A pharmacokinetic evaluation of five H1 antagonists after an oral and intravenous microdose to human subjects

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Normal Sleep Progression

Awake, Alert
- GABA
- Histamine

Sleep Onset
- GABA
- Histamine

Sleep
- GABA
- Histamine
$H_1$ Antagonists Have Hypnotic Effects
Currently marketed H₁ Antagonists

<table>
<thead>
<tr>
<th>H₁ Antagonist</th>
<th>Known Issues</th>
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</table>
| 1<sup>st</sup> Generation (e.g. Diphenhydramine) | • Selectivity (esp. muscarinic – dry mouth, confusion in elderly)  
• Long duration of action - Next day sedation  
• Tolerance |
| 2<sup>nd</sup> Generation (e.g. Loratidine)      | • No CNS penetration (pGP substrates)              |
Optimization of $H_1$ Antagonists

- Poor receptor selectivity leads to side effects
  - Memory and cognition problems, confusion
  - Increased heart rate, blurred vision, urinary retention
- Long plasma half-life gives too long a duration of action (hangover)
The Right PK Profile...

Select for best profile based on prediction of human PK profile
Predicting Human Pharmacokinetics

• *In Vitro* human metabolism
  » Hepatocytes or Human liver microsomes
  » Prediction of clearance only

• Allometric Scaling
  » Need good IV PK data from several animal species
  » Predict clearance and volume of distribution

• Integrated Approach
  » *In vitro* + allometric considerations
  » Uses both in vitro human and in vivo animal data

• Human microdosing
  » Direct measurement of human PK
In vitro to in vivo scaling of clearance

“Liver in a tube”

Considerations:
- Microsomes/Hepatocytes
- Pooled/Individual
- [Substrate]
- Cofactors
- Scaling factors

More considerations:
- Absorption is 100%
- Hepatic uptake
- Protein binding
- Blood/Plasma ratios
- Extrahepatic metabolism
  - Gut
  - Lung, Kidney
- Other clearance mechanisms
  - Bile
  - Renal
Scaling of intrinsic clearance based on in vitro data

Prediction of total clearance based on in vitro microsomal data

Observed Clearance (ml/min/kg)

Predicted Clearance (ml/min/kg)

DPH: Diphenhydramine

Data from Obach, Drug Metab Dispos. 1999; 27; 1350-1359
A relationship exists between body size and physiological parameters:
- Basal metabolic rate (BMR)
- Heart rate
- Breaths/minute
- Life span
- Pharmacokinetics
Rationale for conducting microdose studies

- **Existing human PK prediction methods**
  - **Allometry, in vitro to in vivo extrapolation**
    - OK for predicting FIM dose, clearance, volume of distribution and half life
    - Mean average error for a good prediction is 2 fold
    - No information on the shape of CxT curve
  - **Inadequate for an insomnia program**
    - PK is key determinant of duration of effect
    - Duration of effect is key to marketing success
    - Ideal compound
      - Short t1/2
      - Low variability
        - Low clearance
        - Low volume of distribution
Rationale for microdosing study:
Selecting a Backup

Radio-labeled Compound

Human Microdose Study

Comparators

NBI-1 (Clinical compound)

Radio-label Analysis

Data to Pick Compound

NBI-2/3/4

Diphenhydramine

Benadryl Allergy
Microdosing: Clinical study design

- **Site**
  - PRA International, Stationsweg 163, 9417 AE Zuidlaren, The Netherlands

- **Test articles**
  - \[^{14}\text{C}]-labeled NBI-1, NBI-2, NBI-3, NBI-4 and Diphenhydramine

- **Study Design**
  - N=4 for each compound
  - IV and PO dosing, crossover design, one week wash-out period

- **Dose**
  - 0.1 mg (100 µg)

- **Dose Administration**
  - IV: 10-min IV infusion (flow rate of 0.4 mL/min) of 5% glucose solution containing 100 µg (7400 Bq, 200 nCi) of each \[^{14}\text{C}]-labeled test article
  - PO: Oral intake of 10 mL drinking solution containing 100 µg (7400 Bq, 200 nCi) of each \[^{14}\text{C}]-labeled test article

- **Blood sample collection**
  - Pre-dose and 0.17, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 36 and 48hr post-dose.
Microdosing clinical study: Sample analysis

- **Site**
  - Xceleron Ltd, York Biocentre, Innovation Way, Heslington, York, YO10 5NY, and Sand Hutton, York, YO41 1LZ, United Kingdom.

- **Methodology**
  - High Performance Liquid Chromatography (HPLC) coupled with Accelerator Mass Spectrometry (AMS)
  - Each plasma sample was analyzed for total $^{14}$C content and by HPLC-AMS in order to determine the concentration of the parent drug.

