

## Review Article

# Anti-inflammatory effects of theophylline and selective phosphodiesterase inhibitors

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### ABSTRACT

Theophylline has been used in the treatment of airway diseases, for more than 50 years with benefit thought to be derived from its ability to elicit bronchodilatation. Recent evidence has, however, suggested that theophylline possesses anti-inflammatory activity. The molecular mechanism of action remains unclear, although inhibition of the phosphodiesterase (PDE) enzyme, an enzyme which catalyzes the breakdown of cAMP and cGMP, has been proposed. Theophylline is a relatively weak inhibitor of PDE although there is evidence to suggest that PDE activity is elevated in leukocytes from patients with atopic disease. Thus, an altered responsiveness to PDE inhibitors may partly explain the mechanism of action of theophylline. The PDE enzyme exists as the least of seven different isoenzyme forms which can be characterized on the basis of a number of criteria including substrate specificity, sensitivity to selective inhibitors and the effect of allosteric modulators. The type IV isoenzyme is the predominant isoenzyme in inflammatory cells although it exists together with the type III isoenzyme in T-lymphocytes. There is considerable evidence from *in vitro* and *in vivo* studies suggesting that selective PDE IV inhibitors have anti-inflammatory activity. The following article reviews these studies, together with clinical studies demonstrating that theophylline has anti-inflammatory activity.

### INTRODUCTION

Theophylline and related xanthines have been used in the treatment of diseases of the airways for over 50 years and remain widely prescribed drugs world-wide for the treatment of bronchial asthma.<sup>1–3</sup> The therapeutic effectiveness of this class

of drugs was traditionally thought to be derived from their ability to produce bronchodilatation. However, it is now becoming increasingly apparent that theophylline, in particular, has both immunomodulatory and anti-inflammatory activity. Although theophylline has been used extensively in the treatment of asthma, its molecular mechanism(s) is not clear. Several putative mechanisms of action have been suggested to explain the therapeutic effectiveness of theophylline and related xanthines. One mechanism that has been proposed is that theophylline, through non-selective phosphodiesterase (PDE) inhibition, raises intracellular cyclic 3',5'-adenosine monophosphate (cAMP) and cyclic 3',5'-guanosine monophosphate (cGMP) levels resulting in relaxation of airway smooth muscle and inhibition of inflammatory cell activation. Theophylline is a relatively weak inhibitor of PDE, although there is evidence to suggest that PDE activity is elevated in leukocytes from patients with atopic disease (atopic dermatitis, asthma, allergic rhinitis),<sup>4</sup> and thus an altered responsiveness to PDE inhibitors may partly explain the mechanism of action of theophylline.

Seven gene families of the PDE enzyme have so far been defined, each of which can be distinguished by a variety of criteria including their substrate specificity, sensitivity to selective inhibitors and the effect of allosteric modulators.<sup>5</sup> The type IV isoenzyme appears to be the main isoenzyme of PDE inflammatory cells, although it exists together with the type III isoenzyme in T cells.<sup>6</sup> It is becoming increasingly apparent that theophylline possesses anti-inflammatory and immunomodulatory actions<sup>7–9</sup> that may well contribute to the clinical efficacy of this drug in the treatment of asthma.

### EFFECTS OF THEOPHYLLINE AND SELECTIVE PDE ISOENZYME INHIBITORS ON CELLS INVOLVED IN ASTHMA

The PDE isoenzymes have now been characterized in many inflammatory cells and a variety of studies have examined the potential anti-inflammatory effect of theophylline and selective

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PDE isoenzyme inhibitors on these cells. These include mast cells, basophils, lymphocytes, natural killer cells, eosinophils, neutrophils and macrophages.

