Prophylactic treatment with ALK5 inhibitor SB525334 significantly improves lung function tests and reduces accumulation of myofibroblasts in the mouse model of bleomycin induced pulmonary fibrosis

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Evaluation of novel methodologies in the model of bleomycin induced pulmonary fibrosis through functional and histological readouts may improve its usefulness and clinical predictability. Therefore, the efficacy of a small molecule inhibitor of transforming growth factor-β receptor (TGF-βR)-1/activin receptor-like kinase (ALK5) SB525334 was validated in a prophylactic dosing regimen combining lung function measurements and histopathological evaluation of lung tissue from the same animals at 9 and 14 days post bleomycin challenge.

Pulmonary fibrosis was induced in C57BL/6 male mice by the intranasal application of bleomycin. An ALK5 inhibitor was given orally at a dose of 30 mg/kg twice daily for 15 days, starting prior to bleomycin challenge (day 0). Pulmonary function tests (PFT) were performed on days 9 and 15 using forced pulmonary manoeuvres system BUXCO® DSI. Histopathological evaluation was performed by modified Ashcroft score (Ashcroft, 1988; Matsuse, 1999). The presence of myofibroblasts was evaluated by immunohistochemical staining of alpha smooth muscle actin (aSMA) (Liebler, 1998) and de novo collagen deposition was evaluated using a Fidelta internal scoring on collagen-I (CO1A1) stained slides. Statistical analysis was done using Mann-Whitney test.

Bleomycin challenge induced significant decrease of forced vital capacity (FVC) and forced expiratory volume (FEV) on days 9 and 15 (32-35%). Prophylactic treatment with the ALK5 inhibitor improved FVC and significantly increased FEV on day 9. Statistically significant effects were observed on day 15, on both FVC and FEV in comparison to vehicle controls. Furthermore, lung weights were increased on day 9 due to widespread inflammation, accompanied by accumulation of aSMA positive myofibroblasts and mild deposition of collagen into the extracellular matrix (ECM). At day 15, the fibrosis was further developed with pulmonary structure distorted by the collagen deposits. Active deposition of collagen into ECM was highlighted by groups of large myofibroblasts surrounded by collagen. The ALK5 inhibitor reduced accumulation of myofibroblasts and Ashcroft scores already on day 9, but these effects became statistically significant on day 15, when the fibrosis was fully established. The lung functional tests were similar at both time points.

This study showed that prophylactic treatment with an ALK5 inhibitor significantly improves FEV and FVC and in parallel reduces Ashcroft scores and decreases the number of myofibroblasts within the lungs. These effects could be a consequence of inhibition of epithelial mesenchymal transdifferentiation, as well as commitment and differentiation of aSMA myofibroblasts.