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Application Note R100

Respiratory: Eosinophil; Adhesion Assay; Asthma

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Objectives

To determine novel anti-inflammatory effects of MLK on resting and GM-CSF-stimulated eosinophils using the Cellix VenaFluxTM platform to mimic physiological adhesion to rhVCAM-1

Introduction

Asthma is one of the most common chronic respiratory diseases in developed countries. For most asthmatics their symptoms are satisfactorily controlled by the regular use of inhaled glucocorticoids (GC). However, these drugs are not without side effects and some asthmatic patients derive only partial and in some cases no relief of their symptoms even when using high doses of GC. Clearly, we need more effective therapy for asthma.
Eosinophils are key pro-inflammatory cells in the asthmatic lung where their cytotoxic products cause damage to the airway epithelium, tissue inflammation and airflow obstruction. Eosinophil adhesion to and transmigration through the endothelial cells lining the post-capillary venules are key events in their accumulation in the asthmatic lung. Understanding these mechanisms may lead to the identification of compounds that can blunt eosinophil accumulation. Activation of the cysteinyl leukotriene $cysLT_1$ receptor ($cysLT_1R$) results in eosinophil migration and damage to the mucus layer in the lung. Montelukast (MLK) is part of a new class of anti-asthma drugs that are antagonists to $cysLT_1R$ reducing eosinophil migration. Evidence is accumulating that MLK may have additional anti-inflammatory effects on eosinophil function which were further investigated in this study.

Methods

Refer to Application Note R200 for eosinophil isolation and flow assay details. Concentrations of adhesion proteins, mediators and drugs used were rhVCAM-1; 10 µg mL^{-1}, BSA; 10 µg mL^{-1}, GM-CSF; 10 ng mL^{-1}, LTC4/D4; 100 nM, MLK; 0.1–100 nM, MK571; 100 nM, MK886; 100 nM, Anti-α4β1; 10 µg mL^{-1}, anti-cysLT1R; 10 µg mL^{-1}, Isotype Control; 10 µg mL^{-1}.

Results

MLK (10 and 100 nM) gave partial (~40%) but significant ($P<0.05$) inhibition of unstimulated eosinophil adhesion to rhVCAM-1 at 2 dyne cm^{-2} (Figures 1 and 2). GM-CSF-stimulated eosinophil adhesion under flow was characterised by greater cell flattening with significant ($P<0.05$) inhibition of adherent cell numbers by 100 nM MLK observed (Figure 2). This effect appeared specific for MLK as the analogue MK571 had no significant effect on eosinophil adhesion to VCAM-1 (Figure 3). LTC_4 released from unstimulated or GM-CSF-treated eosinophils did not contribute to their adhesion to VCAM-1 as the leukotriene biosynthesis inhibitor MK886 had no inhibitory effect (Figure 3) while exogenously added LTC_4 did not enhance eosinophil adhesion (Table 1). In contrast, LTD_4, enhanced eosinophil adhesion to VCAM-1; an effect blocked by MLK (Table 1). Comparable observations were also made at 1 dyne cm^{-2} (data not shown).

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Figure 1 Representative experiment illustrating unstimulated eosinophil adhesion at 2 dyne cm-2 to rhVCAM-1 together with the inhibitory effects of pre-incubation with 100nM MLK.

Non-& GM-CSF-stimulated eosinophil adhesion to VCAM-1 at 2 dyne cm-2 montelukast dose response

