Antithrombotic properties of rafigrelide: a phase 1, open-label, non-randomised, single-sequence, crossover study

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Summary
Platelets play a central role in atherothrombotic events. We investigated the effect of a novel platelet-lowering agent, rafigrelide, on thrombus formation and characteristics. In this phase 1, open-label, non-randomised, single-sequence, crossover study, healthy male volunteers received rafigrelide for 14 days (Period 1). Following a ≥6-week washout period, they then received rafigrelide + acetylsalicylic acid (ASA) for 14 days (Period 2). Thrombus formation was assessed ex vivo using the Badimon perfusion chamber, and thrombus characteristics were assessed using thromboelastography. A total of 15 volunteers were enrolled in the study and were assigned to Panel A or Panel B, which had different schedules of assessments. In Panel A, after treatment with rafigrelide alone (Period 1), mean ± standard deviation platelet count was reduced from 283 (± 17) × 10⁹/l at Day 1, to 125 (± 47) × 10⁹/l at Day 14 (n=6) and thrombus area reduced under high and low shear conditions. Reductions in thrombus area under high shear conditions correlated with reductions in platelet count (r²=0.11, p=0.022; n=12). Rafigrelide treatment prolonged clot formation time and reduced clot strength. The addition of ASA to rafigrelide (Period 2) had no additional effect on platelet count or thrombus area under high or low shear conditions. Similar results were seen in Panel B for all parameters. The most common adverse events (≥3 participants per period) were thrombocytopenia and headache. While confirming the platelet-lowering effects of rafigrelide, this early phase study also indicates that rafigrelide has antithrombotic properties under both high and low shear conditions.

Keywords
Rafigrelide, platelet-lowering, thrombus formation, Badimon perfusion chamber, thromboelastography

Introduction
Platelet aggregation and its regulation are central aspects of vascular homeostasis and inflammation. Activated platelets release multiple bioactive mediators that, apart from propagating activation to other platelets, also affect the vascular, inflammatory and blood components of the local and systemic environment (1, 2). This provides a rapid response to injury and helps to initiate tissue repair. However, inappropriate platelet activation can have pathogenic effects in many disorders, such as arteriovenous thrombosis, microangiopathies, atherosclerosis, systemic auto-immunity and cancer metastasis (1). Vessel wall injury also induces platelet activation with increased risk of thrombosis and vessel occlusion (3). The involvement of reactive platelets in disease has long been recognised and a number of platelet-targeting treatments are currently used. As discussed below, these anti-platelet therapies aim either to reduce platelet numbers or to inhibit their aggregation. Many functional assays, such as the Badimon perfusion chamber system (4, 5) and thromboelastography (6, 7), have been developed to study the effects of existing treatments and to aid the search for future therapies. Acetylsalicylic acid (ASA, aspirin) – a cyclooxygenase inhibitor – has been the cornerstone of antiplatelet therapy and is now combined with more powerful agents to inhibit aggregation after platelet activation (8-11) resulting in a reduction in ischaemic events (12-16).

Rafigrelide is a novel, highly selective and potent platelet-lowering agent. It is a chemical analogue of anagrelide, which is used to reduce platelet counts in myeloproliferative disorders (17). Anagrelide has at least two distinct pharmacological activities: 1) inhibition of megakaryocyte maturation and differentiation, resulting in reduced platelets and 2) inhibition of cyclic adenosine triphosphate (cAMP) generation with increased risk of thrombosis and vessel occlusion (3). The involvement of reactive platelets in disease has long been recognised and a number of platelet-targeting treatments are currently used. As discussed below, these anti-platelet therapies aim
monophosphate (cAMP) phosphodiesterases III–V (PDEs III–V), which may lead to vasodilatory effects (18, 19). Compared with anagrelide, rafigrelide has reduced potency against PDE III, which may help to reduce potential side effects (20). The concentration of rafigrelide that produces 50% of the maximum inhibition (IC\(_{50}\)) of PDE III is 164 nM, making it an approximately 200-fold less potent inhibitor of PDE III than 3-hydroxy anagrelide (IC\(_{50}\) 0.9 nM) (Shire Development LLC, data on file).

