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EXPERIMENTAL STUDY

The expression of selected fibrotic factors in COPD and asthma

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ABSTRACT

BACKGROUND: Chronic Obstructive Pulmonary Disease (COPD) and asthma are associated with chronic inflammation leading to airway obstruction and remodelling. There is little information on possible differences in the TGFB signalling pathway in the pathologies compared to less severe chronic bronchitis without airway obstruction.

AIM: To assess the expression of the selected TGFB signalling pathway-associated genes in the pathologies.

METHOD: RT-PCR was used to quantify the mRNAs in bronchoalveolar cells obtained from the Czech patients with chronic bronchitis (n = 26), COPD (n = 22), asthmatic (n = 14) patients.

RESULTS: There was no difference in the BAL cell expression of TGFB1-3, TGFBR1-2, SMAD2,4,5, and 7 between our patients with COPD and those with chronic bronchitis. The expressions were also similar in the patients with asthma and chronic bronchitis. There was no difference between the patients with asthma and COPD.

CONCLUSION: Although we observed no differences in our patients, other studies should investigate the genes and their possible correlation with advanced airway obstruction and emphysematous changes *(Tab. 2, Fig. 3, Ref. 27)*. Text in PDF *www.elis.sk*

KEY WORDS: TGFB signalling pathway, COPD, asthma, chronic bronchitis, bronchoalveolar lavage.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) and bronchial asthma are characterised by airway obstruction that is mainly caused by tobacco smoking and allergen exposure, respectively. The repeated exposures induce the chronic inflammatory response and later the airway remodelling including the fibrotic changes in the affected lungs (1–3).

The key feature of the fibrotic process is the dysregulated activity of the TGFB signalling pathway. This pathway is induced by the TGFB (transforming growth factor-beta) superfamily including TGFBs, BMPs (bone morphogenetic proteins), activin, and other ligands (4). There are three isoforms of TGFB (TGFB1, TGFB2, and TGFB3) in humans. They bind a TGFB cell membrane receptor type I (TGFBRI). There is also TGFB receptor type II (TGFBRII) that is active constitutively on cell membranes. Upon TGFB ligand binding, TGFBRII is brought into proximity to the receptor type I to propagate signal through Smad-dependent (also known as canonical signalling) pathway. In particular, the activated Smads 2 and 3 form the complexes with Co-Smad (Smad 4) and translocate into the nucleus to regulate gene expression. In a negative feedback loop, inhibitory Smad (Smad 7) induced by Smad 3 blocks TGF-ß signalling (5).

Previous clinical studies on COPD and asthma reported the dysregulation of the members of the TGFB signalling pathway with inconsistent results (6–11). To further investigate the members of the TGFB signalling pathway, we measured the intracellular expressions of TGFB1, TGFB2, TGFB3, TGFBR1, TGFBR2, SMURF1, SMAD2,4,5, and 7 in bronchoalveolar lavage obtained from the controls with chronic bronchitis and the patients with COPD and bronchial asthma.

Tab. 1. Characteristics of the patients with chronic bronchitis, COPD and asthma.

	Controls (chronic bronchitis)	COPD	Asthma
n	26	22	14
Mean age (min–max)	45 (22,67)	57 (24,79)	38 (20,69)
Gender, men/women	10/16	11/11	5/9
Smoking history (non-smoker/ex-smoker⁎/current smoker/NA)	15/2/8/1	4/5/10/3	10/1/2/1
Stage (gold 1/2/3/4/NA)	NA	3/7/5/2/5	NA

COPD – chronic obstructive pulmonary disease, NA – not available

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Tab. 2. Subcellular profile of bronchoalveolar lavage.

	controls			COPD			Asthma			Kruskal/Wallis test (p)
	median	min	max	median	min	max	median	min	max	
Total cell count (106/mL)	1.00	0.15	6.80	1.00	0.15	8.10	0.95	0.10	3.90	>0.05
Macrophage relative count (%)	88.25	38.00	98.40	93.00	0.00	100.00	73.50	55.00	97.40	0.03
Lymphocyte relative count (%)	9.00	0.00	28.00	3.00	0.00	22.00	11.50	0.00	28.00	0.004
Neutrophil relative count (%)	4.00	0.00	40.00	1.00	0.00	81.00	4.00	0.30	33.00	>0.05
Eosinophil relative count (%)	0.15	0.00	12.00	0.00	0.00	10.00	1.50	0.00	33.70	0.05
CD3+ relative count (%)	81.00	24.00	95.00	76.00	26.00	91.00	65.50	14.00	89.00	>0.05
CD4+ relative count (%)	44.50	9.00	75.00	44.00	7.00	67.00	32.50	11.00	69.00	>0.05
CD8+ relative count (%)	25.00	12.00	76.00	28.00	5.00	64.00	28.00	0.00	62.00	>0.05
CD19+ relative count (%)	1.00	0.00	3.00	0.00	0.00	5.00	0.00	0.00	4.00	>0.05

