Research Article

In vivo evaluation of a novel zero order drug releasing transdermal system of rotigotine

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Abstract

Background: Rotigotine is a new non-ergolinic dopamine agonist used in the treatment of Parkinson's disorder and restless legs syndrome. It has extensive pre-systemic metabolism and administration of rotigotine through transdermal route is the best way to avoid pre-systemic metabolism and thereby to increase the bioavailability and to deliver the drug in a controlled order. **Objective:** A controlled release transdermal system was developed using a novel hybrid technique including a combination of micro reservoir and adhesive dispersion system. **Materials and Methods:** Transdermal system was prepared by incorporating rotigotine drug in adhesive matrix layer in which microspheres loaded with rotigotine were dispersed. Microspheres were prepared by spray drying technique using poly-e-caprolactone and maltodextrin (1:1 ratio) as carriers in various drug to polymer ratios. **Results:** Microsphere composition comprising drug to polymer ratio 1:9 was found optimal for designing transdermal system. The optimized transdermal formulation comprised of 80 mg of silicon adhesive with 2.5 mg of rotigotine along with 20.1 mg of microspheres containing 2 mg of rotigotine. **Conclusion:** From the PK study conducted in male wistar rats, it was observed that there was 1.679 folds improvement in bioavailability of test formulation when compared to reference (contains 4.5 mg of rotigotine pure crystalline drug in adhesive matrix layer) formulation.

Keywords : Rotigotine, Pre-systemic, Transdermal, Maltodextrin, Poly-e-caprolactone,Microspheres, Bioavailability.

Introduction

Rotigotine is a non-ergolinic dopamine receptor agonist indicated for the treatment of early and advanced-stage Parkinson's disease and moderate-to-severe idiopathic restless legs syndrome (LeWitt et al., 2007; Trenkwalder et al., 2008; Water, 2013; Chen et al., 2009). The Food and Drug Administration (FDA) approved Rotigotine as the transdermal formulation Neupro® in the year 2007. Due to an extensive first pass effect, rotigotine has a very low oral bioavailability i.e., around 1% (Choudhury et al., 2019). Controlled release transdermal formulations may offer stable systemic drug concentrations avoiding first pass metabolism and enhance bioavailability (Pham and Nogid, 2008; Splinter, 2007; Sravanthi et al., 2021). In this study, efforts were put to develop controlled release transdermal patches of Rotigotine using a novel hybrid technique that includes a combination of microreservoir system and adhesive dispersion system.

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Materials and methods

Rotigotine USP (freebase) was received as a gift sample from Neuland Pharma, Hyderabad. Poly-e-caprolactone of (MW 14000) was procured from Sigma Aldrich, Germany. Povidone K90, Vitamin-E TPGS (D-α-tocopheryl polyethylene glycol succinate), Ascorbyl palmitate and D-Alpha tocopherol of USP-NF grade were procured from BASF, Germany. Maltodextrin (Glucidex IT17, USP-NF) was procured form Roquette Pharma, France. Sodium metabisulfite (Ph. Eur.) grade was procured form Merck India. Aluminum vapor coated pigmented polyethylene polyester backing membrane (3M Scotchpack 1109) and Fluoropolyester coated release liner (3M Scotchpack 9744) were received as gift samples from 3M Corporation, USA. Silicon adhesive (7- 4302 BIO PSA, USP-NF) was procured from DOW Corning, USA. Methanol, Ethanol (99.9%), 2-propanol, tert-butyl methyl ether, Acetonitrile, Methanesulfonic acid, ethyl acetate and dichloromethane of HPLC grade were procured from Merck, India.

Formulation of Transdermal Patches

Preparation of rotigotine microspheres

Poly-e-caprolactone and maltodextrin (1:1 ratio) were used

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Table 1. Composition of Optimized Rotigotine Microspheres (1: 9 – Rotigotine: polymer)

Name of the Ingredient	Quantity (mg)
Rotigotine	750
Poly-e-caprolactone	3375
Maltodextrin	3375
Vitamin E TPGS	18.75
Sodium Metabisulfite	18.75

as polymeric drug carriers in the preparation of Rotigotine loaded microspheres by spray drying technique. Microspheres prepared with drug and polymeric drug carriers in the ratio of 1:9 were optimized. The composition of rotigotine microspheres is presented in Table 1. Microspheres were prepared by dissolving poly-e-caprolactone in methanol, maltodextrin, Vitamin-E TPGS sodium metabisulfite in water, and rotigotine in ethanol. Thus three clear solutions were mixed and homogenized (Kinematica® Polytron PT 3100D) at 3000 rpm to form an opaque dispersion. The dispersion was then spray dried in a spray drier of 4 inches internal diameter chamber (JISL, LSD-48) at a flow rate of 2.5 mL to 3.0 mL per minute using 1 mm spray nozzle maintaining inlet temperature, outlet temperature and product temperature at 110°C to 120°C, 60°C to 70°C and below 55°C respectively. The atomization pressure was maintained at 30 PSI ± 3 PSI and aspiration rate of -140 mmwc (1400 rpm in the machine). The dried microspheres obtained were collected and sealed in glass containers (Sravanthi et al., 2021).

