In Vitro and In Vivo Characterisation of Two Contrasting Progesterone Receptor Antagonists

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Progesterone receptor (PR) signalling is involved in the secretory transformation of the endometrial lining of the uterus. Antagonising this process may prove to be a beneficial treatment for gynaecological disorders. Here, we describe the pharmacological properties of both a steroidal and non-steroidal PR antagonist, RU-486 and PF-2413873 (4-{[3-Cyclopropyl-1-(mesylmethyl)-5-methyl-1H-pyrazol-4-yl]oxy}-2,6-dimethylbenzonitrile respectively, in in vitro and in vivo assays.

In cynomolgus macaques, both RU-486 and PF-2413873 induced a reduction in endometrial thickness and BrdU incorporation (proliferation marker), indicative of a PR antagonist effect (Table 1).

<table>
<thead>
<tr>
<th>Effect: % change versus control</th>
<th>endometrial thickness</th>
<th>BrdU incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RU-486 20 mg/kg/day</td>
<td>-34 (p&lt;0.001)</td>
<td>-87 (p&lt;0.01)</td>
</tr>
<tr>
<td>PF-2413873 5 mg/kg/day</td>
<td>-43 (p&lt;0.001)</td>
<td>-15 (NS)</td>
</tr>
<tr>
<td>PF-2413873 20 mg/kg/day</td>
<td>-56 (p&lt;0.001)</td>
<td>-39 (p&lt;0.05)</td>
</tr>
</tbody>
</table>

Table 1. mean % change compared to control, 5 animals per cohort.

The pharmacological mechanism of action of RU-486 and PF-2413873 was evaluated in two in vitro assays, gene transcription and nuclear translocation.

Gene Transcription

Both RU-486 and PF-2413873 inhibited agonist (EC80 progesterone) induced alkaline phosphatase production in the breast cancer cell line, T47D. At high concentrations (>1µM), PF-2413873, in contrast to RU-486, also displayed an apparent agonism (Table 2). To explore this further, Schild experiments were conducted with Lew and Angus non linear regression analysis1 where dextral displacement of a progesterone concentration response curve was observed for both antagonists (Table 2).
### Table 2 Mean or geometric mean of gene transcription data. \( n \geq 3 \), NOA – no observable agonism.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Antagonist</th>
<th>( EC_{50} ) nM (95% C.I.)</th>
<th>Emax (95% C.I.)</th>
<th>Slope (95% C.I.)</th>
<th>( IC_{50} ) nM (95% C.I.)</th>
<th>Slope (95% C.I.)</th>
<th>pKb (95% C.I.)</th>
<th>Slope (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td></td>
<td>2.52 (2.29-2.78)</td>
<td>96.99 (95.07-98.91)</td>
<td>2.17 (2.09-2.24)</td>
<td>-2.27 (-1.98-2.56)</td>
<td>11.59 (11.41-11.76)</td>
<td>1.13 (1.10-1.15)</td>
<td></td>
</tr>
<tr>
<td>RU-486</td>
<td>NOA</td>
<td>NOA</td>
<td>NOA</td>
<td>0.25 (0.13-0.48)</td>
<td>-1.72 (-1.37-2.07)</td>
<td>6.34 (6.09-6.59)</td>
<td>0.74 (0.70-0.79)</td>
<td></td>
</tr>
<tr>
<td>PF-2413873</td>
<td>&gt;1000</td>
<td>≥20</td>
<td>20.00 (11.44-34.99)</td>
<td>-1.72 (-1.37-2.07)</td>
<td>6.34 (6.09-6.59)</td>
<td>0.74 (0.70-0.79)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Nuclear Translocation

Experiments were conducted using a recombinant cell line incorporating the DiscoveRx™ progesterone receptor nuclear translocation PathHunter™ technology. When increasing concentrations of RU-486 and PF-2413873 were incubated alone with this cell line they induced nuclear translocation (Table 3). RU-486 induced nuclear translocation at concentrations similar to those that inhibited gene transcription. PF-2413873 induced nuclear translocation at high concentrations (>1\( \mu \)M) similarly to those that displayed agonism in the gene transcription assay.

\[
\begin{array}{|c|c|c|}
\hline
\text{EC}_{50} \text{ nM (95% C.I.)} & \text{Slope (95% C.I.)} & \text{Emax (95% C.I.)} \\
\hline
\text{progesterone} & 3.69 (2.48-5.48) & 0.86 (0.69-1.02) & 107 (97.9-115.5) \\
\text{RU-486} & 0.78 (0.44-1.46) & 0.94 (0.44-1.46) & 85.4 (69.0-101.7) \\
\text{PF-2413873} & 2541 (2504-4292) & 1.15 (0.55-1.75) & 93.1 (113.7) \\
\hline
\end{array}
\]

Table 3 Mean or geometric mean of nuclear translocation data. \( n \geq 3 \)

While the observed effects of RU-486 and PF-2413873 on endometrial thickness in the macaque appear to be similar, the mechanism of action of RU-486 and PF-2413873 appear to be different. RU-486 facilitates nuclear translocation, yet acts as a competitive antagonist on gene transcription whilst, in contrast, PF-2413873 appears to have complex pharmacology, agonising PR function and facilitating nuclear translocation at high concentrations while behaving as a neutral antagonist at low concentrations. Further work is needed to fully elucidate the mechanism of action of PF-2413873.

### References: