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Magnetic Resonance Imaging (MRI) of the Lung as a Tool for the Non-Invasive Evaluation of Drugs in Rat Models of Airways Diseases

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Summary

Methods currently used to evaluate the efficacy of potential treatments for diseases of the airways in small animal models are generally invasive and terminal. We explored the flexibility of magnetic resonance imaging (MRI) to obtain anatomical and functional information on 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 may be achieved, as well as minimal interference with their well-being and physiological status. Also, MRI has the potential to shorten the overall duration of the observation period after disease onset, since the technique is able to detect changes induced by allergen before these are reflected in secreted parameters of inflammation.

Keywords: asthma, chronic obstructive pulmonary disease, COPD, drug development, lung, magnetic resonance imaging, MRI

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 oedema of the airway mucosa, as well as smooth muscle hypertrophy and mucus plugging, through the release of enzymes and proteins (Kroegel et al., 1994; Barnes, 1996; Moqbel, 1996). In COPD, inflammation of the small airways and lung parenchyma with the involvement of neutrophils, macrophages and cytotoxic (CD8+) 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; Sethi, 2005).

Many species of animals can be used to provide models for airways diseases in humans. Unfortunately, there is no animal model that exactly reproduces the human pathology. Nonetheless, these models are useful for the development of novel therapies and to mimic and study specific aspects of human respiratory diseases (Dawkins and Stockley, 2001; Canning, 2003; Isenberg-Feig et al., 2003). Actively sensitised Brown Norway (BN) rats exposed to allergen develop airway hyperresponsiveness and eosinophilic inflammation together with an increase in activated T cells (CD25+) in the airways (Renzi et al., 1993; Haczku et al., 1997;

Hannon et al., 2001), hence reflecting the key features of asthmatic inflammation. Alternatively, inflammation similar to that observed in COPD patients can be elicited in rodents by the administration of endotoxin (lipopolysaccharide; LPS), a bacterial macromolecular cell wall component. LPS activates mononuclear phagocytes through a receptor-mediated process, leading to the release of a number of cytokines, including tumour necrosis factor-α (TNF-α) (Watson et al., 1994; Yang et al., 1998). TNF-α increases the adherence of neutrophils to endothelial cells, therefore facilitating a massive infiltration of neutrophils into the lung (Albelda et al., 1994). Exposure of BN rats to LPS leads to pulmonary neutrophilia (Tesfaigzi et al., 2000; Tigani et al., 2002) and induces mucus cell metaplasia (Harkema and Hotchkiss, 1992).

Invasive and terminal approaches (broncho-alveolar lavage (BAL) fluid analysis; bronchial biopsies; histology; weighing of lungs) are currently used to analyse such models and to perform preclinical drug studies. Evidently, for ethical reasons, it would be highly desirable to have non-invasive readouts in this research area.

Although *in vivo* MR techniques have been in use in pharmaceutical research for more than 20 years (for reviews, see Rudin et al., 1999; Beckmann et al., 2001a, 2004a), they have only recently been applied to pre-clinical studies in the area of respiratory diseases (Beckmann et al., 2003). Interestingly, a similar time-lag is evident for the clinical application of lung MRI. Probably the main reasons for this delay are the inherent difficulties imposed by the lung tissue on the MR signal properties

and, in the clinical arena, the fact that computerised tomography is the imaging technique of choice for diagnosis of lung diseases. The aim of this chapter is to illustrate how the flexibility of MRI can be exploited to non-invasively derive information on lung inflammation and on its functional status in models of airways diseases in rats, and how this information can be ultimately used to profile compounds in these animal models (Beckmann et al., 2003).

Animals, materials and methods

Procedures are described in detail in (Beckmann et al., 2001b, 2002; Tigani et al., 2002). BN rats weighing 250-300 g were used. Two models of pulmonary inflammation were studied by MRI:

Inflammation models: (i) Allergen-induced pulmonary inflammation in actively sensitised rats, resulting from the intra-tracheal (i.t.) administration of OVA. (ii) Endotoxin-induced pulmonary inflammation in non-sensitised naïve rats, caused by instillation of LPS.

