# Assessment of angiogenesis: dynamic contrast-enhanced MRI with non-targeted ultraparamagnetic nanoparticles compared to Gd-DTPA in a rabbit vx2 tumor model

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**Introduction:** Molecular imaging with highly selective ligand targeted contrast agents (CAs) is emerging as a new tool to image specific molecular pathways in vivo, particularly those that are associated with disease. One example of these new agents are ultraparamagnetic nanoparticles (UPNs) which, when lacking the targeting moieties and injected in much higher quantities, act as a blood pool agent. Since it is possible to assess microcirculatory parameters such as perfusion, blood volume, or microvascular permeability using 3D dynamic contrast-enhanced MRI (DCI) in combination with paramagnetic contrast material [1], it was our aim to evaluate the blood pool effects and potential non-specific leakage of UPNs and compare this to that of Gd-DTPA using a 2 compartment kinetic model [2].

**Material and Methods:** Five white male New Zealand rabbits (mean weight =2.2 kg) underwent 3D dynamic MRI scanning 12-14 days after vx2 tumor implantation. Prior to the exam, all animals were anesthetized using isoflurane and a catheter was inserted into an ear vein for CA injection. All MR imaging was performed on a 1.5T Philips Intera CV system equipped with Master gradients (30 mT/m, slewrate 150mT/m/msec). Animals were positioned prone on a 6cm diameter surface coil placed alongside the hindlimb where tumors could be palpated. DCI was performed using a 3D FFE sequence with the following imaging parameters (TR=8.2, TE=2.4, FOV=70mm, FA=35, 128x128 matrix, 2.5mm slices, $\Delta t$ =8 sec). Two contrast agents were used, Gd-DTPA (Magnevist, Schering) and UPNs which were produced as described previously by Lanza et al. [3], except the moieties for targeting specific ligands were left off. In short, the nanoparticle imaging system, is a lipid-encapsulated liquid perfluorocarbon with a nominal size of 225 nm, which is akin to clinically-available artificial blood substitutes. For MR imaging, lipid-conjugated gadolinium chelates are anchored into the lipid monolayer membrane providing - with approximately 50,000 Gd molecules per nanoparticle - particle-based relaxivities many times greater than typical Gd-DTPA agents [4]. The CA injection scheme was performed by hand as follows: 1) injection of UPNs at 2 ml/kg as a constant infusion over 90s. 2) injection of Gd-DTPA at 0.2ml/kg as a bolus. DCI scan time was around 20 min for Gd-DTPA (1 NSA per volume) and 1hr for UPNs (4 NSA per volume). Data was transferred to an independent WS for analysis. Parametric maps of fractional blood volume (fBV) and permeability (Kps) were calculated as described by Roberts et al [2]. ROIs were selected in the tumor rim, core and nearby skeletal muscle. Mean and STD for both agents were recorded and statistically compared using a 2-tailed paired students t-test.

**Results:** The rapid extravasation of Gd-DTPA is revealed in the considerable overestimation of fractional blood volume (63+/-6%), more representative of the extracellular fluid (ECF) agent's distribution volume as well as the high values of transendothelial permeability constant (Kps = 0.132+/-.022 ml/100g/min). As such, while the agent leads to considerable enhancement and good tumor conspicuity, it is unclear how the dynamic nature of enhancement relates to physiological processes such as angiogenesis. Further, as has been documented in previous studies, it is unclear that these measures would provide any estimate of tumor aggression [5] or response to antiangiogenic therapy [6]. On the other hand, the UPN agent exhibits many of the properties of a blood pool contrast agent, comparable to large macromolecular agents or USPIOs. The fractional blood volume (13.1+/-5.3%) obtained using UPNs was significantly ($p<0.01$) lower than that of Gd-DTPA and much more typical of tumor intravascular fraction. Furthermore, the transendothelial permeability constant was nearly two orders of magnitude lower (0.0025+/-0.0011ml/100g/min) than that of Gd-DTPA ($p<0.01$), consistent with its larger size. Such low permeability is a prerequisite for accurate kinetic modeling (assuming extraction fraction: flow ratio is small is an intrinsic part of the modeling process; if this is violated permeability estimates are said to be "perfusion limited"). Interestingly, the apparent fBV values determined using both agents correlated strongly (r=0.92) albeit with an intercept of 0.48 reflecting the extravascular distribution of Gd-DTPA. Permeability values showed a weaker correlation (r=0.8) again with an intercept of 0.09, suggesting that Gd-DTPA would continue to leak in tissues (e.g. healthy muscle) in which the UPNs exhibited no leakiness.

**Conclusion:**

These results suggest that, the relative area of CA leakage of UPNs is greatly reduced compared to that of a much smaller agent, Gd-DTPA. Furthermore, the areas that are consistent with CA leakage coincide with those areas where angiogenesis, and thus extremely leaky vessels, might be expected - namely near feeding vessels and interfaces between the tumor and neighboring muscle tissues. In conclusion, these results suggest that UPNs can be used as blood pool agents to evaluate vasculature permeability as a putative marker of tumor angiogenesis and thus as a potential objective and early index of biological activity of anti-angiogenic pharmaceuticals.

**References:**

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