

Dear Editor

Theophylline Attenuates the Adhesiveness of Endothelial Cells Augmented with Interferon- γ in the Presence of TNF- α for Blood Eosinophils

Theophylline has been traditionally used to treat asthma. It exerts anti-inflammatory effects, including the induction of eosinophil apoptosis¹ and the reduction of degranulation,² adhesion³ and migration⁴ of these cells in response to a T-helper type 2 (Th2) immune reaction. This anti-inflammatory effect of theophylline leads to reduced eosinophil influx to the airways. However, the effect of theophylline on the functions of eosinophils induced by T-helper type 1 (Th1) cytokines remains ambiguous.

Eosinophils can serve as major effector cells in asthma. Adhesion to vascular endothelium is an initial step for eosinophils to accumulate in bronchial tissue. Eosinophils significantly adhere to vascular endothelium stimulated not only by Th2 cytokines,³ but also Th1 cytokines such as interferons (IFNs) in the presence of tumour necrosis factor- α (TNF- α).⁵ This phenomenon partly attributed to the increase in the expression of adhesion molecules on vascular endothelial cells induced by Th2 cytokines or IFNs.^{3,5}

The aim of the present study was to evaluate whether theophylline modifies the adhesion of eosinophils to human umbilical vein endothelial cells (HUVEC) stimulated by IFNs in the presence of TNF- α .

We performed statistical analysis as follows; A paired *t*-test for comparison of two groups and repeated measures ANOVA with Scheffe's constants for comparison between more than two groups. A *p*-value, 0.05 was considered statistically significant.

Initially we confirmed IFNs augment the adhesiveness of vascular endothelial cells for eosinophils in the presence of TNF- α , compared to HUVEC stimulated by only TNF- α , as previously reported⁵ ($n = 7$, data not shown). Methods were as follows; HUVEC (Clonetics, San Diego, CA, USA) were prepared as previously described.⁶ Confluent monolayers of HUVEC in collagen-coated 96-well tissue culture plates were treated with IFN- γ (100-300 pM) together with TNF- α (10 pM) and cultured in 5% CO₂ at 37°C for 24 h. After that, to block the synthesis of mediators, HUVEC were treated as previously described before use.⁵ Eosinophils were isolated from peripheral blood from normal volunteers by the negative immunomagnetic bead selection method as previously described.⁷ Eosinophils were placed onto the HUVEC monolayers and then incubated at 37°C for 30 min. Eosinophil adhesion to HUVEC was assessed as residual eosinophil peroxidase (EPO) activity of adherent eosino-

phils, as previously described.⁸

Subsequently, we determined whether theophylline modulated the spontaneous adhesion of eosinophils to IFN- γ -stimulated HUVEC. We pre-incubated eosinophils with theophylline (0.1-1 mM) for 30 min at 37°C and then incubated eosinophils placed on HUVEC stimulated by 100 pM IFN- γ in the presence of 10 pM TNF- α , or by only TNF- α , at 37°C for 30 min. After that we treated the reaction wells as described above and measured EPO. Theophylline (1 mM) significantly diminished the adhesion rate of eosinophils to HUVEC activated by IFN- γ in the presence of TNF- α , as well as to HUVEC activated by only TNF- α (Fig. 1).

We next investigated whether theophylline reduced the IFN- γ -promoted expression of intracellular adhesion molecule 1 (ICAM-1) and/or vascular cell adhesion molecule 1 (VCAM-1) on HUVEC in the presence of TNF- α , to determine the inhibitory effect of theophylline on the adhesiveness of HUVEC stimulated by IFN- γ . HUVEC were cultured with IFN- γ (100-300 pM) in the presence or absence of TNF- α (10 pM) for 24 h, and the expression of ICAM-1 and VCAM-1 was measured by cell ELISA.⁵ As previously reported,⁵ we confirmed that in the presence of TNF- α , IFN- γ significantly up-regulated the expression of ICAM-1 and VCAM-1 on HUVEC ($n = 3$, data not shown).