The objectives of this study in healthy volunteers were to assess: (i) the relationship between platelet count and thrombotic response \textit{ex vivo}, using the Badimon perfusion chamber system (4, 5), when using rafigrelide with and without ASA; (ii) the effect of platelet count on thrombus kinetics as determined by thromboelastography; and (iii) the safety and tolerability of rafigrelide administered with and without ASA.

Materials and methods

Study design and population

This study was approved by the Office for Research Ethics Committees Northern Ireland (EudraCT reference: 2010-018445-60) and the Medicines and Healthcare products Regulatory Authority in the UK. All study procedures were performed in compliance with the Declaration of Helsinki (21) and current Good Clinical Practice guidelines (22).

The study protocol used an open-label, non-randomised, single-sequence, crossover design, with two treatment periods separated by a washout period (Figure 1). Healthy male volunteers aged 18–50 years underwent screening, including detailed medical history, physical examination, laboratory blood tests, thromboelastography, 12-lead electrocardiogram (ECG) and a urine toxicology screen. Individuals with a haemoglobin level of at least 12.0 g/dl and a platelet count of 200–400 \(\times 10^9\)/l were eligible. Individuals who had used anti-aggregant, antiplatelet or anticoagulant therapy, including ASA, non-steroidal anti-inflammatory drugs and paracetamol within the 14 days prior to the first dose of the investigational drug were excluded. Individuals with thromboelastography results outside the normal ranges (time from initiation of clot formation (MA): 54.0–72.5 mm; time to initiate clot formation (R time): 4.0–8.5 minutes (min); kinetics of the rate of clot formation (K time): <4.5 min; maximum amplitude of clot formation (MA): 54.0–72.5 mm) were also excluded.

On inclusion, each participant received an oral dose of 4 mg/day of the hydrogen bromide salt form of rafigrelide for 14 days, in Treatment Period 1. Participants (planned sample size 12) were alternately assigned to Test Panels A and B for pharmacokinetic and pharmacodynamic assessments according to the following schedule: Panel A: Days 1, 7, 14, and 21; Panel B: Days 1, 4, 10, and 18. Participants also returned on Days 4 and 10 in Panel A, and Days 7 and 14 in Panel B for additional haematology assessments (Figure 1). A washout period of at least six weeks separated the treatment periods.

Before the second treatment period (Period 2) commenced, participants’ platelet counts were evaluated to confirm that they had returned to pre-treatment levels. In Period 2, in addition to 14 days of treatment with rafigrelide, participants were given 75 mg/day of ASA from Day 1 until Day 20 (Panel A) or from Day 1 until Day 17 (Panel B) (Figure 1). The participants followed the same panel assignment in both treatment periods. At the end of Treatment Period 2, participants returned for a follow-up visit on Day 28 (14 days after the last dose of rafigrelide).

During each treatment period, participants were admitted overnight to the Royal Victoria Infirmary Clinical Research Facility, Newcastle, UK on four occasions, as determined by the panel schedule. They underwent pharmacokinetic and pharmacodynamic assessments after overnight fasting. Rafigrelide was given, and compliance was monitored by the investigator during these visits. Drug dispensing was recorded on a drug accountability log and case report form. During the remainder of the treatment period when not confined in the clinical research facility, participants took the investigational medicinal product (IMP) at home and utilised diary cards to record daily dosing information (date and time), adverse events (AEs) and any concomitant medications taken. Diaries and bottles (containing the IMP) were returned to the clinical research facility at each visit to check compliance.

The IMP was discontinued if: 1) platelet count fell below 100 \(\times 10^9\)/l; or 2) platelet count fell below 150 \(\times 10^9\)/l and decreased by a mean of more than 20 \(\times 10^9\)/l per day. These participants continued to be monitored and to complete all scheduled study assessments.

Blood sampling

An 18-gauge intravenous catheter was placed in the antecubital vein of each participant. After discarding the initial 1 ml blood samples were collected for pharmacokinetic evaluation (in lithium heparin tubes) and evaluation of clot kinetics using thromboelastography (whole blood). Platelet count was determined from a 3 ml blood sample collected on each assessment day. The catheter was then connected to the Badimon perfusion chamber system for the assessment of platelet-dependent thrombus formation.