- **Data Analysis**
  - AMS results expressed as Percent Modern Carbon (pMC)
  - The pMC was converted to units of radioactivity per volume of sample
  - Total $^{14}$C data (dpm/mL) converted to ng equivalents/mL plasma
  - Pharmacokinetic parameters calculated using a validated software package (Pharsight WinNonlin Version 4.1)
Microdosing clinical study:  Clinical supplies

• Synthesis of ‘cold’ AND ‘hot’ $^{14}$C-test articles
  » ~10 g each of ‘cold’ compound was synthesized
  » 100 µCi each of ‘hot’ compound synthesized
    ▪ $^{14}$C was on an ‘easy to synthesize’ spot
    ▪ $^{14}$C was not metabolically stable, hence no metabolites were studied
  » Limited shelf life stability of test articles
  » Level of characterization similar to what is done to support a GLP toxicology study (Not GMP)
  » Same batch of test article used for tox studies
  » Formulation preparation done at the clinical site by the pharmacist
  » Compatibility testing done at the clinical site by the pharmacists
  » Formulation concentration was confirmed
Microdosing clinical study: Preclinical testing

- **Pharmacology studies (non GLP)**
  - Demonstrated compounds to be pharmacologically active in animal model
  - Minimum effective dose in rats was >100-times the dose to be given to humans
  - All compounds passed in vitro criteria established for the program

- **Limited Metabolism and PK data (non GLP)**
  - In vitro metabolism in human liver microsomes similar to the species used for toxicology studies
  - IV/PO PK studies in animals (rodents and nonrodents)

- **Limited Safety assessment (GLP)**
  - Single dose in 1 mammalian species (rat)
    - 5 days of observation
    - Dose >100 times the intended dose; range studied 3-10 mg/kg
    - IV and PO
  - Genotox battery (Ames and mouse lymphoma)
  - Safety pharmacology
    - In vitro-HERG
    - In vivo-Dog single dose
Microdosing clinical study: Regulatory documents

- IMPD for each compound
  » Prepared by Neurocrine with help from PRA’s Pharmacist
- IBs for four novel compounds
  » Prepared by Neurocrine
  » No IB needed for diphenhydramine
- Clinical protocol
  » Prepared by PRA, Neurocrine and Xceleron
- Regulatory submission to IEC (Netherlands)
  » Coordinated by PRA
- Bioanalytical and PK study protocol
  » Prepared by Xceleron and Neurocrine
Diphenhydramine Comparison of CxT curve:
50 mg dose vs. 0.1 mg (Dose Normalized)

50 mg data estimated from Blyden et al., J Clin Pharmacol 1986;26:529-533
Diphenhydramine microdose PK comparison with published literature:
*Data normalized to 50 mg dose where applicable*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Microdose</th>
<th>Spector et al., 1980</th>
<th>Berlinger et al., 1982</th>
<th>Meredith et al., 1984</th>
<th>Blyden et al., 1986</th>
<th>Simons et al., 1990</th>
<th>Scavone et al., 1990</th>
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</thead>
<tbody>
<tr>
<td>Vd (L)</td>
<td>313</td>
<td>480</td>
<td>295</td>
<td>462</td>
<td>317</td>
<td>249</td>
<td></td>
</tr>
<tr>
<td>Cl (L/h)</td>
<td>24</td>
<td>79</td>
<td>42</td>
<td>41</td>
<td>26</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>9.0</td>
<td>4.3</td>
<td>4.9</td>
<td>9.3</td>
<td>8.5</td>
<td>9.2</td>
<td>4.7</td>
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<tr>
<td>PO-%F</td>
<td>35</td>
<td>58</td>
<td></td>
<td></td>
<td>72</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>PO-Cmax (ng/mL)</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
<td>66</td>
<td>76</td>
<td>48</td>
</tr>
<tr>
<td>PO-AUC (ng.h/mL)</td>
<td>675</td>
<td></td>
<td></td>
<td></td>
<td>667</td>
<td>585</td>
<td>270</td>
</tr>
</tbody>
</table>
IV/PO Microdosing PK profiles of four novel compounds

**NBI-1**
- IV: 0.1mg
- PO: 0.1mg

**NBI-2**
- IV: 0.1mg
- PO: 0.1mg

**NBI-3**
- IV: 0.1mg
- PO: 0.1mg

**NBI-4**
- IV: 0.1mg
- PO: 0.1mg
NBI-1 Comparison of CxT curve:
10 mg dose vs. 0.1 mg (Dose Normalized)

10 mg data from Phase I NBI-1-0601 study on file at Neurocrine Biosciences.
Variability in microdose PO PK: All five compounds
Variability in IV PK parameters: All five compounds

- $\text{Cl}$ (L/h)
- $\text{Vss}$ (L)
- $\text{t}/2$ (h)
Mean pharmacokinetic parameters of five compounds following single 0.1 mg dose IV to humans (n=4)

