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NINTEDANIB (TYROSINE-KINASE INHIBITOR) INHIBITS THE TRANSITION OF CIRCULATING FIBROCYTES ISOLATED FROM SYSTEMIC SCLEROSIS PATIENTS INTO MYOFIBROBLASTS: AN IN VITRO STUDY

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Background: Systemic sclerosis (SSc) is a chronic connective tissue disease characterized by microvascular alterations, dysregulated immune response and fibrosis [1,2]. Myofibroblasts are alpha-smooth muscle actin (alphaSMA) positive cells and play a crucial role in fibrosis, through the excessive synthesis and deposition of extracellular matrix (ECM) proteins, in particular fibronectin (FN) and type I collagen (COL1) [3]. Despite myofibroblasts primarily derive from resident fibroblasts transition and differentiation, another important source is represented by circulating fibrocytes [4]. Nintedanib is a tyrosine kinase inhibitor approved for the treatment of idiopathic pulmonary fibrosis that interferes with the signalling pathways involved in the pathogenesis of fibrosis [5].

Objectives: To investigate the possible effects of nintedanib in contrasting the ability of cultured mature fibrocytes from SSC patients to differentiate into profibrotic myofibroblasts.

Methods: Circulating fibrocytes were obtained from peripheral blood mononuclear cells isolated from 5 limited cutaneous SSC patients (mean age 66 +/- 10 years) and then plated on FN-coated tissue culture dishes in growth medium (DMEM at 20% of fetal bovine serum, 1% of penicillin-streptomycin and 1% L-glutamine), to allow the adhesion of fibrocyte precursors. Adherent cells were maintained in growth medium for 8 days in order to allow their differentiation into fibrocytes. Differentially fibrocytes were treated with nintedanib at the concentrations of 100nM and 1000nM for 3 and 24 hours (hrs) or maintained in growth medium without any treatment. The differentiation of fibrocytes into myofibroblasts was determined evaluating the gene expression of alphaSMA, fibroblast specific protein-1 (S100A4) COL1, FN and CXCR4 by quantitative real-time polymerase chain reaction, and the protein synthesis of alphaSMA, COL1 and FN by western blotting.

Results: Nintedanib inhibited alphaSMA and S100A4 gene expression already at the concentration of 100nM in cultured fibrocytes and after 3 hrs of treatment, when compared with untreated cells. Furthermore, both concentrations of nintedanib (100nM and 1000nM) reduced the gene expression of COL1 and FN, whereas only 100nM downregulated the CXCR4 gene expression. At protein level, nintedanib 100nM and 1000nM reduced the synthesis of alphaSMA and COL1 after 24 hrs of treatment, whereas FN synthesis was reduced only by the nintedanib concentration of 1000nM.

Conclusion: The preliminary results show that nintedanib may inhibit the in vitro transition of SSC fibrocytes into myofibroblasts and their profibrotic activity, through the reduction of specific myofibroblast phenotype markers and ECM protein production. The results seem to suggest fibrocytes as further possible target of the antibiotic action of nintedanib in SSC.

References:
Conclusion: PLTs are greatly activated in SSc and this is associated with disease progression. Findings suggest that this activation is greater at less severe patients.

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Disclosure of Interests: None declared
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