The prepared microspheres were characterized for presence or absence of crystallinity by X-ray diffraction technique, Assay and in vitro drug release (Sravanthi et al., 2021), Particle Size by optical microscopy (Rai and Padmini, 2016) and Encapsulation efficiency by HPLC (Venkatesh et al., 2012).

Preparation of transdermal systems

Rotigotine containing adhesive layer was prepared by mixing silicon adhesive with antioxidants (sodium metabisulfate, ascorbyl palmitate, and Vitamin E TPGS), drug substance, methanol, ethyl acetate and polyvinyl pyrrolidone. To the above clear solution weighed amount of Rotigotine microspheres were added and mixed. Then above mixture was casted over a backing membrane and dried for 24h. The release liner was then laid on the adhesive side and the system was cut into patches of suitable size (Sravanthi et al., 2021). The composition of transdermal patches (test and reference formulation) is given in Table 2.

The transdermal patches were evaluated for thickness, weight variation, drug content, moisture content, moisture uptake, flatness and folding endurance (Sravanthi et al., 2021).

Table 2. Composition of Formulated Transdermal Patches

Name of the Ingredient	Test Formulation	Reference Formulation
	Quantity (mg/ patch of 10 cm^2 SA)	
Rotigotine	2.5	4.5
Silicon Adhesive	80	80
Ascorbyl Palmitate	0.5	0.5
Vitamin E TPGS	0.5	0.5
Sodium Metabisulfite	0.25	0.25
Methanol	1 mL	1 mL
Ethyl acetate	0.5 mL	0.5 mL
Polyvinyl Pyrrolidone	10	10
Rotigotine Microspheres	20.1 (contains 2 mg of Rotigotine)	-

Pharmacokinetic Study

Study Design and Sampling Schedule

The bioavailabilities of the optimized transdermal formulation prepared by incorporation of microspheres (TF) and reference transdermal formulation prepared by incorporation of pure crystalline drug (RF) were estimated by conducting pharmacokinetic studies in male wistar rats. All experimental procedures were reviewed and approved by the institutional animal ethical committee (CPCSEA Registration Number – 1230/PO/E/S/08/CPCSEA, Proposal Number – IAE/SKIPS/2016/Mar05/02/Rats-84/Rabbit-12). Male wistar rats weighing 250–350 g were taken for study (3 animals per group). The dose of human was converted to rat equivalent per kg body weight and 3X of the calculated dose was applied as patch. Accordingly, the patch was sized and applied over the depilated skin of the rat. Blood samples were withdrawn by retro-orbital venous plexus puncture at 0, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24h after dose. About 0.5mL of blood samples were withdrawn in eppendorf tubes and centrifuged at 3000 rpm for 30 min to separate the serum. The serum was then transferred to another eppendorf tube and stored at −20°C until analysis.

Serum Sample Extraction and Analysis by HPLC

0.3 mL of serum was taken to which 0.2 mL water containing 5% DMSO was added then 2.5 mL of extraction solvent (2-propanol: tert-butyl methyl ether: Methane sulfonic acid (80:20:0.1)) was added to it. Ensuring the complete mixing of above mixure, 7 mL of 0.1% (v/v) Methanesulfonic acid was added to the resultant mixture and mixed for 2 min. The sample was then centrifuged at 4500 rpm for 15 min. The supernatant liquid was separated and the drug content was analyzed by HPLC using mobile phase comprising acetonitrile: 1mM ammonium acetate (75:25, v/v) with an sample injection volume of 125 microlitres and at flow rate of 2 mL/ minute. (Wang et al., 2016; Swarupa et al., 2015).

