A View on Imaging in Drug Research and Development for Respiratory Diseases

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ABSTRACT

With the incidence of respiratory diseases increasing throughout the world, new therapies are needed. This review provides a short overview of different imaging techniques of interest for drug discovery and development within the pulmonary disease area. The focus is on studies performed in both animals and humans, which are of importance for understanding pathophysiological aspects and evaluating new drugs. Rather than emphasizing particular lung diseases, the noninvasive diagnosis and quantification of a number of characteristics related to several pathological conditions of the lung are addressed: inflammation, mucus secretion and clearance, emphysema, ventilation, perfusion, fibrosis, airway remodeling, and pulmonary arterial hypertension. Techniques are discussed based on their present use or potential future utilization in the context of drug studies.

Introduction

Diseases of the airways such as asthma and chronic obstructive pulmonary disease (COPD) involve a complex interplay of many inflammatory and structural cell types, all of which can release inflammatory mediators including cytokines, chemokines, growth factors, and adhesion molecules. Activated eosinophils are considered particularly important in asthma, contributing to epithelial cell damage, bronchial hyperresponsiveness, plasma exudation, and edema of the airway mucosa, as well as smooth muscle hypertrophy and mucus plugging, through the release of enzymes and proteins (Barnes, 1996). In COPD, inflammation of the small airways and lung parenchyma with the involvement of neutrophils, macrophages, and T lymphocytes results in chronic obstructive bronchitis, destruction of the lung parenchyma by proteolytic enzymes (emphysema), and mucus hypersecretion leading to severe airflow limitation (Barnes, 2002). Pulmonary fibrosis, characterized by fibroblast proliferation and extracellular matrix remodeling, is the end result of diverse types of lung damage including pneumonia (Gross and Hunninghake, 2001).

Spirometry measuring the volume and flow of inhalation and exhalation is the most common approach for diagnosing lung function abnormalities in humans. This method lacks regional information and can have a large range of variation based on patient effort and cooperation. Moreover, subtle changes caused by e.g., treatment may be masked by the global assessment of lung function because nondiseased lung tissue may compensate for the functional impairment of diseased lung tissue.

Many laboratory animal species provide models for airway diseases in humans to help develop novel therapies. Terminal procedures such as bronchoalveolar lavage (BAL) fluid analysis, histology, and weighing of lungs are commonly used to analyze such models. Pulmonary function is assessed either noninvasively in conscious, unrestrained animals (pleth-
Lung Imaging Techniques

Table 1 provides an overview of current imaging modalities of interest for imaging the lungs. Refer to other reviews for detailed descriptions of each method for small-animal applications (Beckmann et al., 2007a,b; De Kemp et al., 2010) or human applications (Harris and Schuster, 2007; Petersson et al., 2007; Ley-Zaporozhan et al., 2008a; Sundaram et al., 2010). In many respects the imaging techniques are complementary; there is no “all-in-one” imaging modality providing optimal sensitivity, specificity, and temporo-spatial resolution. For instance, because of its relatively low sensitivity, MRI is of limited value for detecting molecular processes in vivo; nevertheless, its high spatial resolution provides a good anatomical reference for molecular data obtained with high-sensitivity, low-resolution imaging modalities. Coregistration may be achieved by postprocessing of data obtained in different imaging sessions or simultaneous multimodality imaging such as PET-MRI (Schlemmer et al., 2008), PET-computerized tomography (CT) (Poeppel et al., 2009), and SPECT (Mariani et al., 2010).

A challenge in lung imaging is that cardiac and respiratory motion can cause marked image artifacts. In humans, image acquisition may be performed during breathhold or by gating it by an electrocardiogram. Problems are more evident in small rodents, because of their higher cardiac and respiratory rates. To address this issue, measurements are often performed in artificially ventilated animals to maintain a constant breathing rate, and/or image acquisition is triggered by the electrocardiogram. However, for compound testing in vivo in animal models of airway diseases, it is important to keep acquisition conditions as simple as possible so that repeated measurements interfere minimally with the physiology and the well being of the animals can be performed longitudinally. For instance, one needs to carefully consider possible interferences between the pathophysiology of the disease models and lung injury complications that might potentially be caused by mechanical ventilation (Walder et al., 2005), especially if this is applied repeatedly. Indeed, it has been reported that mechanical ventilation of healthy rats can cause an increase of neutrophils in BAL fluid, pulmonary edema, and even hypoxemia that may lead to progressive circulatory failure and death. Consequently, mechanical ventilation should be avoided whenever possible for the longitudinal investigation of lung disease models with expected inflammatory responses. Signal averaging has been used in lung MRI of spontaneously breathing rats and mice without any gating (Beckmann et al., 2001a; Blé et al., 2008; Zurek et al., 2010) (Fig. 1).

For ethical reasons, animals are kept anesthetized during imaging. One needs to consider what influence anesthesia may have on functional readouts and whether it may interfere with the development of the disease model, especially under repeated inductions. It is recommended to keep the anesthesia time to ≤ 30 min for each session.

