

## Review Article

# Differential effects of corticosteroids and theophylline on the adhesive interaction between eosinophils and endothelial cells

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### ABSTRACT

Corticosteroids and theophylline have been used widely for the treatment of asthma. These two classes of drugs appear to reduce the tissue infiltration of eosinophils, predominant inflammatory cells in the airways of asthmatic patients. Corticosteroids inhibit the generation of both endothelial-activating Th2 cytokines (e.g. interleukin (IL)-4/IL-13) and eosinophil growth factors (e.g. IL-5/granulocyte-macrophage colony stimulating factor) and also attenuate the effects of eosinophil growth factors on the differentiation and prolonged survival of eosinophils. However, corticosteroids modulate directly neither eosinophil adhesiveness nor the expression of adhesion proteins on endothelial cells *in vitro*. Therefore, it is likely that the inhibitory effect of corticosteroids on the tissue infiltration of eosinophils is the consequence of indirect mechanisms, mainly via the inhibition of cytokines. Interestingly, theophylline, which is generally accepted as a bronchodilator, attenuates eosinophil adhesion to endothelial cells *in vitro* at a clinically therapeutic concentration. Furthermore, theophylline inhibits the expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 on endothelial cells that had been stimulated with IL-4 plus tumor necrosis factor- $\alpha$ . Thus, theophylline possibly exerts an inhibitory effect on both the adhesive property of eosinophils and the expression of adhesion molecules on endothelial cells. These findings possibly indicate that theophylline would be adequate to supplement the actions

of corticosteroids in asthmatic airway inflammation, partly via its inhibitory effect on the interaction between blood eosinophils and endothelial cells.

**Key words:** cell adhesion, corticosteroids, endothelial cells, eosinophils, theophylline.

### INTRODUCTION

Corticosteroids and theophylline have been used widely for the treatment of asthma. There is increasing evidence that not only corticosteroids, but also theophylline attenuates eosinophil accumulation in the airways of asthmatic patients.<sup>1–3</sup> For example, Sullivan *et al.*<sup>1</sup> have reported that theophylline attenuates eosinophil airway accumulation in response to allergen inhalation in atopic asthma. More recently, Aizawa *et al.*<sup>2</sup> have shown that theophylline treatment in mild to moderate asthma resulted in a reduction in the percentage of eosinophils from induced sputa. Similarly, Lim *et al.*<sup>3</sup> have found that theophylline reduces the number of eosinophils in sputa, biopsied specimens and bronchoalveolar lavage fluid in mild asthmatic patients.

It is plausible that eosinophils are pivotal effector cells in asthma. For example, recent evidence indicates that the number and activation of eosinophils are reduced by anti-interleukin (IL)-5 monoclonal antibody (mAb), resulting in the significant attenuation of extracellular matrix protein deposition in the airway subepithelium of asthmatic patients.<sup>4</sup> Although the accumulation of eosinophils into the airways of asthmatic patients depends on multiple factors, including migration through tissue and prolongation of cell survival, the initial step is the adhesion of circulating eosinophils to vascular endothelial cells. It is generally accepted that this process is mainly mediated by the interaction between eosinophil integrin

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adhesion molecules, including  $\alpha_4$  integrins such as  $\alpha_4\beta_1$  (CD49d/CD29; VLA-4),  $\beta_2$  integrins such as  $\alpha_L\beta_2$  (CD11a/cd18; LFA-1) and  $\alpha_M\beta_2$  (CD11b/CD18; Mac-1), and their counterligands vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 on endothelial cells.<sup>5,6</sup> In the present review, the mechanisms by which corticosteroids and theophylline modulate eosinophil accumulation into the airways of asthmatic patients are discussed. The author focuses particularly on their effects on the adhesive interaction between blood eosinophils and endothelial cells.