MRI: Measurements were carried out with a spectrometer operating at 4.7 T. For image acquisition, rats were anaesthetised with 2% isoflurane in a mixture of O_2/N_2O, administered via a face mask. All measurements were performed in spontaneously breathing animals; neither cardiac nor respiratory triggering was applied.

Analysis of BAL fluid or histology: Carried out in order to better understand the signals detected by MRI in the lungs. These
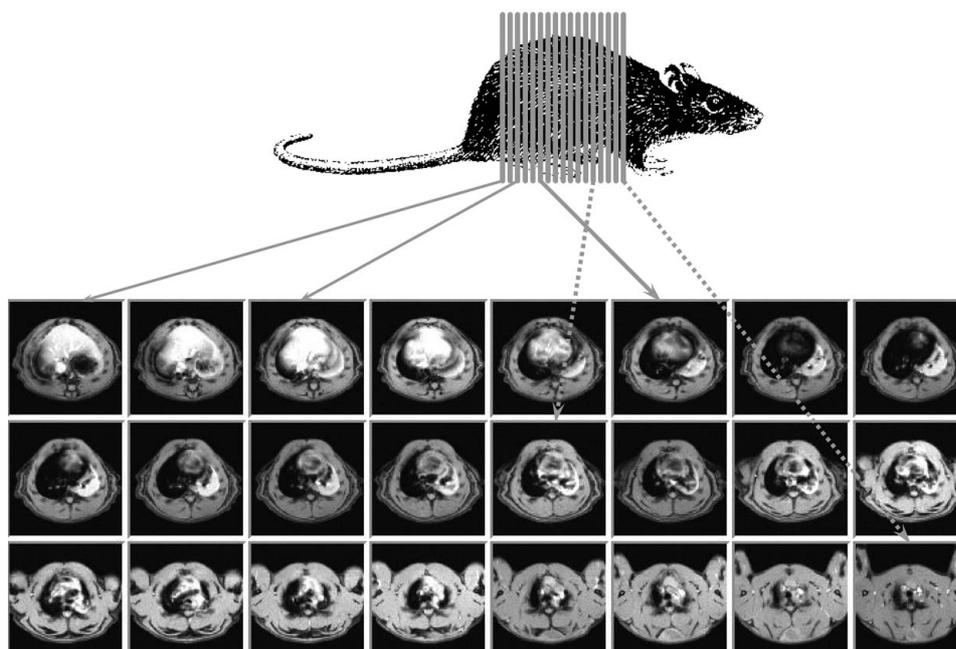
Fig. 1: Axial MR images acquired sequentially through the chest of an actively sensitised BN rat, 24 h after i.t. OVA (0.3 mg/kg) challenge, using a gradient-echo sequence. The acquisition time per image was 60 s. The animal was kept under anaesthesia (isoflurane administered through a mask) and respired spontaneously. Neither respiratory nor cardiac gating was applied during image acquisition. Oedema area is assessed on each image using a semi-automatic segmentation procedure. Total oedema volume is computed by adding the areas obtained for each slice, multiplied by the slice thickness. See Beckmann et al. (2001b) for more details.

terminal methods were only used during the establishment phase of MRI within lung research. The lungs were lavaged and several inflammation parameters were assessed in the BAL, such as cellular infiltration, including eosinophil and neutrophil numbers. Histology was carried out to assess, e.g. perivascular oedema, mucus and goblet cell numbers.

Results

Lung inflammation

A characteristic feature of respiratory diseases such as asthma is oedema of the airways due to an increase in the permeability of the lung microvasculature. The resulting effect is the leakage of fluid containing plasma proteins from the microvascular circulation into the surrounding tissue. Assessment of this fluid can be important for diagnostic purposes and for planning and guiding treatment.

Proton MRI is the natural candidate for trying to detect inflammatory responses in the lungs in models of airways diseases. We used a conventional gradient-echo technique to generate images of the rat thorax in which motion artefacts were suppressed by averaging (Beckmann et al., 2001b). Neither respiratory nor cardiac triggering was applied, and the animals were able to breathe spontaneously during data collection. Under the conditions chosen for acquisition, the signal from the lung parenchyma itself is too weak to be detected at 4.7 T. However, the absence of any detectable lung parenchymal signal in combination with a background devoid of artefacts provided a high contrast-to-noise ratio for the detection of fluid signals associated with the inflammatory process (Beckmann et al., 2001b, 2002).

