Fevipiprant, a Selective Prostaglandin D_2 Receptor 2 Antagonist, Potently Inhibits Chemotaxis and Cytokine Production by Group 2 Innate Lymphoid Cells

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Introduction

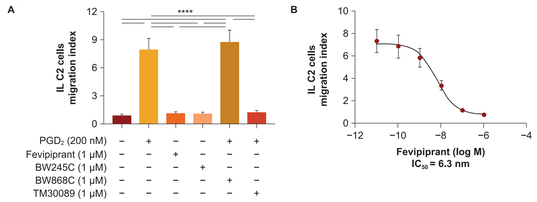
- Activation of the group 2 innate lymphoid cell (ILC2) population leads to production of classical type 2 cytokines, promoting type 2 immunity[1]. Increased ILC2 levels are found in blood and sputum of severe eosinophilic asthmatics in comparison to mild asthma patients[2]. Such elevated ILC2 levels are hypothesized to drive the chronic eosinophilia associated with severe asthma
- Prostaglandin D_2 (PGD_2) is produced mainly by activated mast cells and is increased in asthma, with highest levels in patients with severe disease[3]. PGD_2 is also increased in response to allergen challenge[4]
- The PGD_2 receptor 2 (DP_2; previously known as CRTh2) is expressed on adaptive and innate immune cells such as eosinophils, $CD4^+$ Th2 cells, and ILC2. The DP_2 receptor plays a key role in the pathophysiology of asthma: activation induces and amplifies the inflammatory cascade[5]
- PGD_2 binding to DP_2 induces ILC2 migration and production of type 2 cytokines such as IL 4, IL 5 and IL 13, in addition to other cytokines[6]
- Fevipiprant, a potent selective antagonist of the DP_2 receptor[7,8], reduces eosinophilic airway inflammation in patients with persistent asthma and high sputum eosinophil counts[9]. Fevipiprant is in Phase 3 development as once daily oral treatment of uncontrolled asthma
- Here we characterize the inhibitory effects Fevipiprant on a variety DP_2 pathway mediated ILC2 activation parameters

Methods

- ILC2 cells were isolated and cultured from peripheral blood mononuclear cells derived from healthy volunteers as previously described[10]
- The dose-response inhibitory effect of Fevipiprant on PGD_2 induced ILC2 chemotaxis **(Figure 1)** was determined in a transwell assay using Incucyte live cell analysis
- The inhibitory effect of Fevipiprant on PGD_2 induced ILC2 apoptosis suppression **(Figure 2)** was determined using Annexin V staining
- The inhibitory effect of Fevipiprant on PGD_2 induced ILC2 cell aggregation (**Figure 3A**) was recorded under an EVOS Floid cell imaging station, and on adhesion molecule expression (**Figure 3B**) was determined with qPCR
- The dose-response inhibitory effect of Fevipiprant on PGD_2 induced cytokine production **(Figures 4** and **5)** was determined at the protein level using ELISA (IL 4, IL 5 and IL 13) and Luminex (IL 8, GMCSF and CSF1) and at the mRNA level by Quantitative RT-PCR
- For migration and cytokine production assays, BW245C (DP_1 receptor agonist), BW868C (DP_1 antagonist) and TM30089 (DP_2 antagonist) were used as positive and negative controls
- Data are presented as means with SEM. Data were analysed using one-way ANOVA followed by the Tukey's test. Values of p<0.05 were considered statistically significant

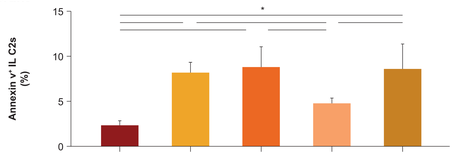
Results

Figure 1. Fevipiprant inhibits DP_2-mediated ILC2 migration



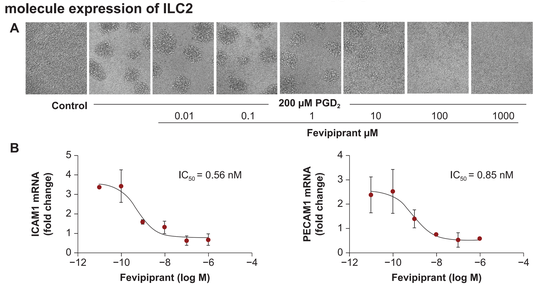
(A) ILC2 migration towards PGD_2 (200nM) was measured in the presence or absence of Fevipiprant (1 μM) or controls, BW245C (1 μM), BW868C (1 μM) or TM30089 (1 μM). (B) ILC2 migration towards 200nM PGD_2 was measured in the presence of increasing concentrations of Fevipiprant. ****p<0.0001, n=10.