## IN VITRO STUDIES

### Effects on mast cells

Human lung mast cells release a variety of mediators including histamine, leukotriene C<sub>4</sub> (LTC<sub>4</sub>) and prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) which are thought to account for the majority of allergen-induced bronchoconstriction in allergic asthmatics. Theophylline has been shown to reduce antigen and anti-IgE receptor antibody-stimulated histamine release from rat peritoneal mast cells<sup>10</sup> and reduce histamine release from human basophils.<sup>11</sup> However, such effects are only seen at concentrations in excess of those achievable *in vivo*. Cultured murine mast cells contain a type IV PDE isoenzyme and selective inhibitors of the type IV isoenzyme have also been shown to inhibit histamine release.<sup>6</sup>

### Effects on lymphocytes

There is evidence to suggest that activated T cells play a key role in the pathogenesis of allergic inflammatory diseases such as bronchial asthma.<sup>12</sup> T cells are likely to play a role in all antigen-driven inflammatory responses since they are the only cell type that directly recognizes and responds to processed antigens. They are involved in many stages of the allergic response including regulation of IgE production by B cells and recruitment of other inflammatory cells such as eosinophils. In the mid 1970s, theophylline was used to produce clones of peripheral blood mononuclear cells with possible suppressor cell activity.<sup>13</sup> In a more recent study, incubation of peripheral blood lymphocytes with theophylline caused them to suppress autologous cell responses.<sup>14</sup> This phenomenon was demonstrated to be due to a subgroup of T cells which were sensitive to *in vitro* stimulation by theophylline.<sup>13</sup> Subsequently a number of *in vitro* investigations have suggested that xanthines have the ability to modify lymphocyte behaviour, in particular the ability to inhibit the lymphocyte proliferation.<sup>15</sup> Two studies reported that theophylline inhibited T cell proliferation following antigenic<sup>16</sup> and mitogenic<sup>17</sup> stimulation and it has also been shown to diminish E rosette formation.<sup>18</sup> The synthesis and release of interleukin 2 (IL-2) is a necessary stimulus for lymphocyte proliferation.<sup>19</sup> It is of interest therefore that theophylline has been found to inhibit interferon-gamma (IFN-gamma)-induced expression of IL-2 receptors on a cultured lymphocyte line.<sup>20</sup> Theophylline can also inhibit production<sup>16</sup> and release<sup>21</sup> of IL-2.

Type III and IV PDE isoenzymes have been characterized in T cells<sup>22,23</sup> and selective inhibitors of the type III and IV PDE isoenzyme have been shown to inhibit phytohaemagglutinin (PHA)-stimulated human<sup>24</sup> and murine<sup>25</sup> T cell proliferation. In a study using rat thymocyte cells, type III, IV and V inhibitors were examined for their ability to inhibit the concavaline A-stimulated pro-

liferative response.<sup>26</sup> The type IV PDE inhibitor, rolipram, was the most effective at inhibiting proliferation whilst the type III and V inhibitors only modestly inhibited at high concentrations. In another study, selective inhibitors of the type IV PDE isoenzyme inhibited anti-CD3-stimulated human lymphocyte proliferation<sup>15</sup> and combined addition of the type III inhibitor, motapizone, and the type IV inhibitor, rolipram, produced a more potent and complete inhibition, an effect which was also seen with the mixed type III-IV inhibitor, zardaverine. Another, very recent study has found that the proliferation and cytokine secretion of T helper (CD4<sup>+</sup>) and T suppressor (CD8<sup>+</sup>) cells is inhibited by type III and IV PDE isoenzyme-selective inhibitors.<sup>27</sup> There is also preliminary evidence to suggest that type IV PDE inhibitors can inhibit expression of IL-4 and IL-5 genes in Th<sub>2</sub> cells.<sup>28</sup>

