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BOOTSTRAP CONFIDENCE INTERVAL APPROACH TO COMPARE BIOAVAILABILITY OF ORAL LEVODOPA CARBIDOPA TABLET VS INTRANASAL LEVODOPA MICROSPHERES

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ABSTRACT

Aim of the present study is pharmacokinetic comparison between nasal levodopa carbidopa formulation, nasal levodopa microspheres (test) and oral levodopa carbidopa tablet in rat. The comparative pharmacokinetic study was carried out on 324 healthy experimental rats in an open label randomized, parallel, three treatment, serial sacrifice design in 54 groups. All PK parameters were calculated using a noncompartmental model. The mean AUCt for each product and time point t of measurement is calculated by using the mean concentrations (Ct) at each time point t to derive the mean profile for each product. The ratio and 90%CI for nasal l-dopa/nasal l-dopa+c-dopa formulation was 96.94(60.05-133.83) for C_{max} and 92.29(66.54-118.04) for AUC_t. Despite 90% CI limit not within the bioequivalence limit of 80% to 125% both formulation are equivalent at ratio test. Since animal sample size were less, 90% CI could not be met otherwise expected to meet at higher number of animal. The above statement is further proved by student t-test as probability of detecting difference between above formulations, at 95% CI was 0.547 which signifies there is no significant difference between two formulations. Since the value were not within the limit of 80-125% the test and reference product were pharmacokinetic nonequivalence and indicated that that this comparative pharmacokinetic study was well designed to conclude that the test formulation and reference formulation were pharmacokinetically non equivalent. Based on the above analysis it was found that nasal microspheres of levodopa were suprabioavailable compared to oral.

KEYWORDS: Parkinson's disease, nasal microspheres, bootstrap, l-dopa, pharmacokinetics.

INTRODUCTION

Parkinson's disease (PD) is a progressive, neurodegenerative disorder of the extrapyramidal nervous system affecting the mobility and control of the skeletal muscular system.[1] This study proposes to develop nasal mucoadhesive microspheres of l-dopa, which would keep releasing it at a predefined sustained rate for an extended period of time. Owing to the unique connection of the nose and the CNS, the intranasal route can deliver therapeutic agents to the brain bypassing the BBB.[2]

The hypothesis of this study is that a constant plasma level of l-dopa would overcome the motor complications (such as dyskinesia) associated with the use of its oral dosage form in Parkinson's patients. Hence, prepared HPMC-Carbopol 934p microspheres of l-dopa were subjected to animal study to compare bioavailability between nasal levodopa (l-dopa) microspheres and oral levodopa+carbidopa(c-dopa) preparations.

Average bioequivalence (ABE) is commonly tested for pharmacokinetic (PK) parameters (e.g. AUC and Cmax) obtained from bioequivalence (BE) studies of crossover or parallel design. Generally, log (AUC) and log (Cmax) values are statistically analyzed using the mixed effect or two-stage linear model. This two stage analysis procedure involves estimation of AUCs & Cmax in first stage for each analysis subject separately, and the second stage uses the individual parameters estimates for statistical inference. Two formulations are claimed to be bioequivalent when the 90% confidence intervals (CIs) of mean log (AUC) differences and log (Cmax) differences fall within the regulatory acceptance limits [log (0.8) to log (1.25)].[3]

The above standard BE measure is possible only when a complete pharmacokinetic(PK) profile is available from the single object and not applicable in sparse sampling situations/ serial sacrifice design where only one sample is available per analysis object.[4-7] The challenge starts when limited samples are available from an object and collection of some numbers of objects provides a complete PK profile to establish BE. There are several instances where such situation arises like, to compare ocular bioavailability, to compare bioavailability in the brain, to access bioavailability in the rodent where only one sample withdrawal is permitted due to safety reason or limited availability of samples from the object.[8]

