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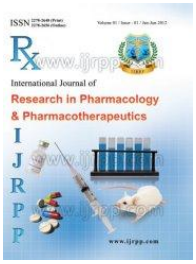

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In-Vivo studies of anti-parkinson activity of Ropinirole Hydrochloride loaded in microsphere for brain targeting by intranasal delivery

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ABSTRACT

Objective

In the present study, we evaluated anti-Parkinson's activity of Ropinirole hydrochloride loaded in microsphere by intranasal delivery for brain targeting.

Material & Method

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).

Result

For *In-Vivo* evaluation of nasal microspheres of Ropinirole Hydrochloride, rabbit was chosen as a model for study because the blood volume of the rabbit is sufficiently large (approximately 300 ml) to permit frequent blood sampling and allow a full characterization of the absorption and determination of the pharmacokinetic profile of the drug. The Cmax after oral dosing was found to be 107.833±1.567 ng/ml and the corresponding Tmax was at 1.86±0.066 hrs.

Conclusion

From all the parameters studied, it can be concluded that combination of Carbopol 974P and guar gum is better mucoadhesive polymer for the formulation of mucoadhesive microspheres of Ropinirole Hydrochloride for intranasal administration. Thus, the formulated microspheres seem to be a potential candidate as intranasal controlled drug delivery system for treatment of Parkinson's disease.

Keywords: Anti-Parkinson activity, Ropinirole Hydrochloride Microsphere, Brain targeting.

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. It has a molecular mass of 296.84 (260.38 free base) and its molecular formula is $C_{16}H_{24}N_{20}$.HCl [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 cyto-chrome 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 [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 [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 [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-4-17phenyl-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 [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 [7, 8].

MATERIAL AND METHODS

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).

Study design

Out of twenty one formulations, two were selected (one is guar gum and another is combination of Carbopol 974P and guar gum based microspheres) for *in vivo* bioavailability studies on the basis of data of the *in-vitro* results. *In-vivo* bioavailability studies were conducted on healthy male rabbits weighing around 2.5 kg.

Six rabbits were divided into three groups and fasted for 24 h. One batch was fed withthe oral tablet preparation (equivalent to 5 mg of drug); while other two batches were given the formulations F15 and F21 (equivalent to 5 mg of drug). Water was given *ad*

libitum during fasting and throughout the experiment. [9]

Processing of samples

Blood samples of 2 ml were collected from the marginal ear vein of the rabbits into heparinised centrifuge tubes at 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 12 h after the drug administration. The blood samples were centrifuged at 3000 rpm for 15 min to obtain the plasma and stored at -20°C until analysis. The extraction of drug from plasma was carried out and then analysis was carried out by using HPLC system.

Chromatographic conditions

The chromatographic separation was performed at ambient temperature with a reverse phase, 150×4 mm base specific column packed with 5 mm C18 silica reversed-phase particles. The mobile phase used was acetonitrile: 80 mm sodium sulphate solution (60: 40) at a flow rate of 1.5 ml/min. Detection was perform at a wavelength of 250 nm.

Pharmacokinetic analysis

Pharmacokinetic parameters were derived from the plasma concentration vs. time plot. The area under the curve (AUC), the peak plasma concentration (Cmax) and the time to attain peak concentration (Tmax) were obtained from the plot. The elimination rate constant (Kel) was determined from the semi-logarithmic plot of plasma concentration vs. time. Elimination half-life ($t^{1/2}$) was calculated using the formula; $t^{1/2} = 0.693/Kel$. [10]

Table 1: Pharmacokinetic parameters of oral tablet and intranasal microspheres of Ropinirole Hydrochloride

Formulation	Tmax (hr)	Cmax (ng/ml)	Kel (hr^{-1})	$T_{1/2}$ (hr)	$(AUC)_{0-12hr}$ (ng.hr/ml)	$(AUC)_{0-\alpha}$ (ng.hr/ml)
oral	1.86±0.066	107.833±1.567	0.263±0.004	2.48±0.02	308.99±4.40	369.82±3.41
F15	5.16±0.166	227.733±1.82	0.177±0.001	4.01±0.042	1365.76±4.472	1492.28±2.93
F21	5.08±0.088	274.866±2.567	0.159±0.002	4.62±0.077	1478.91±2.852	1593.61±3.87

