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Evaluation and invitro gut permeation studies of solid lipid nano carrier mediated drug delivery system of perinodopril

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***Abstract*---**High BP is one of the most predominant causes of heart diseases and cerebrovascular problems. Perinodopril is an ACE inhibitor that is a non sulfhydryl derivative that is used for the treatment of hypertension. To enhance the effect of the drug it was formulated into Lipid based nano carrier system (NLC) to improve the bioavailability and thereby the therapeutic potential. So the objective of the current work was to evaluate the NLC of perinodopril formulation and to evaluate the same. Animal protocol no. PCP/IACE/2019 and Registration no. 1678/PO/a/19/CPCS was approved by the Institutional Animal Ethics Committee (IAEC) at Pallavan Pharmacy College, Kanchipuram, Tamilnadu and their guidelines were followed. The invivo estimation of the activity was carried out on Albino wistar rats that are maintained under room conditions and the formulation was investigated for the Pharamcokinetic and pharmacodynamic paramteres in rat plasma. Also they were tested for their stability invitro. Results show that the nano particles measured as 0.207nm in size. The invitro drug release studies suggest that the formulations were releasing the drug in controlled fashion during 23 hrs. stability studies proves the drug is very stable in the formulation. With R2 = 0.9683, the in vitro-in vivo correlation research clearly shows good agreement between in vitro drug solubilization during lipolysis and in vivo drug absorption during pharmacokinetic studies. This in vitro lipolysis research, which has an R2 close to 1, suggests that this model can match the in vivo dissolution profile in the gut.

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Keywords---perinodopril, antihypertensive, NLC, lipid nanoparticles, carriers.

Introduction

High blood pressure, the most common cause of heart disease, claims the lives of 7 million individuals every year throughout the world. Perinodopril is a nonsulfhydryl prodrug that has been labeled as an ACE inhibitor and is used in the treatment of hypertension [1]. Perinodopril has a specific action on the coronary and skeletal muscle vasculature, according to the manufacturer. Additionally, Perinodopril improves blood flow by inhibiting the synthesis of certain natural compounds that constrict the blood vessels [2]. To achieve these goals, the current research focused on developing an improved Perinodopril- Nano Lipid based carrier (NLC) formulation through the use of Quality by design (QbD), which would further improve the oral bioavailability and, consequently, the therapeutic prospects of Perinodopril [3]. Because of high blood pressure, human heart needs to beat harder in order to pump blood that is enough to keep up with the demands of regular bodily functioning [4]. Untreated diabetes can couple with hypertension cause heart disease and other complications, such as renal disease, brain damage, and vision loss [5]. The objective of the current research is development and optimization of oral NLC formulation of Perinodopril to increase its oral bioavailability the from optimized formulation and to perform invivo studies

In vitro gut permeation study

Intestinal permeability of Perinodopril suspension and Perinodopril -NLC is shown in Table 41 and Fig. 32 A. After 2 h permeability through rat intestine for Perinodopril suspension of marketed formulation and Perinodopril -NLC was found to be 11.23±1.74 and 21.69±2.38 μg/cm2 respectively. There was significant difference in the permeability of Perinodopril -NLC formulation as compared to Perinodopril suspension at $p < 0.05$. These observations suggest that smaller particle size and presence of permeation enhancers (Poloxamer 188 and Tween 80) resulted in higher permeation of drug through Perinodopril -NLC.

Table 1
Serosal concentration of drug from its suspension and NLC formulation at different time points. Data expressed as mean±SD (n = 3)

Time (h)	Drug suspension	NLC
0.5	1.04±0.21	4.875±1.04
1	2.58±0.59	8.71±0.98
1.5	4.94±0.83	13.18±1.31
2	6.97±0.97	19.76±1.87

It's clearly indicates that the presence of verapamil which is a well-known P-gp efflux inhibitor markedly enhanced the permeability of drug from NLC formulation and suspension both by inhibiting the pumping of drug back inside intestine. There was 3.92 fold and 5.65-fold increase of permeability of suspension and

Perinodopril -NLC respectively in the presence of verapamil. Also, permeability of drug from suspension was significantly higher in the presence of Poloxamer 188 and Tween 80 as compared to control which could be explained on the basis of P-gp inhibitory action of both the surfactants.

