Non-Invasive Methods: Investigation of Airways Diseases by MRI in Rats

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Summary
Current techniques to evaluate the efficacy of potential treatments for airways diseases in small animal models are generally invasive and terminal. In this contribution we illustrate the usefulness of magnetic resonance imaging (MRI) to obtain anatomical and functional information of the lung, with the scope of developing a non-invasive approach for the routine testing of drugs in rat models of airways diseases. With MRI, the disease progression can be followed in the same animal. Thus, a significant reduction in the number of animals used for experimentation is achieved, as well as minimal interference with their well-being and physiological status. In addition, MRI has the potential to shorten the duration of the observation period after disease onset since the technique is able to detect changes before these are reflected in invasively determined parameters of inflammation.

Keywords: reduction, refinement, rat, lung, asthma, drug screening, magnetic resonance imaging (MRI), inflammation, non-invasive

Background Information

Rodent models of airways diseases

To study specific aspects of human respiratory diseases and its treatment it is necessary to measure the induced symptoms and the impairment of lung function in living animals. For example, key features of asthmatic inflammation (airway hyperresponsiveness, eosinophilic inflammation together with an increase in activated T cells in the airways) are induced in actively sensitized Brown Norway (BN) rats exposed to allergen (ovalbumin, OVA). Alternatively, an inflammation similar to that observed in chronic obstructive pulmonary disease (COPD) patients (neutrophilia and mucus cell metaplasia) can be elicited in rodents by the administration of endotoxin (lipopolysaccharide, LPS). Emphysema, one of the most critical components of COPD, which results in the destruction of lung parenchymal tissue by a number of proteases (neutrophil elastase or matrix metalloproteinases), is induced in rats by the application of a single dose of porcine pancreatic elastase (PPE). This destruction leads to an enlargement of air spaces and to a loss of lung elasticity, ultimately impairing gas exchange. In all cases the symptoms resemble those seen in patients in the early onset of the disease.

The inflammatory status of the lung is routinely inferred from post mortem analyses of broncho-alveolar lavage (BAL) fluid. Occasionally, time consuming histological analysis is also performed. Lung function is assessed in terminal experiments where animals are treated with a muscle relaxant, tracheotomized and artificially ventilated. Airflow, transpulmonary pressure, and airway resistance are determined for each respiratory cycle. Following these measurements animals are killed by an overdose of anesthetic. Clearly, the invasive character of these procedures precludes repeated assessments in the same animal. Therefore the flexibility of MRI was explored by Nicolau Beckmann and his colleagues to obtain anatomical and functional information of the rat lung, with the scope of developing a non-invasive means of analysis.

MRI: A powerful tool

Based on the use of magnetic fields and radiofrequency, MRI basically maps the distribution of hydrogen nuclei (protons) from water and fat in a region of the body. MRI is primarily a clinical diagnostic tool, however, in the past ten years, significant developments have been achieved in imaging small animals as well.

Because of physical characteristics, the living lung is one of the most challenging organs to image by MRI. Using conventional acquisition techniques, the lung appears dark in the images (Fig. 1). In order to acquire signal from lung parenchyma, special techniques need to be used. A further challenge for lung MRI is that cardiac and respiratory movements may cause marked image artefacts. These problems are more evident in small rodents, because of the higher cardiac and respiratory rates.

For drug testing *in vivo*, it is important to keep the acquisition conditions as simple as possible so that repeated measurements interfering minimally with the physiology and the well-being of the animals can be carried out on a routine basis. Beckmann et al. developed an approach based on a conventional MRI tech-

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nique, which produces sharp images from the chest of a rat respiring spontaneously (Beckmann et al., 2001). The examination time is of approximately 25 min, and during this time the animal is kept anaesthetized by the same anesthetic gas used in the clinic.

Detection of structural and functional changes

With this approach, effects of inflammation (Beckmann et al., 2001), mucus secretion (Beckmann et al., 2002), airway and vascular remodeling (Beckmann et al., 2004; Tigani et al., 2007), and parenchymal destruction (Karmouty et al., 2006) can be assessed in the rat lung serving as important readouts for models covering a variety of respiratory diseases. The disease progression is followed in the same animal. Two examples illustrate the relevance of the information that can be obtained with MRI.