Mean ± S.D, n=3

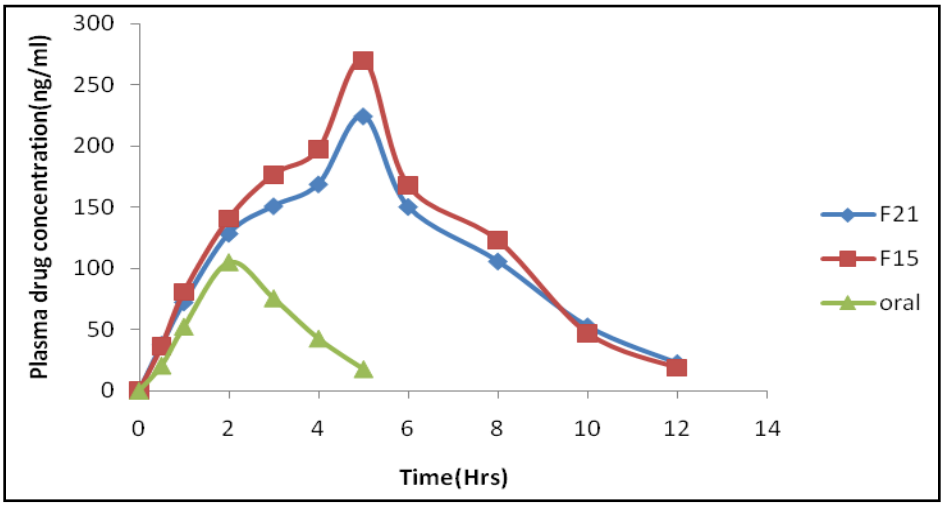

Figure 1: Ropinirole Hydrochloride concentration–time profiles after intranasal administration of Microspheres and oral tablet

Table 2: Ropinirole Hydrochloride concentration–time profiles after intranasal administration of Microspheres and oral tablet

Time (hrs)	Oral Concentration (ng/ml)	F15 Concentration (ng/ml)	F21 Concentration (ng/ml)
0.5	20.7	37.6	36.4
1	52.5	72.2	80.8
2	105.3	128.3	140.7
3	75.8	150.8	176.6
4	42.7	168.7	197.2
5	17.8	224.2	270.1
6	--	150.2	167.7
8	--	105.7	122.9
10	--	52.7	47.1
12	--	22.6	18.7

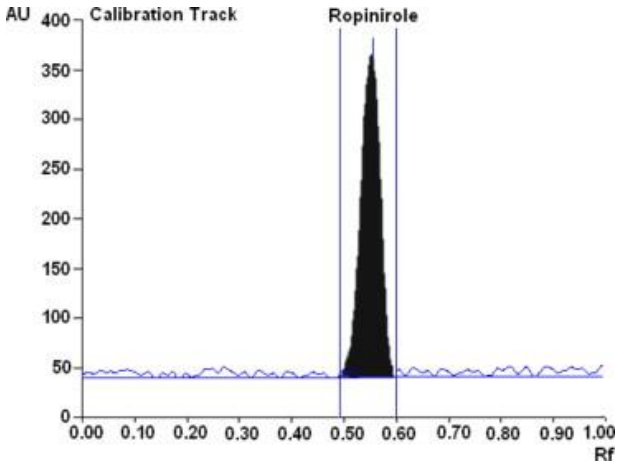

Figure 2: Chromatogram of standard Ropinirole Hydrochloride (500ngspot−1): peak (Rf=0.58) using optimized mobile phase consisting of acetonitrile: 80 mm sodium sulphate solution (60:40)