Table 2
Permeability of perinodopril formulation in the presence of verapamil, poloxamer and Tween 80 and Perinodopril NLC in the presence of verapamil. Data expressed as mean±SD (n = 3)

Formulation	Control	Verapamil	Poloxamer 188	Tween 80
Suspension	2.632±0.809	10.32±1.9	13.13±3.36	10.95±3.24
NLC	7.234±1.49	14.88±2.53	----------	----------

Confocal microscopy

In Fig. 1 A and 1 B depth of penetration of Perinodopril suspension through z-axis were 20 and 64.9 µm respectively, while penetration of Perinodopril -NLC through z-axis as in figure 10 C and 10 D were 35 and 104.9 µm respectively. There was significant penetration of Perinodopril -NLC as compared to Perinodopril suspension through z-axis revealing capability of Perinodopril -NLC to pass through small intestine. Higher penetration of Perinodopril -NLC could be due to smaller size of NLC and due to surfactants which acts as penetration enhancers. This study followed by gut permeation study further confirmed higher diffusion capability of NLC as compared to test suspension across intestine.

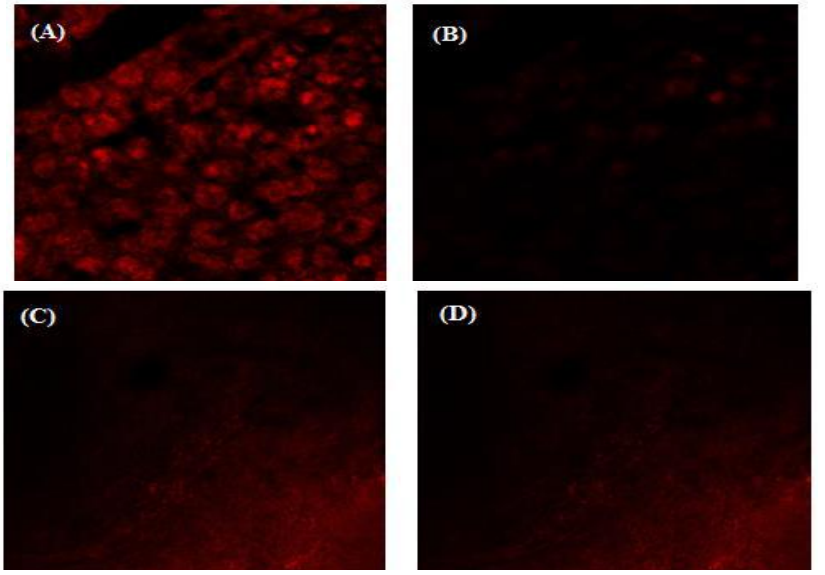

Figure 1. Confocal microscopy of rat small intestine showing depth of penetration A) 20 min after penetration of Perinodopril suspension B) 1 h after penetration of suspension C) 20 min after penetration of NLC D) 1 h after penetration of NLC

Pharmacokinetic study

Plasma drug concentration-time profile of Perinodopril suspension and Perinodopril -NLC with and without cycloheximide are shown in Table 2 and Fig. 2. Difference in the plasma concentration of Perinodopril -NLC as compared to Perinodopril -NLC with cycloheximide and Perinodopril suspension at each time point was significant ($p<0.01$). Pharmacokinetic parameters exhibited by the formulation (Perinodopril-NLC) showed remarkable increase in oral bioavailability as evident from Table 2. Oral bioavailability of Perinodopril -NLC improved by 4.2 fold and 1.9 fold in contrast to Perinodopril suspension and Perinodopril -NLC with Cycloheximide respectively. There was significant difference ($p<0.01$) in Cmax and t1/2 of PERINODOPRIL-LC (6987.64 ng/mL/h and 14.31 h) as compared to Perinodopril -NLC with cycloheximide (1893.66 ng/mL/h and 11.84 h) and Perinodopril suspension (2354.25 ng/mL/h and 8.52 h).

