COMBINATION OF CLOPIDOGREL & MONTELUKAST IN ASTHMA TREATMENT: AN IN VIVO STUDY

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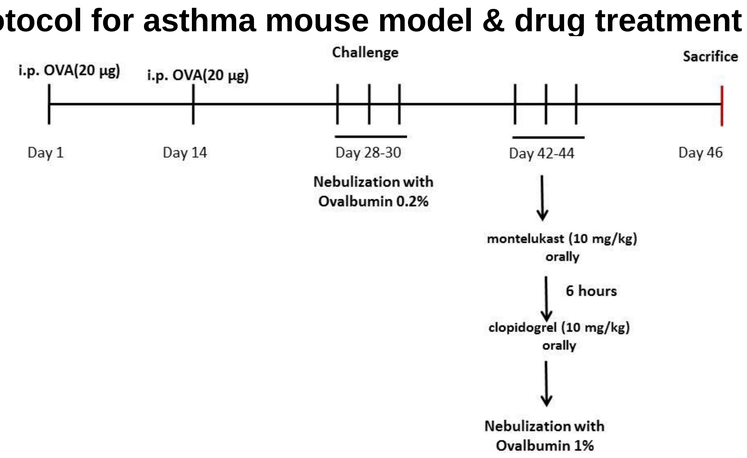
INTRODUCTION

- Clopidogrel, P2Y12 receptor (P2Y12R) antagonist could attenuate airway inflammation in an allergic asthma mouse model.
- We aimed to investigate the effects of addition of clopidogrel to montelukast, a widely prescribed drug in asthma treatment, in an ovalbumin (OVA)-induced eosinophilic asthma mouse model.

MATERIAL & METHODS

- Strains: BALB/c mice, 20 gram, 6 weeks.
- Asthma induction: Ovalbumin, grade V (Sigma)

Protocol for asthma mouse model & drug treatment



- Mice were administered orally with either montelukast (10 mg/kg) or clopidogrel (10 mg/kg) or both
- 48 hours after final challenge, mice were assessed for airway responsiveness (Flexi Vent).
- Bronchoalveolar lavage fluid (BALF) was collected for differential cell count (Hematoxylin & Eosin).
- Platelet-eosinophil complex (PEC) level was measured in peripheral blood (flow cytometry) and BALF (by immunocytochemistry).
- Th2 cytokines (interleukin 4, interleukin 5) were measured by ELISA.

RESULTS

All *P* values were calculated by Mann-Whitney U test.

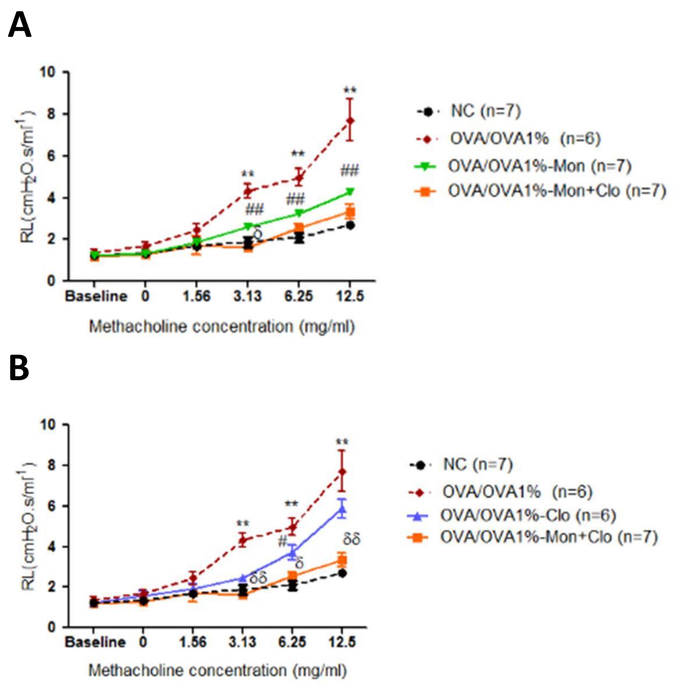


Figure 1. Airway hyperresponsiveness. Changes in lung resistance in response to increasing doses of methacholine (0, 1.56, 3.12, 6.25, 12.5 mg/kg). (A) Mon vs Mon+Clo; (B) Clo vs Mon+Clo. **, ***: *P*<0.01, *P*<0.001 comparing to negative control; #, ##: *P*<0.05, *P*<0.01 comparing to OVA/OVA 1%: δ, δδ: *P*<0.05, *P*<0.01 comparing between Mon+Clo and single treatment with either Mon or Clo.
NC, negative control; OVA/OVA1%, secondary challenge asthma mouse model 1% Clo, clopidogrel; Mon, montelukast; Mon+Clo, montelukast+clopidogrel

