Registered Bioimaging of Nanomaterials for Diagnostic and Therapeutic Monitoring

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Abstract

Nanomedications can be carried by blood borne monocyte-macrophages into the reticuloendothelial system (RES; spleen, liver, lymph nodes) and to end organs. The latter include the lung, RES, and brain and are operative during human immunodeficiency virus type one (HIV-1) infection. Macrophage entry into tissues is notable in areas of active HIV-1 replication and sites of inflammation. In order to assess the potential of macrophages as nanocarriers, superparamagnetic iron-oxide and/or drug laden particles coated with surfactants were parenterally injected into HIV-1 encephalitic mice. This was done to quantitatively assess particle and drug biodistribution. Magnetic resonance imaging (MRI) test results were validated by histological coregistration and enhanced image processing. End organ disease as typified by altered brain histology were assessed by MRI. The demonstration of robust migration of nanoformulations into areas of focal encephalitis provides "proof of concept" for the use of advanced bioimaging techniques to monitor macrophage migration. Importantly, histopathological aberrations in brain correlate with bioimaging parameters making the general utility of MRI in studies of cell distribution in disease feasible. We posit that using such methods can provide a real time index of disease burden and therapeutic efficacy with translational potential to humans.

Protocol

1. Introduction

The selective delivery of drugs and therapeutic macromolecules (peptides, proteins and nucleic acids) to cellular and tissue sites of active disease and ongoing microbial infections will improve pharmaceutical responses during disease1-3. One particular cellular site is the macrophage that is both highly mobile and immune engaging and is a consistent principal target for the human immunodeficiency virus (HIV).4 Importantly, macrophage engaged inflammation also underlies a broad range of disorders that include degenerative, inflammatory, infectious and cancerous diseases; and the cell's mobility to disease sites underlies progression of tissue injuries5-9. Importantly, the use of blood borne macrophages as drug, macromolecule, and signal carriers has gained recent attention for its translational potential. However, a significant obstruction in realizing therapeutic potentials is the blood brain barrier (BBB) amongst other tissue barriers that are impermeable to a spectrum of macromolecules and proteins. These, barriers, nonetheless, do permit cell passage. All together it is projected that in the natural course of disease peripheral macrophages that bypass barriers can carry formulated drugs, markers, and peptides to sites of infection or inflammation. Nonetheless, such technologies remain only in development. It is through our works that cell-mediated delivery can be developed for diagnostic and therapeutic applications and such applications are supported by laboratory and animal models of human disease10-12.

2. Nanomaterial Preparations

Preparation of nanomaterials for drug delivery and biodistribution studies is the topic of a parallel manuscript in this issue (reference parallel manuscript). All procedures for crystalline nanoparticle manufacture are carried out in a laminar flow hood. All surfaces are disinfected prior to use with 70% alcohol. This includes working surface, the exterior of gloves and any spills. All are covered with solution of replicate 70% alcohol immediately with wipes. Gloves are discarded after use and are not worn when entering any other laboratory area. Excipient, drug, sterile water with/containing any/all reagents for manufacture of drug-laden particles are only brought into work areas when needed for procedures. Sterile wrapped pipettes are used only and discarded after use into a biohazard waste container. The wet-wiping apparatus is disinfected with alcohol prior to and following use. Work area is cleaned immediately before and after with 70% alcohol. Nanoparticle solution is tested for pyrogen in accordance with FDA guidelines to assess the absence of bacterial endotoxin in drug particle solutions used for animals. Briefly,

1. Candidate nanoformulations for in-vivo use are replicated by replacing the drug core or droplet with an identical sized particle or milled piece of superparamagnetic iron oxide (SPIO) before coating with the appropriate surfactant.
2. This is followed by measures of size, charge, shape, and cytotoxicity to determine whether the SPIO model system has the same properties as the candidate nanoformulated drug.
3. Finally, cell loading assays are performed by incubation with the candidate SPIO model nanoformulation in order to determine the relaxivity within the cells using phantoms composed of labeled cells suspended in agar gel. Phantoms are prepared in triplicate and are prepared at a series of concentrations in order to quantify the relaxivity due to the SPIO uptake in cells. This provides an index of sensitivity and determines whether the nanoformulations may affect the oxidation state, and hence the visibility of the SPIO in magnetic resonance imaging (MRI) scans.

