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PHARMACOLOGY

BIOEQUIVALENCE STUDY OF 400 AND 100 MG IMATINIB FILM-COATED TABLETS IN HEALTHY VOLUNTEERS

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Abstract: The aim of the study was to investigate the bioavailability of a generic product of 100 mg and 400 mg imatinib film-coated tablets (test) as compared to that of a branded product (reference) at the same strength to determine bioequivalence. The secondary objective of the study was to evaluate tolerability of both products. An open-label, randomized, crossover, two-period, single-dose, comparative study was conducted in 43 (Imatynib-Biofarm 100 mg film-coated tablet) and in 42 (Imatynib-Biofarm 400 mg film-coated tablet), brand name Imatenil, Caucasian healthy volunteers in fed conditions. A single oral dose administration of the test or reference product was separated by 14-day washout period. The imatinib and its metabolite N-desmethyl imatinib concentrations were determined using a validated LC MS/MS method. The results of the single-dose study in healthy volunteers indicated that the film-coated tablets of Imatynib-Biofarm 100 mg and 400 mg film-coated tablets manufactured by Biofarm Sp. z o.o. (test products) are bioequivalent to those of Glivec 100 mg and 400 mg film-coated tablets manufactured by Novartis Pharma GmbH (reference products). Both products in the two doses of imatinib were well tolerated.

Keywords: imatinib, bioequivalence, relative bioavailability, tolerability

Imatinib mesylate is potent revolutionary antineoplastic medication of high specificity (1–3). It functions at the molecular level inhibiting activity of particular tyrosine kinase enzymes, namely bcr-abl kinase, platelet-derived growth factor (PDGF) receptor and receptor for stem cell factor (c-kit receptor) at submicromolar concentrations. In consequence, the signal transduction *via* ligand-stimulated receptor autophosphorylation, inositol phosphate formation and mitogen-activated protein kinase (MAP kinase) activation is selectively inhibited with resultant cell proliferation arrest (4, 5). This abnormal enzyme is associated with the Philadelphia chromosome (Ph^+), which is a consequence of reciprocal translocation of genetic material between chromosomes 9 and 22 with generation of the chimeric BCR-ABL fusion gene. The highly elevated catalytic activity of the enzyme encoded by BCR-ABL fusion gene leads to a resistance to apoptosis, cell transformation and malignancy (including chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL)), and altered cell adhesion. Protein product of BCR-ABL fusion gene is present in 95% of CML and 10–25% of adult ALL cases. Imatinib mesylate competitively blocks the binding of ATP to the activation domain of bcr-abl, which modulates phosphorylation status of the kinase and diminishes its activity (3, 6). In consequence, imatinib mesylate suppresses the proliferation of bcr-abl+ cells (3). The efficacy of imatinib mesylate (STI571) in newly diagnosed chronic Ph^+ CML was evaluated in an open-label, multicentre, international phase III study referred as International Randomized Study of Interferon and STI571 (IRIS)

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(7–9). Moreover, given the literature data, combining imatinib mesylate with conventional chemotherapy for the treatment of newly diagnosed or minimally treated Ph^+ ALL may yield complete remission rates approaching 95%. The agent may be administered either concurrently or sequentially with chemotherapy (10).

Nowadays, it is known that certain other human tumors are considered to be associated with deregulation of c-kit receptor. These are gastrointestinal stromal tumors (GIST), germ cell tumors, mast cell tumors, small-cell lung cancers, melanoma, breast cancer, and neuroblastoma. Abnormal cell growth has been linked to mutation of c-kit, which leads to ligand-independent activation of the receptor (3, 5, 11–13). There are many studies from which it is well documented that imatinib mesylate has been shown to be highly effective in the treatment of unresectable and/or metastatic GIST, resulting in a substantial improvement in the survival rate. Imatinib has, therefore, become the standard of care in patients with advanced GIST (14–17).

Imatinib mesylate was also demonstrated to be effective in the treatment of adult patients with hypereosinophilic syndrome (HES) and/or chronic eosinophilic leukemia (CEL) who are Fip1-like1-platelet-derived growth factor receptor α chain (FIP1L1-PDGFRα) fusion kinase positive (18–21).

Imatinib mesylate has favorable pharmacokinetic profile. It is well absorbed form gastrointestinal tract, with bioavailability of almost 100% (22–24). At clinically relevant concentrations imatinib mesylate is highly bound to plasma protein (ca. 95%), primarily to a1-acid glycoprotein (22, 25). It undergoes a considerable metabolism in the liver, mainly *via* CYP3A4. The main metabolite, CGP 74588, is known to be active, and the elimination of this and other metabolites is more than 90% through the bile (22, 23). The elimination half-life of imatinib mesylate is approximately 18 h, with two- to three-fold accumulation at steady-state (22, 26).

The recommended dose of imatinib mesylate is 400 mg/day for adult patients in chronic phase CML and 100 mg/day with HES/CEL (27). The drug is a generally well tolerated drug, with chronic oral daily dosing.

The reference product in the present study was an already approved and commercially available Glivec (100, 400 mg), film-coated tablets (Novartis Pharma GmbH).