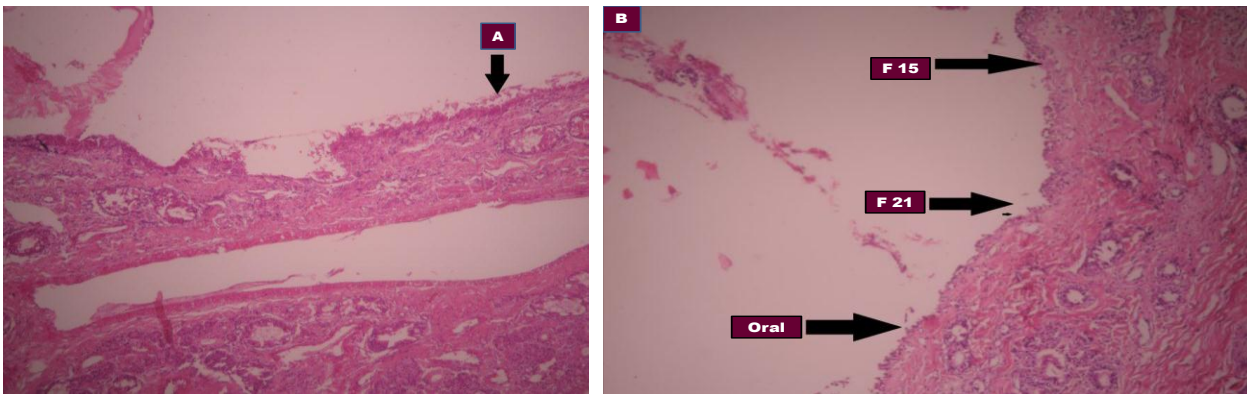

Figure 3: Microscopic images of nasal mucosa treated with (A) Negative control, (B) drug-loaded microsphere F15, F21 and Oral tablet

DISCUSSION

From the *in vitro* release graph, formulations F15 and F21 were selected as the best formulations based on guar gum and combination of Carbopol 974P and guar gum based microspheres respectively as they showed controlled release of drug up to 12 h.

For *in vivo* evaluation of nasal microspheres of Ropinirole Hydrochloride, rabbit was chosen as a model for study because the blood volume of the

rabbit is sufficiently large (approximately 300 ml) to permit frequent blood sampling and allow a full characterization of the absorption and determination of the pharmacokinetic profile of the drug. The Cmax after oral dosing was found to be 107.833±1.567 ng/ml and the corresponding Tmax was at 1.86±0.066 hrs. It was observed that the concentration of drug in the plasma decreased very fast after the Cmax was attained for oral group. The concentration obtained at 5 hr was 17.8 ng/ml. The concentration range in the subsequent hours was in the traces and was out of the limit of detection. The highest concentration was observed in the plasma after intranasal administration, the Cmax was 227.733±1.82 ng/ml and 274.866±2.567 ng/ml for formulations F15 and F21 respectively and the corresponding Tmax was found to be 5.16±0.166 hrs and 5.08±0.088 hrs for both the formulations.

This result shows that the high initial plasma concentration after intranasal administration of microspheres may be as lower transport of Ropinirole Hydrochloride across the BBB by passive diffusion. Based on the AUC data determined over 0–12 hr period, the bioavailability of Ropinirole Hydrochloride nasal microspheres for the formulations F15 and F21 found to have significantly increased 4.51% and 4.90% respectively as compared to oral bioavailability of 55%. This could relate to the rapid absorption and longer residence time of the microspheres in the rat nasal cavity, which provided the opportunity for intranasal delivery to the brain. The Pharmacokinetic parameters of oral tablet and intranasal microspheres of Ropinirole Hydrochloride.

CONCLUSION

The present study has been satisfactorily attempted to formulate a mucoadhesive microspheres system of an antiparkinsons drug like Ropinirole Hydrochloride for intranasal administration with a view of enhancing bioavailability of the drug. Assessment of AUC showed that the relative bioavailability was found to have significantly increased 4.51% and 4.90% for F15 and F21 respectively. From all the parameters studied, it can be concluded that combination of Carbopol 974P and guar gum is better mucoadhesive polymer for the formulation of mucoadhesive microspheres of Ropinirole Hydrochloride for intranasal administration. Thus, the formulated microspheres seem to be a potential candidate as intranasal controlled drug delivery system for treatment of Parkinson's disease.

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