Nephrotoxicity of Dextran Functionalized Graphene Nanoparticles and Their Potential as Magnetic Resonance Imaging Contrast Agents for Renal Abnormalities

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ABSTRACT

Contrast-enhanced magnetic resonance angiography (MRA) employs the use of blood pool contrast agents (CA) such as gadolinium (Gd^{3+}) complexes that allow for delayed phase arterial and venous imaging and improve the sensitivity in detecting small vascular defects. However, recent association of Gd^{3+}-based CA with nephrogenic systemic fibrosis (NSF), a debilitating disease characterized by progressive and severe thickening of the skin, in patients with renal insufficiency has led to restrictions on their clinical use by the FDA. We have developed a dextran functionalized manganese intercalated graphene-based CA with relaxivity values ~20X greater than current clinically approved CAs. In this work, we report the safety and efficacy of our novel CA for renal *in vivo* MRA. The propensity for our contrast agent (at 5 and 50 mg/kg doses) to induce histopathological signs of NSF was evaluated in an experimental rat model of chronic renal failure. *In vivo* studies indicate that Mangradex formulations are safe and show no nephrotoxic effects after chronic exposure at doses ≤ 50 mg/kg. MR imaging results indicate Mangradex's excellent potential as a blood pool CA.

Keywords: MRI, graphene, nanoparticle, contrast agent, renal imaging

1 INTRODUCTION

Renal insufficiency is the loss of normal kidney function due to acute injury (via trauma, toxins, surgery, etc.), or chronic diseases such as diabetes and hypertension. Magnetic resonance angiography is an important imaging technique routinely used in the clinical diagnosis of renal failure and the detection of vascular diseases. Contrast enhanced (CE) MRA employs the use of blood pool contrast agents such as gadolinium (Gd^{3+}) complexes that allow for delayed phase arterial and venous imaging and improve the sensitivity of detecting small vascular defects. However, recent association of Gd^{3+}-based CA (GBCA) with nephrogenic systemic fibrosis, a debilitating disease characterized by progressive and severe thickening of the skin and other organs, in patients with moderate to severe renal insufficiency has fostered concerns from the Food and Drug Administration (FDA), and ultimately led to restrictions on their clinical use.[1,2] To address these safety concerns, we have developed a dextran functionalized manganese (Mn^{2+}; ~0.06 wt %) intercalated graphene-based CA (known herein as Mangradex). We previously reported these nanoparticles' excellent magnetic and relaxometric properties with relaxivity values ~ 20X greater than current clinically approved CAs.[3,4] They are disk-shaped with an average diameter of ~100 nm and thickness of 3-4 nm, hydrophilic and form stable colloidal dispersions in deionized water (up to 100 mg/mL concentration) and biological fluids that are both iso-osmol and iso-viscous to blood.[4] Our previous acute toxicity study in rodents suggested that they are hemocompatible with a maximum tolerated dose (MTD) between 50 mg/kg and 125 mg/kg, and are eliminated mainly through feces within 24 hours after intravenous (IV) administration.[5] In this work, we report the safety and efficacy of Mangradex as a novel CA for renal *in vivo* MRA.

2 MATERIALS AND METHODS

2.1 Animal Model and Treatment

The 5/6 Nephrex Wistar rat (where the left kidney has 2/3 pole regions excised and the right kidney is completely removed) has been used as an experimental animal model for chronic renal failure. Following the guidelines by the Institutional Animal Care and Use Committee (IACUC) at Stony Brook University, 5/6 Nephrex Wistar rats were injected with Mangradex at 5 and 50 mg/kg doses by tail vein IV injection after anesthetized with 5% mixture of air: isoflurane in a 1:1 air - oxygen mixture. Each animal received IV injections of Mangradex either once on the first day of the study (single injection) or five times per week for two weeks or four weeks (multiple injections). Animals were observed for vital parameters, behavior and toxicity-related adverse effects. Controls were sham and animals injected with mannitol (vehicle control) only. At the end of each study, the animals were euthanized by inhalation using 100% CO2. All major organs (heart, lungs, liver, brain,

spleen) as well as the dorsal skin from each animal were harvested and prepared for histopathology analysis.

