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Phase 1 study of novel anti-platelet agent to overcome pharmacogenomic limitations of clopidogrel

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ABSTRACT

Aims Clopidogrel is the most commonly prescribed thienopyridine as part of dual anti-platelet therapy for the treatment of cardiovascular diseases. However, clopidogrel responsiveness shows variability based on CYP2C19 polymorphism. Therefore, we planned a study with an objective of evaluating safety, tolerability, pharmacodynamics and pharmacokinetics of a novel thienopyridine antiplatelet agent AT-10 in healthy Indian subjects compared with standard dosage regimen of clopidogrel based on their CYP2C19 genotyping.

Methods Two CYP2C19 genotype–based groups were identified, that is, poor metabolisers and extensive metabolisers, with 20 subjects in each group (n=40) for participating in a randomised, two-period, crossover study. Each study period lasted 6 days including administration of loading and maintenance doses of AT-10 (40 mg/10 mg) or clopidogrel (300 mg/75 mg). The pharmacokinetics and pharmacodynamics were assessed on day 1 and day 6 at several time intervals.

Results Overall result of pharmacodynamic parameters showed that mean %inhibition of platelet aggregation between AT-10 and clopidogrel in all subjects at 6 hours postdose (loading dose) (AT-10: clopidogrel; 73.30% vs 18.53%) and 6 hours postdose on day 6 (maintenance dose) (AT-10: clopidogrel; 83.41% vs 51.19 %) obtained from the AT-10 group was significantly higher than the clopidogrel group. Further, %inhibition of platelet aggregation from AT-10 treatment in poor metaboliser group was significantly higher than the clopidogrel treatments in extensive metaboliser group.
Overall pharmacokinetic comparison in all subjects indicates that AT-10 gives greater exposure to active Metabolite H4 than clopidogrel.

Conclusion AT-10 showed better inhibition of platelet aggregation in poor metabolizers as compared to Clopidogrel. AT-10 may emerge as a potential alternative to Clopidogrel as an anti-platelet drug. It can be further developed in clinical studies for the unmet medical needs in management of CVDs and overcome the pharmacogenomic limitations of Clopidogrel.

Trial registration number Clinical Trial Registry-India URL: http://ctri.nic.in. Registration number: CTRI/2021/03/032206.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Dual anti-platelet therapy with aspirin and P2Y12 inhibitor is the cornerstone of medical treatment in patients with acute coronary syndrome, those undergoing percutaneous coronary intervention. Among all P2Y12 inhibitors, clopidogrel is the most widely prescribed thienopyridine with acceptable safety profile; however, it has major shortcomings associated with CYP2C19 genetic polymorphism.

WHAT THIS STUDY ADDS

⇒ AT-10 (2-oxo-clopidogrel bisulfate) is a novel thienopyridine P2Y12 inhibitor under clinical development in India. It is a clopidogrel derivative that is metabolically converted to produce the active thiol metabolite similar to clopidogrel. The response to AT-10 was found unaffected by genetic CYP2C19 polymorphisms to the extent of its influence in clopidogrel, thus eliminating the need for characterisation of clopidogrel responsiveness and thereby maintaining effectiveness in all patients, including those patients who are identified as CYP2C19 poor and intermediate metabolisers.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This was a small sample study in healthy volunteers for shorter duration. Further studies are required to investigate the influence of CYP2C19 functional polymorphisms on the response to AT-10 dose in diverse clinical settings, and especially on the risk of recurrent thrombotic events during AT-10 treatment as compared with clopidogrel therapy.

INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of mortality worldwide. Estimated 20.5 million deaths were reported globally due to cardiac conditions in 2021 which showed a marked increase from 12.1 million CVD deaths recorded in 1990.[1] Of these deaths, more than 50% are secondary to coronary artery disease (CAD), with acute coronary syndrome (ACS) accounting for approximately 10% of all admissions presenting to emergency care physicians.[2] Currently, available anti-platelet agents have several limitations including inadequate efficacy, risk of bleeding and variability in individual response.[3] Dual anti-platelet therapy

with aspirin and P2Y12 inhibitors, drugs from thienopyridine class, is therefore the cornerstone in managing patients with ACS, undergoing percutaneous coronary intervention (PCI) and preventing stent thrombosis in both the acute and long-term therapy.[4 5] Many patients continue to have recurrent atherothrombotic events despite receiving dual anti-platelet therapy.[6] While the use of clopidogrel has undoubtedly been a beneficial advancement in treating ACS in both short and long-term morbidity and mortality,[7] it remains a treatment with several shortcomings.

