A Novel Class of Vanadium-based MRI Contrast Agents Specific for Highly Glycolytic Cancer Cells

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Abstract: We have developed a novel class of contrast agents that contain VO^{2+}-chelated organic ligands for magnetic resonance imaging (MRI). These contrast agents provided excellent T_1 and T_2^* contrasts compared to that of Gd-DTPA in high-resolution MR images of rodent tumors. The major objective of this study was to demonstrate that these contrast agents are taken up by highly glycolytically active colon cancer cells and target intracellular signaling pathways. Cells were pretreated with VO^{2+}-chelates and experiments were carried out to measure activations of intracellular kinases. We demonstrated that VO^{2+}-chelates activate kinases involved in glycolysis. MRI and atomic absorption studies of the soluble portion of cell extracts provided unambiguous evidence for the uptake of VO^{2+}-chelates into cancer cells. Furthermore, MRI experiments demonstrated preferential and persistent uptake of the VO^{2+}-chelates in some tumor regions.

Introduction: Improved methods for early detection and metabolic characterization of cancers are highly desirable for treatment of cancer. We developed a novel class of MRI contrast agents that are preferentially taken up by cancer cells. Aggressive cancers have enhanced glycolysis that results in increased uptake of glucose. Here we provide direct experimental evidence from *in vitro* MRI and atomic absorption (AA) studies that VO^{2+}-chelates are taken up by glycolytically active colon cancer cells. These compounds activated intracellular signaling pathways involved in glycolysis. Therefore the VO^{2+}-chelated contrast agents could provide high-resolution *functional* images of tumors that cannot be achieved by conventional, non-specific contrast agents.

Methods: To demonstrate the uptake of VO^{2+}-chelates in cancer cells, we have carried out *in vitro* MRI (Bruker scanner at 4.7 Tesla) and AA (Perkin Elmer) studies with intracellular extracts. For these experiments we chose HCA-7 and Caco-2 cells derived from human colonic adenocarcinomas. The following VO^{2+}-chelates were used: *bis* oxovandium(IV) complexes of acetylacetonato [$VO(acac)_2$], maltolato [$VO(malto)_2$], and *N*-oxide-pyridine-2-thiolato [$VO(OPT)_2$]. Cells were plated on 6-well plates at 250,000 cells/well. Each VO^{2+}-chelate was added to a final concentration of 100µM, 250 µM, or 500 µM and cells incubated for 5 min. The cytosolic fraction of cell extracts was collected for MRI and AA studies. To further asses if VO^{2+}-chelates induce intracellular signals, we examined AKT (protein kinase B) and ERK (extracellular signal receptor kinase) activations by Western blotting using antibodies to phospho-active AKT and ERK. They are both intracellular kinases that play key roles in many cellular processes. In addition, MR images of tumors implanted in the hind-limbs of rats were obtained at 4.7 Tesla before and after I.V. injection of contrast media.

Results: The results are summarized in the figure below. In panel A, we illustrate MR images of soluble extracts after cell treatment with vehicle (Control) or $VO(acac)_2$: water (lane 1); $VO(acac)_2$ (top two spots in lane 2), controls (bottom 2 spots in lane 2); washings (lane 3); and buffer (lane 4). The spin lattice relaxation time T_1 for extracts from $VO(acac)_2$-treated cells was 899 ± 149 msec, which was significantly lower than all other samples (2089 ± 234 msec). This lower T_1 time for extracts from $VO(acac)_2$-treated cells reflects significant paramagnetic vanadyl concentrations in the cytosolic fraction of cell extracts. Panel B summarizes the amount of intracellular vanadium in HCA-7 cells as measured by atomic absorption. The amounts of intracellular vanadyl are indicated in the inset. These results clearly demonstrate there is intracellular uptake of vanadyl into colon cancer cells exposed to VO^{2+}-chelates – but no vanadyl was found in controls. In panel C, we illustrate Western blots of phospho-active AKT (p-AKT) in HCA-7 colon cancer cells treated with 100 µM, 250 µM, and 500 µM of $VO(acac)_2$ (**+**); the expression levels of p-AKT increased 2-fold, 5-fold, and 12-fold, respectively, compared to controls (**-**). Similar results were obtained for two other VO^{2+}-chelates and in Caco-2 cells. MRI of rodent tumors showed preferential and persistent uptake of VO^{2+}-chelates at the tumor rim.

Discussion: Results from *in vitro* MRI and AA studies demonstrate that there is a significant concentration of vanadium in the soluble portion of cell extracts, providing direct experimental evidence that VO^{2+}-chelates accumulate intracellularly. Furthermore, the cell signaling experiments indicate that VO^{2+}-chelates activated key intracellular protein kinases. MRI showed preferentially accumulation of VO^{2+}-chelates in small ROI's along the tumor rim. This is a novel approach to cancer detection and diagnosis with the potential to produce easily tolerated MRI contrast agents that are sensitive to cancer metabolism. [Supported by the American Cancer Society, Illinois Division – 06-18].