Table 3
Pharmacokinetic parameters as obtained after oral dosing of rat with drug suspension, formulation with and without cycloheximide (Cx). Data expressed as mean±SD (n = 4)

Parmameters	PERINODOPRIL suspension	PERINODOPRIL-NLC	PERINODOPRIL-NLC with Cx
AUC0 to t (ng.h/mL)	18710.44±1759.25	78725.72±1948.62	27426.5±12325.28
Cmax (ng/mL)	2354.25±356.28	6987.64±597.9	1893.66±359.64
Tmax (h)	2±0.00	2±0.00	2±0.00
Ka (h-1)	0.081302±0.021	0.048422±0.039	0.061086±0.052
AUC0 to ∞ (ng.h/mL)	20547.06±1124.52	106555.5±34257.51	29024.76±1539.84
AUMC0 to t (ng.h2/mL)	119942.5±27548.29	676093.7±13326.49	201920.17±983.17
AUMC0 to ∞ (ng.h2/mL)	186611.6±18526.91	1918742±22356.47	396370.6±34691.67
T1/2 (h)	8.52±2.13	14.31±4.19	11.34±3.97
Relative bioavailability	-----	4.207	1.907

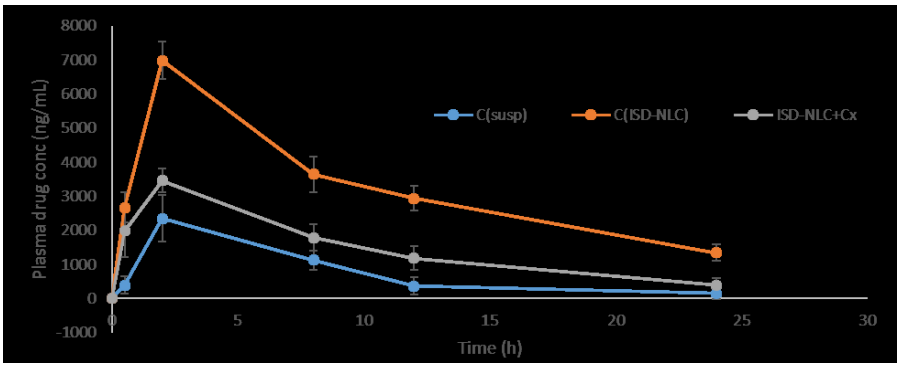

Figure 2. Plasma drug concentration-time profile of Perinodopril suspension, Perinodopril-NLC and formulation with cycloheximide (Cx). Data expressed as mean ± SD, n = 4

This increased oral bioavailability of Perinodopril -NLC as compared to Perinodopril suspension could be attributed due to pre-enterocyte solubilisation and absorption of the drug in lipid nanoformulation as evident from the lipolysis study. Also, it has been found that surfactants like Tween 80 and Poloxamer 188 acts as P-gp efflux inhibitor which could have contributed in marked enhancement of drug level in plasma through NLC as already observed in gut permeation study.

Pharmacodynamic study

In vivo antihypertensive activity was performed for 24 h period. All the animals of group I was found to be hypertensive with mean systolic blood pressure between 161.23-171.34 mmHg. Significant reduction in mean systolic blood pressure ($p<0.001$) was found with treatment groups II and III i.e., 119.35±18.81 mm Hg and 123.74±8.4 mm Hg respectively after 1 h of oral administration as compared to Perinodopril -suspension. Further, Perinodopril -NLC was found to show mean systolic blood pressure less than 130 mmHg for the entire 24 h period.

