DEVELOPMENT AND IN VIVO EVALUATION OF SOLID LIPID NANOPARTICLE FORMULATION OF CERITINIB BY DOE

Gaddam Suvarsha[1*], M. Vijey Anandhi[2], Aduri Prakash Reddy[1]

[1]Department of Pharmaceutics, School of Pharmaceutical Science, Vels Institute of Science, Technology and Advanced Studies, Pallaravam, Chennai-600117, Tamil Nadu, India.

[2]Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Pallaravam, Chennai-600117, Tamil Nadu, India.

*Corresponding author : Gaddam Suvarsha, Department of Pharmaceutics, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, India. E-mail: suvarshagaddam@gmail.com

ABSTRACT

Ceritinib is an anaplastic lymphoma kinase (ALK) inhibitor that exhibit varyingly low water solubility; poor drug compressibility hence depressed bioavailability. The objective of current research is to process ceritinib loaded SLNs for enhancing bioavailability. Box-Behnken design (BBD) was employed to optimize variables used for formulation process of ceritinib loaded SLNs containing 3 factors and evaluated at 3 levels. Three optimised formulations of ceritinib SLN prepared and subjected to physicochemical characterization. The formulation F1 with mean particle size (167.9nm), PDI (0.645), zeta potential (-24.9±1.48mV) and % entrapment efficiency (90.24%) is chosen for further investigation while the SEM study of optimized formulation confirms spherical shape. The *in vitro* studies indicate a maximum drug release of 95.12 % in 360 minutes for F1 which is much higher than control (30.12% in 360 minutes). *In vivo* pharmacokinetic analysis conducted on rats indicated that C_{max} of SLNs (66.233±3.54ng/ml) was significant ($p<0.05$) in comparison to pure drug (20.1±3.41ng/ml). The t_{max} of SLN formulation and pure drug suspension were 2.50±0.01 and 4.00±0.03h respectively. $AUC_{0-\infty}$ for optimized SLN formulation was higher (592.5±5.24 ng. h/ml) than the pure drug suspension formulation (155.7±5.02 ng. h/ml), where the bioavailability was 4 folds increased. These constructive results marked that the proposed SLNs were effective in improving the bioavailability of ceritinib.

KEYWORDS: Ceritinib, lymphoma kinase inhibitor, solubility, Box-Behnken design, In vivo bioavailability studies

DOI Number: 10.14704/nq.2022.20.13.NQ88223 Neuro Quantology 2022; 20(13):1793-1800

I. INTRODUCTION

Solid lipid nanoparticles (SLN) are auxiliary to emulsions, liposomes, micro particles and other drug carrying systems since the early 1990s.Initially, nanoparticles were designed to carry vaccines and anticancer drug with the strategy of enhancing drug targeting. Further coating of nanoparticles with hydrophilic substances reduced the intake of these nano formulations by reticuloendothelial system (RES) cells (Rane et al., 2008; Bargoni et al., 2001). The use of hydrophilic substances like poloxamers, polyvinyl alcohol and PEG further minimize the non-specific interactionof drug and other proteins (Rossi et al., 2006). Ceritinib is a small molecule drug belonging to second generation selective ALK inhibitors with activity 20 times higher than crizotinib. Ceritinib is chemically 5-chloro-N4-[2-[(1-methylethyl) sulfonyl]phenyl]-N2-[5-methyl-2-(1-methyl ethoxy) -4-(4-piperidinyl)phenyl] 2,4-pyrimidine diamine used for treatment of positive lung cancer. Ceritinib is BCS class IV drug exhibiting very low solubility of 0.02mg/ml at room temperature along with low permeability thus making the drug difficult to formulate (Chakraborty et al., 2009). Also,

picking and sticking problems are encountered during the formulation process of ceritinib owing to its physical characteristics thus resulting in poor compression. Ceritinib sticky nature causes a high drug load to further negatively influence the manufacturing of tablets due to enhanced sticking/picking. Clinical effectiveness of ceritinib on administration requires its delivery in a dosage form which would result in high bioavailability in addition to decreased variability within or in between subjects.