In rats actively sensitised to ovalbumin (OVA) and challenged with the antigen, an intense, uniform oedematous signal was detected in the lungs 24 h after challenge. By acquiring approximately 20 images displaced from each other by a distance corresponding to a single slice thickness, the whole thorax of the animal was scanned and the total volume of the oedematous signal determined (fig. 1). The volume of the oedematous signal was dependent on the dose of allergen and reached a maximum 48 h after challenge (fig. 2). The MRI signal could be seen for approximately 100 h and was correlated highly significantly with a variety of inflammatory parameters determined in the BAL fluid recovered from the same animals (Tigani et al., 2002).
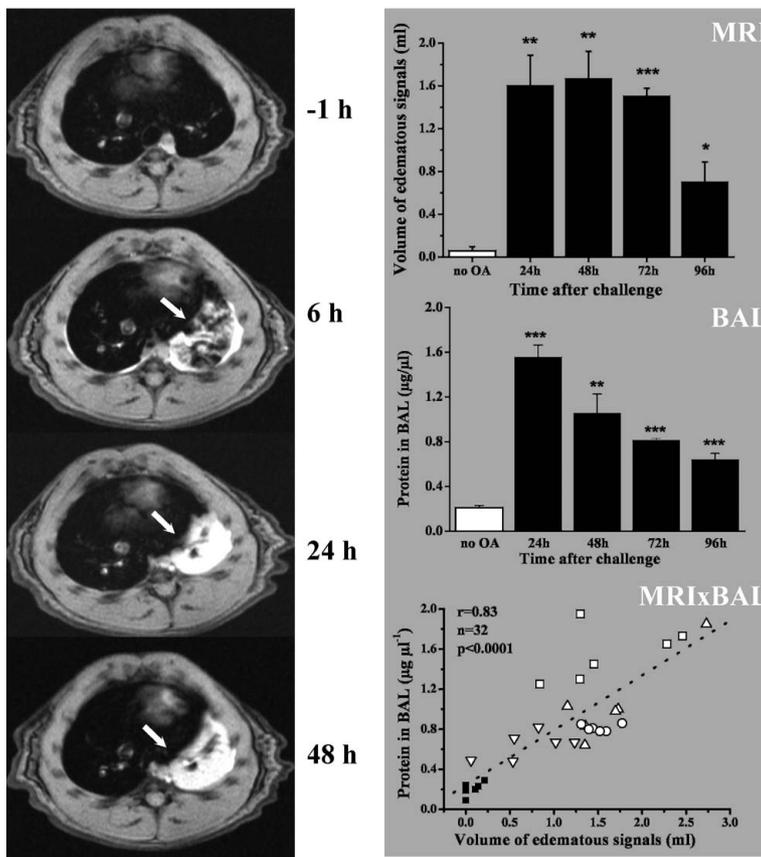
Fig. 2: Axial MR images acquired from an actively sensitised BN rat at different time points with respect to i.t. OVA (0.3 mg/kg) challenge. Prominent oedematous signals were seen already 6 h after challenge. The curves on the right depict the time course of MRI signals (mean ± SEM, n=6), and for the same animals, the protein (mean ± SEM) assessed in BAL fluid. The MRI signals significantly correlated with the protein and several markers of inflammation in the BAL. For more details, see Beckmann et al. (2001b) and Tigani et al. (2002).

Of special interest, the strongest correlations were with the eosinophil numbers, eosinophil peroxidase activity (a marker of eosinophil activation), and the total protein concentration (a marker for plasma extravasation). Importantly, the signal detected by MRI correlated significantly with the perivascular oedema assessed by histology (Tigani et al., 2003a).

