Targeted Contrast Using Gadolinium Labeled G5 Dendrimers

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Synthesis:

A target-specific MRI contrast agent for tumor cells expressing high affinity folate receptor has been synthesized and evaluated. The contrast agent was synthesized using generation five (G5) of polyamidoamine (PAMAM) folate-dendrimer and the bifunctional DOTA chelator (Dow Chemical Company) that forms stable complexes with Gadolinium (Gd). G5 PAMAM dendrimer was functionalized for targeting with folic acid. The remaining terminal primary amines of the dendrimer were conjugated with the bifunctional DOTA chelator. As a control, G5 PAMAM dendrimer was completely surface functionalized with DOTA chelator but without folic acid targeting moiety on the surface. Both dendritic chelators were then complexed with GdCl3. Dendritic chelators were titrated with GdCl3 followed by NMR as well as MRI measurement of their longitudinal relaxation rates (1/T1) of water.

MRI:

Analysis of NMR spectroscopy data revealed that targeted imaging agent (FA-G5-Gd) was loaded with 6.0% of Gd and non-targeted contrast agent (G5-Gd) was loaded with 7.7% of Gd. Both agents were dissolved in sterile saline to equimolar concentrations of Gd at 0.45 mg/ml. The relaxivity of Gd dendrimer was measured and found to be 19.4 ± 0.1 $s^{-1}mM^{-1}$. The increased relaxivity is likely to be due to the increased rotational correlation time of the G5 dendrimer.

A murine tumor model was established in NOD scid mice using human KB tumor cells over-expressing folate receptor (FAR+). Mice were maintained on a folate-deficient diet for 7 days prior to injection of tumor KB cells on both flanks of mice. Subcutaneous KB tumor nodules appeared 11 days post-implantation. Imaging agent was delivered through intravenous injection via the tail vein 30 days post-implantation. Targeted (FA-G5-Gd) and non-targeted (G5-Gd) dendrimers were delivered intravenously in 0.2 ml of saline at 0.029 mmol of Gd/kg. Two-dimensional and three-dimensional MRI images were obtained before and after administration of imaging agent (0.3 h, 4 h, 24h, 48h, and 120h post injection).

Results and Discussion:

The average signal intensity of the uptake and washout of targeted and non-targeted Gd-dendrimer was monitored and results are shown for heart and tumor in Fig. 1. Results in heart showed an increase of contrast immediately after injection (0.3 h), a complete washout within 24h and no difference in uptake between targeted and non-targeted imaging agent (Fig. 1). MRI images of tumor showed an increase of uptake within 24h with a higher retention of targeted Gd-dendrimer in tumor tissue (Fig. 2). The biggest difference between targeted and non-targeted uptake was observed at 24h and was maintained with a slow decline up to 120h (Fig. 1). Post-MRI analysis of cells isolated from tumors showed a high level of FA receptor positive cells using flow cytometry, indicating that the target was present during MR imaging and that the specific binding was possible (data not shown).

Conclusion:

We have synthesized a targeted contrast agent (FA-G5-Gd), demonstrated increased relaxivity compared to conventional contrast agents, shown specific binding of the targeted agent for FA receptor, and demonstrated selective accumulation in vivo of the targeted contrast agent.

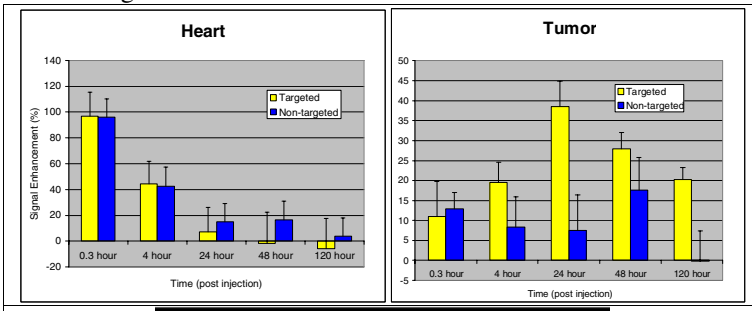

Figure 1 Pharmocokinetics of Gd labeled targeted (yellow) and non-targeted (blue) dendrimer in heart (left) and tumor (right). Data are plotted as the percent change in signal relative to the control image. Data from a region over the heart are representative of blood pool signal intensity. These results show that at 24 hours post injection, both the targeted and non-targeted contrast agents have cleared the blood pool. The data show selective enhancement of the tumor.

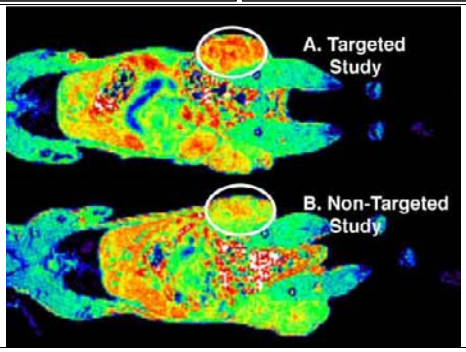

Figure 2. Examples of the gradient echo images of the control mouse (A) and the mouse injected with FA-G5-Gd to target folic acid receptor-positive tumor cells. The image of the targeted mouse was obtained 24 hours post injection of targeted contrast agent. The oval areas drawn on the tumors indicate the regions of interest used to compute the percent signal enhancement quantified in Fig. 1.