Dynamic Lung Morphology of Methacholine-Induced Heterogeneous Bronchoconstriction

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Hyperpolarized (HP) $^3$He dynamic MRI was used to investigate airway response in rats following intravenous (i.v.) bolus administration of a contractile agent, methacholine (MCh). The method provides direct visualization of the ventilated regions within the lung. Heterogeneous bronchoconstriction following the i.v. MCh injection was evident using this technique. These $^3$He dynamic lung images revealed that the inspired fresh air was shunted to the less-constricted regions after the MCh challenge in a similar manner as described by Laplace’s relationship for the stability between adjacent alveoli. The airways in the more-constricted regions became nearly closed, resulting in air trapping, while the airways in the less-constricted regions remained effectively open, leading to over-inflation. These data suggest a lung model of airway constriction partitioned into ventilated and nonventilated regions. These nonventilated regions are heterogeneous distributed in the lung and this distribution cannot be deduced from spirometric measurement of the whole lung. We demonstrate that a combination of functional $^3$He images and anatomical $^1$H images provide an effective method to diagnose regional lung abnormalities in rats. Magn Reson Med 52:1080–1086, 2004. © 2004 Wiley-Liss, Inc.

Key words: MR imaging; hyperpolarized gas; ventilation shunt; regional lung function; air trapping

The increasing incidence of asthma poses a worldwide health problem (1,2), spurring major efforts to understand the mechanism and treatment of the disease. Measurement of pulmonary mechanics using spirometry has been the most common method to evaluate lung function. Unfortunately, this method can only measure global function of the lung. It is generally believed that measurement of regional ventilation within the lung can provide an early diagnosis of lung dysfunction. Since the late 1960s, ventilation distribution has been assessed with the scintillation camera using inert radioactive gases, i.e., $^{133}$Xe and $^{81}$Kr, or aerosol-labeled with $^{131}$I or $^{99m}$Tc (3). These nuclear imaging techniques are still the primary clinical methods to determine regional ventilation abnormalities in obstructive airway diseases. Nevertheless, due to poor resolution and the complicated procedures of these techniques, most basic work on lung morphology has been performed using postmortem lung fixation and high-resolution computed tomography (4). These structural data delineate only an aerated lung and cannot be used to distinguish trapped exhaust air from fresh air in the lung. To further define nonventilated (abnormal) regions within the aerated lung, particularly in a constrictive state, a new technique to assess ventilation distribution as well as lung structure is required.

Hyperpolarized (HP) $^3$He MRI has been shown to provide an alternative nonradioactive approach both in animals (5,6) and patients (7–11). This technique, different from more traditional $^1$H imaging showing aerated lung structure, demonstrates ventilated regions in the lung similar to the radioactive ventilation methods described above (3). Several groups have recently demonstrated dynamic lung imaging with much-improved spatial and temporal resolution to evaluate regional pulmonary function morphologically in rats (12) and in humans (13). Previously, we incorporated a constant flow ventilator and a new scanning scheme with HP $^3$He to acquire quantitative pulmonary images in the rat (14). We report here the use of methacholine (MCh), a provocative agonist used routinely in the clinic, to induce airway constriction in the rat. To rule out the possibility of inhogeneous deposition within the lung by aerosol delivery, MCh was administered intravenously (i.v.). Most studies by other investigators on MCh-induced airway constriction were based on airflow and pressure measurement (15–18). This work interprets changes of lung function using a combination of the ventilation data from HP $^3$He imaging and the structural data from conventional $^1$H imaging in a carefully controlled animal model.

MATERIALS AND METHODS

Animal Preparation

A total of 24 female rats (Sprague Dawley, Charles River, Portage, MI) weighing 220–240 g were used for the MCh study, in addition to the control (physiological saline) study (n = 2). The Institutional Animal Care and Use Committee at Duke University approved all animal procedures. Anesthesia was induced using two separate intraperitoneal injections of ketamine (56 mg/kg) and diazepam (2.8 mg/kg). The rat was intubated under direct laryngoscopic visualization using a shortened catheter (Intravenous Cannula, 14GA, Sherwood Medical, Tullamore, Ireland). Within 3–5 min following induction of anesthesia, the rat was ventilated in a prone position at a rate of 72 breaths/min and a tidal volume (TV) of 8–8.5 ml/kg. To maintain anesthesia, ketamine (10 mg/ml) and diazepam (0.48 mg/ml) were continuously infused via a cannulated tail vein (26GA × 19 mm, Abbocath-T, Abbott Ireland, Sligo, Ireland). The infusion rate was adjusted (40–80 μl/min/kg) to keep the heart rate stable at ~280 beats/min. Body temperature was measured with a rectal probe and maintained at ~37°C via a warm air system.
Methacholine Administration