Figure 2. The suppressive effect of PGD_2 on ILC2 apoptosis is reversed by Fevipiprant



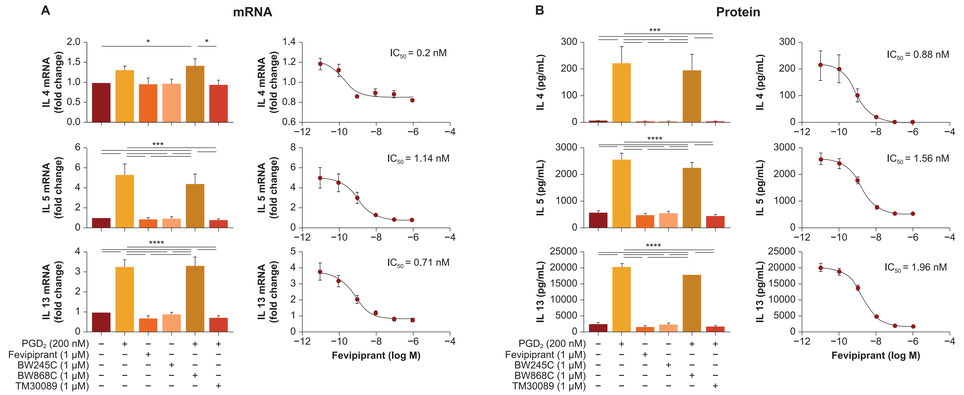
The frequency of Annexin V positive ILC2, induced by deprivation of IL 2 and human serum for 16 h, in the absence or presence of PGD_2 (1 μM) or Fevipiprant (1 μM) was measured by flow cytometry. *p<0.05, n=5.

Figure 3. Fevipiprant inhibits PGD_2 induced cell aggregation and adhesion molecule expression of ILC2



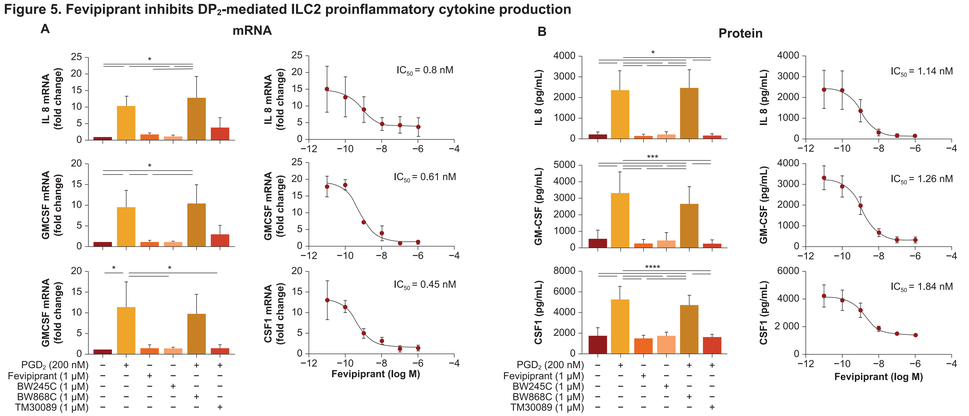
(A) Cell aggregation was observed by microscopy of ILC2 after incubation with or without 200 nM PGD_2 in the presence of increasing concentrations of Fevipiprant for 2 h. (B) Levels of mRNA for Icam1 and Pecam1 in ILC2 treated with 200 nM PGD_2 in the presence of increasing concentration of Fevipiprant for 4 h was measured by qPCR. n=3.

Figure 4. Fevipiprant inhibits DP_2-mediated ILC2 type-2 cytokine production



The effect of Fevipiprant (1 μM) or controls, BW245C (1 μM), BW868C (1 μM) or TM30089 (1 μM) on levels of IL 4, IL 5 and IL 13 mRNA in ILC2 was measured by qPCR (A) and IL 4, IL 5 and IL 13 protein released by ILC2 (B) on incubation with PGD_2 (200 nM) was measured by ELISA (left panels). Right panels show effects of ILC2 incubation with 200nM PGD_2 in the presence of increasing concentrations of Fevipiprant. *p<0.05, ***p<0.001, ****p<0.0001, n=8 except n=4 for IL 4 mRNA and n=6 for IL 5 mRNA.

Figure 5. Fevipiprant inhibits DP_2-mediated ILC2 proinflammatory cytokine production



The effect of Fevipiprant (1 μM) or controls, BW245C (1 μM), BW868C (1 μM) or TM30089 (1 μM) on levels of IL 8, GMCSF and CSF1 mRNA in ILC2 was measured by qPCR (A) and IL 8, GM-CSF and CSF1 protein released by ILC2 (B) on incubation with PGD_2 (200 nM) was measured by Luminex (left panels). Right panels show effects of ILC2 incubation with 200nM PGD_2 in the presence of increasing concentrations of Fevipiprant. *p<0.05, ***p<0.001, ****p<0.0001, n=3.

Conclusions

- Fevipiprant is a potent inhibitor of DP_2 pathway mediated activation of ILC2
- The inhibitory potency on ILC2 migration and cytokine release is comparable with previous data generated for inhibition of PGD_2 induced cytokine release from $CD4^+$ Th2 cells and also with the DP_2 receptor affinity
- Given the emerging important role of ILC2 in asthma, these data support further development of Fevipiprant in this indication and complement prior in vitro characterization data obtained with eosinophils and $CD4^+$ Th2 cells[7,8]

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Disclosures

D.A. Sandham and V.J. Erpenbeck are current employees of Novartis

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