Research Article

In vivo Study of Polymeric Nanoparticles Loaded Transdermal Film for the Treatment of Alzheimer's Disease

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ABSTRACT

In the present investigation, Anti Alzheimer's drug loaded polymeric nanoparticles (PNP's) incorporated transdermal films were made to enhance its uptake to brain via systemic circulation. PNP's were prepared by modified emulsification diffusion method and PNP's incorporated transdermal films were prepared by solvent casting method. The optimized Rivastigmine Tartrate PNP's loaded formulation was evaluated for *in vivo* pharmacokinetic study and dermal toxicity study. *In vivo* studies were performed on New Zealand white rabbits and various pharmacokinetic and dermal toxicity parameters were determined. The pharmacokinetic parameters after administration of Rivastigmine Tartrate PNP's incorporated film were found to be, Tmax 3 h, Cmax 109.16 ± 0.9 ng/mL, AUC0 - ∞ 1491.97±1.87 ng.h/mL, and Ke 0.16 ± 0.031 h-1. The dermal toxicity study was carried out for 3 min, 1 h and 4 h respectively with optimized film and no skin irritation or redness was found. The results highlights that the prepared formulation of PNP's loaded transdermal films were able to deliver a sustained supply of the Rivastigmine Tartrate.

Keywords: Alzheimer disease, Rivastigmine Tartrate, nanoparticles.

INTRODUCTION

Rivastigmine is a slowly reversible, centrally selective dual inhibitor of butyrylcholinesterase and acetylcholinesterase, which improves neurotransmission and enhances the acetylcholine availability levels. It has established efficacy in the symptomatic treatment of Parkinson disease[1-3] and Alzheimer disease4 and it was shown to improve the daily activities like living, cognition, behavior, and global function[5-8]. Dose response relationships studies for cholinesterase inhibitors support better enzyme inhibition, in turn leading to higher efficacy and long-term benefits with higher drug doses[9].

Transdermal film is a medicated device that delivers drugs through the skin for systemic effects at a programmed and controlled rate[10]. The advantages of transdermal drug delivery is, providing controlled release of the drug to the patient and enabling a steady blood level profile, avoidance of first-pass hepatic metabolism and helping in the rapid termination of therapy[11]. Furthermore, the dosage form of transdermal film is user friendly, convenient and offers multi-day dosing.

In general terms, pharmacokinetic (PK) models of dermal absorption of chemicals have been created and reported in a series of publications[12-14]. Skin is frequently represented either as a single compartment or by two compartments separately distinguishing the lipophilic and hydrophilic layers of the skin[15]. Such modeling attempts were generally used in the toxicology rather than clinical pharmacology fields until recently when Polak et al.[16] reported a mechanistic dermal absorption model using simcyp simulator. Still, there is limited information on dermal absorption models that were put into practical use for simulations of drug concentrations in the clinical setting.

In present study *in vivo* studies for Dermal toxicity study and Pharmacokinetics were performed on New Zealand white rabbits for prepared Rivastigmine Tartrate polymeric nanoparticles loaded transdermal films for Alzheimer's disease.

MATERIALS AND METHODS

Rivastigmine Tartrate was obtained as a gift sample from Jubilant life sciences Ltd, India. Acetonitrile was procured from Merck Specialities Pvt Ltd, Mumbai, India. All other solvents, reagents and chemicals used were of analytical grade.

Study was conducted at Institutional Animals Ethics Committee, JSS College of Pharmacy, Mysuru, Karnataka, India. (Proposal No. 215/2017).

Dermal Toxicity Study[17,18]

The *in vivo* test was performed initially using one rabbit and applying the following approach. Up to three test films were applied one after another to the rabbit. After 3 minutes the first film

was removed and if no serious skin reaction was observed, then a second film was applied at a different site and removed after 1 h and the observations at this stage indicated that exposure can humanely be allowed to extend to four hours, a third film was applied and removed

after 4 h, and Dermal toxicity was scored and recorded according to the Draize scoring system depicted in Table 1.

Table 1: Draize scoring system

Description of erythema or edema	Score assigned
Erythema and eschar formation	
➢ Severe erythema with slight eschar formation	4
➢ Moderate to severe erythema	3
➢ Well-defined erythema	2
➢ Very slight erythema	1
➢ No erythema	0
Edema	
➢ Severe edema with raised margin>1 mm and extending beyond the area of exposure	4
➢ Moderate edema with raised margin≈1 mm –	3
➢ Slight edema with raised margin	2
➢ Very slight edema	1
➢ No edema	0

If a corrosive effect was observed after any of the three sequential exposures, the test was immediately terminated. If a corrosive effect was not observed after the last film was removed, the animal was observed for 14 days, unless corrosion develops at an earlier time point. In those cases in which the test chemical was not expected to produce corrosion but may be irritating, a single film was applied to one animal for four hours.

Pharmacokinetic Study[19]

All experiment rabbits were acclimatised to the laboratory conditions for a period of one week prior to the initiation of experiment. The experimental animals were exposed to test drug or reference drug once only. On the day of experiment the transdermal film was applied on the shaven back of the rabbit and blood samples were collected at different time intervals of 0 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h and 24 h from ear vein of the rabbit. Plasma was separated and stored at - 20 °C until analysis. Plasma concentration of drug was determined using high performance liquid chromatography (HPLC) method.