Clopidogrel is a prodrug and its clinical efficacy appears to be a function of the amount of enzymatically derived active thiol metabolite formed. Clopidogrel is first metabolised to the intermediate metabolite, 2-oxo-clopidogrel, followed by metabolism to an active thiol metabolite in vivo. Most of the drug is inactivated by carboxylesterase-1, and only 15% is converted to the active metabolite H4 via a two-step process in the liver, influenced by CYP2C19 polymorphisms. CYP2C19 is crucial in both the first (45%) and second (21%) metabolic steps, while CYP3A4 enzyme plays a significant role in the second step (40%). Therefore, any changes in CYP2C19 activity impacts thiol metabolite formation significantly and hence also the response to treatment.[8 9] The metabolism of clopidogrel to its active metabolite can be impaired by genetic variations in CYP2C19 or by drugs that inhibit CYP2C19, such as omeprazole or esomeprazole.

It has been established that all patients receiving clopidogrel do not benefit equally and poor metabolisers (PM) of CYP2C19 are at increased risk of ischaemic events after PCI,[4 9] suggesting that CYP2C19 polymorphism is a clinically relevant determinant of response to clopidogrel. In 2010, the US FDA had imposed a box warning on clopidogrel bisulfate tablets (Plavix) stating that 'effectiveness of clopidogrel bisulfate depends on conversion to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19 enzyme. Consider use of another platelet P2Y12 inhibitor in patients identified as CYP2C19 poor metabolisers'.[10]

Patients with carriers of reduced-function CYP2C19 alleles (PMs and intermediate metabolisers) produce lower levels of clopidogrel's active metabolite. Such patients show diminished platelet aggregation inhibition and are at higher risk of major adverse cardiovascular events, including a threefold greater risk of stent thrombosis;[11 12] hence, newer anti-platelet agents are preferred in such patients. Although the newer agents like Prasugrel and Ticagrelor are devoid of such problems (genetic polymorphism), the CV benefit comes with the cost of increased bleeding risk that includes fatal intracranial hemorrhage.[13]

There is a considerable heterogeneity observed in the activity of CYP450 enzymes in humans, and it has become apparent that individuals have polymorphisms in CYP2C19 resulting in low or non-responsiveness to clopidogrel known as 'clopidogrel resistance'.[11 14] The frequency of CYP2C19*2 allele associated with PM type has been reported to be 47.23% in CAD patients in India.[15] Studies by Adithan *et al*[16] have reported incidence of CYP2C19*2 of around 37.9% in south Indian general population and 35.5% by Shalia *et al*[17] in western Indian general population.

In the recently published real-world data, comprising of East Asians who had undergone drug-eluting stent implantation and received clopidogrel-based anti-platelet therapy indicated that intermediate metabolisers (IMs) or PMs (~62.1% of the total population evaluated) had a higher risk of cardiac death, myocardial infarction and stent thrombosis at 5-year follow-up compared with normal metabolisers.[18] The most common CYP2C19 LOF allele is *2 with allele frequencies of ~15% in Caucasians and Africans and 29–35% in Asians, indicating the prevalence of IM and PM is more in Asian population than western population.[12 18] This high prevalence of CYP2C19 polymorphism renders clopidogrel ineffectiveness or adds variability in effectiveness in PMs and intermediate metabolisers, respectively.

Hence, to overcome the shortcomings of clopidogrel associated mainly with CYP2C19 metabolism, we have developed a novel anti-platelet agent AT-10 (2-oxo-clopidogrel bisulfate), which is a clopidogrel derivative that is metabolically converted in one CYP-dependent step, to produce the active metabolite similar to the approved listed drug Plavix. The metabolic pathway of clopidogrel and AT-10 is depicted in figure 1.

