Endothelin converting enzyme-1 genetic inhibition prevents bleomycin-induced pulmonary fibrosis in mice

Anggoro B. Hartopo[1], Nur Arfian[1], Dwi A.A. Nugrahaningsih[1], Nicolas Vignon-Zellweger[2], Susi Heiden[2], Kazuhiko Nakayama[2], Keiko Yagi[2], Ken-ichi Hirata[1], Noriaki Emoto[1,2]. [1]Kobe University Graduate School of Medicine, Division of Cardiovascular Medicine, Department of Internal Medicine, Kobe, Japan, [2]Kobe Pharmaceutical University, Department of Clinical Pharmacy, Kobe, Japan.

Progressive fibro-proliferation and excessive extra cellular matrix remodelling are prominent processes in pulmonary fibrosis which culminate to respiratory failure and death. Endothelin system is activated during these processes and promotes profibrotic state. Endothelin converting enzyme-1 (ECE-1) is a key enzyme to produce mature and active endothelin-1 (ET-1) from its precursor, proET-1. In the lung, ECE-1 exerts its role in various cells: endothelial, epithelial and mesenchymal cells as well as alveolar macrophages. These cells contribute in pulmonary injury and fibrosis. To elucidate whether ECE-1 inhibition has advantageous role in pulmonary fibrosis, thus provide its therapeutic effect on this uncured disease, we conducted an experimental study using genetically disrupted ECE-1 mice. Pulmonary fibrosis was induced by instilling bleomycin sulphate (10 μg/g body weight) intratracheally into the lungs of 8- to 10-week-old male ECE-1 heterozygous knockout mice (ECE-$1^{+/-}$) and their wild type (ECE-$1^{+/+}$) littermates (Sv129 background) under general anaesthesia (intraperitoneal pentobarbital). We assessed the fibrotic response in both mice within serial time points: 7, 14, 21 and 28 days after bleomycin instillation. Comparisons and statistical significances were tested with one way ANOVA and Fisher's least significant difference post-hoc analysis. Differences were statistically significant at $p<0.05$. The result of our study showed that after 28 days, the peak time of fibrotic response, ECE-$1^{+/-}$ mice exhibited less pulmonary collagen content (51.5±2.1 vs. 66±2.5 μg, $p<0.05$, n=5 each) and fraction fibrotic area (16.5±1.1 vs. 35±2.5 %, $p<0.01$, n=5 each) than ECE-$1^{+/+}$ mice. Fibroblast accumulation in ECE-$1^{+/-}$ mice was almost three times lower than that in mice (FSP1 (+) cells: 347 vs. 977 cells/field, $p<0.01$, n=4 each). Pulmonary ECE-1 ($p<0.05$) mRNA was markedly upregulated in ECE-$1^{+/+}$ mice but not in ECE-$1^{+/-}$. Up regulation of fundamental profibrotic cytokine, TGFβ, was observed from 7 days in both mice and persisted until 28 days in ECE-$1^{+/+}$ mice but this was prevented in ECE-$1^{+/-}$ mice. In accordance with this finding, Smad3 signalling pathway, a main downstream signal for TGFβ, was activated in bleomycin ECE-$1^{+/+}$ mice but not in ECE-$1^{+/-}$ mice. TGFβ/Smad3 pathway regulates fibroblast activity and matrix production, which implicates to wound healing and fibrotic response. In conclusion, ECE-1 inhibition has advantageous role in preventing bleomycin-induced pulmonary fibrosis in mice through prevention persistent TGFβ/Smad3 signalling.