A characteristic feature of respiratory diseases such as asthma is edema in the airways due to an increased permeability of the lung microvasculature to plasma proteins. Assessment of the fluid leaking out from the microvascular circulation into the surrounding tissue is important for diagnostic purposes. In rats actively sensitized to ovalbumin (OVA) and challenged intra-tracheally (i.t.) with the antigen (OVA, 0.3 mg/kg), an intense, even, fluid signal is detected in the lungs 24 h after challenge (Fig. 2a). Despite the extensive presence of fluid in the lungs, no abnormal behavior of the rats is noticed. The MRI fluid signal that is observed for about 4-5 days, before it resolves spontaneously, significantly correlates with perivascular edema assessed by histology (Tigani et al., 2003).

This correlation provides the basis to address the effects of anti-inflammatory therapies in the allergen model. In one experimental paradigm the drugs are given in a therapeutic regimen 24 h after the challenge with OVA, a time point when an extensive MRI signal is present in the lungs. Treatment with budesonide, a corticosteroid approved for clinical use, accelerates the rate of resolution of the MRI signal (Fig. 2b). The decline in the edematous signal correlates significantly with the reduction in perivascular edema quantified by histology of the lungs. By contrast, BAL fluid markers of inflammation are not affected by budesonide. It seems, accordingly, that the early resolution of MRI edematous signals by the anti-inflammatory drugs does not

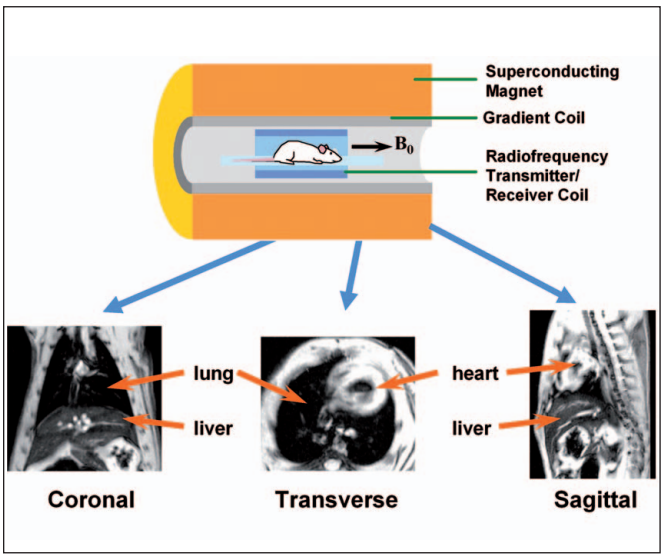

Fig. 1: MRI equipment for small rodent imaging.
Essential elements are a magnet (usually superconducting operating at high B0 field, e.g. 4.7 T) generating a homogeneous constant magnetic field, a gradient system capable of producing a position-dependent magnetic field when data acquisition takes place, and a coil which transmits and receives radio-frequency waves. An image is reconstructed from such waves and represents a weighted distribution of water and fat protons in the body. Images with an arbitrary orientation can be obtained using MRI, for instance coronal, transverse, or sagittal sections, as illustrated. Animals are kept under anaesthesia (e.g. with an anaesthetic gas administered through a mask) during the imaging session.