Table 4
Mean arterial blood pressure in mm Hg in animals with normal saline and treated with Perinodopril -suspension) and Perinodopril -NLC. Values expressed in mean ± S.D. (n=4)

Time (h)	Group I (Normal saline) (mm Hg), n = 4	Group II (Perinodopril-suspension) (mm Hg), n = 4	Group III (Perinodopril-NLC) (mm Hg), n = 4
0	171.34±7.92	168.54±9.88	170.38±9.69
1	169.75±11.19	119.35±18.81	123.74±8.4
2	167.29±13.64	122.3±12.58	121.3±9.51
4	163.59±8.05	131.26±14.08	118.74±9.03
6	166.71±9.74	134.7±10.94	122.8±8.43
9	161.23±9.39	143.67±14.25	121.27±9.94
24	164.18±8.99	158.8±11.5	129.71±8.73

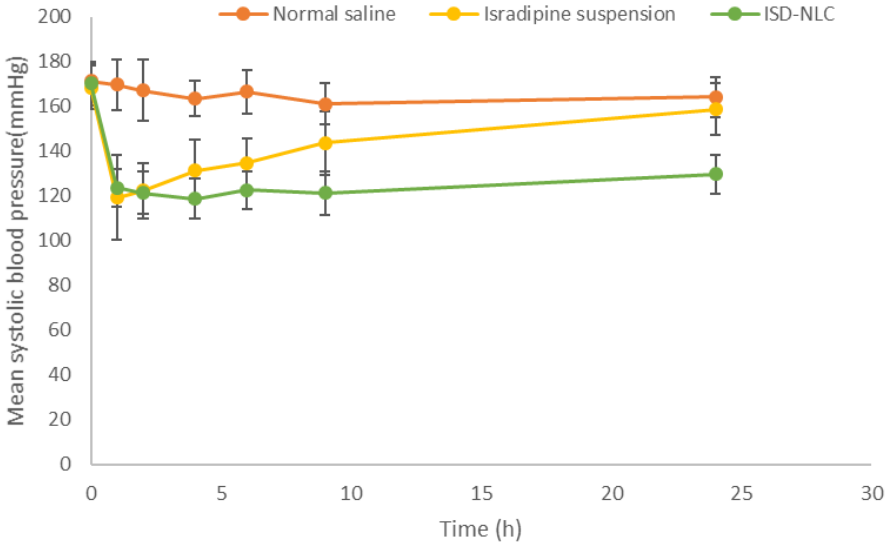

Figure 3. In vivo antihypertensive activity of animals treated with normal saline, Perinodopril-suspension and Perinodopril -NLC. Values expressed in mean ± S.D. (n=4)

Stability Study
Physical stability test of formulation

Results showed no significant difference on physical test at 0 and 6 months after storage at 25 °C ± 2 °C/60% RH ± 5% RH while storing at 40 °C ± 2 °C/75% RH ± 5% RH showed significant difference at 95 % confidence interval. However, this difference was not significant till 3 month of storage. Storing formulation at 25 0C showed better stability than storing it at 40 0C.

Table 5
Degradation profile of Perinodopril at different storage temperature stability test at room temperature and accelerated

Time (months)	Drug content (mg)	% Drug remaining	Log % drug remaining
0	48.32±1.19	100	2
3	48.05±1.27	99.07	1.993
6	46.12±0.98	96	1.972
0	48.32±1.19	100	2
3	47.01±1.07	97.28	1.979
6	45.31±1.17	93.77	1.968
0	48.32±1.19	100	2
3	44.98±1.81	93.08	1.981
6	41.24±0.692	85.34	1.942