To address the issue of serial sacrifice design or sparse sampling techniques, two stage average bioequivalence analysis approach has been modified slightly. The modification was done in a way where a complete plasma profile is generated from the group of objects assigned by the predefined sampling time points. Once complete mean plasma profiles are obtained from the study, PK metrics will be calculated using non compartmental model (NCA) model. Serial sacrifice design (SSD) approach is limited for estimation of standard error of PK metrics, because above NCA model will generate single PK metrics for test and reference product.[9]

To obtain statistical inference from the SSD samples, distribution independent simulation is to be applied to generate numerous PK metrics.[10] Unlike commonly used simulation techniques where prior distribution of interest is simulated to estimate critical value of distribution, bootstrap method use resampling techniques to simulate independent sample for statistical inference. Instead of generating observations from a known theoretical distribution as before, we generate observations from the distribution of the sample itself—the empirical distribution. Each simulation results in a new sample.[11] Each bootstrap sample is typically similar sample size as the original, by randomly selecting (with replacement) individuals from the original sample. With replacement means that at each step in the selection process, every individual from the original sample is again eligible to get selected, whether or not he has already been selected. Thus, in each bootstrap sample, some of the original individuals may not be represented and others may be represented more than once.[12]

Distribution independent simulation is the best option to generate numerous PK metrics from the SSD of same drug. The techniques utilize the original independent sample again and again by replacing one or more sample to generate new sample.[13, 14] Collective PK metrics of all new samples, generated from the original sample can serve the purpose of drawing statistical inference by giving room for analyzing standard error from the several PK metrics.

Since one test and one reference PK metrics is insufficient to estimate SD, or standard error. Boot strap simulation was followed to estimate 90% CI using the formula test/reference +/- 1.645*SE (Standard error)

MATERIALS AND METHODS

Preparation and characterization of HPMC-Carbopol 934p microspheres

Microspheres were prepared by the w/o emulsification solvent evaporation technique. The drug was dissolved in warm water and this solution was added in aqueous solution of

polymeric mixture containing HPMC and carbopol 934p. This solution was stirred for 30min to homogenize. The above solution was added drop wise into mineral oil containing 0.5% w/v span 60 as emulsifying agent. The aqueous phase was emulsified into the oily phase by stirring the system in a beaker. Constant agitation at various RPM was carried out using a homogenizer stirring rod and stirring motor. The flask and its contents were heated by an electrothermal isomantle at 80^0C. Stirring and heating were maintained for 1hr until the aqueous phase was completely removed by evaporation.

Particle size, entrapment ratio, swelling, extent of dissolution and extent of bioadhesion were the dependent variables and their levels were investigated in the preparation of microspheres. A three factor, three-level Box–Behnken design was used for the optimization of levodopa microspheres with drug:polymer ratio, polymer:polymer ratio and agitation speed (rpm) as the independent variables. The observed optimized formulation had swelling index of 9, drug content 99%, dissolution time of 6.5hrs, size of 18 μm and bioadhesion of 80 The prepared microspheres were administered nasally(test), the above microspheres with carbidopa is used nasally(reference) and oral tablets of levodopa and carbidopa were used as reference.

Study design

All animal experiments adhered to the Principles for Biomedical Research involving animals developed by the Council for International Organizations of Medical Sciences. Male Sprague–Dawley (SD) rats weighing between 180–280 g were involved in the study. All surgical procedures were performed under anaesthesia with ether, and an intra peritoneal injection of 0.2 mL/kg xylazine with 0.2 mL/kg tiletamine-xolazepam was given for deeper anaesthesia and immobilization. 324 rats were divided into two groups – nasal(192) and oral(132) group. Nasal group was divided into two groups for test and reference formulations. These groups were further sub grouped with 6 rats per time points. Serial sacrifice design approach was used to collect blood sample. As per design one animal was assigned for only one time point hence at least 6 animals was chosen to establish statistical significant difference between the time points. Oral group was administered with l-dopa and c-dopa tablet (reference) and nasal group was administered with l-dopa and c-dopa nasal formulation (reference) and l-dopa microspheres (test). Serial sacrifice design approach was used to collect blood sample. Due to difficulty in measuring drug concentration in the brain at 8-12 time points in the same animal, Serial sacrifice design was chosen to characterize PK profile

of levodopa. As per design one animal was assigned for only one time point hence at least 6 animals was chosen to establish statistical significant difference between the time points.[15,16]

Blood samples were collected from the caudal vein. Blood collection was terminated by decapitation. The brain was removed quickly and weighed. The brain (1 g) sample being snap-frozen on liquid nitrogen and stored at −70°C until analyzed.

