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Thrombin Exosite 1 Inhibition with JNJ-64179375

Inhibits Thrombus Formation in a Human Translational Model of Thrombosis

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Abstract

Aims

JNJ-64179375 (hereafter JNJ-9375) is a first-in-class, highly specific, large molecule, exosite 1 thrombin inhibitor. In preclinical studies, JNJ-9375 demonstrated robust antithrombotic protection with a wider therapeutic index when compared to apixaban. The purpose of the present study was to examine for the first time the antiplatelet, anticoagulant and antithrombotic effects of JNJ-9375 in a translational model of ex vivo human thrombosis.

Methods and Results

Fifteen healthy volunteers participated in a double-blind randomized crossover study of JNJ-9375 (2.5, 25 and 250 μg/mL), bivalirudin (6 μg/mL; positive control) and matched placebo. Coagulation, platelet activation and thrombus formation were determined using coagulation assays, flow cytometry and an ex vivo perfusion chamber respectively.

JNJ-9375 caused concentration-dependent prolongation of all measures of blood coagulation (prothrombin time, activated partial thromboplastin time, thrombin time; p<0.001 for all) and agonist selective inhibition of thrombin (0.1 U/mL) stimulated platelet p-selectin expression (p<0.001) and platelet-monocyte aggregates (p=0.002). Compared to placebo, JNJ-9375 (250 μg/mL) reduced mean total thrombus area by 41.1% (95% confidence intervals, 22.3 to 55.3%; p<0.001) at low shear and 32.3% (4.9 to 51.8%; p=0.025) at high shear. Under both shear conditions, there was a dose-dependent decrease in fibrin-rich thrombus (p<0.001 for both) but not platelet-rich thrombus (p=ns for both).

Conclusion

Exosite 1 inhibition with JNJ-9375 caused prolongation of blood coagulation, selective inhibition of thrombin-mediated platelet activation, and reductions in ex vivo thrombosis driven
by a decrease in fibrin-rich thrombus formation. JNJ-9375 represents a novel class of anticoagulant with potential therapeutic applications.
Introduction

The coagulation cascade plays a central role in thrombosis and the pathophysiology of thromboembolic events, the leading cause of global mortality.\(^1\) Anticoagulants are of proven benefit in a wide range of thromboembolic disorders, but despite recent improvements, important limitations persist. All the currently licensed agents, including direct oral anticoagulants (DOACs), act to either inhibit thrombin generation or block the active site of the protease directly.\(^2\) Consequently they provide broad inhibition of all thrombin activity, which although efficacious, invariably fails to discriminate between protease interactions relating to thrombosis and those essential to haemostasis. Treatment related bleeding remains a major concern and for many patients this leads to dosing restrictions or exclusion from anticoagulation altogether.\(^3-9\)

JNJ-64179375 (hereafter JNJ-9375) is a first-in-class, recombinant, fully human, IgG4 monoclonal antibody anticoagulant that binds reversibly and with high affinity and specificity to the exosite 1 region of thrombin.\(^10\) Exosite 1 is a positively charged domain on the surface of thrombin that together with exosite 2 serves to regulate enzymatic activity of the protease by providing an initial binding site for substrates, co-factors and inhibitors.\(^11-13\) JNJ-9375 therefore acts to inhibit the interaction of thrombin with its exosite 1 substrates, which include fibrinogen, but retains function of both the active site and exosite 2.\(^10\) This capacity to inhibit fibrinogen binding while preserving other (non-exosite 1) protease interactions offers the potential for a wider therapeutic index, and in preclinical animal models JNJ-9375 was associated with substantially less bleeding when compared to apixaban at doses of equivalent antithrombotic efficacy.\(^10\) In the present study we sought to examine for the first time the anticoagulant and antithrombotic effects of exosite 1 thrombin inhibition with JNJ-9375 in human blood using a translational model of \textit{ex vivo} thrombosis.
Methods