Calculation of Pharmacokinetic Parameters

The concentrations of rotigotine in rat serum samples were obtained from the calibration curve prepared. The

Table 3. Chromatographic Conditions followed for analysis

S.No	Parameter	Details
1	Mobile phase	Acetonitrile: 1mM ammonium acetate (75:25, v/v)
2	Flow rate	2 mL/min
3	Column	LiChrosorb® RP-8 (5 µm)
4	Column Temperature	30°C
5	Injection volume	125 µL
6	UV detection	272 nm (10).
7	Retention time of Rotigotine	3.58 min
8	Retention time of Internal Standard	3.64 min
9	Internal Standard	Rotigotine Free base

pharmacokinetic parameters Cmax, Tmax, AUC0−24, and $t_{1/2}$ were calculated by PK Solver® software. The relative bioavailability (BA) of rotigotine from test to the reference formulation was calculated as follows:

Relative BA = (AUC test x Dose Reference /AUC Reference x Dose test)

Results and discussion

Based on the physico-chemical characteristics, in vitro drug release and presence or absence of crystallinity microspheres prepared with drug to polymer mixture (1:9) were found to be optimal. The Particle Size, Assay and Encapsulation efficiency of the microspheres were found to be 15.66 ±3.24 µm, 99.6 ±2.42% and 96.80 ±2.86% respectively. The drug release from the microspheres within 3h was found to be 99.36 ±5.14%. X-ray diffraction studies indicated the amorphosity of the drug in microspheres.

The optimized microspheres were then incorporated into the adhesive layer of transdermal patch. The optimized transdermal test formulation (TF) comprised of 80 mg of silicon adhesive with 2.5 mg of rotigotine along with 20.1 mg of microspheres containing 2 mg of rotigotine. The thickness, weight variation and drug content of the optimized transdermal patch was found to be 0.167 ± 0.039 mm, 0.101 ± 0.0027 g and 102.29 ± 3.99% respectively. The moisture content and moisture uptake of the patch was found to be 3.673 ± 0.365% and 0.96 ± 0.253% respectively. The flatness and folding endurance of the patch was found to be 100% and greater than 250 respectively indicating patch has no level of immediate constriction and the patch was having good strength, elasticity and can maintain its integrity when applied on to the skin.

Table 4. Rotigotine Plasma Concentration in rat serum from reference and test formulation

Time (h)	Serum Concentration (ng/mL) ± SD	
	Reference Formulation (RF)	Test Formulation (TF) (F10)
0	0 ±0	0.00 ±0
1	2.9 ±0.42	3.13 ±0.36
2	5.5 ±1.1	10.91 ±1.52
4	11.1 ±1.6	15.40 ±0.92
6	25.5 ±2.3	17.89 ±1.36
8	17.1 ±2.36	18.93 ±1.25
10	9.7 ±1.21	21.26 ±2.02
12	8.1 ±0.98	25.38 ±1.26
16	3.9 ±0.35	13.29 ±0.50
20	2.1 ±0.75	4.15 ±1.25
24	0.6 ±0.2	1.19 ±0.61

Table 5. Comparison of pharmacokinetic parameters of rotigotine from transdermal patches in rats -Reference and Test formulation (n= 3)

Pharmacokinetic Parameters	Reference Formulation (RF)	Test Formulation (TF) (F10)
C_{max} (ng/mL) ± SD	25.52± 1.06	25.37 ± 1.25
T_{max} (h)	6.0	12.0
$AUC_{(0-24)}$ (ng/mL)h ± SD	187.45 ± 5.25	314.73 ± 8.26
$AUC_{(0-\infty)}$ (ng/mL)h ± SD	190.51 ± 6.43	318.68 ± 7.892
$t_{1/2}$ (h) ± SD	3.54 ± 0.52	2.29 ± 0.41
MRT (h) ± SD	8.97 ± 0.94	10.55 ± 1.28

The statistical comparison of data was done using unpaired t-test by Minitab® (Version 16), and significance was calculated at P value of 0.05. Significant difference was observed between test and reference formulation in terms of AUC and T_{max}. No significant difference was observed in terms of C_{max}, $t_{1/2}$ and MRT.

The drug in the serum samples was estimated by using HPLC method (Wang et al., 2016; Waters, 2013; Swarupa et al., 2015) and the values are given in Table 4. Various pharmacokinetic parameters obtained for both test and reference formulation are given in Table 5.

