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Original Article

Preparation and *in vitro* Evaluation of Rasagiline Mesylate Hybrid Nanoparticles

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Abstract

Rasagiline is a selective and irreversible inhibitor of monoamine oxidase B (MAO-B) that is effective in the treatment of Parkinson's disease (PD). It had antioxidant and anti-apoptotic activity in experimental models. Moreover, it has low permeability and its oral bioavailability is weak and highly variable due to extensive first-pass hepatic metabolism (35%). This study aimed to formulate rasagiline mesylate (RM) as a lipid-polymer hybrid nanoparticle in order to enhance its permeation and increase its chance to be absorbed by lymphatic circulation to avoid metabolism and control its release. Successful formulation (PCL-2) was reached by the nanoprecipitation method using polycaprolactone with RM in the organic phase and lecithin in the aqueous phase DSPE-PEG. The lipid:polymer ratio of 24% and DSPE: lecithin of 50% resulted in stable nanoparticles having a particle size of 132±4.58 nm, polydispersity index of 0.273±0.02, zeta potential of -25.6±3.3, entrapment efficiency of 46±3.9%, and drug loading of 51.93±6.5. Results showed that the diffusion was more effective on the release profile than the degradation and resulted in a Fickian diffusion mechanism.

Keywords: Nanoparticles, Parkinson's disease, Propargylamine, Rasagiline

1. Introduction

Rasagiline is a selective and irreversible propargylamine, which has a slight chemical difference in side chain structure, compared to selegiline (deprenyl) (1). These compounds are not metabolized to potentially toxic amphetamines. Moreover, they have been shown to have therapeutic effects on neuronal cultures and animal models of Parkinson's disease (PD) by their main metabolite 1-R-amino indane (2). As well as inhibition of monoamine oxidase B (MAO-B), rasagiline mesylate (RM) exhibited antioxidant and anti-apoptotic activities in experimental models, which may potentially have long-term neuroprotective actions (3). The RM initially has extensive hepatic metabolism that results in poor and highly variable oral bioavailability (35%) and exerts linear absorption at doses of 1-10 mg/day (4).

Moreover, in a previous study, it was demonstrated that transdermal application of a melanin-binding drug (which is rasagiline) to an actively growing melanoma decreases the tumor size and tumorigenicity potential (5). The half-life of rasagiline is dose-dependent; however, it is usually 1.5-3.5 h. The oral clearance of the drug is 94.3 L per h, and extrahepatic processes do not contribute to its elimination (6). The therapeutic dosage of RM for Parkinson's disease is 1 mg or 0.5 mg once a day along with L-dopa; if a satisfactory clinical

response is not obtained, the dose should be increased to 1 mg once a day (7).

The RM is a highly soluble drug with low permeability; therefore, it is classified as a class III product according to the Biopharmaceutical Classification System. The kinetics of drug absorption in the gastrointestinal tract is controlled by biopharmaceutical and physiological factors since excipients have no relevant effect on the transport in the gastrointestinal tract and the permeability of the drug (8).

The term "nanoparticles" refers to both nanospheres and nanocapsules; in nanocapsules, the drug is enclosed by a polymeric membrane, whereas in nanospheres, it is embedded inside a polymer matrix. Polymeric nanoparticles are colloidal particles of a nano-scale size that can be designed from a wide variety of natural and synthetic polymers (9). Polymeric nanoparticles have earned more interest due to their distinctive capacities for the control of drug release, entrapment of hydrophilic and hydrophobic compounds, and possession of higher stability than lipid carriers (10).

Nowadays, the use of biological compounds as strong therapeutic agents in the treatment of various diseases is of interest to researchers. These compounds are often unstable and the focus of biomedical nanotechnology is to protect these compounds against premature degradation. In addition to highly selective approaches, targeted drug delivery has also been expanded to limit their side effects (11). Various research groups have investigated the specialized architecture to activate and increase the solubility of this compound for the clinical use of polymeric nanoparticles as carriers of hydrophilic and hydrophobic drugs in the core-shell using biocompatible phospholipids. The two-component structure of these compounds facilitates drug transport and solubility as hydrophilic drugs are encapsulated in the polymer core and lipophilic drugs are placed inside the surface-stabilized lipid shell (12).

