Longitudinal Quantitation Of Ozone Induced Pulmonary Oedema And Lung Volume Changes By MRI

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Synopsis: The aim of this study was to evaluate ozone induced pulmonary oedema using proton MRI. Animals were acutely challenged with ozone and imaged up to 32 days post challenge. At 24 h, large oedematous areas were measured together with corresponding increases in lung volume. Over time the oedematous signal reduced and was replaced by a fibrous looking signal within the pulmonary cavity. Longitudinal assessment of ozone exposed animals may provide important information regarding oxidative-stress induced lung injury and inflammation.

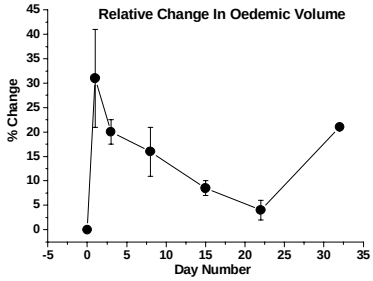
Introduction: Chronic obstructive pulmonary disease (COPD) is a slowly progressing disorder characterised by chronic, poorly reversible, lung airflow obstruction. COPD is believed to be a chronic inflammatory process which produces characteristic lung pathology such as increased mucous production, fibrosis and emphysema. Approximately 80 – 90% of all COPD suferers are smokers as cigarette smoke is a rich source of harmful oxidants to the lung [1]. However, environmental pollutants such as petrochemical car emissions are also thought to be a key factor in contributing to progressive lung disorders and exacerbations. Ozone and nitrogen dioxide are the two most common air pollutants and cause a variety of clinical symptoms ranging from shortness of breath, decreased lung capacity, reduced membrane permeability, as well as airway diffusion deficits and epithelial airway remodelling. On a cellular level, ozone can produce an oedematous inflammatory responses together with production of pro-inflammatory cytokines, neutrophilia [2,3]. Animal studies incorporating ozone exposure provide a model for studying some of the aspects of oxidative-stress induced pulmonary inflammation . In the current study we have characterised the effects of an acute ozone challenge in the rat using MRI. MRI allows longitudinal evaluation of the same animal over a period of 32 days in which changes associated with pre- and post inflammatory processes may be identified.

Method: Animals were housed and maintained and procedures conducted in accordance with the Home Office Animals (Scientific Procedures) Act 1986, UK. Six female Lewis rats were exposed to 3 ppm ozone for a total of 6 h. The ozone chamber consisted of an ozoniser, silica filter, chamber fan, air pump and charcoal scavenger. Two separate sensors mounted independently in the chamber monitored ozone levels. Following exposure animals were imaged on Day 1 (24 h post exposure), 3, 8, 15, 22 and 32. Animals were anaesthetised using isoflurane and the free breathing animals were immediately placed supine in a 2T Bruker Medspec S200, 96 cm bore system, in a 55 mm purpose built coil inside a BGA-12 gradient insert. Animals were homeostatically maintained and respiratory rate monitored under 2% isoflurane anaesthesia (flow rate of 800 ml/min O_2). 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. MRI: Pilot scans were conducted to accurately line up a slice pack of 12 slices (1.5 mm) with anatomical landmarks and online measurements. A multislice (128x128; 6x6 cm^2) respiratory triggered RARE sequence, 8 echoes, inter-echo time 4.2 ms (TE_{eff} 8.4) with a TR of 1 s and 16 averages provided good lung parenchymal visualisation within 6 minutes. Image Analysis Images of lung and oedema were segmented using thresholding and contour following procedures. The thresholds for identifying voxels associated with the lung, and oedema, were computed by placing 6 ROIs of approx 1.2 mm radius in homogeneous areas within the image. Baseline subtracted image thresholds were a function of the mean and SD intensity values. After thresholding, a contour following algorithm was applied to extract lung and oedema boundaries resulting in lung and oedemic volumes (as well as mean and SD intensity of voxels associated with oedema for separate analysis).

Results and Discussion:

Following exposure to ozone all animals showed large bright areas associated with pulmonary oedema within the lung (see Figure 1). Inflammation was widespread and distal, as opposed to that seen after intratracheal dosing, which results in variable and more proximal responses [4]. Over time these intense areas reduced as shown in Graph 1; the early inflammatory process produced a 30% increase ($P < 0.01$) in pulmonary oedema volume (with respect to total lung volume) at 24 h, which was clearly quantifiable by MRI. As time progressed the relativele hyperintensity within the lung decreased and by 15 - 22 days had returned to almost pre-ozone challenge values. Interestingly, by Day 32 distinct increases in lung parenchyma intensity were measured i.e. 21.4% compared with 5% on day 22. The increase in signal intensity may reflect later inflammatory activities or some component of lung remodelling and this phenomenon warrants further investigation. Measurements of lung volume change over the same time points show an increase of approximately 35% ($P < 0.01$) at 24 h (Graph 2), corresponding with the 30% increase in percentage pulmonary oedema volume with respect to total lung volume (Graph 1). Following this on Day 3 and at all subsequent time points, lung volume returned to pre-ozone challenge values. Increases in lung volume indicate ventilatory mechanisms compensating for the depletion in viable airspace due to the inflammatory fluid influx. As pulmonary oedema recedes the compensatory lung volume returns to baseline.

Graph 1



Graph 2

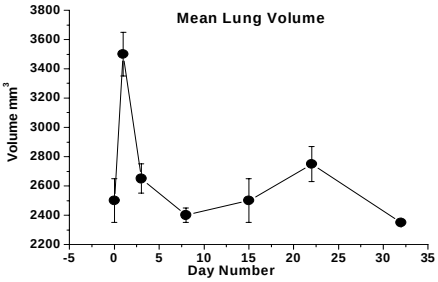
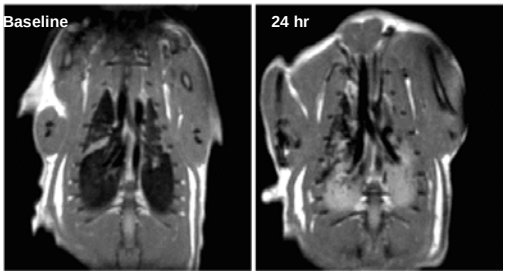


Figure 1



Conclusion:

Ozone-induced pulmonary inflammation can be longitudinally quantified using proton MRI. This may allow the various aspects of inflammatory processes and changes in the lungs to be identified and measured within the same animal. Serial MRI assessments may therefore guide therapy targetted at the various disease stages, and could provide an important tool in the assessment of pharmacological agents for the treatment of inflammatory lung diseases.

References:

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