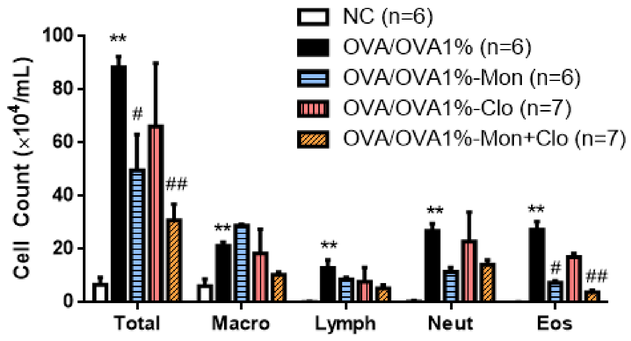


Figure 2. Total & differential cell count. **: *P*<0.01 comparing to negative control; #, ##: *P*<0.05, *P*<0.01 comparing to OVA/OVA 1%.
NC, negative control; OVA/OVA1%, secondary challenge asthma mouse model 1% Clo, clopidogrel; Mon, montelukast; Mon+Clo, montelukast+clopidogrel; total, total cell count; macro: macrophage; lymph: lymphocyte; neut, neutrophils; eos, eosinophils

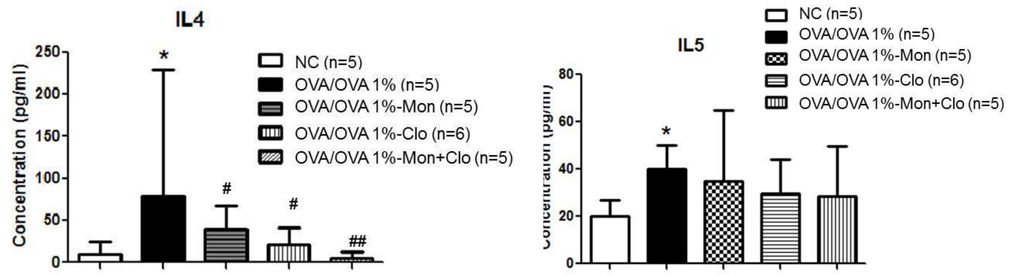


Figure 3. Interleukin (IL)-4, IL5 in BALF, measured by ELISA. *: *P*<0.05 comparing to negative control; #, ##: *P*<0.05, *P*<0.01 comparing to OVA/OVA 1%.
NC, negative control; OVA/OVA1%, secondary challenge asthma mouse model 1% Clo, clopidogrel; Mon, montelukast; Mon+Clo, montelukast+clopidogrel

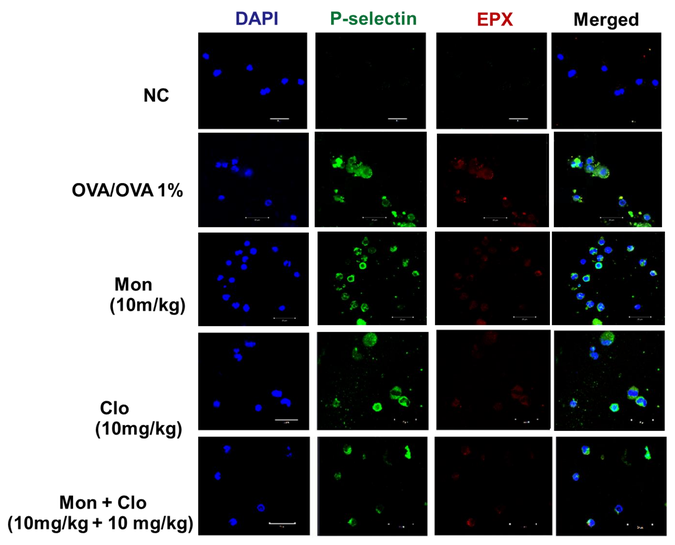


Fig 4. Formation of platelet-eosinophil aggregation in BALF. BALF was labled by eosinophil peroxidase and P-selectin. Cells were visulaized by confocal microscopy

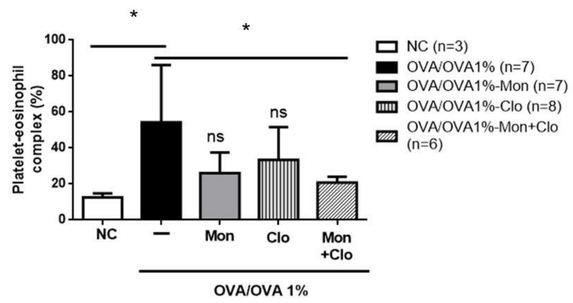


Figure 5. Platelet-eosinophil complex. Peripheral blood was collected and stained for eosinophil marker (Siglec-F), platelet marker (FITC-CD41). Platelet-eosinophil aggregation was analyzed by flow cytometry. *: *P*<0.05 comparing to negative control; ns: no significant. Clo, clopidogrel; Mon, montelukast; NC, negative control; Mon+Clo, montelukast+clopidogrel combination; OVA/OVA 1%, secondary challenge asthma mouse model (1%).

CONCLUSION

- Clopidogrel facilitate montelukast in attenuating airway inflammation.
- Further studies are required to investigate mechanism and effectiveness of this combination in asthma treatment. `