Investigation of an animal model of pulmonary fibrosis - *ex vivo* lung MRI using a perfluorocarbon compound as a contrast agent for hyperpolarized ^{129}Xe

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PURPOSE:

Idiopathic pulmonary fibrosis (IPF) is an interstitial lung disease where the gas transfer from the alveoli, across the alveolar membrane, to the red blood cells is impaired[1]. In the last decade, hyperpolarized (hp) ^{129}Xe has been increasingly used to study the gas transfer through the lung parenchyma[2,3]. The large chemical shift (200ppm) between the ^{129}Xe gas phase and the dissolved phase permits the study of the diffusing capacity in the lungs. However, the peaks associated with the ^{129}Xe in the tissue and in the blood are adjacent and can be difficult to differentiate. It has been shown that ^{129}Xe dissolved in perfluorocarbon (PFC) emulsion resonates at 106ppm[4]. In this study, a PFC is used as a ^{129}Xe contrast agent. The replacement of the blood by the PFC in the lungs will therefore aid the discrimination of the blood peak from the tissue peak and allow selective excitation. To obtain first insights without additional animal suffering and regulatory approval, excised rodent lung imaging has been validated and used to study excised healthy rodent lungs with hp ^{129}Xe [5] and hp ^{83}Kr [6], allowing for the imaging of the lungs prior to histological studies. Gas phase hp ^{129}Xe imaging has been performed to compare the ventilation in control and fibrotic *ex vivo* lungs. Hp ^{129}Xe dissolved in the PFC is used as a probe to study the diffusion kinetics from the alveolar space, through the tissue, to the vasculature in fibrotic and control lungs.

METHODS:

An *ex vivo* rat model of fibrosis was produced by oropharyngeal delivery of bleomycin (500U in 250uL saline 0.9%) to the fibrotic rats or 250uL saline 0.9% to the control rats. Bleomycin is a chemotherapeutic antibiotic well knows for its adverse effect of activating fibroblasts and subsequently inducing fibrosis[7]. The animal lungs were subsequently excised 21 days after treatment with the blood replaced post-mortem by PFC. The whole organ was then suspended in a custom-built ventilation chamber filled with isotonic fluid[5]. Hp ^{129}Xe was produced by batch mode low pressure spin exchange optical pumping (SEOP), recompressed to atmospheric pressure with a piston chamber and delivered to the lungs[8]. Hp ^{129}Xe images were acquired using a 9.4T Bruker Avance III and a custom-built 25 mm birdcage coil tuned to the ^{129}Xe resonance frequency. Ventilation imaging was performed using a Variable Flip Angle (VFA) FLASH sequence (Matrix=128×64, FOV=33.1×47.0 mm^2). Slice selective coronal images (4mm slice thickness) at three locations and non-slice selective images were acquired for each gas delivery to the lungs. Spectroscopy was performed by applying a selective sinc pulse (785us) on the PFC peak and averaged 64 times with a range of TRs (0.01, 0.05, 0.10, 0.20, 0.40, 0.60, 0.80, 1.00s).

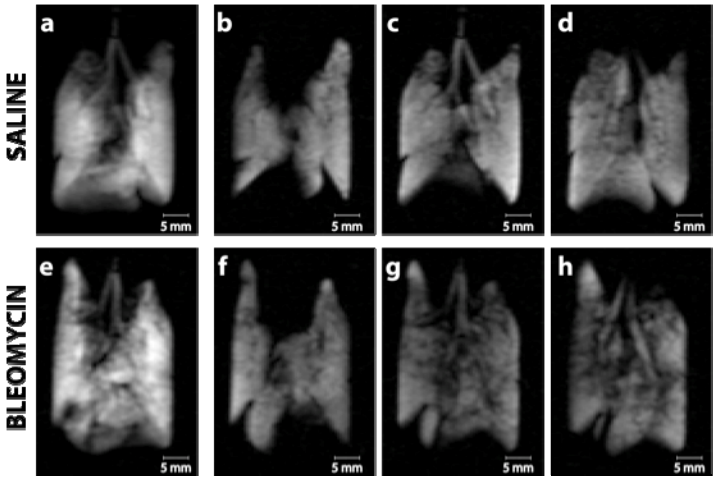

Figure 1: Non-slice selective image (a and e) and three slice selective images (b-d and f-h) in a saline-treated rat and a bleomycin-treated rat.