2.2 Nephrotoxicity

Nephrotoxicity was assessed by examining significant changes in skin thickness and collagen content. Measurement of dorsal skin thickness was performed by two methods: digital calipers and histomorphometry using digitized photomicrographs of H&E stained skin samples of each animal and Image J. Collagen concentration was measured and quantified per skin section obtained from 5/6 Nephrex rats using Masson's trichrome staining and Image J software.

2.3 *In Vivo* MR Imaging

In vivo T_1-weighted MRI was performed on mice using a 7 T Bruker Biospec to compare Mangradex CA (dose of 25 mg/kg, equivalent to 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 with a 7-Tesla Magnex horizontal magnet interfaced to Bruker Biopsec MR Console.

3 RESULTS AND DISCUSSION

3.1 Toxicity and Histopathology

No significant effects were observed in body weights, blood pressure and hematological parameters for all subjects (not shown). Histopathology analysis of the major organs treated with Mangradex revealed the presence of brown pigments only in lungs and liver, suggestive of graphene nanoparticles present in those organs. More brown pigmentation was observed in the rats that received multiple injections than a single injection. No diagnostic abnormalities were observed however in other major organs.

3.2 Nephrotoxicity

FDA guidelines require new pharmaceuticals under development to undergo special toxicity studies in animal models relevant to the human disease they indicated for. Since Gd^{3+}-based contrast agents present a risk to patients with renal insufficiencies, we performed single and repeat dose studies of Mangradex in 5/6 Nephrex rats, a widely used experimental animal model for chronic renal failure. Previous studies of NSF for clinically approved MRI CAs examined toxicity from 5 days to 4 weeks; thus, for short term investigation of Mangradex exposure, 2 week and 4 week time points were selected in this study. Rats administered with single and multiple IV injections of Mangradex were examined for signs of NSF that include skin thickening due to increased dermal fibrosis in the presence of unregulated collagen density.

Analysis of dorsal skin thickness and collagen concentration showed no significant differences between test and control animals. One-way ANOVA of skin thicknesses measured by histomorphometry (Figure 1) and digital calipers (not shown) revealed no statistically significant ($p<0.05$) differences among the sham control and the experimental groups.

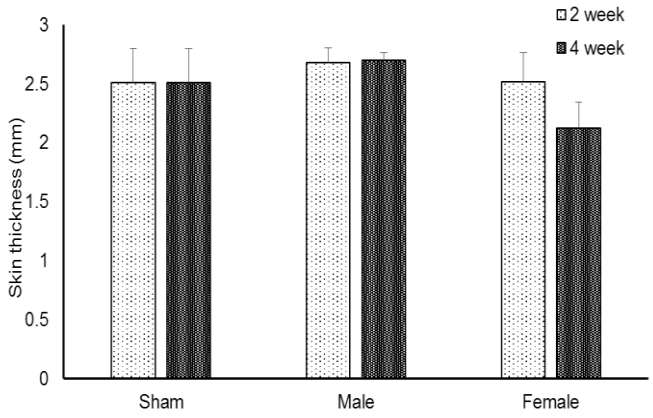
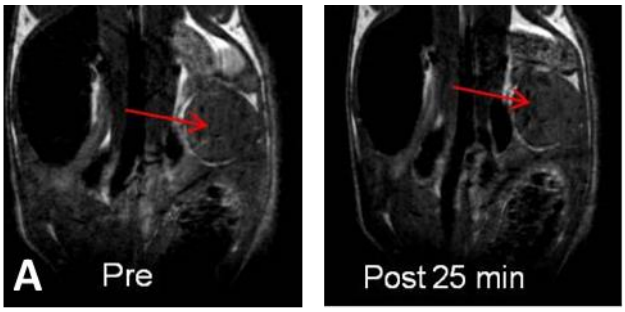


Figure 1: Dorsal skin thicknesses from 5/6 Nephrex rats given multiple doses of 50 mg/kg Mangradex for 2 or 4 weeks by histomorphometric image analysis using Image J software.