With test formulation, the average peak plasma concentration of rotigotine is 25.37 ± 1.25 ng/mL, whereas in the case of reference formulation, the peak plasma concentration is 25.5 ± 1.06 ng/mL. The tmax for both test and reference formulation was found to be 6.0 h and 12.0 h.The plasma concentration-time profiles of test and reference formulation are shown in Figure 2. The test

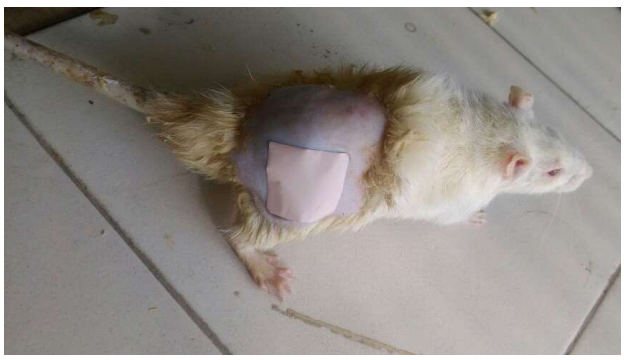

Figure 1. Application of Transdermal Patch to male wistar rat

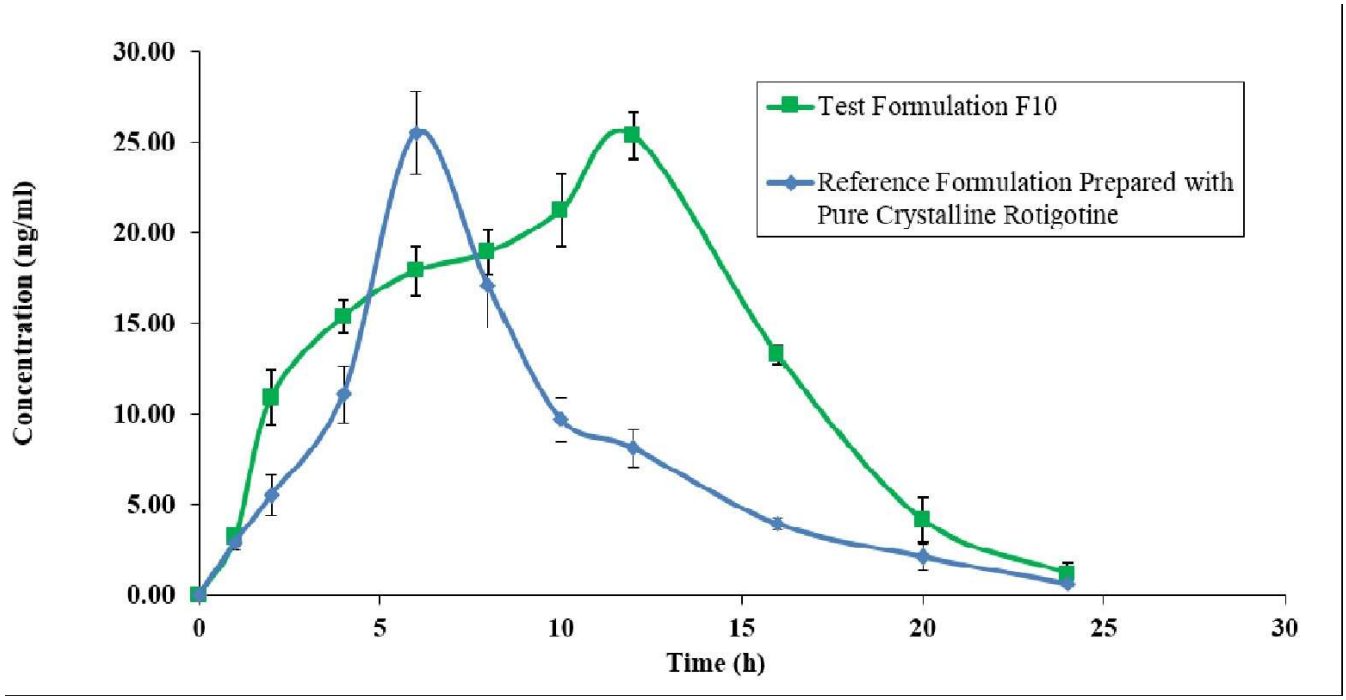

Figure 2. Serum concentration versus time profile of rotigotine from transdermal patch of test formulation (F10) and reference formulation (Pure crystalline rotigotine in adhesive matrix) in rats.

formulation profile is superior to that of reference formulation with respect to AUC. Test formulation AUC (0-24) was found to be 314.73 ± 8.26 ng/mL*h whereas the reference formulation showed 187.45 ± 5.25 ng/mL*h. Test formulation showed 1.679 times higher exposure than reference formulation.

Conclusion

A controlled release transdermal system was developed using a novel hybrid technique including a combination of micro reservoir and adhesive dispersion system. From the PK study conducted in male wistar rats, it was observed that there was 1.679 folds improvement in bioavailability of test formulation when compared to reference formulation.

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