Prior to oral administration, the animals were fasted overnight and were kept under fasted conditions until 4 h. After oral administration of a single dose of l-dopa/C-dopa and levodopa alone; they were allowed water ad libitum. The dosages for oral administration were 80 mg/kg of l-dopa and 20 mg/kg of C-dopa. Blood and brain samples were collected predose and at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 24 and 36 h after oral administration.

The nasal absorption study of l-dopa/c-dopa was conducted using an *in vivo* experimental technique described by Hussain et al.[17] The microspheres of l-dopa (2.5 mg/kg)/C-dopa (0.63 mg/kg) and l-dopa alone (2.5 mg/kg) were administered into one nostril using a microsyringe. Blood and brain samples were collected at predose and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 8 and 12 h. Total 324 rats were used in this study because each rat was assigned only one time point due to decapitation.[18,19]

Study medications

The test product, l-dopa (Divi's lab, Hyderabad, Andhra Pradesh, India) microsphere formulation was developed by East Point College of pharmacy, Bangalore, Karnataka, India and the reference was obtained from product Hetero Drugs Limited, Hyderabad, Andhra Pradesh, India

Drug Analysis

Chromatographic conditions

Samples from pharmacokinetic study were analyzed by high performance liquid chromatography (HPLC). The HPLC system with a mass detector, with a flow rate of 1mL/min. Spherisorb 0DS2 (150 x 4.6 mm i.d., 5µ column with a Bondapack C18 guard column were used. The mobile phase composition was of 16.5 g of potassium phosphate, 1.0mL of 0.1M $EDTANa_2$, 1.2mL of 0.5mM HAS, and 19.5mL of methanol with pH adjusted to 3.4 with phosphoric acid. The mobile phase was filtered through a 0.2µm cellulose membrane.[20]

Plasma and brain preparations

Working stock solutions of l-dopa and d_3 levodopa (internal standard, IS) were prepared in water at a concentration of 1mg/mL. Prior to use, the stock solutions of l-dopa and IS were further diluted with water to obtain working solutions. An appropriate dilution of the working solution with drug-free plasma or brain gave l-dopa concentration between 50 and 1000 ng/mL. Two-hundred microliters of the IS (10 ng/mL aqueous solution) and 100µL of 4M perchloric acid was added to 1mL of plasma, then vortex-mixed for 2min and centrifuged at 9000rpm for 20 min. A 300-µL aliquot of the supernatant was added to 200µL of 2M potassium citrate buffer (pH 3.8) to precipitate the perchlorate. Each tube was vortexed for 1min and then centrifuged as above. 20-µL aliquots of the clear supernatant were injected onto the LC-MS/MS system for analysis.

For the preparation of brain standard solutions or samples, the frozen brains were ground using mortar and pestle. The prepared standard brain or sample brain from the pharmacokinetic study was spiked with 200µL of IS and 300µL of 4M perchloric acid and then the samples were prepared as described above for plasma preparation.

Blank brain extract were prepared from the fresh brain of the rat.

Pharmacokinetics analysis

Individual subject mean concentration data was plotted (using purse sampling technique for serial sacrifice design analysis) against each time points to determine primary pharmacokinetic parameters like C_{max}, AUC_t, AUC_{inf} and secondary pharmacokinetic parameters like T_{max}, $t_{1/2}$ and Kel using non-compartmental model by WinNonlin® (Version 2.1, Pharsight Corporation, Mountain View, CA, USA). The difference between concentration of levodopa in blood and brain by oral and nasal group was compared by 2000 bootstrap samples in order to find the differences between the formulations. The extent of difference between the formulations was determined by 5000 bootstrap samples.