Finally, we determined whether theophylline modulated the expression of ICAM-1 and VCAM-1, when stimulated by IFN- γ in the presence of TNF- α . We cultured HUVEC in the presence or absence of theophylline for 1 h and stimulated them with a combination of IFN- γ (100 pM) and TNF- α (10 pM) for 24 h, and then the expression of ICAM-1 and VCAM-1 were measured by cell ELISA. Theophylline significantly reduced the expression of not VCAM-1 but ICAM-1 on HUVEC stimulated by IFN- γ in the pres-

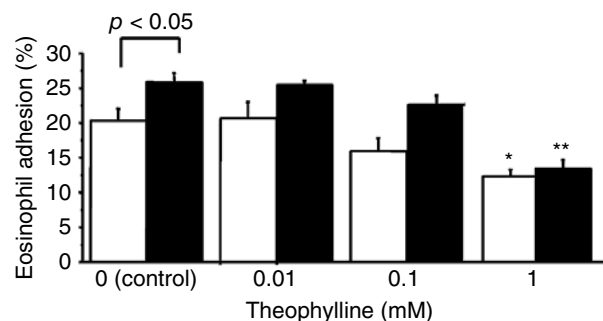


Fig. 1 Effect of theophylline on eosinophil adhesion to HUVEC stimulated by 10 pM TNF- α in the absence of (open column), or in the presence of 100 pM IFN- γ (black closed column). For each bar, the mean $\pm$ SEM of 5 experiments is shown. * $p < 0.01$, compared to control; ** $p < 0.0001$, compared to control.

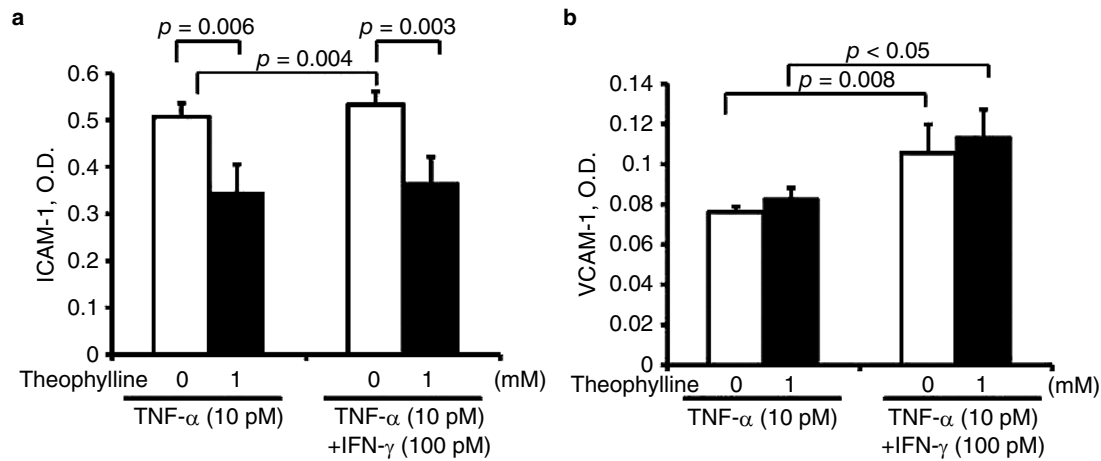


Fig. 2 (a) and (b): Effect of theophylline on the expression of ICAM-1 (a) and VCAM-1 (b) stimulated by TNF- α (10 pM) in the absence of (open column), or in the presence of 100 pM IFN- γ (black closed column). For each bar, the mean $\pm$ SEM of 4 different experiments is shown. OD, optical density.

ence and absence of TNF- α (Fig. 2a, b).

This study showed the reduction of adhesion of eosinophils to IFN- γ -stimulated HUVEC in the presence of TNF- α was attributable to the effect of theophylline on both eosinophils and HUVEC. IFN- γ seemed to enhance the adhesiveness of HUVEC through promotion of the expression of ICAM-1 and VCAM-1 on HUVEC in the presence of TNF- α .⁶ Interaction of eosinophils with VCAM-1 or ICAM-1 seemed to be involved in the enhancement of the effector functions of eosinophils, including the release of radical oxygen species and the production of cysteinyl leukotriene.⁵⁻⁸ The exposure of eosinophils to these molecules can enhance their adhesiveness. Therefore, the reduction of the adhesiveness was partly because theophylline attenuated the expression of ICAM-1 on IFN- γ -stimulated HUVEC. This coincides with the attenuation of ICAM-1 and VCAM-1 on HUVEC stimulated with a combination of interleukin-4 (IL-4) and TNF- α , by theophylline.³ Additionally, the cyclic AMP analogue N 6, 2)-O-dibutyladenosine 3)5)-cyclic monophosphate, attenuated the expression of adhesion molecules on HUVEC stimulated with a combination of IL-4 and TNF- α ,³ suggesting that the reduction of the expression of adhesion molecules by theophylline resulted from the elevation of intracellular cyclic AMP levels.

