# Proton MRI parameters of lung disease following bleomycin induced inflammation in the rat.

J. Hammersley[1], A. L. Busza[1], N. Saeed[1], A. White[1], H. L. Lloyd[1], D. G. Reid[1], M. Salmon[2], D. C. Underwood[2], R. R. Osborne[2], K. K. Changani[1]

[1]ri-MRI Centre, GlaxoSmithKline plc, Welwyn, Hertfordshire, United Kingdom, [2]COPD Biology, GlaxoSmithKline plc, Upper Merion, PA, United States

**Synopsis:**

Proton MRI of lung parenchyma was acquired serially over a 29 day period following induction of fibrotic disease in the rat. Bright, homogeneous signals a few days after challenge suggest oedema; these give way to more diffuse fibrous networks later. T2 values show a gradual progression away from oedema to protein over the time period suggesting, lung fibrosis. This study demonstrates the utility of proton MRI to characterise the development of chronic obstructive pulmonary disease in a rat model. Information from a single imaging session can provide inflammatory volume, compensatory lung volume and T2 value of lung composition changes.

**Introduction:**

Pulmonary fibrosis is characterised by chronic inflammation followed by progressive extracellular collagen deposition in the alveolar walls. The initial reversible fibroproliferative response following reagents such as bleomycin would involve general inflammatory cell recruitment producing alveolitis. After fibroblast proliferation, collagen fibre deposition results in the fibrotic state. Pulmonary function becomes compromised as the collagen based extracellular tissue matrix builds up producing, in some cases, 'honeycomb lung' [1] characterised by irreversible alveolar obliteration and accompanied by pulmonary hypertension and right ventricular hypertrophy. Alterations in ventilation-perfusion relationships representing changes in airway flow, reduced gas diffusion and loss of alveolar-capillary units may result from collagen-based extracellular matrix deposition. This study aimed at assessing the ability of MRI to differentiate the various stages of bleomycin-induced fibrosis by evaluation using simple proton MRI, changes in disease volumes, compensatory lung volume changes and T2 measurements of tissue composition.

**Method:**

Animals were housed and maintained and procedures conducted in accordance with the Home Office Animals (Scientific Procedures) Act 1986, UK. Baseline images were obtained from 18 female Sprague-Dawley rats (n=6 per group). These animals were anaesthetised using isofluorane and the free breathing animals were immediately placed supine in a 2T Bruker Medspec S200, 96cm bore system, using a 55mm purpose built coil in a 22cm gradient insert. Animals were homeostatically maintained and respiratory rate monitored under 2% isofluorane anaesthesia via 800ml/min O2. Specific positioning of the animal within the magnet was precisely adhered to in order to produce similar slice packs through the lung for eventual image registration and analysis of each slice at subsequent time points. Following imaging, animals (n=6) were dosed via intratracheal (it) passage with 1 unit bleomycin (200µl) and both treated and control animals re-imaged on Days 3, 10, 14, 21 and 29. The control group of age-matched rats was not treated. MRI - Pilot scans allowed accurate alignment of a 12 slice pack (1.5 mm) with anatomical landmarks. Multislice (128x128; 6x6 cm2 ) respiratory triggered RARE, 8 echoes, inter-echo time 4.2 or 6ms (TEeff 8.4 or 12ms), TR 1s, 4 averages, provided good lung parenchymal visualisation within 6 minutes. Multislice gradient echo images were also acquired. After the final scan lungs and hearts were removed for hydroxyproline analysis and right ventricular hypertrophy respectively. Analysis - Contours were created using in-house software: COBRAS defined lung in all timepoints, allowing calculation of total volume using QUAIL across 12 slices. Oedema was quantified using un-registered difference images and calculated bright intensity thresholds.