For the registration purposes, the efficacy and safety of this product has been proven already in clinical trials. This drug has therefore served as a reference and a basis for comparison to a imatinib mesylate test product: Imatynib-Biofarm (Imatenil). It is known that imatinib mesylate exists in two polymorphic forms α and β, which differ in their physicochemical properties i.e., melting points (t_{onset} 226°C and 217°C, respectively) or entalphy of thermal events (36). Moreover, the both polymorphs exhibit a tendency to generate amorphous form during some technological processes i.e., grinding (36). There are some suggestions that differences in the apparent solubilities of the various polymorphic forms can affect the drug bioavailability and bioequivalence (37). Since imatinib mesylate in the Imatynib-Biofarm (Imatenil) exists in α form only (38), whereas in Glivec the β form is postulated (39), therefore the differences in their bioavailability cannot be excluded. So, the aim of the study was to investigate the pharmacokinetic properties and the bioequivalence of imatinib from a new preparation: Imatynib-Biofarm 100 and 400 mg film-coated tablets Imatenil (Biofarm Sp. z o.o.) compared with the reference formulation Glivec (100, 400 mg), film-coated tablets (Novartis Pharma GmbH) following a single oral dose administration of 100 or 400 mg under fed conditions. Moreover, its active metabolite derivative – desmethyl imatinib (CGP74588) pharmacokinetics was planned to be evaluated as supportive information. The secondary objective of the study was to compare tolerability of the products.

EXPERIMENTAL

Subjects and Methods

Study products

The test products were: Imatynib-Biofarm 100 mg film-coated tablet (Imatenil) batch no: 030709 and Imatynib-Biofarm 400 mg film-coated tablet (Imatenil) batch no: 011210 manufactured by Biofarm Sp. z o.o. The reference product was Glivec (100 and 400 mg, film-coated tablets, batch no. S0041 and batch no. S0197, respectively, manufactured by Novartis Pharma GmbH.

Subjects

The study was performed as a single centre, open-labeled, randomized, two-period, 2-way crossover, single dose study under fed conditions with a washout period of 14 days between drug administrations in each treatment period. Subjects were selected according to the inclusion and exclusion criteria in order to obtain a low individual variability within the subject group. The demographic characteristics of the study population was shown in

Table 1. Healthy willing male and postmenopausal female subjects (male aged between 21 and 58 years and female aged between 42 and 58 years), able to communicate clearly with the study personnel and able to give written consent for participation in the study, who were non-smokers or no-users of tobacco products for at least ninety (90) days before screening, having body mass index (BMI) between 18.50 kg/m^2 and 26.99 kg/m^2 (the minimum body weight for males was not less than 60 kg, for females – not less than 50 kg), having no significant diseases (current or past), no clinically significant abnormal laboratory values or clinically significant abnormal results of 12-lead ECG, vital signs and chest X-ray, with physical examination without any clinically relevant abnormality were randomized and included to the study.

A single dose of test drug (Imatynib-Biofarm, 100 or 400 mg film-coated tablet) or a single dose of reference drug (Glivec, 100 or 400 mg film-coated tablet) were administered by the oral route for subsequent subjects in the morning on days 1 and 15 (treatment period I and II) in the sitting position, according to the randomization list and under open-label conditions. The tablet was administered, 30 min after standardized breakfast, with 240 mL of boiled water (at room temperature). No other food was allowed until 4 h after drug administration. No fluid intake apart from the fluid given at the time of drug intake was allowed from 2 h before until 2 h after dosing.

Study design

This study was prepared according to the Note for Guidance on the Investigation of Bioavailability and Bioequivalence, CPMP/EWP/QWP/1401/98 (28) and the Note for Guidance on the Investigation of Bioavailability and Bioequivalence, CPMP. EWP/QWP/1401/987/Rev 1 (29). The use of a generic preparation of a therapeutically well-established active drug principle has to be justified by an appropriate bioequivalence study, because the proof of bioequivalence of the test and reference products assures equal therapeutic efficacy. The study was conducted between January 2010 and June 2010 (for the dose of 100 mg) and December 2010 and April 2011 (for the dose of 400 mg) by Lambda Therapeutic Research Sp. z o.o., Centrum Badań Klinicznych NZOZ in Warszawa in compliance with the approved Protocols in adherence to Good Clinical Practices and Ethical Principles, as described in: ICH harmonized Tripartitate Guidelines for Good Clinical Practice and World Medical Association Declaration of Helsinki and its amendments (30, 31). Ethical approval was received from the Independent Ethics Committee (IEC) in Warszawa. The clinical trial registration numbers of the study were KB/693/09 for Imatynib-Biofarm 100 mg film-coated tablet and KB/733/10 for Imatynib-Biofarm 400 mg film-coated tablet. The Ministry of Health approval was obtained on January 21, 2010 for Imatynib-Biofarm 100 mg film-coated tablets (approval number CEBK/0034/10) for Imatynib-Biofarm 400 mg film-coated tablet was obtained on January 11, 2011 (approval number CEBK/0007/11). The studies were registered in EudraCT and obtained the numbers: EudraCT 2009-016180-10 and 2010-021028-91 for the dose of 100 mg and 400 mg, respectively. All eligible subjects provided written informed consent to participate and were free to withdraw from the study at any time without any obligation.

Blood sample collection

Blood samples for determination of imatinib and it's metabolite concentrations were collected up to 96 h after the drug administration in 19 time points in each treatment period.

