# TIOTROPIUM INCREASES MUSCARINIC M3 RECEPTOR EXPRESSION IN INDUCED SPUTUM CELLS OF COPD PATIENTS

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Chronic obstructive pulmonary disease (COPD) is characterized by irreversible progressive airflow limitation related to tobacco smoking. This limitation is caused by chronic inflammation of the airways and lung parenchyma, and is associated with increased activity of parasympathetic system. The most effective bronchodilators in COPD are muscarinic receptor antagonists (MRA), which reverse, at least in part, compromised respiratory function. MRA also contribute to control inflammatory processes via interactions with inflammatory signaling molecules, therefore once daily use of long acting cholinolitic bronchodilatator with high affinity to M3 receptors - Tiotropium is suggested as a first line maintenance treatment in COPD patients. In this study we assessed M3 receptor protein expression in induced sputum of 21 stable COPD patients before and after therapy consisting of 18 mcg once daily Tiotropium for 12 weeks. Lung function tests including spirometry, lung volumes, and DLCO were performed before and after therapy in all COPD patients. All patients were subjected to the sputum induction procedures before and after therapy. Sputum cells were isolated, sample-specific ell profiles were characterized, and cells were processed to isolate pure cytosolic fractions. M3 protein levels were quantified using specific antibodies against human M3 proteins (Santa Cruz Biotechnology) and Western blot with enhanced luminescence detection.M3 protein levels in cytosolic fractions of cells isolated from induced sputum of Tiotropium-treated patients were increased by 29%; ($P<0.05$). Increased M3 protein levels positively correlated with alterations in FEV1 and negatively correlated with lung hyperinflation markers.