AT-10 has the same chemical structure as that of 2-oxo-clopidogrel. It can exist in four chiral isomers: SS, SR, RR and RS. In clopidogrel, one chiral carbon has S-configuration fixed; thus, 2-oxo-clopidogrel metabolite of clopidogrel can exist in two chiral isomers (SS and SR). AT-10 is a single diastereomer of 2-oxo-clopidogrel having 7aS, 2S-configuration that generates the active metabolite H4 similar to clopidogrel. AT-10 is an isomerically purified form (7aS,2'S-configuration) of 2-oxo-clopidogrel, which is a desired intermediate metabolite formed during the in vivo bioactivation of the dosed drug clopidogrel. More recently, similar approach in this therapeutic area has been adopted by some researchers for their novel ester prodrug, Vicagrel as an antiplatelet drug.[19]

The response to AT-10 may not be influenced by genetic CYP2C19 polymorphisms to the extent of its influence in clopidogrel, thus eliminating the need for characterisation of clopidogrel responsiveness and thereby maintaining effectiveness in all patients, including those patients who are identified as CYP2C19 PMs and intermediate metabolisers. Prior to this Indian phase 1 study, one proof of concept clinical study was performed with an objective of evaluating and establishing the dose-response relationship of single and multiple doses of AT-10 ranging from 7.5 mg to 40 mg in healthy human subjects where 40 mg dose of AT-10 was found to be safe, well-tolerated and comparable with the clopidogrel 300 mg dose with regard to % inhibition of platelet aggregation (IPA) at 6 hours following the single loading dose (LD). Hence, AT-10 40 mg was selected as the LD. Since

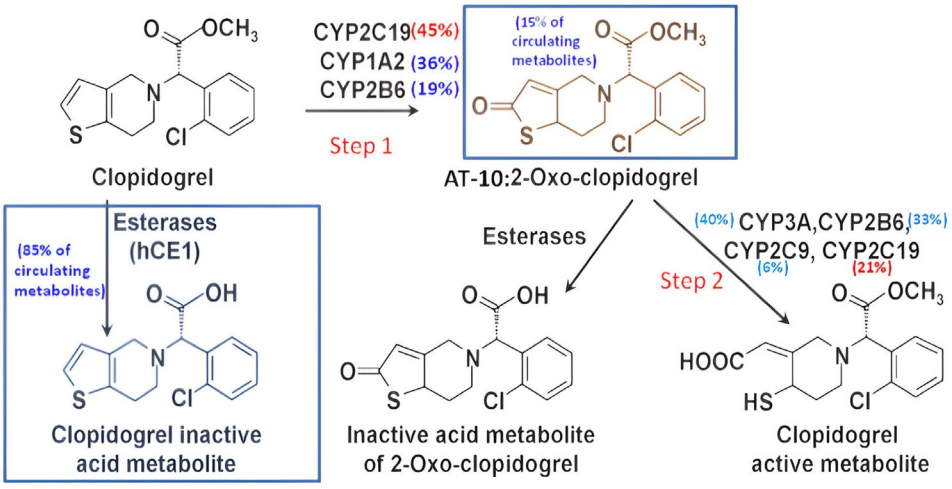

Figure 1 Metabolic pathway of Clopidogrel.

the maintenance dose (MD) of clopidogrel (75 mg) is a quarter of the LD of clopidogrel (300 mg), 10 mg dose of AT-10 (a quarter of the LD, 40 mg) was selected as the MD in this study. Thus, in light of promising data obtained from earlier studies, we first time evaluated safety, tolerability, pharmacodynamics and pharmacokinetics of AT-10 in healthy Indian subjects in comparison with standard dosage regimen of clopidogrel. It is important to note that AT-10 is an intermediate metabolite in the metabolic pathway of clopidogrel to its active metabolite. Therefore, this study does not represent the first exposure of AT-10 in humans. It does, however, represent the direct administration of AT-10 to humans.

METHODS

Study design

This was an open label, unicentric, randomised, two-period, two-sequence, crossover, comparative, single and multiple-dose, pharmacokinetic (PK) and pharmacodynamic (PD) study conducted in healthy human subjects. Two CYP2C19 genotype–based groups were identified, that is, PMs and extensive metabolisers (EMs) with 20 subjects in each group (n=40). Each metaboliser group was further randomised in 1:1 ratio to receive either clopidogrel (Plavix, Sanofi Aventis, USA) or AT-10 (Ipca Laboratories Limited, India) in two sequences. Sequence-1 includes administration of AT-10 on the first period and clopidogrel on the second period, while sequence-2 was the reversal of sequence-1. All subjects underwent a two-period treatment regimen, 6 days each, which included administration of LD of clopidogrel 300 mg or AT-10, 40 mg on day 1 followed by one time per day MD of either clopidogrel 75 mg or AT-10 10 mg from day 2 to day 6. Then, the subjects were crossed over to receive the alternate therapy for the second period. A washout period of at least 14 days was maintained between the study periods.

