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Superparamagnetic iron oxide polyacrylic acid coated γ-Fe_2O_3 nanoparticles do not affect kidney function but cause acute effect on the cardiovascular function in healthy mice

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ABSTRACT

This study describes the distribution of intravenously injected polyacrylic acid (PAA) coated γ-Fe_2O_3 NPs (10 mg kg^{-1}) at the organ, cellular and subcellular levels in healthy BALB/cJ mice and in parallel addresses the effects of NP injection on kidney function, blood pressure and vascular contractility. Magnetic resonance imaging (MRI) and transmission electron microscopy (TEM) showed accumulation of NPs in the liver within 1 h after intravenous infusion, accommodated by intracellular uptake in endothelial and Kupffer cells with subsequent intracellular uptake in renal cells, particularly the cytoplasm of the proximal tubule, in podocytes and mesangial cells. The renofunctional effects of NPs were evaluated by arterial acid–base status and measurements of glomerular filtration rate (GFR) after instrumentation with chronically indwelling catheters. Arterial pH was 7.46 ± 0.02 and 7.41 ± 0.02 in mice 0.5 h after injections of saline or NP, and did not change over the next 12 h. In addition, the injections of NP did not affect arterial PCO_2 or [HCO_3^-] either. Twenty-four and 96 h after NP injections, the GFR averaged 0.35 ± 0.04 and 0.35 ± 0.01 ml min^{-1} g^{-1}, respectively, values which were statistically comparable with controls (0.29 ± 0.02 and 0.33 ± 0.1 ml^{-1} min^{-1} 25 g^{-1}). Mean arterial blood pressure (MAP) decreased 12–24 h after NP injections (111.1 ± 11.5 vs 123.0 ± 6.1 min^{-1}) associated with a decreased contractility of small mesenteric arteries revealed by myography to characterize endothelial function. In conclusion, our study demonstrates that accumulation of superparamagnetic iron oxide nanoparticles does not affect kidney function in healthy mice but temporarily decreases blood pressure.

Introduction

In recent years, the vast clinical potential of nanomedicine has incited the development of various multimodality particles with distinct biological, physical and chemical properties useful for imaging, as well as various biomedical and therapeutic applications (Farokhzad and Langer, 2006; Gu, 2007; Liong et al., 2008; Longmire et al., 2008). Superparamagnetic iron oxide nanoparticles (NP) have been explored for targeted drug delivery, hyperthermia, tissue repair, cell sorting, and contrast agents for magnetic resonance imaging (Gupta et al., 2007; Jain, 2008; Rosen et al., 2012). The NPs are often chemically modified and synthesized to increase stability and safety. In contrast to conventional imaging agents, NPs are relatively stable *in vivo*, exemplified by the recent findings that quantum dots are retained in the body for 100 days (Choi et al., 2007) and even up to two years (Ballou et al., 2007). It is imperative, therefore, to study the specific distribution of magnetic NP to understand the specificity and long-term biodistribution profiles to access a better clinical efficiency and safety (Rosen et al., 2012). It has been shown that excessive release of free ions from NPs that may be toxic (Weir et al., 1984), lead to oxidative stress (Hussain et al., 2005; McCord, 1996) and disturb liver metabolism (Wisse et al., 1991). In addition, cirrhosis and hepatocellular carcinoma can develop when the liver iron concentration exceeds 4 μg g^{-1} wet weight (Neuberger et al., 2005). Hussain et al. (2005) recently demonstrated that high

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concentrations of F_3O_4 NP *in vitro* reduced cell proliferation and caused cell death in rat liver cells.

Inhalation of air-borne particular matter (PM) increases mortality and morbidity from pulmonary mediated cardiac arrhythmias (Samet et al., 2000; Zhu et al., 2008) that seems to be precipitated by disrupted autonomic cardiovascular regulation (Magari et al., 2001). PMs are a complex mixture of organic and inorganic chemicals, including metals and particulates (Mo et al., 2009) and particularly the ultra-fine particles (UFPs) with an aerodynamic diameter less than 100 μm seem to elicit cardiorespiratory malfunctions (Stern and McNeil, 2008). Recent human and animal studies show that inhaled UFPs swiftly enter the systemic circulation (Nemmar et al., 2001; Pope et al., 2004), where they disturb myocardial or vascular endothelial cell function (Dockery et al., 1993; Pekkanen et al., 2002; Pope et al., 2004) by generation of reactive oxygen species (ROS; Mo et al., 2009). Nevertheless, the specific biological interactions leading to the possible cardiovascular effects of PM and UFP remain largely unknown (Pekkanen et al., 2002) precluding successful mitigation of their negative impact. Many of the NPs explored for biomedical use share some physicochemical properties with UFPs and are therefore also believed to have similar detrimental effects on the cardiovascular system (Oberdörster et al., 2002).

Considerable research has been devoted to understand and characterize the distribution and accumulation of various metal and magnetic nanoparticles in healthy organs, but less attention has been drawn to elucidate renal effects following NP injection. It is known that engineered particles, such as iodinated contrast agents, can cause acute kidney injury (Bruce et al., 2009; Byrd and Sherman, 1979; Cochran et al., 1983; Nash et al., 2002) and the incidence of acute kidney injury caused by contrast administration is commonly referred to as contrast-induced nephrotoxicity (Nash et al., 2002). Intrarenal accumulation of NPs may lead to acute kidney injury (Bruce et al., 2009; Byrd and Sherman, 1979; Cochran et al., 1983) followed by a more permanent renal failure (Toprak, 2007), likely mediated by medullary hypoxia due to decreased renal blood flow, secondary to vasoconstriction, tubular obstruction, direct tubular toxicity and oxidative damage (Persson and Tepel, 2006). Furthermore, Chen et al. (2006) observed that mice exposed to nano-copper developed glomerulitis, degeneration and necrosis of renal tubules, and renal inflammation.