With nuclear imaging techniques, exposure to radiation may be a serious limiting factor for longitudinal studies. For patients, a rise in the effective dose from medical diagnostic procedures in the past years has been reported for CT (Chen and Moir, 2010). Thus, effort is being spent to develop high-resolution CT (HRCT) procedures with reduced radiation exposure. Awareness of this fact will affect the use of this technique in clinical drug studies. For animal applications, radiation doses of micro-CT, despite not being lethal, may be high enough to induce changes in the immune response and

<table>
<thead>
<tr>
<th>Technique</th>
<th>Spatial Resolution and Time Scale</th>
<th>Application</th>
<th>Main Characteristics</th>
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<tr>
<td></td>
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<tr>
<td>CT</td>
<td>50–100 μm; seconds to minutes</td>
<td>Anatomical, functional</td>
<td>Ionizing radiation; poor soft tissue contrast</td>
</tr>
<tr>
<td>Scintigraphy</td>
<td>≥1 mm; ~20 min</td>
<td>Functional</td>
<td>Planar information</td>
</tr>
<tr>
<td>SPECT (low-energy γ-rays)</td>
<td>No</td>
<td>Functional</td>
<td>Ionizing radiation; radioisotopes have longer half-lives than those used in PET; sensitivity 10 to 100 times smaller than PET</td>
</tr>
<tr>
<td>PET (high-energy γ-rays)</td>
<td>1–2 mm; ~20 min</td>
<td>Metabolic, functional, molecular</td>
<td>Ionizing radiation; high sensitivity (picomolar concentrations); cyclotron needed</td>
</tr>
<tr>
<td>MRI</td>
<td>80–140 μm; seconds to hours</td>
<td>Anatomical, functional, molecular</td>
<td>High spatial resolution and soft tissue contrast; low sensitivity</td>
</tr>
<tr>
<td>NIRF optical imaging</td>
<td>1–3 mm; seconds to minutes</td>
<td>Molecular</td>
<td>High sensitivity (nanomolar concentrations); maximal penetration depth &lt;10 cm</td>
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other biological pathways, so that experimental outcomes could be affected. Doses similar to those used in clinical medicine have been reported (Ask et al., 2008), thereby restricting considerably the number of repeated measurements on each animal.

**Imaging in Respiratory Diseases: From Animal Models to Patients**

In this section, the use of imaging to noninvasively characterize several aspects of lung diseases will be discussed. Whenever possible, references are made of pharmacological studies involving imaging, either in animal models or human patients. For many applications, however, studies involving compounds have not been reported. Nonetheless, the applications are addressed because of their potential in becoming useful tools for drug discovery in the near future. Summaries of techniques and applications in animals and humans are provided in Tables 2 and 3, respectively.

**Airway Inflammation.** A characteristic feature of lung inflammation is edema in the airways caused by an increase in the permeability of the microvasculature. MRI has been used to quantify edema in the lungs of spontaneously breathing mice (Blé et al., 2008; Conti et al., 2010) or rats (Beckmann et al., 2001a; Tigani et al., 2002; Quintana et al., 2006a) actively sensitized to and challenged with ovalbumin (OVA). The MRI signals after OVA challenge correlated significantly with a variety of inflammatory parameters determined in the BAL fluid recovered from the same animals. It is noteworthy that the fluid signals detected by MRI correlated significantly with the perivascular edema assessed by histology (Tigani et al., 2003a; Blé et al., 2008). Micro-CT also has been used to quantify edema in a rat model of allergic lung inflammation (Jobse et al., 2009).
When assessing the effects of anti-inflammatory drugs administered before disease induction in these models, a dose-related reduction of the MRI signals has been shown for compounds such as the glucocorticosteroids budesonide (Beckmann et al., 2001a; Tigani et al., 2003a; Blé et al., 2009a) (Fig. 2) and mometasone (Tigani et al., 2003a) and a mitogen-activated protein kinase inhibitor (Tigani et al., 2003a). Moreover, the pharmacology of sphingosine-1 receptors has been studied in vivo using MRI (Blé et al., 2009a). Imaging data correlated with changes in the parameters of inflammation assessed in the BAL fluid. MRI was also applied to address the effects of compounds on established allergic inflammation. Treatment with budesonide, mometasone, or a phosphodiesterase-4 inhibitor at 24 h after OVA challenge reduced MRI signals at 3 h after drug administration. The decline in MRI signals correlated significantly with a reduction in perivascular edema quantified by histology (Beckmann et al., 2001a; Tigani et al., 2003a)
TABLE 3
Applications of imaging techniques in clinical lung research

