Bioequivalence Study of Ticagrelor Tablet in Healthy Volunteers

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Abstract

Aim**: To evaluate the comparative oral bioavailability of single dose of Tigemac® Tablets 90 mg (Ticagrelor manufactured by Macleods Pharmaceuticals Ltd., India) with Brilinta® Tablets 90 mg (Ticagrelor manufactured by AstraZeneca Pharmaceuticals LP, USA) in healthy, adult, human volunteers under fasting condition. Additionally, safety and tolerability of test and reference products were also evaluated.**

Methods: **This was an open label, balanced, analyst blind, randomized, two-treatment, two-period, two-sequence, single dose, crossover bioequivalence study with 7 days wash out period in 24 healthy, adult, human volunteers. The study compared oral bioavailability of two formulations Tigemac® (Test Product) versus Brilinta® (Reference product) in a single dose administered in fasting condition.**

Results: **The 90% confidence intervals for the ratio (Test/Reference) of C_{max} and AUC_{0-48} for Tigemac® tablet were within the acceptable limits of bioequivalence 80.00% - 125.00%. The ratios (T/R) of C_{max} and AUC_{0-48} for ticagrelor were found to be 96.88% and 101.84% respectively. Similarly, the ratios (T/R) of C_{max} and AUC_{0-48} for ticagrelor active metabolite (deshydroxyethoxy ticagrelor [ARC124910XX]) were found to be 104.03% and 105.77% respectively. The highest intra subject C.V. for ticagrelor was observed to be 14.95%. No adverse event occurred during the study period while two adverse events were reported during post study assessments. Both test and reference products were safe and well tolerated in fasting condition.**

Conclusion: **Tigemac® the test formulation was found to bioequivalent with the reference product Brilinta® in healthy, adult, human volunteers under fasting condition.**

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Keywords: **Ticagrelor, Bioequivalence, C_{max} and AUC**

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Introduction

The National Institute for Health and Clinical Excellence (NICE) recommends using clopidogrel for the treatment of non-ST-segment elevation acute coronary syndrome (ACS) in people at moderate-to-high risk of myocardial infarction or death.[1,2] However, it was reported that the clopidogrel produce low degree of inhibition of platelet aggregation (IPA) and broad response variability in patients with poor metabolism of clopidogrel.[3,4] Moreover, the associated adverse clinical outcomes with clopidogrel including an increased risk for recurrent ischaemic events are increasing day by day.[4] To address this poor metabolism issue of clopidogrel, another drug called prasugrel was developed. However, it was found that prasugrel is associated with increased risk of major bleeding, including life-threatening and fatal bleeding which limits its use.[5,6] Later, Ticagrelor was developed to address the issue.

Ticagrelor is a reversible antagonist of $P2Y_{12}$ receptor and has shown benefits in the management of ACS. Results from in-vitro study showed that ticagrelor reversibly binds to $P2Y_{12}$ receptor at a distinct site from the adenosine diphosphate (ADP) binding site.[7] Unlike thienopyridines, ticagrelor does not require metabolic activation to exert its effect, which may account for its fast onset of action. Few reported studies showed that, there is a clear separation between ticagrelor's antithrombotic effects and bleeding time in animal models. In a phase II study, ticagrelor showed consistent inhibition of ADP-induced platelet aggregation without an increase in major and minor bleeding.[8] In a randomized double blind clinical trial in ACS patients, ticagrelor was found superior to clopidogrel.[9]

In healthy volunteers, ticagrelor is absorbed rapidly, having a median peak concentration time (T_{max}) of 2–3 hours after oral dosing. Similarly, its active metabolite AR-C124910XX has the median T_{max} of ~2.5-4 hours. After absorption, ticagrelor and AR-C124910XX are highly bound to plasma proteins (more than 99.8%) and largely restricted to the plasma space. The absolute bioavailability of ticagrelor is estimated at 36%, and the steady-state volume of distribution of ticagrelor is 88 L.

Currently, Brilinta® (Ticagrelor) Tablets 90 mg is used widely, and to market any new formulation of ticagrelor, bioequivalence should be established with reference listed drug Brilinta®. The Macleods Pharmaceuticals Ltd. has developed generic Ticagrelor Tablets 90 mg (Tigemac®) as an alternative to the reference listed drug Brilinta®. It is recommended that bioequivalence to the reference listed drug must be evaluated in fasting conditions. Hence, the test product of Tigemac® (Ticagrelor) Tablets 90 mg (Macleods Pharmaceuticals Ltd., India) was compared with Brilinta® (Ticagrelor) Tablets 90 mg (AstraZeneca Pharmaceuticals LP, USA) in healthy, adult, human volunteers under fasting condition.

