In-vivo assessments of mucus dynamics in the lungs using a Gd-Cy5.5-bilabeled contrast agent

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Introduction:

Mucociliary clearance is an important mechanism for removing inhaled particles, secretions and cellular debris from the respiratory tract. Dysfunctions in airway clearance are associated with the accelerated loss of lung function in patients with obstructive lung diseases. In order to obtain information on mucus dynamics using MRI, we developed a Gd- and Cy5.5-bilabeled probe aimed at binding specifically to mucus. Results are reported on the application of the probe to lipopolysaccharide (LPS) challenged rats, which were additionally treated with uridine triphosphate (UTP), a mucolytic agent, or not.

Methods:

LPS challenge: Male Brown Norway (BN) rats were anesthetised with 3% isoflurane (Abbott, Cham, Switzerland) and LPS from Salmonella typhosa (Sigma, Dorset, UK; 1 mg/kg dissolved in 0.2 ml saline) was administered intra-tracheally (i.t.) and the animals allowed to recover.

Probe: The rationale for the probe synthesis resided on the fact that dextran is a polysaccharide that competes with mucous glycoproteins for hydrogen bonding sites (1). Aminodextran was labelled with Gd and Cy5.5. The probe was applied i.t. either as solution (BCR249) or as dry powder (BCR250), 24 h after LPS challenge.

Treatment: UTP (60 mM) or its vehicle (saline) was administered i.t. together with BCR249 or without the probe, at 24 h after LPS.

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 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 LPS. Neither cardiac nor respiratory triggering was applied, and rats respired spontaneously.

Histology: Immediately following an MRI session, rats were killed by an overdose of pentobarbital (250 mg/kg i.p.). After being removed from the thorax, lungs were immersed in 10% neutral formalin buffer for 72 h. Following stainings were applied to lung sections: (i) hematoxylin/eosin to assess general morphology; (ii) PAS/Alcian blue reaction to detect mucus and goblet cells. One slice was left unstained for the detection of Cy5.5 by confocal microscopy.

Near-infrared fluorescence (NIRF): NIRF images were acquired from isolated lungs, harvested immediately after an MRI session. For details, see (2).

Results and Discussion:

Figure 1a shows representative MRI transverse sections through the thoracic region of a BN rat before and at various times following LPS exposure. In agreement with previous studies (3,4), a discrete signal of weak intensity was detected by MRI at 24 h following LPS. Intra-tracheal administration of BCR250 at this time point resulted in a significant increase of MRI signals until 24 h later (Figure 1a). Histology demonstrated that the agent bound specifically to mucin that had been secreted or was present in goblet cells (Figure 1b). Similar enhancement of MRI signals was also observed when BCR249, the agent in solution form, was given 24 h post-LPS. The increased signal was still detected 24 h later and at this time point, NIRF confirmed the presence of the contrast agent in the isolated lungs from the same animal.

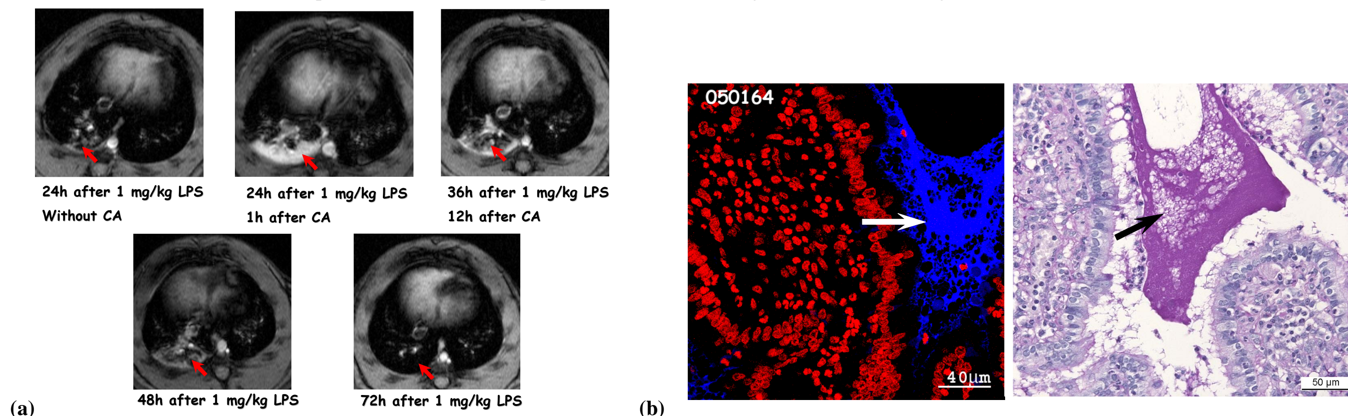

Fig. 1 – (a) *Signal increases in the lung of an LPS-challenged rat, obtained by administering BCR250 24 h after LPS.* **(b)** *Confocal microscopy (mucus: blue; nuclei: red) and PAS/Alcian blue (mucus: violet; nuclei: blue) stained sections of lungs from the same animal, demonstrating that BCR250 bound specifically to mucus.*

Data were then obtained by administering UTP or its vehicle (saline) together with the contrast agent, BCR249, at 24 h after endotoxin. The same experiment was then repeated, but without addition of contrast agent. The dynamics of labeled mucus, obtained by subtracting data collected without from data obtained after administration of contrast agent. Because BCR249 binds specifically to mucus, it can be assumed that the subtraction of fluid signals obtained without the probe from signals detected in the presence of the agent provides a means to measure the kinetics of contrast agent-labeled mucus in the lung. With this approach, it was verified that UTP had an early effect (significant 4 h after its administration) on the clearance of labeled-mucus whereas the vehicle-instilled animals demonstrated maintained probe-specific signals until 24 h. Of note, despite the acceleration of the clearance of labeled mucus by UTP illustrated by the decreased probe-specific signals, the therapeutic effect of the compound was questionable since the original course of MRI signals showed that the overall fluid content 24 h after UTP and later was the same for UTP- and vehicle-treated rats.

Our results indicate the great advantage of using MRI and a probe for labeling specifically mucus: the technique provides at the same time a global view on overall fluid dynamics, as well as specific information on the mucus dynamics. Both views may be fundamental in gaining insights into the therapeutic value of compounds aiming at influencing the dynamics of mucus in the lung.

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