Many studies have examined the effects of selective PDE inhibitors on mononuclear cell preparations. Type IV PDE inhibitors were shown to inhibit N-formyl-met-leu-phe (fMLP)-stimulated arachidonic acid<sup>29</sup> and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ )<sup>30</sup> release from human mononuclear cells. Type IV and mixed type III-IV PDE inhibitors have also been demonstrated to inhibit the PHA-stimulated proliferation of human peripheral blood mononuclear cells (HPBM).<sup>24,31</sup> In a recent study, the atopic PDE IV isoenzyme in mononuclear leukocytes was found to be more sensitive to type IV inhibitors than the type IV isoenzyme from normal subjects<sup>32</sup> and the type IV inhibitor, RO 20-1724, reduced hyper-IgE synthesis by atopic dermatitis cells *in vitro*.<sup>33</sup> Two studies have also demonstrated that therapeutic levels of theophylline (10–20  $\mu$ g/mL) can inhibit the tumoricidal activity of natural killer cells.<sup>34,35</sup>

### Effects on macrophages

Airway macrophages arise from circulating monocytes, which mature into macrophages during their residence in the airways. In asthmatic subjects, macrophages release a variety of chemical mediators in response to allergen challenge including potent eosinophil chemoattractants like platelet activating factor (PAF), leukotriene B<sub>4</sub> (LTB<sub>4</sub>) and cytokines. Studies have demonstrated that after exposure to low therapeutic levels of theophylline, peripheral blood mononuclear cells and alveolar macrophages obtained from bronchoalveolar lavage fluid (BALF) of normal subjects secrete less superoxide anions *in vitro*<sup>36</sup> and theophylline has been shown to inhibit opsonized zymosan-stimulated H<sub>2</sub>O<sub>2</sub> generation from human alveolar macrophages.<sup>37</sup> In addition, theophylline and IBMX inhibit the generation of the potent eosinophil chemoattractants, PAF, complement C2 and LTB<sub>4</sub> from human monocytes,<sup>38,39</sup> and also the synthesis and release of TNF $\alpha$  from human peripheral blood monocytes.<sup>40</sup>

The type IV isoenzyme is the main cAMP PDE isoenzyme that has been identified in murine macrophages.<sup>41</sup> The selective type III PDE inhibitor, motapizone, the type IV inhibitor, rolipram, and

the mixed type III–IV PDE inhibitor, zardaverine, have been shown to inhibit TNF release from human macrophages.<sup>15</sup> In guinea pig and mouse peritoneal macrophages, PDE IV inhibition suppresses lipopolysaccharide (LPS)-stimulated TNF production<sup>42</sup> and superoxide generation.<sup>43</sup>

### Effects on eosinophils

There is now strong evidence to suggest that eosinophils play an important role in lung inflammation caused by asthma through their ability to release a variety of toxic and proinflammatory mediators. These include eicosanoids, reactive oxygen species, cytokines and basic proteins. The basic proteins, major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) are thought to contribute to the damage observed in the epithelial lining of the airways of asthmatics.<sup>44,45</sup> Therapeutic concentrations of theophylline and other cAMP elevating agents (e.g. cAMP analogues) have been demonstrated to inhibit the release of basic proteins such as EDN<sup>46</sup> and also immunoglobulin-induced eosinophil degranulation. In another study, theophylline was found to inhibit the release of ECP and reactive oxygen species from highly purified blood eosinophils<sup>47</sup> with a potency nearly identical to that for inhibition of PDE type IV activity. Superoxide anions and arachidonic acid derivatives are generated by leukocytes and it has been suggested that they may contribute to airway injury, oedema and smooth muscle contraction seen in asthma.<sup>48</sup> In one study by Yukawa *et al.*, concentrations of theophylline which cover the therapeutic range ( $10^{-6}$ – $10^{-5}$  mmol), significantly potentiated opsonized zymosan-induced  $O_2^-$  generation from isolated guinea pig and human eosinophils, whereas at high concentrations there was a significant inhibition of the release.<sup>49</sup> In another study, therapeutic concentrations of theophylline ( $10$ – $50$   $\mu$ mol) have been shown to significantly inhibit the respiratory burst and also arachidonic acid metabolite generation induced by chemotactic peptides such as fMLP and the calcium ionophore, A23187.<sup>50,51</sup>