3. Methods and Procedures: Animal Preparation

1. Injections/Catheters. Depending on time of interest, the injections may require use of a catheter to inject the animal within the MRI. Catheters are prepared using a non-magnetic needle and a tubing extension with minimum diameter to minimize dead space in the injection line. The catheter should be prefilled with either the solution containing the nanomaterial to be injected or saline, depending on the dead space and the total acceptable volume of the injection. If possible, the injection may be followed with a physiological saline flush. If acute times are not of
5. Data Analyses

1. SPIO detection using MRI: SPIO causes signal loss in T2* weighted MRI; and, as such, the MRI signal void is a sensitive, but not specific

2. SPIO detection using MRI: SPIO causes signal loss in T2* weighted MRI. The region of the brain is determined from the localizer scans and prescribed on images acquired during the same imaging session. Anatomical locations are found on images to prescribe the region of interest for acquiring spectra. Once the region is identified, shimming is performed on a region matching the volume of acquisition, checked using a localized water spectrum. Then, the power of the water suppression pulses are optimized, water frequency is measured to ensure on-resonance water signal, and a short test spectrum is acquired to provide quality control. If spectra are of insufficient quality, system settings, including radiofrequency (RF) power and shim settings, are checked. Finally, if quality is still insufficient, a second 3-plane localizer is run to ensure that the animal has not moved from the initial scans. In our experience, this provides a very high degree of reproducibility and accuracy for spectroscopic acquisitions. Finally, the spectra are acquired in short blocks with resetting of the system frequency between acquisitions to eliminate effects of magnetic field drift and to ensure reproducibility and quality of the final scans. At the end of the acquisition, a single pulse water spectrum at a predefined preamplifier gain is used to acquire a quantitative signal amplitude reference.

3. Histology and Blockface Imaging: After the final MRI scanning session in the time series of experiments, the mouse is perfused, the brain is removed and embedded into a block of OCT compound which has been darkened using a drop of India ink. The block is placed into a cryostat for slicing and histological analysis. Digital images are acquired using a digital flatbed scanner (Kodak EKTADigital Rebel 300D with a Canon Ultrasonic EFS 60mm f/2.8 Macro USM lens) mounted to the front of the cryostat. Individual blockface slices were aligned to reconstruct the 3D volume using the Analyze software package (AnalyseDirect, Lexena, KS).

6. Data Analyses

1. SPIO detection using MRI: SPIO causes signal loss in T2* weighted MRI; and, as such, the MRI signal void is a sensitive, but not specific marker for SPIO presence in tissue. The sensitivity is dependent on the spatial resolution of the MRI scan and the size of the SPIO particle, with a single micron sized particle detected with 100 micron isotropic resolution. In these works, 150 micron isotropic resolution with 200 nanometer sized SPIO particles are used. To provide both sensitivity and specificity for the presence of SPIO in brain, mice were scanned prior to injection of the SPIO labeled cells to allow subtraction images to be used for positive identification of the cells in the brain at later time points. 3D MRI scans were subimaged using the constrained level set method developed in our laboratory as previously described17. Subimaged brain volumes were then coregistered, signal intensity normalized, and the volumes subtracted to detect regions within the brain.
6. Representative Results

Examples of DTI and 1H MRS are shown in Figures 1 and 2. Additional examples of 1H MRS and DTI results can be seen in our previous publications.

Figure 1. Depiction of regions analyzed for DTI metrics.

Figure 2. Spectroscopic fitting using QUEST in the jMRUI signal processing suite.
Disclosures

No conflicts of interest declared.

Discussion

The accurate registration of histology with in-vivo imaging results is a critical step in the development of imaging biomarkers for detection and staging of neuronal disease. Some imaging metrics are likely to be correlated with gross morphological changes including changes in magnetic relaxation properties of tissue used for detecting the presence of white matter disease and cancers. Other more subtle methods, such as DTI, are likely to detect early cellular changes that may not be detectable as histological changes caused by the disease do not appear until later the stages of disease. Still other markers, such as spectroscopic markers, may be indicators of early and reversible changes, which precede even the subtlest cellular alterations.

Biodistribution can be determined non-invasively using a variety of methods. The primary non-invasive methods are positron emission tomography (PET), single photon emission computed tomography (SPECT), optical imaging, and MRI. Nuclear medicine based imaging (PET and SPECT) have been used over the years for many biodistribution, but these methods are limited by the half life of the radiotracers used for labeling the compounds or nanomaterials, especially for PET tracers. Optical imaging can be used for small rodents but cannot be translated to human use except for regions easily accessible such as surface tumors due to light absorption and light scattering. In addition, it is difficult to quantify the optical signals for these same reasons. MRI uses persistent tags such as SPIO that can be tracked in the body over a period of weeks. This, too, must be used with caution, as the label can be transferred to different cells or be reabsorbed by the body.

Detection specificity for SPIO in MRI can be provided by a variety of methods. Detection methods, which provide positive as well as negative signals, are used for improving specificity of the MRI for detecting the presence of SPIO in tissue. The subtraction method used in this work has been used by others, as well28. Other approaches include off resonance detection29-31, phase sensitive imaging that produces a particular pattern near SPIO voids32, and zero echo time image that uses T1 weighting to produce a positive signal intensity in the region of SPIO33. The advancement of these methods for improving quantitation of label, sensitivity and specificity is an area of active research today.

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