This study was conducted with following objectives:

i) **Pharmacokinetic:** To evaluate the comparative oral bioavailability of single dose of Tigemac® 90 mg (Macleods Pharmaceuticals Ltd., India) with Brilinta® (Ticagrelor) Tablets 90 mg (AstraZeneca Pharmaceuticals LP, USA) in healthy, adult, human volunteers under fasting condition.

ii) **Safety:** To monitor safety and tolerability of single oral dose of Tigemac® tablets 90 mg when administered in healthy, adult, human volunteers under fasting condition.

Materials and Methods

Formulations in the study

Test Drug: Tigemac® 90 mg tablet manufactured by Macleods Pharmaceuticals Ltd., India.

Reference Drug: Brilinta® 90 mg tablet manufactured by AstraZeneca Pharmaceuticals LP, USA.

Volunteers

A total of 24 healthy volunteers were enrolled in the study and 23 of them completed both periods of the study; one patient was dropped out in period 2 due to personal reason. All the healthy volunteers were aged between 18 to 45 years, both inclusive. The body mass index of all the healthy volunteers was between 18.50 kg/m^2 - 29.99 kg/m^2 (both inclusive) and body weight was equal to or above 50 kg for males and 45 kg for females. No volunteer had significant smoking, alcoholism and drug abuse history. Volunteers were excluded from the study in case of any allergy or hypersensitivity to study treatments. Volunteers were also excluded from the study if they had consumed alcohol or tobacco within 48 hours prior to dosing. A thorough physical and medical examination was done before enrolment of volunteer in the study. Physical examination included assessment of blood pressure, pulse rate, respiratory rate, 12-lead ECG, normal chest X-ray (PA view) and systemic examination. Blood samples of volunteers were collected to perform PT (INR) [Prothrombin Time {International Normalized Ratio}], haematology (leukocyte count, erythrocyte count, PCV [packed cell volume], ESR [erythrocyte sedimentation rate], platelet count, haemoglobin, and

DLC [differential leukocyte count] and Biochemistry (Blood sugar, triglycerides and cholesterol, Hepatic profile: SGOT [serum glutamic oxaloacetic transaminase], SGPT [serum glutamic pyruvic transaminase], Alkaline Phosphatase, GGT [gamma-glutamyl transpeptidase] and serum bilirubin [direct, indirect and total], Renal profile: Serum creatinine, BUN [blood urea nitrogen], serum calcium, serum electrolytes [sodium, potassium, chlorides]).

Obtaining informed consent

Volunteers were informed about the consent form in detail by the designated personnel before the initiation of the study. Volunteers were explained in detail about the purpose of the study, possible benefits, risks and discomforts, alternative treatment and confidentiality. All the queries / clarifications asked by the volunteers were solved so they could freely decide to participate in the study. Volunteers signed the informed consent form with date. Copies of the signed and dated informed consent form were given to the respective volunteers for their reference.

Ethical conduct of the study

The study was conducted in accordance with the protocol and comply with all requirements regarding the obligations of investigators and all other pertinent requirements of international, national and ICH 'Guideline for Good Clinical Practice', Schedule Y of Drug and Cosmetic Act of India and in accordance with USFDA requirement and Declaration of Helsinki (2013).

Study Design

This was an open label, balanced, analyst blind, randomized, two-treatment, two-period, two-sequence, single dose, crossover bioequivalence study on 24 healthy, adult, human volunteers under fasting condition. Healthy volunteers were randomized to receive a single dose of test formulation (one tablet of Tigemac® 90 mg) and single dose of reference formulation (one tablet of Brilinta® 90 mg) separately in each treatment period. There were two treatment sequences and a "7 days" washout between the two treatment periods. The study assessments were performed during the study and at check-in and check-out of each period.

The study was conducted at bioequivalence facility of Macleods Pharmaceuticals Ltd., Mumbai, India after approval from an independent ethics committee. Volunteers had a screening visit within 21 days prior to the first dose of study drug, two treatment periods with each containing a single dose of study drug, followed by 48 hours of serial pharmacokinetic sample collection. The volunteer returned for the next treatment period or for the final follow-up visit, as appropriate. Volunteers were assigned to each of the two treatments randomly as per the randomization schedule.