Study Population

Healthy non-smoking male and female volunteers aged between 18 and 45 years (inclusive) with a body-mass index (BMI) of 18 to 35 kg/m² were enrolled in this study. All volunteers underwent a detailed screening assessment for eligibility. Exclusion criteria included women who were pregnant or still lactating, or any clinically significant coexisting condition including hypertension, hyperlipidaemia, diabetes mellitus, cardiovascular disease, recent infective or inflammatory condition, coagulopathy, known liver disease or screening blood tests indicative of renal, liver, clotting, thyroid or haematological abnormality. Volunteers were not permitted to take any prescription or non-prescription medication (including acetylsalicylic acid, paracetamol, vitamins and herbal supplements) within 14 days of an experimental visit. Prior to each visit, volunteers must have abstained from alcohol for 24 hours and food including caffeine-containing products for 8 hours. Informed written consent was obtained from all volunteers before enrolment. The study was approved by the local research ethics committee (reference 16-HV-025) and conducted in accordance with the Declaration of Helsinki.

Study Design

This was a double-blind randomised controlled five-way crossover study conducted at a single site (Clinical Research Facility, Royal Infirmary of Edinburgh, Scotland) between the 24th May 2016 and 1st July 2016. Study measures were performed during extracorporeal infusion of JNJ-9375 (estimated final concentration of 2.5, 25 and 250 μg/mL), bivalirudin (positive control; estimated final concentration of 6 μg/mL; The Medicines Company, Abingdon, UK) at a dose equivalent to recommendations at the time of percutaneous coronary intervention (PCI), and matched placebo (10 mM phosphate, 8.5% (w/v) sucrose, 0.04 % (w/v) polysorbate 20, 10 μg/mL EDTA, pH 7.1; Janssen Research and Development) upstream of the perfusion chambers. Three perfusion chamber studies were performed at the first experimental visit and two perfusion chamber studies at the second experimental visit.
Study Objectives

The primary objective was to assess the relationship of JNJ-9375 dose concentrations to *ex vivo* thrombus formation under conditions of both low and high shear stress, and to compare these effects with placebo under the same rheological conditions. Bivalirudin, which blocks both exosite 1 and the active site of thrombin, was used as a positive control. Secondary objectives included a similar comparison of compound effects on platelet activation, markers of coagulation, and the fibrin and platelet components of thrombus formation. Finally, correlations between measured chamber concentrations of study drug and pharmacodynamic end-points were explored.

Perfusion Chamber Experiment

Thrombus formation was assessed using the Badimon chamber, a well validated perfusion model for measuring the effect of study drugs on *ex vivo* human thrombus formation.\(^{14,21}\) In brief, a pump was used to draw native (unanticoagulated) blood from an antecubital vein directly through a series of three cylindrical perfusion chambers maintained at 37°C in a water bath. Each chamber contained a strip of porcine aorta from which the intima and a thin layer of media had been removed. Rheological conditions in the first chamber were set to simulate those of patent medium-sized arteries (inner lumen diameter, 2.0 mm; vessel wall shear rate, 212 s\(^{-1}\); mean blood velocity, 5.3 cm/s; Reynolds number: 30), whereas those in the second and third chambers were set to simulate those of mild to moderately stenosed coronary arteries (inner lumen diameter, 1.0 mm; vessel wall shear rate: 1690 s\(^{-1}\); mean blood velocity, 21.2 cm/s; Reynolds number: 60). Shear conditions at the vessel wall were calculated from the theoretical expression for shear rate given for a Newtonian fluid in tube flow.\(^{22,23}\) Each study lasted for exactly 5 min during which flow was maintained at a constant rate of 10 mL/min. All studies were performed using the same perfusion chamber and by the same operator.
Study Outcome Measures

Chamber concentrations of study drug

Blood samples for determination of serum JNJ-9375 and plasma bivalirudin concentrations were taken immediately distal to the perfusion chamber into 3.5 mL serum gel and 2.7 mL sodium citrate (3.2%) tubes (Becton-Dickinson, Cowley, UK). JNJ-9375 samples were allowed to clot for 30 min then centrifuged at 1500 g (20 °C) for 20 min. Bivalirudin samples were centrifuged at 1500 g (15 °C) for 15 min within 1 hour of collection. Samples were then aliquoted and stored immediately at -70 °C before analysis. Concentrations of JNJ-9375 were determined by electrochemiluminescence using the Meso Scale Discovery platform and plate reader (Rockville, Maryland, USA). JNJ-9375 concentrations were regressed from the standard curve in Watson LIMS (version 7.4.1, Thermo, PA, USA) using a five-parameter logistic regression model with $1/Y^2$ weighting.