Platelet-dependent thrombus formation assessment

Platelet-dependent thrombus formation was assessed \textit{ex vivo} using the Badimon perfusion chamber system (4, 5). The system consisted of three small plexiglass chambers in series; each chamber was lined with a piece of porcine aorta stripped of its intimal layer to expose the underlying thrombogenic tunica media. The first chamber was a unit with a low shear rate (inner lumen diameter: 2.0 mm; vessel wall shear rate: 212 sec\(^{-1}\); mean blood velocity: 5.3 cm/sec; Reynolds number: 30) that simulated flow in a normal coronary artery, and the next two were units with a high shear rate (inner lumen diameter: 1.0 mm; vessel wall shear rate: 1690 sec\(^{-1}\); mean blood velocity: 21.2 cm/sec; Reynolds number: 60) that simulated flow conditions in a moderately stenosed coronary artery. A peristaltic pump at the distal end drew blood directly from the intravenous catheter over the porcine aorta at a constant rate of 10 ml/min for 5 min. The aortae with thrombi were then fixed in 10% buffered formalin for 72 hours and stained with modified...
Masson trichrome stain. The stained thrombi were quantified by planimetry using a Leica DM2000 microscope (Leica Mikrosysteme Vertrieb GmbH, Weltzler, Germany) under ×10 magnification with Image ProPlus 4.0 software (Media Cybernetics, Bethesda, MD, USA). Total thrombus area (TTA) was calculated as the mean of individual thrombus area in individual high shear rate and low shear rate chambers, and expressed as micrometres squared per millimetre (µm²/mm) of aorta. All the slides were analysed by one independent observer (KB) with intra-observer coefficient of variation of 4.1%.

**Assessment of thrombus characteristics**

The visco-elastic properties of thrombi during formation and the early phase of autolysis (clot retraction) were assessed by thromboelastography (Haemonetics Corporation, Braintree, MA, USA). The R time, the K time, α-angle and the MA were recorded to provide data on the speed of clot formation and clot strength. Thromboelastography parameters to assess clot lysis were also recorded: clot lysis₃₀ and clot lysis₆₀ (which indicate the percent decline in amplitude from the MA after 30 min and 60 min, respectively) and estimated plasma lysis.

**Pharmacokinetic analysis**

A blood sample for the measurement of rafigrelide plasma concentration was collected before each analysis in the Badimon perfusion chamber system. The blood samples were collected in lithium heparin tubes and placed on ice. Immediately following collection, the samples were centrifuged at approximately 1500 g for 15 min at 4°C. The separated plasma from each sample was divided equally into two clearly labelled polypropylene transfer tubes and stored at −80°C until analysis. Plasma concentrations of rafigrelide were determined at York Bioanalytical Solutions (York, UK). Rafigrelide calibration standards used in the study were prepared fresh in human plasma on the day of analysis at nominal concentrations of 0.5, 1, 5, 25, 75, 125, 225 and 250 ng/ml. Rafigrelide QC samples at low, medium, and high concentrations (1.5, 100, and 200 ng/ml, respectively) were prepared and stored at −80°C prior to the start of sample analysis.

Briefly, 50 µl of the plasma sample was placed in an Eppendorf tube. An aliquot of 1% formic acid in acetonitrile (20 µl) and 20 µl of internal standard (dissolved in ammonium formate, 50 mM, pH native) was added to each sample, as appropriate. After the addition of ammonium formate (50 mM, pH native) (500 µl), the
samples were vortex mixed and an aliquot (200 µl) transferred to a supported liquid extraction plate (SLE+, 200 mg). Elution was performed with tert-butyl methyl ether (2 x 500 µl) into a 96-well plate. Following evaporation to dryness under nitrogen at 35°C, the extracts were reconstituted in acetonitrile: ammonium formate (10 mM, pH 4) (70:30, v/v, 200 µl).