Just before the first blood sampling (the morning of a day of drug administration), a cannula was introduced into a vein and blood samples were collected during the study by means of this cannual till pharmacokinetic blood sample at 72-h post dose in each treatment period. After the cannulation of the vein, a pre-dose PK sample of 7 mL was collected. Blood samples were obtained prior to dosing (baseline) and 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 5.0; 6.0; 8.0; 12.0; 16.0; 24.0; 36.0; 48.0; 72.0 and 96.0 h post-dose (19 samples per subject in each treatment period). Blood was collected from cubital vein or forearm vein or from veins into the tubes with anticoagulant. After collection, the blood samples were immediately cooled in an ice bath and then were centrifuged at 3000 ± 100 rcf or 4200 ± 100 rpm for 5 min below 10^{o}C to separate plasma. The separated plasma in the study for Imatynib-Biofarm 100 mg was transferred to pre-labelled polypropylene tubes in 2 aliquots (around 0.7 mL in case of pre dose sample and rest of the volume in second lot) and stored upright at a temperature –55^{o}C or colder for interim storage until shipment to bioanalytical laboratory, for analysis.

The separated plasma in the study for Imatynib-Biofarm 400 mg was transferred to pre-labelled polypropylene tubes in 2 aliquots (around 1.25 mL in first lot, 1.75 mL in case of pre dose sample and rest of the volume in second lot) and stored upright at a temperature –55^{o}C or colder for

interim storage until shipment to bioanalytical laboratory, for analysis.

Determination of imatinib and its metabolite N-desmethyl imatinib plasma concentrations

Plasma samples of subjects, were assayed for imatinib and its metabolite N-desmethyl imatinib (CGP74588) using a precise and accurate LC-MS/MS method (ACQUITY UPLC BSM binary solvent manager, Acquity UPLC SM sample manager with column heater and Waters Quattro Premier XE mass spectrometer) with imatinib-d_8 & N-desmethyl imatinib-d_8 as the internal standards (ISTD), which is validated according to the international guidelines at Lambda Therapeutic Ltd., UK. The method has been developed and validated for calibration curve ranging from 5.012 to 2999.700 ng/mL for imatinib and from 1.999 to 400.660 ng/mL for N-desmethyl imatinib. Briefly, the analytes and internal standards were extracted from plasma using liquid-liquid extraction method. It means that the frozen calibration curve standards, quality control samples and the study samples were thawed in a water bath maintained at room temperature and vortexed to ensure complete mixing of contents. Two hundred fifty microliters of each of the calibration curve standards, quality control samples and the subject samples were aliquoted into pre-labeled tubes. Fifty microliters of the ISTD dilution mixture (about 200 ng/mL of imatinib-d_8 and about 375 ng/mL of N-desmethyl imatinib-d_8) was added to each tube except for standard blank, Subject blank and blank QC tubes and vortexed for 1 min. Thereafter, 100 µL of 100 mM ammonium formate buffer (pH 7.0) was added to each tube and vortexed for 1 min followed by the addition of 4 mL of extraction solution and vortexed for 10 min. The samples were centrifuged at 3345 ± 150 rcf for 5 min at 10°C using imatinib-d_8 and N-desmethyl imatinib-d_8 as the internal standards. The plasma layer was flash-frozen in alcohol freezing bath and organic layer was transferred into pre-labeled tubes. The contents were evaporated to dryness at room temperature under nitrogen stream and reconstituted with 250 µL of the reconstitution solution and vortexed. Next, the contents were transferred into appropriate autosampler vials for analysis. Ten microliters of each sample was chromatographed on Hypersil GOLD, 150 × 4.6 mm, 5 µm column maintained at 40°C using an binary mode of mobile phase system composed of 70% of acetonitrile and 30% of 2 mM ammonium formate buffer (pH 3.5). Imatinib and ISTD-1 and N-desmethyl imatinib and ISTD-2 were monitored in the positive ion mode using the MRM transitions (m/z 494.22 > 394.10 for imatinib, m/z 502.00 > 394.10 for ISTD-1, m/z 480.20 > 394.10 for N-desmethyl imatinib, m/z 488.44 > 394.15 for ISTD-2) and retention times (imatinib and ISTD-1 – 1.74 min; N-desmethyl imatinib and ISTD-2 – 1.70 min). MassLynx Software Version 4.1 was used for the evaluation of chromatographic data. A linear equation was judged to produce the best fit for the concentration *vs.* area response relationship. The regression type was 1/concentration and peak area ratio for an 8-point calibration curve was found to be linear from 5.012 (lower limit of quantification) to 2999.700 ng/mL for imatinib and from 1.999 (lower limit of quantification) to 400.660 ng/mL for N-desmethyl imatinib with correlation coefficient (r) greater than 0.99 for imatinib and N-desmethyl imatinib during the course of validation. For imatinib, the range of precision and accuracy of the back-calculated concentrations of the standard curve points was from

Table 1. The demographic characteristics of the study population.