This study aimed to enhance the effect of RM by increasing the bioavailability through avoidance of first-pass metabolism, control of drug release, and enhancement of its permeation. These goals can be achieved by formulation as lipid polymer hybrid nanoparticles.

2. Materials and Methods

2.1. Preparation of Rasagiline Mesylate-Loaded Lipid Polymer Hybrid Nanoparticles

Core-shell lipid-polymer hybrid nanoparticles were fabricated using self-assembly of polycaprolactone (PCL), lecithin, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(amino[polyethylene-glycol]-2000) (DSPE-PEG2000) through a single-step nanoprecipitation method: (152), (153).

For the preparation of a clear solution (5 mg\mL), PCL was dissolved in acetonitrile with simple shaking and heating. A solution of lecithin (0.1 mg\mL) and DSPE-PEG2000 (0.1 mg\mL) was prepared by dissolving lecithin and DSPE-PEG2000 in a 4% ethanolic aqueous solution. The ethanolic aqueous solution should be heated beyond the gel-liquid transition temperature for about 65 ˚C. Afterward, the stock solution of the drug was prepared by dissolving RM in acetonitrile. Quantity of the drug was calculated to prepare 1 mg in each 3 mL formula. Drug and polymer solutions were mixed to prepare a drug-polymer mixture that represented the organic solution. Different ratios of lecithin and DSPE-PEG2000 solutions were mixed to prepare the lipid aqueous solution. The organic solution was added to the lipid aqueous solution drop-wise (1 mL/min) under gentle moderate stirring (500 rpm) at 25 ˚C. Different formulations were prepared to study the component effect on formula properties (Table 1).

2.2. Characterization of Rasagiline Mesylate Nanoparticles

2.2.1. Particle Size, Zeta Potential, and Polydispersity

These were performed using dynamic light scattering techniques at a scattering angle of 90 ° at room temperature. For each sample, measurements were achieved in triplicate (13).

2.2.2. Entrapment Efficiency and Drug Loading

The Amicon ultrafiltration device and molecular weight cutoff (MWCO 3kDa) were employed to measure the entrapment efficiency in order to detect the amount of drug incorporated inside the polymer nanoparticles. It was centrifuged for 15 min at 3,000 rpm and the amount of free drug was determined by spectrophotometry. The UV absorbance was 271 nm and the following equation was applied:

$$EE\% = \frac{WT - WF}{WT}$$

Where WT = total $drug$ weight

Wf = $free\ drug$ weight

Moreover, the DL% was calculated using the following equation:

D.L (%) =($W_{\text{loaded drug}}$ / $W_{\text{lipid-polymer}}$)×100.

2.3. *In vitro* Rasagiline Mesylate-Nanoparticles Release Studies

A suitable amount of nanodispersion (containing 1 mg of RM) was placed in dialysis bags (8,000–14,000 D), which were sealed and placed in a 100 mL dissolution medium (phosphate-buffered saline pH 7.4). Release of RM was measured by employing the dissolution apparatus type I at 37 °C±0.5 and 100 rpm. At each time interval of 15, 30, 60, 120, 240, 350, and 480 min, 5 mL aliquots of the sample were withdrawn and fresh buffers were added instead. The collected samples were diluted to a suitable concentration within the calibration curve range and investigated spectrophotometrically at λmax (14). The measurements were performed in triplicate and results were expressed as mean values.

2.4. Analysis of Release Kinetics

The mechanism of drug release from the polymeric nanoparticles was clarified by fitting 60% of the amount released into different kinetic models, which are zero-order model (Eq.1), first-order model (Eq.2), Higuchi model (Eq.3), and Korsmeyer-Peppas model (Eq.4). The model with the highest correlation coefficient was considered to be the best fitted model (15).

