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Characterization of Early Neovascular Response to Acute Lung Ischemia using Simultaneous $^{19}\text{F}/^1\text{H}$ MR Molecular Imaging

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Abstract

Angiogenesis is an important constituent of many inflammatory pulmonary diseases, which has been unappreciated until recently. Early neovascular expansion in the lungs in preclinical models and patients is very difficult to assess noninvasively, particularly quantitatively. The present study demonstrated that $^{19}\text{F}/^1\text{H}$ MR molecular imaging with $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles can be used to directly measure neovascularity in a rat left pulmonary artery ligation (LPAL) model, which was employed to create pulmonary ischemia and induce angiogenesis. In rats 3 days after LPAL, simultaneous $^{19}\text{F}/^1\text{H}$ MR imaging at 3T revealed a marked ^{19}F signal in animals 2 hours following $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles (^{19}F signal (normalized to background)= 0.80 ± 0.2) that was greater ($p=0.007$) than the non-targeted (0.30 ± 0.04) and the sham-operated (0.07 ± 0.09) control groups. Almost no ^{19}F signal was found in control right lung with any treatment. Competitive blockade of the integrin-targeted particles greatly decreased the ^{19}F signal ($p=0.002$) and was equivalent to the non-targeted control group. Fluorescent and light microscopy illustrated heavy decorating of vessel walls in and around large bronchi and large pulmonary vessels. Focal segmental regions of neovessel expansion were also noted in the lung periphery. Our results demonstrate that $^{19}\text{F}/^1\text{H}$ MR molecular imaging with $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles provides a means to assess the extent of systemic neovascularization in the lung.

Keywords

MRI; angiogenesis; molecular imaging; fluorine; lung; nanotechnology

Introduction

The lung is perfused by two circulations with the entire venous return coursing through the right heart and comprising the pulmonary circulation. The bronchial circulation is the systemic circulation to the lung and emerges from the aorta and secondary intercostal arteries. Ninety-nine percent of the total lung blood flow is constituted by deoxygenated

blood delivered to the alveolae for oxygenation and gas exchange through a series of pulmonary arteries and capillaries with the return oxygenated blood flowing back to the left atrium through the pulmonary veins. The bronchial circulation delivers oxygenated blood and nutrients to the walls of the bronchi, the pleura, and the vasa vasorum of the pulmonary vasculature, which accounts for the remaining 1% of total blood flow. In the event of markedly diminished total blood flow, such as by severe pulmonary embolus, the bronchial circulation preserves viability of the lung parenchyma and can increase its blood flow capacity to retain nutrient flow to lung structures. Interestingly, it is the bronchial circulation that has been shown to be pro-angiogenic in pathologic settings. [1]

Several techniques have been employed to study the response of the bronchial arterial network in response to severe lung ischemia induced experimentally by pulmonary artery ligation. The earliest studies utilized bronchovascular casts and microscopy [2], which were later complemented by blood flow and gas exchange studies [3]. These techniques provided initial anatomical and functional insight into circulatory compensation mechanisms present weeks to months following pulmonary artery ligation. Later, Weibel et al[4] using infusion of a black viscous gelatin and microscopy studied earlier time points of circulatory remodeling and suggested that the existing bronchial vessels were dilated by 10 days and continue to gradually increase up to 40 days, while concurrently, a small new capillary bed formed and progressed into a network dense enough to obscure visualization of enlarged peripheral vessels previously recognized. In these early days, the question arose as to whether preexisting arterioles and capillaries were too small to appreciate until they were dilated and transformed into arteries or if the increased vascular density resulted from de novo formation of vessels.