The collection plate was placed in the auto-sampler tray and ≤20 µl of the sample was injected into an HPLC/MS/MS system. The HPLC/MS/MS analysis was carried out with a Sciex 5000 mass spectrometer coupled with an Acquity LC system. The Acquity LC system consisted of an LC pump with two-channel capability (low-pressure mixing) and an auto-sampler. The autosampler tray was kept at room temperature. The chromatographic separation was achieved on a Gemini 3 µm C18, HPLC column, 50 x 4.6 mm, with mobile phase running under isocratic conditions.

The mass spectrometer was operated in positive Turbo Ion-Spray mode and the resolution setting used was unit for both Q1 and Q3. The multiple reactions monitoring (MRM) transition was 284 m/z → 256 m/z for rafigrelide and the MRM transition was 291 m/z → 263 m/z for the internal standard, D7- rafigrelide. Peak area integrations were performed using Analyst software (version 1.4.2) from MDS Sciex-Applied Biosystems. Peak areas were electronically transferred and peak area ratios were calculated in Watson (version 7.1.0.01). Concentrations were calculated using an eight-point curve ranging from 0.5 to 250 ng/mL with weighted (1/x) linear regression. The lower limit of quantification was 0.5 ng/ml.

### Statistical analysis

The Safety Analysis Set was defined as all participants who received at least one dose of the IMP and had at least one post-dose safety assessment. The Pharmacodynamic Analysis Set was defined as all participants from the Safety Analysis Set with at least one evaluable post-dose pharmacodynamic assessment (platelet count, Badimon chamber or thromboelastography evaluation). The Pharmacokinetic Set was defined as all participants who received at least one dose of the IMP and had at least one measurable plasma concentration of the drug. The sample size of this study was not based on statistical power considerations since no formal statistical analyses between treatments were planned. Results are
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Results

Disposition

In total, 15 volunteers received at least one dose of rafigrelide and were included in the Safety, Pharmacodynamic and Pharmacokinetic Analysis Sets for their respective panels across the study period, as illustrated in Figure 2. One participant in Panel A withdrew from the study before the start of Period 2, without giving a specific reason. For two further individuals in Panel A, the drug was withdrawn during Period 2. One withdrawal occurred after Day 12 because of a protocol violation (ingestion of non-steroidal anti-inflammatory drugs) and the participant was not included in the remaining pharmacokinetic and pharmacodynamics assessments. The second withdrawal occurred after Day 10 because the participant developed mild thrombocytopenia on Day 10 (platelet count 140 (10⁹/l); this individual continued to have all study assessments until the end of Period 2 and was considered as having completed the study period. Replacement volunteers were recruited accordingly to ensure that at least six participants completed each of the study periods in each panel. In total, 12 participants completed Period 1 (6 in each panel) and 13 participants completed Period 2 (7 in Panel A and 6 in Panel B).

The mean (± SD) age of all participants was 33.1 ± 6.0 years: 32.1 ± 5.4 years for the nine participants in Panel A and 34.5 ± 7.1 for the six participants in Panel B. Mean (± SD) body mass index was 25.3 ± 2.2 kg/m² for Panel A and 25.3 ± 2.4 kg/m² for Panel B. All of the participants in Panel B were white and non-hispanic, and there were eight white, non-hispanic participants and one Asian participant in Panel A.

Figure 3: Effect of rafigrelide on platelet count. The effect of rafigrelide on (a) platelet count and (b) mean platelet volume during the study when administered alone (Period 1) and in combination with acetylsalicylic acid (Period 2). Treatment with rafigrelide was stopped at Day 14 in both periods for both Test Panels. ASA administration stopped on Day 20 for Test Panel A and day 17 for Test Panel B during Period 2. Data are shown as mean with error bars showing one standard deviation.
Platelet count

Following administration of rafigrelide in Period 1, participants in Panel A demonstrated a reduction in mean (± SD) platelet count from 283 (± 17) × 10^9/l at baseline to 125 (± 47) × 10^9/l at Day 14 (Figure 3a). Rafigrelide was no longer administered after Day 14, and by Day 21 the platelet count had partially recovered [206 (± 48) × 10^9/l], but was still less than at baseline. A similar pattern was seen in Panel A when the drug was co-administered with ASA (Period 2); platelet count decreased from 281 (± 33) × 10^9/l at baseline to 121 (± 29) × 10^9/l at Day 14, and recovered to 239 (± 41) × 10^9/l after treatment cessation, at Day 21.

