Bioinert polymeric MRI contrast agent for *in vivo* living endothelial progenitor cell tracking in rat ischemia model

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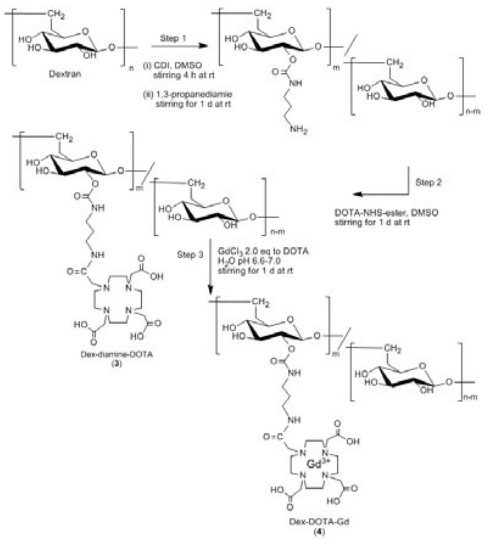
Introduction: Transplantation of autologous stem cells derived from peripheral blood, bone marrow or adipose tissue has been attracting great attention in the clinical stages because of the low risk of infection and rejection. In recent years, noninvasive tracking system for cell transplantation has been studied in order to clarify the mechanism for the cell transplantation therapy (1-3). Fluorescence- or bioluminescence- labeled cells are easily available and effective but they are applicable only for mice or rats due to the weak penetration of the light. In contrast, magnetic resonance imaging (MRI) is more promising system because of the high resolution and no limitation for the animal size. In this decade, superparamagnetic iron oxide particles (SPIO) have been used for labeling the cells. SPIO-based method is very sensitive but one of the biggest problems is the fate of free SPIO which leaks out of the cells due to the exocytosis or cell death. In order to overcome this problem, we recently succeeded to develop a water soluble polymeric contrast agent composed of poly(vinyl alcohol and Gd-chelate for cell labeling (4). In the present study, another water soluble Gd contrast agent using dextran and Gd-DOTA (1,4,7,10-tetraazacyclo-dodecane-N,N',N'',N'''-tetraacetic acid) was developed, and the in vivo fate of endothelial progenitor cells (EPCs) transplanted to rat ischemia limb model were investigated.

Methods: Dex-Gd conjugate was synthesized as follows (Scheme 1); dextran was dissolved in anhydrous DMSO and 1,1'-Carbonylbis-1H-imidazole was added. Reaction proceeded under nitrogen conditions at room temperature for 4 hours. Then, 1,3 propanediamine was added and stirred overnight at rt to give dex-diamine. About 1% of the total amino groups were reacted with FITC for studying the intracellular stability of the agent later. Dex-diamine was reacted with mono-N-succinimidyl 1,4,7,10 Tetraazacyclodecane-1,4,7,10 tetraacetate (DOTA) and treated with 2.2 molar excess of gadolinium chloride solution to give Dex-Gd.

EPC cells were harvested from the bone marrow of F344 rats (4 weeks old, male) after dosing granulocyte colony-stimulating factor (G-CSF). CD34 and FLK-1 positive bone marrow cells were isolated by magnetic beads method. Cells were grown for 2 months. Ten mM of Dex-Gd were added to the culture medium and an electrical pulses were applied to the cells using a CUY-21 electroporator in order to deliver the contrast agent into the cells. Male F344 rats (8 weeks old) were anesthetized and the left femoral artery and vein and their branches were ligated and totally excised. Dex-Gd-labeled EPCs were injected to the femoral muscle in three places. The intracellular stability and the intracellular distribution of



Scheme 1. Synthesis of Dex-Gd

the Dex-Gd were evaluated under the confocal microscope, the blood flow recovery of the ischemia rat was analyzed by laser Doppler method and angiography, and the in vivo cell migration was tracked with 1.5T MRI.

Results: In EPC transplantation group, the blood flow was rapidly recovered and it became to the normal level in 30 days. The angiography revealed that a number of the very small diameter blood vessels were formed in the ischemia region. Under the 1.5T animal MR imaging instrument cells can be clearly detected for 30 days postransplantation. We succeeded to clarify that the transplanted EPCs were migrates toward the ischemia region and that the transplanted cells died with time even for this syngeneic transplantation.

Conclusion: Water soluble dex- Gd contrast agent is very useful for labeling cells by electroporation method due to the very low cytotoxicity and the high intracellular stability. Since the contrast agent did not affect on the growth and differentiation of the living stem cells, it would be very useful for clarifying the detail of the stem cell transplantation therapy.

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