Blood collection method

Rabbits were placed in rabbit holder; blood was collected in glass tube containing 11% tri sodium citrate (100µl) using heparinised capillary tube. Blood sample was processed by centrifugation (REMI, India) to separate serum and was used for HPLC analysis.

Analytical method - HPLC method

A previously reported and validated RP-HPLC method was used to estimate pure Rivastigmine tartrate[20]. The mobile phase consisted mixture of 10 mM concentration of ammonium acetate buffer:Acetonitrile [30: 70% v/v, pH adjusted to 4.0 with orthophosphoric buffer] for 100 mcg/ml with C18 column (250mm ×4.6mm × 5µ). The drug retention time was found to be 4.08 min. Representative chromatogram of Rivastigmine Tartrate obtained at 263 nm. A linear relationship was observed in the concentration range of 50-100 mcg/ml (r2=0.999; n= 3). Typical chromatogram of Rivastigmine Tartrate by RP-HPLC graph is represented in Figure 1. A calibration graph is represented in Figure 2.

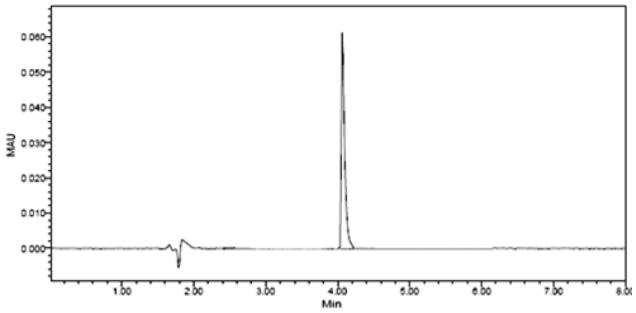

Figure 1: Typical chromatogram of rabbit serum spiked with Rivastigmine Tartrate

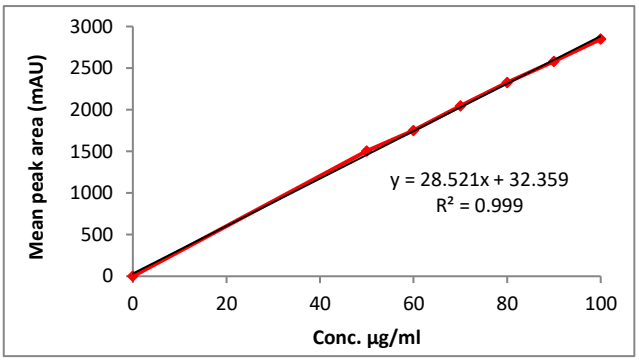

Figure 2: Standard calibration curve of Rivastigmine Tartrate

Bio analytical method

For the bio analytical method of Rivastigmine Tartrate Protein precipitation technique was used as suitable approach for extraction. During extraction procedure supernatant fluid was separated and filtered (0.45 µ filter). The filtered sample was injected into the HPLC and drug retention was observed to be 4.15 min. The chromatogram of Rivastigmine Tartrate is shown in Figure 3. The calibration curve plot of concentration v/s area and their data are shown in Figure 4.

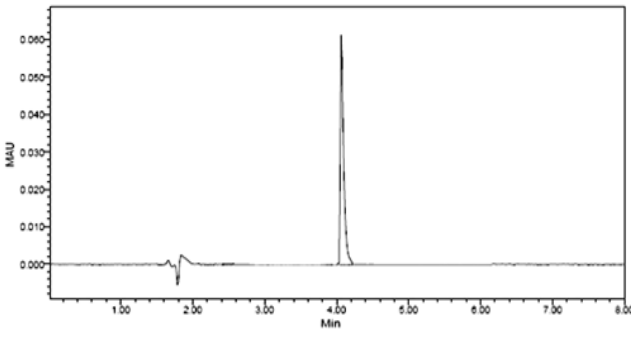

Figure 3: Typical chromatogram of rabbit serum spiked with Rivastigmine Tartrate

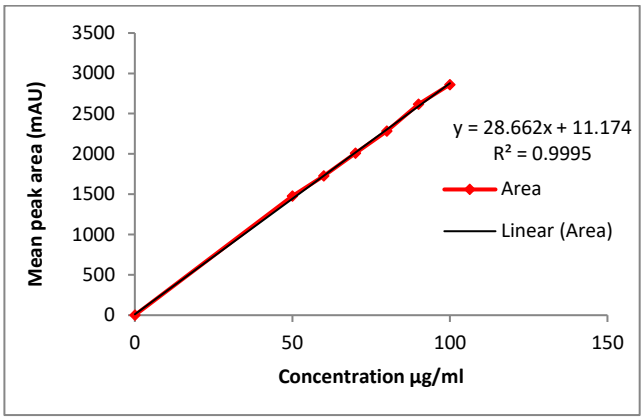

Figure 4: Calibration curve for the estimation of Rivastigmine Tartrate in serum of PNPs

Stability studies

The long term & augmented stability studies were conducted at various temperature and relative humidity (RH) for 6 months. Long term study was conducted at 25±2 °C and 60±5% RH & augmented study was conducted at 40±2 °C and 75±5% RH in stability chambers. Samples were taken at 0 day, 1 month, 3 & 6 months. The physical properties of the formulations and % drug content were determined as represented parameters for stability[21].