Coagulations assays

Blood samples for coagulations assays (prothrombin time, activated partial thromboplastin time and thrombin time (undiluted and diluted)) were collected immediately distal to the final perfusion chamber into 4.5 mL sodium citrate (0.38% final v/v) tubes (Becton-Dickinson). Samples were centrifuged at 1500 g (15 °C) for 20 min within 1 hour of collection. Plasma was then aliquoted and stored immediately at -70 °C before analysis using a STA-Compact-Max analyser (Stago, Parsippany, NJ, USA). The following reagents were used, for prothrombin time, STA-Neoplastine CI Plus, for activated partial thromboplastin time, STA-PTT Automate, and for thrombin time, STA-Thrombin.

Platelet activation

Platelet p-selectin expression and platelet-monocyte aggregates are sensitive markers of in vivo platelet activation. Blood (2.7 mL) was collected immediately distal to the final perfusion chamber into tubes containing 0.3 mL of 3.8% sodium citrate and Pefabloc FG (final
concentration 1.5 mg/mL; Quadratex Diagnostics, Surrey, UK). After 5 min, samples were aliquoted into Eppendorfs pre-filled with or without agonist (adenosine diphosphate 20 μM, Sigma-Aldrich, Gillingham, UK; human alpha thrombin 0.1 U/mL, Enzyme Research Laboratories, Swansea, UK) and the following conjugated monoclonal antibodies: allophycocyanin (APC)-conjugated CD14, phycoerythrin (PE)-conjugated CD62P and fluorescein isothiocyanate (FITC)-conjugated CD42a (Becton-Dickinson). All antibodies were diluted 1:10. Samples were incubated for 15 min at room temperature before fixing with 1 % paraformaldehyde (p-selectin) or FACS-Lyse (Becton-Dickinson; platelet-monocyte aggregates). All samples were analysed within 24 h using a FACSCalibur flow cytometer (Becton-Dickinson). Data analysis was performed using FlowJo v10 (Treestar, Oregon, USA).

**Thrombus Formation**

After each perfusion experiment, the porcine strips with attached thrombus were removed and fixed in 4 % paraformaldehyde for 72 h at 4 ºC prior to being prepared for histological analysis. As thrombus forms longitudinally along the entire length of the exposed porcine aortic strip, the mean cross-sectional area gives a reliable representation of total thrombus formation. Following fixation, the proximal and distal 1 mm of the exposed substrate were discarded and the remainder cut into eight segments. Segments were embedded in paraffin wax and 4-μm sections prepared.

To detect total thrombus area, endogenous hydrogen peroxide activity was blocked using 3 % hydrogen peroxide solution (Leica Microsystems GmbH, Wetzlar, Germany) for 5 minutes. Sections were then incubated at room temperature for 1 hour with polyclonal rabbit anti-human fibrin(ogen) antibody (1.2 μg/mL, Dako, Glostrup, Denmark; Cat. No. A0080) and monoclonal mouse anti-human CD61 antibody (1.28 μg/ml, Dako; Cat. No. M0753). Antigen visualisation was performed using a Bond Polymer refine detection kit (Leica Microsystems GmbH) and treatment with 3,3’-diaminobenzidine substrate chromogen (66 mM, Dako). Finally,
sections were counterstained with a modified Masson’s trichrome (hematoxylin and sirius red 0.1 %).

To examine the effect of study drug(s) on fibrin-rich and platelet-rich thrombus formation, endogenous hydrogen peroxide activity was blocked using 3 % hydrogen peroxide solution (VWR, Radnor, PA, USA) for 10 min and non-specific binding blocked using 20 % normal goat serum (Biosera, Nuaille, France) in Tris-Buffered Saline with 0.01% Tween (TBST). Sections were then incubated with polyclonal rabbit anti-human fibrin(ogen) antibody (1.2 μg/ml) to detect fibrin and CD61 monoclonal mouse anti-human antibody (0.32 μg/ml) to detect platelets. Following TBST washes, goat anti-rabbit peroxidase (1:500; Abcam, Cambridge, UK) was applied and the presence of antigen visualised with Tyramide Cy3 (1:50; Perkin Elmer, Boston, MA, USA; Cat. no. NEL744B001KT) and FITC (1:50; Perkin Elmer, Waltham, MA, USA; Cat. no. NEL741B001KT) before nuclear counterstaining with DAPI (5 μg/ml; Sigma-Aldrich; Cat. No. D9542).

