RESEARCH ARTICLE

Evaluation of Oral Bioavailability and *In-vivo* Anti-leukemic Potential of Dasatinib Loaded Solid Lipid Nanoparticles

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Received: 15th April, 2023; Revised: 20th July, 2023; Accepted: 25th August, 2023; Available Online: 25th September, 2023

ABSTRACT

Dasatinib is one of the second-generation tyrosine kinase inhibitors (TKI) that is taken orally and has antiproliferative activity against chronic myeloid leukemia (CML). Dasatinib/hesperidin loaded-SLNs were synthesized in-house using a high-shear homogenizer and optimized by central composite design (CCD). Oral bioavailability and *in-vivo* anti-leukemic potential of developed dasatinib/hesperidin-loaded-solid lipid nanoparticles (SLNs) were determined using intravenous injection of leukemia cells in mice model. Dasatinib was administered as pure drug (suspension) and SLN formulation in leukemic mice modal. The pharmacokinetic profile was studied and compared with drug suspension using HPLC. Results denoted mean maximum plasma concentrations C_{max} as 184.52 and 390.43 ng/mL, mean T_{max} as 2 and 4 hours, mean half-life as 4.63 and 8.06 hours., for pure drug (suspension) and SLN formulation, respectively. The mean area under the curve (AUC_{last}) was 1080.94 and 3669.49 hr*ng/mL for the same. SLN also showed statistically significant survival. A comparison of SLN and free drugs revealed that SLN was more effective at cytotoxicity. Therefore, the developed dual-targeted SLN formulation of dasatinib demonstrated higher sensitivity of cells to the drug entrapped in SLN than the drug suspension.

Keywords: HL60, Anti-leukemic, Dasatinib, Kaplan-Meier survival, Pharmacokinetics, Solid lipid nanoparticles.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.3.21

How to cite this article: Moinuddin, Neekhra S, Ahmad S, Swarnkar SK, Gupta D, Khunteta A, Jain P, Jain S. Evaluation of Oral Bioavailability and *In-vivo* Anti-leukemic Potential of Dasatinib Loaded Solid Lipid Nanoparticles. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):587-591.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

In order to stop leukemogenesis, dasatinib, an oral tyrosine kinase inhibitor, targets the BCR-ABL chemokine cytoplasmic protein and controls enzyme activity. In cases of intolerance or resistance, it may be used instead of imatinib therapy as first-line treatment for chronic myeloid leukemia (CML). It is also used to treat adult and pediatric patients with Philadelphia chromosome-positive acute lymphoblastic leukemia and CML that has relapsed and proven resistant. Dasatinib has demonstrated clinical success in older individuals, with an overall 5-year survival rate of 18 to 64%.[5] Dasatinib treatment resulted in an 86% overall 5-year survival rate in pediatric patients.[1]

To overcome the problem of solubility and oral bioavailability of the biopharmaceutical classification system (BCS) II and IV drugs, research in the current scenario is focused on increasing bioavailability by using various techniques to increase solubility such as 'solid-liquid compact', micronization using nanosuspension, solid dispersions, using complex and salt formation.[2] Most drugs show first-pass metabolic effects after oral administration, thus reducing the therapeutic concentration required in the systemic circulation. However, by using a lipid-based delivery system, dissolution and first-pass effect issues are resolved and oral absorption is subsequently increased.[3]

Hesperidin can alter tumor cell survival, division, and death mechanisms. Nevertheless, hesperidin does not have wide clinical use due to its decreased solubility in water.[4] Researchers are focusing on overcoming this problem by developing appropriate delivery systems for hesperidin.

