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BIOEQUIVALENCE, SAFETY AND TOLERABILITY OF DASATINIB TABLETS 140 MG IN HEALTHY ADULT VOLUNTEERS UNDER FASTING CONDITIONS

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ABSTRACT

The objective of this study was to determine the comparative pharmacokinetics, safety and tolerability of test formulation Dasatinib tablets 140 mg with reference formulation Sprycel® 140 mg filmtabletten after single dose administration under fasting conditions in 24 healthy adult male and female volunteers. This was an open label, two periods, two treatments, two-way crossover, controlled, block randomized, bioequivalence study in male and female, healthy volunteers, under fasting conditions. Each subject received a Dasatinib tablets 140 mg of test and reference product in each period respectively. Blood samples were collected before dosing and at various time points up to 24 hours after drug administration. A total of 20 subjects received one dose of Dasatinib tablets 140 mg (TEST) or one dose of SPRYCEL® 140 mg Filmtabletten (REFERENCE) in two different occasions separated by a wash-out period of 16 days. Twenty eight (28) adverse events of mild and moderate intensity occurred in fourteen subjects in the study. These were not serious adverse events and the volunteers completely recovered before the end of the study. The statistical analysis of the pharmacokinetic data obtained in this study shows that the two Dasatinib formulations, Dasatinib tablets 140 mg of MSN Laboratories Pvt. Ltd., India – TEST and SPRYCEL® 140 mg Filmtabletten, formulation of BRISTOL-MYERS SQUIBB PHARMA EEIG, United Kingdom – REFERENCE are not bioequivalent, in fasting conditions. The Dasatinib Tablets 140 mg treatments (TEST and REFERENCE), administered in single dose, orally, to male and female healthy volunteers, in fasting conditions were well tolerated by all the subjects in this study.

INTRODUCTION

Dasatinib is a kinase inhibitor. The chemical name for dasatinib is N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, monohydrate. The molecular formula is $C_{22}H_{26}ClN_7O_2S$ •H2O, which corresponds to a formula weight of 506.02 (monohydrate). The anhydrous free base has a molecular weight of 488.01.

Dasatinib, at nanomolar concentrations, inhibits the following kinases: BCR-ABL, SRC family (SRC, LCK, YES, FYN), c-KIT, EPHA2, and PDGFRβ. Based on modeling studies, dasatinib is predicted to bind to multiple conformations of the ABL kinase. *In vitro,* dasatinib was active in leukemic cell lines representing variants of imatinib mesylate sensitive and resistant disease. Dasatinib inhibited the growth of chronic myeloid leukemia (CML)

and acute lymphoblastic leukemia (ALL) cell lines overexpressing BCR-ABL. Under the conditions of the assays, dasatinib was able to overcome imatinib resistance resulting from BCRABL kinase domain mutations, activation of alternate signaling pathways involving the SRC family kinases (LYN, HCK), and multidrug resistance gene overexpression. Dasatinib is indicated for the treatment of adult patients with:

• Newly diagnosed Philadelphia chromosome positive (Ph+) chronic myelogenous leukaemia (CML) in the chronic phase.
• Chronic, accelerated or blast phase CML with resistance or intolerance to prior therapy including imatinib mesilate.
• Ph+ acute lymphoblastic leukaemia (ALL) and lymphoid blast CML with resistance or intolerance to prior therapy.