Parameter (units)	Imatynib-Biofarm, 100 mg and Glivec, 100 mg		Imatynib-Biofarm, 400 mg and Glivec, 400 mg	
	Mean ± SD			
	n = 43# (Subjects who were enrolled into the study)	n = 37 (Subjects who completed all phases of the study)	n = 42* (Subjects who were enrolled into the study)	n = 37 (Subjects who completed all phases of the study
Age (years)	32.1 ± 9.87	32.4 ± 9.84	33.9 ± 11,78	34.1 ± 11.59
Height (cm)	177.1 ± 7.62	177.4 ± 7.12	177.1 ± 8.70	177.4 ± 9.11
Weight (kg)	74.2 ± 8.10	75.0 ± 7.63	75.6 ± 7.28	75.6 ± 7.16
BMI (kg/m^2)	23.63 ± 1.89	23.83 ± 1.84	24.2 ± 1.79	24.1 ± 1.86

Arithmetic mean ± SD. # – 4 females; * – 4 females

1.0 to 5.5% and from 97.0 to 102.7%, respectively, whereas for N-desmethyl imatinib these parameters were from 1.9 to 4.2% and from 96.5 to 102.2%, respectively. Precision and accuracy of imatinib and N-desmethyl imatinib was determined for limit of quantification, low, medium and high concentrations of quality control samples in the biological matrix, based on the expected range. Accuracy (% nominal) for inter-day and intra-day was within 85–115% of the nominal value for all quality control samples except for LOQ QC, which was within 80–120%. For precision, the % CV was ≥ 15% for all quality control samples, except for LOQ QC, which was ≥ 20%.

Pharmacokinetic and statistical analyses

The pharmacokinetic parameters for imatinib and its metabolite N-desmethyl imatinib were determined from the plasma concentration *vs.* time curve with the aid of the WinNonlin Professional Software version 5.3. The parameters selected as primary endpoints of the study were: the area under the plasma concentration *vs.* time curve (AUC_{0-t}), and the maximum plasma concentration of the drug (C_{max}). The time to reach maximum plasma concentration of the drug (T_{max}), the elimination half-life ($t_{1/2}$) and lz as a first order rate constant associated with the terminal (log-linear) portion of the curve. C_{max} and T_{max} were obtained directly from the experimental data. The elimination rate constant (lz) was estimated by linear least squares regression analysis using at least last three or more non-zero plasma concentration values. The $t_{1/2}$ was calculated as ln 2/lz. The AUC_{0-t} was calculated as the area under the plasma concentration *versus* time curve from time zero to the last measurable concentration as calculated by linear trapezoidal method. The $AUC_{0-\infty}$ was calculated where $AUC_{0-\infty} = AUC_{0-t} + C_t/z$, where C_t is the last measurable concentration and z is the terminal elimination rate constant. For metabolite N-desmethyl imatinib: $AUC_{0-t} = AUC_{0-96}$ (truncated at 96 h). The residual area in percentage (AUC_%Extrap_obs) was determined by the formula $[(AUC_{0-\infty} - AUC_{0-t})/ AUC_{0-\infty}] \times 100$. The statistical calculations were performed using the SAS (Version 9.2 or higher) software. The tests for normality of ln-transformed pharmacokinetic parameters were performed with proper tests. The analysis of variance (ANOVA) was performed on the ln-transformed data. The statistical significance of effects was determined on basis of the calculated p-values with value larger than 0.05 meaning no statistical significance.

Based on the ANOVA results, 90% confidence interval (CI) for the μT/μR (ratio of geometric means for the test and the reference product) of the analyzed pharmacokinetic parameters was constructed. Bioequivalence was assumed when 90%

Table 2. Plasma pharmacokinetic parameters of imitanib after a single dose administration of the Imatynib-Biofarm 100 mg (test product) and the Glivec 100 mg (reference product).

Imatynib-Biofarm 100 mg (test product)					
Pharmacokinetic parameter	Arithmetic mean	Median	Geometric mean	SD	CV [%]
T_{max} (h)	2.9	2.5	2.5	1.5	51.7
C_{max} (ng/mL)	427	428	405	149	35.0
AUC_{0-t} (ng × h/mL)	6582	6510	6252	2169	32.9
$AUC_{0-\infty}$ (ng × h/mL)	67009	6558	6365	2244	33.4
λz (1/h)	0.041	0.040	0.041	0.0083	20.1
$t_{1/2}$ (h)	17.4	17.5	17.066	3.2	18.7
AUC_%Extrap_obs (%)	2.036	1.690	1.831	1.04	51.1
Glivec 100 mg (reference product)					
T_{max} (h)	3.2	3.0	2.8	1.5	47.8
C_{max} (ng / mL)	410	370	388	146	35.6
AUC_{0-t} (ng × h / mL)	6418	5999	6012	2344	36.5
$AUC_{0-\infty}$ (ng × h / mL)	6554	6104	6126	2440	37.2
λz (1 / h)	0.041	0.041	0.040	0.0088	21.3
$t_{1/2}$ (h)	17.5	16.9	17.2	3.8	22.0
AUC_%Extrap_obs (%)	2.141	1.715	1.871	1.45	67.7

Table 3. Plasma pharmacokinetic parameters of N-desmethyl imatinib after a single dose administration of the Imatynib-Biofarm 100 mg (test product) and the Glivec 100 mg (reference product).