<table>
<thead>
<tr>
<th>Drug</th>
<th>AUC(_{0-t}) (ng.hr/mL)</th>
<th>AUC(_{0-inf}) (ng.hr/mL)</th>
<th>t(_{1/2}) (h)</th>
<th>Cl (L/h)</th>
<th>V (L)</th>
<th>V(_{ss}) (L)</th>
<th>MRT(_{inf}) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPH</td>
<td>4.46 (11.9)</td>
<td>4.56 (10.2)</td>
<td>9.00 (29.6)</td>
<td>24.0 (9.98)</td>
<td>313 (33.1)</td>
<td>170 (40.8)</td>
<td>7.00 (34.7)</td>
</tr>
<tr>
<td>NBI-1</td>
<td>5.12 (22.2)</td>
<td>5.21 (21.7)</td>
<td>9.39 (31.4)</td>
<td>21.9 (20.6)</td>
<td>297 (35.3)</td>
<td>134 (22.6)</td>
<td>6.36 (31.5)</td>
</tr>
<tr>
<td>NBI-2</td>
<td>10.6 (21.7)</td>
<td>10.8 (21.5)</td>
<td>8.90 (12.3)</td>
<td>10.5 (23.4)</td>
<td>135 (24.6)</td>
<td>79.8 (36.0)</td>
<td>7.49 (21.5)</td>
</tr>
<tr>
<td>NBI-3</td>
<td>7.41 (14.6)</td>
<td>7.74 (15.6)</td>
<td>11.0 (36.8)</td>
<td>14.0 (17.6)</td>
<td>217 (36.8)</td>
<td>155 (40.4)</td>
<td>11.3 (40.0)</td>
</tr>
<tr>
<td>NBI-4</td>
<td>3.11 (64.9)</td>
<td>3.38 (65.2)</td>
<td>15.2 (54.6)</td>
<td>42.1 (51.9)</td>
<td>742 (31.6)</td>
<td>392 (50.0)</td>
<td>10.5 (48.5)</td>
</tr>
</tbody>
</table>

Data are averages of 4 subjects
Data in parentheses are % CV of the averages
### Mean pharmacokinetic parameters of five compounds following single oral 0.1 mg dose to humans (n=4)

<table>
<thead>
<tr>
<th>Drug</th>
<th>(T_{\text{max}}) (h)</th>
<th>(C_{\text{max}}) (ng/mL)</th>
<th>(\text{AUC}_{0-t}) (h.ng/mL)</th>
<th>(\text{AUC}_{0-\text{inf}}) (h.ng/mL)</th>
<th>(t_{1/2}) (h)</th>
<th>MRT(_{\text{inf}}) (h)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPH</td>
<td>2.25 (42.6)</td>
<td>0.191 (16.3)</td>
<td>1.35 (15.3)</td>
<td>1.58 (13.7)</td>
<td>14.2 (22.9)</td>
<td>14.7 (13.3)</td>
<td>0.35 (15.1)</td>
</tr>
<tr>
<td>NBI-1</td>
<td>1.19 (46.7)</td>
<td>0.746 (42.2)</td>
<td>2.92 (37.3)</td>
<td>2.99 (37.4)</td>
<td>6.79 (40.1)</td>
<td>6.44 (20.9)</td>
<td>0.58 (37.8)</td>
</tr>
<tr>
<td>NBI-2</td>
<td>1.44 (72.9)</td>
<td>0.705 (21.9)</td>
<td>6.41 (18.7)</td>
<td>6.53 (18.2)</td>
<td>8.62 (22.5)</td>
<td>10.9 (14.0)</td>
<td>0.62 (16.7)</td>
</tr>
<tr>
<td>NBI-3</td>
<td>1.75 (28.6)</td>
<td>0.364 (23.3)</td>
<td>3.39 (36.5)</td>
<td>3.74 (34.4)</td>
<td>11.6 (34.3)</td>
<td>14.1 (31.9)</td>
<td>0.48 (20.7)</td>
</tr>
<tr>
<td>NBI-4</td>
<td>1.50 (38.5)</td>
<td>0.114 (59.0)</td>
<td>0.835 (85.8)</td>
<td>0.914 (75.4)</td>
<td>11.8 (24.9)</td>
<td>14.9 (47.1)</td>
<td>0.28 (55.2)</td>
</tr>
</tbody>
</table>

Data are averages of 4 subjects
Data in parentheses are % CV of the averages
Comparison of predicted PK parameters:
in vitro and allometry vs. microdosing

Predicted Cl

Predicted Vss

Systemic Clearance (mL/min/Kg)

In vitro
Allometry
Microdosing

Volume of distribution at steady state (L)

Allometry
Microdosing

NBI1
NBI2
NBI3
NBI4

Over-prediction

Variable errors
Conclusions

- Human microdosing PK data provided key information for back-up compound selection for an insomnia program
  - Bioavailability
  - Clearance, volume of distribution
  - Variability in PK
- Based on the data, NBI-2 was selected as the most suitable development candidate
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