### EFFECTS OF CORTICOSTEROIDS ON EOSINOPHILIC INFLAMMATION

Corticosteroids are used as first-line drugs in the treatment of bronchial asthma. Numerous studies have revealed that corticosteroids decrease the number of eosinophils and the expression of inflammatory cytokines in the airways of asthmatic patients.<sup>7</sup> Corticosteroids attenuate the generation of Th2 cytokines, including IL-5 and CC chemokines.<sup>8,9</sup> Corticosteroids also inhibit the differentiation and prolonged survival of eosinophils induced by growth factors such as IL-5<sup>10</sup> and suppress the migration of eosinophils *in vitro*.<sup>11,12</sup> Finally, there is evidence that corticosteroids directly induce apoptosis of eosinophils.<sup>13,14</sup>

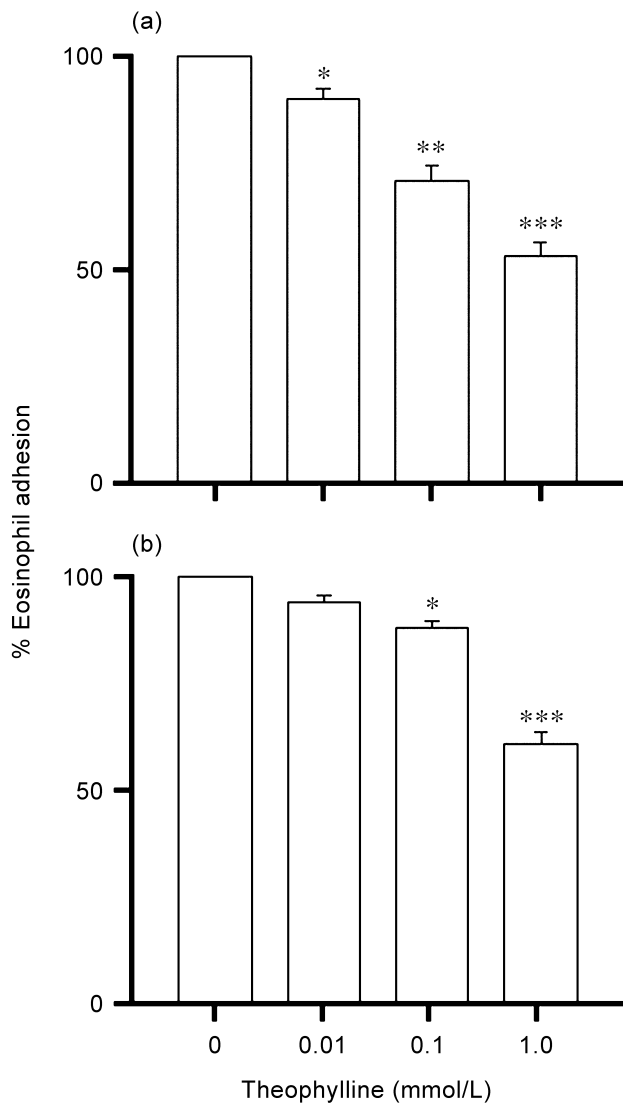
Sutani *et al.*<sup>15</sup> and Kaiser *et al.*<sup>16</sup> have evaluated whether corticosteroids modulate the adhesive interaction between blood eosinophils and endothelial cells. In our study,<sup>15</sup> dexamethasone did not inhibit the spontaneous or *N*-Formyl-methionyl-leucyl-phenylalanine (FMLP)- or IL-5-stimulated adhesion of eosinophils to endothelial cells. These results are consistent with a previous report using budesonide<sup>16</sup> and suggest that corticosteroids are devoid of a direct inhibitory effect on the adhesiveness of eosinophils. We have also observed that dexamethasone does not modify the expression of VCAM-1 or ICAM-1 on human pulmonary microvascular endothelial cells stimulated with IL-4 and tumor necrosis factor (TNF)- $\alpha$ .<sup>15</sup> A previous study has also demonstrated that budesonide fails to modify the expression of adhesion molecules on cytokine-stimulated human umbilical vein endothelial cells.<sup>16</sup> Together, these results suggest that corticosteroids have no ability to modify the expression of adhesion molecules on endothelial cells induced by cytokines. Therefore, it is likely that the inhibitory effect of corticosteroids on the tissue infiltration of eosinophils is the

consequence of other effects, probably inhibition of cytokine generation.

