# Safety and Efficacy Evaluation Of A Novel Graphene-Based Nanoparticles As An MRI Blood Pool Agent

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**Target Audience:** Scientists and clinicians with research interests in MRI contrast agents and CE-MR Angiography

**Purpose:** Magnetic Resonance Angiography (MRA) is an important imaging technique for vascular disease detection. The use of contrast enhanced (CE) MRA with $Gd^{3+}$-based blood pool contrast agents (CA) provide a distinct advantage in widening the imaging window resulting from the extended pharmacokinetics and improves the sensitivity in detecting small vascular defects. However, the resulting extended retention of these $Gd^{3+}$ based CAs (GBCA) impose a greater risk of developing debilitating and possibly life-threatening disease of nephrogenic systemic fibrosis (NSF) in patients with moderate to severe renal insufficiency and vascular or metabolic disorders.[1,2] Thus, there is a need for a $T_1$ MRI CA that is safer, more efficacious, and can allow the same clinical MRA performance at substantially lower dosages than clinical GBCAs. We have developed a novel high-performance carbon nanostructure-based MRI CA by synthesizing graphene nanoplatelets (GNPs; small stacks of graphene sheets) that are intercalated with trace amounts of manganese (~0.06 wt%), and are non-covalently functionalized with the FDA-approved natural polymer dextran (Mn-GNP-Dex (disk shaped, thickness=3-4 nm, diameter ~100 nm)).[3,4] The $r_1$ relaxivity of Mn-GNP-Dex is 92 $mM^{-1}s^{-1}$ (22 MHz proton Larmor frequency); ~20-30 fold greater than clinical MRI CAs.[4] These nanoparticles are hydrophilic, hemocompatible, iso-osmol and is-viscous to blood and form stable colloidal dispersions in deionized water and biological fluids.[3,4] In this work, we report their *in vivo* pre-clinical safety and efficacy evaluation as a blood pool imaging CA.

**Methods:** *Toxicity & Biodistribution*: Wistar rats were injected with Mn-GNP-Dex at 1, 25, 50, 125, 250 and 500 mg/kg (n=8/dose) by IV injection for dose range finding, acute toxicity and biodistribution studies. Nephrotoxicity assessment (histology and histomorphometry) was performed in a renal compromised rat model at potential therapeutic dosages between 1-50 mg/kg (n=8/dose for 15 days). For chronic toxicity, rats were injected at dosages between 1-100 mg/kg (n=8/dose) three times per week for three weeks. Animals injected with Dextran and Mannitol were used as controls. Vital parameters such as blood pressure, body weight, cardiac output, hematological factors (i.e. blood cell count, lipid panel and metabolic panel) and tissue histopathology were analyzed. *MR Imaging*: $T_1$-weighted 3D-GE MRI was performed in mice to compare our Mn-GNP-Dex CA (Dose = 5 nanomoles $Mn^{2+}$ ions/kg body weight) with FDA-approved clinical intravascular agent Ablavar® (Gadofosveset trisodium ) at equivalent $Gd^{3+}$ concentration. All images were acquired on a 7-Tesla Magnex horizontal magnet interfaced to Bruker Biopsec MR console.

**Results and Discussion:** The table summarizes the major findings of the toxicity study on rodents**.** No significant effects were observed in body weights, blood pressure and hematological parameters for all subjects. *In vivo* small animal MRI showed significant contrast enhancement compared to Ablavar® at potential diagnostic dosages (5 nmoles/kg) and high contrast remained in blood vessels for extended periods of time (up to 120 min).

| Parameters | Results |
|---|---|
| $LD_{50}$ | > 500 mg/kg |
| Maximum Tolerated Dose (MTD) | 50 mg/kg<MTD<125 mg/kg for acute toxicity<br>50 mg<MTD<100 mg/kg for chronic toxicity |
| Elimination | Major route-feces Excreted within 24-hrs |
| Blood half-life | < 30 min |
| Histopathology | Changes observed at ≥ 250 mg/kg for acute and at 100 mg/kg for chronic toxicity in the heart, liver, lung, spleen, and kidney. No effect in brain |
| Nephrotoxicity | No increase in skin thickness and collagen contents. No symptoms of NSF |

**Table 1:** Summary of pharmacokinetics, biodistribution and toxicity analysis on rats demonstrating no deleterious effects.

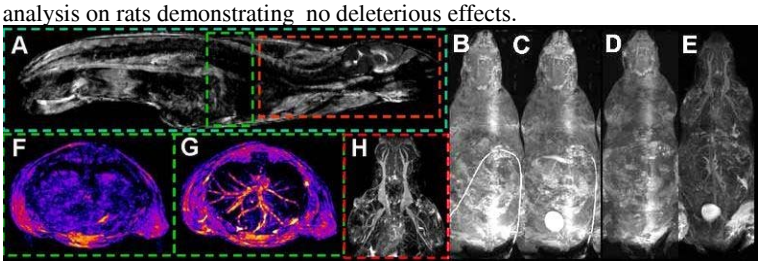


**Figure 1** Illustrates **A)** the whole body coverage achieved using 7-T mouse MRI set up with a homemade RF coil enabling 200-µm isotropic resolution in less than 12-min, (TR=15ms, TE=6.2ms, Nav=3). **B)** to **E)** illustrates the effect of CA injection **B)** pre- & **C)** post-Ablavar® as well as **D)** pre- & **E)** post-Mn-GNP-Dex using MIPs of 3D datasets. Closer view of **F)** pre- & **G)** 120min post-injection MIPs near cardiothoracic region (Transverse) and **H)** head (Horizontal orientation).

**Conclusion:** *In vivo* small animal studies indicate that Mn-GNP-Dex formulations are safe over large range dosages and do not show any toxic effect in the animals with renal insufficiency. Preliminary whole body murine examination at 7-Tesla show great MR imaging potential as a blood pool MRI CA. Taken together these results should establish the basis for further consideration in studying vascular disease models and subsequently into higher species to make its clinical translation closer.

**References**: **1.** Zou Z *et al., JACC Cardiovasc Imag*, 2011; 4(11):1206-16; **2.** Tsushima Y. *et al., Brit J Rad*, 2010; 83: 590–595; **3**. Paratala BS *et al*., PloS one. 2012;7(6):e38185; **4**. Kanakia S *et al., Int J of Nanomedicine*, 2013; 8: 2821–2833.