Today the process is recognized as a combination of flow-induced vascular dilation of preexisting vessels and neovascular expansion through an angiogenesis process. Clinically, invasive aorto-angiography with X-ray contrast could be used to visualize the number and origin of the bronchial arteries. However, noninvasive multi-detector computed tomography (MDCT) or high-resolution spiral CT may be employed to acquire pulmonary and aortic angiograms with relatively high spatial resolution. However, these techniques lack the resolution to visualize a nascent neovasculature, which may lack significant blood flow until it matures into micro- or usually much larger vessels. Primarily in the context of cancer, approaches to assess remodeling of the vascular supply have included measurements of microvessel density (MVD) by CT perfusion [5,6], fluorodeoxyglucose positron emission tomography (^{18}F -FDG PET) [7], and dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) [5]. While these measures have shown potential prognostic uses for some cancer types, none have high correlation with tumor progression outcomes alone or responses to therapy. While PET/CT offers high signal sensitivity, most biomarkers of angiogenesis are not exclusive to the proliferating endothelial cells, and the high tissue penetrance of these tracers allows significant off-target binding to nonendothelial cells, which confounds interpretation. DCE-MRI estimates the permeability of a vascular bed by calculating the kinetic transfer rate coefficient (K^{trans}) of gadolinium-based contrast agent blood pool escape into the interstitium of tumor or tissue. However, the variability of these estimates are highly dependent on many factors and rigorous comparisons in the same animal model have shown that the estimates are markedly different from those obtained from direct MR molecular imaging with a vascular constrained nanoparticle system [8].

A preferred biomarker of angiogenesis is the $\alpha_v\beta_3$ -integrin, a heterodimeric transmembrane glycoprotein, which is differentially up-regulated in proliferating versus quiescent endothelial cells [9]. However, it is also expressed on numerous cell types including: macrophages [10], platelets [11], lymphocytes [11], smooth muscle cells [12] and tumor cells [13]. We have reported that vascular constrained $\alpha_v\beta_3$ -integrin-targeted paramagnetic

nanoparticles bind to intraluminally expressed $\alpha_v\beta_3$ -integrin that is up-regulated in proliferating nonpolarized endothelial cells ($\alpha_v\beta_3^+$, PECAM⁺, Tie-2⁻) on early vessel tubules, sprouts, or bridges in which there was minimal or mostly no established blood flow. In contradistinction, more mature but still nascent microvessels ($\alpha_v\beta_3^-$, PECAM⁺, Tie-2⁺) with polarized endothelium and an established circulation were not targeted [14].

The human lung presents many challenges for MR imaging with typical proton-sensitive techniques because: 1) respiratory motion interferes with the relatively long image acquisition required, 2) intrinsically low proton density inherently translates into low signal strength, and 3) magnetic susceptibility artifacts are generated by the ubiquitous air-tissue interfaces. However, $\alpha_v\beta_3$ -integrin-targeted perfluorocarbon (PFC) nanoparticles would allow direct imaging of the ^{19}F fluorine nuclei that are present within each bound particle at ~60M concentrations. Since fluorine is not inherently present in the body at detectable levels, confounding background tissue signal is eliminated, resulting in high background-to-noise ratio. Further, the assessment of ^{19}F is direct, it can be quantified more precisely and without undo influence of the surrounding microenvironment water availability, than typical proton-influencing contrast agents. Unlike other non-proton nuclei, ^{19}F is a relatively high signal nucleus, giving nearly 85% the signal per nucleus as ^1H . Our laboratory [15] and others [16–18] have previously developed ^{19}F MR imaging techniques that have been used to evaluate non-targeted perfluorocarbon emulsions for MR phantoms, cell tracking, macrophage imaging, and tissue oxygenation assessments. The present paper, for the first time, explores the potential of ^{19}F imaging of sparse up-regulated $\alpha_v\beta_3$ -integrin receptors expressed in the neovasculature of acutely ischemic lung tissue.

In previous LPAL rodent studies, Wagner and colleagues utilized methacrylate casts to clearly visualize asymmetrical vascular expansion in the left lung 28 days following pulmonary artery ligation, which was not appreciated in the uninjured right lung. [19] They further documented a marked reduction in blood flow to the ligated left rat lung versus a sham-operated control and reported that normal respiratory function was retained by the right lung as compared with diminished DL_{CO} in the ischemic left lung [20]. Histological studies in mice following LPAL documented pulmonary remodeling and systemic vascular angiogenesis expansion in the ischemic but not the sham-operated left lung. [21] In the present study, this characterized LPAL ischemic model was used to determine whether ^{19}F MR imaging with $\alpha_v\beta_3$ -integrin-targeted perfluorocarbon nanoparticles could offer sensitive and specific assessments of very early neovascular expansion using a commercial clinical 3T scanner with multinuclear capability.