Participants

Healthy Indian subjects aged 18 to 45 years with body mass index of 18.50 to 29.90 kg/m^2 were enrolled in the study. Prior enrolment, we conducted CYP2C19 genotyping for categorising the subjects as EMs (*1/*1) or PMs (*2/*2). In order to confirm and cross-verify the detected genotypes, the blood samples of all screened subjects were assessed independently at two different laboratories. Subjects with matching alleles only at both the testing laboratories were considered for enrolment in the study. PCR followed by DNA sequence analysis technology was used for genotyping test. Subjects with no evidence of underlying disease on medical history, clinical examination and laboratory investigations performed during the prestudy screening (within 28 days prior to commencement of the study) were included.

The sample size chosen is not based on a statistical calculation of PD effect or PK variability but rather on the number of subjects deemed necessary to assess the safety and tolerability, characterise the PK parameters and generate proof-of-concept data on the pharmacodynamic effect of AT-10 in humans. 40 healthy male and female subjects were enrolled in the study.

The randomiation schedule was generated for 40 subjects for each type of metabolisers, that is, extensive and poor, and for each gender group, that is, 20 for each male and female, respectively, for each type of metabolisers. Total four randomisation schedules were prepared. Each randomisation schedule contained 20 subjects (10 subjects for each sequence).

Ethics

The study was conducted at CBCC Global research, India. It was done in accordance with the ethical principles of Declaration of Helsinki and requirements of Good Clinical Practice. It was approved by Sangini Hospital Ethics Committee and Indian Regulatory Authority (Central Drug Standard Control Organization). The study protoco(online supplemental file 1) l was registered on Clinical Trials Registry India with registration number CTRI/2021/03/032206 (registered on: 23/03/2021).

Consent

Written informed consent was obtained from all the participants' prior to participation in the study. No study-related procedure was undertaken prior to obtaining written informed consent from subject.

Safety assessment

Safety assessments were performed by the investigator throughout the study. Safety evaluations involved assessment of adverse events, coagulation parameters (prothrombin time (PT) and partial thromboplastin time (PTT), predose and postdose vital signs, ECGs, clinical laboratory testing (haematology, blood chemistry, urine analysis), and general physical and clinical examinations. Coagulation parameters were also assessed at screening, at housing during each period, at 8 hours postdose on each dosing-day, and at exit or early termination.

Pharmacodynamic assessment

For pharmacodynamic assessment, blood samples were collected into prelabelled citrate tubes (3.2%). A total of four blood samples (2.7 mL each) were collected, prior to administration of drug, and at 30 min and 2 and 6 hours after the study drug administration on each dosing day. Additionally, one more blood sample was collected on day 7 after 24hours of last MD administration in each period.

Inhibition of platelet aggregation was performed by using adenosine diphosphate (ADP)-induced platelet aggregation from whole blood treated with sodium citrate (3.2%) as an anti-coagulant. The whole blood impedance aggregometry procedure (CHRONO-LOG592, Pennsylvania, USA) was used. Inhibition was calculated using the standard formula. The PD samples were analysed at the clinical site only. Aggregometry was carried out after stimulation of collected blood sample with 20 µM of ADP. As compared with conventional light transmission aggregometry, the impedance aggregometry assesses platelet function under more physiological conditions as it is performed in whole blood, thus enabling other blood elements to influence platelet aggregation. The principle of impedance method is similar to that of light transmission aggregometry except that it is done in whole blood, thus obviating the need for preparation of a platelet rich and poor plasma which may add variability to the test results.

Pharmacokinetic assessment

In each period, blood samples (5 mL each) were collected in prelabelled Na citrate vacutainers at predose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours after study drug administration on days 1 and 6 for assessment of PK parameters. In between days 3, 4 and 5 of each period, predose sample was also collected.

After collection, the blood samples were placed in a refrigerated centrifuge within 45 min of blood sample collection and then spun at 4000 rpm at set 4°C±2°C for 10 min. The plasma was then be separated, transferred to a single prelabelled polypropylene tube and vortexed to mix, and then the plasma was distributed in two prelabelled polypropylene tubes. The separated plasma was kept in the wet ice bath until storage in a freezer at –70°C (±10°C) at clinical facility.