Model-independent analysis

A non-compartmental approach was used for pharmacokinetic (PK) data analysis. The PK parameters were determined using WinNonlin® (Version 2.1, Pharsight Corporation, Mountain View, CA, USA). The area under plasma drug concentration–time curve from time zero to the last sampling time point "t" (AUC_t) was calculated using the trapezoidal method. AUC from time zero to time infinity (AUC_{inf}) was obtained by extrapolation using

elimination rate constants. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined directly from the individual drug concentration against the time curves. The terminal elimination rate constant (Ke) was estimated by linear regression from the points describing the elimination phase on a log-linear plot. Half-life (t1/2) was calculated by 0.693/ke. Total clearance (Cl) was calculated by dose/AUC_{inf}.

Mean brain levodopa Concentration (pg/mL) from nasal microsphere following nasal administration of levodopa + Carbidopa microsphere from group 1 to 10 at various time points (n=6, per group) were found to be 7.89, 37.81, 60.19, 98.46, 63.44, 48.3, 30.45, 29.7, 24.93 and 15.74 respectively.

Mean brain Levodopa Concentration (pg/mL) from oral microsphere following oral administration of levodopa + Carbidopa microsphere from group 11 (time 0, baseline) to group 20 was found to -7.89, 12.39, 52.46, 85.70, 52.20, 37.38, 21.74, 18.14, 14.43 and 11.13 respectively.

Mean plasma Levodopa Concentration (pg/mL) from nasal microsphere following nasal administration of levodopa + Carbidopa from group 21 (time 0, baseline) to group 32 was found to -0.00, 4.95, 26.37, 47.37, 97.99, 80.70, 36.85, 32.08, 27.31, 15.23,2.20 and 0.62.
Mean plasma Levodopa Concentration (pg/mL) from oral microsphere following oral administration of levodopa + Carbidopa microsphere in plasma from group 33 (time 0, baseline) to group 44 was found to -0.00, 6.25, 30.14, 50.06, 22.65, 86.04, 42.19, 37.43, 32.65, 20.58, 7.08 and 23.96 respectively.

Mean brain Levodopa Concentration (pg/mL) from nasal microsphere following nasal administration of levodopa microsphere from group 45 (time 0, baseline) to group 54 was found to be -10.24, 57.42, 55.64, 92.34, 53.20, 32.69, 29.83, 23.89, 24.13 and 19.66 respectively.

Statistical analysis

The comparison of primary PK parameters between test and reference formulations was performed by two way analysis of variance and two one sided Student's t-test for the randomized crossover design. Statistical analysis was performed by means of ANOVA considering treatment and group as independent model terms and log transformed primary PK parameters as dependent variables.

Original sample test to reference ratio was calculated from the ANOVA and confidence interval of original ratio was calculated using bootstrapping techniques. For determining confidence interval 5000 bootstrapping samples was generated and finally bootstrapping confidence interval of the original ratio was calculated for the all primary pharmacokinetics parameters.

To estimate confidence interval, individual PK parameters for test and reference were calculated for each bootstrap sample. Test to reference ratio of each PK metrics was calculated against each bootstrap samples so that 5000 bootsamples produced 5000 individual PK metric ratio. Standard error of the ratio was calculated and finally 90% confidence interval was established using following formula; original ratio +/-1.645* standard error from bootstrap samples. All the statistical analyses were performed using SAS V9.1.3 (SAS Institute Inc., USA). The extrapolated area (%) also was considered, in order to ensure compliance with the required 80% set target limit as per the international applicable guidelines. Formulation and group effects were tested at 5% level of significance.

To establish pharmacokinetic equivalence between two formulations, 90% CIs of the geometric mean ratios (GMR), power of the study and intra-subject variability were obtained. Non parametric Wilcoxon signed-rank test was performed to compare Tmax since it presented additional information in terms of the rate of absorption and the 90% CI of the median of individual difference was determined. The pharmacokinetic parameters of Levodopa following nasal administration in the presence and absence of C-dopa were compared using Student t-test. A p-value of less than 0.05 was considered significant and the differences between formulations were found to be insignificant.