In the current study, ceritinib was formulated as solid lipid nanoparticles (SLNs) to overcome the variably lower oral bioavailability, solubility and protein binding capacity. The formulation and process variables of these SLNs, are optimized by statistically experimental design methodology followed by Box Behnken design (Nagar et al., 2012). Different parameters were evaluated on the optimized SLN formulations such as particle size, PDI, zeta potential, entrapment efficiency, SEM analysis etc. (Journals et al., 2015).The best ceritinib SLN formulation was chosen for *in vivo* studies on rats for therapeutic efficiency evaluation

II. MATERIALS AND METHODS

Materials

Ceritinib was gifted from Caplin point laboratories Limited, Bangalore, India. Glycerylmonostearate (GMS), glyceryltripalmitate, glyceryltristearate, glycerylbehenate, glyceryl palmitostearate, dialysis tubing (molecular weight cut off 12-14kDa), were obtained from Sigma Aldrich (St. Louis, MO, USA). Tween®80was a product from SD Fine Chem Ltd (Mumbai, India). Soya Lecithin, Poloxamer-188 and PVA were gift samples from Dr. Reddy's laboratories ltd., India.

Design of Experiments [DOE]

The Box-Behnken design (BBD) was used to optimize the formulation variables of ceritinib loaded SLNs containing 3 factors and evaluated at 3 levels. Ratio of drug to lipid (A), Concentration of glyceryl monostearate (B) and co surfactant concentration (Ploxamer-188, C) as independent variables while size of particle(PS) (Y1), entrapment efficiency(EE) (Y2) were the dependent responses[Table 1]. The experimentation designed by using DOE software (Stat-Ease Design Expert ® software V8.0.1) by employing one-way ANOVA test at 0.05 levels (Roy, 1990; Myers and Montgomery, 2002).

Table 1:List of factors(*independent variables)* and responses (*dependent variable)*choosen for Box-Behnken design

Factors			***Level***		
Variable	Name	Units	Low	Middle	High
A	Drug - lipid ratio	-	1:10	1:15	1:20
B	glyceryl monostearate concentration	mg	50	75	100
C	poloxamer 188 concentration	mg	20	30	40
Responses			***Goal***		
Y1	Size of Particle	Nm	Minimize		
Y2	EE	%	Maximize		

Preparation of ceritinib SLN formulations

Single emulsification and solvent-evaporation method was employed for SLN of ceritinib. Ceritinib (100 mg), glycerylmonostearate and soya lecithin was taken into 10ml volumetric flask followed by dissolution in 3ml chloroform. The contents then added to 10ml of 1.5% w/v of poloxomer 188 solutions, the dispersion homogenized at 10000 rpm for 6 min and sonicated for 15 min followed by stirring for 3 h at 1000 rpm. The obtained dispersion was further subjected to centrifugation for 45min at 15000 rpm. Nanoparticles were subjected to further purification after the obtained pellets were subjected to 3-4 times washing with milliQ (Pooja et al., 2015).

Characterization of ceritinib loaded SLNs

Ceritinib SLN's formulation that was optimized was subjected to evaluation of various physicochemical parameters.Zeta potential (ZP), size and polydispersity index of the formulated SLN's were analysed by Nano ZS90Zetasizer (Malvern, Worcestershire, UK) (Rajendra et al., 2011; Vijaykumar et al., 2016; Ruan et al., 2003; Zhongcheng et al., 2016). Entrapment efficiency and drug loading are analysed for10 ml of ceritinib loaded SLN at 10 ml of ceritinib loaded SLN. Scanning Electron Microscope (SEM, Hitachi, Tokyo, Japan) adopted for the study of particle surface morphology (Ekambaram et al., 2011).

***In-vitro* drug release evaluation**

In vitro drug release evaluation performed using dialysis membrane having molecular weight in range of 12,000 – 14, 000 that was soaked inwater overnight with 0.01 M HCl as dissolution media. The dialysis membrane consists of both acceptor and donor compartments.150mg of SLN formulation filled in donor compartment while receptor compartment filled with 100ml release medium at 37±0.5°C.About 3ml sample drawn out each time from receiver compartment at intervals of 15, 30, 45, 60, 90, 120, 240, and 360 min followed by dilution with dissolution medium and analysed for UV absorbance at λ_{max} 319.6 nm. Elucidation of mechanism and mode of drug release performed by fitting the dissolution data into different kinetic model equations like zero order, first order, Korsemeyer Peppa's and Higuchi's model. The release data from the nano formulation was determined by curve fitting method.