A semi-automated slide scanner (Axioscan Z1; Zeiss, Jena, Germany) and image analysis software (Definiens, Munich, Germany) were used by a blinded operator to quantify thrombus area and composition. Digital images of each section were acquired at ×20 magnification. High-resolution classifiers based on colour were established to detect total thrombus area, fibrin-rich thrombus area and platelet-rich thrombus area.

Statistical Analysis

After study completion, the database was locked and all statistical analyses carried out by an independent statistician. Categorical variables are expressed as percentages, continuous variables are expressed as mean ± standard deviation (SD). The effects of study compounds on study end-points were assessed by general linear mixed effect models with period and study compound as fixed effects, subjects as random effects. Chamber end-points were log-
transformed and assessed separately by shear rate (low and high). From the models, point and interval estimates for means and mean differences versus placebo (absolute and %) were generated and analysed using the Least Significance Difference (LSD) test. The correlation between plasma JNJ-9375 concentrations and study end-points were determined by Pearson’s (r) or Spearman’s rank-order correlation (ρ) as appropriate. Two-sided p values of ≤0.05 were considered statistically significant. All statistical calculations were performed using SAS version 9.4.
Results

All 15 enrolled volunteers (10 male) completed the study in full, with no safety concerns. Mean age of the volunteers was 26±5 years with a body-mass index of 24±3 kg/m².

Chamber concentrations of study drug

Compound concentrations in the effluent of the perfusion chamber (JNJ-9375 1.93±0.68, 22.3±5.86, and 214.0±20.8 µg/mL; bivalirudin 6.92±11.3 µg/mL) closely matched the targeted concentrations (JNJ-9375 2.5, 25 and 250 µg/mL; bivalirudin 6 µg/mL).

Effect of JNJ-9375 on coagulation assays

JNJ-9375 caused dose-dependent prolongation of all measured blood coagulation markers, with thrombin time the most sensitive to the anticoagulant effect (Table 1). Pearson’s correlation coefficient between chamber plasma concentrations of JNJ-9375 and coagulation assays was 0.98 for prothrombin time, 0.87 for activated partial thromboplastin time, and 0.91 for thrombin time (p<0.001 for all; Supplementary S1).

Effect of JNJ-9375 on ex vivo platelet activation

Compared to placebo, JNJ-9375 2.5, 25 and 250 µg/mL inhibited thrombin (0.1 U/mL) stimulated platelet p-selectin expression (geometric mean fluorescent intensity, GMFI) by 46.5% [95% confidence intervals (CI), 4.6 to 97.5%; p=0.07], 95.2% [95% CI, 43.2 to 147.2%; p<0.001] and 99.0% [95% CI, 46.1 to 151.9%; p<0.001] and platelet-monocyte aggregates (GMFI) by -3.4% [95% CI, -56.1 to 49.4%; p=0.90], 56.3% [95% CI, 2.2 to 110.4%; p=0.04] and 69.9% [95% CI, 16.2 to 123.6%; p=0.01]. Chamber plasma concentrations of JNJ-9375 correlated with both platelet p-selectin expression (ρ=-0.83, p<0.001) and platelet-monocyte aggregates (ρ=-0.64, p<0.001). In contrast, JNJ-9375 had no effect on ADP (20 µM) stimulated platelet activation (p=ns for all). Bivalirudin exhibited a similar selective profile (Table 1; Figure 1).
Effect of JNJ-9375 on ex vivo thrombus formation

Ex vivo total thrombus formation was reduced at both low and high shear stress at the 250 μg/mL concentration (Figure 2). Compared to placebo, JNJ-9375 (2.5, 25 and 250 μg/mL) reduced mean total thrombus area by -7.4% (95% CI, -41.6 to 18.5%; p=0.60), 6.6% (95% CI, -23.1 to 29.2%; p=0.62) and 41.1% (95% CI, 22.3 to 55.3%; p<0.001) at low shear and by 9.8% (95% CI, -26.6 to 35.7%; p=0.54), 3.3% (95% CI, -35.8 to 31.1%; p=0.85) and 32.3% (95% CI, 4.9 to 51.8%; p=0.025) at high shear. Chamber plasma concentrations of JNJ-9375 correlated with total thrombus area at low (ρ=-0.56, p<0.001) and high (ρ=-0.32, p=0.03) shear (Supplementary Figure S1).