Drug Administration

A single dose of test formulation (Tigemac® 90 mg tablet) and reference formulation (Brilinta® 90 mg tablet) was administered orally at 0.00 hours during each period with 240 mL drinking water after 10 hours of fasting. Volunteers were dosed in upright sitting posture and instructed to avoid severe physical exertion following the investigational product administration. Volunteers were kept in supine position at least 9.00 hours after dosing. Blood samples (1 x 5 mL) were collected in 5 mL blood collection tube containing K_2EDTA as anticoagulant during each period. The venous blood samples were withdrawn pre-dose and at 0.17, 0.33, 0.50, 0.75, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 8.00, 12.00, 16.00, 24.00, 30.00, 36.00 and 48.00 hours post-dose (time points being relative to the investigational product dosing). Blood samples were collected at bedside up to 9.00 hours post dose.

Owing to the sensitivity of ticagrelor to light, blood samples were collected and processed under yellow monochromatic light at each time point. The collected blood samples were centrifuged between 4°C to 8°C and at 4000 rpm for 10 minutes to separate plasma. These samples were centrifuged within 30 minutes after collection of last blood sample; for any delay in centrifugation, samples were kept in cold condition. The separated plasma was aliquoted in duplicate prelabelled amber colored polypropylene tubes during each period and then transferred to a deep freezer for storage.

LC-MS/MS Method for Estimation of Ticagrelor and its Active Metabolite Deshydroxyethoxy Ticagrelor (AR-C124910XX) in Human Plasma Samples

A LC-MS/MS method was developed by using liquid-liquid extraction method and Ticagrelor D7 and Ticagrelor metabolite M8 D7 as internal standards. Chromatographic separation was achieved by Kromasil 100-5-C18 (50X4.6) mm, 5µm column with mobile phase consisting methanol, buffer solution (0.5 mM ammonium acetate) and formic acid in volume ratio of 80:20:0.1 (V/V/V). The total run time was 2.20 minutes. Detection was carried out in positive turbo-spray ionization mode using MRM transitions of 523.40/153.00 for ticagrelor, 530.40/153.10 for

ticagrelor D7, 479.20/153.10 for Deshydroxylethoxy Ticagrelor and 486.30/153.10 for Ticagrelor metabolite M8 D7.

The analytical method was validated over the range of concentration range of 4.92-1505.74 ng/mL and 1.98-251.35 ng/mL for ticagrelor and deshydroxyethoxy ticagrelor respectively. The results of validation parameters including selectivity, analytical standard purity, exchange reaction, carryover effect, linearity, precision and accuracy, bench top stability, freeze thaw stability and dilution integrity test were within the acceptance range. The above said analytical method is valid for the estimation of ticagrelor and deshydroxyethoxy ticagrelor in human plasma for above mentioned concentration range.

Pharmacokinetic Assessments

Pharmacokinetics parameters including C_{max}, AUC_{0-48}, and T_{max} were calculated for test formulation, reference formulation and their active metabolite.

Statistical Analysis

Statistical analysis was performed using SAS® version 9.4. Standard ANOVA model was used to analyse the log-transformed pharmacokinetic parameters (C_{max} and AUC_{0-48}) for ticagrelor and deshydroxyethoxy ticagrelor with the main effects of "sequence, period, formulation and volunteer nested within sequence.

The following bioequivalence criteria were established on the protocol: The products claimed to be bioequivalent if the 90% confidence intervals are included in the range of 80.00% -125.00% for C_{max} and AUC_{0-48} log-transformed.[10]

Adverse events

Any untoward reactions during study were planned to be reported.

Results and Discussion

Acute coronary syndrome like myocardial infarction is associated with high rate of morbidity and mortality. In such conditions, therapeutic management should include platelet aggregation inhibitors. However, several studies have reported that platelet aggregation inhibitors like clopidogrel and prasugrel are associated with either compromised metabolic problems causing poor efficacy or serious adverse effects.[3,5] In order to address such issues, ticagrelor was developed which has shown to improve inhibition of platelet aggregation compared to other drugs of same therapeutic category.

In this study, we compared the pharmacokinetic profiles of generic drug formulation Tigemac® and its metabolites to reference listed drug formulation Brilinta® and its active metabolite.

