible to adjust the size of the final contrast agent, when the Gd chelates is linked to the large molecule-targeting agents, such as antibodies. Visualization of blood vessels involves a variety of the physiopathological importance, such as monitoring tumor angiogenesis as well as disease-related vascular disorders. In order to target blood vessels with the MR contrast agents, endothelial cell-specific monoclonal antibodies(mAb), anti-mouse VEGFR2 and anti-mouse ICAM-1 were linked to DTPA using bioconjugation reaction. The DTPA-mAbs were then chelated with GdCl₃ to form the vessel-specific MR contrast agents,

Materials and methods The anti-VEGFR2 or ICAM-1 antibody was purified and conjugated to DTPABA in PBS for 24hrs at 4°C. One part of the DTPA-antibody complexes was then reacted with 20 parts of gadolinium chloride. The Gd-DTPA-antibody complexes (10 μ g/ml) were added to the endothelial and non-endothelial cells(5 x 10⁶) treated with TNF- α (2 nM) and placed at 4°C for 4 hrs. The cells were harvested by scraping and pelleted by centrifugation. MR image of the cell-containing tubes placed at the water-filled chamber was obtained by the 1.5 T module assisted with the 47mm surface coil. MR imaging was performed using spin echo sequence with the following imaging parameter (TR=500, TE=15, FOV=60mm, FA=90, slice thickness 2mm). The expression of VEGFR2 and ICAM-1 in the endothelial cells was also confirmed with immunoblot analysis.

Results The purified anti-VEGFR2 and ICAM-1 antibodies were conjugated with DTPABA. The molar ratio of DTPABA to antibody was 20 :1. Gd-DTPA-anti-VEGFR2 and ICAM-1 complexes were obtained in high yield. The molar ratio of antibody to gadolinium was 1:30. Induction of ICAM-1 by TNF- α resulted in high T1 signal intensity by Gd-DTPA-anti-mouse ICAM-1. Gd-DTPA-anti-mouse VEGFR2 showed relatively high signal intensity in the endothelial cells but not in the non-endothelial cells.



Discussion One of the most important technical elements to develop the cell or tissue-specific targeted MR contrast agents is the size control of the contrast agent to achieve relatively long life-time of circulation as well as successful delivery to the target tissues. The small molecule Gd-DTPA is considered as a useful lanthanide cation for preparation of targeted MR contrast agent using the cell-specific immunoglobulin molecules which have relatively high molecular weight.

Conclusions Our results showed that Gd-DTPA-anti-VEGFR2 antibody conjugates selectively detect the vessel endothelial cells and give endothelial cell-specific MR image. Moreover, Gd-DTPA-anti-ICAM-1 antibody complex appears to specifically bind the ICAM-1-expressing endothelial cells which are the typical characteristics of the inflammation, suggesting that Gd-DTPA-anti-mouse ICAM-1 antibody is a useful MR contrast agent for the inflammatory tissue.

References (1) Ralf C. Zimmermann, Tipton Hartman, Peter Bohlen, Mark V. Sauer, and Jan Kitajewski Preovulatory Treatment of Mice with Anti-VEGF Receptor 2 Antibody inhibits angiogenesis in corpora lutea. *microvascular research* 2001; 62:15-25. (2) D.Shahbazi-gahrouei, M.Williams, S.Rizvi, and B.J.Allen In vivo studies of Gd-DTPA-monoclonal antibody and Gd-porphyrins: Potential magnetic resonance imaging contrast agents for melanoma. *J Magn Reson Imaging* 2001;14:169-173. (3) Shahbazi-Gahrouei D, Rizvi SM, Williams MA, Allen BJ. In vitro studies of gadolinium-DTPA conjugated with monoclonal antibodies as cancer-specific magnetic resonance imaging contrast agents, *Australas Phys Eng Sci Med* 2002;25(1):31-38.