Following challenge of non-sensitised rats with LPS, the signals that appeared in the lungs were uneven and significantly less intense than those detected after OVA administration to actively sensitised animals (fig. 3). They were of long duration, being detectable up to 8 days after dosing (Beckmann et al., 2002). The only parameter in the BAL fluid that correlated significantly with the MRI signal was the mucus concentration (Beckmann et al., 2002; Tigani et al., 2002). Histological analysis indicated a substantial and sustained increase in goblet cell numbers up to 16 days after LPS challenge, and flocculent mucoid material was consistently detected close to the apical surface of epithelial cells (Beckmann et al., 2002). These observations suggest that the long lasting MRI signal following LPS was due to secreted mucus.

Lung ventilation

Gas exchange is the major function of the lungs. The regional pulmonary blood flow (perfusion) and the ventilation in the lungs need to be matched for this process to occur efficiently. In diseases of the airways like asthma and COPD, lung ventilation can be compromised. Asthma is a highly prevalent chronic inflammatory disorder of the airways characterised by periodic and reversible narrowing of the airways making breathing diffi-

cult. Bronchial hyperresponsiveness to various stimuli such as irritants, infection, exercise, cold air, or allergens is a key feature of asthma and is related to an enhanced sensitivity of the airway smooth muscle to contractile stimuli. In COPD, sustained smoking causes chronic inflammation of the airways responsible for mucosal thickening, airway narrowing and loss of elastic recoil.

Pulmonary function tests providing global lung function information, such as forced expired volume in one second (FEV1) or flow-volume pattern, are used to quantify the severity of lung diseases and to evaluate the efficiency of treatment. Invasive measurements of airflow and transpulmonary pressure following stimulus by agents inducing bronchoconstriction are used to monitor lung function in rats (Hannon et al., 2001). However, these tests do not provide any regional information. The following subsections address efforts made to obtain regional information about lung function in rat models non-invasively by using proton MRI.