**Results and Discussion**

Bleomycin administration produced several distinct patterns of signal intensity change within the lung over a period of 29 days (Fig 1). Each image reflects a similar slice through the thorax. Day 0 images (A) reflect no underlying pathology but good parenchymal visualisation. At Day 3 (B) significant areas of high but homogenous intensity are clearly seen, very similar to those following it challenges with allergen or endotoxin [2-4] suggesting oedema/mucus/cell infiltration into the parenchyma. At Day 21 (C) the homogenous signal intensity has assumed an irregular fibrous appearance which is even more marked by Day 29 (D). We believe the network of signal at the latter two timepoints reflects fibrotic tissue; it is clearly differentiable from the early pulmonary fluid retention signal. Control animals showed no significant change in signal intensity or distribution. Fig 2 represents the total lung volume change following bleomycin challenge over a 28 day period. Compared with control lung, the oedemic/fibrotic changes produced a 46-52% increase in lung volume ($p<0.001$ for all time points following bleomycin challenge). Total oedemic/fibrotic change produced a total volume of 1145 ± 117 mm3 compared with a baseline total lung volume of 2318 ± 81.7 mm3 . This indicates a total lung infiltration of 49%, which compares favourably with the percentage lung volume change at the same time point. It would also suggest that oedemic change is followed by comparable fibrotic change. Histology showed clearly defined fibrosis around airways indicated by staining collagen and hydroxyproline increased by approximately 45% also equating to total lung volume change and hence fibrosis at this final time point. Fig 3 illustrates the T2 values (± sem) of lung parenchyma over the time course of disease progression. Values increase significantly from normal lung on days 3 and 7. Thereafter, there is slow decline reflecting gradual tissue composition change. During the early stages ie on Day 3 and 7, most of the T2 increase is associated with fluid such as oedema/mucous. As the fluid component of inflammation regresses the T2 reduces accordingly and correspondingly to the progression of fibrosis.

**Conclusion**:

This study has revealed several MRI markers of disease at the different stages of the evolution of bleomycin-induced lung injury in the rat. These markers include oedemic/mucous volume infiltration, total lung volume changes as well as T2 measurements of lung parenchyma composition. Early changes suggest similar inflammatory processes (oedemic/mucous) to those active in acute models of asthma, such as instillation of LPS via the intratracheal route [2]. Over time the fluid signal reflected in the high T2 characteristic of inflammation recedes, to be replaced by a more fibrous, diffuse signal and hence reduced T2 values. We presume this is associated with deposition of extracellular collagen-based matrix. An interesting observation in this study is the correlation between the lung volume change, the total oedemic volume and the eventual hydroxyproline analysis following instillation of bleomycin. These results indicate that oedemic occupation has been compensated for by a similar increase in resting total lung volume. The increase in hydroxyproline would also indicate that early changes monitored by MRI translate directly to the degree of eventual damage. This study suggests an important role for MRI to differentiate progressive fibrosis in pre-clinical animal models and potentially as a diagnostic clinical tool. Non-invasive evaluation of the progression of fibrosis may allow early diagnosis of disease and appropriate pharmacological intervention.

**References:**

[1] Crouch, Am J Physiol 1990, 259: L159-184, [2] Changani et al Proc ISMRM 2002 p 1986 [3] Beckmann et al NMR Biomed 2001, 14:297-306, [4] Tigani et al Biochem Biophys Res Commun 2002, 292:216-21

**Figure 1**

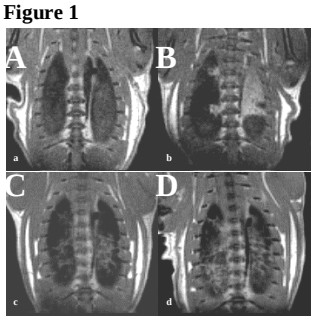


**Figure 2** - Lung Volume changes.

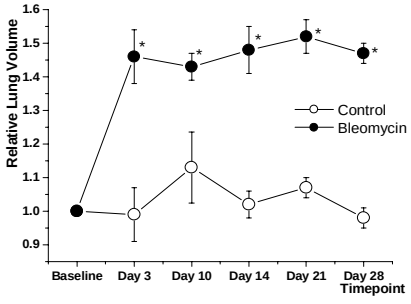


**Figure 3** - T2 relaxation compared with muscle