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Technique</th>
<th>Readout</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Inflammation</td>
<td>[18F]FDG-PET</td>
<td>Deoxyglucose uptake reflecting neutrophil recruitment and activation</td>
<td>Chen et al., 2009</td>
</tr>
<tr>
<td>Mucus secretion and clearance</td>
<td>Scintigraphy</td>
<td>Mucus clearance assessed using 99mTc-labeled sulphur colloids</td>
<td>Sood et al., 2003; Donaldson et al., 2006</td>
</tr>
<tr>
<td>Mucus plugging</td>
<td>HRCT</td>
<td>Mucus detection</td>
<td>Puderbach et al., 2007</td>
</tr>
<tr>
<td>Mucus plugging</td>
<td>MRI</td>
<td>Mucus detection; use of contrast agent holds promise to improve discrimination between bronchial wall thickening and mucus</td>
<td>Puderbach et al., 2007; Ley-Zaporozhan et al., 2008b</td>
</tr>
<tr>
<td>Emphysema</td>
<td>HRCT</td>
<td>Quantification of emphysematous destruction</td>
<td>Stoei and Stolk, 2004; Ley-Zaporozhan et al., 2008n</td>
</tr>
<tr>
<td>Emphysema</td>
<td>HP 3He MRI</td>
<td>ADC</td>
<td>Samee et al., 2003; Mentore et al., 2005; van Beek et al., 2007; Tzeng et al., 2009</td>
</tr>
<tr>
<td>Ventilation, perfusion</td>
<td>SPECT</td>
<td>99mTc-labeled macroaggregated albumin trapped in capillaries</td>
<td>Petersson et al., 2007</td>
</tr>
<tr>
<td>Ventilation</td>
<td>PET</td>
<td>Washout of inhaled 13N-N2 gas</td>
<td>Vidal Melo et al., 2002, 2010; Musch et al., 2004; Harris et al., 2006</td>
</tr>
<tr>
<td></td>
<td>HP 3He-MRI</td>
<td>Gas distribution; bronchoconstriction</td>
<td>Samee et al., 2003; Mentore et al., 2005; van Beek et al., 2007; Tzeng et al., 2009</td>
</tr>
<tr>
<td>Ventilation</td>
<td>Oxygen-enhanced MRI</td>
<td>Contrast change caused by paramagnetic properties of O2</td>
<td>Ohno et al., 2008, 2011</td>
</tr>
<tr>
<td>Perfusion</td>
<td>Contrast-enhanced MRI</td>
<td>Contrast change of dynamic images after administration of paramagnetic contrast material</td>
<td>Eichinger et al., 2006; Ley-Zaporozhan et al., 2007</td>
</tr>
<tr>
<td>Perfusion, ventilation</td>
<td>Fourier decomposition MRI</td>
<td>Lung perfusion and ventilation without administration of contrast agent</td>
<td>Bauman et al., 2009</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>HRCT</td>
<td>Fibrosis-related anatomical changes</td>
<td>Lynch et al., 2005; Best et al., 2008</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>SPECT</td>
<td>Lung uptake of 111In-octreotide</td>
<td>Lebtahi et al., 2006; Ha et al., 2008</td>
</tr>
<tr>
<td>Airway remodeling</td>
<td>HRCT</td>
<td>Airway wall thickness and area</td>
<td>Tschirren et al., 2005; Aysola et al., 2008</td>
</tr>
<tr>
<td>Pulmonary arterial hypertension</td>
<td>Echocardiography</td>
<td>Pulmonary arterial pressure and pulmonary vascular resistance</td>
<td>Bleeker et al., 2006</td>
</tr>
<tr>
<td>Pulmonary arterial hypertension</td>
<td>MRI</td>
<td>Pulmonary arterial pressure; heart right ventricular mass and function</td>
<td>Sanz et al., 2007; Reiter et al., 2008; Nogami et al., 2009; Bradlow et al., 2010; Toshner et al., 2010</td>
</tr>
<tr>
<td>Drug delivery</td>
<td>SPECT</td>
<td>Distribution of 99mTc- or 111In-labeled substrates</td>
<td>Dolovich and Labiris, 2004; Yates et al., 2005</td>
</tr>
<tr>
<td>Drug delivery</td>
<td>PET</td>
<td>Distribution of positron-emitter labeled compounds</td>
<td>Coates et al., 2007, 2008</td>
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2003a,b). No changes in BAL parameters were observed at this early time point. These observations indicate that MRI is more suitable to detect early effects of compounds on established inflammation than the traditional BAL fluid analysis.

Compared with single dosing, repeated OVA challenge in actively sensitized rats induced an attenuation of the inflammatory response as evidenced by MRI and BAL fluid analysis (Tigani et al., 2007). Moreover, vascular remodeling has been detected by MRI in the model: the decrease in vascular permeability assessed by dynamic contrast enhanced-MRI was consistent with the thickening of the vascular wall for vessels of diameter up to 300 μm as revealed histologically (Tigani et al., 2007). Clinically, the assessment of lung edema with MRI is more suitable to detect early effects of compounds on established inflammation than the traditional BAL approach.

PET with [18F]fluorodeoxyglucose ([18F]FDG) has also been used to quantify pulmonary inflammation. [18F]FDG, an analog of glucose, is taken up by the same transporters that transfer glucose into the cell. Studies in rat models of pneumonia and pancreatitis- and sepsis-induced lung inflammation (Jones et al., 1994; Hansson et al., 1999; Hartwig et al., 2002), as well as in a canine model of acute endotoxin-induced lung injury (Chen and Schuster 2004), showed increased [18F]FDG uptake by the lungs after the insults. Tissue autoradiography demonstrated [18F]fluorodeoxyglucose uptake by neutrophils before their entry into the alveolar space. These studies suggest that increased deoxyglucose uptake occurs early in the process of neutrophil recruitment to the lungs. In patients, [18F]FDG PET has been applied to demonstrate anti-inflammatory effects of statin treatment after endotoxin-induced lung inflammation in volunteers (Chen et al., 2009). However, increases in lung [18F]FDG uptake may not necessarily be limited to neutrophils, but may also be caused by macrophages, eosinophils, or tumors. Thus, efforts are underway to synthesize neutrophil-specific PET agents, such as cFLFLFK-PEG-64Cu, a highly potent 64Cu-labeled peptide that targets the formyl peptide receptor on neutrophils (Zhang et al., 2007; Locke et al., 2009) (Fig. 3).

Radiolabeled annexin V has been proposed for the noninvasive detection and monitoring of active pulmonary inflammation using SPECT (Blankenberg, 2009). Annexin V is a ubiquitous intracellular protein with nanomolar affinity for the phospholipid phosphatidylinerine, which is selectively ex-
pressed on the surface of apoptotic or physiologically stressed cells.

Another promising approach is the use of $^{99m}$Tc-labeled interleukin-8, a SPECT probe that binds to neutrophil CXC1 and CXC2 receptors. It has been successfully applied to image different lung infections in rabbits (Rennen et al., 2004) as well as various inflammatory processes in human patients (Bleeker-Rovers et al., 2007), but is not yet in use for human lung inflammation. The clinically most widely used in vivo neutrophil radiolabeling agent, $^{99m}$Tc-sulesomab or Leuko-Scan, an NCA-90 antibody Fab fragment, has not been applied in pulmonary studies to date.