For collagen quantification, histology sections of 5/6 Nephrex rat skin were stained with Masson's Trichrome to mark collagen fibers brilliant blue. Image J software was then used to select and measure the concentration of collagen in each skin section. Analysis shows no statistically significant differences in collagen concentration in skin among test and control animals (not shown).

3.3 *In Vivo* MR Imaging

In vivo small animal MRI showed significant contrast enhancement compared to Ablavar® at potential diagnostic dosages (5 nmoles/kg) and high contrast remained in the vasculature for extended periods of time up to 85 min (not shown).



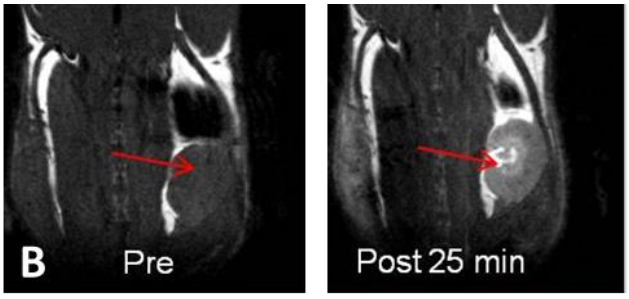


Figure 2: Representative *in vivo* MR images of pelvic region that show the nephrectomized rat kidney (red arrow) before (left) and 25 minutes after (right images) injection of Ablavar (A); before (left) and 25 minutes after (right) injection of Mangradex (B).

Compared to Ablavar®, Mangradex showed significant SNR and CNR up to 25 minutes and continued until 85 minutes post injection (not shown). The drop in intensity between the two contrast agents is likely due to the half-life of Mangradex being much longer than Ablavar®, as previously determined from our other studies. The enhanced and prolonged contrast observed in blood vessels suggests that Mangradex has great potential to be used for renal artery imaging.

4 CONCLUSIONS

In vivo small animal studies indicate that Mangradex formulations are safe and do not show any nephrotoxic effects in the animals after chronic exposure at doses ≤ 50 mg/kg. Examination of the renal vasculature at 7-Tesla indicates excellent potential of Mangradex as a blood pool MRI CA. In conclusion, these results lay the foundation for preclinical safety and efficacy studies of Mangradex in a large animals, necessary for their eventual clinical translation.

5 DECLARATION OF FINANCIAL INTEREST

Stony Brook University and the investigators have filed patents related to the technology reported in this article. If licensing or commercialization occurs, the researchers are entitled to standard royalties. S.B. has a financial interest in Theragnostic Technologies Inc., which however did not directly support this work.

6 ACKNOWLEDGEMENTS

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REFERENCES

[1] Zou, Z., Zhang, H. L., Roditi, G. H., Leiner, T., Kucharczyk, W., & Prince, M. R. (2011). Nephrogenic systemic fibrosis: review of 370 biopsy-confirmed cases. *JACC: Cardiovascular Imaging*, *4*(11), 1206-1216.

[2] Tsushima, Y., Kanal, E., & Thomsen, H. S. (2010). Nephrogenic systemic fibrosis: risk factors suggested from Japanese published cases. *The British Journal of Radiology*, *83*(991), 590–595.

[3] Paratala, B. S., Jacobson, B. D., Kanakia, S., Francis, L. D., & Sitharaman, B. (2012). Physicochemical characterization, and relaxometry studies of micro-graphite oxide, graphene nanoplatelets, and nanoribbons. *PloS one*, *7*(6), e38185.

[4] Kanakia, S., Toussaint, J. D., Chowdhury, S. M., Lalwani, G., Tembulkar, T., Button, T., ... & Sitharaman, B. (2013). Physicochemical characterization of a novel graphene-based magnetic resonance imaging contrast agent.*International journal of nanomedicine*, *8*, 2821.

[5] Kanakia, S., Toussaint, J. D., Chowdhury, S. M., Tembulkar, T., Lee, S., Jiang, Y. P., ... & Sitharaman, B. (2014). Dose ranging, expanded acute toxicity and safety pharmacology studies for intravenously administered functionalized graphene nanoparticle formulations. *Biomaterials*, *35*(25), 7022-7031.