RESULTS:

Non-slice selective hp ^{129}Xe gas phase images of saline and bleomycin-treated lungs are displayed in **Fig. 1a and e**. Three slice-selective images are displayed for each lung (**Fig. 1b-d and f-h**). Hp ^{129}Xe dissolved in degassed PFC shows a peak at 92ppm whereas the tissue peak is located at 197ppm. The PFC phase has a T_1 = 97.10±13.46s (*in vitro* measurement, n=4). This long T_1 allows us to perform chemical shift spectroscopy on the *ex vivo* lungs. **Fig. 2a** shows an example of the integrated hp ^{129}Xe signal in PFC at different TRs. **Fig. 2b** shows an illustration of a series of spectra (64 averages), acquired with the different TRs.

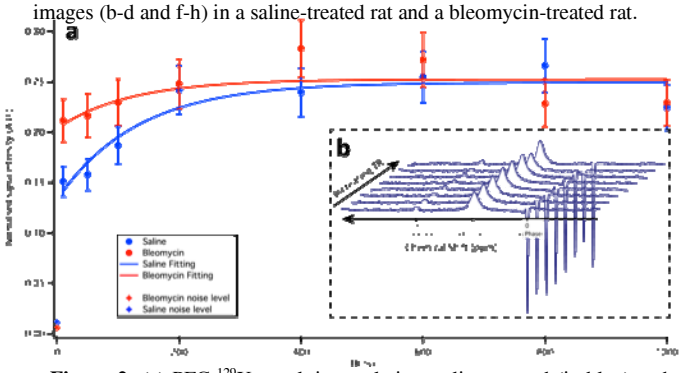

Figure 2: (a) PFC ^{129}Xe peak integrals in a saline-treated (in blue) and a bleomycin-treated rats (in red) and an illustration of a set of chemical shift selective spectra acquired on a bleomycin-treated rat with increasing TR (64 averages) and their respective noise levels.

DISCUSSION:

Fibrotic lungs show ventilation defects (**Fig. 1**), mostly located around the main airways as expected with the bleomycin model of fibrosis, confirming disease induction in the animals. Spectroscopy of hp ^{129}Xe in PFC (**Fig. 2**) allows for a quantitative comparison of the bleomycin-treated lung and the saline-treated lung. The diffusion in the bleomycin-treated lung from the tissue to the vasculature appears to be faster than in the saline-treated lung in agreement with previous reports[9]. Respective curve fits are displayed in **Fig. 2a**. The selective destruction of the PFC signal preserves the tissue and gas phase signal, which can then provide insight into the tissue-blood diffusion properties. The method enables exchange measurement of hp ^{129}Xe diffusion from the gas to the tissue and the vascular phases but also passage of hp ^{129}Xe from the tissue to the vascular liquid. The relaxivity of hp ^{129}Xe in PFC ($R_1 = 0.097788 + 0.002976 \times C_{O_2}$) will permit the mixing of hp gas with oxygen before inhalation for preclinical *in vivo* studies, however, the minimum volume of PFC to be injected has yet to be determined for sufficient contrast in the lungs.

CONCLUSION:

Ex vivo lung imaging of fibrotic lungs demonstrates a faster diffusion from the tissue to the capillaries compared to control lungs, in accordance with previously published work[9]. The difficulty of identifying the hp ^{129}Xe signal from the tissue phase and the red blood cells phase raises first insights in the usefulness of PFC as a contrast agent for ^{129}Xe dissolved phase spectroscopy or imaging. These results with PFC will be used to design *in vivo* protocols.

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