The analytes were quantitated using a solid phase extraction (SPE) method. 0.200 mL aliquot of calibration curve standards, QC samples and study samples were spiked with internal standard mix working solution. Sample preparation was carried out in wet ice bath. These samples were extracted using SPE cartridges (Agilent Bond Elute C18, 100 mg, 1 mL). The samples were applied to the preconditioned SPE cartridges, and thereafter each cartridge was washed and eluted using elution solution. Following extraction, 15.0 µL of resulting solution was injected onto liquid chromatography-mass spectrometry (LC-MS)/MS system for further analysis.

Plasma concentrations of 2-oxo-clopidogrel and derivatised active metabolite H4 (analysed as MP-H4) were determined using a validated LC-MS/MS method. The PK analysis was performed using a model-independent software programme (WinNonlin Professional V.8.3). Maximum concentration (C_{max}) and T_{max} were obtained from plasma concentration time curves.

Statistical analysis

For PD analyses, %IPA generated by aggregometry at different time points were assessed. Maximum %IPA for each group was compared using analyses of variance (ANOVA) with sequence, treatment, period and subject (sequence) as fixed effects. For the comparison between types of metabolisers, the ANOVA model with types of metabolisers as fixed effect was used. Least-squares means with 95% CI was calculated by ANOVA for each comparison.

To evaluate the PK parameters, statistical comparison of the overall PK (day 1 and day 6), single-dose and multiple-dose PK parameters of peak (Cmax) and total (AUCt, AUCinf) exposure of H4 were made between the AT-10 and clopidogrel dose groups: between and within metaboliser groups. Geometric means were calculated for Cmax, AUCt and AUCinf. ANOVA was performed on the ln-transformed PK parameters Cmax, AUCt and AUCinf. Geometric means and its 90% CI were given for all parameters in each phenotype.

Data were presented using descriptive statistics, that is, n(%) for categorical variables and mean, SD, minimum and maximum for continuous variables, according to the regulatory guidelines. Significance was calculated using P values; $p<0.05$ was considered statistically significant. All the statistical analyses were performed using SAS V.9.4.

RESULTS

Subject enrollment and demographics characteristics

A total of 40 subjects were enrolled in the study from June 2021 to August 2021. Two CYP2C19 genotype–based groups were identified, that is, PM and EMs with 20 subjects in each group. Out of 40 enrolled subjects,

Table 1 Demographic and baseline characteristics of the enrolled subjects

Characteristic	Treatment		Total
	Sequence AB (n=20)	Sequence BA (n=20)	
Gender			
Female	6 (30.0)	6 (30.0)	12 (30.0)
Male	14 (70.0)	14 (70.0)	28 (70.0)
Age (years)	32.6±6.36	34.9±5.21	33.7±5.85
BMI (kg/m^2)	23.41±3.47	24.51±2.67	23.96±3.11
Height (cm)	166.2±10.20	162.0±7.23	164.1±8.99
Weight (kg)	64.91±12.67	64.08±9.07	64.50±10.89
Race			
Asian	20 (100)	20 (100)	40 (100)

Treatment A: AT-10 (2-oxo-clopidogrel bisulfate) tablets 40 mg/10 mg.
Treatment B: Plavix (clopidogrel bisulfate) tablets 300 mg/75 mg.
Data presented as n (%).
Data presented as mean±SD.
BMI, body mass index.

37 subjects had completed the study. Remaining three subjects were discontinued from the study due to adverse event (n=2) and consent withdrawal (n=1) (online supplemental figure 1). A total of 40 subjects were analysed for safety. PK/PD data from the 37 subjects who completed the study were used in the PK/PD data analyses. The two treatment groups were well balanced with regard to all baseline characteristics. Demographic characteristics of these subjects are given in table 1.

Pharmacodynamic results

The ADP-induced platelet aggregation was assessed for change from baseline in each treatment group at each time point assessed on each dosing day for PD analysis. The inhibition of ADP-induced platelet aggregation occurred rapidly and reaches the peak at around 6 hours, and inhibition was sustained until 24 hours postdose. The mean %IPA over 6 days of drug administration (LD and MD) is shown in figure 2A for both AT-10 and clopidogrel.

The mean %IPA between PMs and EMs in AT-10 group showed that %IPA at 6 hours postdose on day 6 obtained from EMs group appeared to be higher than PMs group. However, the difference between the AT-10 treatments in EMs versus PMs was found to be statistically insignificant at 6 hours postdose LD and 24 hours postdose after day 6. It means AT-10 produces equivalent PD effect in both EMs and PMs, and the anti-platelet effect of AT-10 is not affected by the genotyping metabolising status of the volunteers (online supplemental table 1).

