Esmethadone (REL-1017) Compares With NMDA Receptor Antagonists in FLIPR-Calcium Assay

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INTRODUCTION

• N-methyl-D-aspartate receptor (NMDAR) channel blockers such as ketamine and sotakukan are emerging as a new drug class with potentially rapid and effective antidepressant activity.

• However, the adoption of intravenous ketamine and intranasal sotakukan has been limited by dissociative psychotomimetic effects requiring careful patient supervision during and post-administration.

• Esmethadone (REL-1017; dexemethadone; DXT) is a low affinity, low potency NMDAR channel blocker. It binds to the MK-801 site of the NMDAR with low micromolar IC50 values.1 Esmethadone has 20 fold lower affinity for mu opioid receptors (MORs) compared to levomethadone2 and does not appear to contribute in a clinically meaningful way to the opioid effects of racemic methadone.3 According to a recent DEA statement on racemic methadone, esmethadone "lacks significant respiratory depressant action and abuse liability."3

• In a recent phase 2 MDD trial, esmethadone showed robust, rapid, and sustained antidepressant effects and very favorable safety, tolerability, and pharmacokinetic (PK) profiles in patients with inadequate responses to standard antidepressant treatments.

OBJECTIVES

• To characterize esmethadone in vitro functional effect on heterodimeric NMDA receptors, by calculating esmethadone IC50 values in presence of 10 µM L-glutamate, as well as by estimating Ki, the equilibrium dissociation constant, in FLIPR calcium assay.

METHODS

• Fluorometric imaging plate reader (FLIPR, Molecular Devices) cell-based assays was performed in 384 well plate format, using Fura-4 fluorescent indicator of intracellular free calcium ion concentration.

• Assay buffer composition included 145 mM NaCl, 5 mM KCl, 2 mM CaCl2, 10 mM HEPES, 10 mM D-glucose, 20 mM HEPES (pH adjusted to 7.3 with NaOH).

• Test items were added, without pre-incubation, together with indicated concentration of L-glutamate and 10 µM glycine, but in absence of magnesium.

• Area under the curve (AUC) of fluorescence readings were measured for 5 minutes after L-glutamate addition, and normalized to readings obtained in presence of 10 µM L-glutamate plus 10 µM glycine (100%) and buffer (0%).

• In FLIPR concentration response curve (CRC) experiments, every test item was assayed at 11 final concentrations: 100-33.1-1.7 µM, then 12.5-17 -5.1-1.7 µM. L-Glutamate and glycine were both used at 10 µM final concentration in CRC experiments.

• Apopt CHD cell lines, expressing human heterodimeric NMDA receptors, were used: hGluN1/hGluN2A, hGluN1/hGluN2B, hGluN1/hGluN2C, and hGluN1/hGluN2D.

• Protein name and accession number of NMDAR subunits are:

  - hGluN1: NP_015566
  - hGluN2A: NP_000824
  - hGluN2B: NP_000825
  - hGluN2C: NP_000826
  - hGluN2D: NP_000827

• Four parameter logistic equation were used to calculate L-glutamate (EC50) or test item pIC50.

• Operational equation for allosteric modulators 4 was created in Prisen 8 (GraphPad) software to estimate Kd and α parameters for every test item:

  \[ Y = I_{max} \left( \frac{[E]{IC50} + E}{[E]{IC50} + E} \right)^{\alpha} \]

  Y is % effect of L-glutamate in presence of test item. [A] is EC50, IC50 and (efficacy value) are L-glutamate parameters, while [E] and Kd are test item parameters, corresponding to test item molar concentration, estimated test item equilibrium dissociation constant, and α or cooperativity term, respectively.

RESULTS

Figure 1 – L-glutamate concentration response curve (CRC) was obtained to characterize the four NMDA cell lines by calculating L-glutamate EC50 in the different cell lines. L-glutamate CRC was performed in FLIPR assay using 0.1 µM to 10 µM L-glutamate. The sigmoidal curves representing different heterodimeric human NMDA receptors, L-glutamate CRC included following 10 final concentrations: 1 µM, 10 µM, 50 µM, 33.1 µM, 1.7 µM, 12.5 µM, 17 µM, 50 µM, 111 µM, 450 µM. L-glutamate EC50 measured (D, 1.0; B, 0.97 and 0.92 µM on hGluN1/hGluN2A, hGluN1/hGluN2B, hGluN1/hGluN2C, hGluN1/hGluN2D receptors, respectively.

Figure 2 – Esmethadone CRC

Esmethadone CRC was performed to calculate CRC curve in five selected NMDA channel blockers included in Figure 2. For hGluN1/hGluN2A, esmethadone EC50 value indicates low potency for test item, since it means that higher concentration of the test item are required to elicit a 50% inhibition of agonist response.4

Table 1 – NMDAR channel blockers IC50 values

<table>
<thead>
<tr>
<th>Test Item IC50 (µM)</th>
<th>NMDAR type</th>
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</thead>
<tbody>
<tr>
<td>Esmethadone IC50</td>
<td>hGluN1/hGluN2A</td>
</tr>
<tr>
<td>Memantine IC50</td>
<td>hGluN1/hGluN2B</td>
</tr>
<tr>
<td>(+)-Ketamine IC50</td>
<td>hGluN1/hGluN2C</td>
</tr>
<tr>
<td>(+-)MK 801 IC50</td>
<td>hGluN1/hGluN2D</td>
</tr>
<tr>
<td>Dextromethorphan IC50</td>
<td>hGluN1/hGluN2E</td>
</tr>
</tbody>
</table>

Table 2 - NMDAR channel blockers estimated Ki values

<table>
<thead>
<tr>
<th>Test Item Ki (µM)</th>
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<tbody>
<tr>
<td>Esmethadone Ki</td>
<td>hGluN1/hGluN2A</td>
</tr>
<tr>
<td>Memantine Ki</td>
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</tr>
<tr>
<td>(+)-Ketamine Ki</td>
<td>hGluN1/hGluN2C</td>
</tr>
<tr>
<td>(+-)MK 801 Ki</td>
<td>hGluN1/hGluN2D</td>
</tr>
<tr>
<td>Dextromethorphan Ki</td>
<td>hGluN1/hGluN2E</td>
</tr>
</tbody>
</table>

CONCLUSIONS

• Esmethadone inhibited NMDAR response to 10 µM L-glutamate in all four different tested receptor combinations, with similar potency (IC50 values). Esmethadone resulted with following IC50 rank order: hGluN2DbGluN1bGluN2BhGluN2D (Figure 2 and Table 1).

• Remaining test items, i.e. (+)-ketamine, memantine, (+)MK 801, (+)ketamine, memantine and dextromethorphan, all showed IC50 values in line with their reported potencies, and with limited subunit preferences (Table 1).

• Esmethadone showed (Figure 3) an unsurmountable profile, when tested in presence of different L-glutamate concentrations, typical of NMDAR pore blockers.

• Esmethadone resulted (Table 2) with estimated Kd in the micromolar range with any of the studied NMDAR isoforms.

• Esmethadone potency range at different NMDARs, together with a favourable PK profile may be a key to its observed antidepressant effect, devoid of psychotomimetic side effects.

REFERENCES


DISCLOSURES

• This research was sponsored by Relmada Therapeutics, Inc. Drs. Inturrisi, Stahl, Pappagallo, and Manfredi are paid consultants for Relmada Therapeutics. Drs. Inturrisi and Manfredi are inventors on esmethadone patents and other patents and patent applications.