ACTIVITY OF HISTONE DEACETYLASE IN CELLS ISOLATED FROM INDUCED SPUTUM OF COPD PATIENTS TREATED WITH TIOTROPIUM

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Chronic obstructive pulmonary disease (COPD) is an irreversible and progressive illness in which reduced lung function is associated with local and systemic inflammation. COPD patients are treated with bronchodilators such as long-acting beta2-agonists (LABA) frequently combined with long-acting antimuscarinic agents (LAMA) like tiotropium bromide or/and steroids producing respiratory benefits and better patient survival. Signaling pathways involved in inflammatory processes in COPD and inflammatory response to the therapy are not known. Our aim was to isolate cells from induced sputum of COPD patients treated with LABA or LABA+LAMA and assess enzymatic activity of histone deacetylases (HDACs) and expression of inducible nitric oxide synthase (iNOS). HDACs are enzymes that remove acetyl groups from histone ε-N-acetyl lysines allowing the histones to tightly wrap the DNA and to limit or even block DNA transcription important in signal transduction, inflammation and cell growth and death. iNOS is generating nitric oxide (NO) a molecule relevant to blood pressure regulation, inflammation and infections. Thirty stable COPD patients (21 males and 9 females, mean age 67yrs) receiving 12 ug BID formoterol were assayed before and after three months add-on therapy consisting of and 18 ug QID tiotropium. In all patients spirometry, lung volumes, and DLCO were performed before and after tiotropium therapy and all patients were subjected to the sputum induction. Sputum cells were isolated and processed to obtain cytosolic and nuclear fractions. HDAC activity was measured in nuclear fraction using colorimetric assay (Enzo Life Sci. HDAC kit, Switzerland). Expression of iNOS was quantified using Western blot and specific antibodies (Abcam, UK). In patients receiving both drugs significantly improved FEV1 and lung volumes were observed comparing to formoterol–only treated patients. Mean HDAC activity was slightly decreased ($P<0.05$), while iNOS expression was significantly elevated (increase by about 77%, $P<0,01$). Our data show that beneficial effects of tiotropium in add on therapy to formoterol may be related to increased expression of iNOS and possibly also to altered HDAC activity.