Materials and methods

Paramagnetic nanoparticle synthesis

Paramagnetic perfluorocarbon nanoparticles (NP) were prepared similarly to previous reports [22,23]. The emulsions were comprised of 20% (v/v) of perfluorooctylbromide (PFOB; Elf Atochem), 2% (w/v) of a surfactant commixture, 1.7% (w/v) glycerin, and tartaric acid buffer (7.5mM, pH 5.5) representing the balance. The surfactant commixture consisted of 99.9 mole% lecithin (E80, Lipoid, Germany), 0.1mg/ml of an $\alpha_v\beta_3$ -integrin antagonist conjugated to PEG₂₀₀₀-phosphatidylethanolamine (Kereos, Inc., St. Louis, MO, USA). This was combined with PFOB, buffer, and glycerin, and the mixture was emulsified (Microfluidics, Newton, MA, USA). Non-targeted nanoparticles excluded the $\alpha_v\beta_3$ -integrin antagonist, which was replaced with equimolar phosphatidylethanolamine. Alexafluor 594 coupled to phosphatidylethanolamine (0.2 mole%) was incorporated into the lipid surfactant for fluorescent microscopic imaging. Nominal particle sizes obtained by dynamic light scattering (DLS) were 220 80nm (Brookhaven Instrument Corp., Holtsville, NY, USA) with polydispersity indexes < 0.2. (Figure 1)

The $\alpha_v\beta_3$ -integrin antagonist was a quinalone nonpeptide developed by Bristol-Myers Squibb Medical Imaging (US patent 6,511,648 and related patents) and initially reported and characterized as the ^{111}In -DOTA conjugate RP478 and cyan 5.5 homologue TA145 [24]. PFC nanoparticles present ~300 ligands/particle with an IC_{50} of 50 pM for the Mn^{2+} -activated $\alpha_v\beta_3$ -integrin (unpublished data from Kereos, Inc., St. Louis, MO, USA)[22]. Homing specificity of the vascular constrained agent has been demonstrated through *in vivo* competition studies in numerous reports [25–28], and Matrigel™ plug models [14]. Pharmacokinetic and biodistribution studies of ^{111}In -labeled $\alpha_v\beta_3$ -targeted nanoparticles were previously reported [29].

Left pulmonary artery ligation (LPAL)

All animal research was conducted under a protocol approved by the Washington University Animal Studies Committee. Left pulmonary artery ligation was performed on Sprague Dawley male rats (n=20 total; 300 g; Harlan, Indianapolis, IN) as previously described [30,20]. Rats were weighed and anesthetized with isoflurane/oxygen, shaved, intubated, mechanically ventilated and maintained under anesthesia to effect. Positive end-expiratory pressure (PEEP) was maintained at 5 cmH₂O during surgery and increased momentarily from 5 to 20–25cmH₂O just prior to closing the thorax. The left thoracotomy surgical site was aseptically prepped, sterile draped, and infiltrated with bupivacaine. An incision was made over the third intercostal space; the muscle layers were separated with blunt dissection to expose the underlying ribs. The left lung was retracted through the spread ribs and the left pulmonary artery was ligated with prolene suture. Following ligation, the chest cavity was closed, the lungs re-inflated, and the ribs apposed using absorbable suture. The muscle layers were closed in layers with absorbable suture and the skin closed using tissue glue. The animal was extubated and recovered, at which time buprenorphine (0.02–0.05 mg/kg SC) was given and treatment was repeated every 12 hours for 48 hours after surgery for pain management.