Pharmacokinetic study of ceritinib

Wistar rats weighing between 150-180 g that were healthy during the period of the experiment were chosen for the experiment performed at controlled temperature of 25^0C, 45% RH and 12h alternate cycle of light and dark .The animal room is facilitated with 100% fresh air exchange with continuous supply of power and water (Vijayanand et al., 2018). Rats fed with standard diet and *waterad libitum.* The approval for conducting protocol as obtained from institutional animal ethics committee with IAEC NO: 1447/PO/Re/S/11/CPCSEA-48/A.

Study Design

The wistar rats were categorized into two groups that were offered with food post four hours of dosing. Group 1 was administrated with pure drug (ceritinib) suspension in methanol while Group 2 was administrated with the optimized ceritinib SLN formulations by oral route at a dose of 10mg/kg. 500 µL blood samples collected from the femoral artery of the animals at varying time intervals of 0, 0.50, 1, 1.50, 2, 2.50, 3, 4, 5, 6, 8, 12, 16, 20, 24h post dose. The samples transferred into Eppendorf tubes containing heparin to prevent clotting. Plasma was separated by centrifugation of the blood at 5000 rpm, cooling centrifuge for 5-10 minutes and stored frozen at −20°C until analysis.

Pharmacokinetic analysis

The pharmacokinetic parameters evaluate were C_{max} (maximum plasma concentration), T_{max} (time to attain C_{max}), $AUC_{0\text{-}t}$ (area under plasma concentration–time curve from zero to the last sampling time), $AUC_{0\text{-}\infty}$ (area under plasma concentration–time curve from zero to ∞) and $t_{1/2}$.

III. RESULTS AND DISCUSSION

Design of experiment

On the basis of Box–Behnken design, designing of about 17 experiments conducted as shown in table 1 and 2. The regression equations are a representation quantifiable effect of drug - lipid ratio (A), glycerylmonostearate concentration (B) and concentration of Poloxamer 188 (C) on particle size (Y1) and entrapment efficiency (Y2).Interaction terms and quadratic relationship are represented by one factor and higher factor order coefficients. Synergistic and antagonistic effects are indicated by positive and negative signs respectively. Data fitting to quadratic model was done using a backward elimination process. Both the polynomial equationswere significant statistically (P\0.01), as determined using ANOVA as per the provisions of Design Expert software.

Table 2: Experimental observations of factors and responses

Run	A	B	C	Response Y1	Response Y2
1	1:15	100	20	188.32	89.13
2	1:20	75	20	402.42	90.76
-3	1:15	75	30	161.82	91.23
4	1:15	50	40	213.62	78.12
5	1:20	100	30	412.56	89.82
6	1:10	50	30	160.12	69.36
7	1:15	100	40	333.72	67.46
8	1:20	75	40	394.24	90.36
9	1:15	75	30	162.24	90.86
10	1:20	50	30	383.56	93.82
11	1:15	75	30	162.06	89.76
12	1:10	75	20	174.32	74.32
13	1:10	75	40	176.72	79.62
14	1:15	75	30	162.96	89.32
15	1:15	50	20	253.46	71.72
16	1:15	75	30	161.32	91.36
17	1:10	100	30	182.62	88.93

Effects on particle size (Y1)

Table 2 shows the nanoparticles particle size that ranged from 160.12-412.56 nm.Fig 1 indicates an increase in Y1 values from 160.12 nm to 182.62 nm and from 383.56 nm to 412.56 nm is seen at low (drug to lipid ratio) and high levels of A.

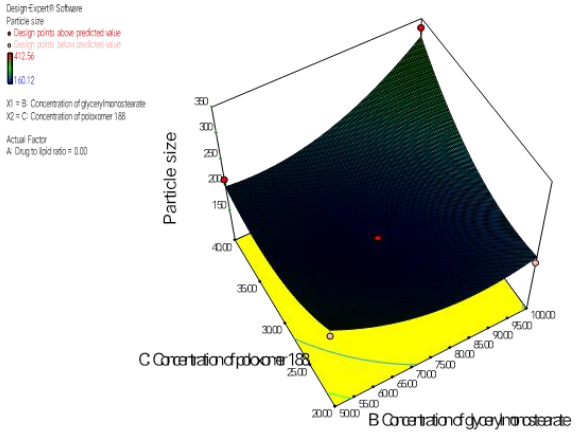

Fig 1: 3D plots indicating the influence of glyceryl monostearate and poloxamer 188 concentration on particle size