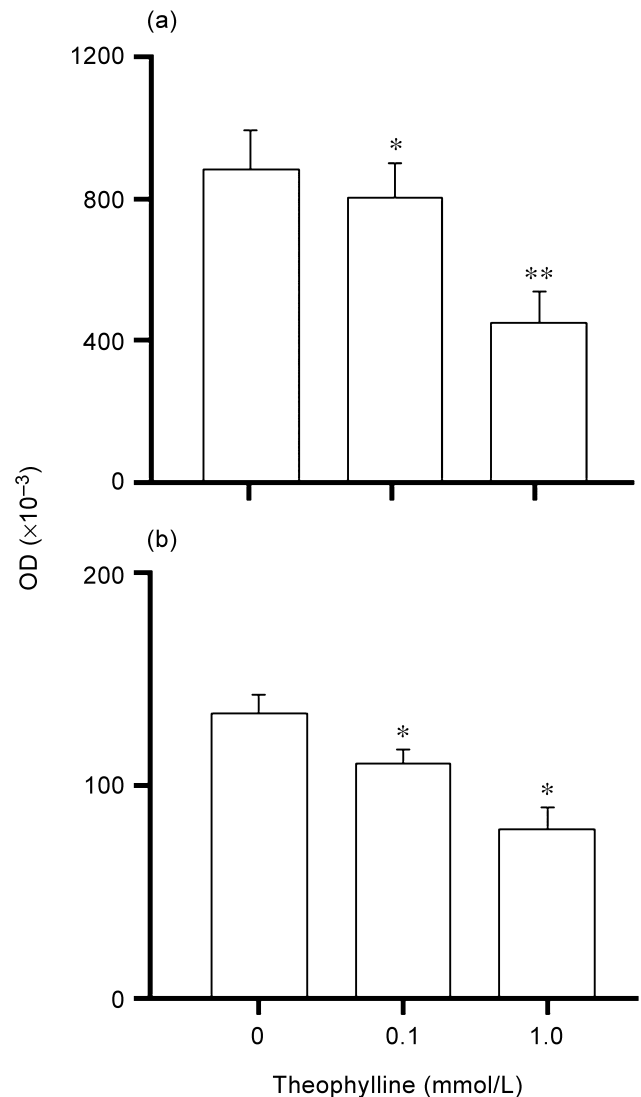
### THEOPHYLLINE ATTENUATES ADHESIVE INTERACTION BETWEEN EOSINOPHILS AND ENDOTHELIAL CELLS

Traditionally, theophylline has been used as a bronchodilator to control the tone of airway smooth muscle in asthmatic patients. More recently, theophylline has been shown to induce a variety of inhibitory effects on inflammatory cells involved in asthmatic inflammation. In eosinophils, theophylline accelerated apoptotic events<sup>17</sup> and attenuated both the release of specific granule proteins<sup>18,19</sup> and the surface expression of CD11b.<sup>19</sup> In this context, we evaluated whether theophylline modifies the adhesive properties of blood eosinophils and the expression of adhesion molecules on human umbilical vein endothelial cells (HUVEC).<sup>20</sup> Consequently, we found that theophylline attenuated the IL-5- or FMLP-stimulated adhesion of eosinophils to endothelial cells (Fig. 1). This anti-inflammatory effect of theophylline seems to be due, at least in part, to the inhibition of type IV phosphodiesterase (PDE) because rolipram, a selective type IV PDE inhibitor, and *N*6,2'-*O*-dibutyladenosine (db)-cAMP, a cAMP analog, also attenuated eosinophil adhesion to endothelial cells.<sup>20</sup> We also observed that theophylline attenuated the expression of ICAM-1 or VCAM-1 on HUVEC that had been induced by IL-4 plus TNF- $\alpha$  (Fig. 2). These effects seemed to be observed at therapeutically relevant concentrations of theophylline. Collectively, these results suggest the potentially important inhibitory effect of theophylline on the adhesive interaction between circulating eosinophils and vascular endothelial cells.

