Redox-Sensitive Contrast Agents for MRI Based on Reversible Binding of Thiols to Serum Albumin

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Introduction. The thiol/disulfide redox status of the extracellular milieu is known to affect tumor sensitivity to radiotherapy and platinum-based anticancer drugs. Emerging evidence indicates the presence of highly reducing microenvironments in tumors, and that these conditions promote tumor cell survival and proliferation. New drugs have been designed to target cells in reducing regions. Methods for imaging tumor redox status would allow non-invasive assessment of this potential biomarker of tumor sensitivity to existing and novel platinum-based and redox-active therapies as well as radiotherapy. We have designed and tested gadolinium chelates which bind reversibly and spontaneously to circulating plasma albumin by means of covalent disulfide bonds. We present *in vitro* and *in vivo* evidence of the redox-sensitivity of this binding.

Methods. Two representatives of the family of gadolinium chelates with the general structure shown in **figure 1**, with X = $(CH_2)_3$ and $(CH_2)_6$, were synthesized, purified and characterized *in vitro* and *in vivo*. Solutions of Gd-DO3A-NH-$(CH_2)_3$-SH and Gd-DO3A-NH-$(CH_2)_6$-SH were made in phosphate-buffered saline (PBS), PBS containing 0-2 mM homocysteine, PBS containing 0.66 mM Human Serum Albumin (HSA), and PBS containing 0.66 mM HSA and 0-2 mM homocysteine, to final gadolinium concentrations of 0-1.0 mM. All solutions also contained 10 mM sodium azide to inhibit microbial growth, and final pH of all solutions was 7.35-7.40 at room temperature. HSA-bound and unbound gadolinium were separated by ultrafiltration by the method of Caravan et al. [*Inorg Chem* **40:**6580-6587, 2001], and the apparent equilibrium binding constant (*K*) for each complex was calculated from the Law of Mass Action assuming a single binding site. Measurements of the longitudinal water-proton relaxivities of these solutions of Gd-DO3A-NH-$(CH_2)_3$-SH and Gd-DO3A-NH-$(CH_2)_6$-SH were made at 37°C on a 4.7 T Bruker Biospec MR Instrument (Bruker Biospin, Billerica, MA) by spin-echo with recycle times (TR) ranging between 50-8000 ms and an echo time (TE) of 6 ms. The kinetics of MR image enhancement and washout of the two complexes, and the influence of a chase bolus of homocysteine on the washout kinetics of these thiol complexes of gadolinium, were examined in mice by dynamic contrast-enhanced MRI (DCE-MRI) using a radial spin-echo sequence (TR = 100 ms, TE = 9 ms). Pre-contrast axial images through the kidneys were acquired continuously for ≈8.5 min, at which point gadolinium solution was manually administered via a tail-vein cannula at a dose of 0.025-0.1 mmole/Kg with continuous imaging. After 30 min, mice were administered a chase bolus of either saline or 1 mmole/Kg homocysteine with continuous imaging out to 60 min total.

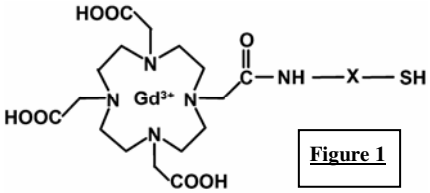

Figure 1

Results. The apparent binding constant *K* of Gd-DO3A-NH-$(CH_2)_3$-SH for HSA decreased from 5.0±1.5 mM^{-1} in the absence of homocysteine to 0.87±0.05 mM^{-1} in the presence of 2 mM homocysteine. The apparent binding constant *K* of Gd-DO3A-NH-$(CH_2)_6$-SH for HSA decreased from 64±16 mM^{-1} in the absence of homocysteine to 3.6±0.23 mM^{-1} in the presence of 2 mM homocysteine. The relaxivity of Gd-DO3A-NH-$(CH_2)_3$-SH bound to HSA was calculated to be 4.5±0.29 mM^{-1} s^{-1}, while the relaxivity of unbound Gd-DO3A-NH-$(CH_2)_3$-SH was measured to be 2.9±0.11 mM^{-1} s^{-1}. The relaxivity of Gd-DO3A-NH-$(CH_2)_6$-SH bound to HSA was calculated to be 5.3±0.34 mM^{-1} s^{-1}, while the relaxivity of unbound Gd-DO3A-NH-$(CH_2)_6$-SH was measured to be 2.3±0.15 mM^{-1} s^{-1}. Regions-of-interest (ROIs) containing the renal pelvis were manually drawn inside images of the kidneys, and washout kinetics of the two complexes as well as Gd-DTPA were analyzed. As seen in **figure 2**, the washout of GdDTPA (dotted, black) in the renal pelvis was unaffected by a homocysteine chase (vertical line at ≈2300 s). This was also the case when a saline chase was used (not shown). By contrast, the washout curves of both Gd-DO3A-NH-$(CH_2)_3$-SH (solid, gray) and Gd-DO3A-NH-$(CH_2)_6$-SH (solid, black) show an upslope immediately following the homocysteine bolus, indicating the arrival of freshly filtered gadolinium in the renal pelvis. A saline chase of either thiol complex of Gd did not produce this increase (dashed gray & black). Taken together, these data lend support to the hypothesis that both Gd-DO3A-NH-$(CH_2)_3$-SH and Gd-DO3A-NH-$(CH_2)_6$-SH spontaneously bind circulating albumin upon intravenous injection into the mice. The fraction of gadolinium which is albumin-bound at any time is excluded from glomerular filtration. However, homocysteine in the chase bolus competes for binding to albumin, releasing free Gd-DO3A-NH-$(CH_2)_3$-SH or Gd-DO3A-NH-$(CH_2)_6$-SH which are then filtered and appear in the renal pelvis. We hypothesize that competitive binding by endogenous thiols in highly reducing regions in tumors will release Gd-DO3A-NH-$(CH_2)_n$-SH bound to HSA. Extravasation of unbound Gd-DO3A-NH-$(CH_2)_n$-SH will highlight reducing regions on MRI images, and we are working to develop such a technique for imaging tumor redox.

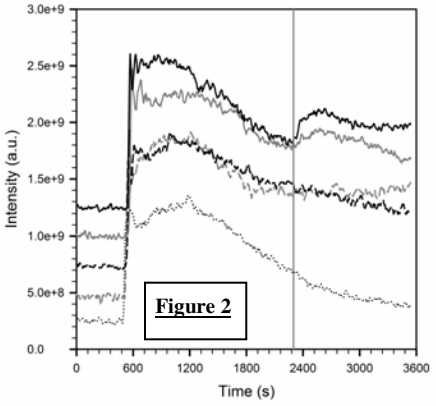

Figure 2