On the contrary, following loading and maintenance 6 hours postdose on day 6 in clopidogrel treatment group, the mean %IPA was found to be significantly higher for EMs as compared with PMs. In PMs, %IPA observed was half of that observed in EM. This shows the influence of CYP2C19 polymorphisms on PD effect in PMs (online supplemental table 1).

Further, when we compared the mean %IPA between AT-10 PMs and clopidogrel EMs, the %IPA at 6 hours postdose obtained from the AT-10 in PM group was statistically significant than clopidogrel in EMs group. However, %IPA at 6 hours postdose on day 6 and 24 hours postdose after day 6 obtained from the AT-10 treatment in PM group appears to be statistically insignificant than the clopidogrel treatments in EMs group. This indicates that the response to AT-10 is not influenced by CYP2C19 polymorphisms; hence, patients including those who are CYP2C19 PMs may receive clinical benefits from AT-10 treatment (online supplemental table 1).

Moreover, the overall result of PD parameters showed that the mean %IPA between AT-10 and clopidogrel in all subjects at 6 hours postdose 6 (LD), 6 hours postdose and 24 hours postdose after day 6 obtained from the AT-10 group was significantly higher than the clopidogrel group. Similarly, following LD and MD, the mean %IPA for AT-10 treatment group was significantly higher than that observed with clopidogrel treatment group in both PMs and EMs (figure 2A).

Pharmacokinetic results

AT-10 is a clopidogrel derivative that is metabolically converted to produce the active metabolite (H4) of the approved listed drug Plavix. The PK parameters for 2-oxo-clopidogrel and H4 for all subjects have been presented in online supplemental table 1 after administration of AT-10 and Clopidogrel.

Analysis of H4 between AT-10 and clopidogrel in all subjects at day 6 showed that Cmax (AT-10: clopidogrel; 2.68 ng/mL vs 1.07 ng/mL, p<0.0001) and AUCt (AT-10: clopidogrel; 3.27 hour*ng/mL vs 1.09 hour*ng/mL, p<0.0001) of pharmacologically active metabolite H4 are significantly higher in AT-10 treatment than clopidogrel; this indicates that AT-10 at 10 mg gives greater exposure of H4 metabolite than clopidogrel 75 mg. H4 metabolite is