On day 3 following surgery, LPAL rats were placed in a Decapicone™ restrainer and administered $\alpha_v\beta_3$ -targeted PFOB NPs (1.0 ml i.v./kg, n=5), non-targeted PFOB NPs (n=4), or a combination of $\alpha_v\beta_3$ -targeted oil NPs and $\alpha_v\beta_3$ -targeted PFOB NPs (3.0 ml:1.0 ml i.v./kg, respectively, n=6) via jugular vein catheter. As additional controls, sham thoracic surgery was performed on rats without the final ligation step, and these animals were dosed with either $\alpha_v\beta_3$ -targeted PFOB NPs (n=2) or non-targeted PFOB NPs (n=2) 3 days after surgery. Two hours post NP injection, the animals were anesthetized with a non-fluorinated anesthetic (ketamine/xylazine; 85/13mg/kg) and imaged with simultaneous $^{19}\text{F}/^1\text{H}$ MRI. Images were acquired at 3T (Philips Achieva) using an in-house, custom dual-tuned open semi-birdcage transmit-receive coil [31]. Simultaneous 3D $^{19}\text{F}/^1\text{H}$ imaging was used employing a novel balanced steady state ultrashort echo time (UTE) technique with the frequencies set to the resonance of ^1H and the CF_2 groups of the PFOB spectrum (offset 6328Hz from ^{19}F ; representing 12 of 17 total ^{19}F nuclei) [32–35]. Imaging parameters were as follows: FOV=140mm, matrix 112^3 , isotropic voxel $\Delta x=1.25$ mm, $\alpha=30^\circ$, excitation bandwidth $\text{exBW}=9$ kHz centered on the PFOB- CF_2 line group, pixel bandwidth $\text{pBW}=900$ Hz, $\text{TR}=2.0$ ms, $\text{TE}=100\mu\text{s}$ (FID sampling), $\text{NSA}=10$. Using a highly oversampled 3D radial readout scheme, the $^{19}\text{F}/^1\text{H}$ image datasets were reconstructed, *post facto*, by modifying the Nyquist weighting factor ($n=0.2$) applied to samples in k-space to obtain an optimal balance between the signal-to-noise ratio (SNR) and image resolution for the different ^{19}F and ^1H signal levels [28] [36]. Uniform ^{19}F weighting was applied to all images from all animals, and voxel size was set to the original $1.25\times 1.25\times 1.25$ mm³. In this anesthetized rat model, neither mathematical correction nor respiratory gating were required to adjust for motion, which was of the same order of magnitude as the pixel resolution. Typical total scan time was 28min.

MR data sets were imported into MATLAB[®] for quantification of the ¹⁹F signal. The ¹H anatomical image was used to manually draw regions-of interest (ROIs) on the left and right lungs, and these masks were copied onto the fluorine images. For each animal, the fluorine signal intensity was normalized by dividing by the average background signal (i.e., air) of the same ROI area. The mean signal intensity was calculated for both the left and right lung ROIs, and then plotted in arbitrary units as (left signal)-(right signal).

Histology

Following the MR imaging session rats (n=11: $\alpha_v\beta_3$ -targeted PFOB NP, n=5; non-targeted PFOB NPs n=3) were deeply anesthetized and underwent thoracotomy. Dylight-488 *Lycopersicon esculentum* (tomato) lectin was infused through the right ventricle, allowed to circulate for 2 min, then flushed with 20ml of saline to remove unbound nanoparticles from the vascular bed. The euthanized animal airway passages were infused with OCT at 25 cmH₂O to preserve lung architecture. Tissues were snap frozen in OCT media and cryosections (8 μ m) were produced for qualitative corroborative histological analysis. A minimum of 5 high-powered fields (100 $\times$) from each of 5 sections obtained from the left lung parenchyma and the proximal hilar peri-injury region were reviewed for fluorescent imaging of the vascular-bound lectin and the Alexafluor 594-enhanced nanoparticles in each animal. Further sections were obtained near these for routine H&E staining with acetone fixation. Multiple digital images were acquired with an Olympus BX61 fluorescence microscopy imaging system with Biological Suite software for instrument control and image processing (Olympus America, Inc, Center Valley, PA), using Color View II camera for optical image digitization, and F-View II B&W CCD camera for fluorescent images.