Imatynib-Biofarm 100 mg (test product)					
Pharmacokinetic parameter	Arithmetic mean	Median	Geometric mean	SD	CV [%]
T_{max} (h)	3.2	3.0	2.7	1.8	57.0
C_{max} (ng/mL)	40.6	37.1	38.4	14.9	36.6
AUC_{0-t} (ng × h/mL)	937	861	898	282	30.1
$AUC_{0-\infty}$ (ng × h/mL)	1136	1016	1088	355	31.3
λz (1/h)	0.017	0.017	0.017	0.0035	20.6
$t_{1/2}$ (h)	17.3	16.0	16.6	5.2	30.0
AUC_%Extrap_obs (%)	17.3	16.0	16.6	5.2	30.0
Glivec 100 mg (reference product)					
T_{max} (h)	3.3	3.0	2.9	1.7	52.1
C_{max} (ng / mL)	39.0	38.2	37	12.8	32.9
AUC_{0-t} (ng × h / mL)	9434	833	889	335	35.5
$AUC_{0-\infty}$ (ng × h / mL)	1153	1018	1084	418	36.2
λz (1 / h)	0.016	0.016	0.016	0.0033	20.0
$t_{1/2}$ (h)	44.0	44.0	43.0	10.2	23.2
AUC_%Extrap_obs (%)	17.8	16.5	17.1	5.6	31.3

CI of the point estimate (test over reference products) for AUC_{0-t}, $AUC_{0-\neq}$ and for C_{max} falls within the 80.00–125.00% range and when the Schuirmannis TOST test (two one-sided *t*-test) was complied ($p < 0.05$) (28, 29, 32, 33). The statistical analysis for T_{max} was performed on the untransformed data using the non-parametric Wilcoxon test.

Tolerability/safety analysis

In order to prevent the occurrence of an adverse events during the study, the following measures have been taken: the drug administration was limited to a single oral dose of 100 or 400 mg/study period; only healthy adult volunteers with no history of hypersensitivity reactions to the drug or other related molecules were enrolled; the investigator has checked each volunteer's well being prior to his/her discharge from the clinic. Tolerability and safety were determined by monitoring vital signs (blood pressure, heart rate, body temperature) at baseline and at the end of each period. Laboratory results (hematology, urinalysis, blood biochemistry) collected before and after the study of all the subjects were also considered. The participants were interviewed by the physician as well as nonspecific questioning. All the subjects were advised to report any adverse event or undesirable sign or symptom at any time during the study period.

RESULTS

Study population

The study was conducted in 43 (Imatynib-Biofarm 100 mg, film coated tablet) and in 42 (Imatynib-Biofarm 400 mg, film coated- tablet) Caucasian non-smoking healthy male and female subjects. In each studies 37 subjects completed all phases of the study. The results of the physical examination for all subjects during the pre-study visit and post-study visit were found to be normal. No subject abandoned the study for any reason. The summary of the demographic data of the population is presented in Table 1. The clinical part of the study with 100 mg of Imatynib-Biofarm was completed without deaths, serious adverse events and suspected unexpected serious adverse reactions. During the study, a total number of adverse events was 35. During the whole study period, 35 non-serious adverse events were reported in 18 subjects. No deaths or serious adverse events were reported during the study. Twelve adverse events occurred in treatment period I, 1 adverse event in wash-out period, 15 in treatment period II and 7 adverse events occurred during the follow-up examination. Twenty adverse events were of mild intensity and 15 of moderate. There was 1 adverse event classified as significant adverse event – allergic reaction. Moreover, there were 6 adverse

events in post-study laboratory values estimated as clinically relevant abnormalities. One adverse event was related to the study drug, 20 adverse events were possible related and 14 were not related to the study drug. All adverse events were resolved.

In the study for Imatynib-Biofarm 400 mg dose, the 43 non-serious adverse events were reported in 21 subjects for both product (test and reference). No death or serious adverse events were reported during the study. Twenty three adverse events occurred in treatment period I, 2 adverse events in wash-out period, 8 in treatment period II and 10 adverse events occurred during the follow-up examination. Thirty one adverse events were of mild nature, 11 moderate and 1 severe. Twenty nine adverse events were assessed as possibly related to the study drug and 14 as not related to the study drug. All adverse events were resolved. The adverse events were: ALT increased (4 events), vascular access complication (4 events), blood glucose increased (3 events), blood triglycerides increased (3 events), constipation (3 events), AST increased (2 events), CRF increased (2 events), weakness (2 events), abnormal body temperature (1 event), back pain (1 event), bad feeling (1 event), blood cholesterol increased (1 event), blood pressure increased (1 event), cough (1 event), diarrhea (1 event), dry skin (1 event), faint (1 event), headache (1 event), loose stools (1 event), muscle pain (1 event), nausea (1 event), pain of orchis (1 event), proteinuria (1 event), rhinitis (1 event), sleep disturbances (1 event), WBC abnormal (1 event), wind (1 event) and vertigo (1 event).

