IN-VIVO STUDIES OF ANTI-PARKINSON ACTIVITY OF ROPINIROLE HYDROCHLORIDE LOADED IN MICROEMULSION FOR BRAIN TARGETING BY INTRANASAL DELIVERY

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ABSTRACT
In the present study, we evaluated anti-Parkinson’s activity of Ropinirole hydrochloride loaded in microemulsion via intranasal delivery for brain targeting. All animal experiments were approved and performed in Malla Reddy College of Pharmacy accordance with the guidelines of Institutional Animal Ethics Committee (CPCSEA Registration No: 1217/PO/RE/S/2008). The results of biodistribution studies showed the time profile of Ropinirole Hydrochloride concentration in brain and plasma higher after intranasal (IN) administration of drug-loaded ME as compared to intravenous (IV) administration of PDS. The first finding of our study was that intranasal administration allowed Ropinirole Hydrochloride uptake into the CNS. After the initial 20 min, the drug concentration in the brain was found higher for intranasal delivered microemulsion (0.9334±0.0292µg/ml) than the Intravenous administered PDS (0.1567±0.023 µg/ml). In vivo studies data suggest that the nasal route could exploit to increase the availability of Ropinirole Hydrochloride inside the brain. However, clinical benefits of the formulation developed in this investigation will decide its appropriateness in the clinical practice for the treatment of Parkinson’s disease.

KEYWORDS: Anti-Parkinson activity, Ropinirole Hydrochloride Microemulsion, Brain targeting.

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INTRODUCTION

Ropinirole hydrochloride (RH), commonly used for Parkinson’s and restless legs syndrome, is administered orally as dopamine receptor agonist. Ropinirole hydrochloride is identified chemically as 4-[2-(dipropylamino) ethyl]-1,3-dihydro-2Hindol-2- one hydrochloride. The chemical structure of RH is shown in Figure 1.14. It has a molecular mass of 296.84 (260.38 free base) and its molecular formula is C_{16}H_{24}N_{2}0.HCl. \cite{1}

As mentioned above, Ropinirole is used to treat Parkinson’s disease by alleviating the dopamine deficiency. It is a non-ergoline dopamine D2/D3 receptor agonist that stimulates striatal dopamine receptors. Ropinirole binds to central and peripheral dopamine receptors with an order of receptor affinity similar to that of dopamine. It is highly selective D3 rather than D2. Ropinirole is 20 times more selective for D3 receptors than D2 receptors and about 50 times more selective for D3 than D4 receptors, with negligible affinity for D1 receptors. It has little or no affinity for β-adrenoceptors or adrenergic, serotonergic, GABA or benzodiazepine receptors. Ropinirole acts on postsynaptic dopamine receptors in the CNS associated with Parkinson’s disease. Ropinirole has about 50% bioavailability since it undergoes first pass effect after absorption. The main metabolic pathway is the cytochrome P450 (CYP) isozyme CYP1A2, with a minor contribution from CYP3A. 10% of a ropinirole dose is excreted unchanged in urine. The distribution volume of ropinirole is 7 L/kg, with plasma protein binding of 10–40%. Ropinirole has an average elimination half-life of approximately 6 hours. Variety of devices can be used for the nasal administration of dosage forms. Nasal drops and nasal sprays are delivery devices for liquids formulations, while nasal insufflators are delivery devices for powders.\cite{2,3}

Parkinson’s disease (PD) is a progressive neurodegenerative disease resulting from the destruction of dopaminergic neurons of the A9 nigrostriatal pathway originating in the midbrain. PD is a debilitating condition, which causes motor dysfunction, akinesia, and eventually death. The currently available treatment strategies for PD do not arrest disease progression and only provide patients with temporary symptomatic relief. \cite{4}

Neurotrophic factors are proteins that promote the growth, regeneration, and survival of Neurons. Several neurotoxin animal models have been developed to better understand the pathogenesis of PD, and to study potential therapeutic agents. The most commonly used neurotoxins for the development of PD animal models are 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, and 6-hydroxydopamine (6-OHDA). All three agents
are believed to induce the death of dopamine neurons by generating reactive oxygen species, and by inhibiting mitochondrial respiration.\textsuperscript{[5]}

In the 1980’s, MPTP emerged as the cause of a PD outbreak amongst young drug addicts. MPTP was accidentally synthesized from the illicit manufacture of 1-methyl-4-17 phenyl-4-propionoxypiperidine (MPPP), an analog of meperidine. MPTP was found to cause severe and irreversible symptoms of PD in humans, primates, and in some strains of mice. However, rats were unaffected by this neurotoxin. MPTP readily crosses the BBB, is oxidized to MPP+ (its active form) by monoamine oxidase B (MAO-B), and then undergoes uptake into presynaptic dopamine neurons by the dopamine transporter. Once inside the dopamine neuron, MPP+ inhibits mitochondrial complex I of the electron transport chain, depletes the neuron of ATP, and stimulates the production of reactive oxygen species.\textsuperscript{[6]}