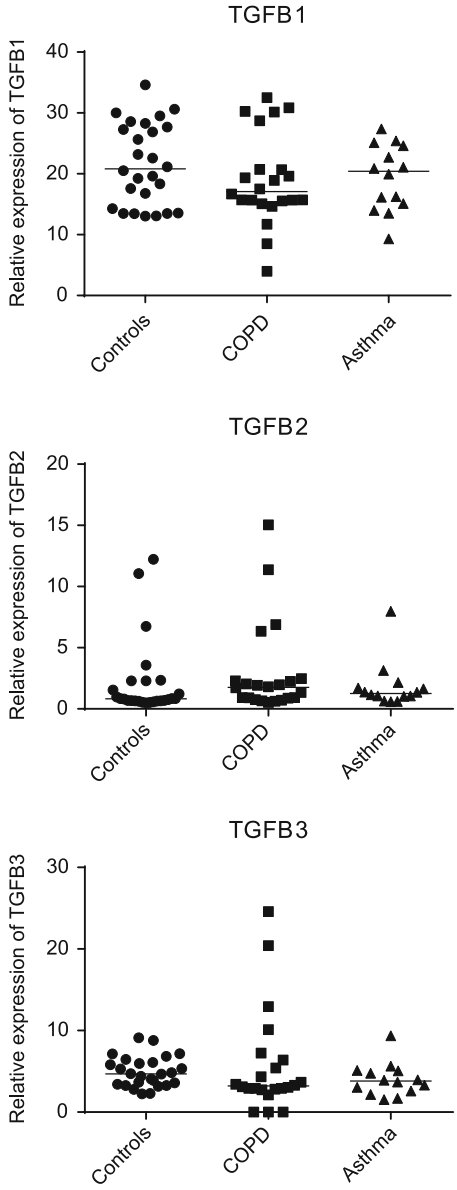

Fig. 1. The bronchoalveolar mRNA expression of TGFB1-3 in the control patients (chronic bronchitis) in comparison with COPD and asthma.

Methods

Subjects

Stable COPD and asthma were defined according to the criteria of the Global initiative for chronic Obstructive Lung Disease (GOLD) and Global Initiative for Asthma (GINA), respectively (12, 13). Chronic bronchitis is defined by the presence of cough and sputum production for at least 3 months in each of two consecutive years according to Global Initiative for Chronic Obstructive Lung Disease criteria (http://www.goldcopd.org). The patients´ characteristics and sub-cellular profile are listed in Tables 1 and 2.

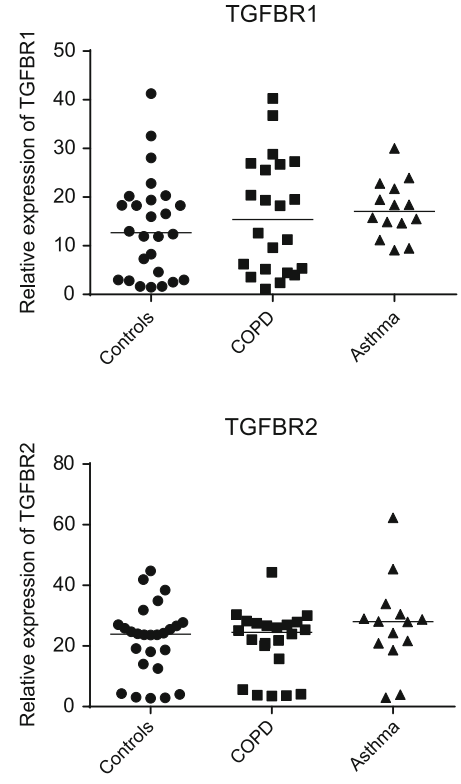

Fig. 2. The bronchoalveolar mRNA expression of TGFBR1-2 in the control patients (chronic bronchitis) in comparison with COPD and asthma.