Zero-order equation Q0-Qt=k0 t (Eq.1)

First-order equation lnQt=ln Q0 - k1 t (Eq.2)

Higuchi equation Qt=kh$t$1/2 (Eq.3)

Korsmeyer-Peppas equation Mt/M∞=kt n (Eq.4)

Where Qt is the amount of drug released in time (t), Q0 is the initial amount of drug in the polymeric nanoparticles, and k values are rate constant. For the Korsemeyer-Peppas equation, Mt/M∞ is the fraction of drug released at the time (t) and n is the release exponent which can be used to characterize the exact mechanism of drug release (16).

2.5. Statistical Analysis

In order to statistically analyze the obtained data, the mean±standard deviation of triplicate samples was analyzed based on a one-way analysis of variance. It should be noted that a *P* value of less than 0.05 was considered statistically significant.

Table 1. Composition of different formulas prepared lipid polymer hybrid nanoparticles

Formula Code	Amount (mg)			Volume (mL)		Percent (wt/wt)	
	PCL	Lecithin	DSPE-PEG2000	ACTN	Water	Lipid\ polymer	DSPE-PEG2000 \Lecithin
PCL-1	5	1	0.5	2	20	30	50
PCL-2	5	0.8	0.4	2	20	24	50
PCL-3	5	0.6	0.3	2	20	18	50
PCL-4	5	0.6	0.6	2	20	24	100
PCL-5	5	0.4	0.8	2	20	24	200
PCL-6	5	0.8	0.4	1	20	24	50

3. Results and Discussion

Table 2 summarizes the measured and calculated particle size, polydispersibilty index, zeta potential, entrapment efficiency, and drug loading of prepared hybrid nanoparticles evaluation.

3.1. Influence of Lipid:Polymer Ratio

Table 2 tabulates the effect of the lipid:polymer ratio on Zeta potential. Accordingly, an increase in the ratio leads to a significant reduction ($P<0.05$) in the magnitude of zeta potential as the cationic lipid neutralizes the negative charge of PCL. This is similar to what was observed in PCL-1 with a lipid:polymer ratio of 30% having a zeta potential of -20.3, compared to PCL-3 with a zeta potential of -27.9. Effect of the ratio on particle size was also studied. It was found that an increase in the lipid content led to an increase in the particle size which might be due to the formation of lipid multilayer around the particles which results in a larger diameter.

Similar results were obtained during the preparation of PCL-lipid hybrid nanoparticles to enhance the antibacterial activity of ciprofloxacin. The largest size was observed with the formula containing a 1:2 stearic acid-to-PCL ratio. Reduction of the ratio to almost 1:3 and 1:4 resulted in a significant reduction in particle size (17). The lipid concentration optimization is critical for the stability of hybrid nanoparticles as high lipid content may reach the critical micelle concentration and lead to the formation of liposomes in addition to the nanoparticles. However, low concentrations may lead to the aggregation of particles due to insufficient surface coverage (18). The formula with a lipid:polymer ratio of 24% was found to be more stable with an acceptable particle size and zeta potential; hence, it was selected as the best ratio for formulation.

3.2. Influence of DSPE-PEG2000:Lecithin Ratio

To study the effect of DSPE-PEG2000\lecithin ratio on hybrid nanoparticle properties, PCL-2 with a ratio of 50% was compared with two other formulas. The two other formulas were PCL-4 which contained equal quantities of both and PCL-5, in which the quantity of DSPE-PEG2000 was twice the quantity of lecithin.

A significant increase ($P<0.05$) in particle size was observed with an increase in mass ratio. The PCL-4 and PCL-5 having ζ -27.1 and -27.8 mv with constant L:P ratio of 24% and DSPE-PEG2000:lecithin mass ratios of 200% and 50%, respectively, indicated insignificant changes in ζ that were the results of the changing mass ratio. A decrease of lecithin of less than 0.6 mg led to aggregation after storage in the refrigerator for 2 days. These results suggest that the molar ratio of lecithin:lipid-PEG is an effective factor for the control of the physicochemical properties of the hybrid nanoparticles (19).

3.3. Influence of Organic Phase Volume

By fixing all other factors, like lipid:polymer and DSPE-PEG2000:Lecithin ratios, significant changes ($P<0.05$) in ζ and size were observed by decreasing the volume of the organic phase and increasing the concentration of polymer and drug. This finding was in line with those of previous studies regarding the high viscosity of the organic solvent resulting from the usage of high amounts of PCL. Accordingly, the droplet will be bigger from a highly viscous solution and thereby, large particles will be produced (20).