Fig. 2A

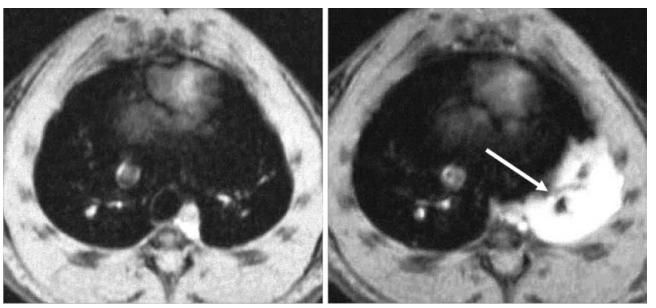

Fig. 2B

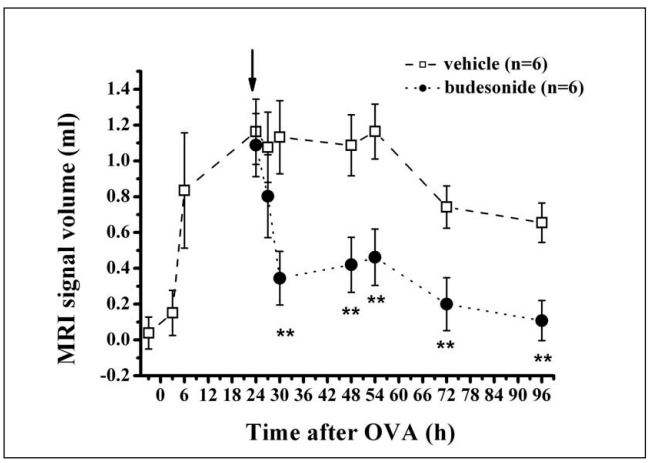

Fig. 2: Allergen challenge.
(A) Axial images through the chest of an actively sensitized BN rat, acquired before (left) and 24 h after intra-tracheal (i.t.) OVA instillation (right). Note that the lung parenchyma appears dark; however, following allergen, a prominent MRI fluid signal is seen (arrow). This signal is due to edema. The acquisition time for one image is of 1 min.
(B) Volume of fluid signals detected by MRI in the lungs for an OVA dose of 3 mg/kg (i.t.). Administration of the corticosteroid budesonide (1 mg/kg i.t.) 24 h after OVA (arrow) leads to an acceleration of the resolution of the MRI signals, suggesting a rapid effect of the compound. This effect is not detectable by conventional BAL fluid analysis. Data are expressed as means ± SEM (n=6 rats per group).

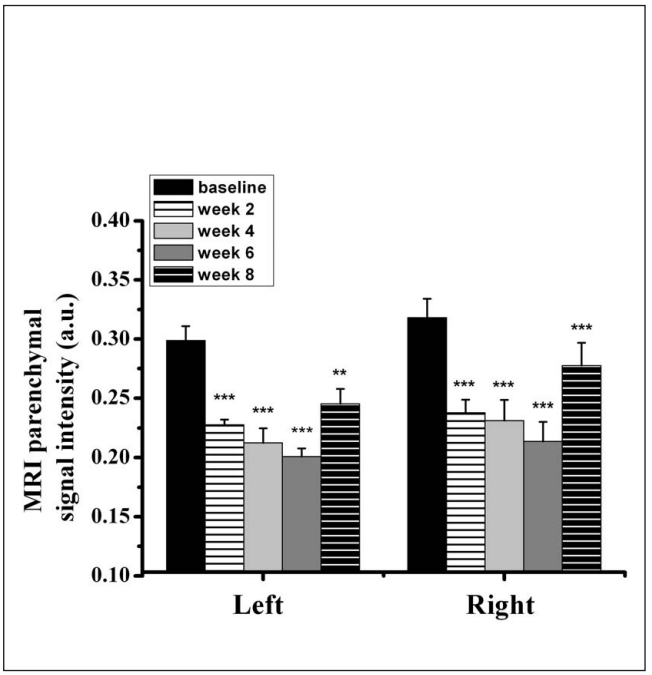

Fig. 3A

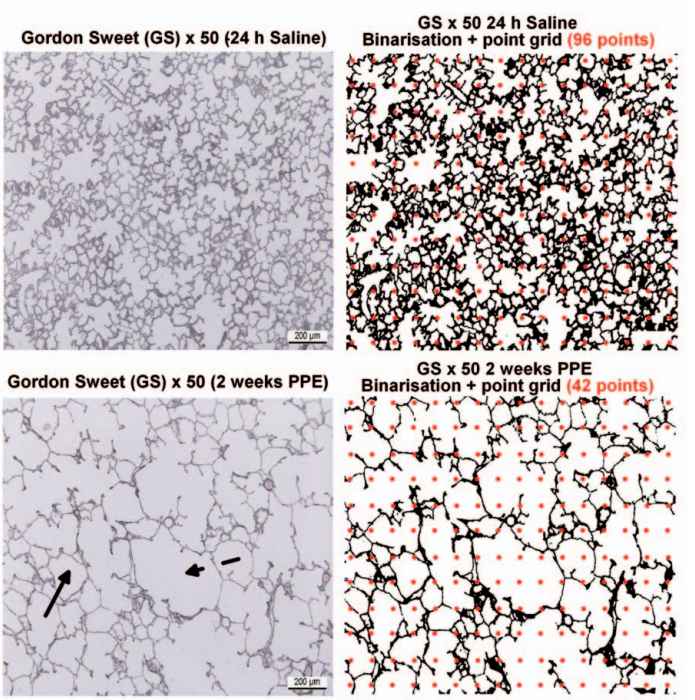

Fig. 3B

involve general suppression of the inflammatory response at least as monitored by BAL fluid analysis (Tigani et al., 2003).

A second example concerns the use of MRI in a rat model of emphysema (Quintana et al., 2006). For animals treated with PPE administered i.t., the parenchymal signal intensity was decreased in the first 6 weeks following PPE (Fig. 3a). Consistent with this, extensive enlargement of the alveoli was observed in alveoli rich sections of histological slices (Fig. 3b). A tendency towards recovery of the MRI signal intensity was apparent at week 8, which correlated with a reduction of the emphysematous damage assessed histologically by point morphometry. Related to this reduction in damage could be the fact that following PPE the elastin content initially decreases, but appreciable elastic fiber deposition, and granulation of the alveolar airspaces containing fibroblasts, endothelial cells and a provisional collagen matrix are observed weeks after injury. The significant negative correlation between the MRI signal intensity of lung parenchyma and the percentile alveolar content determined by histology (Fig. 3c) indicates that proton MRI is sensitive to non-invasively detect structural changes of the lung parenchyma related to the development of emphysema in this model.