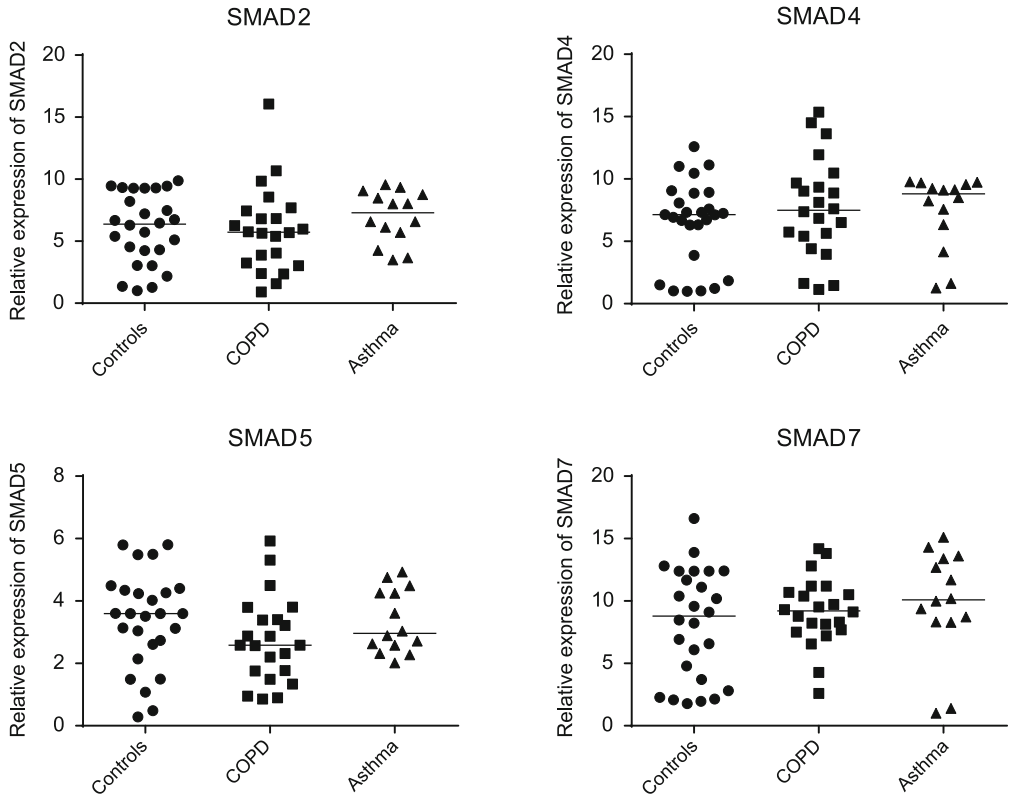

Fig. 3. The bronchoalveolar mRNA expression of SMADs in the control patients (chronic bronchitis) in comparison with COPD and asthma.

All the patients were recruited from the patients of the Department of Respiratory Medicine, University Hospital in Olomouc, the Czech Republic. The study was performed with the approval of the Ethical committees of Medical Faculty PU & University Hospital, Olomouc. Informed consent for the anonymous usage of BAL samples for the purposes of the study was obtained from all enrolled subjects.

BAL cells processing, RNA isolation, and real-time PCR

BAL cells were separated from the fluid by centrifugation as described previously (14). The total RNA was isolated with mirVana™ miRNA Isolation Kit (Life Technologies, USA). Reverse transcription was performed by SuperScript™ VILO™ cDNA Synthesis Kit (Life Technologies, USA). REALIST DX analyser (GeneTiCA, the Czech Republic) was used to assess a relative expression. RT-PCR reaction conditions and a reference gene PSMB2 are described elsewhere (15).

Statistics

Kruskal–Wallis test ($p_{K\text{-}W}$) and Mann–Whitney U-test ($p_{M\text{-}W}$) were used to detect possible differences between the patient groups (GraphPad Prism; GraphPad, La Jolla, CA USA). The p value < 0.05 was considered significant.

Results

The relative expressions of TGFB1, TGFB2, TGFB3, TGFBR1, TGFBR2, SMURF1, SMAD2,4,5 and 7 did not differ between our COPD patients and the patients with chronic bronchitis (for all genes $p_{K\text{-}W} > 0.05$, $p_{M\text{-}W} > 0.05$) (Figs 1–3). The relative expressions were also similar in the patients with asthma and chronic bronchitis ($p_{K\text{-}W} > 0.05$ and $p_{M\text{-}W} > 0.05$). There was no difference between the patients with asthma and COPD ($p_{K\text{-}W} > 0.05$ and $p_{M\text{-}W} > 0.05$).

