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Research Article

ENHANCEMENT OF ORAL BIOAVAILABILITY VIA SOLID LIPID NANOPARTICLES OF ANTICANCER DRUG DASATINIB - AN *IN VITRO* CYTOTOXICITY AND PHARMACOKINETIC STUDY

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ABSTRACT

Objective: Dasatinib (DST) is a Biopharmaceutics Classification System Class II drug having very low solubility and high permeability. Low aqueous solubility and poor dissolution of DST lead to poor bioavailability, Thus, limited aqueous solubility is the bottleneck for the therapeutic outcome of DST. Animal data suggest that the absolute bioavailability of DST is about 14–34% due to an extensive first-pass effect. To overcome hepatic first-pass metabolism and to enhance oral bioavailability, lipid-based drug delivery systems such as solid lipid nanoparticles (SLNs) can be used.

Methods: SLNs are submicron colloidal carriers having a size range of 50–1000 nm. These are prepared with physiological lipid and dispersed in water or aqueous surfactant solution. DST can be conveniently loaded into SLNs to improve the oral bioavailability by exploiting the intestinal lymphatic transport. An optimal system was evaluated for bioavailability study in rats compared with that of DST suspension (SUS).

Results: An *in vitro* cytotoxicity study was done by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay method through ATCC cell lines; the percent inhibition was more in SLN when compared with SUS. The pharmacokinetics of DST-SLNs after oral administration in male Wistar rats was studied. The bioavailability of DST was increased by 2.28 fold when compared with that of a DST SUS.

Conclusion: The results are indicative of SLNs as suitable lipid-based carrier system for improving the oral bioavailability of DST.

Keywords: Dasatinib, Solid lipid nanoparticles, *In vitro* cytotoxicity, Pharmacokinetic study.

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INTRODUCTION

The drugs with poor oral bioavailability are unable to reach the minimum effective concentration to exhibit therapeutic action. Some of the reasons for poor bioavailability include poor solubility, inappropriate partition coefficient as it influences the permeation of drug through lipid membrane, first-pass metabolism, P-glycoprotein mediated efflux, and degradation of drug in the gastrointestinal (GI) tract due to pH of the stomach or enzymatic degradation or by chemical [1].

Solid lipid nanoparticles (SLNs) are sub-micron colloidal carriers having a size range of 50–1000 nm. These are prepared with physiological lipid and dispersed in water or aqueous surfactant solution. SLNs were developed in the past decade as an alternative system to the existing traditional carriers, i.e., emulsions, liposomes, and polymeric nanoparticles [2,3]. These are related to emulsions, where the liquid lipid oil is substituted by a solid lipid. SLNs offer unique properties such as small size, large surface area, and high drug loading and are attractive for their potential to improve the performance of active pharmaceutical ingredients. The advantages of SLNs include drug targeting, biocompatibility, nontoxicity, drug release modulation, and small-scale production [4].

SLNs are also useful for the improvement of bioavailability of poorly water-soluble drugs, such as cyclosporine A [5], and to prolong the release of lipophilic drugs, such as camptothecin [6]. The mechanism proposed for enhancement of bioavailability of poorly water-soluble drugs by use of oral lipids includes promotion of lymphatic transport, which delivers drug directly to the systemic circulation while avoiding hepatic first-pass metabolism and by increasing GI membrane permeability [7,8]. It is known that drug delivery through SLNs improved the pharmacokinetic (PK) behavior. SLNs of dasatinib (DST) were not yet reported till now; hence, there is a need to improve the solubility and bioavailability through this delivery system.

MATERIALS

DST was obtained as gift sample from Aurobindo labs, Hyderabad. ATCC-Leukemia Cell Line Panel was obtained from Sigma-Aldrich, USA. Culture media contains Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin from Sigma-Aldrich, USA. Male Wistar rats were obtained from M/s. Sri Venketeswara Enterprises, Bangalore.

METHODS

In vitro cytotoxicity studies

Leukemia Cell Line Panel (ATCC® TCP-1010™) cells are used for the cytotoxicity of Dasatinib (DS)-SLNs. ATCC is a perpetual cell line consisting of human leukemia cells, derived from the genomic mutations in one or more of the following genes: CDKN2A, KDM6A, TP53, NRAS, NOTCH1, PTEN, FBXW7, FLT3, KRAS, MLH1, and PIK3R1. ATCC was cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a 5% CO_2 incubator. This cell line has been well characterized for its relevance to the toxicity models in human.

