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SERIES 'THEOPHYLLINE AND PHOSPHODIESTERASE INHIBITORS'
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Theophylline and selective phosphodiesterase inhibitors as anti-inflammatory drugs in the treatment of bronchial asthma

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Theophylline and selective phosphodiestrase inhibitors as anti-inflammatory drugs in the treatment of bronchial asthma. K.H. Banner, C.P. Page. ©ERS Journals Ltd 1995.
ABSTRACT: Theophylline has been in clinical use for the treatment of bronchial asthma and other respiratory diseases for well over 50 yrs. Over this time, a considerable body of evidence has accumulated to show that this drug has a wide range of pharmacological actions, in addition to the well-recognized action on airway smooth muscle function. Current evidence suggests that part of the therapeutic value of theophylline in the treatment of asthma is by virtue of an anti-inflammatory or immunomodulatory effect, although the actual mechanism of action remains unclear. It has been proposed that the observed anti-inflammatory effects of theophylline could be attributed to phosphodiesterase (PDE) inhibition, and recently the type III and IV isoenzymes have been characterized in a number of inflammatory cells.

This article reviews the evidence that theophylline and the newer more selective type IV PDE isoenzyme inhibitors can inhibit the activation of inflammatory cell types, such as T-lymphocytes, eosinophils, mast cells and macrophages, *in vitro*. The evidence supporting the ability of theophylline and selective PDE IV isoenzyme inhibitors to modify allergic inflammation both in animal models and clinical asthma is also discussed. We conclude that theophylline possesses important anti-inflammatory and immunomodulatory activity and that, in light of this evidence, it is timely to reconsider the place of theophylline in the treatment of asthma.
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Background: effects of theophylline and selective isoenzyme inhibitors on cells involved in the inflammatory response

Xanthines have long been recognized for their use in the treatment of airways disease, largely from the standpoint of the ability of these drugs to elicit bronchodilatation [1–3]. It has also been suggested, for some 20 yrs, that theophylline and related xanthines may owe part of this therapeutic activity to an inhibition of phosphodiesterase (PDE) enzymes. Recently, it has become apparent that a number of inflammatory cells specifically possess the PDE isoenzyme IV [4], raising the possibility that modulation of this enzyme may lead to anti-inflammatory effects. In support of this hypothesis, it is increasingly apparent that xanthines, such as theophylline, and more selective PDE inhibitors, possess anti-inflammatory and immunomodulatory actions [5–7], that may well contribute to the clinical effects of such drugs.

Effects of theophylline and selective PDE isoenzyme inhibitors on cells involved in the immune response

In vitro *studies*

In the mid 1970s, theophylline was used to produce clones of peripheral blood mononuclear cells with possible suppressor cell activity [8]. Subsequently, a number of investigations *in vitro* have suggested that selective PDE inhibitors possess the ability to modify lymphocyte behaviour, in particular the ability to inhibit lymphocyte proliferation [9–10]. A variety of studies have examined the potential anti-inflammatory effect of theophylline and isoenzyme selective PDE inhibitors on different cell types which have been postulated to have a role in the inflammatory response. These include mast cells, basophils, lymphocytes, eosinophils, neutrophils, monocytes and natural killer cells.

Human lung mast cells release a variety of bronchoconstrictor mediators including histamine, leukotriene C_4 and prostaglandin D_2, and these mediators have been suggested to be responsible for the majority of allergen-induced bronchoconstriction observed in allergic asthmatic patients. Whilst theophylline has been shown to reduce both antigen and anti-immunoglobulin E (IgE) receptor antibody-stimulated histamine release from rat peritoneal mast cells [11], and to reduce histamine release from human basophils [12], these effects are only seen at very high concentrations.