The type IV PDE isoenzyme is the only PDE isoenzyme which has been characterized in guinea pig eosinophils<sup>52</sup> and a number of studies have examined the effect of isoenzyme selective inhibitors on the respiratory burst and the release of cationic proteins from guinea pig eosinophils. The type IV PDE inhibitors, rolipram and RO 20-1724, at concentrations which inhibited degradation of cAMP have also been found to inhibit the leukocyte respiratory burst in both activated guinea pig peritoneal and human eosinophils.<sup>52–55</sup> Another selective type IV PDE inhibitor, RP 73401, inhibited superoxide generation as well as  $LTB_4$ -stimulated MBP and ECP release from guinea pig eosinophils, however, these were dose dependent.<sup>56</sup>

### Effects on neutrophils

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. Incubation of neutrophils with theophylline concentrations which cover the therapeutic range inhibited  $LTB_4$  generation by 90%. In three studies by Nielson *et al.*, therapeutic concentrations of theophylline inhibited neutrophil activation and potentiated the effect of isoprenaline.<sup>50–53</sup> In contrast, two separate studies have reported an enhancing effect of therapeutic concentrations of theophylline on human neutrophil superoxide production.<sup>57–59</sup> The reason for these discrepancies is not yet known. Like eosinophils, the type IV isoenzyme has been characterized in neutrophils. Rolipram and RO 20-1724 can inhibit the respiratory burst in neutrophils,<sup>53</sup> an effect that can be inhibited by the protein kinase A antagonist, H89.<sup>59</sup> Inhibition of PDE isoenzymes by selective inhibitors has also been shown to decrease the chemotaxis and chemokinesis of human blood neutrophils *in vitro*.<sup>60</sup>

### Effects on endothelial and epithelial cells

The endothelium provides the major permeability barrier of the vessel wall. Polymorphonuclear leukocytes may damage endothelial cells by releasing agents, such as  $H_2O_2$ , resulting in an increase in vascular permeability. There is evidence to suggest that type III and IV PDE inhibitors abolish the increase in permeability of cultured porcine endothelial cells induced by  $H_2O_2$ , suggesting that PDE inhibition may result in a decrease in microvascular permeability.<sup>61</sup>

Epithelial cells not only act as a protective barrier to the external environment but also represent a metabolically active secreting tissue which may actively interact with inflammatory cells. Human airway epithelial cells produce PAF, granulocyte macrophage colony stimulating factor (GM-CSF), prostaglandin  $E_2$  ( $PGE_2$ ),  $PGF_{2\alpha}$  and 15-lipoxygenase pathway-derived eicosanoids, all of which may influence other inflammatory cell types. IBMX, a non-selective PDE inhibitor has been found to inhibit the bradykinin-stimulated generation of  $PGE_2$  by epithelial cells.<sup>62</sup>

### IN VIVO STUDIES

The 'anti-inflammatory' properties of theophylline and selective PDE inhibitors has been examined in a number of studies utilizing different animal models. Exposure of sensitized animals to antigen results in acute airway obstruction and an influx of inflammatory cells into the airway lumen. These animal models have been used to examine the effect of both currently prescribed anti-asthma drugs and also novel anti-asthma agents. In rat<sup>8,63</sup> and guinea pig models,<sup>64</sup> xanthines have been shown to inhibit the inflammatory and hyper-responsive components induced by allergen. Xanthines have also been shown to attenuate the late response<sup>65</sup> and the eosinophil-infiltration induced by allergen<sup>66</sup> in allergic rabbits.