Near-IR fluorescence (NIRF) molecular tomography imaging in combination with probes targeting proteases (Ntziachristos, 2009) or matrix metalloproteinases (Cortez-Retamozo et al., 2008) has also been successfully applied to image lung inflammation and the effects of dexamethasone treatment in OVA- or lipopolysaccharide (LPS)-induced lung inflammation in mice. Because of the low penetration of the near-IR radiation (a few centimeters), this technique is limited to small rodent applications.

**Mucus Secretion and Clearance.** Chronic mucus hypersecretion and dysfunctions in mucociliary clearance are associated with the accelerated loss of lung function in several respiratory diseases. Measurements of mucus clearance in the mouse lungs have been performed using scintigraphy in combination with the administration of $^{99m}$Tc-labeled sulfur colloid (Foster et al., 2001). The same approach has been applied to monitor the retention and clearance of radiolabeled human serum albumin and sulfur colloids in dogs (Lay et al., 2003). Also clinically, radiolabeled inert particles have been widely used to assess mucociliary clearance. From the ratio of particle deposition in central versus peripheral regions of the lungs it could, for instance, be clearly demonstrated that treatment of patients with cystic fibrosis (CF) with hypertonic saline...
significantly increased mucociliary clearance (Donaldson et al., 2006).

Exposure of Brown Norway rats to LPS induces mucus release (Tesfai et al., 2000). MRI can detect secreted mucus in the lungs of spontaneously respiring rats up to 8 days after a single LPS challenge (Beckmann et al., 2002; Tigani et al., 2002). Bilabeled amino dextran-based probes binding specifically to mucus have been synthesized to extract information on mucus dynamics in this model (Blé et al., 2009b).

An up-regulation of sensory-efferent neural pathways is implicated in asthma and COPD. The acute effects of sensory nerve stimulation by capsaicin in the rat lung have been studied by MRI (Karmouty-Quintana et al., 2007a). Capsaicin-induced MRI signals reflected the release of mucus after activation of sensory nerves. The transient receptor potential vanilloid-1 antagonist, capsazepine, the dual neurokinin-1,2 receptor antagonist, DNK333 \( \text{N} \) vanilloid-1 antagonist, capsazepine, the dual neurokinin-1,2 activation of sensory nerves. The transient receptor potential cin-induced MRI signals reflected the release of mucus after nerve stimulation by capsaicin in the rat lung have been implicated in asthma and COPD. The acute effects of sensory mation on mucus dynamics in this model (Ble ´ et al., 2009b).

Specifically to mucus have been synthesized to extract inform- et al., 2002). Bilabeled amino dextran-based probes binding after a single LPS challenge (Beckmann et al., 2002; Tigani after inflammation (see Airway Inflammation), hypoxic vasoconstriction (see Perfusion), and pulmonary arterial hypertension (see Pulmonary Arte- rial Hypertension) that accompany emphysema, MRI shows more clinical promise.

In contrast, MRI has been applied successfully in animal studies. Using a single-point imaging technique to achieve short echo times, Olsson et al. (2007) determined the relaxation time \( T_2^* \) to detect emphysematous changes in the lungs of tight-skin mice, which spontaneously develop emphysema-like alveolar enlargement. Tight-skin mice displayed significantly shorter \( T_2^* \) values than control, age-matched mice, because their larger alveoli result in an increased air/tissue ratio and hence an increase in the internal susceptibility gradients. The \( T_2^* \) of the lung parenchyma at 4.7 T was approximately 0.46 ms for control mice, being in excellent agreement with the 0.48 ms reported earlier (Beckmann et al., 2001b). Also ultra-short echo time MRI has been used to compare normal and emphysematous lungs in mutant mice (Takahashi et al., 2010).

Gradient-echo proton MRI has been used to detect elastase-induced changes in the lungs of spontaneously breathing rats (Quintana et al., 2006b). Reductions in MRI signal intensity of the lung parenchyma detected from 2 to 8 weeks after the insult correlated significantly with the loss of alveolar structure assessed by histology, suggesting that the MRI signal reflected elastase-induced alveolar destruction (Beck- mann et al., 2007a). Treatment with retinoic acid did not elic a reversal of lung damage as measured by MRI and histology.

Emphysema has also been studied in small rodents and clinically using hyperpolarized \(^3\)He-diffusion MRI. The apparent diffusion coefficient (ADC) is a sensitive measure for the airspace size. Significantly increased ADC values have been obtained in elastase-induced emphysema in rats and mice (Chen et al., 2000; Peces-Barba et al., 2003; Dugas

**Fig. 4.** \( T_1 \)-weighted MRI of a 43-year-old patient with CF before (A) and after (B) contrast agent administration. The contrast-enhanced images demonstrate extensive bronchial wall enhancement and permit differentiation of a thickened wall from intrabronchial secretions, with intra-bronchial fluid showing an air-fluid level (arrow). [Repro-duced from Ley-Zaporozhan J, Puderbach M, and Kauczor HU (2008) MR for the evaluation of obstructive pulmonary disease. Magn Reson Imaging Clin N Am 16:291–308, ix. Copyright © 2008 Elsevier Inc. Used with permission.]
et al., 2004) and humans. Good correlations between ADC values and histology have been reported in humans (Woods et al., 2006). The initial enthusiasm for this approach has been slightly dampened by two longitudinal studies. In a 1-year study on patients with COPD, disease progression was apparent from an increase in functional residual capacity, which was not matched by an increase in 3He ADC values or by a significant change in forced expiratory volume in 1 s or diffusion of CO. However, lacunarity, a measure of the inhomogeneity in distribution of ADC values, did correlate with disease progression (Diaz et al., 2009). In a 2-year study in α-1-antitrypsin-deficient patients, significant changes were reported for forced expiratory volume in 1 s, diffusion of CO, tissue density, and emphysema index from CT, but the increase in 3He ADC values just missed significance (Stavngaard et al., 2009).