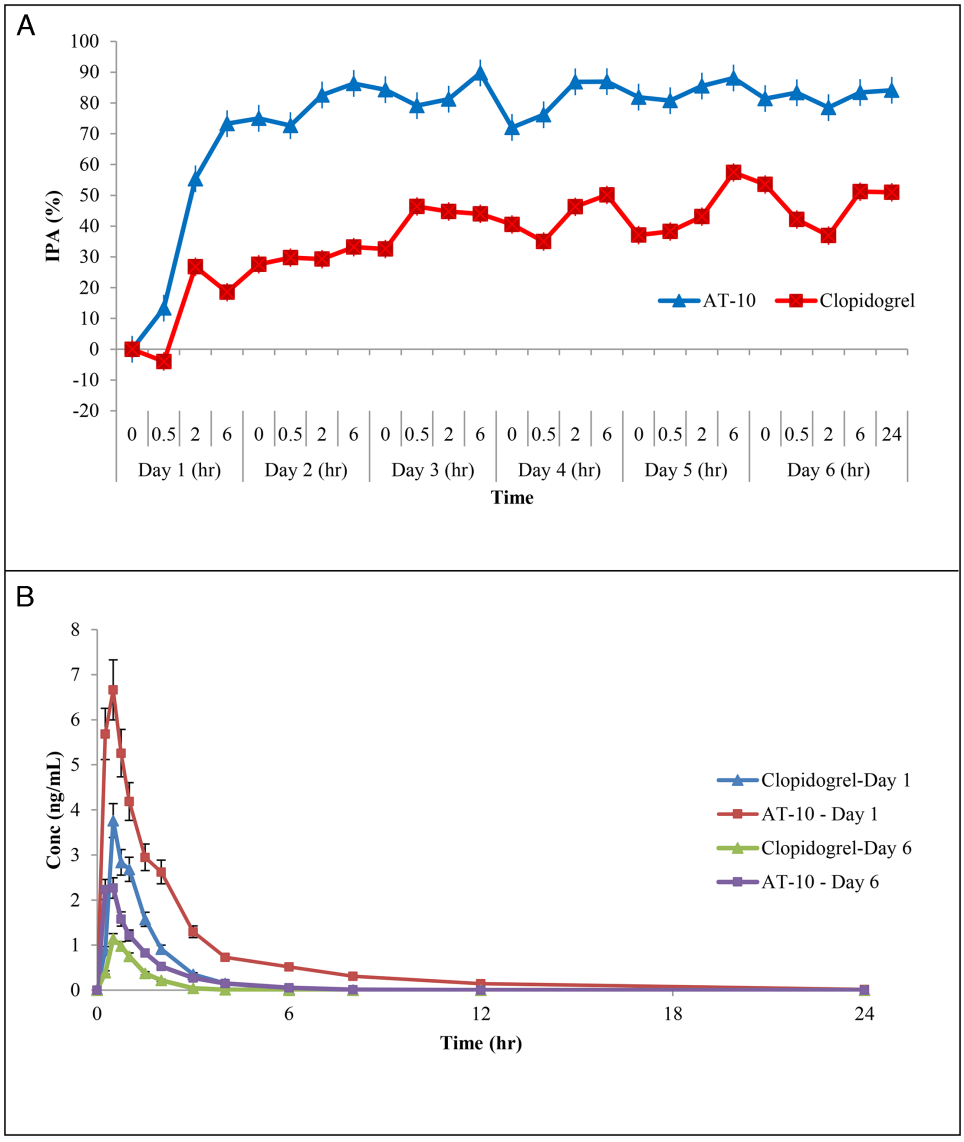

Figure 2 Overall %IPA and H4 of participants receiving AT-10 vs clopidogrel. (A) Overall %IPA of AT-10 versus clopidogrel. (B) H4 of AT-10 and clopidogrel on day 1 and day 6 (overall subjects).

responsible for pharmacological and therapeutic action; hence, pharmacodynamic effect (anti-platelet effect) expected at steady state with AT-10 can be greater than clopidogrel regardless of genotyping status. The mean plasma concentration curve after administration of LD and MD of AT-10 and clopidogrel is shown in figure 2B.

Overall, there is a statistically significant difference observed between AT-10 and clopidogrel treatment for assessed pharmacokinetic parameters.

Safety assessment

All enrolled subjects were included in the safety analysis. The study documented 11 treatment emergent adverse events in seven participants, with five mild, one moderate and five severe cases. Nine events were possibly related to the study drug, while two were unlikely (table 2). No serious adverse events were reported, indicating both treatments were safe and well-tolerated across all participants despite differences in genotyping status.

DISCUSSION

Despite availability of newer anti-platelet agents (P2Y12 inhibitors), clopidogrel is the most widely prescribed thienopyridine with acceptable safety profile; however, it has major shortcomings associated with CYP2C19 genetic polymorphism.

Ipca has developed a new anti-platelet drug, AT-10, to address the limitations of clopidogrel associated with CYP2C19 metabolism. AT-10 is metabolically converted to produce the active metabolite of clopidogrel. A major advantage of AT-10 is its more efficient metabolism as compared with the two-step process for clopidogrel. This study evaluates the PD, PK, safety and tolerability of AT-10 in Indian subjects emphasising on the impact of CYP genotype on the pharmacological profiles of both AT-10 (test) and clopidogrel (reference) as previously

Table 2 Summary of treatment emergent adverse events

Events	Treatment		Overall
	A (n=40)	B(n=39)	(n=40)
Gastrointestinal disorders	2 (0.9)	3 (1.3)3	5 (12.5)5
Nausea	2 (0.9)	3 (1.3)3	5 (12.5)5
General disorders and administration site conditions	00.0 (00.0)	1 (0.4)3	1 (2.5)3
Pain	00.0 (00.0)	1 (0.4)1	1 (2.5)1
Fever	00.0 (00.0)	1 (0.4)1	1 (2.5)1
Swelling	00.0 (00.0)	1 (0.4)1	1 (2.5)1
Psychiatric disorders	1 (0.4)1	00.0 (00.0)	1 (2.5)1
Anxiety	1 (0.4)1	00.0 (00.0)	1 (2.5)1
Respiratory, thoracic and mediastinal disorders	1 (0.4)1	00.0 (00.0)	1 (2.5)1
Cough	1 (0.4)1	00.0 (00.0)	1 (2.5)1
Skin and subcutaneous tissue disorders	00.0 (00.0)	1 (0.4)1	1 (2.5)1
Erythema	00.0 (00.0)	1 (0.4)1	1 (2.5)1