Acute administration of theophylline and isbutylline has been reported to reduce eosinophil infiltration induced by allergen

challenge in actively sensitized guinea pigs.<sup>64,67</sup> A number of studies have also demonstrated that acute administration of type IV and mixed type III–IV PDE inhibitors is effective at inhibiting eosinophil recruitment into the lungs of sensitized guinea pigs. The type IV PDE inhibitor, rolipram,<sup>68</sup> and the mixed type III–IV PDE inhibitor, zardaverine,<sup>69</sup> given before antigen challenge have also been found to inhibit eosinophil accumulation in the airways. In a separate study, prior administration of the mixed type III–IV inhibitor, benzafentrine, prevented eosinophil accumulation in pulmonary airways of guinea pigs<sup>70</sup> induced by human recombinant GM-CSF, IL-3 and mouse TNF $\alpha$ . Theophylline and the type IV inhibitors, rolipram and RO 20-1724, significantly reduced antigen and PAF-induced eosinophil recruitment.<sup>71</sup> RP 73401 inhibited antigen induced bronchospasm and eosinophil recruitment in previously sensitized conscious guinea pigs.<sup>72</sup> In a study by Turner *et al.*, rolipram given 1 h before antigen challenge inhibited both eosinophil recruitment and the development of airway hyper-responsiveness in allergic monkeys.<sup>3</sup>

However, most studies which have examined the effect of theophylline and selective PDE isoenzyme inhibitors on eosinophil accumulation are not directly relevant to the clinical use of theophylline for two reasons; firstly the doses which have been used in these studies have been given as one acute dose, before antigen challenge, and secondly because the doses used in these studies are often much higher than those used clinically. Two studies conducted by Sanjar *et al.* found that 7-day administration of either theophylline or the mixed type III–IV PDE inhibitor, benzafentrine, inhibited both PAF<sup>74</sup> and allergen-induced<sup>75</sup> pulmonary eosinophil accumulation in guinea pig at doses equivalent to those used clinically. Furthermore we have recently shown that much lower concentrations of the mixed PDE type III–IV inhibitor, zardaverine, are required to inhibit eosinophil infiltration when the drug is administered chronically compared with when the drug is administered acutely.<sup>76</sup> In addition, rolipram given twice daily for 3 days before antigen challenge attenuated antigen-induced airway hyper-responsiveness and pulmonary eosinophilia in the allergic rabbit.<sup>66</sup> The type IV inhibitor, rolipram, has also been shown to prevent recruitment of eosinophils to the skin.<sup>77</sup>

Microvascular leakage in asthmatic airways leads to plasma exudation into the airway lumen and plasma exudation is a consistent indicator of airway inflammation.<sup>77</sup> Theophylline has been demonstrated to reduce plasma exudation in the guinea pig tracheobronchial mucosa induced by local administration of inflammatory stimuli.<sup>79–82</sup> In a separate study, theophylline and rolipram inhibited PAF-induced leakage into bronchial walls as well as into BALF.<sup>83</sup> A PDE type IV inhibitor has also been shown to be effective at inhibiting airway microvascular leakage induced by PAF and allergen in guinea pigs.<sup>84</sup>

## Clinical studies

Plasma exudation is a consistent indicator of ongoing inflammatory processes in both asthma and allergic rhinitis, and is used as a clinical model of airway inflammation to study the effects of anti-asthma agents. Treating patients with allergic rhinitis with theophylline has been demonstrated to reduce nasal plasma exudate secretion.<sup>7,85</sup> Several studies have reported that theophylline has a minimal effect on acute allergic bronchoconstriction which suggests that theophylline is not likely to inhibit lung mast cells *in vivo* in any major way.<sup>9,86</sup> This conclusion is supported by a recent study showing that treatment with theophylline for 5 weeks did not alter the increased levels of the mast-cell-derived mediators, PGD<sub>2</sub> and histamine, in BAL fluid.<sup>87</sup>

However, in another study, a 1 week course of theophylline treatment caused a reduction in histamine release in subjects with allergic rhinitis following antigen challenge.<sup>85</sup> 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.