RESULTS AND DISCUSSION

Dermal toxicity study

Dermal toxicity study of Rivastigmine PNP's loaded transdermal films were performed on 3 male New zealand rabbits using 3 different films for 4 h and the results are shown in Table 2.

Table 2: Dermal toxicity study results

Sl.No	Film	Score (Erythema)	Score (Edema)
1.	First film	0	0
2.	Second film	0	0
3.	Third film	0	0

Pharmacokinetic Study

Pharmacokinetic study of formulation shows significant improvement of C_{max} and bioavailability of the drug compared to the pure drug. The pharmacokinetic parameters of Rivastigmine Tartrate PNP's loaded film absorption are summarized in Table 3. Figure 5 depicts the mean serum concentration profile as a function of time obtained by the pharmacokinetic studies carried out in rabbits for Rivastigmine Tartrate PNP's loaded film formulation and pure drug. The serum level profiles were significantly increased for Rivastigmine Tartrate PNP's loaded film formulation compared to pure drug.

The pharmacokinetic results of pure drug Rivastigmine PNPs film showed the C_{max} value of 142.26 ng/mL and T_{max} of 2h. Similarly the C_{max} value of formulation K-10 was 109.16 ng/mL and T_{max} of 3h. The Pharmacokinetic study shows that polymeric nanoparticles shows significant improvement of bioavailability of the drug while compared to its pure form.

Table 3: Pharmacokinetic parameters of pure Rivastigmine Tartrate pure and optimized PNP's loaded film.

Product	C_{max} (ng/ml) *	T_{max} (h) *	K_{el} (h^{-1}) *	$T_{1/2}$ (h) *	$(AUC)_0^t$ (ng/ml×h) *
Pure drug	142.26±1.2	2	0.1	3.4	1462.56±3.53
PNPs Film	109.16±0.9	3	0.16	4.3	1491.97±1.87

* mean ± SD, n=3

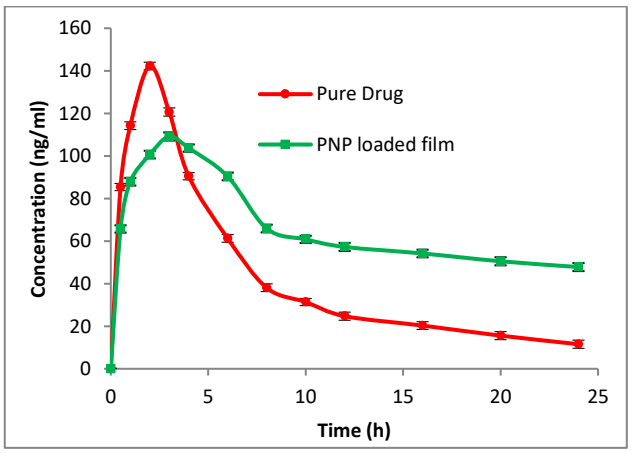

Figure 5: Serum drug level profiles of pure drug and optimized PNP's loaded film formulation

Stability studies

The prepared optimized formulation of Rivastigmine Tartrate PNP's loaded transdermal films were kept in stability chamber at different temperature and different humidity and tested the samples at different time intervals. The results obtained for optimized formulation was within the limit. The results are shown in the Table 4, which indicates no significant changes in the parameter even when it was subjected to stress testing for a period of six months.

Table 4: Stability study data of optimized formulation

Stability condition	Sampling interval (months)	Physical appearance	% Drug content (mean ± S.D*)
25±2 °C/ 60±5% RH	0	No change	97.73±0.38
	3	No change	96.37±0.46
	6	No change	98.65±0.25
30±2 °C/ 65±5% RH	0	No change	95.97±0.18
	3	No change	97.29±0.72
	6	No change	96.46±0.50
40±2 °C/ 75±5% RH	0	No change	97.18±0.63
	3	No change	98.07±0.27
	6	No change	98.38±0.74

* mean ± SD, n=3

CONCLUSION

Optimized formulation of Rivastigmine Tartrate PNP's loaded transdermal film was subject to dermal toxicity study and Pharmacokinetic studies, successfully done on New zealand white rabbits. No serious skin reaction was observed in dermal toxicity study. Rivastigmine Tartrate PNP's film shows the retention time of 4.15 min. The analytical method conducted using RP-HPLC was found to be highly sensitive, selective and reproducible for measurement of Rivastigmine Tartrate. The pharmacokinetic results of pure drug Rivastigmine Tartrate showed the C_{max} value 142.26 ng/mL and T_{max} 2 h. Similarly the C_{max} value of optimized PNP's loaded transdemal film showed 109.16 ng/mL and T_{max} 3 h. The Pharmacokinetic study shows that PNP's loaded film shows significant improvement of bioavailability of the drug while compared to its pure form.

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