Effect on Entrapment efficiency (Y2)

An increase in Y2 from 69.36 % to 88.93 % and from 89.82 % to 93.82 % was observed at low and high levels of A respectively. Similarly, an increase in Y2 from 71.72 % to 93.82 % and from 67.46 % to 89.82% was observed at high and low levels of B respectively. On the other hand, a reduction in Y2 value from 90.76 % to 71.72 % at low levels of C and 90.36 % to 67.46 % at high levels of C was observed (Fig 2A and 2B)

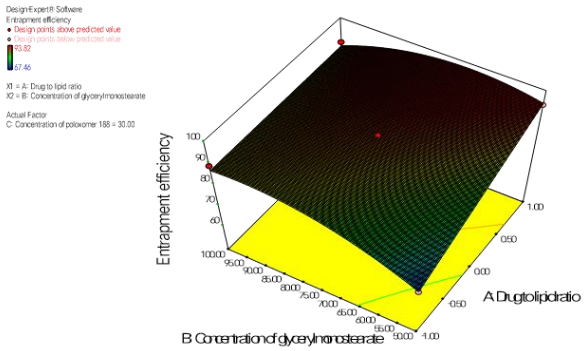

Fig 2A: Response surface plot indicating effect of ration of drug to lipid and glycerylmonostearate concentration on entrapment efficiency keeping C as constant

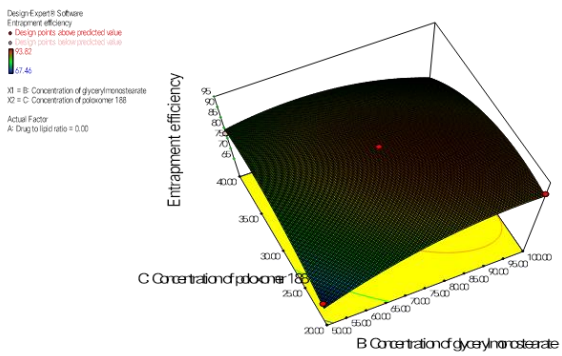

Fig 2B: Response surface plot demonstrating the effect of glycerylmonostearate and poloxomer188 concentration on entrapment efficiency by keep A as constant

Characterization of optimized formulations

All optimized formulations were characterized for particle size distribution, entrapment efficiency and ZP. An avaerage of 167.9 ±12.9 to 172.4 ± 9.9 nm as mean particle size and 89.46 to 90.24% as entrapment efficiency was observed for all formulations (Table 3). F1 formulation shown less particle size and more entrapment efficiency. A broad range of size distribution wa indicated by PDI that ranged from 0.586 to 0.645. Inclusion of ceritinib in SLN's lipid matrix was indicated by the negative surface charge of prepared formulations that is a key regulating factor for particle stability.This zeta potential ranged between -0.6± 5.48 mV to -24.9 ± 2.89 mV for the prepared formulations. (Table 4)

Table 3: Predicted and observed values for restraints applied on Y1, and Y2

Independent variable	Nominal	Predicted		Observed		
		(Y1)	(Y2)	Batch	(Y1)	(Y2)
Drug -lipid ratio (A)	1:12.5	160.12	91.35	1	167.9	90.24
				2	172.4	89.46
Conc. of glycerylmonostearate (B)	82.17			3	169.6	89.72
Conc. of poloxomer 188 (C)	27.69					

Table 4: Observed responses of optimized ceritinib SLNs

Batch	MPS ± SD (nm)	PDI	ZP ± SD (mV)	% EE ± SD
1	167.9 ±12.9	0.645	-24.9 ± 1.48	90.24 ± 0.28
2	172.4 ±9.9	0.629	-6.0 ± 3.22	89.46 ± 0.17
3	169.6 ± 3.8	0.586	-7.2 ± 3.89	89.72 ± 0.42

n = 3 ($p < 0.05$)

In vitro drug release study

Fig 3 illustrates SLN formulated with ceritinib dissolution profile which was about 94.91to 95.63% at 6 hours end, whereas 30.12% release was found in case of pure drug suspension which can be due to hydrophobicity of the drug that results in decreased solubility which is enhanced in case of nanoparticles formulation (Table 5). Release order kinetics **v**alue of n equal to 0.83847 in Korsmeyer-Peppas plots indicated non Fickian (anomalous) diffusion with drug release by erosion and coupled diffusion.