The MCh bolus injection (30 μg in ~0.1 ml saline) was slowly administered over 30–60 sec. Following the injection, the TV decreased as peak inspiratory pressure (PIP) increased. In preliminary experiments, the rat expired immediately when attempting to maintain the TV during this period. Lung images were acquired before and after the MCh challenge as indicated (double-head arrows). The observed decreases in PIP during imaging were caused by the 3He/oxygen mixed gas for ventilation.

Image Acquisition and Ventilation

A 2.0 T magnet (Oxford Instruments, Oxford, UK) with shielded gradients (180 mT/m) and a Signa console (Epic 5X, GE Medical Systems, Milwaukee, WI) were used for imaging. A 7-cm-diameter birdcage RF coil was constructed to operate at 85.5 MHz and 64.8 MHz, which permitted acquisition of registered 1H and 3He images (6). A custom-made constant flow ventilator was used to provide a breathing pattern of a positive-pressure forced inhalation and a passive exhalation (14,19). Dynamic lung imaging was acquired during inhalation period using a ventilator-gated, 2D RA-CINE technique (12,14). The acquisition window was divided evenly into eight adjacent time frames (28.5 ms). Three different scans, described in detail previously (14), were acquired: a 1H scan (flip angle = 12° and no skipping scheme) to provide anatomical landmarks of the chest, a 3He scan (flip angle = 24° and no skipping scheme) focusing on the major airway branches, and a 3He scan (flip angle = 12° and skip factor = 2) focusing on the lung periphery. The 1H images were acquired using field of view (FOV) = 100 mm, TR = 4.75 ms, and TE = 1 ms, then regridded onto a 512 × 512 array and cropped to a 256 × 256 array covering a 50-mm FOV (14). The 3He lung images were acquired using FOV = 50 mm, TR = 4.75 ms, and TE = 1 ms for a 256 × 256 array. A skipping scheme was applied: one in every second frame in the case of skip factor = 2. These imaging parameters were chosen to ensure a linear relationship between signal intensity and the flow to the regions of interest. RF spoilers were applied at the end of each breath to eliminate any residual magnetization. During the 3He scans, 70% of N2 in the inspired gas (a mixture of 23% O2 and 77% N2) was replaced with the HP 3He gas. A total of 150 breaths were used to obtain 900 radial samples of Fourier space for each image. As illustrated in Fig. 2, coronal images were selected to cover most of the major airways. Axial images were selected at the bottom of the heart to sample more of the distal airspace. The 1H and 3He images, each including coronal and axial views, were acquired prior to and ~10 min after the MCh challenge.
Helium Hyperpolarization

A polarizer (IGL.9600.He, Amersham Health, Durham, NC) with gas capacity of ~1200 ml was used to polarize 3He gas. The technique for spin-exchange optical pumping polarization has been described in detail by Happer et al. (20) and Moller et al. (21). The system achieved 30–40% nuclear polarization in 8 hr.

RESULTS

After the i.v. saline injection, PIP was unchanged. Compared to the images acquired prior to the saline injection as shown in Fig. 3, no noticeable changes in major airway size or peripheral ventilation distribution were observed. Airway responses measured by airway pressure after a bolus (~0.1 ml) of i.v. MCh (30 μg) are shown in Fig. 1. The reactivity varied between rats. Two main patterns of changes in PIP were observed (Fig. 1a,b).

In a short-reacting case (Fig. 1a), the PIP increased up to ~120% as the TV decreased by ~30% immediately after the MCh challenge. The rapid changes in the PIP and the TV diminished within 2 min. This pattern of response was observed in 20 of the 24 total MCh-tested rats with various degrees of constriction. Serial coronal 3He images before and after the MCh challenge in this pattern are shown in Fig. 4. Despite the fact that the post-MCh images were acquired after the PIP returned to the baseline value (Fig. 1), inhomogeneous ventilation distribution was observed in the post-MCh coronal images (Fig. 4e–h) as compared to the pre-MCh coronal images (Fig. 4a–d). Similar results were also observed in the axial images (Fig. 5).

