Internalization of MR Contrast Agent MGd by Vascular Endothelial and Smooth Muscle Cells

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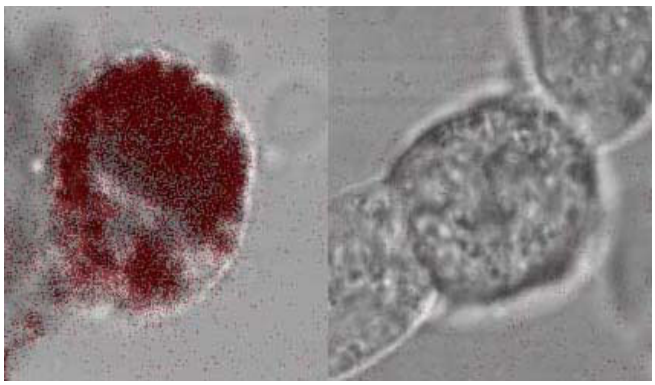
Abstract
We investigated the internalization of Motexafin Gadolinium (MGd, Xyctrin®, Phamacyclics Inc.), an MR contrast agent, by vascular endothelial and smooth muscle cells, and demonstrated MR signal enhancement of cells containing MGd. Cells were exposed to a range of MGd concentrations and were washed to remove MGd from the cell surface. Taking advantage of MGd's fluorescence properties, exposed cells were imaged with confocal microscopy to demonstrate internalization. These cells were also imaged with a 1.5T MR scanner to demonstrate increased signal intensity.

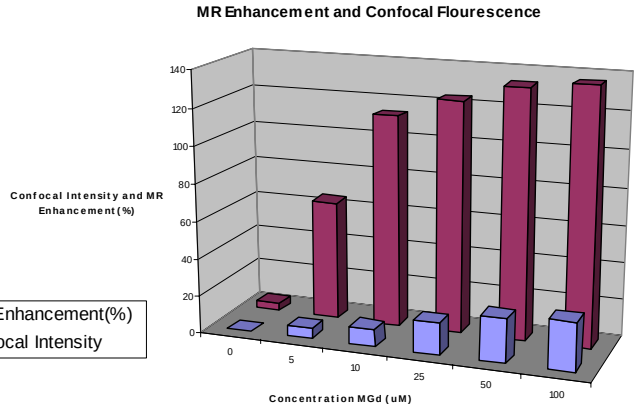
Introduction
Atherosclerotic ischemic heart disease is the leading cause of death in the USA. Angiography, which measures the degree of stenosis, is currently the clinical gold standard. However, plaque composition is a better predictor of clinical events than the degree of stenosis [1]. For example, a large lipid core with a thin fibrous cap is thought to indicate "vulnerable" plaque. The goal of this study was to investigate the internalization of an MR contrast agent by cells of the vascular system, and demonstrate signal enhancement of these cells. An MR contrast agent that is selectively internalized by vascular cells could generate contrast between different tissue types of atherosclerotic plaque, and thereby may provide a method for plaque characterization.

Methods
We investigated Motexafin Gadolinium. MGd is a texaphyrin molecule, a ring-shaped molecule that binds Gadolinium in the center. It functions as an MR contrast agent, and it fluoresces with excitation at 470nm and emission at 740nm. Vascular endothelial cells and smooth muscle cells were exposed to 0, 5, 10, 25, 50, and 100uM MGd in media for 24 hours in a 6-well plate. Cells were then washed 5 times to remove residual MGd and were allowed to culture for an additional 24 hours. The cells were then fixed in formalin on microscope slides, and imaged with a confocal microscope with excitation at 470nm, and emission long-pass filter of 700nm. Cells were also suspended in cell culture media in a 96-well plate, which was then imaged using a 1.5T GE MR scanner with T1-weighting.

Results
Confocal imaging showed internalization of MGd in both endothelial cells and smooth muscle cells. Flourescence was detected in exposed cells and was contained within the cellular membrane as shown in the figure1. Signal intensity vs concentration curves were created for confocal and MR imaging. All curves were similar in shape and showed nearly maximal intensity at 50uM concentration as shown in Figure 2. This concentration corresponds to an in vivo dose of 0.004mmol/kg MGd.



(**Figure1**: Confocal images of endothelial cells exposed to 50uM MGd (left) and unexposed cells(right) pseudo-red color indicates fluorescence)



(**Figure2**: Confocal signal intensity and MR signal enhancement of endothelial cells exposed to various concentrations of MGd)

Discussion and Conclusions
Our experiments demonstrated the internalization of MGd by vascular endothelial and smooth muscle cells using confocal microscopy, and demonstrated an increased MR signal intensity for cells with internalized MGd. We also determined a suitable in vivo dosage (0.004mmol/kg) of MGd for near optimal internalization and enhancement. Vascular cells are shown to maintain a high level of internalized MGd for at least 24 hours post-contrast enhancement. These findings suggest that MGd can generate contrast between various vascular tissue types involved in atherosclerotic plaque, and may be useful for plaque characterization using high-resolution, contrast-enhanced MR vessel wall imaging. An in vivo study examining the efficacy of MGd for atherosclerotic plaque characterization is currently underway using a heritable-hyperlipidemic Watanabe rabbit model.

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References
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