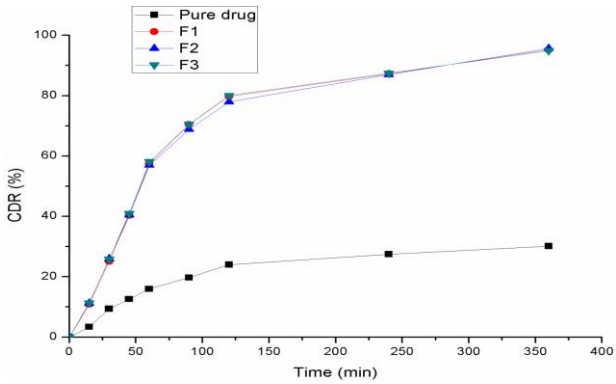

Fig 3: In-vitro release of ceritinib from nanoparticles.

In vivo drug bioavailability data

Fig 4 shows the plasma concentration–time curve in Wistar rats after a single oral dose of ceritinib solid lipid nanoparticles formulation as compared to ceritinib pure suspension. At all the indicated time points, the ceritinib plasma concentrations in rats treated with solid lipid nanoparticles formulation was significantly higher than those treated with pure drug. Pharmacokinetic parameters of ceritinib post administration in wistar rats are tabulated in Table 5. C_{max} of ceritinib SLN (66.23±3.54ng/ml) was significant ($p<0.05$) as compared to the pure drug suspension formulation (20.1±3.41ng/ml). T_{max} of SLN preparation and pure drug was 2.50±0.01 and 4.00±0.03h, respectively. $AUC_{0-\infty}$ infinity for solid lipid nanoparticles formulation was higher (592.5±5.24ng. h/ml) than the pure drug suspension 155.7±5.02ng. h/ml. The ceritinib SLN formulation bioavailability was higher and faster than that of the pure drug.

Table 5: Pharmacokinetic Parameters of optimized ceritinib solid lipid nanoparticles formulation and pure drug

Pharmacokinetic parameters	Ceritinib Pure drug	Ceritinib SLN
C_{max} (ng/ml)	20.1±3.41	66.233±3.54
AUC_{0-t} (ng. h/ml)	132.2±3.22	474.4±2.51
AUC_{0-inf} (ng. h/ml)	155.7±5.02	592.5±5.24
T_{max}(h)	4.00±0.03	2.50±0.01
$t_{1/2}$ (h)	7.50±0.02	5.50±0.02

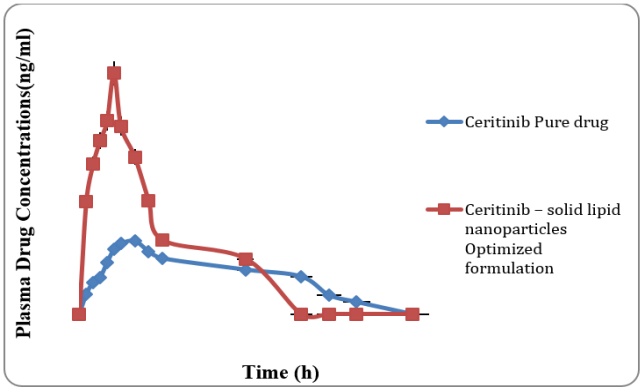

Fig 4: Plasma concentration profiles of optimized Ceritinib solid lipid nanoparticles and pure drug