Results

Figure 2A presents a low-power (40 $\times$) light image of the ischemic left lung tissue sampled near the pulmonary artery ligation 2 hours following IV administration of $\alpha_v\beta_3$ -targeted or non-targeted PFOB NPs incorporating a fluorescent 594nm dye (red). Fluorescent 488nm tomato lectin (green) was given 2 hours after the nanoparticles as a vascular constrained high affinity vascular endothelium marker. Two block insets are shown near a large airway (top) and adjacent to a large pulmonary vein (bottom). Figure 2 B–D are fluorescent microscopy images from the large airway region of interest. Figure 2B reveals strong co-staining (i.e., yellow) of $\alpha_v\beta_3$ -targeted PFOB NPs (red, Figure 2C) and the vascular bound green lectin (Figure 2D), which occurred in high density within the lamina propria subjacent to the bronchial epithelium. Figures 2 E–G corresponded to the lower region-of-interest adjacent to a large pulmonary vein. Figure 2E shows the $\alpha_v\beta_3$ -targeted PFOB NP bound densely to small vessels near the PV and co-localized with the lectin. Fluorescent particles were not observed within the lumen of large vessels indicating adequacy of washout, yet they were prominent in the vascular wall adventitia, consistent with expectations for neovascular expansion.

The periphery of the same ischemic lung lobe, seen as a low power (40 $\times$) bright-light image in Figure 3A, showed a well-inflated lung parenchyma. Higher power fluorescent microscopy, Figure 3B–C, presents a lacy pattern of green tomato lectin decorating the vessels throughout the tissue. Co-localized with the lectin stain, adjacent to the air-spaces, are numerous examples of $\alpha_v\beta_3$ -targeted PFOB (red 594nm) NPs. Co-localization of integrin-targeted particles and endothelial-specific lectin was appreciated throughout the ischemic apical lung lobes.

Simultaneous ¹⁹F/¹H imaging of the LPAL rats three days after the induction of ischemia using a clinical 3T MR scanner showed clear signal from $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles localized to the left lung at the levels of the heart and in the periphery (Figure

4 A–C). As anticipated, obvious signal derived from the liver and spleen, primary reticuloendothelial clearance organs for perfluorocarbon nanoparticles, was observed. (Figure 4 A, B, D, E) Of note, little ^{19}F contrast was visually appreciated in the right lung, indicating that the nanoparticles did not accumulate in normal pulmonary tissues through aggregation or entrapment and that off-target binding to mature vessel endothelium or other cellular constituents, such as intravascular macrophages, was negligible. Simultaneous $^{19}\text{F}/^1\text{H}$ imaging of the LPAL rats following injection of the non-targeted particles, Figure 4 D–F, showed no visible accumulation in the ischemic left lung or control right lung, although particle clearance into the liver and spleen were readily apparent. These data further indicate that passive entrapment of nanoparticles in the neovasculature or phagocytosis of particles by pulmonary macrophages or other inflammatory cells homing into the ischemic lung were minimal. Figure 5 presents the quantitative ^{19}F signal difference between the ischemic and normal lungs of rats after LPAL that had been administered $\alpha_v\beta_3$ -targeted nanoparticles. As apparent from the MRI images, the ^{19}F signal derived from the $\alpha_v\beta_3$ -nanoparticles targeted to the ischemic left lung (2.35 ± 0.30) far exceeded the signal derived from the contralateral control lung (1.55 ± 0.22 ; $p=0.009$). These data provide the first examples of direct, noninvasive, and quantitative neovascular imaging in an ischemic lung using MRI, more specifically dual $^{19}\text{F}/^1\text{H}$ MRI with a clinical 3T multinuclear scanner.