The objectives of this study were to determine: a) the destiny of intravenously injected polyacrylic acid (PAA) coated γ-Fe_2O_3 particles at a clinical relevant dose (Ma et al., 2012) for hyperthermic cancer therapy with particular emphasis on the renal and hepatic accumulation, b) the renal function (systemic acid/base status and GFR) following exposure to these particular NPs and c) the effect of the NPs on cardiovascular functions (MAP and vascular contractility).

Methods

Experimental animals. BALB/cJ mice of both sexes (7–10 weeks) were purchased from Taconic or Harlan (Denmark) and transported to the animal facility at Department of Biological Sciences, Aarhus University. In total 104 mice were used divided into the following studies to address the a) distribution of polyacrylic acid (PAA) coated γ-Fe_2O_3 particles (MR n = 9, TEM n = 8), b) the renal function (systemic acid/base status n = 21 and GFR n = 18), c) cardiovascular effects (MAP n = 12 and vascular contractility, n = 36).

The mice used for GFR measurements were housed at the Biomedical Laboratory, University of Southern Denmark. At both institutions, mice were kept under standard light (12:12 dark–light) and temperature conditions with free access to rodent chow and tap water. Animal care followed the guidelines of the National Institutes of Health and the experimental protocol was approved by the Danish Animal Experiments Inspectorate.

Magnetic resonance imaging. A clinical 1.5 T Phillips Achiva MRI system (Philips Medical Systems, Best, Netherlands) was used to visualize the *in vivo* distribution of NPs. NPs were administered directly to the tail vein 1 h (n = 3) or 96 h (n = 6) prior to MRI, while a third group of three mice received a sham injection of saline. All mice were euthanized with an over-dose of pentobarbital (10 mg kg^{-1}) immediately before positioned in the scanner.

The iron-containing NPs cause a dephasing of the nearby spins of water, thereby decreasing the signal intensity on a T2*-weighted images. Thus, we applied a standard T2*-weighted gradient-echo sequence using the following parameters: field-of-view = 260 × 260 mm^2, matrix = 384 × 384, 10 slices with a thickness of 1 mm, repetition time = 800 ms, echo time = 20 ms, excitation angle = 90, and number of averages nt = 3. A spin-echo sequence, for T2 relaxation mapping, was applied: field-of-view = 260 × 260 mm^2, matrix = 384 × 384, 10 slices with a thickness of 1 mm, repetition time = 2000 ms, array of echo times = 20,40, 60, 80, 100, 120, 140, and 160 ms, and number of averages nt = 2.

All data were exported in DICOM format to the Mistar (Apollo Imaging Technology, Melbourne, Australia) and ImageJ (a public domain, Java-based image processing program developed at the National Institutes of Health) analysis software. Relaxometric analysis was used to generate parametric T2 map based on the acquired multi-echo spin-echo images. A global intensity profile was computed for all 10 slices, and NP accumulation was confirmed by reduction in signal intensity in kidney and liver regions.

Transmission electron microscopy (TEM). γ-Fe_2O_3 particles (10 mg kg^{-1}) were given in the venous catheter in two experimental groups with different exposure duration (24 h and 96 h, n = 3 in each), while additionally two mice received saline and thereby served as controls. After 24 h and 96 h, respectively, the mice were deeply anesthetized by intraperitoneal injections of pentobarbital (50 mg ml^{-1}, 0.01 ml g^{-1}) and perfused transcardially with a 2% paraformaldehyde and 2.5% glutaraldehyde fixative in a 0.1 M phosphate buffer (pH 7.4) for 5 min until all organs became pale. Liver and kidney were transferred to individual glass vials with the fixative and subsequently post fixated in 4% osmium tetroxide dissolved in milli Q water. After 24 h, the tissue blocks were dehydrated in a series of increasing concentrations of ethanol (70, 90, 96 and 99.9%), immersed in the organic intermedium propylene oxide and embedded in EMBed-812 (Electron Microscopy Sciences, Hatfiled, PA). Ultrathin sections for TEM were cut on a Leica Ultracut UCT ultramicrotome (Leica Mikrosysteme GmbH, Vienna Austria) with a 45 degree angled diamond knife (Diatome, Biel, Switzerland). The ultrathin sections were stained with uranylacetate and inspected in a CM 100 FEI transmission electron microscope (FEI, North America NanoPort, Hillsboro, Oregon 97124, USA) and photographed with a CCD camera (1K MegaView, Olympus Soft Imaging Solutions GmbH, Münster, Germany), followed by image processing in AnalySIS (Olympus, Germany).

Electron Energy Loss Spectroscopy (EELS; Gatan Quantum SE/963 spectroscope and camera (Gatan, Inc. Pieasanton, CA 94588) on a Titan Krios 80-300 microscope (FEI, North America NanoPort, Hillsboro, Oregon 97124, USA)) were used to determine the elemental constitution of the particles imaged in TEM. The presence of the Fe_2O_3 in the particles was evaluated by comparing the position of the Fe-L2 and Fe-L3 edges in the obtained EELS spectrum with the positions of Fe-L2 and Fe-L3 edges from Fe_2O_3 in the EELS Atlas. A 30 eV slit was applied for acquisition of pre-edge (slit center 683 eV) and post-edge (slit center 728 eV) images. Jump-ratio was calculated from these recordings using Gatan software. The size of the particles was measured using ImageJ on the TEM-images from the for EELS analysis.

Insertion of arterial and venous catheters for GFR measurements. The eighteen mice used for determination of glomerular filtration rate