In a long-reacting case (Fig. 1b), PIP increased rapidly up to ~100% as the TV decreased by >50% after the MCh injection, but remained ~25% above the baseline value after the primary constriction. It appears that these rats were hyperreactive to MCh, even though these rats had not been previously sensitized. As shown in Fig. 6, a significantly increased portion of inspired air was confined in the major airway branches as the TV was maintained at the same rate. Note that some of inspired air reaching the peripheral lung was not observed in the selected coronal images since the slice thickness was only 4 mm. Major airway branches were expanded up to 2–3 times and delay in inspired airflow (Fig. 6e–h) was consistent with the increase in PIP (Fig. 1b), suggesting severe airway constriction. As the anatomical dead space increased after severe constriction (Fig. 6), the rat was hypoventilated using the same TV. Efforts to maintain oxygenation using a larger TV

FIG. 3. The 3He images at the end of inspiration are used to indicate ventilation distribution before (a) and after (b) the saline bolus injection. Images were acquired using the scan sequence identical to that used for images shown in the other figures.

FIG. 4. Serial inspired coronal 3He images (at 57 ms intervals) in the short-reacting airway response after the MCh challenge are shown. As compared with the pre-MCh images (a–d), delay of the inspired airflow and increases in the airway size were noticeable in the post-MCh images (e–h). Ventilation deficiency was observed in the regions indicated (arrows). These images provide direct evidence of heterogeneous constriction induced by MCh.
resulted in a pneumothorax. Four of the 24 total MCh-tested rats reacted in this fashion and died within 60 min.

An increase in lung patency after the MCh challenge was observed in the $^1$H images in the example of short-reacting airway response (Fig. 7). These data suggest air trapped inside the lung, as reported by high-resolution computed tomography (4). The site of air trapping in the upper-left lobe of the lung can be seen in the combined $^1$H/$^3$He coronal lung images (Fig. 7b vs. 7a). Also, the images showed more air shunting into the less-constricted right lung. These data demonstrate heterogeneous constriction induced by the i.v. MCh bolus injection.

**DISCUSSION**

We have demonstrated direct visualization of heterogeneous constriction induced by i.v. MCh bolus injection in the rat. Although the degree of constriction after the MCh challenge varied between rats, inhomogeneous $^3$He distribution in the peripheral lung was observed in both pat-

![FIG. 5. Serial axial inspired $^3$He images (at 57 ms intervals) in the short-reacting airway response after the MCh challenge are shown. As compared with the pre-MCh images (a–d), much brighter signals were detected in the peripheral regions marked with arrows in the post-MCh images (e–h). These data demonstrate that more inspired air was distributed into the regions indicated after the MCh challenge.](image1)

![FIG. 6. Serial coronal $^3$He images (at 57 ms intervals) in the long-reacting airway response for pre- (a–d) and post- (e–h) MCh exposure are demonstrated. Distended major airways and lack of ventilation within the peripheral regions were observed in the post-MCh images, suggesting constriction of the small airways (after the first two major airways). These data are consistent with the increase in the PIP during the imaging period, as shown in Fig. 1.](image2)
Airway closure has been suggested to occur after MCh challenge. Brown et al. (23) showed that airway closure could occur even at relatively high peak end-expiration pressure. Wright et al. (24) also reported that the MCh-induced constrictions occurred primarily in the medium- and larger-sized bronchioles (>0.32 mm) using lung explants in rats. A decrease in lung density, an indicator of air content, was observed by using computed tomography after the MCh challenge (4). Yet the observed decrease in lung density could only suggest occurrences of air trapping. To locate the sites of action, we compared the 1H images as an indicator of air content in the lung (patency) with the 3He images as an indicator of ventilated regions. As shown in the registered 1H and 3He images in Fig. 7, the left upper lobe was not ventilated, but remained aerated after MCh challenge. Compared with the pre-MCh 1H images (top, Fig. 7a), the post-MCh 1H images (top, Fig. 7b) show an increase of air content in the upper left lobe. Since static lung elastic recoil increased after the constric- tor stimuli (25), to keep the aerated region from shrinking in the constrictive state, the small airways must close partially or completely. The combined in vivo images shown here indicate partial/complete airway closure in the lung after the MCh challenge. Our results are consistent with a model proposed by Anaï and Wilson (26), which describes constriction heterogeneity as two stable states of airways—one effectively open and one nearly closed. This model provided an excellent rationale to explain the dependence of airway constriction on the TV and the peak end-expiration pressure (27–30).