Airway remodelling and hyporesponsiveness induced by inflammation
Inflammation of the airways leads to pathophysiological changes in the structure of the lung tissue, including thickening of the airway smooth muscle (Ebina et al., 1993), which may influence the responsiveness to bronchospasmogens (Martin et al., 2000) and alter ventilation. The progressive structural change known as airway remodelling, which is driven by chronic local inflammation, is a fundamental component in the development of airway hyperresponsiveness (for a recent review, see Halayko and Amrani, 2003).
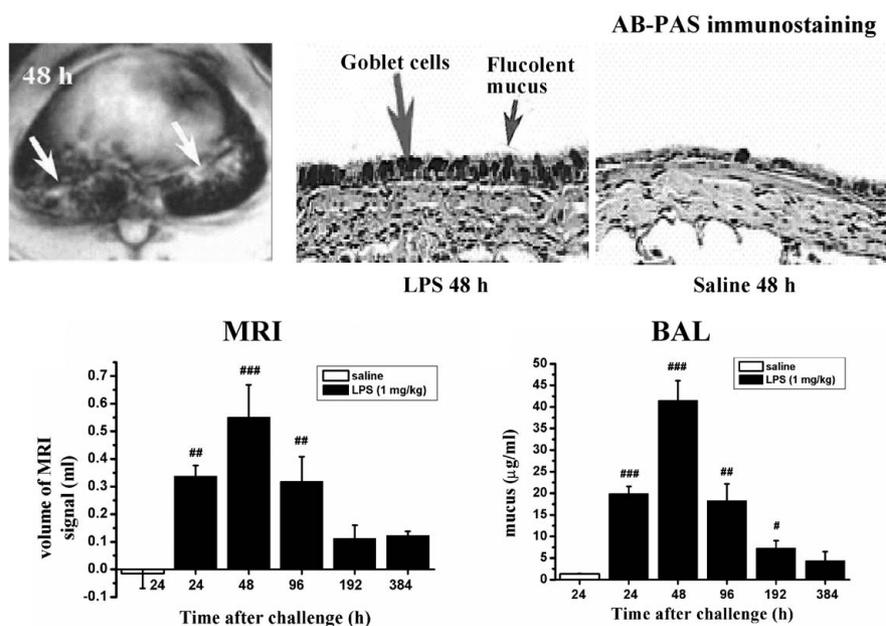
Fig. 3: Axial image through the chest of a naïve BN rat, 48 h after i.t. instillation of LPS (1 mg/kg). Histology and BAL fluid analysis revealed that the MR signal reflected secreted mucus. For details, see Beckmann et al. (2002) and Tigani et al. (2002).
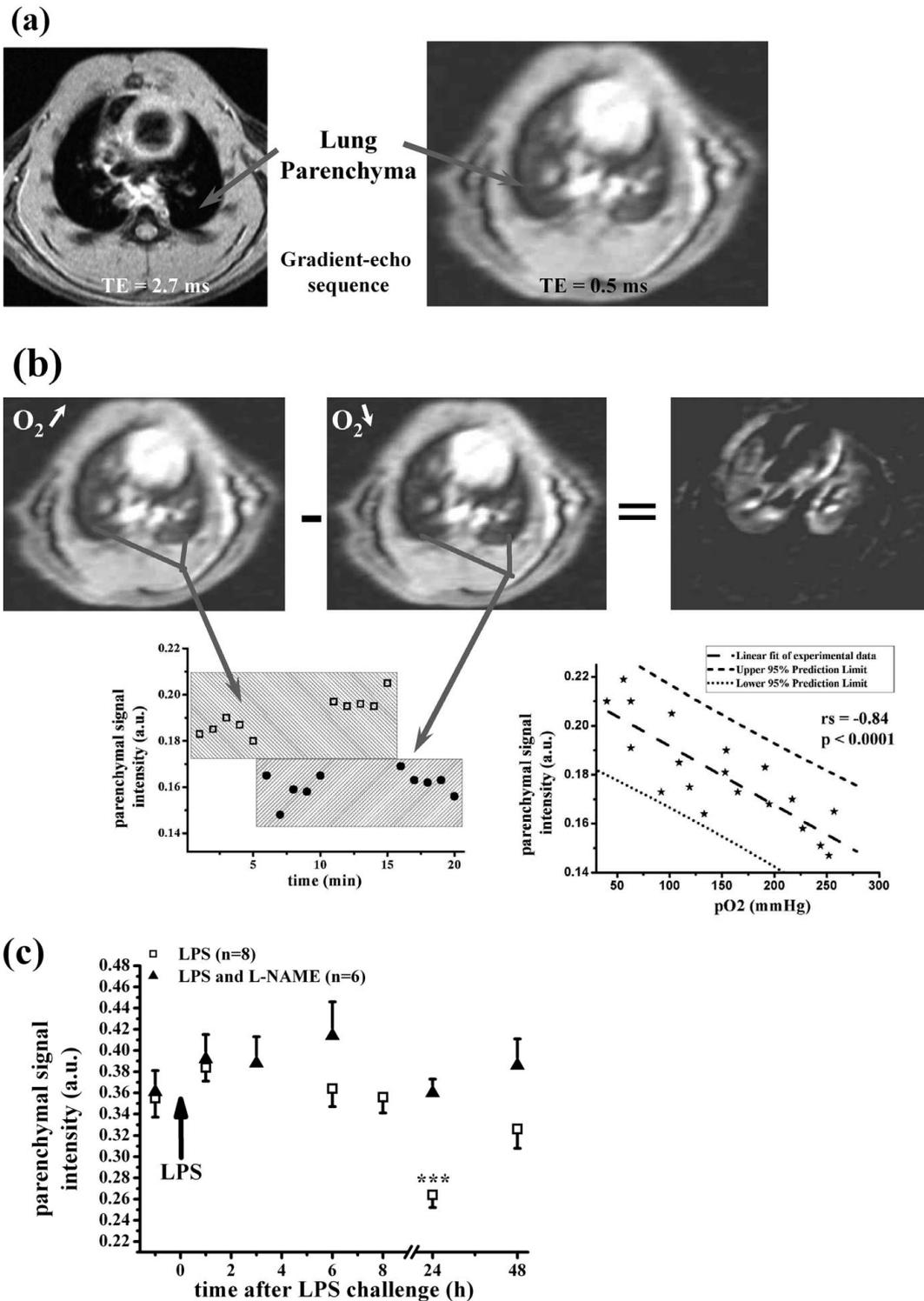
Fig. 4: (a) By changing the acquisition parameters of the gradient-echo sequence, signals from lung parenchyma become more evident. The rat could respire spontaneously, and neither cardiac nor respiratory gating was applied during image acquisition. (b) The parenchymal signal intensity is inversely dependent on the amount of O_2 in the lung. The reason is that molecular O_2 is paramagnetic, thus acting as a natural contrast agent. We found a significant negative correlation between the parenchymal signal intensity and the partial oxygen pressure in blood for different amounts of oxygen administered (between 21% and 65%). (c) Course of lung parenchymal signal (mean ± SEM) following challenge with LPS (1 mg/kg i.t.). Some of the animals were pre-treated with L-NAME (10 mg/kg i.v.) 10 min before the challenge. The significance level corresponds to comparisons with baseline signal intensities before challenge (*** p<0.001). See Beckmann et al. (2004b) for more details.

