Abstract# 265
Radiosynthesis and Characterization in non-Human Primates of Three Enantiomerically Pure PET Radioligands for Imaging the GluN2B Subunit of the NMDA Receptor Complex

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• \(^{18}\)F-OF-Me-NB1 is a promising PET tracer for GluN2B subunit of NMDA receptors in rodents;

• Selected in MHPRD for monkey studies and potential human use. [https://medicine.yale.edu/pet/mhprd/]
Objectives

- Preparation of three forms of $^{18}$F-OF-Me-NB1
- Baseline and blocking scans in rhesus monkeys
- Metabolite analysis and input function measurement
- Modeling analysis
Table 1. Summary of $^{11}$C and $^{18}$F tracers in QC analysis

<table>
<thead>
<tr>
<th></th>
<th>$^{11}$C (n=3)</th>
<th>$^{18}$F (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molar activity (MBq/nmol)</td>
<td>518 ± 185</td>
<td>129.5 ± 44.4</td>
</tr>
<tr>
<td>RCP</td>
<td>&gt;98%</td>
<td>&gt;98%</td>
</tr>
<tr>
<td>ee</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
</tr>
</tbody>
</table>

(R)-OF-NB1 (precursor)  
(R)-$^{11}$C-OF-Me-NB1  
(R)-BPin precursor  
(R)-$^{18}$F-OF-Me-NB1  
(S)-BPin precursor  
(S)-$^{18}$F-OF-Me-NB1
Metabolite Analysis in Monkey

**R-^{18}\text{F-OF-Me-NB1}**  30% parent fraction @ 30 min p.i.
(Similar pattern for **R-^{11}\text{C-OF-Me-NB1}**)

**S-^{18}\text{F-OF-Me-NB1}**  18% Parent fraction @ 30 min p.i.
PET Image & TACs-Baseline

Summed SUV Images, 10-30 min

(R)-¹¹C-OF-Me-NB1 baseline
(R)-¹⁸F-OF-Me-NB1 baseline
(S)-¹⁸F-OF-Me-NB1 baseline

Higher brain uptake for (R)-enantiomer
PET Images & TACs-Blocking

Summed SUV Images, 10-30 min

(R)-$^{18}$F-OF-Me-NB1 Baseline

(R)-$^{18}$F-OF-Me-NB1 Blocking with 0.25 mg/kg Co101244

Occupancy: 77%
$V_{ND}$: 6.4
## Regional $V_T$ and $BP_{ND}$

### Table 2: Derived Regional $V_T$ and $BP_{ND}$ of The Three Radioligands in Different Monkey Brain Regions

<table>
<thead>
<tr>
<th>Tracer/regions</th>
<th>Cerebellum</th>
<th>Frontal cortex</th>
<th>Hippocampus</th>
<th>Occipital cortex</th>
<th>Putamen</th>
<th>Cingulate cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R^{\text{11}}$C-OF-Me-NB1</td>
<td>9.6</td>
<td>11.8</td>
<td>11.8</td>
<td>9.0</td>
<td>11.4</td>
<td>13.9</td>
</tr>
<tr>
<td>$R^{\text{18}}$F-OF-Me-NB1 (BP_{ND})</td>
<td>8.7 (0.37)</td>
<td>10.6 (0.67)</td>
<td>10.9 (0.71)</td>
<td>8.2 (0.29)</td>
<td>10.9 (0.72)</td>
<td>12.8 (1.00)</td>
</tr>
<tr>
<td>$S^{\text{18}}$F-OF-Me-NB1</td>
<td>10.9</td>
<td>12.1</td>
<td>13.6</td>
<td>10.8</td>
<td>13.3</td>
<td>15.6</td>
</tr>
<tr>
<td>$R^{\text{18}}$F-OF-Me-NB1 (Blocking)</td>
<td>6.7</td>
<td>6.7</td>
<td>6.8</td>
<td>6.7</td>
<td>8.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

$BP_{ND} = (V_T - V_{ND})/ V_{ND}$

Regional $BP_{ND}$ for $(R)^{18}$F-OF-Me-NB1: 0.29-1.00
(R)- vs. (S)-$^{18}$F-OF-Me-NB1

- Binding potential for (R)-enantiomer is slightly higher than the (S)-enantiomer.
Conclusions

✓ All three tracers were successfully prepared and evaluated in a rhesus monkey;

✓ Regional TACs were fitted well with the one-tissue compartment model (1TC) to obtain regional $V_T$ values using the metabolite-corrected arterial input function.

✓ In vivo binding of $(R)$-^{18}F-OF-Me-NB1 is specific to the GluN2B subunit of NMDA receptors, as demonstrated by blocking study with the GluN2B specific antagonist Co-101244.

✓ Comparison of regional $V_T$ values for $(R)$-^{18}F-OF-Me-NB1 and $(S)$-^{18}F-OF-Me-NB1 indicates that $(R)$-^{18}F-OF-Me-NB1 has slightly higher $BP_{ND}$ values

✓ $(R)$-^{18}F-OF-Me-NB1 provides good specific binding signals ($BP_{ND} = 0.29 – 1.00$) and is a promising radiotracer for PET imaging of the GluN2B subunit.
Acknowledgement

Abstract #263 (Ahmed et al.)