Cell culture and treatment

Cells were seeded at 2, 50, 000 cells/flask in a total volume of 10 mL. When confluent, cells were trypsinized and seeded in 96 well plates at the rate of 1.0×10^4 cells/0.1 mL. All the cell cultures were maintained in a 5% CO_2 incubator at 37°C. The control and SLNs were prepared as dispersions in different concentrations (10–100 µg/mL) in phosphate-buffered saline as a solvent with <1% polyethylene glycol as a stabilizer and used for different assays.

Cell viability assay

The effect of DST-SLNs on the cellular proliferation and viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay method. The procedure for this assay

is as follows: Briefly, the cells were seeded in 96 well plates and to it, SLN and control suspension (SUS) (100 µL, in the triplicate) in media were added. The microtiter plates were then incubated at 37°C for 48 h in 5% CO_2 incubator, and 20 µL of MTT (5 mg/mL) was added to each well. The plate was again incubated for 2 h, and dimethyl sulfoxide (80 µL) was added to each well and was wrapped in aluminum foil to prevent the oxidation of the dye. The plate was placed on a rotary shaker for 2 h for thorough mixing. The absorbance was recorded on the enzyme-linked immunosorbent assay reader at 321 nm. The absorbance of the test was compared with that of solvent control to get the cell viability [9,10].

***In vivo* bioavailability study**

Male Wistar rats were obtained from M/s. Sri Venketeswara Enterprises, Bangalore. The animals were quarantined in the animal house. Rats had free access to food and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee (P.COL/15/2018 IAEC.VMCP). Twelve male Wistar rats weighing 210–230 g were kept for overnight fasting with free access to water. The animals were divided into two groups (six animals per group), optimized DST-SLN formulation for one group and another one with DST-SUS was administered by oral route [11].

Oral administration

Two groups of rats were administered with their respective formulations using oral feeding needle based on body weight 10 mg/kg. For comparison, Dasatinib suspension was administered to the second group. Rats were anesthetized and blood samples (0.5 ml) were collected from the retro-orbital venous puncture at 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h after drug administration. The blood samples were centrifuged (5000 rpm, 15 min) and serum was collected and stored at −20°C until high-performance liquid chromatography (HPLC) analysis [12].

Serum drug analysis by HPLC

For the determination of Dasatinib in 100 µl of serum, add 25 µl of methanol and 25 µl of internal standard (Imatinib, 1 µg/ml) was added and vortexed for 2 min. The samples were made alkaline by the addition of 100 µl of 1N NaOH and vortexed for 3 min. To this sample, 750 µl of dichloromethane was added and vortexed for 5 min and then centrifuged at 4000 rpm for 15 min. The organic layer was separated and evaporated. The residue was reconstituted with mobile phase and a volume of 20 µl of sample was injected into HPLC (LC 10AT, Shimadzu, Japan) with ultraviolet detector at 321 nm (SPD 10A, Shimadzu, Japan) using Lichrospher C_{18} (250 mm × 4.6 mm, i. e., 5 µm particle size) analytical column for the analysis. Acetonitrile:water at a ratio 66:34 v/v was used as mobile phase with a flow rate of 1 ml/min. Retention time was 10.9 min for DS and 7.2 min for internal standard. The standard graph was plotted in the linearity range of 0.1–10 µg/ml concentration with r^2>0.995 [13].

Calculation of PK parameters and statistical significance

PK parameters such as C_{max}, T_{max}, area under the curve (AUC), $t_1/_2$, and mean residence time (MRT) were calculated using Kinetica software (version 5.0). The statistical significance of observed differences in C_{max}, AUC, $t_1/_2$, and MRT of different groups was assessed by ANOVA test using GraphPad Prism software. All the data were expressed as mean ±SD. p <0.05 was considered to be statistically significant [14,15].

