Penta-DOPA, a New Highly Soluble L-DOPA Ester for Continuous Dopaminergic Stimulation (CDS) in the Treatment of Parkinson´s Disease

Contributors

Berlirem GmbH, Berlin
M. Bräutigam
M. Hümpel
D. Palla
J. Tack
C. Völkel

INNOVENT e.V., Jena
T. Laube
M. Schnabelrauch
R. Wyrwa

Berlin, March 2020
Penta-DOPA in Parkinson`s Disease

ABSTRACT

Introduction/Background
Continuous Dopaminergic Stimulation (CDS) based therapy is still an unmet medical need to further improve the therapy of Parkinson`s disease (PD). Depending on the severity of PD, constant L-DOPA plasma levels of ≥ 800 ng/ml will be effective in CDS [1]. Recently a L-DOPA prodrug [2] of high water-solubility or new L-DOPA formulations [3] for continuous s.c. infusion have emerged for development.

[1] Le Witt PA Review: Levodopa Therapy for Parkinson’s Disease: Pharmacokinetics and Pharmacodynamics; Published online 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.26082
ABSTRACT (continued)

Strategy BERLIREM
• To follow the pro-drug concept of polyalcohol-esters of L-DOPA.

• To synthesize the L-DOPA glycol ester Gly-DOPA and the respective pentaerythritol ester Penta-DOPA.

• To investigate their chemistry, solubility, and in vitro stability.

• To study their first subcutaneous infusions in man.
Animal Study with Gly-DOPA

The L-DOPA glycerol ester (Gly-DOPA) was tested in the s.c. minipig model for PK and local tolerance.

Favourably constant L-DOPA plasma levels and very good local tolerance even at highly concentrated solutions infused showed the general suitability of this pro-drug concept (data not shown).
Penta-DOPA in Parkinson`s Disease

ABSTRACT (continued)

Human pharmacology: 4 hours s.c. infusion

• Gly-DOPA and Penta-DOPA were tested for PK and tolerance in three male subjects (intra-individual comparison) during four hours s.c. infusion.

• Local tolerance was found to be excellent and L-Dopa plasma levels showed low variability between individuals in case of Penta-DOPA. Penta-DOPA was selected for further studies.
ABSTRACT (continued)

Human pharmacology: 12-24 hours s.c. infusion

• Local tolerance and L-DOPA levels during a 12-24 hours constant infusion was tested in the same three individuals.

• Infusions were accompanied by oral COMT- and decarboxylase inhibition (opicapone, benserazide).

• After 12 to 24 hours of s.c. infusion individual L-DOPA plasma levels had increased clearly above the CDS-threshold level of 0.8 µg/ml.
ABSTRACT (continued)
Human pharmacology: 12-24 hours s.c. infusion

• The systemic tolerance was very good with some expected transient episodes of dizziness.
• Local tolerance again was good with some transient tenderness on palpation during and after infusion (2/3) or no side effects (1/3).

CONCLUSION:
By human pharmacology studies Penta-DOPA had been selected for further pro-drug development.
Objectives (main)

- To overcome the low solubility of L-DOPA preventing its infusion in PD,
- To achieve constant L-DOPA levels because On/Off episodes in PD are directly connected to fluctuating levels typically present in oral therapies [4],
- To synthesize highly water-soluble pro-drugs of L-DOPA as a prerequisite for s.c. infusion and constant L-DOPA levels,
- To construct pro-drugs as esters liberating L-DOPA by various esterase activities ubiquitously present in tissues and organs.

Penta-DOPA in Parkinson`s Disease

Objectives (secondary)

• To safeguard drug stability,

• To develop a suitable preparation,

• To secure a good local tolerance of mini-pump treatments,

• To demonstrate sufficiently high and constant L-DOPA plasma levels of $\geq 0.8 \, \mu g/ml$ in humans.
Penta-DOPA in Parkinson`s Disease

METHODS

Chemistry

• Synthesis of a L-DOPA derivative in which the amino and phenolic functions were protected.
• The derivative was subsequently coupled with the primary alcoholic function of glycerol or pentaerythritol.
• As the last step, Gly-DOPA and Penta-DOPA were synthesized by removing the protecting groups by hydrogenation [5].
• Compounds were purified by crystallisation or preparative chromatography.