Lung Ventilation and Perfusion. Ventilation and perfusion distribution in the lung form the foundation of pulmonary physiology and remain cornerstones in pathology. Efficient gas exchange in the lungs can occur only through intimate matching of regional ventilation and perfusion. The nonuniform distribution of regional lung blood flow and ventilation were first demonstrated using radioactive tracers and external scintillation detectors that registered the distribution of radioactivity within the lung. After intravenous injection, particles larger than red blood cells (RBCs) are trapped in the first capillary bed that they encounter. This is the principle of lung perfusion imaging in nuclear medicine, where macroaggregated albumin is radiolabeled with 99mTc, infused, and then imaged with γ-scintigraphy (Petersson et al., 2007). The gaseous pharmaceutical most commonly used for studying pulmonary circulation is 133Xe. The gas is dissolved in saline and injected intravenously. The γ-camera records perfusion while the patient holds his breath. The xenon passes almost immediately into the alveoli. As a result, during subsequent respiration the distribution of xenon in all areas of ventilation can be recorded. Xenon is cleared from the lungs in 3 to 4 min in normally ventilated areas, but is delayed in poorly ventilated regions (air trapping).

With the development of SPECT, three-dimensional distributions of regional blood flow or ventilation became accessible (Petersson et al., 2007). Regional ventilation-to-perfusion (V/Q) ratios can be obtained using steady-state intravenous infusion of 133Xe (Almquist et al., 1999). However, most clinical γ-cameras have low spatial resolution (>10 mm). Therefore, the majority of animal studies using this technique have focused on large species (Bajc et al., 2002; Kozian et al., 2008). SPECT with pinhole collimation has enabled lung perfusion studies in mice (Häfeli et al., 2010). During recent years, other imaging techniques have been used to image regional lung blood flow and ventilation, some of which are described next.

Ventilation. Ventilation imaging has been shown to be sensitive to a variety of lung disease models, including asthma in mice (Haczku et al., 2005), emphysema in rats (Spector et al., 2005), and pulmonary embolism in sheep (Wellman et al., 2010). Ventilation can be quantified from the dynamic change in image signal after application of an inhaled contrast agent. For instance, ventilation imaging has been demonstrated in animals with stable Xe-enhanced CT (Lam et al., 2007), 13N-N2 washout with PET (Vidal Melo et al., 2003), fluorescent microspheres using luminescence spectrometry (Robertson et al., 2005), and 3He MRI (Haczku et al., 2005).

Continuous Xe-enhanced micro-CT enabled assessing regional ventilation in mechanically ventilated rats during the washin and subsequent washout of xenon (Lam et al., 2007). Lung tissue density changes caused by xenon attenuation of x-rays were assessed at different phases of the respiratory cycle. This technique, however, requires repeated measurements and, therefore, repeated radiation exposure.

In PET studies, a closed-circuit rebreathing system is used to administer 13N-N2 gas. Because of its low solubility in water and tissue, inhaled 13N-N2 remains confined to alveolar airspaces and conducting airways, with the tracer concentration in a region after equilibration being proportional to the gas volume in that region. Washout scans of the inhaled 13N-N2 after a switch of the breathing system to tracer-free gas are then acquired to assess regional ventilation. The method has been applied to assess ventilation in pigs (Richard et al., 2005) and sheep (Wellman et al., 2010). In humans, this approach has been explored mainly in a setting of acute lung injury (Vidal Melo et al., 2002; Musch et al., 2004).

Hyperpolarized 3He-MRI has been used to measure the fractional ventilation in artificially ventilated small rodents (Deninger et al., 2002; Emami et al., 2008). The approach detected early changes of lung function and structure in a rat model of elastase-induced emphysema at mild and moderate severities (Emami et al., 2008). The fractional ventilation declined primarily in the first 5 weeks, whereas enlargement of alveolar diameters appeared primarily between the 5th and 10th weeks after elastase. Further HP 3He-MRI studies focused on airway constriction induced chemically. High-resolution 3He-MRI was used to depict regional ventilation changes and airway narrowing in artificially ventilated mice (Mistry et al., 2010) or rats (Chen and Johnson, 2004) challenged with methacholine. Mosbah et al. (2010) demonstrated in a spatially resolved manner the effects of serotonin-induced bronchoconstriction on lung ventilation in spontaneously breathing rats. Dynamic ventilation 3He MR images spanning a respiratory cycle were obtained using a retrospective cinematic (CINE) image reconstruction procedure (Stupar et al., 2007). Clinical applications of HP noble gas MRI have also slowly matured. In particular, 3He-MRI has been used to characterize ventilation heterogeneity and defects in patients with asthma (Fain et al., 2008), cystic fibrosis (Mentore et al., 2005; van Beek et al., 2007) (Fig. 5), and COPD (van Beek et al., 2004). Patients with asthma demonstrated ventilation defects both before and after a challenge with methacholine or exercise (Samee et al., 2003; Tzeng et al., 2009), and a significant overlap of 3He defects and hyperlucency on HRCT was found (Fain et al., 2008). In patients with COPD, primarily 3He diffusion MRI has been applied to examine alveolar air space size as mentioned previously (see Emphysema). However, this parameter can be assessed only in reasonably well ventilated lung tissue. To date, very few studies using 3He MRI have reported on therapeutic interventions: preliminary results of bronchodilator therapy in asthmatics (Samee et al., 2003) and immediate effects of standard chest physiotherapy on regional ventilation with spirometry unchanged in patients with CF (Woodhouse et al., 2009). Because of a potential shortage of 3He, improved polarization technology for 129Xe and the solubility of Xe in tissue, enabling simultaneous ventilation and dis-
solved phase images, have renewed interest in clinical $^{129}$Xe MRI (Patz et al., 2008; Cleveland et al., 2010). However, the anesthetic properties of Xe are of some concern.