Dasatinib is known as rapidly absorbed following oral administration, with peak concentrations between 0.5-3 hours. Following oral administration, the increase in the mean exposure (AUCτ) is approximately proportional to the dose increment across doses ranging from 25 mg to 120 mg twice daily. The overall mean terminal half-life of dasatinib is approximately 5-6 hours. Data from healthy subjects administered a single 100 mg dose of dasatinib 30 minutes after a high-fat meal indicated a 14% increase in the mean AUC of dasatinib. A low-fat meal 30 minutes prior to administration of dasatinib resulted in a 21% increase in the mean AUC of dasatinib. The observed food effects do not represent clinically relevant changes in exposure. In patients, dasatinib has a large apparent volume of distribution (2,505 l) suggesting that the medicinal product is extensively distributed in the extravascular space. At clinically relevant concentrations of dasatinib, binding to plasma proteins was approximately 96% based on in vitro experiments. Dasatinib is known as extensively metabolised in humans with multiple enzymes involved in the generation of the metabolites. In healthy subjects administered 100 mg of [14C]-labelled dasatinib, unchanged dasatinib represented 29% of circulating radioactivity in plasma. Plasma concentration and measured in vitro activity indicate that metabolites of dasatinib are unlikely to play a major role in the observed pharmacology of the product. CYP3A4 is a major enzyme responsible for the metabolism of dasatinib. Elimination of dasatinib is predominantly in the faeces, mostly as metabolites. Following a single oral dose of [14C]-labelled dasatinib, approximately 89% of the dose was eliminated within 10 days, with 4% and 85% of the radioactivity recovered in the urine and faeces, respectively. Unchanged dasatinib accounted for 0.1% and 19% of the dose in urine and faeces, respectively, with the remainder of the dose as metabolites [1,2]. This study was designed to assess comparative pharmacokinetics, safety and tolerability of newly developed oral test immediate release formulation against a registered and marketed reference product (Sprycel (Dasatinib) 140 mg Film Coated Tablets of BRISTOL-MYERS SQUIBB PHARMA EEIG, UK) in healthy, adult volunteers under fasting conditions according to EMA & ICH guidelines [3,4,5].

PARTICIPANTS AND METHODS

Study subjects

The study protocol was approved by the National Ethics Committee and the Medicines and Medical Devices Agency, Chisinau, The Moldavian Republic and conducted in compliance with the study protocol, the guideline for Good Clinical Practice (GCP), the Directive 2001/20/EC and the Declaration of Helsinki from 2013. Twenty four (24) healthy adult male and female volunteers were scheduled to participate in the present study under fasting conditions. Subjects underwent a screening procedure at least 21 days before the first day of dosing. All subjects were provided with a volunteer information leaflet and written informed consent to participate in the study was obtained from all subjects prior to enrolment. The subjects were free to withdraw their participation at any time during the entire study. Subjects were eligible to participate if they were 18 to 55 years old with a Body Mass Index (BMI) of 18.0 to 30.0 kg/m^2. Furthermore subjects needed to be healthy volunteers as per screening criteria with a willingness to sign the informed consent form and to adhere to protocol throughout the study. Into this study non-smoker or ex-smokers were enrolled only. Subjects needed to agree to abstain from alcohol 48 hours before initiation of study and during the study period. All enrolled subjects needed to agree to use acceptable contraceptive regimen from day 1 of the study until follow-up examination.

Subjects were excluded from this study based on history of hypersensitivity to the study drug, to any of the excipients of the formulations or to drugs belonging to the same pharmacological and chemical class. Furthermore intake of any prescribed or non-prescribed drug during the 28 days preceding the study or throughout the study led to non-enrolment of the subjects. Subjects with history or presence of any relevant medical condition including cancer, significant disease of the renal, hepatic, gastrointestinal, respiratory, cardiovascular, endocrine or locomotor systems and any metabolic, hematological or neurological disorder were excluded from this study. Subject with heart rate outside the normal range of 50-100 beats per minute or a body temperature outside the normal range of 35.5-37.4°C or a respiratory rate outside the normal range of 14-20 breaths per minute or a sitting blood pressure less than 90/60 mmHg or more than 140/90 mmHg at the screening examination were not included into this study. Furthermore ECG evidence of any clinically significant abnormalities, any recent history (within the last two years) of drug or alcohol abuse, recent psychiatric disorder or use of psychotropic medicines, or positive results to the HIV or hepatitis C or hepatitis B tests led to non-enrolment of the subjects. Breast feeding females or female subjects with a positive pregnancy test were excluded from enrolment.

Test formulation:

Dasatinib Tablets 140 mg (manufactured by MSN Laboratories Pvt Ltd, India).

Reference formulation:

SPRYCEL® 140 mg Filmtabletten (MAH: BRISTOL-MYERS SQUIBB PHARMA EEIG, UK).

Subject sample size determination

Subject sample size was based on estimates obtained from reported literature, assuming a true Test/Reference ratio between 95% and 105% and an intra-subject variability (CV%) of about 40%, with 5% significance level and power of the test of at least of 30%, bioequivalence limit between 80% and 125%. To account for subject withdrawal and dropouts due to adverse events, and non-compliance or personal reasons, 24 subjects were selected, randomized and enrolled into the study.