Discussion

The dysregulation of the TGFB signalling pathway is associated with COPD and asthma (6, 7, 9, 11). We observed no significant difference in the BAL cell expression of TGFB1, TGFB2, TGFB3, TGFBR1, TGFBR2, SMURF1, SMAD2,4,5, and 7 between our patients with COPD and those with chronic bronchitis. Previous studies reported both increased (8, 10, 16) and decreased (6, 11) expression of TGFB1 in COPD and asthma. Other studies reported no difference (6, 17–21). The relevance of the TGFB signalling pathway is further supported by several studies on other members of this pathway. The study by I Wang (2007) showed both TGFB2 and TGFBRII being related to % parenchyma in COPD lung (9).

The study by HA Golpon (2004) demonstrated TGFBRIII and another member of the TGF-beta receptor family (activin A receptor II) was decreased in emphysematous lungs (22). V Batra et al reported decreased TGFB2 in asthmatic patients (19).

Regarding signalling molecules, downregulation of SMAD6 and Smad 7 was observed in COPD (11, 13). However, Antonino Di Stefano et al (2018) confirmed neither SMAD7 nor other SMADs was dysregulated in various biological materials from COPD patients (6).

Several factors can explain the inconsistency in the current observations. First, various biological materials were used in the previous studies. The whole-genome gene expression studies usually analysed the lung tissue (8, 22). Our study obtained unseparated cells from bronchoalveolar lavage that comprises mostly alveolar macrophages, neutrophils, lymphocytes, and others.

In COPD, alveolar macrophages were earlier investigated by Antonino Di Stefano (2018) who assessed mostly the same members of the TGFB signalling pathway (TGFB1, TGFB2, TGFB3, TGFBR1, TGFBR2, SMAD2, and 7) and others. Of 20 molecules, they reported only TGFb1 positive alveolar macrophages were downregulated in COPD (6). Another study confirmed ex vivo the downregulation in COPD alveolar macrophages (7).

However, both studies compared stable COPD patients with healthy smokers (6, 7). By contrast, our controls were the patients with chronic bronchitis. They were symptomatic controls without airway obstruction. We can speculate that non-significant differences between our COPD patients and the controls can be explained by both pathologies being associated with the same dysregulation in comparison with healthy smokers. Our hypothesis is not supported by Wen Ning et al (2004), who compared two groups of COPD patients with GOLD2 (airway obstruction) and GOLD0 stage (non-obstruction) (8). They observed dysregulation and even increased TGFB1 expression in the lung tissue obtained from COPD patients with airway obstruction. In the lung tissue unlike BAL, however, other cellular sources of TGFB1 are the fibroblast and myofibroblast which contribute to the total TGFB1 expression. Regarding bronchoalveolar lavage, bronchiolar epithelium cells and bronchial fibroblasts contribute to the total TGFB1 level. Hence, the intracellular expression does not have to be in line with the TGFB1 level in BA lavage (10).

Our study had several limitations. Although our patients were well-characterised, there were no smoking history-matched groups and knowledge on the asthmatic stage in our study. In asthma, the increased TGFB1 and TGFB2 have been reported in severe asthma (20), and other studies observed association with atopic asthma (10), or after allergen challenge (19).

Another source of heterogeneity among current clinical studies could be the therapy that modulates key inflammatory targets and pathways in the lung of the patients with COPD and chronic bronchitis. In the study by Mirco Govoni (2020), the patients commenced three, 32-day treatment periods during which they received inhaled PDE4 inhibitor CHF6001 (total daily doses of 1600 or 3200 μg) or a matching placebo. The treatment with a higher dose reduced sputum TGFB1 and other molecules relative to placebo (24). By contrast, inhaled steroids seemed to rather interfere with the biological effects of TGFB than affect its expression (25, 26). Future studies should therefore investigate the possible therapeutic effect. Besides, they should consider sample size to reach sufficient statistical power. In this study, we present pilot data because our negative results can be affected by the small number of the patients.

In conclusion, our patients with COPD and chronic bronchitis showed similar bronchoalveolar cell expressions of the selected genes of the TGFB signalling pathway. Our observation does not exclude the possibility that airway obstruction or/and emphysema are associated with dysregulated expression of the genes in other biological materials. In addition, COPD is associated with other genes that are involved in the activation of TGFB (9). Despite being negative, our data contribute to the current knowledge in this area and they could help other investigators in designing studies to target the real biomarkers associated with COPD and asthma. In this context, dissemination of our results is therefore valuable (27).

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