Reductions in total thrombus area were driven by a dose-dependent decrease in fibrin-rich thrombus deposition under both shear conditions (Figure 3). At peak dose (250 μg/mL), JNJ-9375 reduced fibrin-rich thrombus area by 59.5% [95% CI, 37.8 to 73.7%; p<0.001] at low shear and 51.8% [95% CI, 37.7 to 62.7%; p<0.001] at high shear. There was no reduction in platelet-rich thrombus area (p=ns for all). Chamber plasma concentrations of JNJ-9375 correlated with fibrin-rich thrombus area at low (ρ=-0.66, p<0.001) and high (ρ=-0.70, p<0.001) shear (Supplementary Figure S1).

Effect of bivalirudin on ex vivo thrombus formation

Bivalirudin reduced total thrombus area at both low and high shear, also driven by a decrease in fibrin-rich thrombus formation (Figures 2 and 3). In contrast to JNJ-9375, there was a modest reduction (p=0.01) in platelet-rich thrombus formation at high shear (Figure 3).
Discussion

In this double-blind randomised controlled crossover study, *ex vivo* administration of JNJ-9375, a highly specific exosite 1 thrombin inhibitor, resulted in dose-dependent prolongation of blood coagulation and selective inhibition of thrombin-stimulated platelet activation. Thrombosis was reduced under rheological conditions of both low and high shear stress, driven principally by a reduction in fibrin-rich thrombus formation. We conclude that JNJ-9375 holds promise as an anticoagulant for the prevention and treatment of thromboembolic events, and our results provide further insights into the role of exosite 1 in human thrombogenesis.

The outstanding challenge in anticoagulation is the development of drugs that can provide equivalent (or superior) antithrombotic efficacy but with a significantly lower bleeding risk. While the safety of JNJ-9375 has yet to demonstrated in clinical trials, several lines of evidence indicate the potential for favourable outcomes. On a mechanistic level, selective inhibition of thrombin through exosite 1 specific antagonism is attractive because of the potential to inhibit fibrinogen binding without overly interfering with other (active site and exosite 2 dependent) protease interactions relating to haemostasis. For example, both the active site and exosite 2 are involved in catalytic feedback activation of clotting cofactors V, VIII, XI and XIII, with deficiencies of each of these factors associated with bleeding diatheses.28-31

Thrombin is also a potent platelet agonist, and whilst over-aggregation may lead to pathological events, early platelet responses are central to haemostasis. Thrombin activates platelets through binding to platelet surface GPIb and protease-activated receptors 1 (PAR1) and 4 (PAR4).32 Exosite 1 interacts with PAR1 to facilitate efficient receptor cleavage,33 whereas PAR4 activation and GPIb binding are largely dependent on the active site and exosite 2 respectively.34,35 In the present study, JNJ-9375 selectively inhibited thrombin stimulated platelet activation but was not associated with a reduction in platelet deposition. This is consistent with
previous reports that exosite 1 inhibition only weakly inhibits thrombin-induced platelet aggregation and does not affect platelet collagen binding.\textsuperscript{36,37} Collectively, these results suggest potentially favourable differential effects on thrombin-platelet responses, that could be especially useful in clinical situations where combined treatment with an antiplatelet is required.\textsuperscript{38,39} This is speculative and requires further exploration. Future studies examining the effects of JNJ-9375 on platelet adhesion, thrombosis and bleeding, alone and in combination with existing antiplatelet agents would be of interest.