There was also a reduction in platelet count in treatment Period 1, in Panel B, from 250 (± 25) × 10^9/l at baseline to 160 (± 51) × 10^9/l at Day 14. Platelet counts were taken until Day 18 for volunteers in Panel B, and no recovery was seen after treatment cessation at this time point. Similar results were seen in Period 2, after co-treatment of rafigrelide and ASA.

For both panels, at the final follow-up that occurred 14 days after the last dose of rafigrelide (Day 28), platelet counts had generally returned to baseline levels (data not shown).

Mean platelet volume

Mean platelet volume (MPV) was assessed along with platelet count during all the visits. In Treatment Period 1, following administration of rafigrelide, participants in Panel A demonstrated an increase in mean (± SD) MPV from 10.7 (± 0.9) fL at baseline to 12.2 (± 0.8) fL at day 14 (Figure 3b). Rafigrelide was no longer administered after Day 14, and by Day 21 mean MPV was 12 (± 0.9) fL, higher than at baseline. A similar pattern was seen in Panel A when the drug was co-administered with ASA (Period 2); mean MPV increased from 10.7 (± 0.6) fL at baseline to 12.2 (± 0.9) fL at Day 14, and reduced to 11.8 (± 0.8) fL after treatment cessation, at day 21.

There was also an increase in mean MPV in Treatment Period 1, in Panel B, from 10.1 (± 0.6) fL at baseline to 11.5 (± 1.1) fL at day 14. MPVs were taken until day 18 for volunteers in Panel B,
and no recovery was seen after treatment cessation at this time point. Similar results were seen in Period 2, after co-treatment of rafigrelide and ASA.

MPVs had generally returned to baseline levels for both panels at the final follow-up that occurred up to 14 days after the last dose of rafigrelide (Day 28) (data not shown).

Platelet-dependent thrombus formation

Under both high and low shear stress conditions, treatment with rafigrelide generally led to reductions in TTA (Figure 4). These reductions in TTA tended to occur in conjunction with reductions in platelet count under both high and low shear conditions, with moderate correlations between the parameters (Figure 5). Under high shear conditions, there was a reduction from baseline in mean TTA at Day 14 in Panel A after administration of rafigrelide alone [mean (± SD) at baseline of 22,613.3 ± 1,220.9 μm²/mm aorta vs 11,930.0 ± 5,348.2 μm²/mm aorta on Day 14; Figure 4a]. Although there was a slight increase in TTA from Day 1 to Day 4 in Panel B (15,893.7 ± 10,052.1 μm²/mm on Day 1 to 19,413.7 ± 7,869.7 μm²/mm on Day 4), by Day 10 a reduction was seen (14,618.3 ± 6,312.1 μm²/mm on Day 10). There was no clear difference between rafigrelide administered alone or in combination with ASA in either panel. Under low shear conditions, there were generally small reductions in TTA after rafigrelide was administered with or without ASA in both panels, and no apparent recovery after treatment stopped at Day 14 (Figure 4b).

Thrombus characteristics

Administration of rafigrelide alone in Panel A increased time to initiate clot formation (R time). In this group, the mean (± SD) R time increased from 5.17 ± 0.80 min at baseline to 8.25 ± 2.33 min on Day 14 (Figure 6a). In Panel B, R time increased from 6.35 ± 1.29 min at baseline to 7.05 ± 2.09 min on Day 10. After drug withdrawal, R time returned towards baseline to 6.07 ± 1.93 min on Day 21 for Panel A, but remained constant until Day 18 in Panel B (7.10 ± 2.24 min). A similar pattern was seen for Panel A when rafigrelide was co-administered with ASA; R time increased from baseline to Day 14 and decreased after drug withdrawal. In
Panel B, treatment with a combination of ASA and rafigrelide had little effect on mean R time between Days 1 and 10 (8.48 ± 1.09 min on Day 1; 8.20 ± 1.78 min on Day 10) but there was a small reduction in R time by Day 18 in these participants (7.02 ± 2.65 min). Similar patterns were seen for K time and α-angle (see Suppl. Table 1, available online at www.thrombosis-online.com).