According to the Committee for Pharmaceutical and Medicinal Products (CPMP) bioequivalence guideline the 80% to 125% decision rule was applied to the 90% confidence interval for log transformed primary PK parameters.

RESULTS

A total of 324 animals had completed both periods of the study. The method demonstrated acceptable performance and was therefore suitable for the determination of l-dopa in animal plasma and brain in the present bioequivalence study.

Under the described analytical conditions, the relationship between the concentration and peak area ratio was linear form 0.05 to 20 pg/mL (LLOQ, 0.05 pg/mL) (l-dopa, Y= 0.000915X–0.000429, R^2= 0.9995).

The linear calibration curve of the peak area ratio (analyte to internal standard) vs. concentration is shown in Fig.1.

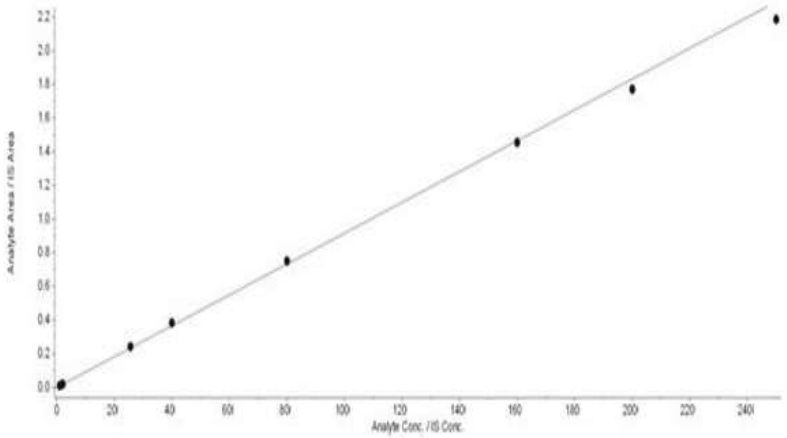

Fig. 1: Standard calibration curve of levodopa concentration vs. mean drug to internal standard (IS) peak area ratio (n = 6).

Linear curve equation is Y= 0.000915X–0.000429, R^2= 0.9995 where Y stands for drug to IS peak area ratio, X stands for levodopa concentration (pg/mL) and R^2 is the regression coefficient.

The mean AUC_t for each product and time point "t" of measurement is calculated by using the mean concentrations (C^t) at each time point "t" to derive the mean profile for each product. On the basis of the trapezoid rule, mean AUC_t is computed as the weighted linear combination of these mean concentrations at each time point through time "t".

Nasal microspheres of l-dopa(test) are considered to be pharmacokinetically equivalent, i.e. bioequivalent to oral l-dopa c-dopa formulation (reference), if the 90% CI of the test and reference geometric mean ratios of the AUCs and C_{max} fall within 80% to 125%. However, in certain cases with consideration of small sample size this range may be widened.

The ANOVA tests, followed by bootstrap, were used to assess the statistical significance of the differences between the results following nasal and oral modes of administration. A *P*

value of less than 0.05 was considered significant. Data are presented as mean±S.E.M., unless stated otherwise.

Pharmacokinetic properties of nasal and orally administered l-dopa formulations are depicted in table 1 and Fig. 2.

Table 1-Comparitive Pharmacokinetics properties for levodopa nasal microspheres(E-test) and other formulations of l-dopa+c-dopa through nasal and oral route(A,B,C and D- reference).

