The use of MRI to track the migration of labelled neutrophils to lung inflammation in the rat.

S. Hotee[1], K. Changani[2], A. White[2], K. Bhakoo[3], J. Bell[1]

[1]Molecular Imaging Group, Imaging Sciences Department, MRC Clinical Sciences Centre, London, United Kingdom, [2]GlaxoSmithKline PLC, Welwyn Garden City, United Kingdom, [3]Stem Cell Imaging, Imaging Sciences Department, MRC Clinical Sciences Centre, London, United Kingdom

Background

Neutrophils are known to cause host tissue damage and are reported to be involved in a spectra of human chronic lung diseases, which involve tissue damage and inappropriate tissue repair, such as acute respiratory distress syndrome[1], emphesema[2] and chronic obstructive pulmonary disease[3]. Lipopolysaccaride (LPS) is used to produce lung inflammation in the rat[4] and is known to initially induce a mainly neutrophilic response. The objective of this study was to develop methods to track the migration of neutrophils, using magnetic resonance imaging, to an area of inflammation in the lung. Here we investigated the effects of magnetic-iron-oxide-nanoparticle coated with secondary antibody (microbead) labelled neutrophils on the intensity of lung oedema in the rat.

Methods

Neutrophil isolation and labelling*:* Neutrophils were purified from rat bone marrow using a modified discontinuous Percoll gradient protocol[5]. Neutrophils were removed and labelled with granulocyte specific RP1 anitbody at manufacturers (BD Biosciences) instructions for 15 minutes. Secondary labelling with microbead was performed using manufacturer recommended concentrations (MACS) for 1 hour. Cells were washed thrice before use.

LPS administration: Lung inflammation was induced in 18 female Sprague Dawley rats (200g-250g) with LPS i.t. (400µg/kg) and oedema was allowed to develop for five hours.

MRI imaging: Animals were then scanned continuously by MRI (Bruker Biospec 7T horizontal bore) to determine when the oedema content of the lung had stabilised. After administration of neutrophils (labelled or unlabelled), animals were scanned up to 140 minutes thereafter, using a snap-shot flash sequence (FOV 6 x 6cm, slice thickness 1mm, TR/TE 10/1.1, 16 averages, receiver gain 5000, 15 slices, 6 min scan time)

Cells administration: Labelled and unlabeled neutrophils were administered after oedema stabilisation via a tail vein cannulation. Animal remained unmoved in the magnet. Animals received 300µl PBS plus either unlabelled neutrophils (n=6), microbead-labelled neutrophils (n=6) or microbead alone (2µg/300µl). The cannula was flushed with an extra 300µl saline to ensure no residual cells.

Image analysis: Images were acquired and analysed using Paravision software. Signal intensity of the regions of interest (reduced signal) were quantified relative to the baseline images (before cell administration).

Results

LPS induces MRI visible oedema which, although generally homogeneous, had regions of both low and high signal intensity (figure 1a). These areas of heterogeneity may arise from variable increases in fluid content of the oedemic regions. Administration of unlabelled-neutrophil showed no significant changes in signal intensity during the time course of the experiment (figure 2). In labelled-neutrophil treated animals, areas of low intensity developed through the course of the study and remain at fixed locations (figure 1). Temporal measurements show a consistent decrease in intensity in these regions (figure 2), which is significantly different to the response measured for unlabelled neutrophils ($P>0.05$).

Conclusions

There is significant signal intensity reduction in oedemic regions of the lung upon administration of microbead labelled-neutrophils compared to unlabelled neutrophils. This suggests that MRI can be used to monitor the migration of microbead-labelled neutrophils to inflamed regions of the lung in a quantitative and reproducible manner. Further work is underway to determine the temporal and regional characteristics of neutrophil migration within the areas of lung inflammation.

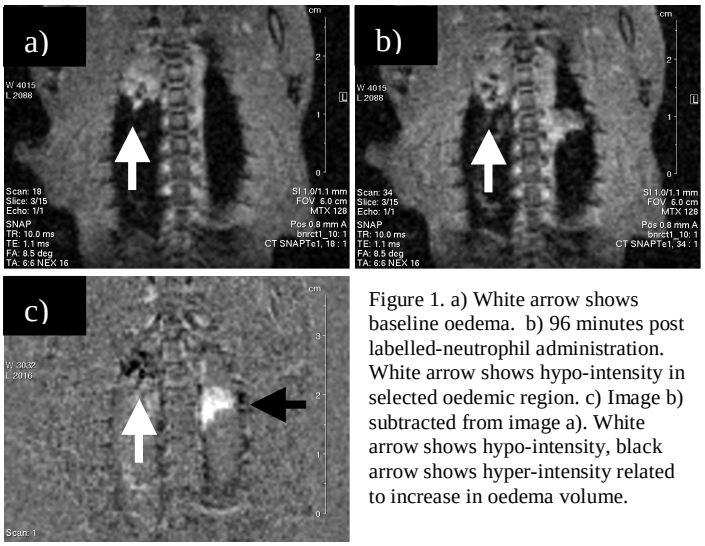

Figure 1. a) White arrow shows baseline oedema. b) 96 minutes post labelled-neutrophil administration. White arrow shows hypo-intensity in selected oedemic region. c) Image b) subtracted from image a). White arrow shows hypo-intensity, black arrow shows hyper-intensity related to increase in oedema volume.

The effect of microbead-labelled neutrophils on oedema intensity

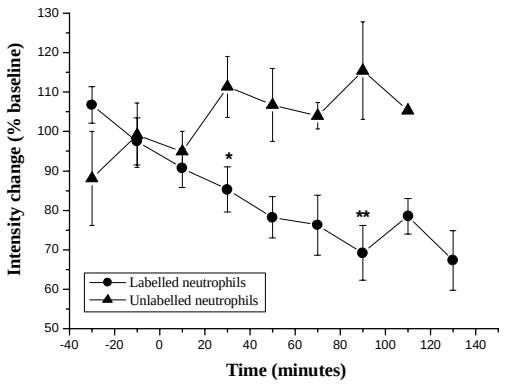

Figure 2. Intensity measured as % intensity of baseline scan (before cell administration) ±se. Labelled or unlabelled cells administered in 300µl PBS at time 0 and flushed with 300µl saline.

References

1) Ognibene FP *et al.* N Engl J Med 1986. **315**(9):547-51.
2) Betsuyaku T *et al.* Chest 2000. **117**(5):302S-3S.
3) Sethi S Chest 2000. **117** (5):286S-291S.
4) Changani et al Proc ISMRM 2002 p 1986
5) Sugawara T *et al*. J Pharmacol Toxicol Methods 1995. **33**(2):91-100.