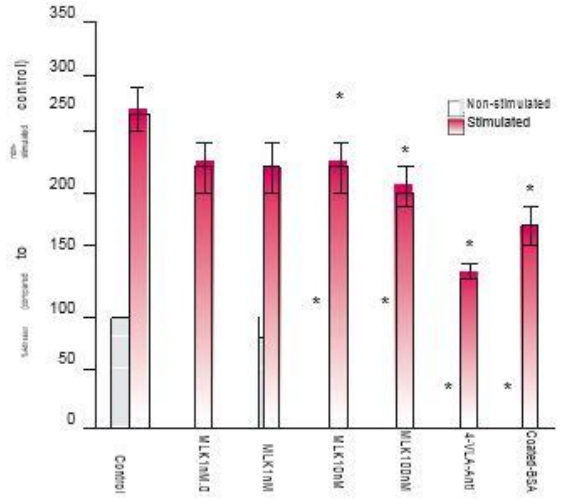

*Figure 2 Dose response of the effect of MLK on non-stimulated and GM-CSF-stimulated eosinophil adhesion to rhVCAM-1 under flow conditions of 2 dyne cm^{-2} (n=4, *P<0.05).*

Non- & GM-CSF-stimulated eosinophil adhesion to VCAM-1 at 2 dyne cm^{-2} effect of MK571, MK 886 and CysLT1 Ab

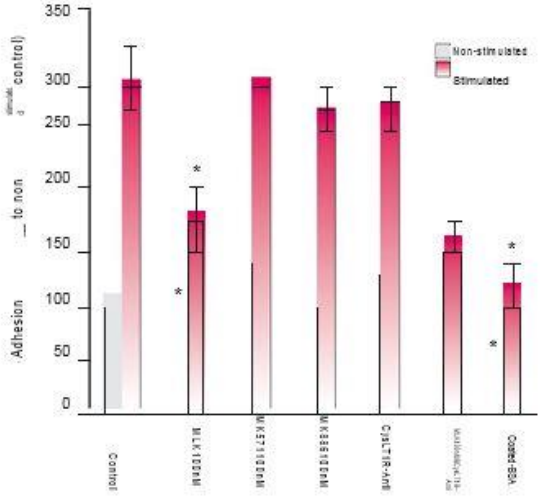

*Figure 3 Effect of MLK, the montelukast an MK571 and the leukotriene biosynthesis inhibitor MK886, on unstimulated or GM-CSF-stimulated eosinophil adhesion to rhVCAM-1. Data expressed as a percentage of non-stimulated eosinophil adhesion at 2 dyne cm^{-2} (n=4, *P<0.05).*

Conditions	Change in adhesion at 2 dyne cm-2 (%)
Nil	100 ± 0.0
100 nM LTC4	111.8 ± 40.6
100 nM LTD4	187.1 ± 7.4*
100 nM LTD4 + 10 nM MLK	103.3 ± 31.7**
100 nM LTD4 + 100 nM MLK	43.8 ± 19.4**
100 nM LTD4 + anti-CysLT1R	111.6 ± 35.9**
100 nM LTD4 + isotope control Ab	155.1 ± 10.4

Table 1: The effect of MLK and an anti-CysLT1R antibody on non-stimulated and LTC4/D4-stimulated eosinophil adhesion to rhVCAM-1 at dyne cm^{-2}. (n=4).

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Conclusions

- Physiologically relevant concentrations of MLK inhibited resting and GM-CSF-stimulated eosinophil adhesion to VCAM-1 in an in vitro model of the post-capillary venules.
- Inhibitory effects by MLK appeared independent of cysLT1R blockade.
- Our study confirms a previous report that MLK inhibited transmigration of eosinophils across human umbilical vein endothelial cells under static conditions2.
- These findings may provide important clues for developing novel therapy aimed at blunting eosinophil-induced inflammation in allergic-based disease.

References

[1] Robinson A.J., Kashanin D, O'Dowd F, Williams V. and Walsh G.M. (2008) Montelukast inhibition of resting and GM-CSF -stimulated eosinophil adhesion to VCAM-1 under flow conditions appears independent of CysLT1 antagonism Journal of Leukocyte Biology (in press).

[2] Virchow Jr., J.C., Faehndrich, S., Nassenstein, C., Bock, S., Matthys, H., & Luttman, W. (2001). Effect of a specific cysteinyl leukotriene receptor antagonist (montelukast) on the transmigration of eosinophils across human umbilical vein endothelial cells. Clin. Exp. Allergy, 31, 836-844.