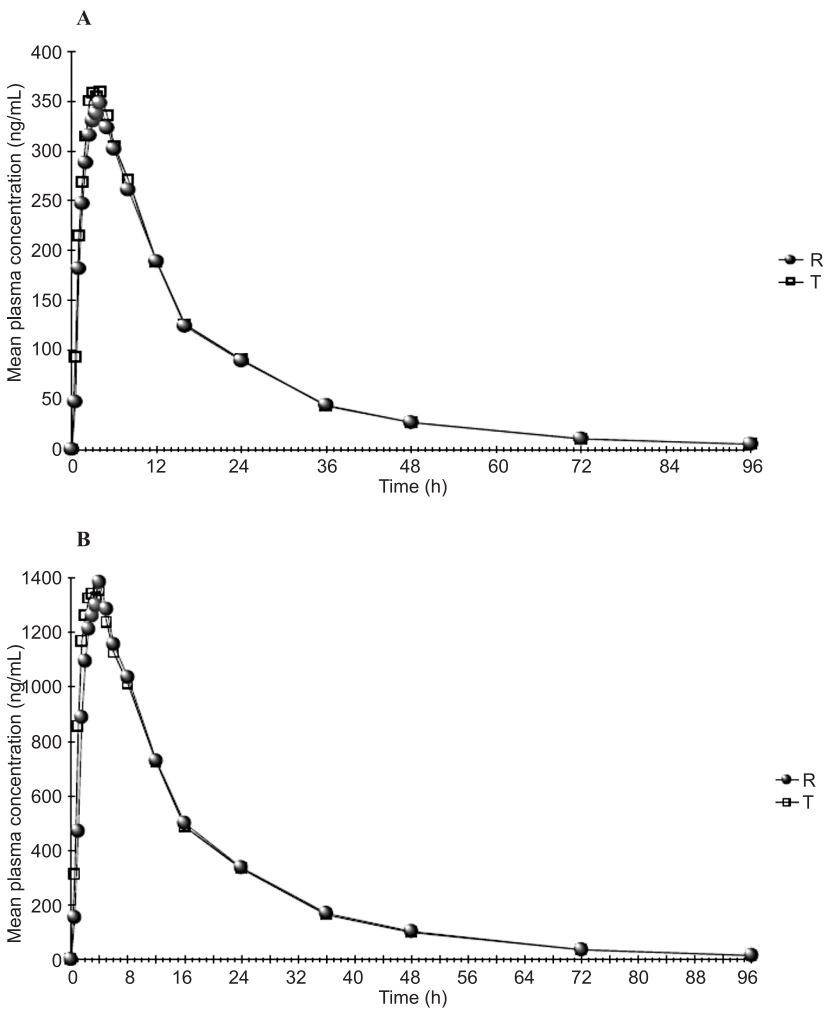

Figure 1. Linear plot of mean plasma concentration of imatinib *versus* time curves after administration of reference product- R (Glivec) and test product-T (Imatynib-Biofarm) under fed conditions in healthy volunteers. **A**: Glivec – 100 mg, Imatynib-Biofaam – 100 mg; **B**: Glivec – 400 mg, Imatynib-Biofarm – 400 mg

Pharmacokinetics and bioequivalence analysis

The mean plasma concentrations *vs*. time profiles after a single oral administration of both products with imatinib in two doses are shown in Figure 1, whereas its metabolite, N-desmethyl imatinib, in Figure 2. The descriptive statistics of pharmacokinetic parameters is shown in Tables 2, 4 for imatinib and in Tables 3, 5 for metabolite – N-desmethyl imatinib, respectively. It appeared that one cannot reject the hypothesis on the ln-normal distribution of the AUC_{0-t}, $AUC_{0-\infty}$, AUC_%Extrap _obs, C_{max}, $t_{1/2}$ and lz for both test and reference products at the significance level $\alpha = 0.05$. The $T_{max(s)}$ distribution values were significantly different from the normal distribution; therefore, in the subsequent analysis nonparametric tests were used for the evaluation. All primary pharmacokinetic parameters, i.e., AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}, met the bioequivalence regulatory criteria and there were no statistical differences between proper values of the pharmacokinetic parameters (Tables 6, 7). The descriptive statistics for /λz and $t_{1/2}$ were similar for the test and reference products and there were no statistical differences between proper values of these pharmacokinetic parameters (Tables 2–5). The descriptive statistics of T_{max} of imatinib were similar for the test and reference products (Tables 2, 4), but lower variability in T_{max} was observed for the test product (CV = 47.8% (100 mg) and 39.6% (400 mg)) than for the reference product (CV = 51.7% (100 mg) and 58.6% (400 mg)), respectively. However, since p-value for Wilcoxon-Signed-Rank Test was $p > 0.05$, therefore

