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 VO2+-chelated organic ligands, namely VO(acac)2. As contrast agents, vanadyl (VO2+) chelates provided excellent T1 and T2* 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 VO2+-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 (Kd) 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 VO2+, 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 VO2+-based contrast agents could provide high-resolution functional images of tumors that cannot be achieved by non-specific Gd-based agents.

Methods: In vivo MRI 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 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 VO2+-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 (Kd) 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.

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Methods: In vivo MRI 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 Kd of ~2.5 ± 0.7 x 10^-7 M and with n=1.03 ± 0.04.

Discussion: Based on the Kd 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)]