Introduction

Prostaglandin E2 (PGE2) production and the expression of its retroperitoneal receptors, including EP2, correlates with the progression of many epithelial cancers. PGE2 may play a role in reducing inflammatory cytokine release in the tumour microenvironment resulting in tumour growth.

The reversal of PGE2 inhibition of tumour necrosis factor α (TNFα) in lipopolysaccharide (LPS) stimulated human whole blood (LPS-HWB) has been used as a target engagement marker for antagonists of the prostaglandin receptor 4 (EP4). 

However, there is conflicting data as to whether the LPS-HWB assay can also measure an EP2 response; therefore, the aim of this study was to characterise the primary receptor response in the LPS-HWB.

THP-1 cells, an immortalised monocytic cell line, were used to aid this pharmacological characterisation. The use of these cells allowed functional signalling to be measured alongside the LPS-HWB assay.

Methods

CAMP functional assays: Recombinant HEK293 EP4/EP2 cAMP assay: HEK293 cells were infected with 0.05% EP2 or 0.05% EP2 BacMam (5 k cells/well). THP-1 functional cAMP Assay: THP-1 cells (7.5 k cells/well). In both assays cells were treated with compound only for agonist mode (30 min incubation), or followed by ECP50, PGE2 (3 mM) for antagonist mode (30 min incubation). HTRF G, dynamic CAMP assay was performed to manufacturer’s instructions (Cisbio).

THP-1 receptor expression: Relative expression of prostaglandin receptors was characterised using qRT-PCR.

LPS-challenge whole blood assay: Human whole blood was sourced from Cambridge Biosciences. The LPS-HWB was performed following literature methods with antagonists pre-incubated (37°C at 5% CO2) for 30 mins prior to agonist addition. 4 h after LPS addition (10 µg/mL). TNFα concentration was measured via ELISA following manufacturer’s conditions (DY210 R&D systems DuoSet).

Data analysis: All data were analysed using GraphPad Prism v8. Agonist data were fitted using a sigmoidal four-parameter fit. Functional antagonist data were fitted to Cheng-Prusoff equation, corrected using agonist pEC50.

Results

Recombinant HEK293

Table 1. Data table summarising the activity of the compounds used in recombinant HEK293 EP4/EP2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>pEC50</th>
<th>KE293</th>
<th>pEC50</th>
<th>KE293</th>
<th>pEC50</th>
<th>KE293</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE2</td>
<td>9.8±0.5</td>
<td>NA</td>
<td>9.2±0.3</td>
<td>NA</td>
<td>9.0±0.1</td>
<td>NA</td>
<td>Non-selective agonist</td>
</tr>
<tr>
<td>KAG308</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>Selective EP4 agonist</td>
</tr>
<tr>
<td>AH13205</td>
<td>5.6±0.1</td>
<td>NA</td>
<td>8.0±0.4</td>
<td>NA</td>
<td>6.0±0.1</td>
<td>NA</td>
<td>EP2 agonist</td>
</tr>
</tbody>
</table>

Data shown are pooled (mean ± SD) of at least 3 independent experiments.


t* literature PK, value.

Immortalised cell line - THP-1

Figure 3. Human EP2 receptor expression was shown to be 50 fold higher vs. EP2 in THP-1. Data shown are pooled (mean ± SD) of 3 independent experiments, normalised to expression relative to GAPDH.

Results continued...

Human whole blood data

Figure 4. THP-1 cells dose-dependently respond to EP2 agonists, KAG308 and PGE2, with a minimal response to EP2 agonist AH13205.

THP-1 cells responded to KAG308 with nM potency whereas AH13205 response was >1 µM. Data shown are pooled (mean ± SD) of 3 independent experiments, normalised to percentage of the maximal PGE response.

Figure 5. EP2 agonist, ONO-AE3-208 has a greater potency in the presence of ECP50, PGE2 over EP2 agonist, PF-04418948 in THP-1 cells.

Data shown are pooled (mean ± SD) of 3 independent experiments, normalised as above.

Figure 6. In THP-1 cells a single concentration of ONO-AE3-208, an EP2 agonist, antagonises PGE2 response 200 fold greater than PF-04418948, an EP2 agonist.

Data shown are pooled (mean ± SD) of 3 independent experiments, normalised as above.

Figure 7. TNFα levels in HWB dose-dependently increased in response to KAG308 but not AH13205, recapitulating functional data in THP-1.

Data shown is pooled (mean ± SD) of 3 independent experiments, normalised to average LPS response, and basal TNFα levels. PGE2 slope is > 1 suggesting potential for involvement of multiple receptor responses. However, limited AH13205 response suggests a lack of EP2 response.

Figure 8. The PGE2 response in HWB was blocked by ONO-AE3-208 but not PF-04418948, suggesting the major response being detected was mediated via EP2.

Data shown are pooled (mean ± SD) of 3 independent experiments, normalised to maximum LPS response and basal TNFα.

Conclusions

These data suggest in both the THP-1 cells and in the LPS-HWB assay, EP2 agonists and antagonists have little activity, in contrast to published data1.

The THP-1 data consistently replicates the LPS-HWB assay findings, suggesting that the assays are comparative to each other.

Overall, the LPS-HWB assay is an appropriate assay to measure EP2 responses.

The lack of EP2 response questions the need for an EP2/EP2 dual antagonist for the treatment of DPC.

Future directions include measuring human plasma protein binding to allow calculation of the free fraction.

References


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