The Development of Micro-CT for Imaging and Measuring Experimental Pulmonary Fibrosis

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Aims

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive lung disease for which there is currently no effective treatment. After instillation of Bleomycin into the mouse lung fibrosis develops, which models pulmonary fibrosis, and may be used to test new therapies. The ability to view and measure structures in 3D throughout the lung, and the use of CT analysis in the clinic, has led us to test micro-CT analysis of fibrosis in this model, as an alternative to histological or biochemical measurement of collagen deposition.

Method

Bleomycin or saline was instilled into the mouse lung through either the intra-tracheal or oropharyngeal routes. Four days to 3 months later small groups of 1-3 mice were killed at different timepoints and the lungs prepared for ex-vivo micro-CT scanning.

The lungs were fixed by inflation with paraformaldehyde fixative. They were then dehydrated and chemically dried for micro-CT scanning using a method kindly given to us by Jeroen Hostens, SkyScan. The lungs were enclosed in a tightly fitting expanded polystyrene plastic container and scanned in the SkyScan 1072 scanner at 40kV 100mA without filtration and with 11µm voxel resolution, and 1,100 sections per lung were reconstructed with the SkyScan NRecon software.

For the analysis of the micro-CT sections we tested a new method using pattern recognition to identify different structures within the images without having to define regions of interest. Inform software (CRI Inc.) was trained to recognize fibrotic and normal lung parenchyma, large airways and section background, by defining a few training regions in 3 sections from each lung. After checking the accuracy of the segmentation, the trained software was used to automatically find and measure the volume and density of fibrotic and normal lung tissue throughout all the sections of all lungs in the study.

Results

The fibrotic lung showed large regions of increased density around bronchioles (arrowed in the example micro-CT sections shown below) and in the peripheral lung parenchyma, and these dense regions correlated well with mature collagen staining by histology. They often also contained enlarged airspaces, particularly at 3 months after Bleomycin. In contrast the only dense structures found in the saline control lung were airway walls and vessels.
Example micro-CT sections at mid-lung level, 21 days after Bleomycin or saline. The 3D images show fibrosis located mainly in the central lung bordering the larger airways with smaller patches of fibrosis in the periphery of the lung, which models the focal distribution found in the clinic.
Measurements of relative volume of dense fibrotic lung tissue taken at different times after Bleomycin instillation into the lung (above) showed that fibrosis developed rapidly and persists through 3 months after bleomycin in 20% of the total lung volume, without resolution, in contrast to the common perception of this as a resolving model. A large window of measurement was found between the bleomycin and control lungs at the 21 days and 3 months timepoints.

**Conclusion**
These initial pilot studies showed that micro-CT followed by pattern recognition is a good method for imaging and objective measurement of fibrosis. The micro-CT analysis of fibrosis has been so successful that we have been able to justify the purchase of an in-vivo micro-CT scanner which will refine the experiments by allowing longitudinal studies of the effects of therapy and reduce animal numbers. Experiments are now underway using micro-CT to measure the effects of therapeutic compounds and also to compare micro-CT measurements with those made by the gold standard hydroxyproline assay for collagen.