T-lymphocytes are likely to play a role in all antigen-driven inflammatory responses, since they are the only cell type that directly recognize and respond to processed antigens and they are known to be involved in many stages of the allergic response, including the regulation of IgE production by B-lymphocytes and also the

recruitment of other inflammatory cells, such as eosinophils, into the airways. Incubation of peripheral blood lymphocytes with theophylline causes them to suppress autologous cell responses [13], and this phenomenon has been shown to be due to a subgroup of T-cells which is sensitive to *in vitro* stimulation by theophylline [8]. In 1988, theophylline (15–30 $\mu g \cdot mL^{-1}$) was demonstrated to inhibit antigen-stimulated T-cell proliferation and interleukin-2 (IL-2) production [14]. More recently, selective inhibitors of the type III PDE isoenzyme and the type IV isoenzyme have been shown to inhibit human lymphocyte proliferation with biphasic concentration inhibition curves [9, 10]. Combined addition of a type III and type IV inhibitor produced a more potent and complete inhibition, an effect which was also seen with a mixed III/IV inhibitor. Therapeutic levels of theophylline (10–20 $\mu g \cdot mL^{-1}$) have been shown to inhibit the tumoricidal activity of natural killer cells [15].

A considerable body of evidence now exists to suggest that eosinophils play an important role in the lung inflammation characteristic of bronchial asthma through their ability to release a variety of toxic and proinflammatory mediators, including major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN), which have been suggested to contribute to the damage observed in the epithelial lining of the airways of asthmatics [16, 17]. Theophylline and other drugs capable of elevating cyclic adenosine monophosphate (cAMP) levels, such as cAMP analogues, have been found to inhibit immunoglobulin-induced eosinophil degranulation *in vitro* [18]. Leucocytes are able to generate superoxide anion and arachidonic acid derivatives, which may contribute to airway injury, oedema and smooth muscle contraction seen in asthma [19]. In one study by YUKAWA *et al*. [20], concentrations of theophylline which cover the therapeutic range (1–10 μM) significantly potentiated opsonized zymosan-induced O_2^- generation from isolated guinea-pig and human eosinophils; whereas, at high concentrations a significant inhibition of the release was seen [20]. In a separate study, therapeutic concentrations of theophylline (10–50 μM) have been shown to inhibit the respiratory burst and also arachidonic acid metabolite generation induced by chemotactic peptides, such as N-formyl-met-leu-phe (fMLP) and the calcium ionophore, A23187 [21]. Type IV PDE inhibitors, at concentrations which inhibited the degradation of cAMP, have also been found to inhibit the leucocyte respiratory burst [22, 23].

Airway macrophages arise from circulating monocytes, which mature into macrophages during their residence in the airways. The airways both of normal subjects and asthmatics contain macrophages. In atopic subjects, exposure to allergen causes macrophages to release a variety of chemical mediators, such as potent eosinophil chemoattractants like platelet-activating factor (PAF), leukotriene B_4 (LTB_4) and cytokines [19]. Therapeutic concentrations of theophylline have been found to cause a 90% inhibition of LTB_4 generation by neutrophils [24]. Selective type III and IV PDE inhibitors and a mixed type III/IV PDE inhibitor have been demonstrated to inhibit the release of tumour necrosis factor (TNF) from human macrophages [9]. Studies have also demonstrated that peripheral blood mononuclear cells and alveolar macrophages obtained from bronchoalveolar lavage (BAL) fluid of normal subjects exposed to low therapeutic levels of theophylline secreted less superoxide anions *in vitro* [25].

Neutrophils, like eosinophils, have the potential to damage the airways and exacerbate the inflammatory process through their ability to release reactive oxygen species, cationic proteins and lipid inflammatory mediators. It has also been shown that PDE inhibitors can decrease the chemotaxis and chemokinesis of human blood neutrophils *in vitro* [26].

In vivo *studies*

A number of studies have examined the "anti-inflammatory" properties of theophylline and selective PDE inhibitors in different animal models of airways inflammation. Exposure of sensitized animals to antigen results in acute obstruction of the airways and an influx of inflammatory cells into the airway lumen. These animal models have been utilized to examine the effect both of currently prescribed anti-asthma drugs and novel anti-asthma agents for their ability to reduce inflammatory cell accumulation. In rat [6] and guinea-pig models [27] of airways inflammation and hyperresponsiveness, xanthines have been shown to inhibit both the inflammation and hyperresponsiveness induced by allergen. Xanthines have also been demonstrated to attenuate the late response in allergic rabbits [28].

