Bleomycin-induced lung injury assessed non-invasively and in spontaneously breathing rats by proton MRI

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Introduction:

Bleomycin (BLM), an antibiotic that possesses chemotherapeutic properties, produces a dose-dependent pulmonary fibrosis in many patients [1]. A single intra-tracheal (i.t.) instillation of BLM is commonly used to induce experimental pulmonary fibrosis in rodents [2,3]. The antibiotic instillation causes fibrotic changes that resemble human fibrotic lung disease both histologically and physiologically. In the present study proton MRI was used to assess in male Brown Norway (BN) rats, structural changes following a single i.t. application of BLM.

Methods:

BLM: Rats were anaesthetised (4% isoflurane; Abbott, Cham, Switzerland) in a chamber and then treated with BLM hydrochloride (Euro Nippon Kayaku Gmbh, 7.5 U/ kg dissolved in 0.2 ml saline; n=56) [3] or vehicle (0.2 ml saline; n=20) administered i.t. before the bifurcation of the carina.

MRI: Rats were anaesthetized with isoflurane (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 either modulations of parenchymal signals (TR = 2 ms; TE = 0.55 ms; FOV = 6x6 cm^2; matrix = 36x128; slice = 2 mm; 80 image averages with an interval of 500 ms between each image acquisition) or 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 BLM. Neither cardiac nor respiratory gating was applied for image acquisition.

Results and Discussion:

No changes in MRI parameters (fluid volume or parenchymal signal intensity) were observed 6 and 24 h after BLM (fig. 1). Bronchoalveolar lavage (BAL) fluid analysis at 24 h following BLM revealed a significant increase of inflammatory cells, but protein concentration and eosinophil peroxidase (EPO) and myeloperoxidase (MPO), markers for eosinophil and neutrophil activities, respectively, were not changed. One week after BLM, prominent MRI signals were observed in the lung (fig. 1, left). These findings correlated with increased inflammatory cell infiltration, as well as with raised levels of protein, and EPO and MPO activities in BAL fluid. Histological evaluation of BLM-treated lungs (left and right caudal lobes) 1 week after treatment revealed gross perivascular edema, goblet cell metaplasia and severe pulmonary fibrosis. Additionally, in MR images of BLM-treated rats the heart appeared to have displaced towards the left side of the thorax. In these animals an increase in parenchymal signal intensity was observed 1 week after challenge, but as a result of the extensive edematous response observed in the lungs at this time point, such observations were excluded from the statistical analysis. For those BLM-treated rats scanned up to week 8, localized signals having a volume of approximately 0.45 ml were present in the left side of the lung from week 2 onwards; additionally, in 60% of the animals, the heart appeared to have been displaced towards the left side, as observed in rats imaged 1 week after BLM (fig. 1, right). These changes were attributed to the severe fibrosis and deterioration of the left lobe that were clearly visible with the unaided eye at necropsy 8 weeks in BLM-exposed rats. Because of this parenchymal signal intensity was assessed only in the right side of the lung. An increase in signal intensity of the parenchyma, respective to baseline levels, was observed in MR images at week 2, that declined progressively towards normal levels in the right transversal and upper right coronal regions of the lung (fig. 2). Histology of the right caudal lobe 8 weeks after BLM administration showed a clear reduction of fibrosis and evidence of alveolar wall thinning and airspace enlargement correlating with the decline in parenchymal signal intensity observed by MRI.

Our results are consistent with data from the literature demonstrating that the initial response to BLM instillation is the induction of an inflammatory response characterized by gross perivascular infiltration and edema [3,4]. The major fibro-proliferative phase induced by BLM in rats and mice occurs within the first week following its administration and this process co-exists with inflammation [3,4]. The initial inflammatory reaction typically resolves between 10 and 14 days after administration of BLM [4], and this explains the reduction in MRI signal volume observed here between 1 and 2 weeks following BLM. Furthermore, the extent of BLM induced fibrosis progressively declines over time in rats [3]. Our histological observations demonstrated a much more reduced extent of fibrotic tissue in the right lobes at 8 weeks post BLM, compared to that observed at one week after instillation of the antibiotic. In line with this, the intensity of parenchymal MRI signal in the same areas began to decline at week 3, reaching near normal levels at 8 weeks after BLM exposure. Exactly his observation points to the opportunity of exploring proton MRI as a tool to evaluate effects of therapeutic regimens in experimental models of lung fibrosis in rats.

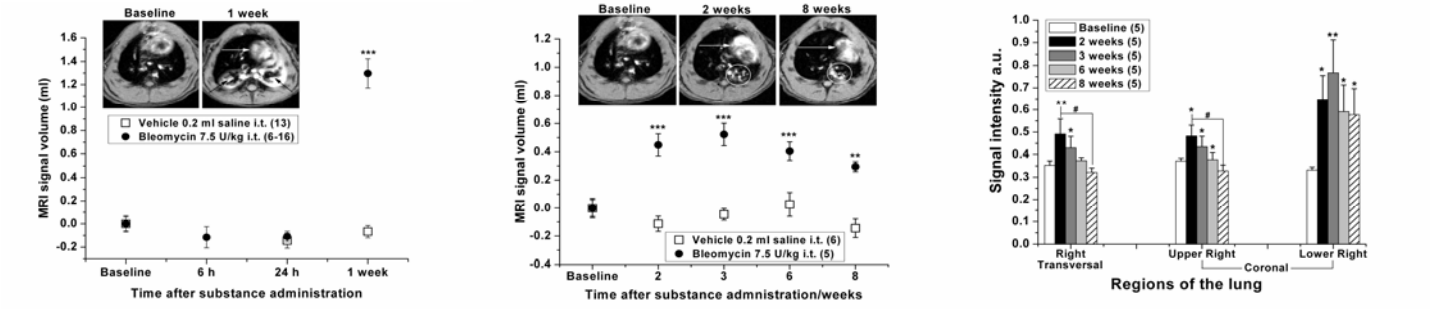

Fig. 1 – *Initial* ***(left)*** *and late* ***(middle)*** *effects of BLM.* ***(right)*** *Signal intensities (means ± SEM) of lung parenchyma normalized to muscle from coronal slices corresponding to the upper right, upper left, lower right and lower left anatomical sides.*

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