Monitoring of Cellular Uptake of Gadollinium by Dendritic Cells Useful For Tracking the Cell Trafficking in Tumor using MRI.

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Adoptive immunotherapy of tumors using dendritic cells (DCs) has gathered much interest due to its capability of harnessing the body's own immune system to fight against the tumor. In this technique autologous DCs are injected into a patient, which traffic into the tumor and trigger the immune response. Despite successful experimental clinical applications, the mechanism of action of DCs and their trafficking is poorly understood. We have initiated a project to track the trafficking of DCs into tumors using magnetic resonance imaging (MRI). Using a modification of the method developed by Crich et al.[1] , we chose a clinically approved formulation of a contrast agent (CA): Gd(III)-HP-DO3A in the form of Prohance™, to label DCs. Immature DCs were incubated with varying concentrations of ProHance™. The uptake of Gd(III) was determined by incubating DCs in 10% HNO_3 for 48 hours and comparing the T1 relaxivity of the supernatant at 20 MHz against a standard curve. The Gd-uptake was further validated by ICP-MassSpec analyses of the supernatant. Fluorescent activated cell sorting was used to assay MHC class I and II, CD 80, and 86 antigens. DC mediated enhancement of NK cell killing was assayed using NK:DC ratios (1.25:1; 2.5:1; 5:1; and 10:1). Co-cultured NK cells were then assayed for lytic activity against YAC-1 target cells in standard 4 hr, 51Cr-release assays at 100:1 and 50:1 Effector:Target ratios.

The cellular uptake of the CA was dependent on the extracellular concentration of the CA and resulted in a significant amount of incorporation at the higher CA concentration. The intracellular concentration of Gd(III) was approximately 5, 15, and 25 mM for cells incubated with extracellular ProHance concentrations of 6.25, 25, and 50 mM respectively. Gelatin suspended DCs loaded with CA showed significant differences in the signal intensity in both T1 and T2 weighted images relative to unloaded DCs (Figure 1), and the percent contrast enhancement of T_1 weighted images exceeded 50% even for cells treated with an extracellular ProHance™ of 6.25 mM .

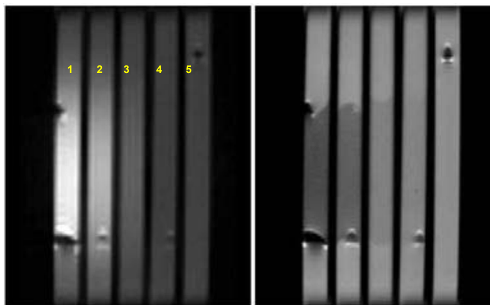

Figure 1. T1-weighted (left panel, TR = 350 ms and TE = 6.67 ms) and T2-weighted (right panel, TR = 4s and TE = 150 ms) imaging of ProHance labeled iDCs. Tubes: 1 = cells loaded with 50 mM ProHance; 2 = cells loaded with 25 mM Prohance; 3 = cells loaded with 6.25 mM Prohance; 4 = unloaded control cells; and 5 = gelatin.

Loading of DCs with ProHance™ did not induce a change in the expression of MHC Class I, Class II, CD80, or CD86 (Table 1). In terms of the effects DC loading with ProHance™ has on NK:DC reciprocal co-activation, we observed that there was no reduction in induced lytic activity at any of the ProHance™ concentrations tested. These data indicate that this CA does not inhibit DC function. The use of the clinically approved version of Gd(III)-HP-DO3A in this technique and its effective loading into DCs signifies the potential for its rapid translation into clinical applications to monitor biological cancer therapy.

[Prohance][a]	MHC Class I[b]	MHC Class II	CD80	CD86
0	61.7	61.9	13.9	56.5
50	76.0	74.5	35.9	76.0
25	72.7	68.0	23.3	70.2
12.5	74.4	61.1	19.7	79.9
6.25	84.2	59.9	26.9	75.4

Table 1. Effects of ProHance Labeling on the Expression of MHC Class I and Class II, and Co-Stimulatory Molecules on Dendritic Cells.

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[1] Crich SG, Biancone L, Cantaluppi, Duo D, Esposito G, Russo S, Camussi G, and Aime S, "Improved Route for the Visualization of Stem Cells Labeled with a Gd(III)-/Eu(III)-Chelate as a Dual (MRI and Fluorescence) Agent" Magn Reson Med 51: 938-944 (2004).

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