Other approaches not relying on the administration of gases have been used to derive information on ventilation. Regional lung ventilation and volume were measured with electrical impedance tomography in piglets (Richard et al., 2009). Proton MRI detected, in spontaneously breathing rats, the effects of broncho-modulating agents (Beckmann et al., 2006) or inflammation-induced airway remodeling and hyporesponsiveness in OVA- or LPS-challenged animals, respectively (Beckmann et al., 2004). The approach consists of detecting modulations of lung parenchymal proton signals induced by changes in oxygenation levels, increases in parenchymal signal being linked with a reduced oxygen level, and vice versa (Edelman et al., 1996). Oxygen-enhanced MRI has been further explored clinically in patients with smoking-induced COPD (Ohno et al., 2008) and asthmatics (Fig. 6). It needs to be emphasized that the oxygen-enhanced MRI maps show not only correlation with ventilation limitations in the lungs, but also with oxygen transport capacity.

**Perfusion.** Clinically, regional lung perfusion is assessed primarily with radionuclide scintigraphy, which, however, suffers from limited spatial and temporal resolution. Three-dimensional (3D) MRI has provided the required high spatial and temporal resolution to analyze the first passage through the lungs of a bolus of MRI contrast agent. MRI lung perfusion has been used to demonstrate perfusion abnormalities in patients with CF (Eichinger et al., 2006) and emphysema (Ley-Zaporozhan et al., 2007). Fourier decomposition MRI has been introduced, enabling lung perfusion and ventilation imaging without the administration of contrast agents (Bau- man et al., 2009) (Fig. 7).

The combined challenges of high temporal and spatial resolution have rendered routine quantitative perfusion imaging difficult in small rodents. MRI perfusion assessments in animals have been accomplished primarily using contrast-enhanced techniques comprising the dynamic acquisition of...
images in combination with the administration of a paramagnetic contrast agent (Neeb et al., 2009). Such an approach has been used to analyze a rabbit model of pulmonary embolism (Keilholz et al., 2009) and a newborn piglet model of pulmonary hypertension (Ryhammer et al., 2007). Mistry et al. (2008) developed a cinematic (CINE) technique based on the acquisition of radial images during repeated contrast agent injection matched to the physiology of the animal using a microinjector, enabling perfusion imaging at high spatial and temporal resolution in artificially ventilated small rodents. The feasibility of lung perfusion with arterial spin labeling precluding the administration of contrast material has been demonstrated in rabbit models of pulmonary embolism (Altes et al., 2005) and during repeated balloon occlusion of a segmental pulmonary artery as well as during pharmacological stimulation in pigs (Roberts et al., 2001).

Driehuys et al. (2009) showed in artificially ventilated rats that regional evaluation of pulmonary perfusion and gas exchange can be obtained by intravenous injection of saline saturated with HP $^{129}$Xe and subsequent MRI of the gas phase in the alveolar airspaces. After a single injection, the emerging $^{129}$Xe gas could be detected separately from $^{129}$Xe remaining in the blood because of chemical shift differences. Micro-CT for perfusion imaging in rodents has been demonstrated (Badea et al., 2010). Reproducibility was ensured by the integration of the imaging components with a microinjector and a mechanical ventilator.

Ventilation/perfusion. An intravenous bolus of $^{13}$N-$N_2$-saline provides the opportunity to assess V/Q with PET. Because of the aforementioned low solubility of $N_2$ in blood, virtually all $^{13}$N-$N_2$ arriving in the lungs during apnea will diffuse into the alveolar air space in proportion to regional perfusion, thus providing a perfusion map. Subsequent washout imaging highlights tracer retention in poorly ventilated areas. In asthmatic patients at baseline a characteristic vertical perfusion gradient from dorsal to ventral and an almost complete tracer washout was observed. However, after a methacholine challenge, a marked heterogeneity in perfusion occurred, with the lowest perfusion in areas that were best perfused at baseline, suggesting that already bronchoconstricted areas were protected from methacholine. Washout was poor in areas of low perfusion. Because of perfusion redistribution, the V/Q mismatch was reduced (Harris et al., 2006). Compared with healthy volunteers, perfusion heterogeneity was also greater in patients with mild to moderate COPD, as was the heterogeneity in V/Q, which was primarily caused by the heterogeneity in perfusion and not so much in ventilation (Vidal Melo et al., 2010).

Lung Fibrosis and Airway Remodeling. Ask et al. (2008) demonstrated that the progression of pulmonary fibrosis induced in rats by adenoviral gene transfer of transforming growth factor β can be reliably assessed by micro-CT (Fig. 8). The technique has also been used to image bleomycin-induced fibrotic changes in mice (Shofer et al., 2007). This approach directly translates to the management of patients with idiopathic pulmonary fibrosis and allows the monitoring of therapeutic effects in drug intervention studies.