Table-1A: Comparative Mean Pharmacokinetic Data of Test Formulation: Ticagrelor

Pharmacokinetic Parameters	Test Formulation						
	N	Mean	Median	S.D.	C.V.	Minimum	Maximum
C_{max} (ng/mL)	23	751.59	684.68	170.68	22.71	560.37	1174.64
AUC_{0-48} (ng*hrs/mL)	23	6026.76	6025.32	1619.41	26.87	3269.77	9836.96
T_{max} (hrs)	23	2.53	2.33	1.15	45.53	1.33	4.50

Table-1B: Comparative Mean Pharmacokinetic Data of Reference Formulation: Ticagrelor

Pharmacokinetic Parameters	Reference Formulation						
	N	Mean	Median	S.D.	C.V.	Minimum	Maximum
C_{max} (ng/mL)	23	778.61	785.08	168.17	21.60	489.13	1083.28
AUC_{0-48} (ng*hrs/mL)	23	5921.21	5876.45	1506.46	25.44	2801.50	9044.22
T_{max} (hrs)	23	2.30	2.00	1.27	55.08	1.00	5.00

Table-1C: Comparative Mean Pharmacokinetic Data of Test Formulation: Ticagrelor active metabolite (Deshydroxyethoxy Ticagrelor)

Pharmacokinetic Parameters	Test Formulation						
	N	Mean	Median	S.D.	C.V.	Minimum	Maximum
C_{max} (ng/mL)	23	146.65	139.53	25.92	17.68	117.89	216.40
AUC_{0-48} (ng*hrs/mL)	23	1675.59	1624.69	330.49	19.72	1133.79	2689.08
T_{max} (hrs)	23	3.45	3.00	0.96	27.73	2.00	4.50

Table-1D: Comparative Mean Pharmacokinetic Data of Reference Formulation: Ticagrelor Active Metabolite (Deshydroxyethoxy Ticagrelor)

Pharmacokinetic Parameters	Reference Formulation						
	N	Mean	Median	S.D.	C.V.	Minimum	Maximum
C_{max} (ng/mL)	23	141.87	133.36	30.15	21.25	98.21	203.55
AUC_{0-48} (ng*hrs/mL)	23	1590.45	1524.69	364.27	22.90	1110.63	2676.55
T_{max} (hrs)	23	3.13	2.67	1.16	37.09	1.67	5.00

Table-2A: Ln-Transformed Parameters for Ticagrelor in Test Formulation and Reference Formulation

Geometric Mean, Ratio, Intra-subject C.V., Power & 90% Confidence Interval for Ticagrelor							
Pharmacokinetic Parameters	Geometric Mean		Ratio (T/R) (%)	Intra Subject C.V. (%)	Power (%)	90% Confidence Interval (%)	
	Reference (R)	Test (T)				Lower	Upper
C_{max} (ng/mL)	760.49	736.79	96.88	14.95	99.66	89.84	104.48
AUC_{0-48} (ng*hrs/mL)	5719.96	5825.09	101.84	12.37	99.97	95.66	108.41

Table-2B: Ln-Transformed Parameters for Ticagrelor active metabolite (Deshydroxyethoxy Ticagrelor) in Test Formulation and Reference Formulation

Geometric Mean & Ratio for Deshydroxyethoxy Ticagrelor			
Pharmacokinetic Parameters	Geometric Mean		Ratio (T/R) (%)
	Reference (R)	Test (T)	
C_{max} (ng/mL)	139.03	144.63	104.03
AUC_{0-48} (ng*hrs/mL)	1556.20	1646.01	105.77

Results of the study showed that a single dose of test formulation had similar pharmacokinetic profile to that of reference drug. The comparative pharmacokinetic profiles of both test and reference products are shown in Table 1A, Table 1B, Table 1C and Table 1D. The graphical representations of mean plasma concentration verse time are shown in Figure-1A and Figure-1B. The C_{max} for ticagrelor was 751.59 ng/mL for test formulation compared to 778.61 ng/mL for reference formulation. Similarly, the C_{max} for active metabolite of ticagrelor (Deshydroxyethoxy Ticagrelor) was 146.65 ng/mL for test formulation compared to 141.87 ng/mL for reference formulation.

The AUC_{0-48} for ticagrelor was found to be 6026.76 ng*hrs/mL for test formulation compared to 5921.21 ng*hrs/mL for reference formulation. Similarly, AUC_{0-48} for active metabolite of ticagrelor (Deshydroxyethoxy Ticagrelor) was 1675.59 ng*hrs/mL for test formulation compared to 1590.45 ng*hrs/mL for reference formulation. The median T_{max} for ticagrelor was found to be 2.33 hrs for test formulation compared to 2.00 hrs for reference formulation. Similarly, median T_{max} for active metabolite of ticagrelor (Deshydroxyethoxy Ticagrelor) was 3.00 hrs for test formulation compared to 2.67 hrs for reference formulation.

The T/R ratio (90% confidence interval) for Test/Reference of C_{max} and AUC_{0-48} of ticagrelor in test and reference products were 96.88% (89.84-104.48%) and 101.84% (95.66-108.41%) respectively. The highest intra subject C.V. (coefficient of variation) for ticagrelor was observed to be 14.95%. The power of C_{max} and AUC_{0-48} for ticagrelor was observed to be 99.66% and 99.97% respectively.