In recent years, the intranasal route of administration has emerged as an attractive method for delivering brain impermeable drugs and proteins to the CNS. This is because intranasal drug administration is generally well tolerated, non-invasive, and because the olfactory route of administration completely bypasses the blood brain barrier. As a result, drugs and/or proteins can be transported directly from the nasal epithelium into the brain.\textsuperscript{[7,8]}

\textbf{MATERIAL AND METHODS}

\textbf{Biodistribution studies}

All animal experiments were approved and performed in Malla Reddy College of Pharmacy accordance with the guidelines of Institutional Animal Ethics Committee (CPCSEA Registration No: 1217/PO/RE/S/2008).

\textbf{Study design}

Male Sprague-Dawley rats weighing 250–270gm were selected for the biodistribution studies which were divided into two groups, one for intranasal and another for intravenous administration, respectively. The rats are anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg) and kept on a heating pad to maintain the body temperature. Group I, 50 ml of the formulation (2.5 mg/ml Ropinirole Hydrochloride loaded NE) were instilled into the nostrils with the help of intranasal route, (Meter dose pump VP7, Aptar Pharma India Pvt. Ltd) at the delivery site. The rats are held from the back in slanted position during intranasal administration. For the i.v. administration, the PDS delivered (dose equivalent to 2.5 mg/ml) through the tail. The rats sacrificed humanely at different time intervals and the
blood was collected using cardiac puncture. The animals decapitated immediately after blood
collection and the skull was open, the brain was carefully excise. Each brain tissue was
quickly rinse with saline and blotted up with filter paper to get rid of blood taint and
macroscopic blood vessels as much as possible and weighed. After weighing, the brain tissue
samples homogenized with one volume of saline in a tissue homogenizer (Teflon
homogenizer). Blood samples were anti-coagulated with heparin and centrifuged at 3000 rpm
for 10 min to obtain plasma. All plasma samples and brain homogenates were stored for up to
48 h in a deep freezer (-70°C) until HPLC analysis. [9]

**Processing of samples**

To a 200 µl plasma samples and 500 µl of brain homogenate, 25 µl of the IS (40 µg/ml, Ropinirole Hydrochloride) was spiked and vortex mixed for 30s. Then, 0.5 ml of acetonitrile
was added and vortex-mixed for 1 min. The samples are centrifuged at 8000 rpm for 5 min in
a centrifuge. The supernatant layer (0.75 ml) transferred to a 15ml of glass test tube, and then
4.5 ml of extraction solvent, methyl t-butyl ether– n-hexane (9:1) added. The sample was
mixed for 3 min using a multi-tube vortex mixer. The organic layer (4 ml) was quantitatively
transferred to a 6 ml glass tube and evaporated to dryness using an evaporator at 40°C under a
stream of nitrogen. Then the dried extract was reconstituted in 100 ml of water–methanol
(50:50, v/v; diluents) and a 20 ml aliquot was injected into chromatographic system. The
whole procedure was carried out at room temperature.

**Chromatographic conditions**

The chromatographic separation was performed at ambient temperature with a reverse phase,
150x4mm base specific column packed with 5 mm C18 silica reversed-phase particles. The
mobile phase was a mixture of 10 mm ammonium acetate buffer–acetonitrile (45:55, v/v)
pumped at a flow rate of 1.0 ml/min. Detection was perform at a wavelength of 250 nm.

**Data analysis**

All the data reported as mean ±S.D and the differences between the groups were tested at the
level of p< 0.05. All concentration data were dose and weight normalized. Pharmacokinetic
parameters for Ropinirole Hydrochloride microemulsion formulations were calculated using
Kinetica software. The Cmax and Tmax values of the intranasal and intravenous
administration read directly from the concentration–time profile. The area under the
concentration–time curve (AUC<sub>0-<i>t</i></sub>) was calculated by the trapezoidal rule.. The brain
targeting after nasal dosing, was evaluated by following two indexes
(i) **Drug targeting efficiency (DTE)** – represent a time average partitioning ratio.

\[
\%\text{DTE} = \left( \frac{\text{AUC}_{\text{brain}}}{\text{AUC}_{\text{blood}}} \right)_{\text{i:n}} \times \left( \frac{\text{AUC}_{\text{brain}}}{\text{AUC}_{\text{blood}}} \right)_{\text{i:v}} \times 100
\]