Mechanistic evidence that exosite 1 thrombin inhibition may be associated with a low haemorrhagic potential is supported by data from animal studies of thrombosis and bleeding. Using a baboon arteriovenous shunt model, Cadroy and colleagues found that exosite 1 thrombin inhibition prevented thrombus formation, but did not affect the ability to form haemostatic plugs.\textsuperscript{37} More recently, JNJ-9375 demonstrated a substantially wider therapeutic index when compared to apixaban in rats and cynomolgus monkeys.\textsuperscript{10} Further insight comes from the case report of an anti-exosite 1 thrombin IgA antibody (from which JNJ-937 was subsequently synthesised to mimic) identified in a patient presenting with a large traumatic sub-dural haematoma and persistently abnormal clotting studies.\textsuperscript{40} Despite evidence of intense anticoagulation (prothrombin time, 40 s; activated partial thromboplastin time, 240 s; thrombin time with bovine thrombin, 173 s), the patient made a full recovery without surgical intervention and had no abnormal bleeding events during 8 years of follow up.

Anticoagulants must in addition to avoiding unwanted bleeding provide clinically efficacious antithrombotic protection. Examination of the effect of exosite 1 thrombin inhibition on human thrombosis has previously been limited to studies using heparinised blood in a rabbit aortic angioplasty model\textsuperscript{41} and cone and plate chamber.\textsuperscript{42} This is the first description of the \textit{ex vivo} antithrombotic effects of exosite 1 thrombin inhibition in native human blood under flow conditions. At a dose of 250 µg/mL, JNJ-9375 reduced total thrombus area by over 40% and
30% at low and high shear respectively. Under the same conditions, high dose bivalirudin (equivalent to that used at the time of PCI) reduced thrombus formation by 65% at low shear and 56% at high shear; while in previous studies reductions of 14% with heparin (70 IU/kg bolus plus 15 IU/kg/h infusion),\(^\text{19}\) 26-28% with oral edoxaban (60 mg)\(^\text{43}\) and up to 40% with serial dosing of the parenteral direct factor Xa inhibitor, DX-9065a,\(^\text{20}\) were reported. Importantly therefore, we have shown that exosite 1 thrombin antagonism alone with JNJ-9375 substantially reduces \textit{ex vivo} human thrombus formation. Moreover, reductions were comparable (if not superior) in magnitude to the clinically approved anticoagulant edoxaban suggesting a high probability of \textit{in vivo} antithrombotic efficacy.

JNJ-9375 resulted in dose-dependent prolongation of prothrombin time, activated partial thromboplastin time and thrombin time. As expected, thrombin time was most sensitive to the anticoagulant effect. Although direct oral anticoagulants are licensed for use without the need for routine monitoring, there are clinical situations in which readily available assays to measure anticoagulant activity may be useful. Our data suggests that if indicated, thrombin time, and to a lesser extent prothrombin time and activated partial thromboplastin time, may provide a useful assay for measuring the effect of exosite 1 inhibition and JNJ-9375 activity.

Our study has some potential limitations. First, only a modest number of volunteers were studied. However, problems associated with intra-group variability were minimised by the crossover design that allowed each volunteer to serve as their own control. Second, although the exposed porcine aortic media used in the perfusion model presents many of the common constituents of an injured human blood vessel (including type I collagen), it is unlikely to contain tissue factor (TF).\(^\text{44-46}\) Tissue factor (TF) activates the coagulation cascade and is an important contributor to thrombogenicity.\(^\text{47,48}\) Nevertheless, this does not overly limit our model for the assessment of thrombosis because binding of blood borne circulating TF is sufficient to allow activation of the coagulation cascade and thrombus propagation.\(^\text{44,45,49-51}\) Indeed, previous studies have confirmed that thrombus formed from human blood perfused over porcine tunica...
media (devoid of TF) stains heavily for TF.\textsuperscript{44,45} Third, we used an anti-fibrinogen antibody, which recognises both fibrinogen and fibrin, to examine the fibrin component of thrombus formation. However, the chamber is perfused by saline at the end of the experiment washing away unbound cells, proteins and other molecules, such as fibrinogen, leaving only adherent thrombus. Thus, histomorphometric quantification of fibrin-rich thrombus area is unlikely to be affected by this cross-reacting antibody and our findings are consistent with previous studies using the same immunohistochemical approach.\textsuperscript{52-55} Fourth, while we have shown that exosite 1 thrombin inhibition reduces fibrin-rich thrombus formation, determining how JNJ-9375 alters the dynamics of clot development, stabilisation and dissolution might further inform therapeutic potential and are areas for future exploration. Finally, the study included \textit{ex vivo} experiments only and thus lacked hard clinical end-points necessary to draw any conclusions regarding the safety or efficacy of this novel anticoagulant in practice. However, given this was a translational study designed to examine for the first time the effects of exosite 1 thrombin inhibition with JNJ-9375, we felt our study design appropriate.

