Elastase induced emphysematous development assessed by proton MRI in spontaneously breathing rats

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Introduction:

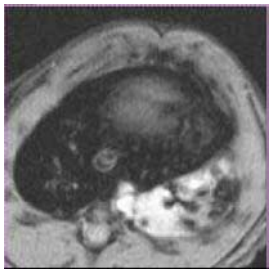
Emphysema is characterized by airspace enlargement in the lung parenchyma as a result of macrophage activation inducing the release of elastase. Measurements of diffusion of hyperpolarized ^{3}He have been used to detect emphysema in human lungs (1) and in rat models (2). All-trans-retinoic acid (ATRA) has been shown to reverse PPE induced emphysema in Sprague-Dawley rats (3). Our aim was to assess whether experimental emphysema could be detected non-invasively by proton MRI and if treatment with ATRA was able to reverse the changes induced in the model.

Methods:

Animals: Male Brown Norway (BN) rats were treated with a single dose of porcine pancreatic elastase (PPE; 75U/100g body weight) or saline and imaged at 4.7 T prior to (baseline), and at 24 h, 2, 4, 6, and 8 weeks after challenge. Histological analysis to determine edema and morphological changes were carried out at all time points. To determine whether ATRA could reverse the damage induced by PPE, rats were treated with ATRA (500 µg/kg i.p.) or its vehicle (triglyceride oil) starting on day 21 after elastase administration and for 12 days thereafter. In this second experiment imaging took place prior to (baseline) and 14, 21, 28, and 32 days after PPE. Histological analysis to assess morphological changes was performed at the end of the study.

MRI: Rats were anaesthetized with forene (1.5-2.0%) in a mixture of O_2/N_2O (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 either modulations of parenchymal signals (TR = 2 ms; TE = 0.55 ms; FOV = 6x6 cm^2; matrix = 36x128; slice = 2 mm; 80 image averages with an interval of 500 ms between each image acquisition) or fluid signals (TR = 5.6 ms; TE = 2.7 ms; FOV = 6x6 cm^2; matrix = 256x128; slice = 1.5 mm; 45 image averages with an interval of 530 ms between each image acquisition) induced by PPE. Neither cardiac nor respiratory triggering was applied, and rats respired spontaneously. For details of image acquisition, see (4,5).

Results and Discussion:



Edematous signals were detected by MRI 24 h after PPE. Areas of very low signal intensity, as a result of haemorrhage (6), were present among the fluid signals following PPE (fig. 1). The fluid signals resolved completely two weeks later.

Fig. 1 – *Transversal section through the chest of a rat treated with PPE and imaged 24 h after. Histology confirmed the presence of haemorrhage at this time point.*

As early as two weeks after enzyme administration, once the acute edematous response had subsided, significant reductions of parenchymal signal intensity (consistent with air trapping, fig 2.) and increases in lung volume, were detected (fig. 3). For all regions analysed, no significant differences were found when signal intensities 2, 4, 6 and 8 weeks after elastase administration were compared between each other. Changes in the MRI parenchymal signal intensity and lung volume remained uniform throughout the study. Histology revealed a considerable reduction in the total number of alveoli and a marked increase in the size of individual alveoli and bronchi. The extent of emphysematous development at 2, 4 and 8 weeks did not differ significantly from each other. The data suggest that, in the BN rat, emphysema is induced early following instillation of the enzyme. They agree with observations made in rabbits showing that changes in lung function and morphology occurred 24 h after challenge and persisted for 8 weeks (7). ATRA did not cause a reversal of elastase induced damage in MRI or histological parameters (fig. 3).

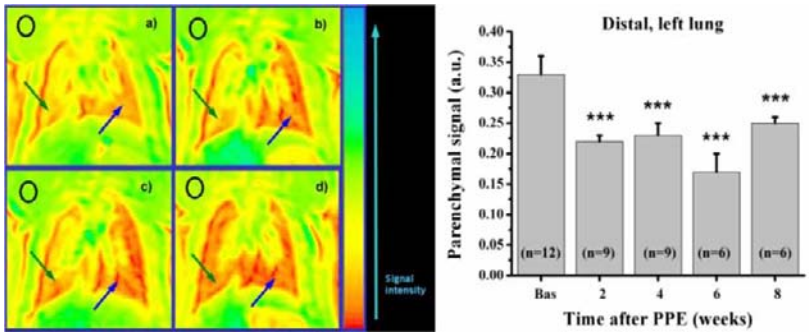


Fig. 2 – *Coronal sections from one rat, acquired at* **(a)** *baseline and* **(b)** *2 weeks,* **(c)** *4 weeks and* **(d)** *8 weeks after PPE. The parenchymal signal (mean ± SEM) for the number n of rats specified was significantly decreased in several regions of the lungs (arrows) The levels of significance (**0.001<p<0.01, *** p<0.001) refer to Anova comparisons with respect to baseline values.*

The increase of air spaces and the destruction of elastic fibres by PPE, compromising the elastic recoil of the lung, result in severe impairment of gas exchange that leads to air entrapment and a local increase of molecular oxygen in the enlarged alveoli, which does not diffuse into the lungs (8). It is believed that these morphological and physiological alterations contributed to the decline in MR signal from the parenchyma following PPE administration. Both MRI and histological parameters of emphysematous development showed that ATRA had no effect in the present model in BN rats.

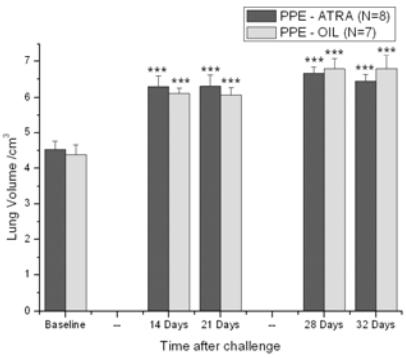


Fig. 3 –*Changes in lung volume assessed by MRI (cm^3, mean ± SEM) between ATRA treated animals and those receiving vehicle (triglyceride oil) The levels of significance (*** p<0.001) refer to Anova comparisons comparisons with respect to baseline values.*

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