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The effects of theophylline on mucosal inflammation in asthmatic airways: biopsy results

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The effects of theophylline on mucosal inflammation in asthmatic airways: biopsy results. R. Djukanović, J.P. Finnerty, C. Lee, S. Wilson, J. Madden, S.T. Holgate. ©ERS Journals Ltd 1995.

ABSTRACT: Theophylline, a nonspecific phosphodiesterase inhibitor, has only recently been reconsidered as a potential anti-inflammatory drug. Its ability to inhibit late asthmatic responses has pointed to possible inhibition of mechanisms regulating the influx and activity of inflammatory cells into the airways.

Increasing evidence points to an anti-inflammatory action of theophylline at doses lower than those necessary for a bronchodilator effect. Withdrawal of theophylline from regular treatment results in an increase both in CD4+ and CD8+ T-cells in the bronchial mucosa and a concomitant decrease in the blood, suggesting that theophylline prevents T-cell trafficking from blood into the airways. Furthermore, pretreatment with theophylline significantly attenuates the influx of eosinophils into the airways associated with an allergen-induced late asthmatic response. In keeping with these observations, in a double-blind, placebo-controlled trial involving mild to moderately severe atopic asthmatics, treatment with theophylline resulted in a significant reduction in the numbers of epithelial CD8+ T-cells. In addition, the numbers of cells containing cytokines, interleukin 4 and 5 (IL-4 and IL-5), decreased in the theophylline-treated group and increased in the placebo-treated group, with the difference between the changes being significant.

It would, therefore, appear that theophylline may contribute to asthma control due to its ability to reduce the suppressor/cytotoxic T-cells and cytokines which are relevant to allergic mucosal responses.

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Atopic asthma is a disease characterized by a specific form of airways mucosal inflammation, which is believed to result from an interaction between inflammatory cells and inhaled allergen, as well as additional triggers, such as air pollutants and viral infections [1]. Because of their ability to inhibit the actions of the majority of inflammatory cells, corticosteroids, delivered either by the inhaled or oral route, have been the mainstay of asthma treatment [2], although new forms of alternative therapies are increasingly being sought to either supplant or supplement this form of treatment.

Theophylline, a nonspecific phosphodiesterase, has been used for decades for its bronchodilator effects to treat asthma. It has been known for some time that theophylline has an inhibitory effect on the allergen-induced late asthmatic airways response (LAR) [3], and the associated increase in airways hyperresponsiveness [4]. The LAR has been shown to be associated with an influx of inflammatory cells, degranulation both of mast cells and eosinophils through the production of chemotactic factors, and upregulation of adhesion molecules in the mucosal postcapillary venules [5, 6]. However, before the advent of fibreoptic bronchoscopy, which has enabled direct study of airways pathology, it has been unclear whether the inhibitory action of theophylline results from functional antagonism of this bronchodilator or reflects its anti-inflammatory effects.

Early indication of possible effects of theophylline on inflammatory cells came from the observation that the numbers of circulating CD8+ T-lymphocytes can be increased with treatment [7]. This has been interpreted as a suppressive effect of this T-cell subset on allergic inflammatory responses. However, the role of CD8+ T-cells has been a matter of considerable controversy, and more recently it has been shown that these cells may be conditioned to produce cytokines of the T-helper (Th)2 type, such as interleukin (IL)-5, and in diseased tissues may be divided into distinct subtypes with cytokine profiles similar to those of the Th1 and Th2 CD4+ T-cells [8].

Studies using fibreoptic bronchoscopy to sample the airways mucosa, utilizing immunohistochemistry to enumerate the extent of cellular infiltration, have shown that withdrawal of theophylline ultimately leads to an increase

in CD4+ and CD8+ T-cells infiltrating the airways, suggesting that this treatment is preventing the recruitment of lymphocytes into the airways [9]. It is unclear whether T-cells in fact proliferate in the airways mucosa, but in view of the ability of theophylline to inhibit T-cell proliferation [10], it remains a distinct possibility that this mechanism of T-cell accumulation in the mucosa may also be affected by theophylline.

