Gadolinium-catalyzed single walled carbon nanotubes as advanced magnetic resonance imaging contrast agents: cell labeling and biodistribution studies

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Introduction: Gadolinium (Gd)-chelate-based compounds are widely used as clinical contrast agents (CAs) for magnetic resonance imaging (MRI). However, for advanced applications such as non-invasive cellular MRI, there is a need to develop CAs that show higher relaxivities, with increased cellular delivery and accumulation, and are biologically inert and nontoxic. In this work, we present *in vitro* magnetic cell labeling studies and *in vivo* biological response of a recently synthesized nanoparticle; Gd nanoparticle-catalyzed single walled carbon nanotubes (Gd-SWCNTs) [2] towards their development as advanced MRI CAs.

Methods: The synthesis method for nanotube involves chemical vapor deposition (CVD) of carbon feedstock on gadolinium nanoparticles as catalysts prepared by a block copolymer templating technique [2]. The Gd-SWCNTs were water-solubilized by dispersing them in N-(Carbonyl-methoxypolyethyleneglycol-5000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG). Phantom MRI studies of Gd-SWCNTs in gelatin were performed on a 3T Philips MR scanner. NIH 3T3 cells were treated with the water soluble Gd-SWCNT for 12, 24 and 48 hrs. Ultrastructural incorporation and localization of Gd-SWCNTs in the cells was analyzed using Transmission Electron Microscope (TEM) operating at 80 keV. Gd-SWCNTs concentrations in cells were analyzed using inductively-coupled plasma-mass spectroscope (ICP-MS). The cellular response towards Gd-SWCNTs was analyzed using cell viability, lactate dehydrogenase, micronuclei formation by staining with acridine orange, changes in the cell cycle (propidium iodide staining) and apoptosis (FITC-annexin-V vs propidium iodide) by flow cytometry. The biodistribution and biological response in various organs of rats was assessed by intravenous injections of Gd-SWCNTs (0.5 mg/kg body weight).

Results & discussion: MRI relaxivities (an important measure of efficacy) of the Gd-SWCNTs at 1.5 T were 126 $mM^{-1}s^{-1}$; 25 fold greater than the current, clinically used Gd^{3+} based contrast agent Magnevist (4.5 $mM^{-1}s^{-1}$) at 0.2 mM [Gd^{3+}]. Representative T_1 weighted MRI images of the Gd-SWCNT in 4% gelatin in water and a control (4% gelatin) are shown in **Figure 1**. At 0.22 mM concentration, Gd-SWCNTs showed significant enhancements (SNR > 100) compared to Magnevist and pure water. The Gd-SWCNTs at incubation concentrations up to 10 µg/ml had no effect on the cell viability profiles up to 48 hrs (**Figure 2**). TEM showed that these nanoparticles are efficiently translocated into the cellular vacuoles (**Figure 3**). The ICP-MS analysis showed that Gd-SWCNTs are uptaken by the cells up to a concentration of 3µg/ml [Gd^{3+}]. No significant changes in the cell viability (trypan blue and live/dead cell assay) was observed in the concentration range of 7ng - 10µg/ml and decreased by 10 – 15% in the concentrations range of 50-100 µg/ml up to 48 hrs. Lactate dehydrogenase (LDH) assay, a cytoplasmic enzyme marker that reflects the membrane damage, showed no significant changes up to 25µg/ml of Gd-SWNCT treatment for 48 hrs. The absence at concentrations 25µg/ml of Gd-SWCNT-induced externalization of PS (a preapoptotic condition), and increased caspase-3 activity (point of no return in apoptosis) further corroborated that cells remain viable up to these Gd-SWCNT concentrations. To overcome any cytotoxic conditions, the cells regulate the cell cycle to trigger the survival signals. Gd-SWCNTs treated up to 25µg/ml did not alter the normal cell cycle and maintained the cellular homeostasis up to 24 hrs. The *in vivo* studies in rats reveal that Gd-SWCNTs injected intravenously at a dose of 0.5 mg/kg are distributed in lungs, liver, kidney and brain and show no signs of inflammation or damage to the tissue architecture. No significant changes in the plasma lipid peroxidation and proinflammatory cytokine (TNF-α) reveal their short-term biological inertness, and their ability to maintain physiological homeostasis.

Conclusion: In conclusion, MRI phantom studies on the Gd-SWCNT show extremely large signal enhancement with intensities up to 100 times larger than Magnevist and pure water at low concentrations of gadolinium. The ability of these nanoprobes to be internalized into cells at sufficient concentration without any cytotoxic effects and to distribute in various organs without any damage to the tissue architecture indicates potential for their future development as efficient magnetic labels for cellular MRI.

References (1) Merbach AE, Toth, E, Helm L, Editors, The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging, John Wiley and Sons, Chichester. (2) Swierczewska M, Rusakova I, Sitharaman B. Carbon 2009, 47; 3137-3142.

Figure 1: T1-weighted MR image of Gd-SWCNTs prepared in 4% gelatin (green), Magnevist (orange) and only 4% gelatin (purple). MR signal is enhanced by the addition of 0.2mM [Gd^{3+}] of Gd-SWCNTs as compared to Magnevist.

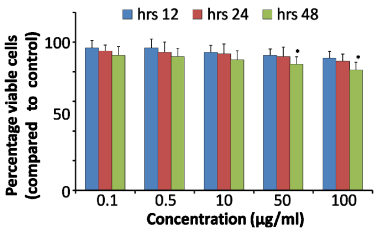

FIGURE 2: Cells viability after treatment with various concentrations of Gd-SWCNT for 48 hrs. No significant change in cell viability with 50µg/ml till 24 hrs. *P<0.05 w.r.t control.

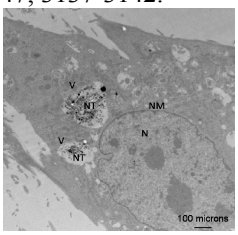

Figure 3: Localization of Gd-SWCNTs in NIH 3T3 cells after treatment for 48 hrs. V: vacuoles, N: nucleus, NM: nuclear membrane, NT: nanotubes