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Impact of theophylline/corticosteroid combination therapy on sputum hydrogen sulfide levels in patients with COPD

To the Editor:

Hydrogen sulfide (H₂S) has emerged as a new and important endogenous regulator of inflammation in recent years [1] and may also protect from emphysema induced by cigarette smoke exposure [2]. We have also recently shown that H₂S can inhibit airway smooth muscle cell proliferation and inflammatory mediator release *in vitro* [3]. Serum levels of H₂S positively correlate with the decline in lung function in chronic obstructive pulmonary disease (COPD) and were significantly lower in Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage III patients compared with those in GOLD I [4]. Existing therapies for COPD, such as corticosteroids or long-acting anticholinergic agents, may reduce the exacerbation rate but do not significantly slow disease progression. A previous study has shown that theophylline alone had no impact on serum H₂S levels and is of limited value in the management of stable COPD [5]. Interestingly, sputum H₂S measured in patients with asthma correlated with sputum neutrophil counts and the degree of airflow obstruction measured by forced expiratory volume in 1 s (FEV₁) % predicted [6]. Moreover, combination therapy of an inhaled glucocorticoid with low-dose theophylline has been shown to attenuate airway inflammation in patients with COPD and reverse glucocorticoid resistance [7]. We therefore investigated whether the combination of inhaled corticosteroid and low-dose theophylline, as opposed to low-dose theophylline alone, would modulate H₂S levels in the lungs of COPD patients. We now report the levels of H₂S assayed in sputum samples collected during this study (www.clinicaltrials.gov identifier NCT00241631), details of which have already been published [7].

FIGURE 1 The impact of low-dose theophylline alone or in combination with inhaled fluticasone on sputum hydrogen sulfide (H_2S) levels a) in all chronic obstructive pulmonary disease (COPD) subjects, b) only in Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage II COPD subjects, c) only in GOLD III COPD subjects and d) in all COPD patients stratified according to the degree of improvement in forced expiratory volume in 1 s (ΔFEV_1) following theophylline/fluticasone combination therapy. Data are shown for the baseline visit (no theophylline) *versus* the final visit (after theophylline administration) and displayed either as a–c) paired data between the two visits for each subject in each arm of the study (placebo *versus* fluticasone) or d) the mean in a box and whisker plot for the fluticasone arm of the study only. *: $p < 0.05$; ***: $p < 0.001$.

Briefly, a total of 29 patients with stable COPD, 18 in GOLD II and 11 in GOLD III, provided sputum for the measurement of H_2S . After an initial 2-week washout during which all inhaled corticosteroid was ceased, subjects were randomised into two treatment arms, one with inhaled fluticasone propionate (500 mg twice daily) the other without for 4 weeks; both arms were given placebo theophylline capsules. After another 2-week washout period, this was followed by a further 4-week period where both arms were given active slow-release theophylline capsules (250 mg twice daily). Induced sputum samples were obtained at the end of the initial run-in period and at the end of each 4-week treatment period. The supernatant fraction was analysed for H_2S content using a method that accounts for all H_2S species and those derived from it at physiological pH, as described previously [8]. The data were analysed using the statistical package SPSS (version 8; IBM, Armonk, NY, USA) incorporating mixed models to account for nonconstant variability across visits and correlation between visits within patient groups. Over the course of the study, COPD subjects as a group exhibited a significant decrease in sputum H_2S levels in the placebo arm compared with the fluticasone propionate arms (fig. 1a). When patients were analysed according to disease severity, as defined by GOLD status, those patents in GOLD II showed no change in sputum H_2S levels in either the placebo or fluticasone arms (fig. 1b). As a group, GOLD III COPD subjects in the placebo arm (no fluticasone) had a reduced level of sputum H_2S after 4 weeks treatment with theophylline only compared with baseline (fig. 1c). In contrast, GOLD III subjects as a group in the fluticasone arm exhibited no changes in sputum H_2S levels after theophylline treatment (fig. 1c). Interestingly, when sputum H_2S levels were analysed within the context of changes in FEV_1 , those COPD patients who exhibited the greatest increase in FEV_1 upon the addition of theophylline to fluticasone had very low initial sputum H_2S levels. H_2S levels rose significantly for all subjects in this subgroup upon addition of theophylline to

fluticasone (fig. 1d). This effect was not apparent in either the placebo arm of the study or those subjects in the fluticasone arm that had an elevated baseline H₂S level.

The significance of decreased sputum H₂S levels in GOLD III is not clear. It may simply reflect the greater degree of oxidative burden in the GOLD III COPD lung [9] as H₂S, or species derived from it at physiological pH, readily interacts with detrimental oxidant species, such as nitric oxide, peroxynitrite and hypohalous acids. Alternatively, as corticosteroids appear to induce the synthesis of endogenous H₂S synthesis enzymes [10], our results may simply reflect a lower level of endogenous H₂S in more severe COPD in the absence of corticosteroid. The most surprising finding was that those subjects in the fluticasone study arm who had low baseline levels of sputum H₂S demonstrated the greatest increase in FEV₁ upon the addition of theophylline. This suggests that low sputum H₂S levels could act as a biomarker for those COPD subjects who could benefit the most from theophylline/corticosteroid combination therapy. Clearly, this small study is hypothesis generating, requires replication and should be interpreted with great caution. However, our data confirm previous evidence suggesting that theophylline alone does not regulate H₂S levels [5] and also suggest that the combination of theophylline plus fluticasone at least maintains H₂S levels in patients with GOLD II or III disease. Increased H₂S levels in the fluticasone arm may be due to increased expression of H₂S-synthesising enzymes [10]. This effect would be independent of the transrepression actions associated with loss of histone deacetylase 2 [9] and may suggest that transactivation by corticosteroids is unaffected in COPD. In conclusion, monotherapy with theophylline does not appear to have any impact on maintaining or further increasing sputum H₂S levels. In combination with inhaled corticosteroid, however, while there is a trend towards increasing sputum H₂S levels, this is most evident in those COPD subjects who had very low baseline sputum H₂S levels and, furthermore, appeared to benefit most in relation to increases in FEV₁.

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COPD patients with low airway H₂S benefit from theophylline/corticosteroid therapy with increased FEV₁ and H₂S levels <http://ow.ly/sx2u4>

Paul A. Kirkham^{1,2}, Matthew Whiteman³, Paul G. Winyard³, Gaetano Caramori⁴, Fabiana Gordon⁵, Paul A. Ford¹, Peter J. Barnes¹, Ian M. Adcock¹ and Kian Fan Chung¹

¹Airways Diseases, National Heart and Lung Institute, Imperial College London, London, ²Dept of Biomedical Sciences, University of Wolverhampton, Wolverhampton, ³University of Exeter Medical School, Exeter, and ⁵Statistical Advisory Service, Imperial College London, London, UK. ⁴Dipartimento di Scienze Mediche, Centro Interdipartimentale per lo Studio delle Malattie Infiammatorie delle Vie Aeree e Patologie Fumo-correlate (CEMICEF), Sezione di Medicina Interna e Cardiorespiratoria, Università di Ferrara, Ferrara, Italy.

Correspondence: P.A. Kirkham, Dept of Biomedical Sciences, Faculty of Science and Engineering, University of Wolverhampton, Wulfruna Street, Wolverhampton, WV1 1LY, UK. Email: P.Kirkham@wlv.ac.uk

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