Route	Formulation	Measurement in	C_{max}	AUC_t	AUC_{inf}	T_{max}	T_{half}	Kel
Nasal	L+C (C)	Blood	97.9915	573.766	578.332	1.00	5.15	0.1346
Oral	L+C (D)		122.655	877.863	1357.66	1.00	4.88	0.0499
Nasal	L+C (A)	Brain	98.4590	351.178	549.548	0.25	8.23	0.0794
Oral	L+C (B)		85.6975	234.968	417.422	1.00	7.86	0.0610
Nasal	L (E)	Brain	92.3433	322.140	604.001	0.25	7.99	0.0408
	L+C (B)		98.4590	351.178	549.549	0.25	8.23	0.0794

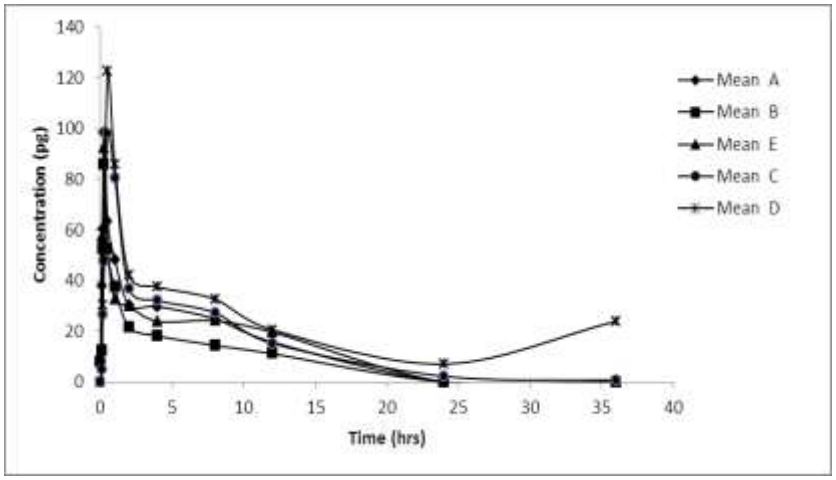

Fig. 2- Comparison of pk profiles of levodopa alone in brain from nasal microsphere of l-dopa and l-dopa concentration in brain after l-dopa+c-dopa formulation when administered by nasal and oral route

Figure 2: Mean A is l-dopa concentration in brain after l-dopa+c-dopa combined microsphere administration through nasal route Mean B is l-dopa concentration in brain after l-dopa+c-dopa combined administration through oral route Mean C is l-dopa concentration in plasma after l-dopa+c-dopa nasal microsphere administration Mean D is l-dopa concentration in plasma after l-dopa+c-dopa oral administration Mean E is l-dopa concentration in brain after l-dopa a alone administration through nasal route

The C_{max} and AUC_t in plasma and brain for oral administration were found to be 122.65 pg/mL, 877.8 pg*hr/mL and 85.7 pg/mL, 234.96 pg*hr/mL respectively. AUC_{inf} in plasma and brain after oral administration of l-dopa and c-dopa was 1357 pg*hr/mL and 417 pg*hr/mL respectively. The C_{max} and AUC_t in plasma and brain for nasal administration in presence of c-dopa were found to be 97.99 pg/mL, 573.7 pg*hr/mL and 98.46 pg/mL, 351.17 pg*hr/mL respectively. The AUC_{inf} in plasma and brain after nasal administration of l-dopa and c-dopa was 578 pg*hr/mL and 549 pg*hr/mL respectively. The C_{max} and AUC_t in brain for nasal administration in absence of c-dopa were found to be 92.34pg/mL and 322.14 pg*hr/mL respectively. AUC_{inf} in brain when l-dopa was given alone was 604 pg*hr/mL. The C_{max} and AUC_t in plasma were not reported since negligible amount of l-dopa in plasma.

Table 2-Geometric mean ratio and 90% Confidence interval for primary PK metrics of Brain l-dopa nasal (A)/ l-dopa oral(B) from l-dopa+c-dopa formulation

PK metrics	Ratio GMR	Standard Deviation	Lower limit	Upper limit
Cmax	115.86	±0.2405	76.30	155.42
T_{max}	101.15	±0.1817	71.26	131.04
AUC5	229.88	±0.8651	87.56	372.20
AUC10	172.06	±0.5058	88.85	255.27
AUC15	134.68	±0.2604	91.84	177.53
AUC30	123.50	±0.1831	93.38	153.62
AUC60	124.22	±0.1576	98.28	150.16
AUC120	127.95	±0.1414	104.69	151.22
AUC240	136.25	±0.1619	109.62	162.89
AUC480	148.63	±0.2262	111.41	185.86
AUC720	151.26	±0.2209	114.92	187.60

PK-Pharmacokinetic, GMR-Geometric Mean Ratio.