SLNs are composed of an essential solid lipid core holding a monolayer surfactant shell. In comparison, SLNs

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were found to be a safer option than other nanosystems.[5] They typically avoid certain significant difficulties, such as liposomes' poor durability, decreased loading capacity, potentially high biotoxicity, and the presence of residual organic solvent when using polymeric nanoparticles.[6] Because of its lipidic components, SLN has been shown to solubilize highly lipophilic pharmaceuticals and has the advantage of holding them in a much better stable solution, avoiding the use of significant quantities of surfactants, and aiding in enlightening biopharmaceutical performance following various administration methods. Moreover, through lymphatic system targeting made possible by SLNs, medicines are protected from hepatic first-pass metabolism, their bioavailability is increased, and hepatotoxicity is decreased.[7]

In the present study, we specifically aimed in determination of oral bioavailability and *in-vivo* anti-leukemic potential of in-house developed dasatinib/hesperidin-loaded-solid lipid nanoparticles (SLNs) for CML.[8] To the best of my knowledge, none of the previous studies have detailed the usage of co-loaded nano-carriers in this way.

MATERIALS AND METHODS

Selection of Animals

Healthy mice of age group 6 to 8 weeks of either sex were selected and acclimatized for at least 5 days. Animal study was performed at Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad, Uttar Pradesh, India. IAEC of Dabur Research Foundation duly approved study protocol with reference no. IAEC/79/1532.[9-17]

Cell Line Expansion and Preparation

Selected luciferase-tagged cancer cells were cultured in CO_2 incubator as per the recommended media and condition. Cell numbers were counted and adjusted by adding phosphate buffered saline (PBS) to achieve desired concentration, i.e., 100 M per mL. Cells were diluted such that the concentration of the cell will be 10 million cells/100 µL of PBS.[18]

Evaluation of *In-vivo* Efficacy

The SLN formulation was evaluated for *in-vivo* anti-leukemic potential using intravenous injection of leukemia cells in mice model. Human cancer cell lines were injected intravenously to induce the cancer.[19]

Animal Protocol

Animal were divided into four groups as G1-G4 and categorized as normal control (placebo), Disease control (Leukemia), dasatinib Suspension and SLN Formulation group with 5 animals in each. Normal saline was used as vehicle. Drug concentration was taken as 1-mg/mL suspension to make the dose of 5 mg/kg body weight, thereby dosing used for this purpose was 5 mL/kg body weight par oral for G1, G3 and G4; and 5 mL/kg for disease control (Leukemia) group.

Insertion of Human Leukemia Cell Line

A microtiter plate (96 flat bottom well) was used for seeding Human leukemia cell line (HL60) with maximum density upto 1104 cells/well and incubated for 36 hours to grow. Post acclimatization, 10 million leukemia cells/100 µL PBS was injected intravenously to each mouse (G2, G3 and G4, n=15) on day "0". The animals were selected and randomized into the remaining 3 groups (G2-G4) based on the CD45+ levels. Animals from G1 served as normal control. G2 was serve as disease control group and receive normal saline at a dose volume of 5 mL/kg. Group G3 and G4 were treated as suspension and SLN formulation, respectively.

During disease induction phase, body weight was recorded daily. During survival period, clinical signs of leukemia and mortality were observed. Body weight, survival analysis (Kaplan Meir's Curve) and %ILS (Increased Life Span) were used as end point parameters.

Pharmacokinetic Evaluation

The dasatinib, pure drug (suspension) and SLN were administered by oral routes and carried out to study the pharmacokinetics in mice. First of all, single dose of dasatinib was administered and further, Plasma samples were collected at definite time interval (0.25, 0.5, 1, 2, 4, 6, 8 and 24 hours). Three animals were bled for blood collection at each the time point in each group. Blood was collected through retro-orbital vein under mild anesthesia in a micro-centrifuge tube containing saturated solution of Na_2 EDTA as an anticoagulant. Further blood sample was centrifuged at 4000 rpm for 5 minutes to separate the blood plasma. Plasma samples were immediately transferred to micro tubes and stored at freezer until analysis.