# Methods

## Table 1: Physico-chemical data of two L-DOPA-esters

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gly-DOPA</th>
<th>Penta-DOPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW&lt;sup&gt;1&lt;/sup&gt;</td>
<td>273.3</td>
<td>315.3</td>
</tr>
<tr>
<td>LogP&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-0.71</td>
<td>-1.35</td>
</tr>
<tr>
<td>pKa:-NH&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.7</td>
<td>6.6</td>
</tr>
<tr>
<td>Stability H&lt;sub&gt;2&lt;/sub&gt;O&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 2.7</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>pH 4.6</td>
<td>3.5</td>
<td>2.1</td>
</tr>
<tr>
<td>pH 6.0</td>
<td>10.3</td>
<td>9.2</td>
</tr>
<tr>
<td>Solubility&lt;sup&gt;5&lt;/sup&gt; (g/ml H&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>≥ 1.0</td>
<td>≥ 2.0</td>
</tr>
</tbody>
</table>

<sup>1</sup>= molecular weight, add 36.5 DA for the respective HCl salt;  
<sup>2</sup>= calculated;  
<sup>3</sup>= calculated for amino group;  
<sup>4</sup>= % degradation after 24 hour at RT (pH 6 interpolated);  
<sup>5</sup>= for HCl salt at ambient temperature.
Methods

Stability *in vitro*
(model: human plasma)

- 2.0 ml plasma (n=3) were mixed with 40 µg Gly-DOPA or L-DOPA ethyl ester (DOPA-EE) and incubated at 37 °C for two hours.

- Samples were taken after 2, 15, 33, 45, 60, and 120 minutes, proteins precipitated and concentrations of Gly-DOPA, DOPA-EE and L-DOPA were analysed by UPLC-PDA-MS/MS.
Methods

Study in the (Göttingen-) minipig-model [6]

• At weekly intervals three animals were dosed with vehicle (10mM phosphate buffer pH 4), 360 mg Gly-DOPA HCl, 720 mg Gly-DOPA HCl, or 720 mg Gly-DOPA HCl plus oral 115 mg benserazide per day for three days.

• Treatments were subcutaneously using a CRONO pump with a flow of 0.3 ml/hour.

• Local tolerance was assessed and PK blood samples were taken for analysis of L-DOPA (and other analytes).

[6] Biotrial OBRIT 16: Evaluation of pharmacokinetics and skin tolerability of L-DOPA glycerin ester hydrochloride after continuous subcutaneous infusion in Göttingen minipigs; March 14, 2019
Penta-DOPA in Parkinson`s Disease

Methods

Human pharmacological studies

• Three studies were carried out in own responsibility of the three volunteers and under medical control and supervision following a written study protocol (Table 2).

• Local tolerance was assessed for 5 days after start of treatment and subjects reported any palpable changes at the site of injection for another 2 weeks.

• Local effects were scored with the 5-point Draize reaction score on erythema and edema.
Methods

Human pharmacological studies (continued)

• Systemic side effects were spontaneously reported by the subjects or reported in written form after leaving the unit.

• L-DOPA plasma/urine levels were quantified using validated LC/MS methods.