Geometric mean ratio (nasal/oral) and 90%CI (GMR±1.645*SD, SD was calculated after 5000 bootstrap) in brain for C_{max} and AUC from l-dopa+c-dopa formulation were estimated to be 115.86(76.30-155.42) and 151.26(114.92-187.60) (table 2) and its p value was found to be 0.024 which signifies there is a significant difference between the formulations.

Whereas same parameters in plasma were found to be 81.57(65.37-97.77) and 67.56(41.46-93.66) as seen in table 3 and its p value was found to be 0.0014 which signifies there is a significant difference between the formulations

Table 3-Geometric mean ratio and 90% Confidence interval for primary PK metrics of plasma l-dopa nasal(C) / l-dopa oral(D) from l-dopa+c-dopa formulation

PK metrics	Ratio GMR	Standard Deviation	Lower limit	Upper limit
Cmax	81.57	±0.0985	65.37	97.77
T_{max}	115.56	±0.3981	50.06	181.05
AUC15	86.47	±0.5360	1.70	174.64
AUC30	88.36	±0.3050	38.18	138.54
AUC45	90.96	±0.1931	59.19	122.74
AUC60	86.66	±0.1116	68.29	105.02
AUC120	86.31	±0.1111	68.02	104.59
AUC240	89.60	±0.1378	66.92	112.28
AUC360	88.76	±0.1162	69.64	107.88
AUC480	88.07	±0.1050	70.79	105.35
AUC720	86.65	±0.1078	68.92	104.39
AUC1440	82.08	±0.1614	55.53	108.64
AUC2160	67.56	±0.1586	41.46	93.66

Geometric mean ratio (nasal l-dopa alone/nasal l-dopa+c-dopa) and 90%CI in brain for C_{max} and AUC were estimated to be 96.94(60.05-133.83) and 92.29(66.54-118.04) as in table 4 and its p value was found to be 0.547 which signifies there is no significant difference between the formulations. Comparable brain l-dopa concentration was observed following equivalent dose of nasal l-dopa+c-dopa formulation. However, in absence of c-dopa in nasal formulation also provided the similar concentration in brain as compared to equivalent dose of oral l-dopa+c-dopa. Hence it can be concluded that l-dopa alone is an ideal candidate for nasal microsphere to achieve therapeutic goal by bypassing peripheral decarboxylation.

Table 4-Geometric mean ratio and 90% Confidence interval for primary PK metrics of l-dopa alone (E)/ l-dopa+c-dopa from nasal microspheres

PK metrics	Ratio GMR	Standard Deviation	Lower limit	Upper limit
Cmax	81.57	±0.0985	65.37	97.77
T_{max}	115.56	±0.3981	50.06	181.05
AUC15	86.47	±0.5360	1.70	174.64
AUC30	88.36	±0.3050	38.18	138.54
AUC45	90.96	±0.1931	59.19	122.74
AUC60	86.66	±0.1116	68.29	105.02
AUC120	86.31	±0.1111	68.02	104.59
AUC240	89.60	±0.1378	66.92	112.28
AUC360	88.76	±0.1162	69.64	107.88
AUC480	88.07	±0.1050	70.79	105.35
AUC720	86.65	±0.1078	68.92	104.39
AUC1440	82.08	±0.1614	55.53	108.64
AUC2160	67.56	±0.1586	41.46	93.66

Based on our results, the l-dopa nasal delivery system could be used as prn (as needed) dosing and as a good adjuvant therapy for PD patients who experience symptom fluctuation by l-dopa oral administration.

