Decreased reticuloendothelial system clearance and increased blood half-life and immune cell labeling for nano- and micron-sized superparamagnetic iron-oxide particles upon pre-treatment with Intralipid

Li Liu [a], T. Kevin Hitchens [a], Qing Ye [a], Yijen Wu [a], Brent Barbe [a], Devin E. Prior [a,1], Wendy F. Li [a], Fang-Cheng Yeh [a,b], Lesley M. Foley [a], Daniel J. Bain [c], Chien Ho [a,*]

[a] Pittsburgh NMR Center for Biomedical Research and Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, USA
[b] Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA, USA
[c] Department of Geology and Planetary Science, University of Pittsburgh, Pittsburgh, PA, USA

ARTICLE INFO

ABSTRACT

Background: Superparamagnetic iron-oxide nanoparticles are useful as contrast agents for anatomical, functional and cellular MRI, drug delivery agents, and diagnostic biosensors. Nanoparticles are generally cleared by the reticuloendothelial system (RES), in particular taken up by Kupffer cells in the liver, limiting particle bioavailability and in-vivo applications. Strategies that decrease the RES clearance and prolong the circulation residence time of particles can improve the in-vivo targeting efficiency.
Methods: Intralipid 20.0%, an FDA approved nutritional supplement, was intravenously administered in rats at the clinical dose (2 g/kg) 1 h before intravenous injection of ultra-small superparamagnetic iron-oxide (USPIO) or micron-sized paramagnetic iron-oxide (MPIO) particles. Blood half-life, monocyte labeling efficiency, and particle biodistribution were assessed by magnetic resonance relaxometry, flow cytometry, inductively-coupled plasma MS, and histology.
Results: Pre-treatment with Intralipid resulted in a 3.1-fold increase in USPIO blood half-life and a 2-fold increase in USPIO-labeled monocytes. A 2.5-fold increase in MPIO blood half-life and a 5-fold increase in MPIO-labeled monocytes were observed following Intralipid pre-treatment, with a 3.2-fold increase in mean iron content up to 2.60 pg Fe/monocyte. With Intralipid, there was a 49.2% and 45.1% reduction in liver uptake vs. untreated controls at 48 h for USPIO and MPIO, respectively.
Conclusions: Intralipid pre-treatment significantly decreases initial RES uptake and increases in-vivo circulation and blood monocyte labeling efficiency for nano- and micron-sized superparamagnetic iron-oxide particles.
General significance: Our findings can have broad applications for imaging and drug delivery applications, increasing the bioavailability of nano- and micron-sized particles for target sites other than the liver.

1. Introduction

Iron-oxide particles are useful tools for various biomedical applications including magnetic resonance imaging (MRI) as contrast agents

[1–12], targeted drug delivery [13,14], diagnosis [15–17], cell sorting [18,19], and therapy [20,21]. However, in-vivo uses of iron-oxide particles can be hampered by their rapid clearance from circulation by the reticuloendothelial system (RES) [22]. Many studies have reported that the majority of injected iron-oxide particles are taken up by the RES, in particular by the liver Kupffer cells [23,24]. In order to improve the usefulness of these particles for imaging and drug delivery applications, it is essential to minimize the clearance by the RES and improve the in-vivo circulation time.

Non-invasive in-vivo MRI of monocytes/macrophages labeled with iron-oxide particles may lead to a better understanding of the pathogenesis of many diseases, including graft rejection [2,10,25,26], atherosclerotic plaques [27,28], tumors [29,30], abdominal aortic aneurysm [31], renal ischemia [32], Alzheimer's disease [33], etc. Due to the fact that monocytes/macrophages are phagocytes, they can be labeled in

vivo by a direct injection of suitable iron-oxide particles. Strategies that reduce liver uptake and prolong the circulation residence time of these particles can improve the in-vivo labeling efficiency of monocytes/macrophages and lower the required effective dose.

Many studies have been conducted to decrease the RES clearance and increase the circulation lifetime of iron-oxide particles by modifying particle characteristics, such as the size, charge, surface property, and composition. For example, larger-sized particles are eliminated from bloodstream faster than smaller-sized particles [22]. Neutral and zwitterionic nanoparticles exhibit longer circulation time than negatively and positively charged nanoparticles [34]. In addition, a surface coating of polyethylene glycol (PEG) and modifications of nanoparticles with liposomes can reduce uptake by the RES, thus prolonging their circulation in the bloodstream [35,36]. Modifying the particle characteristics is effective in reducing RES clearance; however, each new modification calls for thorough toxicity and biomechanics studies before any possibility for translation to a clinical setting. Our strategy is to target the RES, and in particular Kupffer cells, to temporarily blunt particle clearance. In this study, we set out to find a U.S. Food and Drug Administration (FDA) approved agent that could achieve this goal.

Intralipid was approved by FDA in 1972 as a source of parenteral nutrition for patients. Intralipid 20.0% is composed of 20% soybean oil, 1.2% egg-yolk phospholipids, and 2.25% glycerol. Kupffer cells in the liver play an important role in the uptake and metabolism of Intralipid [37]. Intralipid infusion has been reported to inhibit RES function by possibly inhibiting peritoneal clearance and impairing the phagocytic activity of Kupffer cells [38]. Our hypothesis is that the clearance of iron-oxide particles by the RES can be reduced by using agents, such as Intralipid, which is also cleared by Kupffer cells and inhibits their phagocytic activities, prior to injection of the particles. In this study, two types of iron-oxide particles were applied to test our hypothesis: nano-sized ultra-small superparamagnetic iron-oxide (USPIO, with particle size ~30 nm in diameter) and micron-sized paramagnetic iron-oxide (MPIO, ~0.9 μm in diameter) particles.

