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

To the Editor:

Hydrogen sulfide (H_2S) 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 H_2S can inhibit airway smooth muscle cell proliferation and inflammatory mediator release *in vitro* [3]. Serum levels of H_2S 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 H_2S levels and is of limited value in the management of stable COPD [5]. Interestingly, sputum H_2S measured in patients with asthma correlated with sputum neutrophil counts and the degree of airflow obstruction measured by forced expiratory volume in 1 s (FEV_1) % 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 H_2S levels in the lungs of COPD patients. We now report the levels of H_2S assayed in sputum samples collected during this study (www.clinicaltrials.gov identifier NCT00241631), details of which have already been published [7].

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 H_2S level.

The significance of decreased sputum H_2S 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 H_2S, 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 H_2S synthesis enzymes [10], our results may simply reflect a lower level of endogenous H_2S 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 H_2S demonstrated the greatest increase in

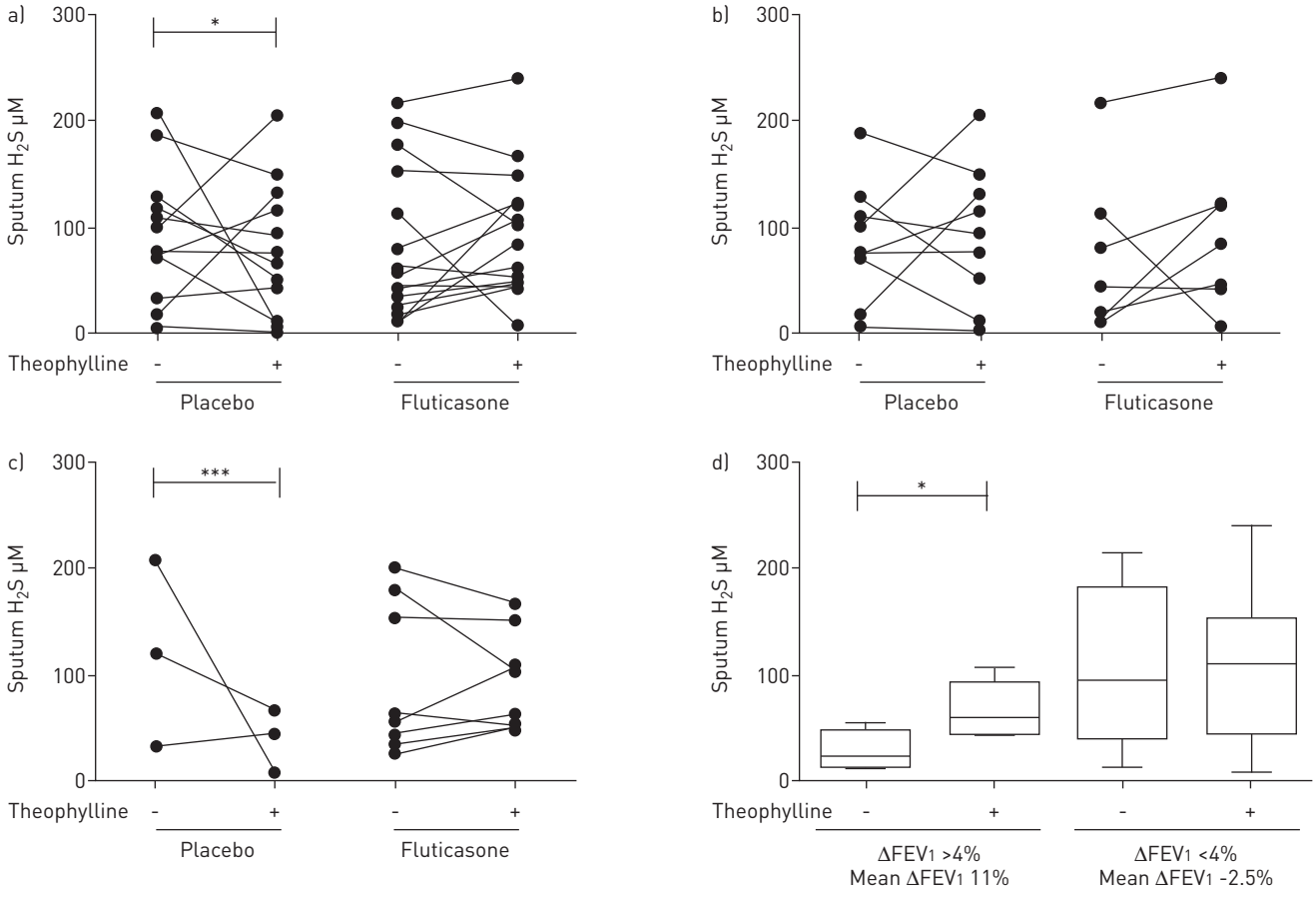

FIGURE 1 The impact of low-dose theophylline alone or in combination with inhaled fluticasone on sputum hydrogen sulfide (H_2S) levels a) in all chronic obstructive pulmonary disease (COPD) subjects, b) only in Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage II COPD subjects, c) only 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 (ΔFEV1) 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. *: $p<0.05$; ***: $p<0.001$.

FEV1 upon the addition of theophylline. This suggests that low sputum H_2S 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 H_2S levels [5] and also suggest that the combination of theophylline plus fluticasone at least maintains H_2S levels in patients with GOLD II or III disease. Increased H_2S levels in the fluticasone arm may be due to increased expression of H_2S-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 H_2S levels. In combination with inhaled corticosteroid, however, while there is a trend towards increasing sputum H_2S levels, this is most evident in those COPD subjects who had very low baseline sputum H_2S levels and, furthermore, appeared to benefit most in relation to increases in FEV1.

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COPD patients with low airway H_2S benefit from theophylline/corticosteroid therapy with increased FEV1 and H_2S levels http://ow.ly/sx2u4

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