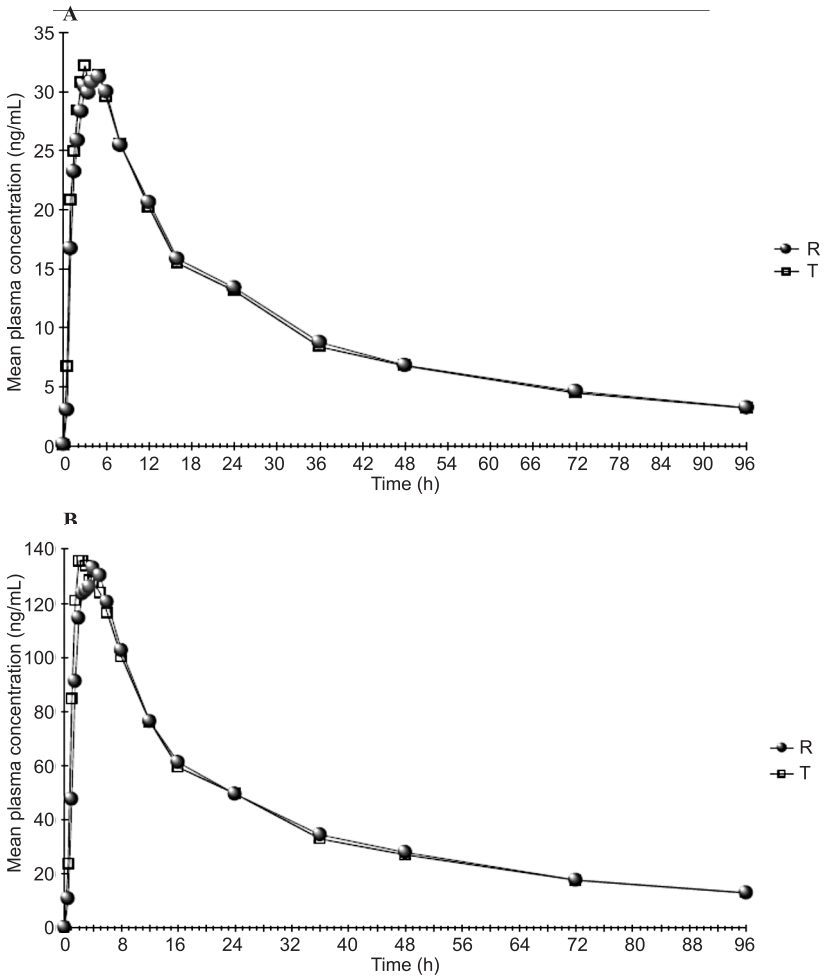

Figure 2. Linear plot of mean plasma concentration of N-desmethyl imatinib *versus* time curves after administration of reference product – R (Glivec) and test product –T (Imatynib-Biofarm) under fed conditions in healthy volunteers. **A**: Glivec – 100 mg, Imatynib-Biofarm – 100 mg; **B**: Glivec – 400 mg, Imatynib-Biofarm – 400 mg

the null hypothesis was not rejected and led to conclude that the difference between the two treatments R and T (both for 100 and 400 mg) with respect to pharmacokinetic parameter T_{max} was not significant. Similarly, there were not significant differences between T_{max} of N-desmethyl imatinib for test and reference products both in the dose of 100 and 400 mg (Tables 3, 5).

Tolerability/safety

Both products in two doses were well tolerated. Thirty seven subjects completed the study without any significant serious adverse events. No clinically significant abnormalities on physical examination including vital signs measurement, ECG recordings and laboratory results were observed.

DISCUSSION

The aim of the study was to evaluate the bioequivalence between the test (Imatynib-Biofarm manufactured by Biofarm Sp. z o.o.) and the reference (Glivec manufactured by Novartis Pharma GmbH) products. The clinical part of the study was designed in compliance with the respective EMA guidelines (28, 29). Based on the imatinib elimination half-life of 18 h (22, 26), and taking into account imatinib metabolite long half-life (about 40 h) (34) and that to avoid carryover effect in second study period washout period should be at least 5 times of the half-life long, wash-out period of 14 days was chosen in that study**.** For all profiles, the AUC_{0-t} was at least 80% of the AUC_{0-8}, which confirmed the proper duration of sampling. The current study demonstrated comparable bioavailability of Imatynib-Biofarm 100 and 400 mg film-coated tablets with Glivec 100 and 400 mg film-coated tablets, respectively. The pharmacokinetic values (C_{max}, T_{max}, and AUC) for N-desmethyl imatinib were also found to be nearly identical for both the test and reference dose forms of imatinib. If the 90% confidence interval of two one-sided tests for ratio of geometric means (test formulation Imatynib-Biofarm over reference formulation Glivec) for both C_{max} and AUC_{0-t} of imatinib were included entirely in the acceptance range of 80.00–125.00%, the test formulation (Imatynib-Biofarm) was assumed as bioequivalent to the reference formulation (Glivec). The similar pharmacokinetic property of the metabolite further supports the bioequivalence of the tablet product. This study indicated relatively rapid absorption of the 100 and 400 mg tablet (median T_{max} 2.5 and 2.5 h, respectively) after oral administration that was comparable with that of Glivec tablets (T_{max} 3.0 and 4.0, respectively). The coefficient for varia-

Table 4. Plasma pharmacokinetic parameters of imitanib after a single dose administration of the Imatynib-Biofarm 400 mg (test product) and the Glivec 400 mg (reference product).