• In study 1, the renal excretion of Gly-DOPA was followed using a validated LC/MS method. 3-O-methyl-DOPA (OMD) was additionally analyzed in order to control the COMT-inhibition effect of opicapone (Ongentys®).
# Methods

**Table 2: Summary of study protocols**

1 = given as mg L-DOPA equivalents;  
2= Catechol-O-methyltransferase inhibition by 50 mg opicapone;  
3= Decarboxylase inhibition by benserazide 50 mg or hourly 25 mg;  
4= with 0 as start of treatment;  
5 = P means plasma, U urine

<table>
<thead>
<tr>
<th>Study</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug</strong></td>
<td>Gly-DOPA</td>
<td>Penta-DOPA</td>
<td>Penta-DOPA</td>
</tr>
<tr>
<td><strong>Subjects</strong></td>
<td>3M</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>88±9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>184±5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>71±3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Duration (h)</strong></td>
<td>4</td>
<td>4</td>
<td>12/24</td>
</tr>
<tr>
<td><strong>Dose1/h</strong></td>
<td>19.2</td>
<td>19.2</td>
<td>19.2</td>
</tr>
<tr>
<td><strong>Volume (ml/h)</strong></td>
<td>0.30</td>
<td>1.37</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>Total Dose1</strong></td>
<td>76.9</td>
<td>76.9</td>
<td>231/461</td>
</tr>
<tr>
<td><strong>COMT-Inh.²</strong></td>
<td>-24h, -2h</td>
<td>-24h, -2h</td>
<td>-24h, -2h, +12h⁴</td>
</tr>
<tr>
<td><strong>Dinh.³</strong></td>
<td>-1h, +2h</td>
<td>-1h to 4h</td>
<td>-1 to 12/24h⁴</td>
</tr>
<tr>
<td><strong>Samples⁵</strong></td>
<td>P, U</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>

- Pump System:  
  - Study 1: Omnipod  
  - Study 2: Cane Crono Pump  
  - Study 3: Cane Crono Pump
RESULTS

Synthesis, physicochemical properties:

- The chemical synthesis of Gly-DOPA and Penta-DOPA had been established.
- Figure 1 gives the structural formulas. Gly-DOPA is the ester of L-Dopa with Glycerol. Penta-DOPA is the ester of L-Dopa with Pentaerythritol.
- Gly-DOPA is a mixture of primary and secondary glycerol esters. The primary glycerol ester has two stereoisomers.
- The slightly basic compounds were isolated as HCl salts.
- Solubility in water was 1-2 g/ml.
- The hydrolytic stability of esters was pH dependent with negligible ester cleavage at pH 4-5.
- Compounds were dissolved and administered in 10mM phosphate buffer.
RESULTS

Figure 1: Structural formulas

Gly-DOPA

Penta-DOPA

* chiral center
RESULTS

Synthesis, *in vitro* properties:

- *In vitro* enzymatic cleavage of Gly-DOPA and L-DOPA-EE was tested in the model of fresh human plasma.
- Ester cleavage was shown to proceed at an identical rate and to the same extent for both the tested L-DOPA esters, Gly-DOPA and Ethyl-DOPA.
- Cleavage was by 50% after two hours and the respective amounts of L-DOPA were generated (Figure 2).
RESULTS
Figure 2: Cleavage of ester bond by fresh human plasma

Dotted lines: L-DOPA
Blue: from Gly-DOPA
Orange: from L-DOPA-EE
Full lines: esters
Penta-DOPA in Parkinson`s Disease

RESULTS

Gly-DOPA in minipigs after s.c.-infusion for 72 hours:

• The animal model is a widely accepted model.
• The local tolerance of the formulation was very good.
• The Gly-DOPA treatments induced very slight erythemas and edemas which resolved within 3-days after infusions.
• Gly-DOPA itself was cleared from plasma at an extremely high rate (estimated to 100 ml/min/kg) pointing to a mostly extra-hepatic de-esterification.
RESULTS

Gly-DOPA in minipigs after s.c.-infusion for 72 hours:
• L-DOPA levels reached steady-state within the first 12 hours of treatment and co-administration of benserazide markedly increased plasma levels.
• OMD-levels were found to be higher than respective L-DOPA levels. With time, ratios increased from 1.5 to 3 (Figure 3).

