Non-Invasive Assessment of Therapy-Induced Tumor Necrosis Using DTPA-Gd-Poly(L-Glutamic Acid)

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Introduction: Therapy-induced tumor necrosis is frequently associated with long-term treatment response. MR contrast agents that selectively accumulate in necrotic tissues would provide a means of non-invasively monitoring the formation of necrosis with high spatial resolution, thus allowing the assessment of early treatment response in cancer patients. We have previously reported the synthesis and characterization of polymeric DTPA-Gd-poly(L-glutamic acid) (PG-Gd) as a potential blood-pool MR imaging agent[1]. In this study, we show that PG-Gd can be used to image regions of tumor necrosis, and may provide evidence for the "enhanced permeability and retention" (EPR) mechanism of action previously proposed as a means of explaining the therapeutic advantage of poly(L-glutamic acid)-paclitaxel over paclitaxel.

Methods and Materials: Mice bearing murine ovarian OCA-1 tumors (~8-mm diameter) were treated on Day 1 with poly(L-glutamic acid)-paclitaxel (PG-TXL) (120 mg equiv./kg) (known to induce significant tumor necrosis in this tumor model[2]) and subsequently injected with PG-Gd, Magnevist™, or a degradation product of PG-Gd (PG-Gd pretreated with cathepsin B). Each agent was given intravenously at a dose of 0.2 mmol Gd/kg. Pre-contrast agent administration axial T_2- and T_1-weighted images and post-contrast agent administration T_1-weighted images were acquired on Day 0 (baseline scan prior to treatment), and subsequently on Days 3 and 5. (No contrast agent was administered on any day except Day 0.) All images were acquired at 4.7T (Bruker BioSpin 200, Billerica, MA) using a 5.7 cm i.d., 950 mT/m, 19,000 T/m-s slew rate gradient coil set and a 3.5 cm i.d. linear resonator. Following the final MR scan on Day 5, each animal was sacrificed and the tumor harvested and sectioned axially at the central MR scan section. Half of the tumor was frozen in liquid nitrogen and the other half in formalin. Subsequently, the samples were sectioned at each of the MR scan planes and stained with H&E and Factor-VIII. Each MR image was analyzed with custom IDL and Matlab routines that allowed for computation of the volume of contrast-enhanced tumor (using the signal intensity measures of normal muscle to set thresholding levels).

Results: Rapid enhancement of the OCA-1 tumors was noted following Magnevist™ and degraded PG-Gd injections. This enhancement was not sustained at Days 3 or 5 (Figure 1). Given the large molecular weight (~100 kD) of PG-Gd, very minimal contrast enhancement of the tumor was noted immediately after its injection (Figure 2). However, a heterogeneous enhancement pattern was observed within the tumors on Days 3 and 5 (without re-administration of contrast agent). Comparison of MR images and microphotography of matched tumor sections stained with H&E clearly showed that areas of contrast enhancement in the MR images corresponded well with regions of tumor necrosis. Neither Magnevist™ nor the degradation product of PG-Gd yielded increased tumor enhancement on images obtained on Day 3 or 5 (Figure 3).

Conclusions: These data strongly suggest that the polymeric form of PG-Gd is required for the agent to accumulate in the necrotic areas, and that further studies are warranted to elucidate the specific mechanism of action. Our data suggest that the enhanced permeability and retention effect of poly(L-glutamic acid)-based polymeric chemotherapeutic agents may be attributed to their selective accumulation and retention in the necrotic areas of the tumors, and that MR imaging of the extent of necrosis with PG-DTPA-Gd may be a useful technique for noninvasive characterization of preexisting necrosis and for monitoring early treatment response.

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References: 1) Wen, X., Jackson, E.F., Price, R.E. *et al*., *Bioconjugate Chem*. **15**:1408-15, 2004, 2) Li C., Yu D.F., Newman R.A., *et al*.. *Cancer Res* **58**:2404-09, 1998.

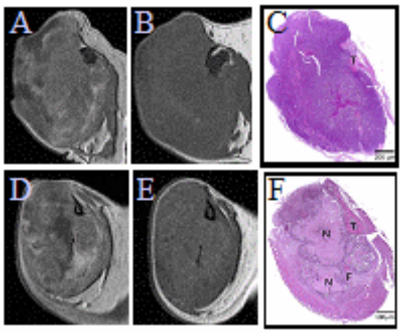

Figure 1 (left): T_1-weighted images at 2 min post-injection of oligo-PG-Gd (A) or Magnevist (B). Images B and E were obtained on Day 3 and show very minimal to no retention of contrast agent. Images C and F are the matched H&E sections.

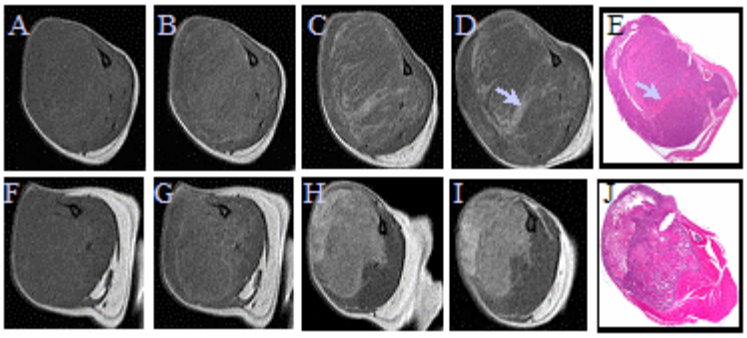

Figure 2 (above): T_1-weighted images from animals that were untreated (top row) or treated with PG-paclitaxel (bottom row). Time points were baseline (A,F), 2 min post-injection of PG-Gd (B,G), Day 3 (C, H), and Day 5 (D, I). Images E and J are the matched H&E sections and demonstrate good agreement of areas of contrast agent retention with regions of tumor necrosis (arrows).

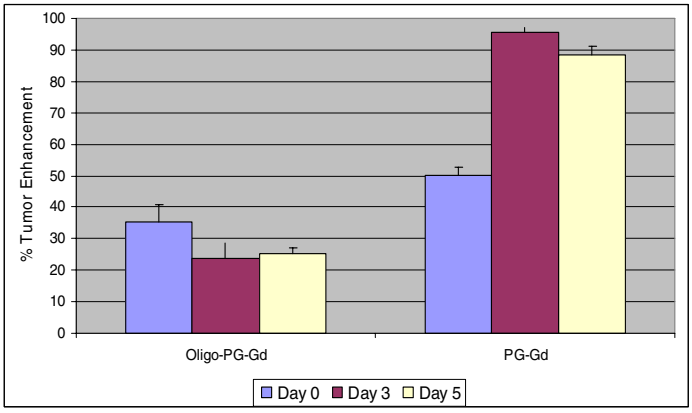

Figure 3 (left): Percent of tumor that enhanced (above a threshold of two times the standard deviation of the mean muscle signal intensity) following injection of PG-Gd degradation product (oligo-PG-Gd) or PG-Gd. Note the substantial increase in tumor enhancement at days 3 and 5 following injection of PG-Gd at Day 0. A similar increase was not seen with degraded PG-Gd or Magnevist (data not shown). Error bars are one standard error of the mean.