EXPERIMENTAL DESIGN

The study was designed as open-labeled, balanced, randomized, two- treatment, two-sequence, two-period, single dose, crossover comparative bioequivalence study between test formulation (Dasatinib Tablets 140 mg) and reference product (SPRYCEL® 140 mg Filmtabletten) with a wash-out period of at least seven (07) days in healthy, adult volunteers under fasting conditions. Subjects were housed in the clinical facility 11.00 hours before drug administration until 24.00 hours after drug administration in each period. Before enrollment, each volunteer was determined to be in good health basis of their vital signs, physical examination and laboratory test results including serum chemistries, complete blood count with differential, 12-lead electrocardiogram (ECG) and chest X-ray. The alcohol breath test, urine drug of abuse test and urine pregnancy test (for female subjects only) were performed on the day of admission of each period of the study. Out of thirty six (36) subjects screened, a total of twenty four (24) subjects were enrolled in the study. All subjects were randomized as per the block randomization schedule. All subjects were fasted overnight at least 10 hours before administration of study medication. Each subject received a single oral dose of test or reference products with 240 ml of still bottled water in each period as per randomization schedule and continued fasting for 4 hours after drug administration. Subjects were instructed not to chew, break or touch the study drug at the time of administration. Mouth check was performed using a flash light and tongue depressor to ensure that the dose was taken entirely. All subjects were dosed at the fixed time and were remained in semi-reclined position for the first 4 hours following drug administration.

Blood sample collection, Separation and Storage

Blood samples were collected at scheduled time points given in the protocol, before the dasatinib administration time 0.0 (5 ml+ reserve blank sample 20 mL) and at 0.167, 0.33, 0.5, 0.667, 0.83, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 14.0, 18.0 and 24.0 hours post dose (5 ml sample per each time point), after each administration. Blood sampling schedule was planned to provide an adequate estimation of Cmax and to cover the con-

centration-time curve long enough to provide a reliable estimate of extent of absorption. The collected blood samples were centrifuged at 3000 rpm for 10 minutes at 4°C. The plasma was separated in to test tubes and stored at -20°Cuntil analysis.

Method of sample analysis

The identification and quantification of dasatinib in plasma was performed by Liquid Chromatography/ Mass Spectrometry (HPLC/MS/MS) method. The analytical method was validated before the start of the analysis of the plasma samples. The analyst was kept blind in respect of the treatment assigned.

Pharmacokinetic variables

The following primary pharmacokinetic parameters were calculated for Dasatinib:

- AUC_{0-t}: area under the curve
- C_{max}: peak drug concentration

The following additional pharmacokinetic parameters were calculated for Dasatinib:

- T_{max}: time of the peak drug concentration
- AUC_{0-inf} (from time 0 to infinite): area under the curve integrated from the plasma
  concentrations extrapolating the terminal elimination period
- % extrapolated AUC: The ratio ($AUC_{0-inf} - AUC_{0-t}$)/ AUC_{0-inf} * 100
- $T_{1/2}$: plasma half life
- MRT: mean residence time

Pharmacokinetic parameters and their statistical analysis

The comparative statistics of the pharmacokinetic data was performed by comparing the TEST formulation vs. REFERENCE formulation in fasting conditions. All statistical calculations were performed using SAS version 9.4 software.

Descriptive statistics was done for all pharmacokinetic parameters (arithmetic mean, harmonic mean, geometric mean, SEM, standard deviation, median, range).

Bioequivalence comparison

The bioavailability comparison has been carried out on the Dasatinib pharmacokinetic parameters: C_{max} and AUC_{0-t} (primary pharmacokinetic parameters). The confidence interval for the ratio of the population means was calculated considering a classic 90% standard confidence interval. The two one-sided parametric T-tests according to Schuirmann (ln-transformed values) was applied with the null hypothesis of bioinequivalence at the 5% significance level. The bioequivalence acceptance interval was set to 80% - 125% for Dasatinib. In accordance with the guidelines of the European Committee for Proprietary Medicinal Products (CPMP) bioequivalence was concluded if the calculated 90% confidence interval of the ratios was completely within the accepted bioequivalence range.