The specificity of $\alpha_v\beta_3$ -nanoparticle targeting to the neovasculature of the ischemic lung was further studied by comparing the ^{19}F signal in the left lung minus the right lung as previously discussed (Figure 6). The net increase in left lung ^{19}F MR contrast signal in LPAL rats receiving the $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles (0.80 ± 0.2) was approximately 250% higher ($p=0.005$) than animals given the non-targeted nanoparticles (0.30 ± 0.04). ^{19}F signal in the sham-operated lung receiving $\alpha_v\beta_3$ -targeted PFC nanoparticles (0.03 ± 0.05) and non-targeted nanoparticles (0.10 ± 0.21) did not differ from each other or the control right lung. Moreover, competitive inhibition of the $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles with $\alpha_v\beta_3$ -targeted oil nanoparticles (i.e. devoid of ^{19}F) showed a ~90% decrease ($p=0.002$) in ^{19}F signal relative to rats receiving the $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticle treatment and to those animals given the non-targeted perfluorocarbon agent (~65% decrease, $p>0.05$). These results further showed that $^{19}\text{F}/^1\text{H}$ molecular imaging of neovasculature was specific, and readily blocked by competition in vivo. Positive ^{19}F signal was often observed at the surgery site presumably associated with wound healing. However, this area was consistently outside the lung ROI chosen for signal analysis, as confirmed by the sham control results and illustrated in Supplemental Figure 1, and did not impact the assessment of pulmonary angiogenesis. These results revealed variable levels of signal at the wound site, independent of treatment.

Discussion

The involvement of angiogenesis in the context of pulmonary disease is receiving increased attention as its involvement in the complex pathophysiology of bronchial asthma[37], angioproliferative pulmonary hypertension[38–40], and acute respiratory distress syndrome[41] is better recognized. Moreover, even the paucity of normal alveolar-associated angiogenesis capacity may significantly contribute to bullae development in chronic obstructive pulmonary diseases [42]. In general, these diseases have progressive natural histories with limited medical approaches available for patients with moderate to severe disease. In fact, medical therapy in these patients is directed toward acute symptomatic relief of clinical exacerbations and the use of mitigating anti-inflammatory medicants, such as steroids, to slow the rate of progression and or reduce the incidence of relapse. New innovative diagnostic and therapy approaches are needed to quantitatively assess patients early in the clinical course and as needed subsequently to improve long-term management.

The present study demonstrated that $^{19}\text{F}/^1\text{H}$ MR molecular imaging with $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles can be used to directly assess angiogenesis in a rodent using a clinical 3T scanner. A rat left pulmonary artery ligation model was employed to create pulmonary ischemia and induce angiogenesis. Following the administration of $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles on day 3 after the induction of left pulmonary artery ischemia, fluorescent and light microscopy illustrated that the nanoparticles were constrained to the vasculature, co-staining with tomato lectin, and heavily decorating vessels in and around larger bronchi and large pulmonary vessels. In the ischemic lung periphery, the particles were also observed throughout the tissue associated with the lectin, suggesting an ongoing angiogenesis process distal from the ligation as well.

Using simultaneous $^{19}\text{F}/^1\text{H}$ MRI with a clinical scanner at 3T, a marked ^{19}F signal was appreciated in the 3 day ischemic left lung 2 hours following $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles when compared to the contrast measured with the non-targeted control nanoparticles. Almost no ^{19}F signal was appreciated in the control right lung with either treatment. Furthermore, competitive blockade of the integrin-targeted particles greatly decreased the ^{19}F signal in the ischemic lungs relative to the $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles similar to the non-targeted control group. These imaging data, acquired only 3 days after ischemia was induced in rats, were the first examples of direct bronchial neovascular MR molecular imaging in a preclinical animal model. Since these results were achieved with techniques developed for a medical 3T MR scanner, the potential for rat to human clinical translation is very high.

