Simultaneous 19F/1H MR Molecular Imaging of Neovascularization in Pulmonary Inflammation

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Purpose: Asthma is one of the most common chronic diseases, with an estimated 300 million individuals affected worldwide[1]. The symptoms have a dramatic impact on the quality of patients' lives and cause a significant economic burden on healthcare systems. Increases in the number and size of vessels within the airway wall have long been recognized as an element of asthma remodeling, but the process is poorly understood and has been unappreciated until recently[2]. While it is generally assumed the expanded blood volume *sustains the lung*, speculation exists that hyperpermeable, dilated vessels in the airways *actually contribute to airway obstruction and decreased lung function*, through edema and inflammation. Early neovascular expansion in the lungs in preclinical models and patients is very difficult to assess noninvasively, particularly quantitatively. We have previously shown MR imaging of angiogenesis in several animal models by targeting the neovascular biomarker αvβ3-integrin, which is upregulated on proliferating versus quiescent endothelial cells[3,4,5]. We recently demonstrated that αvβ3-integrin targeted perfluorocarbon nanoparticles may be used for high resolution, dual 19F/1H 3D MR molecular imaging at 3T to directly measure neovascularity in a rat left pulmonary artery ligation (LPAL) model, which was employed to create pulmonary ischemia and induce angiogenesis[6]. Fig. 1 shows a cast of the lung vasculature 21 days after LPAL surgery where increased vascularity in the left lung is clearly visible[7]. We used this model to show spatial and temporal neovascular progression by MR beginning just 3 days after LPAL. In the current study, this approach was assessed on another complementary and highly clinically relevant animal model of asthma. We hypothesize that 19F/1H MR molecular imaging may be used 1) to noninvasively quantify pulmonary angiogenesis in asthma and 2) to deliver acute antiangiogenic therapy to reduce airway remodeling and improve pulmonary function.

Methods: Perfluorocarbon (PFC) nanoparticles (NPs) were prepared as previously described[8]: 20% (v/v) perfluorooctylbromide (PFOB, Exfluor Inc., Round Rock, TX), 2.0% (w/v) of a surfactant comixture, and 1.7% (w/v) glycerin in pH 6.5 carbonate buffer. The surfactant co-mixture of the NPs consisted of ~ 98 mole% lecithin, 1.7 mole% phosphatidylethanolamine (PE), 0.2 mole% AlexaFluor488 (Invitrogen) coupled to PE, and 0.1 mole % of a peptidomimetic αvβ3-integrin antagonist[9]. The surfactant components were combined with the PFOB, buffer, and glycerin with pH adjusted to 6.5, and the mixtures were emulsified. Nominal particle sizes measured by dynamic light scattering was ~200 nm (Brookhaven Instrument Corp.) A Brown Norway rat (BN) house dust mite (HDM) sensitization model was used to study pulmonary angiogenesis in asthma. Briefly, HDM(100µg/ challenge, Greer Laboratories) was delivered twice weekly for up to 3 weeks by intra-nasal aspiration to BN rats (Charles River). Control rats were given the same volume of PBS. In preliminary studies, we showed this challenge regimen to cause a 200% increase in airway vascularity, measured by MVD, and enhanced methacholine responsiveness (unpublished data). At 7, 14, or 21 days after the start of HDM or saline treatment, rats (n=3-4/group) were administered αvβ3-targeted PFOB NPs (1.0 ml i.v./kg). After allowing the NPs to circulate for 2 hours, rats were imaged with high-resolution 19F/1H MRI. Images were acquired at 3T (Philips Achieva) using an in-house, custom dual-tuned open birdcage transmit-receive coil. Simultaneous 3D 1H/19F imaging was used employing a novel steady state ultrashort echo time (UTE) technique (TE/TR=0.1ms/1.96ms) with the frequencies set to the resonance of 1H and the CF2 groups of the PFOB spectrum (representing 12 of 17 total 19F nuclei)[10]. Using a highly oversampled 3D radial readout scheme, the reconstructed image datasets have a nominal resolution of 1.25mm3, but can be reconstructed, post facto, at lower resolutions if required to optimize the signal-to-noise ratio (SNR). Typical total scan time was 28min.

Results: Fluorine images were reconstructed offline at a range of resolutions to optimize SNR. A Nyquist value of 0.20 was chosen for all images. Fluorine signal was distributed heterogeneously throughout the lung of HDM treated rats and was most obvious at day 7 and 14 (avg. normalized 19F signal = 74, 73 respectively), and was significantly greater than that in the saline control, which remained nearly constant (~34). The 19F signal at 21 days decreased slightly (avg. = 56). A representative image is shown in Fig. 3. The fluorine signal on the MR images revealed angiogenesis distributed throughout the lung. Other prominent sites of signal were appreciated in the spleen, liver and intestine, associated with reticuloendothelial clearance and biliary excretion.

Conclusion: Unique k-space data acquisition techniques supported multi-resolution, multi-sensitivity retrospective reconstruction of the simultaneously acquired 1H/19F data. This novel application of dual 1H/19F MR molecular imaging is a clinically translatable approach for noninvasive temporal-spatial assessment of lung angiogenesis, which may provide a better understanding of the role of pulmonary angiogenesis in asthma. These particles may also be used for delivery of acute antiangiogenic therapy to reduce airway remodeling and improve pulmonary function.
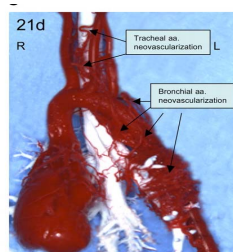

Fig. 1. *Cast of rat bronchial neo-vasculature 21d after LPAL. Systemic vasculature-red. Lung and airways -white. Note the proliferation and enlarged left (L) lung bronchial vessels*
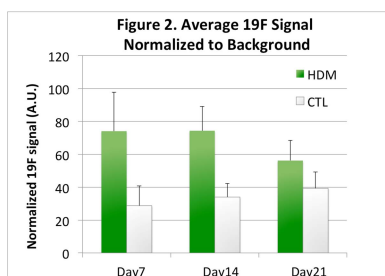

Figure 2. Average 19F Signal Normalized to Background

Fig. 3. *Fluorine signal (green) overlaid on proton image.*

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