Effects of airway remodelling and hyporesponsiveness following respectively allergen or endotoxin challenges were monitored non-invasively in spontaneously breathing rats with a gradient-echo sequence as described by Beckmann et al. (2001c) (fig. 4a). The basis of the approach consisted in detecting modulations of proton signals of lung parenchyma induced by changes in oxygenation levels. An increased parenchymal signal should be consistent with a reduced oxygen level and *vice versa* (Beckmann et al., 2004b) (fig. 4b). This hypothesis has been verified in the allergen and endotoxin models of airways inflammation in the rat (Beckmann et al., 2004b). In actively sensitised rats, an increased parenchymal signal intensity (in areas devoid of oedematous signals) was detected at 6 h and up to 180 h after challenge, at a time when oedematous signals reflecting inflammation had completely subsided. Histological analysis revealed airway remodelling in the lungs of OVA-challenged rats characterised as an increased bronchial epithelium thickness and smooth muscle area, as well as bronchial goblet cell hyperplasia. Thus, the increased parenchymal signal in lung images of rats treated with allergen was consistent with a significant reduction of air space determined by histology, pointing to impaired lung ventilation in these animals. The ventilation defect was still observed after the oedematous signals detected by MRI were completely resolved (Beckmann et al., 2004b).

In a second model, significantly decreased parenchymal signal intensity was detected 24 h after intra-tracheal instillation of LPS (Beckmann et al., 2004b) (fig. 4c). The effect was abolished by pre-treatment with N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide (NO) synthase (NOS). A possible role of NO in the inflammatory response elicited by endotoxin has been demonstrated by Pauwels et al. (1990), who showed that a period of significant hyporesponsiveness, characterised by reduced pulmonary resistance due to increased airway calibre, followed from 9 to 12 h after exposure of rats to aerosolised LPS. In the same model, LPS-induced airway hyporesponsiveness was eliminated by L-NAME (Kips et al., 1995). Moreover, a marked expression of inducible NOS in rat macrophages recovered from the airways has been demonstrated 16 h after local LPS instillation (Kobzik et al., 1993). Therefore, endogenous synthesis of NO, a potent bronchodilator (Abderrahmane et al., 1998), induced by endotoxin might have been responsible for increased oxygenation of the lung tissue, thus contributing to a reduction of the parenchymal signal in the gradient-echo images 24 h following LPS administration.

Airway smooth muscle contraction

Proton MRI was also used to detect the effects of bronchoconstrictor and bronchodilator compounds in spontaneously breathing rats. For instance, a significant increase of the parenchymal signal intensity was observed in the upper airways from the first minutes following *i.v.* administration of a compound eliciting bronchoconstriction. The long-lasting signal increase was reversed by application of a bronchodilator agent, consistent with an increase in oxygenation. Airway resistance measures derived invasively in anaesthetised, paralysed, and artificially ventilated rats showed the same time profile as that of the MRI signal. These observations suggest that the MRI signal changes in the upper airways were due to contraction of airway smooth muscle.

Drug treatment analysis

A variety of new compounds for the treatment of respiratory diseases are under development, many of which are designed as anti-inflammatory therapies (Barnes, 2002, 2004). Since oedema is an integral component of experimental pulmonary inflammation, MRI has the potential to provide a non-invasive means of monitoring the course of the inflammatory response and the consequence of therapy with anti-inflammatory drugs.

