Vascular remodelling in the rat lung detected non-invasively by MRI in a model of chronic inflammation induced by repeated allergen administration

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Introduction:

Remodelling of the airway wall is a distinctive feature of asthma, characterized by hypertrophy and hyperplasia of airway smooth muscle, increase in mucous glands, thickening of the reticular basement membrane, and qualitative and quantitative changes of airway blood vessels (1,2). Despite increasing interest in the understanding and characterization of vasculature remodelling associated with chronic airway inflammation, studies using MRI approaches for the analysis of this feature are yet rare. Our specific aims were *(i)* to develop an experimental method for the non-invasive analysis of vascular remodelling associated with chronic airway inflammation; and *(ii)* to characterize vascular remodelling by histology.

Methods:

Challenge: Male Brown Norway rats weighing ca. 250 g were actively sensitised to OA as previously described (3), and divided into two groups. The first group was challenged intra-tracheally (i.t.) once with OA and killed 24 h later for histology. Animals from the second group were challenged four times with OA at 0, 96, 192 and 288 h and killed 24 h after the last challenge for histological analysis. Additional animals were used as controls and subjected to either single or repeated vehicle challenge.

MRI: Measurements were carried out at 4.7 T on a Bruker Biospec system. A gradient-echo sequence and the following parameters were used: FOV 6x10 cm^2; matrix 64x128; slice 6 mm; TR 2.21 ms; TE 0.62 ms; 28 averages. The acquisition time per image was of 7.9 s. Twenty images were acquired sequentially. Immediately after acquiring the 10^{th} image, a bolus of Gd-DOTA (Dotarem diluted 1:16, 0.5 ml/kg) was injected i.v. The permeability surface-area product (fig. 1) was estimated from the initial slope of the tracer uptake curves (4). All measurements were performed on spontaneously breathing rats.

Histology: Immediately after MRI acquisitions, rats were killed by an overdose of pentobarbital. Lungs were fixed by slow *in-situ* inflation with 5 ml of 10 % phosphate-buffered neutral formalin (BNF) via a tracheal cannula. After removal from the thorax, lungs remained in BNF for a maximum of 72 h. Following fixation, lungs were trimmed and sections cut longitudinally through the left lobe and transversally through the right bottom lobe so as to include the main bronchi as well as the pulmonary alveoli. After dehydration and processing to paraffin wax, 3-µm-slices of each section were stained with Hematoxylin/Eosin for the counting of vessels and with Acid Fuchsin Orange G to assess the thickness of the pulmonary arteries.

Results and Discussion:

The permeability assessed from the Gd-DOTA uptake curves was significantly decreased in the proximal and distal lung regions of rats challenged 4x with OA in comparison to those challenged 4x with saline (fig. 2). In addition, no difference in permeability was detected between repeatedly vehicle-challenged rats and both singly OA- and vehicle-challenged animals. This observation was consistent with histology, which revealed that repeatedly OA-challenged animals exhibited a marked increase in vascular wall thickness compared to rats which received single or repeated vehicle administration, as well as to singly OA-treated rats (fig. 3). The number of vessels with diameters ranging from 15 to 30 µm, assessed in the lung tissue from repeatedly OVA-challenged rats, was increased when compared with lung tissue from singly treated animals. By contrast, the number of vessels of diameter larger than 30 µm was similar in all groups, independently of the treatment regimen (data not shown).

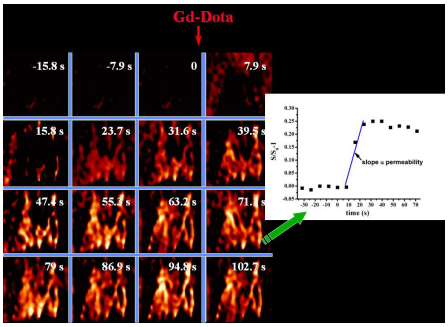

Fig. 1 *Gd-DOTA uptake (difference images)*

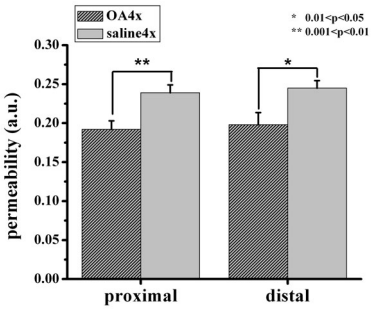

Fig. 2 *MRI permeability indices*

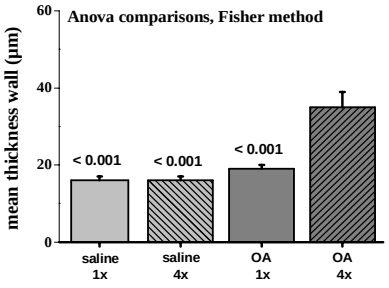

Fig. 3 *Histological assessment of wall thickness*

These data demonstrate that MRI can be used to non-invasively monitor changes in the vascular component of airway remodelling associated to chronic airway inflammation. This approach represents a suitable means of profiling drugs whose aim is to reverse both inflammatory and structural changes in asthma.

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