Quantification of dasatinib in study samples was performed with fit for purpose HPLC-UV method (4,8). The pharmacokinetic parameters were evaluated by using Phoenix WinNonlin Pro, version 8.3 Pharsight, Inc.,USA. Chromatographic conditions included- Zorbax Eclipse XDB C-8 (250 x 4.6 mm, 5 µm particle size) at 30°C column oven temperature; mobile phase A (Methanol), mobile phase B (2.75 g Potassium Dihydrogen Phosphate in 1000 mL milli-Q water - pH 4.5) with flow rate 1.0 mL/minutes; Injection volume- 10 µL, run time 15 minutes; Gradient fixed for 48:52 for mobile phase A: mobile phase B for whole run time; detection wavelength 323 nm (for dasatinib).[9-11,20-26,31]

From time zero to the peak (last measurable) concentration, the linear trapezoidal rule was used to determine the area under the plasma concentration-time curve (AUC_{last}). AUC_{inf} is obtained by adding the extrapolation area defined by AUC_{last} and C_{last}/K_{el}. Peak plasma concentration (C_{max}) and time to peak plasma concentration (T_{max}) are observed values, while other parameters such as half-life (HL) are evaluated and presented. Data are reported using MS-Excel to two decimal places or rounded to the nearest number where appropriate.[25-26,30]

In-vivo Efficacy (Disease model) Parameters

Body weight, clinical signs and mortality were recorded weekly once during disease induction phase (2 weeks, post cell injection) and daily during dosing period (5 weeks).[27-29,32-34,35]

RESULTS AND DISCUSSION

Pharmacokinetic Parameters

Drug suspension and formulation (SLN) were administered through oral route to mice. Dasatinib's mean maximum plasma concentrations C_{max} was 184.52 and 390.43 ng/mL, mean T_{max} was 2 and 4 hours, mean half-life was 4.63 and 8.06 hours following pure drug (suspension) and SLN formulation, respectively. Mean area under curve (AUC_{last}) was 1080.94 and 3669.49 hr*ng/mL for pure drug (suspension) and SLN formulation, respectively. (Table 1; Figures 1 and 2)

G4 (SLN formulation) showed very high C_{max} as compared to G3 (drug suspension), although time taken to achieve maximum concentration was double for G4 which makes it slow release with higher bioavailability. Total amount of drug reached in circulation was also found as significantly high (approximately 3 times).

In-vivo Efficacy (Disease model) Parameters

Body weight, clinical signs and mortality were recorded weekly once during disease induction phase (2 weeks, post cell injection) and daily during dosing period (5 weeks). Animals from disease control group (G2) reference drug treated group (G3, G4) showed Leukemic signs like weight loss, lethargy, and pilo-erection.

Mortalities were observed in all the treatment groups (G2-G4) as follows:

- Five mortalities from group G2 were observed on day 25 (n=1), 32 (n=1), 45 (n=1), 52 (n=1), 59 (n=1).
- Two mortalities from group G3 were observed on day 35 (n=1), 42 (n=1).
- Two mortalities from group G4 were observed on day 52 (n=1) and day 59 (n=1).

Disease control and treatment groups, i.e. G2, G3 (suspension), G4 (SLN formulation) showed body weight changes of -34.62% (n=5), -12.5% (n=5), -11.11% (n=5), respectively on day 21 (Figure 3).

G3 and G4 showed almost same change in percentage weight. Hence no benefit in terms of type of formulation observed for weight change.

Animals from treatment groups G3 and G4 showed survival benefits of 60%, respectively when compared to G2 disease control group with 0% survival benefit. Animals from treatment groups G3, G4 showed an increase in life span of -41.66 and -13.33%, respectively when compared to disease control group G2 which is -58.33%. Survival was compared statistically

Table 1: Pharmacokinetic parameters of dasatinib in plasma following Suspension and SLN formulation

Parameters	*Units*	*Suspension (G3)*	*SLN Formulation (G4)*
C_{max}	(ng/mL)	184.52	390.43
T_{max}	(hr)	2	4
$HL_{\_Lambda\_z}$	(hr)	4.63	8.06
AUC_{last}	(hr*ng/mL)	1080.94	3669.46
AUC_{INF_obs}	(hr*ng/mL)	1120.36	4205.05