There was no statistical significant difference observed in pharmacokinetic parameters between test and reference products.

No adverse events were experienced by any of

Figure 1A: Comparative Linear Plot of Ticagrelor Mean Plasma Concentration (ng/ml) Vs Time (hrs)

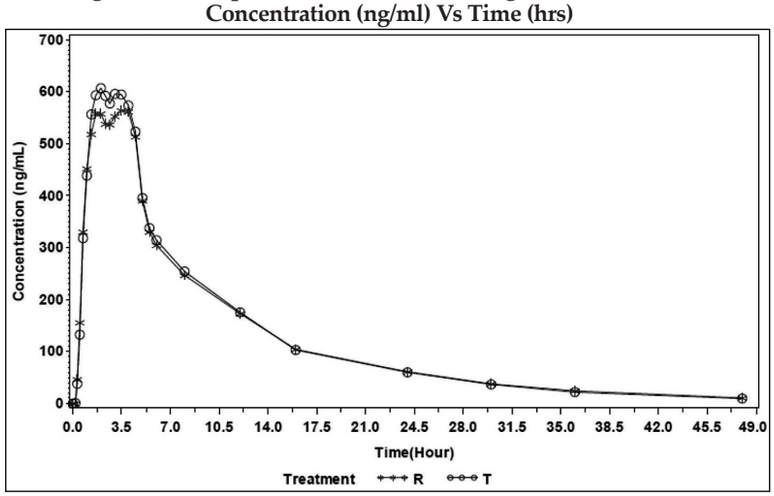
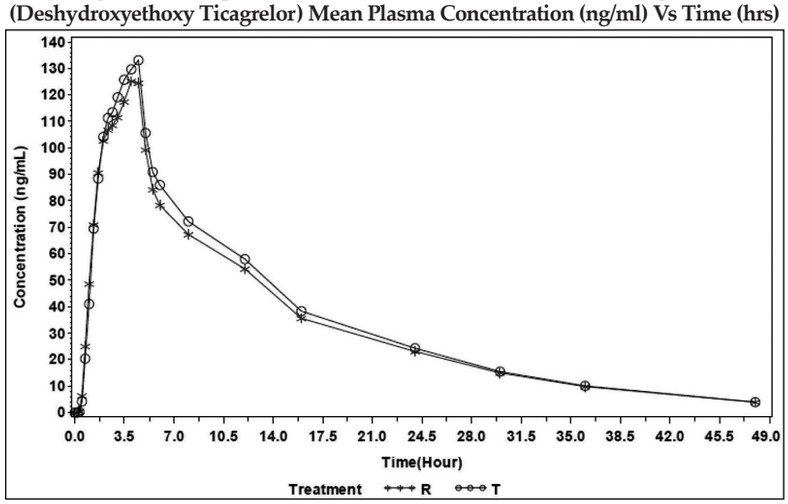


Figure 1B: Comparative Linear Plot of active metabolite of ticagrelor (Deshydroxyethoxy Ticagrelor) Mean Plasma Concentration (ng/ml) Vs Time (hrs)



the patient during study period. However, during post study assessments, two AEs were observed. One volunteer had decreased erythrocyte count and other volunteer had reduced haemoglobin levels (0.5 g/dL from base line). The change in erythrocyte count was unlikely to the study treatment while reduction in haemoglobin level was found to be possibly related to study treatment. The volunteer who suffered haemoglobin drop had baseline haemoglobin level as 11.8 g/dL and post study haemoglobin level as 11.3 g/dL, which is outside of the acceptable range for the study (acceptable range: 11.7 g/dL) and hence reported to be clinically significant.

The innovator's prescribing information does not mention haemoglobin drop as an adverse event. No other signs and symptoms related to decrease in haemoglobin level were observed at the time of post study safety assessment. The volunteer was unwilling to visit the facility for post study follow-up, however during frequent telephonic reminders to visit the facility, the volunteer confirmed that there were no health-related issues and was doing fine. Thus, by the mention of 'no health concern' from volunteer and his unwillingness to visit the facility, no clinical intervention was done for the event and volunteer was reported lost to follow-up.

Conclusion

The test formulation of Ticagrelor (Tigemac®) of Macleods Pharmaceuticals Ltd., India and reference formulation (Brilinta®) of AstraZeneca Pharmaceuticals LP, USA demonstrated the 90% confidence interval for the ratio test/reference of pharmacokinetic parameters of C_{max} & AUC_{0-48} within the acceptable limits (80.00%-125.00%), thereby establishing bioequivalence between both the formulations.

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