Proton MRI has also been used to follow bleomycin-induced injury in mice and rats (Karmouty-Quintana et al., 2007b; Jacob et al., 2010; Babin et al., 2011) (Fig. 8, C and D). The initial response in rats, in the first 2 weeks after insult, characterized predominantly by diffuse MRI signals, was primarily inflammation-related. At later time points, up to 70 days after bleomycin, increased MRI signals reflected tissue remodeling involved in fibrosis development, as suggested by histology revealing prominent collagen deposition in the same areas where MRI signals had been detected in vivo. Topical administration of budesonide showed an effect on inflammation but not on fibrosis.

Effective pulmonary gas exchange relies on the free diffusion of gases across the thin tissue barrier separating airspace from the capillary RBCs. An increased blood-gas barrier thickness is present in pathologies as inflammation, fibrosis, and edema. Driehuys et al. (2006) demonstrated in a rat model of unilateral bleomycin lung injury the feasibility of detecting such impairment by using $^{129}$Xe-MRI and by exploiting the fact that $^{129}$Xe resonates at three distinct frequencies in the airspace, tissue barrier, and RBC compartments. Based on a simple diffusion model, they estimated that this MRI method for measuring $^{129}$Xe alveolar-capillary transfer is sensitive to changes in blood-gas barrier thickness of ~5 μm.

Assessment of airway remodeling in patients is the almost exclusive domain of HRCT, which can characterize anatomic details as small as 200 to 300 μm. Several sophisticated postprocessing tools are available, that, based on volumetric HRCT data sets, can automatically segment the airways down to the eighth generation and reconstruct airway trees (Tschirren et al., 2005). Using such quantitative evaluation it was found that severe asthmatics had thicker airway walls than patients with mild asthma or healthy volunteers, which also correlated with biopsy measures of remodeling and the
degree of airflow obstruction (Aysola et al., 2008). An exploratory method worth mentioning is human in vivo fluorescence microimaging. It provides tremendous detail of the airways up to alveolar ducts and sacs (Thiberville et al., 2009). However, the technique requires bronchoscopy and minute tissue puncture, but without any reported adverse effects.

HRCT has also been used extensively to characterize lung fibrotic disease in patients (Lynch et al., 2005; Best et al., 2008). The images provide a plethora of information, which, however, is difficult to quantify and differential diagnosis is not straightforward. Most common features are ground glass opacity, most likely reflecting interstitial fibrosis, reticular opacity representing fibrosis and thickening of the interlobular septa, honeycombing representing fibrotic, cystic dilation of bronchioles, and traction bronchiectasis representing irregular, fibrotic dilatation of bronchi.

Although the above studies suggest that CT and MRI can be confidently used to localize pulmonary inflammation and fibrosis, they lack specificity. Efforts are underway to search for molecular imaging markers of fibrosis. A modulation of fibroblast activation has been observed with octreotide (Türkcapar et al., 2003), a synthetic somatostatin analog with strong affinity for the somatostatin receptor subtype 2 that is overexpressed in idiopathic pulmonary fibrosis. Lung uptake of $^{111}$In-octreotide in fibrosis patients correlated with lung function alterations and with the intensity of alveolitis evaluated radiologically (Lebtahi et al., 2006; Ha et al., 2008).

**Pulmonary Arterial Hypertension.** Right heart catheterization (RHC) is the gold standard for determination of pulmonary arterial pressure (PAP) and vascular resistance, but is invasive and not attractive for monitoring drug therapy. Echocardiography is the most widely used noninvasive modality to measure elevated PAP and pulmonary vascular resistance and monitor progression of ensuing right heart failure. However, ~50% of patients cannot be analyzed because of failure to trace the (entire) right ventricle (Bleecker et al., 2006), and PAP can only be assessed by Doppler echocardiography when significant tricuspid regurgitation is present. In contrast, MRI is less patient-dependent and has better reproducibility and lower intraobserver variability than echocardiography (Bradlow et al., 2010). Sanz et al. (2007) found strong correlations of RHC with mean PAP, systolic PAP, and pulmonary vascular resistance index derived from average velocity in the pulmonary artery obtained with phase-contrast MRI. Using related methods, Reiter et al. (2008) demonstrated that the duration of a flow vortex in the pulmonary artery had even better correlation with RHC-derived mean PAP. Although only possible in the presence of tricuspid regurgitation, Nogami et al. (2009) found MRI-assessed regurgitation velocity and PAP to be better correlated with RHC than echocardiography-derived PAP. In addition, the distensibility of the proximal pulmonary artery can be assessed with MRI, and its relative area change was the only baseline parameter that correlated with the 6-min walk test after 1 year of treatment of chronic thromboembolic pulmonary hypertension patients with sildenafil (Toshner et al., 2010).

Comparable technology has been introduced for studying a rat model of monocrotaline-induced pulmonary hypertension. Flow-derived PAP and right ventricular mass and function determined with MRI correlated better with RHC than echocardiography-assessed parameters (Urboniene et al., 2010). In the same model, treatment with antioxidant reduced the 3-fold increase in end-systolic right ventricle volume by 42% (Redout et al., 2010).