CONCLUSION

➢ Confirmation of the pro-drug strategy for L-DOPA.
➢ Constant L-DOPA levels in vivo by s.c. Gly-DOPA.
➢ Treatment with effective doses by s.c. infusion.
RESULTS

Gly-DOPA in minipigs after s.c.-infusion for 72 hours

Figure 3:
Mean plasma levels of Gly-DOPA (low), L-DOPA (middle) and 3OMD (top) in mini-pigs during and after 3-days s.c. infusion of 720 mg Gly-DOPA/d and oral coadministration of 115 mg benzerazide/d given within the first 12 hours of each treatment day.
RESULTS

Human pharmacological studies

Study 1: Gly-DOPA for four hours

- L-DOPA levels rather differed between individuals (Figure 4).
- $\text{AUC}_{0-4h}$ values were 960, 555, and 233 ng*h/ml.
- Less than 1% of dose were excreted with the urine as parent.
- Gly-DOPA plasma levels showed a similar high scatter as L-DOPA levels.
- Individual clearance rates of parent compound were 17, 40 and 156 ml/min/kg.
- Mean half-life of disposition was 2.1 hours (Figure 5)
RESULTS

Human pharmacological studies

Study 1: Gly-DOPA for four hours (continued)

Figure 4:
L-DOPA plasma levels in three male subjects during a 4 hourly subcutaneous infusion of Gly-DOPA; the L-DOPA equivalent dose was 19.2 mg/h; study included oral treatments to inhibit COMT and decarboxylase activity.
RESULTS

Human pharmacological studies

*Study 1: Gly-DOPA for four hours*

![Graph showing Mean L-DOPA Plasma Levels over time.]

- Figure 5:
  - Mean L-DOPA plasma levels of three male subjects (study 1); note that last oral benserazide was taken at +2 hours and the last opicapone dose was at -2 hours;
  - half-life of post-treatment disposition was 2.1 hours (calculated from means)
Human pharmacological studies

Study 1: Gly-DOPA for four hours (continued)

• OMD-plasma levels were below the detection limit proving the inhibition of COMT activity by opicapone.
• A moderate to severe local side effect was present in one subject, i.e. swelling, elevated skin temperature, small hematomas and pain (6-7 cm circle around injection).
• The other two subjects only showed mild and transient erythemas.
• All effects had resolved completely after 5 days.
RESULTS

Human pharmacological studies

*Study 2: Penta-DOPA for four hours*

- Individual L-DOPA levels showed almost identical plasma levels.
- Increase rates seemed to follow a zero-order kinetics (Figure 6).
- $\text{AUC}_{0-4\text{h}}$ values were 920, 686, and 828 ng*h/ml.
- Mean AUC ($N=3$) was $811 \pm 117$ ng*h/ml.
- Mean L-DOPA plasma levels over the complete follow up are displayed in Figure 7.
RESULTS

Human pharmacological studies

*Study 2: Penta-DOPA for four hours (continued)*

- OMD plasma levels were either below the detection limit, below 25 % of respective L-DOPA levels or increasing up to L-DOPA-levels at later time points.
- Local tolerance was excellent with no side effects in any of the three subjects.
- As in study 1, one subject reported some episodes of dizziness after about 3 hours of infusion.
- No systemic effects were reported by the other two subjects in both the two studies.
RESULTS

Human pharmacological studies

Study 2: Penta-DOPA for four hours

Figure 6:
L-DOPA plasma levels in the same three subjects as in study 1 during a 4 hourly subcutaneous infusion of Penta-DOPA; the L-DOPA equivalent dose was 19.2 mg/h; study included oral treatments to inhibit COMT and decarboxylase activity

Berlin, March 2020
RESULTS

Human pharmacological studies

Study 2: Penta-DOPA for four hours

Figure 7:
Mean (N=3) L-DOPA plasma levels during and after a 4 hours s.c. infusion of Penta-DOPA; note that oral benserazide was taken up to 7 hours and the last opicapone dose was at -2 hours; no half-life of post-treatment disposition was estimated.
RESULTS

Human pharmacological studies

Study 3: Penta-DOPA for 12 to 24 hours

• S.C.-infusion of Penta-DOPA to the same subjects as in studies 1 and 2 for 12 (N=1) or 24 hours (N=2) (Figure 8).