Where,

\[
\left( \frac{\text{AUC}_{\text{brain}}}{\text{AUC}_{\text{blood}}} \right)_{\text{i:n}} = \text{ratio of area under curve for Ropinirole Hydrochloride concentration in brain and blood after intranasal administration.}
\]

\[
\left( \frac{\text{AUC}_{\text{brain}}}{\text{AUC}_{\text{blood}}} \right)_{\text{i:v}} = \text{ratio of area under curve for Ropinirole Hydrochloride concentration in brain and blood after intravenous administration.}
\]

(ii) **Direct transport percentage (DTP)** – clarify nose to brain direct transport

\[
\%\text{DTP} = \frac{B_{\text{i:n}} - B_x}{B_{\text{i:n}}} \times 100 \quad \text{and} \quad B_x = \frac{B_{\text{i:n}}}{P_{\text{i:v}}} \times P_{\text{i:n}}.
\]

Where,

\(B_x\) - is the brain AUC fraction contributed by systemic circulation through the BBB following intranasal administration,

\(B_{\text{i:v}}\) – \(\text{AUC}_{0-60}\) (Ropinirole Hydrochloride concentration in brain) following intravenous administration,

\(P_{\text{i:v}}\) – \(\text{AUC}_{0-60}\) (Ropinirole Hydrochloride concentration in blood) following intravenous administration,

\(B_{\text{i:n}}\) – \(\text{AUC}_{0-60}\) (Ropinirole Hydrochloride concentration in brain) following intranasal administration,

\(P_{\text{i:n}}\) – \(\text{AUC}_{0-60}\) (Ropinirole Hydrochloride concentration in blood) following intranasal administration.\(^{[10]}\)

**RESULTS AND DISCUSSION**

**In vivo biodistribution studies**

The results of biodistribution studies showed the time profile of Ropinirole Hydrochloride concentration in brain and plasma higher after intranasal (IN) administration of drug-loaded ME as compared to intravenous (IV) administration of PDS. The first finding of our study was that intranasal administration allowed Ropinirole Hydrochloride uptake into the CNS. The profiles of Ropinirole Hydrochloride level in brain and plasma displayed an initial absorption phase and maximum concentration was achieved after about 40 min in brain and plasma after intranasal administration.

After the initial 20 min, the drug concentration in the brain was found higher for intranasal delivered microemulsion (0.9334±0.0292µg/ml) than the Intravenous administered PDS (0.1567±0.023 µg/ml).
As time increase, the concentration increased and thus, after 40 min, Intranasal delivered microemulsion showed higher accumulation (1.9313±0.0192µg/ml) of drug in the brain compared to intravenous administered PDS (0.275±0.09 µg/ml). The presence of Ropinirole Hydrochloride in plasma expected since this route can also lead to systemic drug absorption, but the measured plasma concentrations were well below those found in the brain. The highest concentration was observed in the plasma after Intravenous administration, the Cmax was 0.3966±0.006 µg/ml at Tmax of 9.91±0.62 min, where as the Cmax was 1.4133±0.0353 µg/ml at Tmax of (40±0.00), after intranasl administration.

This result shows that the high initial plasma concentration after Intravenous administration may be as lower transport of Ropinirole Hydrochloride across the BBB by passive diffusion. Based on the AUC data determined over 0–60 min period, the bioavailability of Ropinirole Hydrochloride nasal microemulsion found to be 68.47% for the doses examined compared to oral bioavailability of 55%. This could relate to the rapid absorption and longer residence time of the microemulsion in the rat nasal cavity, which provided the opportunity for intranasal delivery to the brain.

In addition, their smaller size potentially allows microemulsion to be transported transcellularly through olfactory neurons to the brain via the various endocytic pathways of sustentacular or neuronal cells in the olfactory membrane.

The excipients used Tween 80 and PEG 400 which hold the promise of significantly improving the nasal absorption of poorly soluble and absorbed drugs as a result of P-gp inhibition, and thus to enhance the bioavailability of these drugs.

The results of the present investigation prove that drug could transport directly to the CNS after intranasal delivery. Microscopic images illustrate the histopathological condition of nasal mucosa after 2hr exposure of (A, negative control) PBS pH 6.4; (B, positive control) IPA; (C) drug-loaded Microemulsion.

Ropinirole Hydrochloride concentration–time profiles after intranasal administration of Microemulsion and Intravenous administration of PDS at 2.5 mg doses in rats brain (A) and blood (B).
In order to more clearly present nose-to-brain direct transport following Intranasal delivered microemulsion, we introduced a term of DTP and DTE. The % DTP represents the percentage of drug directly transported to the brain via the olfactory pathway.