Data presented as n (%) E.
Treatment A: AT-10 (2-oxo-clopidogrel bisulfate) tablets 40 mg/10 mg.
Treatment B: Plavix (clopidogrel bisulfate) tablets 300 mg/75 mg.
E, number of events; n, number of subjects with adverse event with particular category.

published studies have shown impact of CYP2C19 polymorphism on clopidogrel responsiveness up to a major extent.[11 20 21]

This study met its primary objective and showed AT-10 is safe and well-tolerated. There were no significant trends or abnormalities in safety measures or in liver function tests, clinical chemistry, haematology and coagulation. Adverse events observed were mostly minor and unlikely related to the study drug.

After LD and MD, AT-10 showed significantly higher platelet inhibition than clopidogrel in not only EMs but also in PMs. This suggests AT-10 may benefit all patients, regardless of genotype, by providing superior platelet inhibition.

The pharmacokinetics of the active metabolite H4 was studied in single and multiple doses. The data showed that the concentration and extent of exposure (Cmax and AUC) of H4 were higher in the AT-10 treatment group compared with the clopidogrel treatment group in both PMs and EMs following LD and MD. It means the pharmacokinetics of clopidogrel is influenced by genetic CYP2C19 polymorphisms to a greater extent than its influence in AT-10 treatment group.

The findings from HOST-EXAM Extended study[22] and clinical practice guidelines[23] (Guideline for the Management of Patients with Chronic Coronary Disease by American Heart Association /American College of Cardiology (AHA/ACC) Joint Committee, in patients with chronic coronary disease treated with PCI) support the use of clopidogrel as the preferred anti-platelet agent over aspirin for the secondary prevention of CAD. This means clopidogrel still has an important place in therapy and thus AT-10 will also have an important place as it overcomes the shortcomings of clopidogrel associated mainly with CYP2C19 polymorphism.

It is of due importance that CYP2C19 remains the predictor of variability in response of clopidogrel and has been clearly evident in previous clinical evaluations.[20] This study also found that participants with CYP2C19 LOF alleles had poor platelet inhibition with clopidogrel, while AT-10 showed consistent platelet inhibition regardless of participants' phenotypes. CYP2C19 polymorphism did not impact the effectiveness of AT-10, suggesting it can be used in all patients unlike clopidogrel, which is affected by 'clopidogrel resistance'. Being a clopidogrel derivative, AT-10 may be a safer alternative for managing cerebrovascular and peripheral vascular diseases.

Since the concentration and extent of exposure of H4 and %IPA are significantly higher in the AT-10 treatment group compared with clopidogrel in both EMs and PMs, there is a need to optimise the dose of AT-10 in target population. Further studies can be planned to determine the appropriate PD equivalent dose of AT-10.

Limitations of investigation

This was a healthy volunteer study with limited sample size. The study examined the effects of AT-10 on PMs and EMs. Further research is needed to evaluate the utility of AT-10 in intermediate, rapid and ultra rapid metabolisers in general patient population. Although we have not evaluated the anti-platelet effect in other metabolisers like rapid/ultra rapid, but we believe that AT-10 is likely to offer similar clinical benefit to clopidogrel.

Further larger studies are required to investigate the influence of CYP2C19 functional polymorphisms on the response to AT-10 dose in the clinical setting and especially

on the risk of recurrent thrombotic events during AT-10 treatment including bleeding risk as compared with clopidogrel therapy.

CONCLUSION

AT-10 showed better inhibition of platelet aggregation in poor metabolizers as compared to Clopidogrel. AT-10 may emerge as a potential alternative to Clopidogrel as an anti-platelet drug. It can be further developed in clinical studies for the unmet medical needs in management of CVDs and overcome the pharmacogenomic limitations of Clopidogrel.

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Competing interests AP is the President of Medical Affairs and Clinical Research Department in the pharmaceutical company Ipca Laboratories Limited. NC is the Senior Vice President of Clinical Research and Development in the pharmaceutical company Ipca Laboratories Limited. VR is currently the Deputy General Manager in Clinical Research and Development department at Ipca Laboratories Limited. KN is the Assistant General Manager in Clinical Data Management and Statistical Analysis at Ipca Laboratories Limited. All authors are employees of Ipca Laboratories Limited that developed AT-10, and all authors were involved in conceptualisation, coordination and execution of the study.

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