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- 1. Swiss National Science Foundation;
- 2. NIH grant U01MH107803

- Yale PET Center Zoom Style Group Photo (next slide)

ePoster at the end of slides
The NMDA receptors are involved in diseases of the central nervous system including Alzheimer’s disease, ischemic brain injury, and schizophrenia. To date, there is no suitable PET radioligands for the GluN2B binding sites of brain NMDA receptors in humans. We have previously reported the evaluation of the novel radioligands for the GluN2B binding sites of brain NMDA receptors in humans. We have previously reported the evaluation of the novel radioligands in vitro of the novel radioligands containing NMDA receptor in rodents [1]. The data from in vitro autoradiography and in vivo baseline and blocking studies indicate this tracer is specifically and selectively binding to the NMDA GluN2B in rodent brain.

1. Radiosynthesis of three enantiomer pure PET tracers R-[11C]-OF-Me-NB1, R-[18F]-OF-Me-NB1, S-[18F]-OF-Me-NB1; 2. Evaluate each tracer in rhesus monkey with arterial blood sampling and metabolite analysis; 3. Blocking study with Co101244 to test the binding specificity; 4. T and MA1 modeling, and comparison of R and S-[11C]-OF-Me-NB1.

All target compounds were obtained in ~95% radiochemical and enantiomeric purity, with molar activity of 14 ± 5 mCi/mmol for 11C-OF-Me-NB1 (n=3) and 3.5 ± 1.2 mCi/mmol for 18F-OF-Me-NB1 (n=5) at the end of the synthesis (Fig.1). Metabolism was fast with ~30% parent compound for (R)-11C-OF-Me-NB1 and (R)-11C-OF-Me-NB1 at 30 min after injection, and ~18% for (S)-11C-OF-Me-NB1(Fig.2). Plasma free fraction for all three forms of the radiotracer was ~2%. In the monkey brain both (R)-11C-OF-Me-NB1 and (R)-11C-OF-Me-NB1 displayed very similar pattern of fast uptake and clearance, while (S)-11C-OF-Me-NB1 showed lower brain uptake and faster clearance in all brain regions (Fig. 3). Radioactivity uptake was high in the putamen, hippocampus and thalamus, medium in the occipital cortex and cerebellum, and low in the white matter (centrum semiovale)(Fig.4). Both the 1TC model and MA1 method fitted the TACs well and provided reliable VT estimates, ranging from 8.2 in the Occipital cortex to 13.9 in the cingulate cortex for (R)-11C-OF-Me-NB1 and (R)-11C-OF-Me-NB1, compared to 10.8 in the occipital cortex to 15.6 in the cingulate cortex for (S)-11C-OF-Me-NB1 (Table 1). The binding potential for the (R)-11C-OF-Me-NB1 ranges from 0.29 to 1.00. Blocking study with 0.25 mg/kg of Co1012444 for (R)-11C-OF-Me-NB1 results in 77% of occupancy indicates the in vivo binding is specific. (R)-11C-OF-Me-NB1 provides good specific binding signals (BBPB = 0.29 – 1.00) and is a promising radiotracer for PET imaging of the GluN2B subunit of the NMDA receptors.

References

Table 1: VT Derived Regional VT and BPB of the Three Crosstowners in Different Monkey Brain Regions.

<table>
<thead>
<tr>
<th>Tissue Region</th>
<th>Baseline</th>
<th>Baseline</th>
<th>Hippocampus</th>
<th>Caudate</th>
<th>Putamen</th>
<th>Thalamus</th>
<th>Cingulate</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT (mL/cm³)</td>
<td>9.6</td>
<td>11.8</td>
<td>11.8</td>
<td>9.0</td>
<td>11.4</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>BBPB</td>
<td>11.0</td>
<td>10.6</td>
<td>10.9</td>
<td>8.2</td>
<td>10.9</td>
<td>12.8</td>
<td>12.8</td>
</tr>
<tr>
<td>VT (mL/cm³)</td>
<td>10.9</td>
<td>12.1</td>
<td>13.6</td>
<td>10.8</td>
<td>13.3</td>
<td>15.6</td>
<td></td>
</tr>
</tbody>
</table>

Fig 1

Fig 2

Fig 3

Fig 4

Fig 5

Fig 6

We have successfully synthesized and evaluated three enantiomerically pure radioligands for targeting the GluN2B subunit of the NMDA receptor complex. The (R)-11C-OF-Me-NB1 and the (R)-18F-OF-Me-NB1 have similar in vivo behavior in the white matter (centrum semiovale)(Fig.4). Both the 1TC model and MA1 method fitted the TACs well and provided reliable VT estimates, ranging from 8.2 in the Occipital cortex to 13.9 in the cingulate cortex for (R)-11C-OF-Me-NB1 and (R)-11C-OF-Me-NB1, compared to 10.8 in the occipital cortex to 15.6 in the cingulate cortex for (S)-11C-OF-Me-NB1 (Table 1). The binding potential for the (R)-11C-OF-Me-NB1 ranges from 0.29 to 1.00. Blocking study with 0.25 mg/kg of Co101244 for (R)-11C-OF-Me-NB1 results in 77% of occupancy indicates the in vivo binding is specific. (R)-11C-OF-Me-NB1 provides good specific binding signals (BBPB = 0.29 – 1.00) and is a promising radiotracer for PET imaging of the GluN2B subunit of the NMDA receptors.

1. Swiss National Science Foundation; 2. NIH grant U01MH1107863.