The ME showed the highest DTE% (267.15±0.443) and DTP% (62.83±0.665) suggesting that ME has better brain targeting efficiency mainly because of substantial DTP via the olfactory region of the nasal cavity.

Table 1: Ropinirole Hydrochloride concentration–time profiles after intranasal administration of Micro emulsion and IV administration of PDS in rats brain (A)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Intranasal administration, Micro emulsion Conc(µg/ml)</th>
<th>IV administration, PDS Conc(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0.262</td>
<td>0.445</td>
</tr>
<tr>
<td>20</td>
<td>0.296</td>
<td>0.396</td>
</tr>
<tr>
<td>30</td>
<td>1.198</td>
<td>0.277</td>
</tr>
<tr>
<td>40</td>
<td>1.457</td>
<td>0.239</td>
</tr>
<tr>
<td>50</td>
<td>0.687</td>
<td>0.093</td>
</tr>
<tr>
<td>60</td>
<td>0.283</td>
<td>0.054</td>
</tr>
</tbody>
</table>

Table 2: Ropinirole Hydrochloride concentration–time profiles after intranasal administration of Micro emulsion and IV administration of PDS in rats blood (B).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Intranasal administration, Micro emulsion Conc (µg/ml)</th>
<th>IV administration, PDS Conc (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0.242</td>
<td>0.045</td>
</tr>
<tr>
<td>20</td>
<td>0.886</td>
<td>0.126</td>
</tr>
<tr>
<td>30</td>
<td>1.292</td>
<td>0.285</td>
</tr>
<tr>
<td>40</td>
<td>1.953</td>
<td>0.134</td>
</tr>
<tr>
<td>50</td>
<td>0.895</td>
<td>0.097</td>
</tr>
<tr>
<td>60</td>
<td>0.486</td>
<td>0.064</td>
</tr>
</tbody>
</table>
Figure 1: Ropinirole Hydrochloride concentration–time profiles after intranasal administration of Micro emulsion and IV administration of PDS (plain drug suspension) in rats brain (A) and blood (B).

Figure 2: Chromatogram of standard Ropinirole HCl (500ngspot−1): peak (Rf=0.58) using optimized mobile phase consisting of toluene–ethyl acetate–6M ammonia solution (5:6:0.5, v/v/v).

Table 3: Pharmacokinetics parameters of Ropinirole Hydrochloride following nasal and intravenous administration

<table>
<thead>
<tr>
<th>Formulation and route of administration</th>
<th>Organ/Tissue</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;(µg/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (min)</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt; (min)</th>
<th>AUC&lt;sub&gt;0-60min&lt;/sub&gt; (µg/ml*min)</th>
<th>AUC&lt;sub&gt;0-α&lt;/sub&gt; (µg/ml*min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microemulsion (nasal)</td>
<td>Brain</td>
<td>1.9313±0.0192</td>
<td>40±0.0</td>
<td>26.31±0.493</td>
<td>55.2266±0.120</td>
<td>58.0988±0.838</td>
</tr>
<tr>
<td>Microemulsion (nasal)</td>
<td>Blood</td>
<td>1.4133±0.0353</td>
<td>40±0.0</td>
<td>19.566±0.225</td>
<td>39.8466±0.420</td>
<td>41.9446±0.686</td>
</tr>
<tr>
<td>PDS (intra venous)</td>
<td>Brain</td>
<td>0.275±0.09</td>
<td>29.5±0.5</td>
<td>18.37±0.121</td>
<td>8.0643±0.7721</td>
<td>10.2131±0.979</td>
</tr>
<tr>
<td>PDS (intra venous)</td>
<td>Blood</td>
<td>0.3966±0.006</td>
<td>9.91±0.62</td>
<td>30.27±0.163</td>
<td>15.5793±0.612</td>
<td>17.4212±0.918</td>
</tr>
</tbody>
</table>

Mean ±S.D, n=3
CONCLUSION

The result of present investigation shows that drug loaded oil in-water microemulsion for intranasal administration may be very promising approach for delivering anti-retroviral agent in order to achieve CNS targeting for the treatment of Parkinson’s disease. The physical form microemulsion in which Ropinirole Hydrochloride has given had a significant effect on the measured brain concentrations. In vivo studies data suggest that the nasal route could exploit to increase the availability of Ropinirole Hydrochloride inside the brain. However, clinical benefits of the formulation developed in this investigation will decide its appropriateness in the clinical practice for the treatment of Parkinson’s disease.

REFERENCE