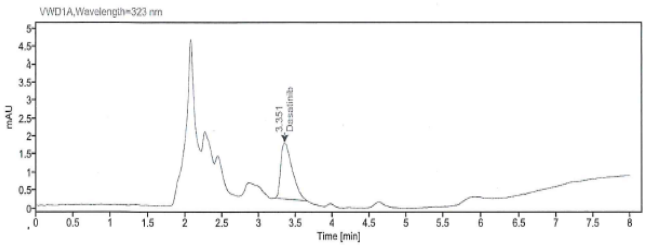

Figure 1: Chromatogram of dasatinb in HPLC

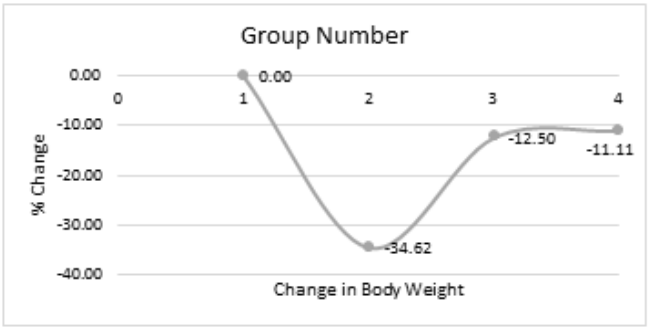

Figure 3: Variation in body weight in various groups

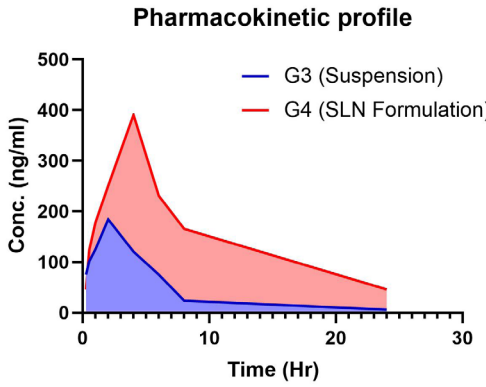

Figure 2: Concentration-time curve for dasatinib suspension and SLN formulation

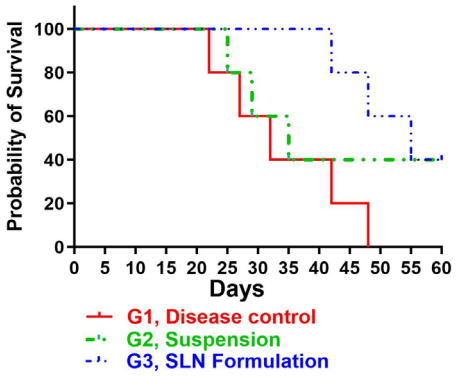

Figure 4: Kaplan-Meier survival analysis.

using Log-rank (Mantel-Cox) test as depicted by Kaplan-Meier survival analysis curve (Figure 4). SLN formulation showed significant survival as compared to dasatinib suspension.

CONCLUSION

SLN was formulated and evaluated *in-vivo* for its oral bio-availability for anti-leukemic activity.

In this study, the method was optimized to determine that the concentration of the drug dastinib in rat plasma is simple, rapid and accurate. This bioanalytical method was efficaciously used to evaluate the pharmacokinetic comparison between dasatinib suspension and SLN formulation in mice. Dasatinib significantly increased efficacy with the presence of SLN formulation. In conclusion of *in-vivo* efficacy data, the biological end points such as percent survival analysis, %increased life span, indicate that dasatinib in SLN formulation shows prolonged results as compare to suspension of pure dasatinib drug.

ACKNOWLEDGEMENTS

Authors acknowledge Dabur Research Foundation, Ghaziabad and Maharishi University of Information Technology, Lucknow, India for jointly providing opportunity to work at their facilities.

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