Safety and tolerability assessment

The general clinical safety was assessed via medical history, clinical examination (physical and systemic examination), 12-lead ECG and vital signs (blood pressure, heart rate, respiratory rate and body temperature) and laboratory parameters were examined at screening. On the arrival at the study centre, before hospitalization, arterial blood pressure in sitting position after 5 minutes of rest (dominant arm), heart rate, body temperature and respiratory frequency have been measured. Based on the results of these tests the Investigator concluded if volunteer could have been hospitalized. In the morning before the study drug administration in both study periods subjects were asked about their well-being and possible intake of other medication. A pregnancy test was performed for all female subjects. Determination of hemoglobin, hematocrit, absolute neutrophil count and platelets was performed during the wash-out period between Period I and Period II in order to assess eventual signs of myelosuppression. During the study treatment days (Period I and II), blood pressure , heart rate and body temperature were checked at rest, before study drug administration and, only blood pressure and heart rate, at approximately 1, 2, 4, 12 and 24 hours post dose and whenever considered necessary. ECG determinations with QT and QTc interval measurement were also done before dose and at approximately 2, 4 and 8 hours post-dose. After administration, the subjects were requested to stay on their beds for the first eight (08) hours following dosing in a semi-reclined position only for the next four (04) hours following dosing and to stand up only if necessary.

Table 1:

N = 24	AGE (years)	WEIGHT (kg)	HEIGHT (cm)	BMI (kg/m2)
MEAN	31.21	70.83	167.50	25.3
SD	5.23	9.63	7.50	3.17
Minimum	21	54	155	19.3
Maximum	41	88	183	29.8

Figure 1:

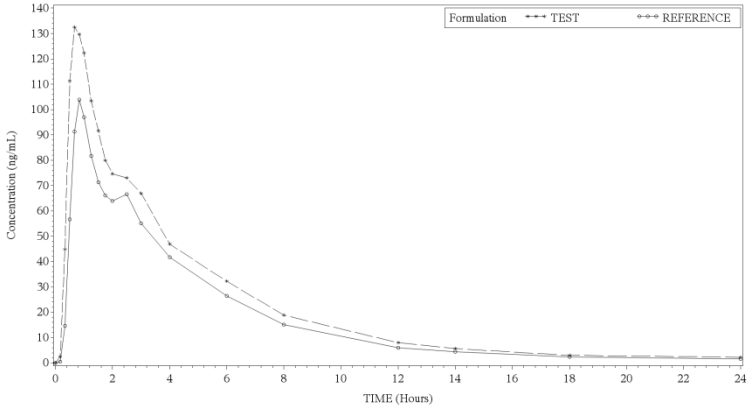

Table 2:

Adverse Events	Mild		Moderate		Total		Total
	R	NR	R	NR	R	NR	
Reference treatment							
Nervous system disorders							
Headache	-	-	6 (28.57%)	-	6 (28.57%)	-	6 (28.57%)
Dizziness	-	-	1 (4.76%)	-	1 (4.76%)	-	1 (4.76%)
Gastrointestinal disorders							
Nausea	1 (4.76%)	-	-	-	1 (4.76%)	-	1 (4.76%)
Vomiting	1 (4.76%)	-	-	-	1 (4.76%)	-	1 (4.76%)
Skin and subcutaneous tissue disorders							
Itching	-	-	1 (4.76%)	-	1 (4.76%)	-	1 (4.76%)
Erythema	-	-	1 (4.76%)	-	1 (4.76%)	-	1 (4.76%)
Test treatment							
Nervous system disorders							
Headache	2 (8.69%)	-	8(34.78%)	-	10 43.48%)	-	10 43.48%)
Drowsiness	1 (4.35%)	-	1 (4.35%)	-	2 (8.69%)	-	2 (8.69%)
Dizziness	-	-	1 (4.35%)	-	1 (4.35%)	-	1 (4.35%)
Gastrointestinal disorders							
Nausea	1 (4.35%)	-	-	-	1 (4.35%)	-	1 (4.35%)
Diarrhoea	-	-	1 (4.35%)	-	1 (4.35%)	-	1 (4.35%)
Vomiting	2 (8.69%)	-	-	-	2 (8.69%)	-	2 (8.69%)

If this was the case, they did this very slowly and under the supervision of one of the staff member. They were advised to refrain from vigorous, laborious exercise during the whole hospitalized period. The subjects were not allowed to develop sporty activities while at the study center. Throughout the study period, volunteers were questioned on adverse events occurrence. The second study period started after a washout period of sixteen (16) days, and the same procedures were followed as for the first study period. Within ten (10) days after the last treatment, subjects underwent a study exit examination that consisted of the same medical examinations as for the screening, except for hepatitis B, C and HIV tests, respiratory frequency, body temperature and for pregnancy test for female subjects.