Imatynib-Biofarm 400 mg (test product)					
Pharmacokinetic parameter	Arithmetic mean	Median	Geometric mean	SD	CV [%]
T_{max} (h)	2.9	2.5	2.5	1.7	58.6
C_{max} (ng/mL)	15477	1549	1452	579	37.4
AUC_{0-t} (ng × h/mL)	24705	22562	23295	8902	36.0
$AUC_{0-\infty}$ (ng × h/mL)	25065	22808	23640.	9005	35.9
λz (1/h)	0.043	0.043	0.043	0.0065	15.1
$t_{1/2}$ (h)	16.4	16.0	16.2	2.6	15.9
AUC_%Extrap_obs (%)	1.45	1.14	1.27	0.86	59.0
Glivec 400 mg (reference product)					
T_{max} (h)	3.5	4.0	3.2	1.38	39.6
C_{max} (ng / mL)	1506	1439	1443	459	30.5
AUC_{0-t} (ng × h / mL)	24545	24149	23437	778	31.7
$AUC_{0-\infty}$ (ng × h / mL)	24913	24304	23772	7957	31.9
λz (1 / h)	0.044	0.044	0.044	0.007	15.6
$t_{1/2}$ (h)	15.9	15.9	15.8	2.44	15.3
AUC_%Extrap_obs (%)	1.41	1.20	1.19	0.88	62.7

Table 5. Plasma pharmacokinetic parameters of N-desmethyl imatinib after a single dose ad-ministration of the Imatynib-Biofarm 400 mg (test product) and the Glivec 400 mg (reference product).

Imatynib-Biofarm 400 mg (test product)					
Pharmacokinetic parameter	Arithmetic mean	Median	Geometric mean	SD	CV [%]
T_{max} (h)	2.6	2.0	2.3	1.7	61.7
C_{max} (ng/mL)	168	151	160	55	32.8
AUC_{0-t} (ng × h/mL)	3698	3477	3476	1333	36.0
$AUC_{0-\infty}$ (ng × h/mL)	4561	4318	4273	1691	37.1
λz (1/h)	0.017	0.017	0.016	0.0036	21.6
$t_{1/2}$ (h)	43.9	40.8	42.6	11.8220	26.9
AUC_%Extrap_obs (%)	18.4	16.7	17.4	6.5798	35.8
Glivec 400 mg (reference product)					
T_{max} (h)	3.2	2.5	2.9	1.7	53.8
C_{max} (ng / mL)	162	150	153	55	34.0
AUC_{0-t} (ng × h / mL)	3705	3796	3447	1446	39.0
$AUC_{0-\infty}$ (ng × h / mL)	4481	4311	4151	1852	41.3
λz (1 / h)	0.018	0.018	0.017	0.0030	16.7
$t_{1/2}$ (h)	40.3	39.4	39.7	7.1	17.7
AUC_%Extrap_obs (%)	16.8	16.1	16.2	4.8	28.8

Table 6. The 90% confidence intervals based on the Schuirmannis TOST test and using mean square error estimated from ANOVA analysis of pharmacokinetic parameters for imatinib.

100 mg			
Pharmacokinetic parameter	Point estimate [%]	90% confidence interval [%]	Estimated intrasubject CV [%]
AUC_{0-t}	104.2	99.3–109.2	12.1
$AUC_{0-\infty}$	104.1	99.2–109.1	12.2
C_{max}	104.6	98.4–111.2	15.7
400 mg			
AUC_{0-t}	99.6	94.8–104.7	12.7
$AUC_{0-\infty}$	99.7	94.8–104.8	12.7
C_{max}	100.7	94.8–107.0	15.5

tion for T_{max}, C_{max} and AUCs showed considerable intersubject variability (up to 15.7%). Nikolova et al. (34) reported that the intra subject variability for C_{max} was on the level of 20%. Although the cause of this was not clear, it is below observed in the above mentioned study and may be attributed to intersubject differences in plasma proteins binding to the parent compound or to variations in CYP3A4, the major CYP isoenzyme involved in the microsomal metabolism of imatinib. It is known that variability in CYP3A activity between individuals is large (35) and may in part have contributed to the large intersubject variability.

There were no serious adverse events reported during the conduct of the trials. Only 1 adverse event was assessed as significant. Based on the clinical results for the parent drug, the study clearly demonstrated that new formulations of imatinib (Imatynib-Biofarm 100 and 400 mg film-coated tablet) were tolerated in the same way as reference drug (Glivec 100 and 400 mg film-coated tablet).

Table 7. The 90% confidence intervals based on the Schuirmannis TOST test and using mean square error estimated from ANOVA analysis of pharmacokinetic parameters for N-desmethyl imatinib.

100 mg			
Pharmacokinetic parameter	Point estimate [%]	90% confidence interval [%]	Estimated intrasubject CV [%]
AUC_{0-t}	101.2	96.9-105.6	10.9
$AUC_{0-\infty}$	100.6	96.6-104.7	10.3
C_{max}	103.7	96.8-111.0	17.5
400 mg			
AUC_{0-t}	104.2	95.4-107.3	15.0
$AUC_{0-\infty}$	103.4	96.6-110.6	17.4
C_{max}	104.3	97.6-111.4	16.9

CONCLUSION

The results of this single-dose study in healthy white volunteers indicated that Imatynib-Biofarm 100 and 400 mg film-coated tablets manufactured by Biofarm Sp. z o.o. (test products) are bioequivalent to Glivec 100 and 400 mg film-coated tablets manufactured by (Novartis Pharma GmbH) (reference products), even though the imatinib mesylate as the active substance in these preparations differs in polymorphic forms, as it was mentioned earlier. Both products were well tolerated. It should be noted that the results for AUC (for imatinib and N-desmethyl imatinib in the study for 100 mg and 400 mg) are generally fitting in the limited acceptance interval for drug in narrow therapeutic index 90.00–111.11%. It is an important premise allowing to conclude the high quality of tested products in term of bioequivalence.

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