Pharmacokinetics of a candidate prototype

From the results of the present thesis, DMPI proved itself to be a promising lead for the following reasons:

i. Marked increase in anti-inflammatory T_{H2} polarization
ii. Least binding energy and best fit in AMPKα docking
iii. Highest AMPKα phosphorylation
iv. Non-ulcerogenic, reduced total and free acidity

Thus, DMPI may be a potential non-ulcerogenic anti-inflammatory lead molecule for further investigation in this direction. Additionally DMPI has been found to be a peripheral analgesic as potent as ibuprofen (*Unpublished results*). Thus, DMPI seems to have a pleiotropic action across several targets. However, DMPI was far more effective in mechanistic evaluation rather than *in-vitro/in-vivo*. In this context and owing to the fact that DMPI is the only patent-filed molecule (2300/CHE/2010), among the test compounds, we conducted a preliminary pharmacokinetic evaluation to compare the PK parameters with that of the parent analog and proven AMPK activator, namely, DHPO (Kandadi et al., 2010).

**Method**

PK was assessed in overnight fasted male BALB/c mice (25g, n=3) after administering a single dose of 200mg/kg *p.o*. Blood was withdrawn from the retro-orbital plexus at various time-points (0min, 15min, 30min, 1h, 1.5h, 2h, 10h, 24h) and the plasma concentration of DMPI was determined by HPLC. Briefly, aliquots of plasma (100μL) were extracted with ACN (300μL), centrifuged (14000rpm for 10 min,) and 20μL was injected into the HPLC system equipped with a PDA detector. Mobile Phase: formic acid buffer (0.1%) and acetonitrile in gradient elution, Stationary Phase: a C-18 reverse phase column, flow rate: 1.0 mL/min, retention time (RT) : 7.46min, \( \lambda_{max} \) : 263nm, Internal Standard (IS) used: 4-hydroxybenzaldehyde, RT : 11.27min, \( \lambda_{max} \) : 276nm ( 5μL of IS (25μg/ml) for 100μL of plasma). Two standard plots were obtained, namely, an analytical plot (500-0.488ng) and a bioanalytical plot (500-0.976ng). Plasma concentrations at various time-points were calculated from the standard plot and the results are represented as a plasma concentration-time profile using Graph Pad Prism Software (Version 5.02 – Demo, Graph Pad Software, Inc., San Diego, CA, USA).
Results

**Figure:** Plasma concentration-time profile of DMPI after 200mg/kg oral administration to BALB/c mice (mean ± SEM; n=3).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DMPI 200mg/kg (p.o.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>23.03 ± 2.60 µg/mL</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>431.79 ± 12.18 min</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>60.00 ± 0.00 min</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;(0-1)&lt;/sub&gt;</td>
<td>78.50 ± 5.32 µg*h/ml</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;(0-α)&lt;/sub&gt;</td>
<td>88.70 ± 5.27 µg*h/ml</td>
</tr>
<tr>
<td>Elimination rate constant</td>
<td>0.09647 ± 0.00265 h&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Volume of distribution (V&lt;sub&gt;d&lt;/sub&gt;)</td>
<td>23582.86±1563.49 ml/kg</td>
</tr>
<tr>
<td>Clearance (CL)</td>
<td>2270.87±135.88ml/min/kg</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± SEM; n=3*

**Table:** Pharmacokinetic profile of DMPI

PK of DMPI was assessed in overnight fasted male BALB/c mice (n=3) after administering a single dose of 200mg/kg DMPI p.o. Blood was withdrawn at various time-points (0min, 15min, 30min, 1h, 1.5h, 2h, 10h, 24h) and the plasma concentration of DMPI was determined by HPLC.

**Discussion**

A preliminary pharmacokinetic evaluation of DMPI was performed at the most effective dose viz. 200mg/kg p.o. in mice. A T<sub>max</sub> of 60 min suggests that the compound is quickly absorbed orally like DHPO (20mg/kg p.o.) (Kandadi et al., 2010). The elimination half-life of DMPI was 7.2 ± 0.2 hours (DHPO 1.53 ± 0.41 hours (Kandadi et al., 2010)). This may be in part due to the clearance, which was ten
times lower than DHPO. The V_d of DMPI, being 50% lower than DHPO, could be the combined result of one or more of the following: low lipid solubility/greater ionization/greater plasma protein binding. This could also underlie the relatively non-toxic nature of DMPI in comparison to DHPO. We also found from a preliminary study that at least a portion of the oral dose is excreted unchanged in urine, suggesting a low possibility of DMPI accumulating in the body. A lower V_d (by 50%) along with a lower clearance (longer t_{1/2}), in comparison to DHPO, suggests that DMPI enjoys an extended duration of action, probably resulting from high plasma protein binding.

DMPI is not likely to accumulate in the body because of low V_d and relatively rapid clearance. Thus, we now have a patent-filed derivative of DHPO with a probably better safety profile, which may be suitably modified pharmaceutically to enhance its efficacy. Extensive toxicity studies and pharmaceutical innovation would be required before taking this molecule further.

Reference


Figure: Analytical standard plot of DMPI
**Figure:** Bio-analytical standard plot of DMPI

**Figure:** Representative HPLC Chromatogram of blank
Figure: Representative HPLC Chromatogram of DMPI
**Figure:** Representative HPLC Chromatogram of IS (plasma)
Figure: Representative HPLC Chromatogram of DMPI + IS (plasma)