RESULTS

Subjects participated in the study

Out of thirty six (36) subjects screened, a total of twenty four (24) subjects were enrolled in the study in healthy conditions. The demographic profiles of all twenty four (24) subjects participated in Dasatinib fasting study is shown in Table 1. A total twenty four (24) subjects were enrolled into the fasting study and twenty (20) subjects completed the full sequence of Test and Reference. Weight and height of the subjects were within the limit of the normal range according to normal values for the BMI ranges from 19.30 to 29.80 Kg/m^2. Age of the subjects was within range of 21 to 41 years. All enrolled subjects were abstained from alcohol consumptions 48 hours before initiation of study and during the study period. A total twenty four (24) subjects in period-I and twenty (20) subjects in period-II were dosed with either test or reference product as per the randomized schedule in the study.

Pharmacokinetic and statistical analysis

The pharmacokinetic and statistical parameters were analyzed from twenty (20) subject's data sets that completed both periods of the study. The ratio of geometric least square means for the (T/R) of AUC_{0-t} and C_{max} were 160.08% and 163.08% under fasting condition. The 90% confidence interval for the (T/R) of AUC_{0-t} and C_{max} were 114.29% - 224.24% and 108.85% - 244.36% under fasting condition.

The intra-subject variability for ln-transformed data of the AUC_{0-t} and C_{max} were 67.33 % and 84.44 %. The power of the test for the ln-transformed pharmacokinetic parameters AUC_{0-t} and C_{max} were found to be 29.46% and 23,46%. The result shows that the point estimate and upper 90% CIs ofAUC_{0-t} and C_{max} are not within the acceptable range in the study and not meeting the predetermined criteria for bioequivalence ranges of 80- 125% suggested by the EMA bioequivalence guideline. Graphical representation of mean concentration profile was given in Figure-1.

Safety and tolerability

A total of twenty eight (28) adverse events were reported in fourteen (14) subjects. Out of the occurred adverse events eight (08) were of mild intensity and twenty (20) were of moderate intensity. None of these adverse events were classified as serious adverse events and the volunteers completely recovered from all adverse events before the end of the study. From the total of fourteen (14) subjects having experienced adverse events after study drug administration, 71.43% of the subjects experienced adverse events (AEs) after the TEST treatment (10) and 42.86% of them experienced AEs after REFERENCE treatment (06). *Note: The overall sum of the percentages presented is 114.29% instead of 100.00% because there were two subjects who experienced AEs after both Test and Reference. From the total number of adverse events (28) that occurred in the present study after study drug administration, 60.71% were after the TEST treatment (17) and 39.28% after the REFERENCE treatment (11).

DISCUSSION

Present bioequivalence study was conducted to show bioequivalence between AET Dasatinib 140 mg tablets and BRISTOL-MYERS SQUIBB PHARMA EEIG SPRYCEL® 140 mg tablets in healthy volunteers. The results of this study demonstrate that the test product was not bioequivalent when compared with reference product. The intra-subject variability for primary pharmacokinetic parameters was high and the study was underpowered. Out of twenty four (24) subjects, twenty (20) subjects completed both the periods. Two subjects withdrawn due to vomiting, one subject withdrawn due to AE and one subject was absent for Period-II admission.

A total of twenty (20) subjects completed both the periods and included in pharmacokinetic and statistical analysis. The adverse events were mild to moderate in nature. No serious adverse events (SAE's) and deaths were observed. All occurred adverse events were resolved after follow-up.

CONCLUSION

The purpose of establishing proof of pharmacokinetic, safety and tolerability to reference product was mandatory for newly developed generic dasatinib tablets. The results of the present study suggest that the test product (Dasatinib Tablets 140 mg) was not bioequivalent to the reference product (SPRYCEL® 140 mg Filmtabletten) based on the rate and extent of absorption in fasting conditions. The reported adverse events were mild to moderate in nature and all resolved after follow up. The tested generic formulation of Dasatinib 140 mg has proven it's safety and tolerability in healthy volunteers. There are no serious adverse events and no deaths occurred in the study.

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