Dabigatran etexilate, an oral direct thrombin inhibitor, represses fibrotic changes in a murine model of pulmonary fibrosis

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RATIONALE: Activation of the coagulation cascade and generation of thrombin has been extensively documented in pulmonary fibrosis, both in acute and chronic lung injury, and in animal models of lung injury and fibrosis. The oral direct thrombin inhibitor (DTI), dabigatran etexilate, modulates the coagulation cascade and inhibits thrombin-induced profibrotic signaling in lung fibroblasts. This study tested whether thrombin inhibition by dabigatran etexilate attenuates bleomycin-induced pulmonary fibrosis in a murine model of lung injury.

METHODS: Lung injury was induced in 6-8 week old female C57BL/6 mice by intratracheal instillation of bleomycin. Dabigatran etexilate was given as supplemented chow (10 mg/g chow) or as matching placebo beginning on day 8 following bleomycin. Two and three weeks after bleomycin instillation mice were euthanized, and lungs, bronchoalveolar lavage fluid (BALF) and plasma were collected. Lung collagen was measured by hydroxyproline assays; dabigatran concentration by LC-MS/MS; thrombin activity by fluorometric assays; TGF-β1 concentrations by ELISA; connective tissue growth factor (CTGF) and smooth muscle α-actin (α-SMA) were assessed by immunoblotting. The association between hydroxyproline levels in lung tissue and dabigatran concentration in plasma was tested using Spearman rank correlation test.

RESULTS: In BALF we observed significant reduction of active thrombin and TGF-β1 from 46.0519.4ng/ml and 54.9±6.1pg/ml in bleomycin-placebo-treated mice to 11.95±4.4ng/ml ($p< 0.001$) and 31.144±8.7pg/ml ($p<0.01$) respectively in bleomycin-dabigatran etexilate-treated mice. Dabigatran treatment was also associated with two-fold decrease in the absolute number of cells in BALF of bleomycin-treated mice. A quantitative evaluation of histopathology by Ashcroft scale demonstrated a significant decrease in fibrosis of dabigatran-treated mice (5.76±1.64 vs. 2.98±0.88, $p<0.05$). A strong negative correlation between hydroxyproline levels in lung tissue and dabigatran concentration in plasma was observed in mice with bleomycin-induced lung fibrosis ($R=-0.96$, $p=0.0005$). There was no correlation between hydroxyproline and dabigatran in control sham-injured mice. Additionally, dabigatran reduced CTGF 9-fold and α-SMA 2.5-fold in mice with bleomycin-induced lung fibrosis, whereas it did not interfere with basal levels of the proteins.

CONCLUSIONS : Inhibition of thrombin using the oral DTI dabigatran etexilate has marked anti-fibrotic effects in a bleomycin-induced mouse model of pulmonary fibrosis. Dabigatran etexilate treatment reduces collagens, TGF-β1, CTGF, and α-SMA induced

by tissue injury, while not interfering with basal levels of these proteins in normal lung tissue. Our data suggest that dabigatran etexilate may be beneficial in the treatment of fibrosing lung diseases, e.g. scleroderma lung disease and idiopathic pulmonary fibrosis.