IV. CONCLUSION

The present research demonstrated the application of Box–Behnken design at 3-factors and 3-levels, for optimizing variables of formulation for ceritinib SLNs nanoparticles preparation. The independent variables include ratio of drug to lipid (A), concentration of glycerylmonostearate (B) and concentration of cosurfactant (Ploxamer-188,C) while particle size (Y1), entrapment efficiency (Y2) were designated as dependent variables. The SLNs prepared by single emulsification-solvent evaporation method were evaluated for least particle size with highest entrapment efficiency. Formulation F1 was found to be less particle size, more entrapment efficiency and higher drug release. *In vivo* pharmacokinetic analysis conducted on rats indicated that C_{max} of SLNs (66.233±3.54ng/ml) was significant ($p<0.05$) in comparison to that of pure drug (20.1±3.41ng/ml). The T_{max} of SLN formulation and pure drug suspension were 2.50±0.01 and 4.00±0.03h respectively. $AUC_{0-\infty}$ for optimized SLN formulation was higher (592.5±5.24 ng. h/ml) than the pure drug suspension formulation (155.7±5.02 ng. h/ml), where the bioavailability was 4 folds increased. These results were indicative that the ceritinib SLN formulation bioavailability was higher and faster than that of the pure drug.

REFERENCES

1. Rane SS, Anderson BD. What determines drug solubility in lipid vehicles: is it predictable. *Adv Drug Deliv Rev*. 2008;60:638-656.
2. Bargoni A, Cavalli R, Zara GP, Fundaro A, Caputo O, Gasco MR. Transmucosal transport of tobramycin incorporated in solid lipid nanoparticles (SLN) after duodenal administration to rats. Part II-tissue distribution. *Pharmacol Res*. 2001;43(5):497-502.
3. De Rossi E, Ainsa JA, Riccardi G. Role of mycobacterial efflux transporters in drug resistance: an unresolved question. *FEMS Microbiol Rev*. 2006;30(1):36-52.
4. Chakraborty S, Shukla D, Mishra B, Singh S. Lipid–an emerging platform for oral delivery of drugs with poor bioavailability. *Eur J Pharm Biopharm*. 2009;73:1-15.
5. Nagar M, Panwar KS, Chopra VS, Bala I, Trivedi P. Quality by design: a systematic approach to pharmaceutical development. *Der Pharmacia Lettre Journal*. 2012;2(2):111–130.
6. Journals H, Article R, Parameters P, Kakodka SSS, Gajare P. Pharmaceutical Quality-by-Design (QbD): basic principles. *Int J Res Methodol*. 2015;1(1):1– 25.
7. Roy RA. Primer on the Taguchi Method, New York: Van Nostrand Reinhold; 1990.
8. Myers RH, Montgomery DC. Response Surface Methodology: Product and Process Optimization Using Designed Experiments. 2nd Edition, New York John Wiley & Sons; 2002.
9. Deep Pooja, Kulhari H, Tunki L, Chinde S, Kuncha M, Grover P, Rachamalla SS, Sistla R. Nanomedicines for targeted delivery of etoposide to non-small cell lung cancer using transferrin functionalized nanoparticles. *RSC Advances*. 2015;5:49122–49131.
10. Rajendra C, Harish P, Jain A K, Himesh, Saraogi G K. Preparation and evaluation of the self emulsifying drug delivery system containing atorvastatin HMG-COA inhibiter. *Int J Phar and Pharm Sci*. 2011;3(3):147-152.
11. Vijaykumar N, Zhijun W, Guru VB. Pharmacokinetic Evaluation of Improved Oral Bioavailability of Valsartan: Proliposomes versus Self-nanoemulsifying drug delivery systems. *AAPS Pharm Sci Tech*. 2016;17(4):851-862.
12. Ruan G, Feng SS. Preparation and Characterization of Poly (lactic acid) - poly (ethylene Glycol)-poly lactic acid (PLA-PEG-PLA) microspheres for the controlled release of Paclitaxel. *Biomater*. 2003;24:5307-44.
13. Zhongcheng K, Xuefeng H, Xiao-bin J. Design and optimization of self-nanoemulsifying drug delivery systems for improved bioavailability of cyclovirobuxine D. Drug Design Dev Therapy. 2016;10:2049-2060.
14. Ekambaram P, Abdul Hasan Sathali. Formulation and Evaluation of Solid Lipid Nanoparticles of Ramipril. J Young Pharm. 2011;3(3):216–220.

15. Vijayanand P, Jyothi V, Aditya N , Mounika A. Development and Characterization of Solid Lipid Nanoparticles Containing Herbal Extract: In Vivo Antidepressant Activity. J Drug Deliv. 2018;1-7.