Acute administration of theophylline has been reported to reduce allergen-induced eosinophil infiltration in actively sensitized guinea-pigs [29]. In separate studies, theophylline [27, 30, 31] and isbufylline [27] have been shown to inhibit eosinophil recruitment into the airways after challenge with a range of stimuli. Type IV and a mixed type III/IV PDE inhibitor given prior to antigen challenge have also been found to inhibit eosinophil accumulation in the airways in a variety of species, including allergic primates (fig. 1) [32–35].

However, the majority of studies which have examined the effects of theophylline and selective PDE isoenzyme inhibitors on eosinophil recruitment are probably not relevant to what is occurring when theophylline is used clinically, for two reasons; firstly, the doses used in these studies have been given as a single dose acutely, prior to antigen challenge; and secondly, the doses employed in these studies are much higher than those used clinically. However, two studies conducted by SANJAR and co-workers [30, 31] found that 7 day administration of either theophylline or a mixed III/IV PDE inhibitor inhibited both PAF [30] and allergen-induced [31] pulmonary eosinophil accumulation in the guinea-pig at doses equivalent to those used clinically. Furthermore, we have recently shown that much lower concentrations of a mixed PDE III/IV inhibitor are required to inhibit eosinophil infiltration when the drug is administered chronically (fig. 1) [33] than when the drug is administered acutely.

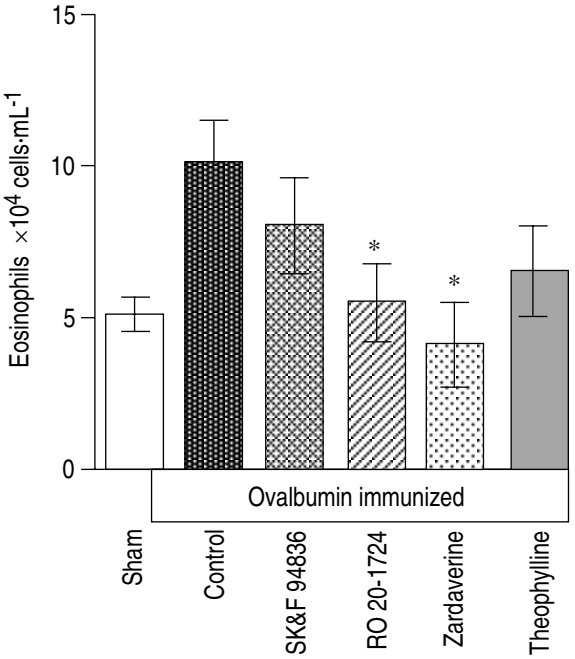

Fig. 1. – Effect of chronic treatment (3 mg·kg^{-1} *b.i.d.* for 7 days prior to antigen challenge) with PDE inhibitors on antigen-induced eosinophil accumulation in the BAL fluid of immunized guinea-pig. Values are mean±SEM. Shading of histograms is designed to correspond with drug specificity of action on PDE isoenzymes. *: p<0.05 *vs* time-matched controls (note that all time-matched control groups have been combined for simplicity of graphic presentation). PDE: phosphodiesterase; BAL: bronchoalveolar lavage. (This figure is adapted from [33]).

Clinical studies

Several studies have examined the effect of chronic treatment with theophylline on the behaviour and number of different inflammatory cell types found in the blood or BAL fluid from asthmatic subjects.

Asthmatics who are on long-term theophylline therapy have more peripheral T-cells that are able to suppress plaque formation in lymphocyte cultures and a reduced *in vitro* graft *versus* host response than do asthmatics who are on other treatments [13]. There is evidence from two separate studies in asthmatic children that there is a decrease in the number of peripheral blood T-lymphocytes which express suppressor activity [36, 37], and one of these studies found that there was a correlation between T-suppressor cell numbers and the severity of asthma. In both studies, following one month of theophylline treatment, the number of T-suppressor cells returned to the normal level found in the control group. In another study, chronic treatment with theophylline resulted in an increase both in the number and the activity of T-suppressor cells found in asthmatic subjects [38]. There is evidence from two studies that withdrawing chronic theophylline treatment leads to a deterioration in asthma [39, 40], and an alteration in inflammatory cells [39] in the blood, with a significant increase in T-lymphocytes found in the airways [41].

