Near-Infrared Fluorescence Reflectance (NIRF) Imaging and Histology Confirm Anomalous Edematous Signal Distribution Detected in the Rat Lungs by MRI After Allergen Challenge

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Introduction:

MRI can be used to follow the development of an edematous signal, a key parameter of airway inflammation, in the lungs of actively sensitized Brown Norway (BN) rats challenged with OVA (1). A critical aspect of the MRI signal is its predominant location on the left anatomical side of the animals. We report on the feasibility of using NIRF imaging to detect the biodistribution of an intra-tracheally (i.t.) instilled fluorescent dye, Cy5.5, for studying the relationship between deposition of the dye and the development of pulmonary inflammation.

Methods:

Challenge: Male BN rats were sensitized to allergen and challenged with OA (0.3 mg/kg i.t.) as described in (1).

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 (TR = 5.6 ms; TE = 2.7 ms; FOV = 6x6 cm^2; matrix = 256x128; slice = 1.5 mm; 45 averages) was used throughout the study. Neither cardiac nor respiratory triggering were applied, and the rats respired freely during data collection.

NIRF: Measurements performed on isolated lungs using Cy5.5 (Amersham, Freiburg, Germany) as fluorescent dye. Excitation was accomplished with 3 laser diodes at 660 nm and a power of 10 mW/cm^2. The fluorescent light emitted from the samples was detected by a CCD camera (Hamamatsu, Schüpfen, Switzerland) equipped with a focusing lens system (macro lens 60 mm, 1:2.8, Nikon). The matrix size of the images was 532×256 pixels. A hard filter selected the wavelength at detection (700 nm). Data acquisition times ranged from 0.5 to 2.0 s. The experiment was controlled by a PC using the Siemens SYNGO® software, which was used as well for image analysis.

Histology: Verhoeff staining was applied for assessment of perivascular edema.

Results and Discussion: Fig. 1a shows an axial image from the thorax of a BN rat, 24 h after i.t. administration of OA. Despite the fact that the allergen had been instilled before the bifurcation of the trachea, strong and continuous edematous signals are prominently present at the left side of the body, a fact that was confirmed histologically (fig. 1b). Such a feature is in agreement with previous observations (2). A representative image from the extracted lungs of an animal that had received an i.t. instillation of the fluorescent dye is shown in fig. 2. Significant signal intensity differences were observed between the right and left sides of the lungs.

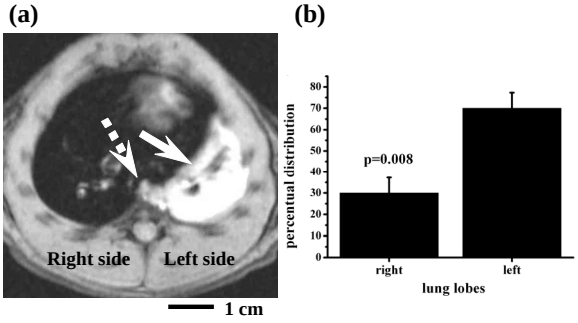

Fig. 1 – (a) *Intense edematous signal (continuous arrow) is seen prominently on the left anatomical side 24h afterOA. The signal indicated by the dashed arrow has been likely contributed by the right post-caval lobe.* **(b)** *Distribution of perivascular edema (means ± s.e.m.; n=7 animals/group) quantified by histology.*

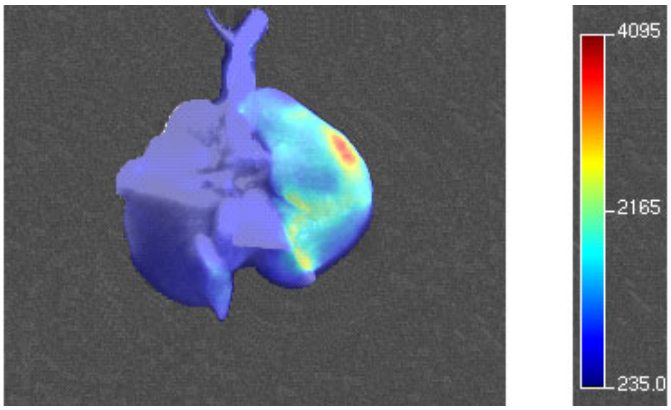

Fig. 2

The distribution of i.t. instilled particles has been shown to be determined primarily by the properties and volume of the carrier fluid rather than by the properties of the particles (3). Thus, since Cy5.5 and OA were both diluted in saline and the volume instilled was the same, it is reasonable to assume that the dye and OA were equally distributed in the different lung lobes. The difference in the distribution may be due to the inherent geometry of the bronchus of each lobe with respect to the left and right primary bronchi. Indeed, the bronchioalveolar tree is more developed in the left rat lung (4).

In summary, the distribution of an i.t. instilled fluorescent dye assessed by NIRF helped to understand the uneven development of pulmonary inflammation in the rat lungs induced by allergen challenge and detected as oedematous signal by MRI. Histological quantification of perivascular oedema confirmed that MRI primarily reflects what happens at the tissue level rather than in the lumen.

1. Beckmann N, et al. Magn Res Med 2001;45:88-95.
2.Tigani B, et al. Br J Pharmacol 2003;140:239-246.
3. Brain JD, et al. Environ Res 1976;11:13-33.
4. Yeh HC, et al. Anat Rec 1979;195:483-492.