DISCUSSION

Pharmacokinetic profiles of l-dopa in brain run parallel to those in plasma with rapid distribution to the brain. The AUC_{inf} in plasma and brain after nasal administration of l-dopa and c-dopa was 578 pg*hr/mL and 549 pg*hr/mL respectively and the AUC_{inf} in plasma and brain after oral administration of l-dopa and c-dopa was 1357 pg*hr/mL and 417 pg*hr/mL respectively, in both the cases AUC_{inf} in plasma was greater than brain which may be due to rapid distribution. AUC_{inf} in brain when l-dopa was given alone was 604 pg*hr/mL, this increased AUC_{inf} may be attributed to bioadhesive property of microspheres which increases residence time of drug in nose increasing transport across olfactory epithelium. Therefore, the absolute extent that reaches the circulation and the brain in the present nasal delivery system might be promising.

Nasal microspheres of l-dopa(test) are considered to be pharmacokinetically equivalent, i.e. bioequivalent to oral l-dopa c-dopa formulation (reference), if the 90% CI of the test and reference geometric mean ratios of the AUCs and C_{max} fall within 80% to 125%. However, in certain cases with consideration of small sample size this range may be widened.

The ANOVA tests, followed by bootstrap, were used to assess the statistical significance of the differences between the results following nasal and oral modes of administration. A *P* value of less than 0.05 was considered significant. Data are presented as mean±S.E.M., unless stated otherwise.

P value of two independent sample student t test (after 2000 bootstrap) AUC_t and C_{max} for nasal and oral administration in brain was found be 0.002 and 0.034 respectively, which signifies there is a significant difference between two formulation with respect to C_{max} and AUC_t for both the formulations. In order to compare bioequivalence 90%CI(after 5000 bootstrapping) of geometric mean ratio(nasal/oral l-dopa + c-dopa in brain) of PK metrics, C_{max} and AUC were estimated and found to be 115.86(76.30-155.42) and 151.26(114.92-187.60) and its p value was found to be 0.024 which signifies there is a significant difference between the formulations.

P value of two independent sample student t test (after 2000 bootstrap) AUC_t and C_{max} for nasal and oral administration in plasma was found be 0.025 and 0.042 respectively, which signifies there is a significant difference between two formulations w.r.t C_{max} and AUC_t. In order to compare bioequivalence 90%CI(after 5000 bootstrapping) of geometric mean ratio(nasal l-dopa/nasal l-dopa+c-dopa in brain) of PK metrics, C_{max} and AUC were estimated and found to be 96.94(60.05-133.83) and 92.29(66.54-118.04) respectively and its p value was found to be 0.547 which signifies there is no significant difference between the formulations. Based on the above analysis it was found that nasal microspheres were suprabioavailable compared to oral.

P value of two independent sample student t test (after 2000 bootstrap) AUC_t and C_{max} for nasal and oral administration in plasma was found be 0.004 and 0.021 respectively, which signifies there is a significant difference between two formulations w.r.t. C_{max} and AUC_t. In order to compare bioequivalence 90%CI (after 5000 bootstrapping) of geometric mean ratio (nasal l-dopa /oral l-dopa + c-dopa) of PK metrics, C_{max} and AUC were estimated and found to be 81.57(65.37-97.77) and 67.56(41.46-93.66) respectively and its p value was found to be 0.0014. Based on the above analysis it was found that nasal microspheres were suprabioavailable compared to oral.

Based on our results, the l-dopa nasal delivery system could be used as prn (as needed) dosing and as a good adjuvant therapy for PD patients who experience symptom fluctuation by l-dopa oral administration.

CONCLUSION

Post formulation animal study result substantiate the importance of nasal microsphere over oral formulation for the treatment of PD, as brain bioavailability of the LD was substantially higher than the oral dose.Hence it can be concluded that bioadhesive nasal microspheres containing LD can be successfully used in treatment of PD.

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CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest with respect to financial disclosure, patent ownership, stock ownership, consultancies, speaker's fees, personal, political, intellectual, or religious interests among us. This study was conducted by self funding; no external agency was involved in the funding. The study was conducted independently with the approval of an institutional review board and it was research purpose only.

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