Pulmonary artery ligation induced lung ischemia in the ventilated lung has been shown to stimulate systemic angiogenesis despite the preservation of normoxia. While the underlying mechanisms are poorly understood, the onset of angiogenesis is suggested to involve early and transient up-regulation of reactive oxygen species (ROS) [43]. In addition, neovascular response in the lung is likely mediated via glutamic acid-leucine-arginine (ELR) CXC chemokines and their G protein-coupled receptor, CXCR2, since antibody-mediated neutralization of CXCR2 significantly decreased vascularity assessed with a methacrylate vascular cast and total bronchial blood flow estimated with radiolabeled microspheres at 21 days after ischemia was induced [19]. From the perspective of on-going preclinical research into the biochemical mechanisms contributing to bronchial angiogenesis, $^{19}\text{F}/^1\text{H}$ MR molecular imaging with $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles could offer a quantitative and sensitive “snap-shot” of angiogenesis status at much earlier time points. This is highly significant because current methods of angiogenesis quantification in the lung assess the mature perfusing microvascular beds formed.

Several previous studies have demonstrated that the bronchial circulation dilates and expands to compensate for the dramatic loss in total lung blood flow during pulmonary ischemia. Under normal circumstances the subcarinal bronchial circulation has been shown to anastomose with pulmonary vessels at capillary and post-capillary sites and drain through pulmonary veins to the left atrium. In the event of severe pulmonary embolism or pulmonary artery ligation, the interruption of pulmonary blood flow and altered pulmonary vascular pressures might allow for an immediate compensatory increase in bronchial arterial flow through the pulmonary capillary network. Weibel, in his careful histologic assessment of the rat lung days after LPAL, showed increased anastomotic or collateral circulation that verified an increase in bronchial to pulmonary blood flow [4]. We speculate that $\alpha_v\beta_3$ -targeted nanoparticles observed in the lung periphery likely relate to the onset of these new collateral networks.

Finally, this paper presents the first report of quantitative simultaneous dual $^{19}\text{F}/^1\text{H}$ MR molecular imaging of the lung neovascularization. Simultaneous transmit and receive

radiofrequency energy at both 120 MHz (^{19}F) and 126 MHz (^1H) has many benefits over executing sequential ^{19}F and ^1H acquisitions. As previously mentioned, ^{19}F imaging assesses the perfluorocarbon nanoparticles directly and the signal conveys the amount of fluorine present, whereas for ^1H contrast enhanced MR images, both T1 (bright) or T2 (dark) are assessed by measuring the effect of the agent on surrounding water molecules, wherein the magnitude of signal becomes only relative and highly dependent on the surrounding water exchange environment. In clinical translation, ^{19}F interrogation will be much less influenced by the local microenvironment but, like the ^1H signal, respiratory motion can cause blurring or ghosting of signal of one voxel across many and may necessitate temporal gating to optimize image quality at the expense of a greatly lengthened time of acquisition. The simultaneous $^{19}\text{F}/^1\text{H}$ acquisition of both the fluorine and proton images, as applied in the present study, imparts the ability to mathematically correct for motion without gating, retaining proper co-registration and eliminating signal smearing [33]. However, mathematical motion correction was not required in this study because the motion was of the same order of magnitude as pixel resolution. The elimination of motion artifacts increases quantitative ^{19}F molecular imaging accuracy and reduces total scan times.

Conclusion

In this study, we have used simultaneous $^{19}\text{F}/^1\text{H}$ MR molecular imaging with $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles to quantitatively assess neovascular expansion of the bronchial arteries following pulmonary artery ligation. The study, conducted in rats using a clinical 3T scanner, provided the earliest noninvasive assessments of angiogenesis in a preclinical model, 3 days after the induction of ischemia. Beyond the high sensitivity of the technique, homing was found to be very specific based upon comparisons to the contralateral normal lung, to non-targeted nanoparticle signal in the ischemic lung, to the sham-operated lung, and following in vivo competitive inhibition. Fluorescent microscopy confirmed that neovascular signal derived after 3 days of ischemia originated prominently from tissues near the ligation site surrounding the large airways and large pulmonary vessels. Peripheral lung showed diffuse binding of nanoparticles suggestive of early peripheral endothelial cell activation. $^{19}\text{F}/^1\text{H}$ MR molecular imaging with $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles offers a unique noninvasive mechanism to understanding bronchial vessel expansion and subsequent pulmonary perfusion compensation, which could be employed to quantify the early progression several lung pathologies with prominent angiogenic features, such as asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Diagrammatic illustration of perfluorocarbon nanoparticle structure with $\alpha_v\beta_3$ -integrin targeting ligand and red fluorescent dye label.