Less discomfort, shorter experiments

With MRI one has the potential to shorten the overall experimental duration after injury onset since the technique is able to detect changes before these are reflected in parameters of inflammation present in BAL fluid. This results in less discomfort for the animals.

Less animals and more relevant data

The non-invasive MRI approach results in a significant reduction in the number of animals used for experimentation. Depending on the application, a reduction between 80 to 90% is estimated. Since repeated measurements are feasible, each animal can serve as its own control, thereby reducing the variability of the data. As acquisitions are performed on spontaneously breathing rats, interferences with their well-being and physiological status are minimized. MRI is able to provide data on rapid effects of anti-inflammatory compounds on established inflammation, an information that is not accessible to conventional, *post mortem* BAL fluid analysis. Thus, data that are more relevant to address therapeutic effects can be obtained using MRI. Anesthesia is the primary limiting factor of the approach. However, following an examination, rats recover from anesthesia within 10-15 minutes.

Overall, MRI provides a global picture of the disease status in the animal model. As this imaging technique is largely available in hospitals, there is potential to address translational aspects from the models in rats to the human situation.

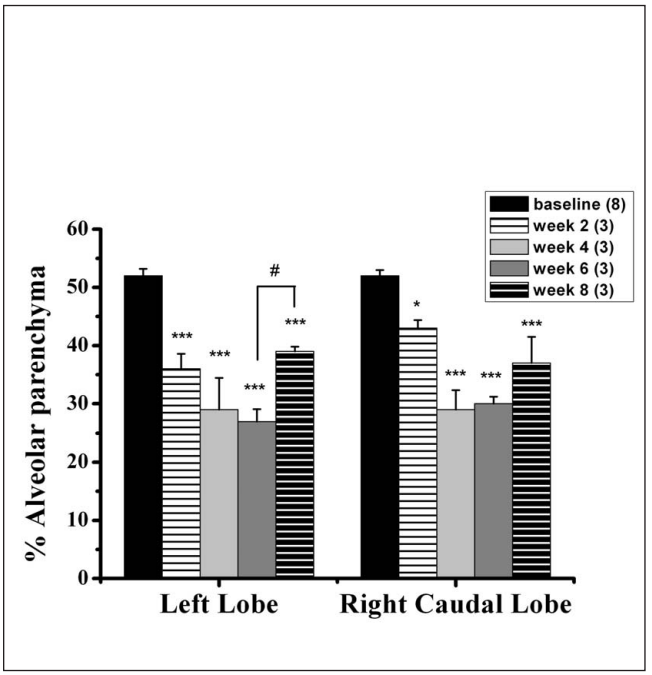

Fig. 3C

Fig. 3: Elastase challenge.
(A) Signal intensities (means ± SEM) from coronal slices corresponding to the lower right and lower left anatomical sides of the lung acquired prior to (baseline) and at 2, 4, 6 and 8 weeks after treatment with elastase. Significance levels *0.01<p<0.05, **0.001<p<0.01, ***p<0.001 refer to Anova comparisons (Bonferroni test vs. control) between the different time points and baseline values, for each region.
(B) Histological slices and corresponding binarization with a 192 point-grid used for analysis showing the alveolar area from the left lobe of rats administered with saline or with PPE. The black arrow shows the increased area of alveoli and the dotted arrow the increased terminal bronchi in the PPE-administered rat.
(C) Percent alveolar parenchyma (means ± SEM) assessed histologically from the left and the right caudal lobes from animals treated with PPE. Significance levels *0.01<p<0.05, and ***p<0.001 refer to Anova comparisons (Bonferroni test pair wise) between the different time points and baseline values, for each lobe. For the left lobe, a significant difference (#0.01<p<0.05) was found in the percentile alveolar area at 6 and 8 weeks. Remarkable is the resemblance between the curves representing the MRI signal intensity of parenchyma in the left and right sides, and the percentile alveolar area assessed in the left and in the right caudal lobes, reflected in a significant correlation (R=0.84, p<0.001) between both values. Histological samples corresponded to the same animals analyzed by MRI. This result suggests that the MRI signal reflects changes induced by PPE at the parenchymal level.

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