RESULTS AND DISCUSSION

***In vitro* cytotoxicity studies**

MTT assay method was used for the determination of cytotoxicity of DST-SLNs in ATCC cell lines. In this method, the yellow tetrazolium salt was reduced by dehydrogenase enzymes present in mitochondria of metabolically active cells, to generate reducing equivalents, NADH, and NADPH. The formazan product has low aqueous solubility and was observable as purple crystals. The resulting formazan was dissolved using suitable detergent, permitted the convenient quantification of product formation. The intensity of the color of the product was measured at 321 nm. From the results, the *in vitro* cytotoxicity of DST-SLN and DST-SUS was dose dependent up to the concentration of 10–100 µg/mL. The percentage inhibition was more (89%) with SLN formulation when compared to SUS (42%) (Fig.1).

***In vivo*-bioavailability studies**

Oral bioavailability studies of DST-SLN and DST-SUS formulation were conducted in male albino Wistar rats. The serum concentration levels of DST-SLN and DST-SUS are represented in Tables 1-3. The PK parameters were calculated using Kinetica software and the profiles are represented in Tables 4-6 and Fig. 2. From the results, the peak plasma concentration and peak time concentration were doubled compared with DST-SUS formulation. This is the indication of prolonged DST levels in the serum. Further, the MRT of DST-SLN was

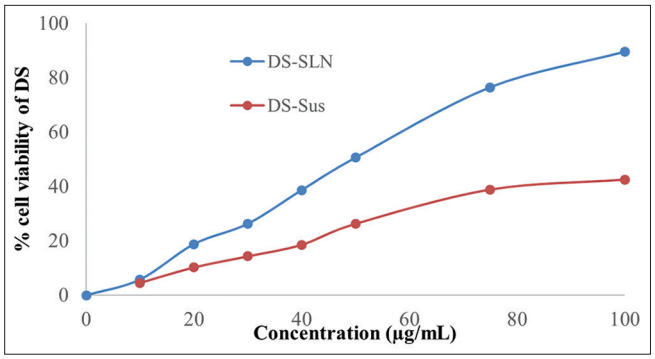

Fig. 1: *In vitro* cytotoxicity profiles of Dasatinib (DST)-Solid lipid nanoparticle and DST-Suspension in ATCC cell lines (mean±standard deviation, n=3)

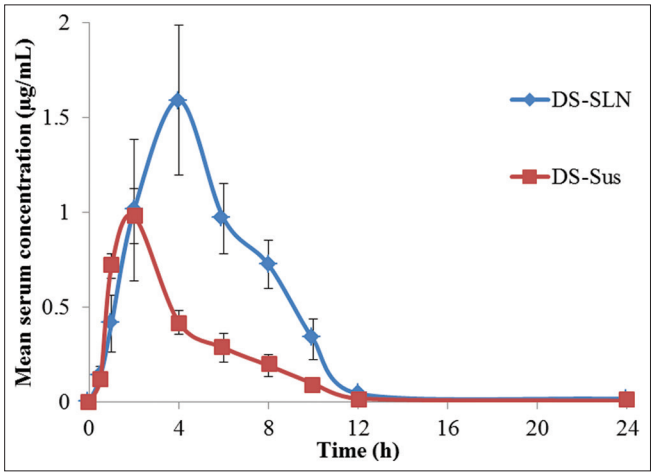

Fig. 2: Mean serum concentration-time profiles of Dasatinib (DST) from DST-Solid lipid nanoparticles and DST-Suspension in rats (Mean±standard deviation, n=6)

Table 1: Serum concentrations of DST-SLN in rats

Time (h)	Serum levels of DST (µg/mL)					
	R1	R2	R3	R4	R5	R6
0.5	0.09	0.11	0.24	0.13	0.13	0.1
1	0.26	0.38	0.604	0.53	0.47	0.23
2	0.82	1.1	1.62	1.12	0.88	0.51
4	2.06	1.16	1.66	1.33	1.28	2.05
6	0.91	1.02	0.86	0.79	0.91	1.31
8	0.85	0.71	0.68	0.52	0.71	0.87
10	0.48	0.33	0.29	0.23	0.43	0.21
12	0.03	0.04	0.06	0.04	0.05	0.04
24	0.01	0.02	0.01	0.01	0.03	0.01