Figure 2.

Neovascularity near the hilar pulmonary artery ligation site. A). Low power H&E showing a large bronchus, pulmonary vein and cartilage. Top region-of-interest (ROI) inset is continued as fluorescent images in panels B, C, and D. Lower ROI is continued in panels E, F, and G. B). Merged images of panel C and D showing co-localization of the red integrin bound nanoparticles (C) with the green vascular tomato lectin (D) in the wall of a bronchus. E). Presents merged images of red integrin bound nanoparticles (F) with the green vascular tomato lectin (G) in the surrounding adventitia of a large pulmonary vein.

Figure 3.

Light and fluorescent images of the ischemic left lung apex. A) Low power (40×) H&E image of the apex of ischemic left lung showing the preservation of airways and modest lung inflammation. B) Fluorescent merged image of integrin-targeted nanoparticles (red) and vascular-targeted lectin (green) in the injured lung apex (100×). C) Higher power (200×) image showing co-localization of nanoparticles and lectin in focal segments.

Figure 4.

Whole body $^{19}\text{F}/^1\text{H}$ images of rat thorax and abdomen 2 hours after injection of $\alpha_v\beta_3$ -targeted or non-targeted (NT) perfluorocarbon nanoparticles (green). A. Coronal plane of rat following $\alpha_v\beta_3$ -targeted nanoparticles showing accumulation in left lung near the pulmonary artery ligation site (adjacent to heart) and diffuse accumulation in the lung parenchyma. Marked accumulation of particles through the reticuloendothelial system (RES) in the liver and spleen is apparent. Minimal signal was detected in the right lung. B. Sagittal view of targeted nanoparticle accumulation in the left lung. C. Transverse view at the level of the heart showing bound nanoparticles in the left but not in the right lung. D. Coronal plane of rat following NT-nanoparticles showing no visual accumulation in the injured left lung adjacent to heart, but with significant RES clearance of particles into the liver and spleen. No signal is appreciated in the right lung. Similar images were observed in the sham and competition groups (i.e. little to no ^{19}F signal in lungs). In all of the images, no off-target contrast was appreciated in surrounding musculature. The ^1H images shown above were obtained at higher resolution using a clinical 3T scanner for higher thoracic anatomical clarity. Yellow arrows indicate examples of ringing artifact recognized in this particular MRI imaging sequence, common to high signal areas (i.e. liver and spleen). ^{19}F signal was occasionally detected in the heart, unrelated to NP treatment and most likely due to blood pool effect. The heart was consistently excluded from the lung ROI analysis.

Figure 5. Comparison of left and right lung ^{19}F signal normalized to background noise within injured rats receiving $\alpha\text{v}\beta\text{3}$ -targeted perfluorocarbon nanoparticles. Left lung ^{19}F signal is significantly greater ($p=0.009$) than right lung contrast.

Figure 6.

Comparison of left-right lung signal in day 3 LPAL rats, and sham-operated rats, administered nontargeted, $\alpha_v\beta_3$ -targeted, or competitive-inhibited $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles after two hours. LPAL rats receiving $\alpha_v\beta_3$ -targeted nanoparticles had the greatest ^{19}F left lung signal compared to animals given the nontargeted perfluorocarbon particles ($p=0.005$), and competitive inhibition of the $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticles with $\alpha_v\beta_3$ -targeted oil nanoparticles greatly decreased lung ^{19}F signal relative to $\alpha_v\beta_3$ -targeted perfluorocarbon nanoparticle group ($p=0.0001$) but also lowered the signal level relative to the nontargeted control rats ($p=\text{NS}$). ^{19}F signal in sham-operated control rats was also greatly decreased ($p=0.002$).