Theophylline inhibits the up-regulation of β 2 integrin expression on activated eosinophils through the elevation of intracellular cyclic AMP levels.^{2,3,9} Human eosinophils normally express β 2 integrins. Thus theophylline may inhibit function of β 2 integrin on eosinophils, leading to a reduction in the adhesion to IFN- γ -stimulated HUVEC. Theophylline attenuates the expression of L-selectin, which is also involved in extravasation and is increased by IFN- γ .⁹ It may contribute to the reduction of eosinophil adhesion to HU-

VEC.

Our results suggest that theophylline exerts potent anti-inflammatory effects on bronchial inflammation arising from the action of Th1 cytokines. Th1 cytokines contribute to bronchial inflammation in various situations, including resistance to corticosteroids seen in severe asthma, and viral infections leading to asthma exacerbation. Theophylline supplements the anti-inflammatory effects of corticosteroids in asthma.¹⁰ Consequently, theophylline can be a feasible medication option, in addition to corticosteroids, especially for asthma modified by a Th1 immune response.

Tomoyuki Soma^{1,2}, Takehito Kobayashi², Akemi Yokote^{1,2} and Makoto Nagata^{1,2}

¹Department of Respiratory Medicine and ²Allergy Center, Saitama Medical University, Saitama, Japan

Email: tsoma@saitama-med.ac.jp

Conflict of interest: No potential conflict of interest was disclosed.

REFERENCES

- Ohta K, Sawamoto S, Nakajima M *et al*. The prolonged survival of human eosinophils with interleukin-5 and its inhibition by theophylline via apoptosis. *Clin Exp Allergy* 1996;**26**:10-5.
- Kita H, Abu-Ghazaleh RI, Gleich GJ, Abraham RT. Regulation of Ig-induced eosinophil degranulation by adenosine 3',5'-cyclic monophosphate. *J Immunol* 1991;**146**: 2712-8.
- Choo JH, Nagata M, Sutani A, Kikuchi I, Sakamoto Y. Theophylline attenuates the adhesion of eosinophils to endothelial cells. *Int Arch Allergy Immunol* 2003;**131** (S1):40-5.
- Tsukadaira A, Okubo Y, Horie S, Koyama S. Theophylline inhibits TNF-alpha-induced CD4 expression on human eosinophils and CD4+ eosinophil migration. *Int Arch Al-*

- ergy Immunol* 2001;**125**:334-43.
5. Kobayashi T, Takaku Y, Yokote A *et al.* Interferon-beta augments eosinophil adhesion-inducing activity of endothelial cells. *Eur Respir J* 2008;**32**:1540-7.
6. Nagata M, Sedgwick JB, Vrtis R, Busse WW. Endothelial cells upregulate eosinophil superoxide generation via VCAM-1 expression. *Clin Exp Allergy* 1999;**29**:550-61.
7. Nagata M, Saito K, Tsuchiya K, Sakamoto Y. Leukotriene D4 upregulates eosinophil adhesion via the cysteinyl leukotriene 1 receptor. *J Allergy Clin Immunol* 2002;**109**:676-80.
8. Nagata M, Yamamoto H, Shibasaki M, Sakamoto Y, Matsuo H. Hydrogen peroxide augments eosinophil adhesion via β 2 integrin. *Immunology* 2000;**101**:412-8.
9. Spoelstra FM, Berends C, Dijkhuizen B, de Monchy JG, Kauffman HF. Effect of theophylline on CD11b and L-selectin expression and density of eosinophils and neutrophils in vitro. *Eur Respir J* 1998;**12**:585-91.
10. Evans DJ, Taylor DA, Zetterstrom O, Chung KF, O'Connor BJ, Barnes PJ. A comparison of low-dose inhaled budesonide plus theophylline and high-dose inhaled budesonide for moderate asthma. *N Engl J Med* 1997;**337**:1412-8.