In our previous study, we demonstrated that the airflow (velocity) in the major airways is proportional to the signal intensity normalized with projected airway diameter, as shown in the coronal 3He dynamic images (14). In the current study, we investigated the airflow profile within the conducting airway after the MCh challenge (Table 1). The signal intensities in the regions of the trachea (M1), the right major airway branch (M2R), and the left major airway branch (M2L) were measured at the end of inhalation and were normalized with respect to the projected airway diameter for comparisons (Table 1). In a control experiment after saline injection, the airway diameter remained unchanged, but there was a trend of decrease in the normalized signal intensity (NSI), which averaged ~14% in the M1, M2R, and M2L. Since the two scans were 30 min apart, this decrease was mainly caused by the $T_1$ decay of the polarization. In a short-reacting example (Table 1), the NSI in M1, M2R, and M2L increased by an average of ~6%, and the airway size decreased by an average of ~11% after the MCh challenge. A similar relationship was also observed in a long-reacting example. The NSI decreased by an average of ~35% as the airway size increased by an average of ~67% (Table 1). These results suggest that, under positive pressure ventilation, the airflow in the airways decreased in response to increases of the airway diameter after the MCh challenge. Local airflow profile could be predicted using airway diameter taken from lung images.

The attractive feature of the HP 3He dynamic MR imaging technique is its unique ability to define ventilating regions within the lung that cannot be assessed by post-mortem lung fixation or high-resolution computed tomog-
Dynamic lung morphology of bronchoconstriction

Table 1
Summary of Regional Function Measurement

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Coronal scan</th>
<th>Control</th>
<th>Methacholine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>D (mm)</td>
<td>2.5</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>(\Delta D^2)%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M1</td>
<td>3830 ± 291</td>
<td>3391 ± 351</td>
<td>6452 ± 438</td>
</tr>
<tr>
<td>NSI</td>
<td>1508</td>
<td>1335</td>
<td>1843</td>
</tr>
<tr>
<td>(\Delta NSI)%</td>
<td>-11%</td>
<td>-7%</td>
<td>-7%</td>
</tr>
<tr>
<td>D (mm)</td>
<td>2.2</td>
<td>2.2</td>
<td>2.7</td>
</tr>
<tr>
<td>(\Delta D^2)%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M2R</td>
<td>3692 ± 357</td>
<td>3201 ± 486</td>
<td>5403 ± 394</td>
</tr>
<tr>
<td>NSI</td>
<td>1717</td>
<td>1489</td>
<td>2001</td>
</tr>
<tr>
<td>(\Delta NSI)%</td>
<td>-13%</td>
<td>5%</td>
<td>-13%</td>
</tr>
<tr>
<td>D (mm)</td>
<td>1.5</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>(\Delta D^2)%</td>
<td>0</td>
<td>0</td>
<td>-19%</td>
</tr>
<tr>
<td>M2L</td>
<td>2715 ± 334</td>
<td>2253 ± 210</td>
<td>3943 ± 246</td>
</tr>
<tr>
<td>NSI</td>
<td>1834</td>
<td>1522</td>
<td>1972</td>
</tr>
<tr>
<td>(\Delta NSI)%</td>
<td>-17%</td>
<td>20%</td>
<td>-17%</td>
</tr>
<tr>
<td>BG Noise</td>
<td>422 ± 189</td>
<td>413 ± 222</td>
<td>471 ± 267</td>
</tr>
</tbody>
</table>

The regions of interest located in trachea (M1), the right major airway branch (M2R), and the left major airway branch (M2L) were selected for analysis. D: the projected airway diameter; \(\Delta D^2\)%: changes of airway cross-section in percentage after the MCh challenge; NSI: the SI normalized with the projected airway diameter; \(\Delta NSI\)%: changes of the NSI in percentage after the MCh challenge; BG: the background.

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REFERENCES