Treatment of asthmatic children for 10 days with theophylline has also been found to have an inhibitory effect on the chemotaxis of neutrophils and mononuclear cells *ex-vivo* obtained from their blood [42].

Alveolar macrophages have been shown to generate less superoxide *ex-vivo* after *in vivo* theophylline treatment. Oral theophylline treatment also reduces alveolar macrophage intracellular killing, bactericidal killing and hydrogen peroxide release, and the degree of impairment correlates well with BAL theophylline concentrations [43].

In one study, after a one week course of theophylline treatment, a reduction in histamine release was demonstrated when subjects with allergic rhinitis were challenged with antigen [44]. A reduction in the early response to allergen in the skin during treatment with theophylline, with no change in the response of the skin to injected histamine, was also found, suggesting that theophylline may be affecting endogenous histamine release. However, another recent clinical study treating allergic asthmatics with low dose theophylline for 6 weeks has found no evidence for theophylline inhibiting the histamine levels in BAL fluid following allergen challenge [45]. Furthermore, the effect of theophylline on acute allergic bronchoconstriction is minimal, which suggests that theophylline is not likely to inhibit lung mast cells *in vivo* in any major way [7, 46].

Plasma exudation is one consistent indicator of the ongoing inflammatory process both in asthma and allergic rhinitis. It is of interest that treating allergic rhinitis patients with theophylline can reduce the nasal plasma exudate [5, 44].

The late asthmatic reaction (LAR) to allergen exposure is a model of airway inflammation which is commonly used for the evaluation of the anti-inflammatory response, and several studies have now reported that theophylline will inhibit the LAR [7, 46–49]. Interestingly, if allergic subjects are challenged with antigen after a period of chronic treatment with theophylline, this results in an inhibition of the LAR at subbronchodilator plasma concentrations [7, 49]. Similar results have been found during oral theophylline therapy after challenge with toluene diisocyanate in sensitive asthmatics [50].

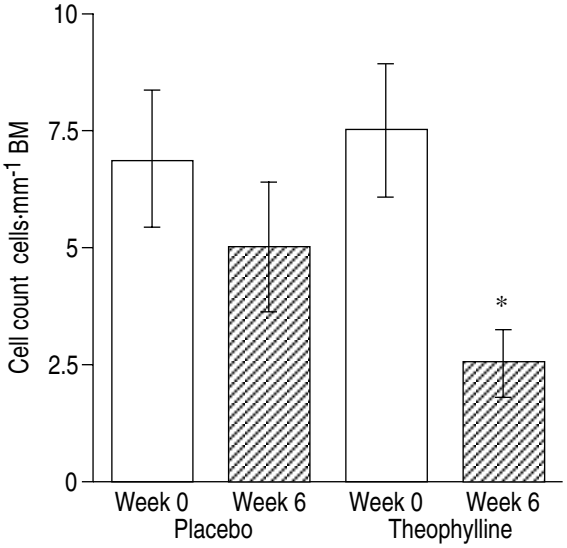

Fig. 2. – EG2+ cell count as cell·mm^{-1} basement membrane (BM) to a depth of 60 µm. *: p<0.05 for paired comparison with pre-treatment value (Wilcoxon rank sign test). Mean values±SEM. (This figure is adapted from [49]).

Furthermore, a recent study has shown that treatment of mild allergic subjects with theophylline for 6 weeks significantly reduced the number of EG2+ eosinophils in biopsies obtained from allergic subjects (fig. 2) [49], and the number of CD4+ lymphocytes in BAL fluid [45]. These effects occurred with plasma levels of 6.6 $mg \cdot L^{-1}$, which are considered subbronchodilator, and suggest that the anti-inflammatory and immunomodulatory actions of theophylline may occur at lower concentrations than those suggested to induce bronchodilatation [49]. Such findings may have significant implications for the future use of xanthines and selective PDE isoenzyme inhibitors, as side-effects appear not to be important at these lower plasma levels when compared with placebo.

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