2. Material and methods

2.1. Materials and animals

Intralipid 20.0% was purchased from Fresenius Kabi (Bad Homburg, Germany). "Molday IONC6 Amine" (USPIO-NH$_2$) particles were purchased from BioPAL (Worcester, MA). MPIO particles coated with polystyrene/divinyl benzene (0.9 μm in size) were obtained from Bangs Laboratories (Fishers, IN). Fluorescein isothiocyanate (FITC) was obtained from Sigma-Aldrich Co. (St. Louis, MO). Phosphate-buffered-saline (PBS) was obtained from Mediatech, Inc. (Manassas, VA). Mouse anti-rat ED1: Alexa Fluor 647 antibody and BUF09 were purchased from AbD SeroTec (Oxford, UK).

Brown Norway (BN; RT1^n) rats were purchased from Harlan (Indianapolis, IN). All experiments involving animal subjects were approved by the Institutional Animal Care and Use Committee of Carnegie Mellon University. Animal care was provided in accordance with the Guide for the Care and Use of Laboratory Animals.

2.2. Synthesis and characterization of USPIO-NH$_2$-FITC

Detailed information on USPIO-NH$_2$-FITC preparation and characterization is described in Supplementary materials.

2.3. Animal studies

Male BN rats, with body weights between 250 and 280 g, were used in this study. Intralipid 20.0% was administered by intravenous injection at a dose of 2 g/kg (n = 30). PBS was administered to control animals (n = 30). After 1 h, iron-oxide particles were injected intravenously. Two types of iron-oxide particles were tested in this study. Nano-sized USPIO-NH$_2$-FITC particles were injected at a dose of 4.5 mg Fe/kg body weight (n = 12 for Intralipid pre-treatment group and n = 12 for control group). MPIO particles were injected at a dose of 6 mg Fe/kg body weight (n = 18 for each group). MPIO particles contain a magnetite core as well as a fluorescent dye (Dragon Green) [39]. Blood samples were collected at different time points to determine the blood half-life of the particles and labeling efficiency of monocytes. 48 h post injection of iron-oxide particles, various tissues (liver, spleen, kidney, lung, and heart) were collected for the iron-level determination and histological analysis.

2.4. Biodistribution and iron levels in different tissues

The wet weight of each tissue sample (both USPIO-NH$_2$ and MPIO treatment groups: n = 6 for Intralipid pre-treatment group and n = 6 for control group) was recorded and 1 mL of tissue homogenate was lypholized in a test tube for 72 h. 1 mL of 6 N HCl was added to each dry tissue sample and the samples were heated overnight in an incubator set at 55 °C. The samples were centrifuged at 500 g for 15 min and the supernatant was collected in a separate test tube. Suitable dilutions of the sample solution were prepared to reach the final concentration in the range of 0.02 to 1 part per million (ppm) with respect to iron. Samples were analyzed for iron concentrations by inductively coupled plasma-mass spectrometry (ICP-MS) (NexION 300X, PerkinElmer Inc., Waltham, MA). Suitable dilutions of standard solutions purchased from CPI International (Santa Rosa, CA) were prepared and a standard curve in the range of 0.02 to 1 ppm Fe was prepared. ^{57}Fe isotope counts were used to determine the Fe content.

The iron levels in different tissues were also determined by transverse relaxation rate (R_2). The R_2 values of tissue samples were measured at 20 MHz using a Bruker Minispec mq20 NMR Analyzer.

2.5. Blood half-life

The R_2 values of blood samples collected at different time points (USPIO-NH$_2$ treatment group: n = 12 for Intralipid pre-treatment group and n = 12 for control group; MPIO treatment group: n = 18 for each group) were measured using a Bruker Minispec mq20 NMR Analyzer. Blood clearance half-life was determined by fitting the R_2 values to a mono-exponential decay using KaleidaGraph 4.1 (Synergy Software, Reading, PA).

2.6. Flow cytometry

Red blood cells in the blood samples were lysed with ACK lysing buffer (Life Technologies, Carlsbad, CA). Mouse anti-rat ED1: Alexa Fluor 647 antibody was used to label monocytes and macrophages and BUF09 was used as a permeabilization reagent for ED1 detection. Flow cytometry was performed on a FACSVantage (Becton Dickinson, Franklin Lakes, NJ). The data were processed with the use of FlowJo software (TreeStar, Ashland, OR).

2.7. Iron content of labeled-monocytes

48 h following injection of USPIO-NH$_2$-FITC particles (n = 6 for Intralipid pre-treatment group and n = 6 for control group) and 20 min (n = 6 for each group) or 48 h (n = 6 for each group) after MPIO injection, 7 mL of blood was collected from each rat. Blood monocytes were stained as described above. The USPIO-NH$_2$-FITC or MPIO-labeled monocytes were sorted with the use of flow cytometry and the numbers of cells were recorded. The collected cells were dissolved in 50 μL of 70% nitric acid. The solutions were then subjected to ICP-MS analysis.