A number of studies have examined the effect of chronic treatment with theophylline on the behaviour and number of different inflammatory cell types found in human blood or BALF. In one study, after asthmatic children were treated for 10 days with oral theophylline, a reduction in neutrophil and mononuclear cell chemotaxis was observed.<sup>88</sup> Alveolar macrophages have been shown to generate less superoxide *ex vivo* after *in vivo* oral theophylline treatment. A reduction in alveolar macrophage intracellular killing, bactericidal killing and H<sub>2</sub>O<sub>2</sub> release was also observed, and the degree of impairment was found to correlate well with BAL theophylline concentrations.<sup>89</sup> In a separate study, the effect of theophylline on eosinophil and neutrophil activity was compared with that of budesonide in a 1 year double blind trial. Both drugs had similar effects in reducing eosinophil and neutrophil activation as well as having comparable clinical efficacy.<sup>90</sup>

The late asthmatic reaction (LAR) to allergen exposure has commonly been used as a model of airway inflammation and this model has been used to evaluate anti-inflammatory drugs. Several studies have now reported that theophylline can inhibit the LAR.<sup>86,91,92</sup> Interestingly, if allergic subjects are challenged with antigen after a period of chronic treatment with theophylline, an inhibition of the LAR at subbronchodilator plasma concentrations occurs.<sup>91,93</sup> Similar results have been found during oral theophylline therapy after challenge with toluene diisocyanate in sensitive asthmatics.<sup>94</sup> Theophylline has also been reported to reduce the overnight fall in forced expiratory volume in 1 s (FEV<sub>1</sub>) and also attenuated the bronchoalveolar lavage (BAL) neutrophil accumulation observed in patients with nocturnal asthma.<sup>95</sup>

There is evidence from two separate studies on asthmatic children that a decrease in the number of peripheral blood T

cells which express suppressor activity occurs<sup>96,97</sup> and one of these studies found a correlation between T suppressor cell numbers and asthma severity. In both studies, after 1 month of theophylline treatment, the number of T suppressor cells returned to the normal level of the control group. In another study, chronic treatment with theophylline resulted in an increase in the number and activity of T suppressor cells found in asthmatic subjects.<sup>98</sup> Asthmatics who are on long-term theophylline treatment have more peripheral T cells that are able to suppress plaque formation in lymphocyte cultures and have a reduced *in vitro* graft versus host response than do asthmatics who are on other treatments.<sup>14</sup> In a study performed by Fink *et al.*, asthmatics were chronically treated with theophylline. Theophylline therapy was then withdrawn and clinical, functional and immunological parameters were studied. A clinical deterioration and fall in lung function was seen together with a fall in activated T helper (CD4<sup>+</sup>) and T suppressor (CD8<sup>+</sup>) peripheral blood lymphocytes.<sup>99</sup> Bronchial biopsies which were performed in a subgroup of patients showed an increase in the number of CD4<sup>+</sup> and CD8<sup>+</sup> T cells during theophylline withdrawal suggesting that theophylline may inhibit T cell trafficking from the circulation into the airways.<sup>100</sup> In this study the mean plasma theophylline concentration was <10 mg/L demonstrating that modulatory effects may be observed at low plasma concentrations. In a study by Ward *et al.*, theophylline at a serum concentration of 7.8 mg/L inhibited the late asthmatic reaction following allergen challenge and suppressed the allergen-induced increase in CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes in peripheral blood,<sup>9</sup> 48h after allergen challenge.

Finally, a recent study has shown that treatment of mild allergic subjects with theophylline for 6 weeks significantly reduced the number of EG2<sup>+</sup> eosinophils in biopsies obtained from allergic subjects and the number of CD4<sup>+</sup> lymphocytes in BALF.<sup>87,93</sup> These effects occurred with plasma levels of 6.7 mg/L 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.<sup>93</sup> These findings have recently been confirmed by measuring EG2<sup>+</sup> eosinophils in BAL fluid/sputum in patients undergoing bronchial provocation.<sup>101</sup> Such findings may have significant implications on the future use of xanthines and selective PDE isoenzyme inhibitors as toxicity appears unimportant at lower plasma levels. Furthermore, these findings suggest that theophylline should be considered as having important anti-inflammatory actions in the lung and should be re-evaluated in the treatment of asthma.

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