We consider first the classic approach of analysing the effects of anti-inflammatory drugs administered prior to allergen challenge (fig. 5a). A clear dose-related reduction of the oedematous signal has been shown for compounds such as the glucocorticosteroids budesonide (Beckmann et al., 2001b; Tigani et al., 2003a) and mometasone (Tigani et al., 2003b), and for a mitogen-activated protein kinase inhibitor (Tigani et al., 2003b). The effects correlated with changes in the parameters of inflammation assessed in the BAL fluid (Tigani et al., 2002). Repeated measurements allowed information on the duration of action to be easily defined.

The MRI technique was also applied to address the effects of drugs administered after the allergic inflammatory response had developed. In one experimental paradigm the drugs were given 24 h after OVA challenge, a time point when an extensive MRI signal was present in the rat lung (fig. 5b). Treatment with budesonide, mometasone or with a selective inhibitor of phosphodiesterase type 4 (PDE4), which is also a powerful inhibitor of allergic pulmonary inflammation in the rat (Trifilieff et al., 2002), accelerated the rate of resolution of the MRI signal (Beckmann et al., 2001b; Tigani et al., 2003a,b). For these compounds, a clear trend towards a reduction in the oedematous signal was observed as early as 3 h after drug administration, and the effect was statistically significant from 6 to 72 h. The decline in the oedematous signal correlated significantly with the reduction in perivascular oedema quantified by histology of the lungs (Tigani et al., 2003a). This suggests that suppression of perivascular oedema following "therapeutic" treatment with budesonide, mometasone or the PDE4 inhibitor caused the decrease in the MRI signal. By contrast, BAL fluid markers of inflammation were not affected by any compound 6 h after treatment (Tigani et al., 2003a). It seems, accordingly, that the early resolution of MRI oedematous signals by the anti-inflammatory drugs did not involve general suppression of the inflammatory response, at least as monitored by BAL fluid analysis. At 48 h following treatment with the steroids or with a PDE4 inhibitor, MPO activity and protein concentrations were significantly reduced and, in animals treated with the PDE4 inhibitor, eosinophil number and EPO activity were also significantly diminished (Tigani et al., 2003a). These changes may be the mechanistic basis for the sustained resolution of MRI signals.

In addition to profiling anti-inflammatory compounds, MRI can also be used to address the effects of compounds designed

to improve lung function. For instance, the examples discussed above suggest that proton MRI approaches have the potential to profile bronchodilator compounds in models of airways obstruction.

Discussion and conclusions

The main advantage of using MRI for the characterisation of animal models of diseases is its non-invasive nature, which allows repetitive measurements to be carried out in the same animal. This is of relevance in longitudinal studies, since the inter-individual variance is eliminated and the number of animals required to reach statistical significance is much smaller. Also, non-invasiveness is a major asset when studying chronic diseases. The flexibility of MRI makes it a widely applicable method. We are currently applying MRI techniques to non-invasively assess drug effects in several disease models, e.g. arthritis, transplantation, neurodegeneration, and stroke (Rudin et al., 1999; Beckmann et al., 2001a, 2004a).

In the case of rat models of asthma, we estimate a reduction by approximately 80-90% in the number of animals used in the studies as compared to BAL fluid analysis or histology. Although providing comprehensive information at the cellular level, those methods have the drawback of being terminal. Here, we have demonstrated how MRI can provide complementary information with a fundamental asset: its non-invasive character. Repeated measurements can be carried out on the same animal, and the time courses of events becomes easily accessible. The significant correlation between the MRI signals and the perivascular oedema determined histologically provides solid evidence for the non-invasive assessment of a key component of inflammation in the allergen model, enabling rapid effects of drugs to be detected *in vivo* by monitoring the rate at which oedematous signals resolve. Also, the prospect of using MRI to detect non-invasively a sustained mucus hypersecretory phenotype induced by endotoxin provides an important new perspective for animal models of COPD. Thus, despite being a macroscopic technique, MRI allows an overall assessment of the time-related behaviour of compounds in models of lung inflammation in rats. With this information it becomes easier to choose the time point for carrying out BAL fluid or histological analysis in order to obtain more specific information on the drug mechanism.

