

Fevipirant, a Selective Prostaglandin D₂ Receptor 2 Antagonist, Potently Inhibits Chemotaxis and Cytokine Production by Tc2 cells (type-2 CD8⁺ lymphocytes)

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Rationale

Tc2 cells are type-2 cytokine-secreting CD8⁺ T lymphocytes that highly express the prostaglandin D₂ (PGD₂) receptor 2 (DP₂). Tc2s are enriched in peripheral blood and airways specifically in patients with severe asthma and persistent corticosteroid-insensitive eosinophilia in two independent cohorts. Activation with PGD₂ leads to cell migration and pro-inflammatory protein production, which promotes eosinophil recruitment and survival directly or indirectly, suggesting an important role for Tc2 cells in the pathogenesis of eosinophilic asthma and potential contribution to airway eosinophilia. Fevipirant is a potent selective DP₂ receptor antagonist that reduces airway inflammation in patients with persistent asthma and elevated sputum eosinophil counts. The drug is currently in Phase III clinical development for treatment of uncontrolled asthma. Here, we characterize the inhibition of Fevipirant on DP₂ pathway activated Tc2 cell migration and cytokine release, including *ex vivo* studies on samples from severe eosinophilic asthmatics.

Methods

CD3⁺CD8⁺CRTH2⁺ Tc2 cells were isolated from mononuclear cells of healthy volunteer blood and cultured. For chemotaxis assays, cell migration induced with PGD₂ (100 nM) in the presence of Fevipirant was determined using the IncuCyte live cell analysis system. Protein and mRNA levels of cytokines IL-4, IL-5 and IL-13 after treatment with PGD₂ (200 nM) in the presence of Fevipirant for 4 h were measured with ELISA and qPCR respectively. Negative controls DP₁ receptor agonist BW245C and DP₁ antagonist BW868A confirmed the DP₂ specificity of the effect; DP₂ antagonist TM30089 was a positive control. For *ex-vivo* studies, patients meeting ATS/ERS severe asthma definition with sputum eosinophil count >3% were recruited. Peripheral blood was collected and treated with PGD₂ (1 μM) in the presence or absence of Fevipirant (1 μM) for 6 h followed by intracellular staining (ICS) for IL-5/13 for flow cytometry analysis. (Ethical approval: Leicestershire, Northamptonshire Research Ethics Committee 08/H0406/189)

Results

Fevipirant specifically inhibited DP₂-mediated chemotaxis (Fig. 1A) and type-2 cytokine production (Fig. 1B) in cultured Tc2 with low nM potency, and showed significant inhibition of type-2 cytokine production *ex vivo* (Fig. 1C). Representative data is presented in the Figure.

Conclusions

Fevipirant is a potent inhibitor of DP₂ pathway mediated activation of Tc2 cells. The inhibitory potency on Tc2 migration is comparable with previous data on inhibition of PGD₂ induced activation in human ILC2 and Th2 cells. Given the potential role of Tc2s in uncontrolled eosinophilic asthma, these data support further development of Fevipirant and complement prior characterization with ILC2s, eosinophils and Th2 cells.

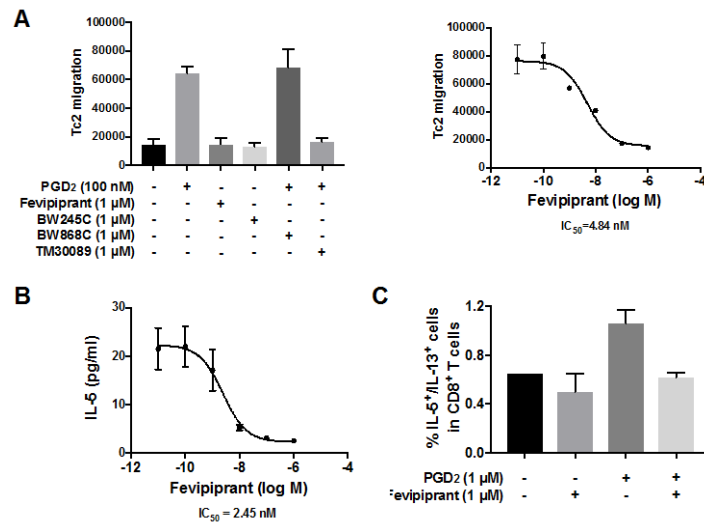


Figure 1. Feviprant inhibition of DP₂-mediated Tc2 cell activation. **A.** Cultured Tc2 cell migration in response to 100 nM PGD₂ is mediated by DP₂ and was inhibited by Feviprant; **B.** IL-5 production in response to 200 nM PGD₂ in cultured Tc2 cells was inhibited by Feviprant; **C.** IL-5/IL-13⁺CD8⁺ T cells detected in fresh PBMC in response to 1 μ M PGD₂ were reduced by Feviprant.