**Drug Delivery.** Determining the topical dose and distribution of inhaled therapies can be accomplished using radio-labeled tracers and imaging. The most widely used techniques in humans (Dolovich and Labiris, 2004) and small animals (Merkel et al., 2009) use two-dimensional planar gamma-cameras and 3D SPECT cameras. It is important that there is no significant change in the in vitro performance of the
labeled formulation compared with the reference formulation. Moreover, the distribution of the radioactivity should represent the distribution of the active drug substance of the reference product. Some confirmation that this is indeed the case can be obtained from measurements with cascade impactors, although generally steady flow conditions are used, which poorly reflect the patient’s inhalation flow profile. Once this condition is satisfied, imaging can be used to determine relative drug deposition in lungs, larger airways, mediastinum, and stomach, when for instance comparing different drug delivery systems as well as mucociliary clearance (Coates et al., 2007).

Micro-SPECT/CT has the sensitivity and resolution to track the fate of compounds in living mice. For instance, Saatchi and Häfeli (2009) studied the distribution of 99mTc-radiolabeled biodegradable microspheres. Hoang et al. (2009) reported a strong correlation between image-based region-of-interest analysis and biodistribution data of an 111In-labeled amphiphilic diblock copolymer micelle formulation. Micro-SPECT has also been used to determine the biodistribution of 111In- or 99mTc-labeled siRNA polyplexes (Merkel et al., 2009).

Using scintigraphy, it has been shown that inhalation of the combination formulation beclomethasone dipropionate/formoterol, dissolved in hydrofluoroalkane and labeled with 99mTc, produced a high and homogeneous compound deposition in the airways of healthy subjects, asthmatics, and patients with COPD, regardless of the pathophysiological condition (De Backer et al., 2010). In addition, lung deposition of inhaled tobramycin has been assessed in healthy people and patients with CF (Fig. 9).

PET provides an attractive alternative to assess the biodistribution of compounds without affecting their pharmacokinetics, as has been demonstrated in vivo in animals for 18F-labeled liposome-encapsulated hemoglobin (Urakami et al., 2009). However, because the compounds need to be labeled directly with positron emitters, the main challenges are the relatively short half-life of commonly used tracers, the introduction of the labeled compound in a regularly functioning inhalation device, the high costs, and the need for a cyclotron to synthesize the PET tracers. Nevertheless, zolmitriptan, used for migraine treatment, was labeled with 11C (half-life 20.4 min) to be chemically identical to unlabeled zolmitriptan (Yates et al., 2005). Immediately after administration in a nasal spray, almost 100% of the compound was found in the nasopharynx. Minimal signal was detected in the lungs, but some radioactivity was found in the brain, indicating that this route of administration presented an interesting alternative to the often problematic oral route. Labeling of inhaled biologies is less constrained, because chelation can be used to bind longer-lived metallic isotopes, without affecting the molecular properties too much.

NIRF imaging is a simple alternative for obtaining rapid information on the delivery of Cy5.5-labeled large molecular weight compounds and powders, whose distribution is affected only marginally by the fluorescent label size, to the lungs of small rodents (Blé et al., 2009).

**Final Remarks**

In the past few years, imaging methods have been considerably refined, allowing examinations of the lungs in humans and small rodents at the anatomical, functional, and even molecular or target levels at high spatial and temporal resolution. The main challenge in using such techniques within the framework of drug discovery and development is 2-fold:

1) Like any biomarker, imaging signatures need to be properly validated and qualified. In animal models, this involves a proper characterization of the readouts against invasive markers like cell influx into BAL fluid and histology. Then, the ability of modulating a given imaging signature by reference pharmacological agents of known activity needs to be demonstrated to verify the sensitivity of the potential biomarker;

2) It needs to be shown that the increased technical capabilities of imaging translate into improved disease diagnosis compared with standard approaches. The hope is that with imaging, earlier phases of the disease can be diagnosed, thus enhancing the chance of pharmacological interventions. Moreover, imaging could facilitate patient stratification in clinical trials.

It cannot be expected that all imaging readouts satisfy these requirements. It may happen that, for a certain indication, imaging can be used at the experimental level in both small rodents and humans, thus facilitating the examination of translational aspects. The other situations may also happen, i.e., imaging being used exclusively in animals or humans or not being adopted at all. In general terms, preclinical and clinical activities should be mutually supportive. For instance, small-animal imaging may help to improve the characterization of clinical readouts and, conversely, imaging in humans may support the improvement of animal models. Both for regulatory and cost reasons imaging will be used primarily in proof-of-concept studies involving a small patient population, rather than in large phase 3 clinical trials.

Given the plethora of imaging techniques, the advantages and limitations of each approach have to be understood for every application. In addition, different modalities might be

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Fig. 9. Deposition patterns of inhaled tobramycin labeled with 99mTc-diethylene triamine penta acetic acid using two-dimensional scintigraphic imaging methodology. Six concentric lung-shaped regions radiating from the hilum were adopted to determine the amount of aerosolized tobramycin deposited in each zone. [Reproduced from Lenney W, Edenshorough F, Kho P, and Kovarik JM (2011) Lung deposition of inhaled tobramycin with eFlow rapid/LC Plus jet nebulizer in healthy and cystic fibrosis subjects. J Cyst Fibros 10:9–14. Copyright © 2010 European Cystic Fibrosis Society. Used with permission.]
used in small animals and humans. For instance, a certain molecular probe may be labeled for fluorescence optical imaging in mice and rats, and then optical fluorescence imaging can be applied for key molecular imaging in patients. In addition, particular care needs to be taken about radiation exposure when using nuclear imaging in pharmacological studies. Therefore, although HRCT is currently the clinical imaging of choice for diagnostic assessment of pulmonary diseases, there is room for improvement of MRI approaches.

The efforts in developing imaging biomarkers for respiratory diseases are going to benefit both drug development and diagnosis. Patients will certainly profit from advancements in this field of research.

**References**


