

# MRI as a non-X ray based imaging alternative to study experimental lung fibrosis induced by bleomycin in rats

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## Introduction:

Computerized tomography (CT) is the standard imaging technique to examine the human lung. At the preclinical level, micro-CT has been shown to be useful in characterizing anatomical changes related to lung fibrosis models in rats [1]. However, radiation doses are an issue both in the clinics and in experimental studies, and repetitive measurements are limited. Recent clinical activities have demonstrated that MRI is comparable to CT for lung examinations [2]. In the present work, we show that proton MRI can be used to follow longitudinally in spontaneously breathing rats the development of structural changes related to lung fibrosis induced by bleomycin (BLM) administration [3], and thus MRI represents a non-X ray based imaging alternative to study experimental fibrosis.

## Materials and Methods:

Animal handling, care, and experimental use were conducted in line with the Swiss Federal Law for animal protection (animal license BS No. 1989).

**Animals:** Male Sprague-Dawley (SD) rats weighing 280-320 g were supplied by SPF Harlan CPB.

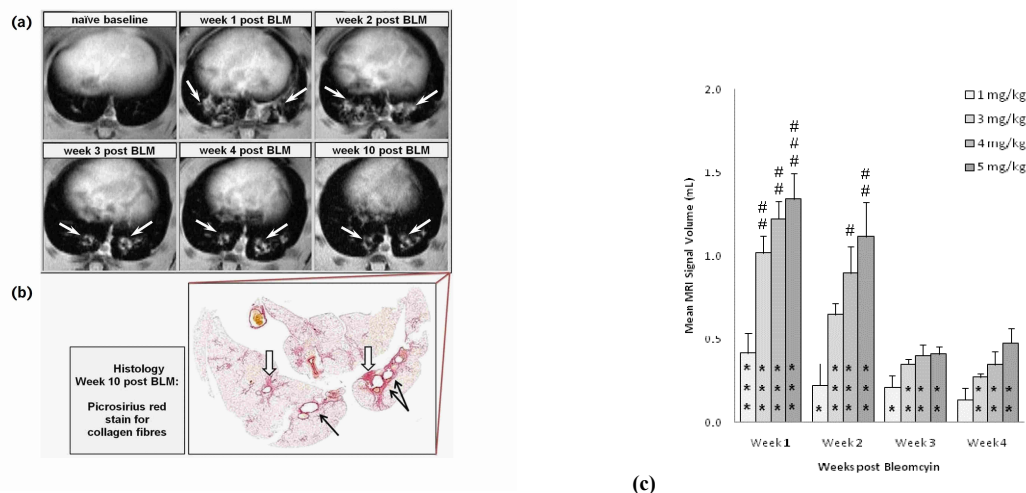
**BLM administration:** Rats were anesthetized (4% isoflurane; Abbott, Cham, Switzerland) in a chamber and then treated with BLM hydrochloride (Euro Nippon Kayaku GmbH, dissolved in 0.2 ml saline) [3] administered intra-tracheally (i.t.) before the bifurcation of the carina. BLM was dosed at 1, 3, 4, or 5 mg/kg on day 0 of the experiment.

**MRI:** Rats were anesthetized with isoflurane (2.0%) in a mixture of O<sub>2</sub>/N<sub>2</sub>O (1:2), administered via a face mask. Measurements were carried out with a Bruker Biospec 47/40 system. A gradient-echo sequence was used throughout the study for detecting fluid signals (TR = 5.6 ms; TE = 2.7 ms; FOV = 6x6 cm<sup>2</sup>; matrix = 256x128; slice = 1.5 mm; 45 image averages with an interval of 530 ms between each image acquisition). Neither cardiac nor respiratory triggering was applied, and rats respired spontaneously.

**Image analysis:** The volume of the MRI signals in the lung was quantified by applying a semi-automatic segmentation procedure as described previously [4].

## Results and Discussion:

MRI detected an increase in signal intensity compared to baseline in all rats treated with BLM, which was detectable through week 10 (Fig. 1a). Histology showed the presence of fibrosis (collagen deposition) and of airways remodeling at this time point (Fig. 1b). Lung signal volume was highest in the first week post BLM, declining thereafter though without complete resolution (Fig. 1c). At week 4 after BLM instillation, rats continued to display increased MRI signal volume in the lung, but this increase was significant only for the 3, 4, and 5 mg/kg groups (Fig. 1c). Histology confirmed that, whereas initial signals reflected a mix of edema and collagen deposition, later signals corresponded to more fibrotic tissue changes. In addition, higher doses of bleomycin resulted in more extensive lung injury, supporting the dose-response relationship seen with MRI.



**Fig. 1 – (a)** Transverse MRI scans show the rat lungs at naïve baseline and at different time points after BLM 4 mg/kg. **(b)** Histology week 10 post BLM identified areas of dense staining for collagen (white arrows), in addition to thickening of vessel and airway walls linked to bronchiectasis (black arrows). **(c)** MRI signal volumes displayed a dose-response to acute BLM ( $n=6$  rats/group). Compared to respective baselines: \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ . Compared to BLM 1 mg/kg: # $P<0.05$ , ## $P<0.01$ , ### $P<0.001$ .

Our results are consistent with data from the literature demonstrating that the initial response to BLM instillation is the induction of an inflammatory response characterized by gross perivascular infiltration and edema [4,6]. The major fibro-proliferative phase induced by BLM in rats and mice occurs within the first week following its administration and this process co-exists with inflammation [3,5]. The initial inflammatory reaction typically resolves at approximately 14-18 days after administration of BLM [6], and this explains the reduction in MRI signal volume observed here between 1 and 3 weeks following BLM.

The main finding of the present work was the ability of MRI to detect the presence of signals, prominent at the level of the hilus and apparent through 10 weeks after BLM. Consistent with the histological analysis and the literature, these signals appear to represent the development of bronchiectasis, a type of tissue remodeling considered to be a general marker for fibrosis [6]. Interestingly, similar findings have been obtained using micro-CT in another rat model of lung fibrosis [1]. Thus, MRI becomes an interesting non-X ray based imaging alternative for the study of experimental fibrosis and for the *in vivo* investigation of anti-fibrotic compounds. Moreover, the MR images from BLM-treated rats strikingly resemble MRI examinations of cystic fibrosis patients [2], revealing bronchiectasis at the hilus level. Similarities among these images suggest the translational value of the technique.

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