New Vanadium-based Magnetic Resonance Imaging Probes Target Glycolytic Tumors

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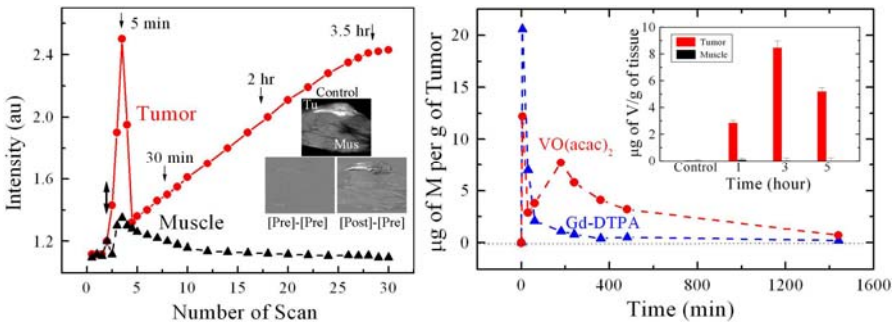
Abstract: We have developed a new class of MRI contrast agents that contain VO^{2+}-chelated organic ligands, namely $VO(acac)_2$. As contrast agents, vanadyl (VO^{2+}) chelates provided excellent T_1 and T_2^* contrasts compared to that of Gd-DTPA in high-resolution MR images of rodent tumors. This class of contrast agents is taken up by cancer cells that are highly glycolytic. *In vivo* MRI experiments demonstrated preferential and persistent uptake of the VO^{2+}-chelates in some tumor regions; these results were corroborated by *in vitro* atomic absorption (AA) studies of extracted tumor and muscle tissues following contrast injection. We have determined the dissociation constant (K_d) and binding stoichiometry (n) of $VO(acac)_2$ and serum albumin, and characterized their binding interactions using isothermal titration calorimetric (ITC) and spectrofluorometric (SF) methods. We have demonstrated that $VO(acac)_2$ has a longer washout rate compared to that of non-specific Gd-based contrast agents, making it an excellent blood-pool agent with a prolonged serum half-life.

Introduction: Improved methods for early detection and metabolic characterization of cancers are highly desirable. Current Gd-based MRI contrast agents quickly wash out of the blood and are not cancer-specific. Vanadyl chelates (VCs), on the other hand, interact with intracellular glycolytic enzymes and, therefore, selectively accumulate in highly glycolytic cancer cells. They also bind to serum proteins, giving them a longer serum half-life, and resulting in selective leakage from hyperpermeable tumor vasculature. The aims of this study were: (1) to characterize the binding interactions between $VO(acac)_2$, a *bis*-acetylacetonato complex of VO^{2+}, and serum albumin; (2) to investigate its effectiveness as a cancer-specific contrast agent for the enhancement of MR images of tumors; and (3) to compare *in vivo* MRI results with quantitative *in vitro* AA results of extracted tissues. Here, we have demonstrated that VO^{2+}-based contrast agents could provide high-resolution *functional* images of tumors that cannot be achieved by non-specific Gd-based agents.

Methods: *In vivo* MR images of highly invasive, metastatic AT6.1 tumors implanted in the hind limbs of rats were acquired at 4.7 T; after acquiring 6-8 pre-contrast reference MR images, we injected 0.15 mmol/kg of $VO(acac)_2$ over 5 minutes before acquiring more images over 3 hours (n=10). *In vitro* studies quantifying the uptake of Gd and $VO(acac)_2$ were done using AA (Perkin Elmer) of extracted tissues as a function of time following injection of contrast agents (0.15 mmol/kg). In ITC and SF studies, we titrated serum albumin as a function of [$VO(acac)_2$] to measure the heat of binding and changes in fluorescence signal, respectively, both of which correlated with the amount of binding.

Results: The figure (left panel) shows the accumulation of $VO(acac)_2$ in the tumor and surrounding muscles over time, in five *in vivo* MRI experiments, all producing similar results. The double-sided arrow in bold indicates when the contrast agent was injected; the inset is a high-resolution pre-contrast image indicating the tumor and muscle area, a control image ([pre-contrast] – [pre-contrast]), and a difference image ([post-contrast] – [pre-contrast]) (with the post-contrast image taken at 2.5 hrs after the administration of $VO(acac)_2$). The uptake and clearance of $VO(acac)_2$ from muscle and tumor are compared in the plots of signal intensity vs. time. As shown in the right panel, the amounts of vanadium (in red circles) and Gd (in blue triangles) in micrograms of metal per gram of tumor are plotted as a function of time, measured by AA studies (n=4). The dose of contrast agents was 0.15 mmol/kg body weight. In the inset, intensities from atomic absorption for $VO(acac)_2$ in tumor (in red) and muscle (in black) samples are illustrated at 1, 3 and 5 hours after injecting $VO(acac)_2$ (dose = 0.15 mmol/kg). In control sets, no contrast agent was added (n=3). ITC and SF showed that $VO(acac)_2$ binds to albumin with a K_d of ~$2.5 \pm 0.7 \times 10^{-7}$ M and with n=1.03 ± 0.04.



Discussion: Based on the K_d and n determined from binding studies, we can conclude that $VO(acac)_2$ binds to albumin in such a way that the VC has a prolonged serum half-life, but not so tightly that it is unable to dissociate from the serum protein and accumulate in cancer cells. Both *in vivo* and *in vitro* studies, as presented here, confirm the preferential uptake of $VO(acac)_2$ by tumor tissues and its superior specificity for glycolytic cancer cells over that of Gd-based contrast agents. Its cancer-targeting and blood-pool properties make $VO(acac)_2$ an ideal, practical, and easily tolerated, MRI contrast agent for the preferential enhancement of MR images of rodent tumors. [Supported by grants from the American Cancer Society, Illinois Division (#06-18 and #08-45)]