On assessment of the effects of rafigrelide on MA (a measure of clot strength), in Panel A, MA decreased with time after initiation of treatment in both Periods 1 and 2, and increased again after treatment cessation indicating a reduction in clot strength with drug treatment that recovers after drug withdrawal (Figure 6b). In Panel B, treatment with rafigrelide, both alone and when co-administered with ASA, did not have a marked effect on MA over time up to Day 18 of the study.

Rafigrelide with or without ASA had no discernible effect on lysis as measured by clot lysis30, clot lysis60 and estimated plasma lysis (see Suppl. Table 2, available online at www.thrombosis-online.com).

Pharmacokinetics

Plasma concentrations of rafigrelide were generally similar in both panels when administered alone (Period 1) or in combination with ASA (Period 2; Suppl. Table 3, available online at www.thrombosis-online.com).

Tolerability, adverse events and safety

A summary of the AEs recorded during the study is shown in Table 1. Most of the AEs during both treatment periods were mild to moderate in severity, and were considered to be related to the IMP, but rarely led to drug withdrawal. Although the overall incidence and number of AEs was slightly higher when rafigrelide was administered in combination with ASA, this was primarily due to single event instances, in different volunteers, of palpitations, nasopharyngitis, contusion, migraine, cough, nasal congestion and four instances (in one participant) of mild photophobia. The most common AEs (occurring in at least three individuals during any period) for rafigrelide both as monotherapy and in

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Figure 6: Effect of rafigrelide on clot formation parameters. The effect of rafigrelide alone (Period 1) and in combination with acetylsalicylic acid (Period 2), on clot formation parameters R time (a) and maximum amplitude (MA; b), as measured by thromboelastography. Treatment with rafigrelide was stopped at Day 14 in both periods for both Test Panels. ASA administration stopped on Day 20 for Test Panel A and Day 17 for Test Panel B during Period 2. Data are shown as mean with error bars showing one standard deviation.
combination with ASA were thrombocytopenia (defined as a platelet count <150 × 10^9/l; 9 and 10 events, respectively) and headache (11 and 14 events, respectively).

One participant developed severe thrombocytopenia with platelet count falling below the normal range (150–450 × 10^9/l) to 82 × 10^9/l on Day 14 (last dosing day). The platelet count continued to fall to below the clinically important level of 75 × 10^9/l to 50 × 10^9/l on Day 17 and to 39 × 10^9/l on Day 18. This was reported as a serious AE although the participant remained asymptomatic. Without intervention his platelet count increased to 75 × 10^9/l on Day 20, 144 × 10^9/l on Day 21 and 410 × 10^9/l on Day 27. In another participant, rafigrelide was withdrawn on Day 10 because his reduced platelet count (140 × 10^9/l) met the protocol-defined level for cessation of the IMP (platelet count below 150 × 10^9/l or reduction of ≥60 × 10^9/l over 3 days). Subsequently, this platelet count recovered to baseline levels without intervention. AEs of each participant have been provided separately (Suppl. Table 4, available online at www.thrombosis-online.com).

There were no changes in routine laboratory assessment results including electrolytes, and kidney and liver function tests, and no clinically relevant changes to vital signs, physical examination results or ECG that were considered as causally related to the IMP.

### Discussion

In this early phase study of rafigrelide, a novel platelet lowering agent, we report a reduction in circulating platelets and thrombus size together with delayed initiation of clot formation and reduced clot strength. The addition of ASA to rafigrelide did not affect any of the recorded parameters.

The Badimon perfusion chamber system is a validated ex vivo model of vessel injury and thrombosis (4, 23), and has previously been used to evaluate the effects of novel antithrombotic agents in human participants in different disease states (24-26). The model enables the measurement of thrombus formation in native (non-anticoagulated) whole blood triggered by exposure to physiologically relevant substrate (collagen in tunica media), and under different rheological conditions mimicking flow in stenosed coronary arteries. We have used this perfusion system to evaluate the effects of anti-platelet therapy on thrombus formation in various high-risk populations in our laboratory (27-32). Thromboelastography is a point of care assay that assesses visco-elastic forces in whole blood under low shear conditions during coagulation in vitro. It measures the strength of the thrombus (fibrin-platelet binding) as it forms (33) and is widely used in different clinical areas (33-35).