In conclusion, JNJ-9375, a highly specific exosite 1 thrombin inhibitor, demonstrated substantial reductions in \textit{ex vivo} thrombosis in native human blood under flow conditions. These reductions were driven by a decrease in fibrin-rich thrombus formation and were comparable in magnitude to clinically approved anticoagulants. Our findings suggest JNJ-9375 represents a promising novel class of anticoagulant, and that further clinical studies are warranted. A phase 2 trial comparing the safety and efficacy of JNJ-9375 to apixaban in patients undergoing elective total knee replacement surgery is currently underway (ClinicalTrials.gov, NCT03251482).
Funding

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Conflicts of Interest

SJW and DEN were supported by, and have undertaken consultancy for, Janssen. TMC, GP, AG, and MJ are employees of Janssen.
References


Figure Legends

Figure 1. The effect of study compound on ex vivo platelet activation.
Extra-corporeal administration of JNJ-9375 inhibited thrombin-simulated [A] p-selectin expression and [B] platelet-monocyte aggregates in a dose-dependent manner, but had no effect on ADP activity. Data shown are the adjusted means (± 95% confidence intervals) and individual points. Statistical comparisons (Least Significance Difference test) versus placebo are represented above each plot: * p<0.05, ** p<0.01, *** p<0.001. Abbreviations used: ADP, adenosine diphosphate; PMA, platelet-monocyte aggregates; GMFI, geometric mean fluorescent intensity.

Figure 2. The effect of study compound on ex vivo total thrombus formation
Extra-corporeal administration of JNJ-9375 inhibited total thrombus formation in a dose-dependent manner at both [A] low shear stress (212 s⁻¹) and [B] high shear stress (1690 s⁻¹) shear stress. Data shown are the adjusted means (± 95% confidence intervals) for [Log] total thrombus area (μm²/mm) and individual points. Statistical comparisons (Least Significance Difference test) versus placebo are represented above each plot: * p<0.05, ** p<0.01, *** p<0.001. Abbreviations used: 9375, JNJ-9375.

Figure 3. The effect of study compound on the components of thrombus formation
Extra-corporeal administration of JNJ-9375 inhibited fibrin-rich thrombus deposition in a dose-dependent manner at both [A] low shear stress (212 s⁻¹) and [C] high shear stress (1690 s⁻¹) shear stress, as compared to placebo. JNJ-9375 had no effect on platelet-rich thrombus deposition at either shear stress. Bivalirudin reduced fibrin-rich thrombus deposition at low and high shear stress, and platelet-rich thrombus deposition at high shear stress. Data shown are the adjusted means (± 95% confidence intervals) for [Log] fibrin- or platelet-rich thrombus area (μm²/mm) and individual points. Statistical comparisons (Least Significance Difference test) versus placebo are represented above each plot: * p<0.05, ** p<0.01, ***
p<0.001. Abbreviations used: 9375, JNJ-9375.
<table>
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<th>Table 1. Summary of means from statistical analysis of end-points</th>
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<tr>
<td><strong>Placebo</strong></td>
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<td>APTT (secs)</td>
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<td>Thrombin 0.1 U/ml</td>
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<td>Total Thrombus area (µm²/mm²)</td>
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<td>Platelet-rich thrombus area (µm²/mm²)</td>
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<td>Fibrin-rich thrombus area (µm²/mm²)</td>
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Data shown are means with 95% confidence intervals. *14 of 15 results > 501 s. Abbreviations used: PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time; GMFI, geometric mean fluorescent intensity; ADP, adenosine diphosphate; and LLOQ, less than lower limit of quantification.
Figure 1

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