• L-DOPA levels showed identical increases as in the 4 hour study.

• Normalized plasma levels were identical to ABBV-951 [3] (Figure 9).

• Mean L-DOPA plasma levels rapidly increased during the first 4 hours followed by a slower increase up to 12 or 24 hours.

RESULTS

Human pharmacological studies

Study 3: Penta-DOPA for 12 to 24 hours

Figure 8: Individual (yellow, blue, green) and mean (red squares) L-DOPA plasma levels during 12 to 24 hours subcutaneous infusion with Penta-DOPA (L-DOPA equivalent dose is 19.2 mg/hour); inhibition of COMT and decarboxylase activity was continued up to 12 or 24 hours, respectively.
RESULTS

Human pharmacological studies

Study 3: Penta-DOPA for 12 to 24 hours

Figure 9: Comparison of mean L-DOPA plasma levels during the first 4 hours of subcutaneous infusion of Penta-DOPA (study 2: orange squares; study 3: blue circles) and L-DOPA phosphate (green triangles; ABBV-951; L-DOPA levels were linearly normalised for 25 mg L-DOPA equivalents/h.)
RESULTS

Human pharmacological studies

*Study 3: Penta-DOPA for 12 to 24 hours*

- On average, steady-state levels of about 900 ng/ml can be anticipated after infusion of an equivalent L-DOPA dose of 460 mg/d. Steady-state is reached after one day.

- Local tolerance was good with some transient tenderness on palpation during and after infusion (2/3) or no side effects (1/3).

- Systemic tolerance was very good with some expected transient episodes of dizziness.
CONCLUSIONS (1)

• Two new L-DOPA poly-alcohol esters were synthesized and showed suitable physico-chemical properties, i.e. very high water-solubility and high hydrolytic stability at moderate pH.

• In vitro experiments showed rapid enzymatic ester cleavage in human plasma which was confirmed by the extremely high clearance of Gly-DOPA in the minipig-model.

• The high solubility of the two new pro-drugs of L-DOPA enables a subcutaneous infusion of pro-drug solutions in a relatively small volume.

• The solution will contain a sufficient daily L-DOPA equivalent dose (≥ 460 mg/d).
Penta-DOPA in Parkinson`s Disease

CONCLUSIONS (2)

• Results from the study in the minipig model served as stable basis for human pharmacological studies with Gly-DOPA and the sister molecule, Penta-DOPA.
• Compared by two 4-hours s.c.-infusion studies Penta-DOPA was selected for further development.
• Main reasons were higher L-DOPA levels, a lower scatter in plasma levels and the absence of local side effects.
Penta-DOPA in Parkinson`s Disease

CONCLUSIONS (3)

• The 12-24h infusion of Penta-DOPA resulted in highly reproducible (study 2) and stable L-DOPA plasma levels.

• Under inhibition of oral COMT and decarboxylase activity, steady-state L-DOPA plasma levels of about 900 ng/ml will be achieved 24 hours after start of treatment. The necessary L-DOPA equivalent dose is 460 mg/day.
CONCLUSIONS (4)

- Known systemic side effects were transient and mild.
- Local side effects were mild and resolved during treatment or shortly thereafter.
- Present L-DOPA plasma level for Penta-DOPA were not different from published data for ABBV-951 (levodopa phosphate in combination with carbidopa phosphate).
Penta-DOPA in Parkinson`s Disease

Penta-DOPA is a promising development candidate to fill the gap of Continuous Dopaminergic Stimulation in Parkinson`s Disease treatment.
In order to get further information, don’t hesitate to contact:

Dr. Johannes Tack
j.tack@berlirem.com
www.berlirem.com