DST: Dasatinib, SLN: Solid lipid nanoparticles

Table 2: Serum concentrations of Dasatinib-suspension in rats

Time (h)	Serum levels of DST (µg/mL)					
	R1	R2	R3	R4	R5	R6
0.5	0.09	0.08	0.17	0.12	0.14	0.11
1	0.76	0.65	0.81	0.69	0.73	0.65
2	1.01	1.21	0.85	0.91	0.83	1.06
4	0.38	0.42	0.35	0.47	0.38	0.51
6	0.28	0.33	0.21	0.29	0.2	0.4
8	0.18	0.26	0.18	0.14	0.13	0.26
10	0.1	0.08	0.12	0.09	0.05	0.14
12	0.02	0.02	0.01	0.02	0.015	0.02
24	0.007	0.005	0.007	0.006	0.001	0.005

DST: Dasatinib, DST-SUS: Dasatinib-suspension

Table 3: Mean serum concentration-time profiles of DST-SLN and DST-SUS in rats

Time (h)	DS-SLN	DST-SUS
	Mean±SD	Mean±SD
0	0±0	0±0
0.5	0.13±0.05	0.12±0.03
1	0.41±0.15	0.72±0.06
2	1.01±0.37	0.98±0.14
4	1.59±0.40	0.42±0.06
6	0.97±0.18	0.29±0.08
8	0.72±0.13	0.19±0.06
10	0.33±0.11	0.10±0.03
12	0.04±0.01	0.02±0.00
24	0.015±0.01	0.005166667±0.00

DST: Dasatinib, SLN: Solid lipid nanoparticles, DS-Dasatinib, SUS: Suspension, SD: Standard deviation

Table 4: PK parameters of DST-SLN in rats

Parameter	R1	R2	R3	R4	R5	R6
C-max (µg/ml)	2.06	1.16	1.66	1.336	1.288	2.05
t-max (h)	4	4	4	4	4	4
AUC_{total} (µg/ml) h	10	8.75	10.21	8.06	8.91	9.94
t1/2 (h)	3.39	3.83	3.08	3.19	4.29	3.52
MRT (h)	5.72	5.91	5.14	5.25	6.42	5.78

DST: Dasatinib, SLN: Solid lipid nanoparticles, MRT: Mean residence time, AUC: Area under the curve, PK: Pharmacokinetic

Table 5: PK parameters of DST-SUS in rats

Parameter	R1	R2	R3	R4	R5	R6
C-max (µg/ml)	1.01	1.21	0.854	0.91	0.83	1.06
t-max (h)	2	2	2	2	2	2
AUC_{total} (µg/ml) h	4.06	4.5	3.69	4	3.36	4.79
t1/2 (h)	3.36	3.43	3.42	3.47	2.53	3.38
MRT (h)	4.57	4.48	4.37	4.47	3.83	4.7

DST: Dasatinib, SUS: Suspension, MRT: Mean residence time AUC: Area under the curve, PK: Pharmacokinetic

Table 6: PK parameters of DST from DST-SLN and DST-SUS in rats (Mean±standard deviation, n=6)

Parameter	DST-SLN	DST-SUS
	Mean±SD	Mean±SD
C-max (µg/mL)	1.59±0.39	0.98±0.14
t-max (h)	4.00±0.00	2.00±0.00
AUC_{total} (µg/ml) h	9.31±0.86	4.07±0.52
t1/2 (h)	3.55±0.45	3.27±0.36
MRT (h)	5.70±0.47	4.40±0.30

DST: Dasatinib, SLN: Solid lipid nanoparticles, SUS: Suspension, MRT: Mean residence time, AUC: Area under the curve

almost more compared with DST-SUS, confirmed the increased residence time and it is responsible for sustained release behavior from the *in vitro* release studies also.

CONCLUSION

DST is a Biopharmaceutics Classification System Class II drug having very low solubility and high permeability. Low aqueous solubility and poor dissolution of DST lead to poor bioavailability, Thus, limited aqueous solubility is the bottleneck for the therapeutic outcome of DST. Animal data suggest that the absolute bioavailability of DST is about 14–34% due to an extensive first-pass effect. In the *in vitro* cytotoxicity study, the percentage inhibition was more with DST-SLN formulation when compared to DST-SUS. The indication of the enhanced oral bioavailability is evidenced from the AUC of the test and control formulation. DST-SLN and DST-SUS shows the AUC_{total} levels at 9.31±0.86 (µg/ml) h and 4.07±0.52 (µg/ml) h, respectively. From the AUC parameter, about 2.28-fold enhancement in the BA was observed in DST-SLN compared with DST-SUS, which is statistically significant at a level of $p<0.05$.

CONFLICT OF INTEREST

No.

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