Physical stability study at 25 °C ± 2 °C/60% RH ± 5% RH						
Time (month)	Physical appearance	Phase separation	Caking	Size (nm)	PDI	% Entrapment efficiency
0	No Change	No	No	85.7±7.3	0.207±0.029	87.4±3.29
3	No Change	No	No	89±5.8	0.284±0.019	83.61±3.8
6	No Change	No	No	96±6.51	0.391±0.038	78.4±4.19
Physical stability study at 25 °C ± 2 °C/60% RH ± 5% RH						
0	No Change	No	No	85.7±7.3	0.207±0.029	87.4±3.29
3	No Change	No	No	96.8±5.45	0.314±0.035	81.8±8.47
6	No Change	Separation	No	117±8.98	0.549±0.048	74.62±5.88

Accelerated stability test and shelf life determination

Log % drug remaining at different time interval and at different temperature is shown in Table 48. The order of degradation of perinodopril was found to follow first order kinetics. A graph was plotted between log K vs absolute temperature.

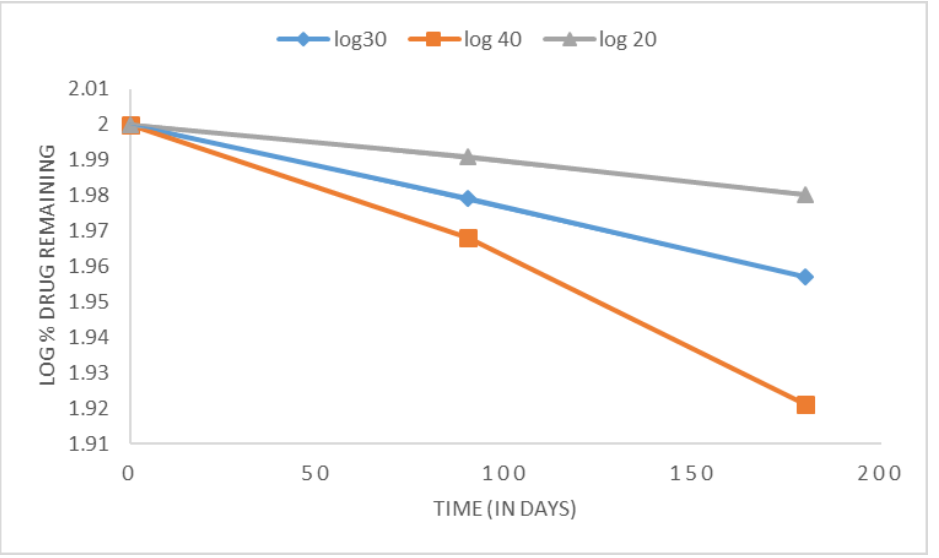

Figure 4. Log % drug remaining at 0, 30, 60 and 90 days of storage

Summary and Conclusion

Confocal microscopy was performed to assess the depth of penetration of drug through intestine. After treating Perinodopril suspension and Perinodopril-NLC with rhodamine 123, formulations were placed into the 5-6 cm segments of small intestine. Fluorescence signal was detected at different depth of the intestine after 20 min and 1 h of treatment. Filling of formulation into intestine, dissolution media and maintenance of temperature was same as for *in vitro* gut permeation

study. After 20 min and 1 h of treatment small intestine was cut open and positioned on the slide such that inner part of intestine faces up. The optimized Perinodopril Formulation was found to be stable at different simulated gastric fluid. Confocal study depicted maximum deposition of Perinodopril into the depth of the small intestine. Perinodopril displayed significantly higher permeation across small intestine due to of P-gp inhibiting activity of the chosen excipients resulting in sufficient improvement in the oral bioavailability of Perinodopril. A significantly higher drug partition into aqueous phase of drug from Perinodopril anticipated that Perinodopril might have higher absorption *in vivo*. This was further confirmed by *in vivo* pharmacokinetic study which demonstrated that Perinodopril could improve oral bioavailability of drug which could be attributed to the lipid based excipients utilized in NLC. *In vivo* antihypertensive activity clearly demonstrated that oral administration of NLC of Perinodopril in rats are a suitable approach to maintain blood pressure for over 24 h duration. Thus, oral administration of NLC loaded Perinodopril has proved to be a remarkable delivery system in the management of hypertension.

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