Since MRI studies are conducted on spontaneously breathing rats, the well-being of the animals during experimentation is improved, as invasive procedures like tracheotomy and/or intubation are avoided. Thus, repetitive measurements can be carried out more easily, and the information obtained from time courses provides a better picture of disease development and treatment. Furthermore, our aim is to replace currently adopted methods of ventilation assessment involving the use of radioactive materials. Finally, we estimate that by using MRI the duration of the experimental period can be reduced in some of the applications as compared to conventional approaches.

Overall, we are confident that MRI and other imaging techniques will play an increasingly important role in pre-clinical research on small rodents in the area of airways diseases. The non-invasive character of the approaches should facilitate not only drug assessments in animal models, but also provide relevant data for the transition to the clinic.
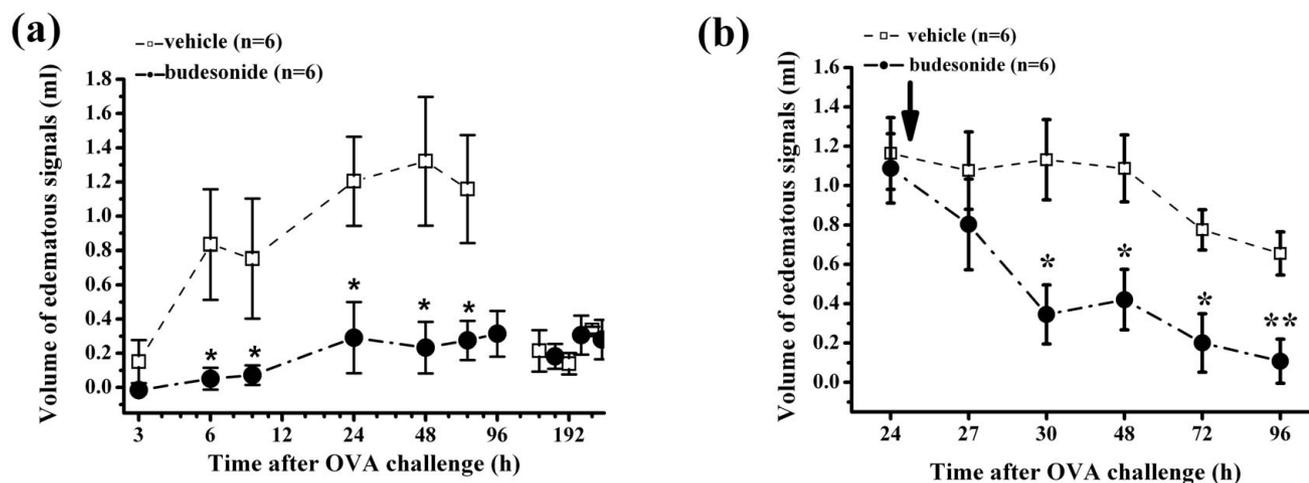
Fig. 5: Course of oedematous signals (mean ± SEM) following OVA (0.3 mg/kg i.t.) challenge in actively sensitised BN rats.
(a) Pre-treatment: animals received budesonide (1 mg/kg i.t.), a glucocorticosteroid, or its vehicle (saline) 1 h before and 24 h after OVA.
(b) Post-treatment: rats received budesonide (1 mg/kg i.t.) or its vehicle 24 h after OVA. The significance levels *p<0.05 and **p<0.01 refer to t-test comparisons made between budesonide- and saline-treated animals, at each time point. For more details, see Beckmann et al. (2001b) and Tigani et al. (2003a).

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Abbreviations

BAL, broncho-alveolar lavage; BN, Brown Norway; COPD, chronic obstructive pulmonary disease; i.t., intra-tracheal; L-NAME, N^G-nitro-L-arginine methyl ester; LPS, lipopolysaccharide; MAP, mitogen activated protein; MR, magnetic resonance; MRI, magnetic resonance imaging; NO, nitric oxide; NOS, nitric oxide synthase; OVA, ovalbumin; PDE4, phosphodiesterase-4; TNF-α, tumor-necrosis factor-α

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