The adhesion molecule responsible for the effect of theophylline on eosinophil adhesion is likely to be a  $\beta_2$ -integrin, because we have confirmed that FMLP- and eosinophil growth factor-activated eosinophil adhesions are  $\beta_2$ -integrin dependent.<sup>21</sup> In this context, theophylline has been shown to attenuate the expression of CD11b, a  $\beta_2$ -integrin molecule on eosinophils.<sup>19</sup> However, in the  $\beta_2$ -integrin-dependent adhesion of leukocytes, the activation state of this integrin may be more relevant than the number of molecules expressed on the cell surface. Therefore, we speculate that theophylline modulates either the enhanced expression or conformational change of  $\beta_2$ -integrin that increases eosinophil ability for cell adhesion.



**Fig. 1** Effect of theophylline on eosinophil adhesion to human umbilical vein endothelial cells induced by (a) 1  $\mu$ mol/L *N*-Formyl-methionyl-leucyl-phenylalanine or (b) 100 pmol/L interleukin-5. Data are the mean  $\pm$  SEM ( $n = 7$ ). \* $P < 0.05$  compared with control; \*\* $P < 0.001$  compared with control and 0.01 mmol/L theophylline; \*\*\* $P < 0.001$  compared with the other three conditions. (Reproduced with the permission of S Karger AG, Basel, from Choo *et al.*<sup>20</sup>)



**Fig. 2** Effect of theophylline on the expression of (a) intercellular adhesion molecule-1 or (b) vascular cell adhesion molecule-1 on human umbilical vein endothelial cells stimulated by the combination of interleukin-4 and tumor necrosis factor- $\alpha$  (both at 100 pmol/L). Data are the mean  $\pm$  SEM ( $n = 5$ ). \* $P < 0.05$ , \*\* $P < 0.01$  compared with control;  $P < 0.05$  compared with 0.01 mmol/L theophylline. OD, optical density. (Reproduced with the permission of S Karger AG, Basel, from Choo *et al.*<sup>20</sup>)

### CLINICAL IMPLICATIONS OF THE DIFFERENTIAL EFFECT OF CORTICOSTEROIDS AND THEOPHYLLINE ON EOSINOPHILIC INFLAMMATION

Our observations and evidence suggest that corticosteroids attenuate eosinophilic inflammation in asthma mainly via the downregulation of cytokine production,

but have no direct action on eosinophil adhesiveness or endothelial adhesion molecule expression. In contrast, the direct modulatory effect of theophylline on the interaction between circulating eosinophils and endothelial cells may be a novel mechanism for the attenuation of eosinophil accumulation in the airways of asthmatic patients. Although the exact impact of these inflammatory

properties on the clinical effects of theophylline remains to be clarified, it is probable that the effects of theophylline in asthma manifest eventually through a combination of bronchodilatory and anti-inflammatory properties.

The combination of inhaled corticosteroids and theophylline has been shown to provide additive clinical and physiological effects in moderate asthma,<sup>22</sup> suggesting that these two classes of drugs act via differential mechanisms to modulate airway changes in asthma. Minoguchi *et al.*<sup>23</sup> have reported that the withdrawal of theophylline in moderate asthmatic patients treated with inhaled corticosteroids resulted in an increase in sputum eosinophils, raising the possibility that these two classes of drugs specifically and differentially act to attenuate eosinophilic infiltration. Theophylline would alter both the bronchial smooth muscle tone and the accumulation and/or functional state of eosinophils, thus potentially providing additive benefits in asthmatic patients treated with inhaled corticosteroids.

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