The role of platelets in thrombus formation is well established. Platelets become activated when they come into contact with a thrombogenic surface such as injured endothelium or an artificial surface such as a stent, vascular graft, or cardiopulmonary or haemodialysis equipment (35). This in turn triggers a complex cascade involving cellular and non-cellular components leading to thrombus formation at the site of injury (36). The role of platelets as mediators and regulators of inflammation in thrombotic events (37), and the contribution of P-selectin-mediated platelet-leukocyte aggregates (38) and tissue-factor-positive platelet-derived microparticles (39) in atherothrombosis, has been studied extensively.

Successful antithrombotic therapy balances improvements in ischaemic outcomes whilst minimising bleeding risk. In spite of optimal therapy with modern antiplatelet drugs (40, 41), patients presenting with atherothrombotic disease typically continue to
have high rates of recurrent events around one year after initiating therapy. Hence there is a need for improvement in the currently available antithrombotic therapies. Newer therapies such as factor Xa inhibitors, direct thrombin inhibitors, nitric oxide donors, and collagen- and ristocetin-mediated platelet aggregation inhibitors are in various stages of development (42-46).

Our data show rafigrelide to have platelet-lowering properties and that these translate into a reduction in platelet-dependent thrombosis. Although mean platelet counts were similar to baseline at Day 4, steady reductions were observed thereafter until treatment cessation. Following abrupt cessation of rafigrelide treatment on Day 14, the platelet count remained low for 3-4 days before increasing toward baseline levels. Platelet counts had generally returned to baseline levels at the final follow-up visit.

The reduction in platelet count was associated with a reduction in mean thrombus area in both low- and high-shear environments, mimicking rheologies of arterial and venous flow conditions, respectively. Under high-shear conditions, the reduction in thrombus area was more pronounced and there was a moderate correlation between platelet count and thrombus area, as would be expected given the greater role of platelets in arterial thrombosis (platelet-rich) compared with thrombus formation in veins (fibrin-rich) under low-shear conditions.

Seven days after the last dose of rafigrelide, reversal of the antithrombotic effect was evident. With the addition of ASA, there were no discernible additional effects on platelet count or TTA beyond that seen with rafigrelide alone. Rafigrelide delayed initiation of clot formation (R time) and provided small reductions in MA, a measure of clot strength.

The adverse events reported with rafigrelide over each two-week treatment period were mild to moderate in nature, although one serious case of thrombocytopenia was observed, which resolved after IMP cessation and without any additional intervention. The platelet count fell below the protocol-defined threshold for study termination in only two individuals. There were no bleeding complications in any of the participants during the treatment or follow-up periods. The occurrence of photophobia, reported in one individual in this study, is another AE that may be of interest in future studies. Overall, in this early phase study of the pharmacodynamic and pharmacokinetic effects of rafigrelide on healthy volunteers, the product was found to be well tolerated.

In conclusion, the new platelet-lowering agent rafigrelide showed antithrombotic properties during a two-week treatment period in healthy male volunteers. Importantly, the reductions in thrombus formation were seen in surrogate models of both high and low shear rates suggesting drug efficacy in different arterial conditions. The antithrombotic effect of rafigrelide in this early phase study suggests that further clinical studies involving different doses of rafigrelide, longer therapy duration and measuring other elements of coagulation system to confirm its mechanism of action are warranted.

**Limitations**

In addition to platelets, a number of cellular and non-cellular components of the coagulation system are also involved in thrombus formation and these were not measured in our study. Though we measured platelet numbers and MPV, neither platelet reactivity nor fibrinogen were measured.

**Conflicts of interest**

Medical writing and editorial support was provided by Dr Rosalind Morley from PharmaGenesis™ London, UK and Dr Steven Inglis from Oxford PharmaGenesis™ Ltd, Oxford, UK, and was funded by Shire Pharmaceuticals. J. Dragone, R. Grose-Hodge, P. Martin, S. Troy and P. Preston are employees of Shire Pharmaceuticals.

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