3.4. *In vitro* Release of Rasagiline Mesylate from Nanoparticles

The selected formula, which was chosen according to the measured parameters (particle size, zeta potential, polydispersity index, entrapment efficiency, and drug loading), was subjected to further investigations for *in vitro* release profile determination.

Figure 1 shows the release of PCL-2, where the cumulative amount of the released drug is plotted versus time.

For the determination of the drug release mechanism of the prepared nanoparticles, the cumulative released drug up to 60% was fitted to different release kinetic models, namely Higuchi, First-order, and Zero-order, while the mechanism of release was predicted by fitting to Korsmeyer-peppas equation and calculation of the N value. Results of the release profile of PCL-2 are tabulated in Table 3.

Table 2. Particle size, polydispersibilty index, zeta potential, entrapment efficiency and drug loading of prepared hybrid nanoparticles

Formula code	Particle size (nm)	Polydispersity index	Zeta potential (mV)	Entrapment Efficiency%	Drug loading %
PCL-1	144±5.32	0.26±0.05	-20.3±2.5	51±5.3	54.92±6.3
PCL-2	132±4.58	0.273±0.02	-25.6±3.3	46±3.9	51.93±6.5
PCL-3	121±6.23	0.11±0.08	-27.9±3.6	48±4.2	56.94±3.3
PCL-4	153±3.66	0.091±0.01	-27.1±4.4	42±3.6	47.41±4.1
PCL-5	172±8.34	0.082±0.04	-27.8±4.6	53±6.7	59.83±7.6
PCL-7	153±3.12	0.113±0.02	-22±5.85	61±6.3	68.87±4.3

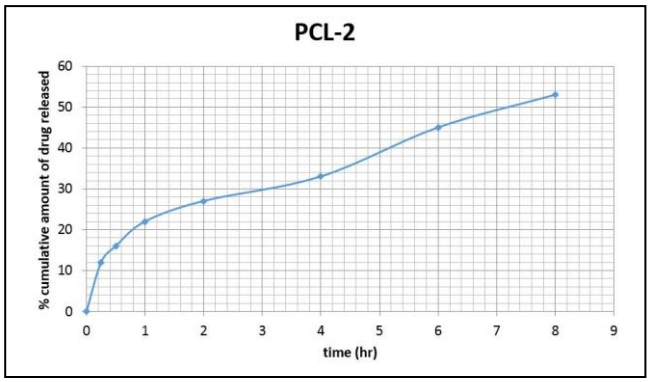

Figure 1. The release of PCL-2 formulation

Table 3. Release kinetic of rasagiline polymeric nanoparticles formula

Formula	Zero order		First order		Higuchi		Korsmeyer-peppas	
	K_0	R^2	K_1	R^2	K_H	R^2	R^2	n
PCL-2	0.0559	0.9126	0.0821	0.9571	17.426	0.9847	0.9866	0.4086

The obtained results showed that the diffusion mechanism was mainly achieved by the diffusion phenomenon. Due to the high hydrophobicity of PCL, the release process was more related to diffusion than degradation, leading to a Fickian diffusion mechanism. It can be concluded that single-step nanoprecipitation has the potential to formulate homogenous nanosuspensions with uniform-sized stable nanoparticles of RM.

The limited oral bioavailability of RM is due to its poor permeability and extensive first-pass metabolism. Therefore, the enhanced permeability by nanosized particles and decreased metabolism by the lipid coat of hybrid may improve the oral bioavailability of RM. The preparations were found to become physically stable with PCL, lecithin, and DSPE-PEG.

Authors' Contribution

Study concept and design: B. W. M.
Acquisition of data: A. A. H.
Analysis and interpretation of data: A. S. S.
Drafting of the manuscript: B. W. M.
Critical revision of the manuscript for important intellectual content: B. W. M.
Statistical analysis: A. A. H.
Administrative, technical, and material support: A. S. S.

Conflict of Interest

The authors declare that they have no conflict of interest.

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