We have recently conducted a double-blind, placebo-controlled parallel group study [11] of the effects of oral theophylline given at doses achieving a mean (SD) serum concentration of 10.9 (6.0) $\mu g \cdot mL^{-1}$ of the drug. The subjects studied were mild to moderately severe atopic asthmatics, some of whom required inhaled corticosteroids for disease control. A substantial proportion of the subjects also had nocturnal asthma symptoms. These subjects would, thus, be considered as being insufficiently well-controlled and would be expected to benefit from the addition of theophylline, a drug which is commonly added in the treatment of nocturnal asthma. By comparison with the placebo-treated group of subjects, the group that received theophylline was noted to have an increase in peak expiratory flow rates (PEFRs) associated with an improvement in nocturnal asthma. The clinical improvement was not associated with any significant changes in the number of effector inflammatory cells, eosinophils and mast cells, nor were there any changes in total CD3+ T-cells or their CD4+ subset. However, there was a significant decrease in epithelial CD8+ cells. In addition, the numbers of cells staining for granular IL-4 and IL-5 were reduced in the actively treated subjects as compared with those receiving placebo.

This study of mild and moderately severe atopic asthmatics has, therefore, suggested that theophylline may be downregulating the genes encoding for IL-4 and IL-5 clustered on chromosome 5. The mechanisms responsible for this effect remain unclear, but it would appear that theophylline may be a potentially important addition to the group of drugs which act on cytokine production. In this study, most of the IL-4 and IL-5 protein detected by immunohistochemistry was localized to mast cells, although it is possible that the activity of theophylline is not restricted to these cells but may also extend to T-cells and eosinophils, two other producers of IL-4 and IL-5.

The mast cell has attracted renewed interest amongst those conducting research into asthma pathogenesis. It is now clear that, in addition to producing a source of vaso- and bronchoactive mediators, mast cells can produce cytokines, such as IL-4, IL-5, IL-6 and tumour necrosis factor-α (TNF-α) [12]. That the finding of IL-4 and IL-5 in the mast cell granules is not a result of endocytosis, but reflects active production, is clearly shown in studies using reverse transcription-polymerase chain reaction (RT-PCR) to demonstrate messenger ribonucleic acid (mRNA) transcription of both these cytokines in highly purified lung mast cells (Okayama, personal communication). In addition, cross-linking of surface immunoglobulin E (IgE) by anti-IgE antibodies results in IL-4 secretion in parallel with histamine release (Okayama, personal communication); although, detection of IL-4 is hampered by rapid degradation by mast cell proteases.

The implications of these findings remain to be elucidated, but they point to the possibility that theophylline may be a valuable form of adjunctive treatment in atopic asthma. IL-4 is central to the function of Th2-type T-cells [13], and acting *via* upregulation of the vascular cell adhesion molecule-1 (VCAM-1) it can participate in the recruitment of very late activation antigen-4 (VLA-4) bearing eosinophils and T-cells [14, 15]. Similarly, a decrease in IL-5 could contribute to reduced eosinophil activation and recruitment, and in this respect the data presented are consistent with the observation that theophylline treatment reduces the levels of serum eosinophil cationic protein (ECP) [16], which reflects eosinophil activation and has been shown to correlate with disease activity. Furthermore, the data are in keeping with the observations of KIDNEY *et al.* [9] of an increase in T-cell infiltration of the bronchial mucosa after theophylline withdrawal. The fact that the reduction in IL-4 and IL-5 was not associated with a reduction in the number of T-cells in the airways mucosa possibly reflects the fact that most subjects had relatively stable asthma, or were using inhaled corticosteroids which are known to reduce eosinophil numbers [17]. In addition, chemokines such as lymphocyte chemotactic factor (LCF) may not have been sufficiently reduced to lead to detectable changes. It is tempting to hypothesize that the reduction in the number of mast cells producing IL-4 and IL-5 could prevent the influx of eosinophils which is seen after allergen exposure, and that theophylline may, therefore, be playing a prophylactic role against exposure to allergen, as suggested by the study of SULLIVAN *et al.* [18], in which theophylline was found to significantly reduce the influx of activated EG2+ eosinophils following allergen challenge.

Taken together studies utilizing the fibreoptic bronchoscope yield plausible evidence for a potential anti-inflammatory role for theophylline. Further studies need to be conducted to clarify the exact mechanisms of action and possible role of theophylline and, indeed, the newer specific phosphodiesterase inhibitors, in the treatment of asthma. Although the mechanisms whereby theophylline may inhibit the production of IL-4 and IL-5 remain to be elucidated (in this case most probably largely attributable to effects on mast cells), the available data suggests a potential novel action of theophylline.

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