Vorapaxar for HIV-associated inflammation and coagulopathy (ADVICE): a randomised, double-blind, placebo-controlled trial

*ADVICE study group**

Summary

Background Increased D-dimer concentrations are associated with poor cardiovascular and other clinical outcomes in people with treated HIV infection. Proteinase activated receptor-1 (PAR-1) is activated by thrombin and overexpressed by immune cells from HIV-infected people. We aimed to study the efficacy of vorapaxar, a licensed inhibitor of PAR-1, in reducing HIV-associated hypercoagulation and inflammation.

Methods This was a multicentre, double-blind, randomised, placebo-controlled trial done in seven hospital clinics in Australia and the USA. Eligible participants were HIV-infected, aviraemic, were receiving stable antiretroviral therapy, and had D-dimer concentrations greater than 200 ng/mL. We randomly assigned participants (1:1) using computer-generated block lists of size two to receive vorapaxar (2·5 mg orally daily) or matched placebo for 12 weeks. Participants were reviewed and had a blood sample taken at weeks 1, 4, 8, and 12 during treatment, and at a final visit at week 18. The primary endpoint was treatment group difference in changes from baseline D-dimer concentrations after 8–12 weeks of treatment, and was assessed in the modified intention-to-treat population (participants who had at least one dose of study drug or one follow-up visit). This trial is registered with ClinicalTrials.gov, number NCT02394730, and is closed to new participants.

Findings Between Oct 21, 2015, and July 14, 2017, 65 eligible patients were randomly assigned to the placebo group (n=31) or vorapaxar group (n=34). One patient from the vorapaxar group did not receive any study drug, and the modified intention-to-treat population was comprised of 33 patients. D-dimer concentrations after 8–12 weeks of treatment did not differ significantly between groups (difference –0·02 $\log_{10}$ ng/mL, 95% CI –0·10 to 0·05; p=0·56). Vorapaxar treatment was safe and well tolerated in this cohort. There were 161 adverse events (n=84 in the placebo group and n=77 in the vorapaxar group), and five protocol-defined serious adverse events that required hospital admission for more than 24 h (n=2 in the placebo group and n=3 in the vorapaxar group). One patient ceased taking vorapaxar because of an adverse event. There were 25 bleeding events, 23 of which were mild, one was moderate, and one was severe.

Interpretation Vorapaxar had no effect on D-dimer concentrations in HIV-infected patients receiving stable antiretroviral therapy but at risk of poor outcomes. Alternative approaches are needed to reduce hypercoagulation, inflammation, and adverse long-term outcomes in patients with treated HIV infection.

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See **Online** for appendix

Introduction

Increased expression of D-dimer (a marker of coagulopathy) and increased high-sensitivity C-reactive protein (hs-CRP) and interleukin 6 (IL-6; markers of immune activation and inflammation) are associated with increased risk of death and serious end-organ diseases among people with HIV infection.[1–3] These markers are increased in untreated HIV replication, but even among people with well controlled HIV receiving combination antiretroviral therapy, there is a consistent relationship between higher D-dimer concentrations and poorer clinical outcome.[4–7] Although antiretroviral therapy reduces concentrations of D-dimer, it does not result in normalisation.[6,8,9] Among patients with suppressed plasma HIV RNA concentrations, expression of D-dimer and inflammation markers is higher than in age-matched populations without HIV infection.[10] Interventions to reduce either hypercoagulation or immune activation might both permit a clearer understanding of the underlying pathogenesis and be of therapeutic benefit.

The relationship between coagulopathic disorder and immune activation is an evolving area of research interest.[11–13] Tissue injury results in the release of tissue factor that promotes the coagulation cascade, resulting in thrombus formation. T cells differentially express receptors linked to this cascade and are activated when tissue injury has occurred. A novel observation suggests that CD8 T lymphocytes from HIV-infected people

Research in context

Evidence before this study

Cardiovascular disease is approximately 50% more common in HIV-infected people, despite antiretroviral therapy. Standard cardiovascular risk factors remain important in the context of HIV infection. Additionally, biomarkers in blood that correspond to increased coagulation and immune activation, particularly D-dimer but also interleukin 6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP), seem to be important markers of HIV-related cardiovascular disease risk. It is not clear if the associated biomarker changes are causal or consequential. The physiological mechanisms underpinning poor clinical outcomes remain unclear. Vorapaxar is a novel oral anticoagulant recently licensed for secondary prevention of cardiovascular disease, which in HIV infection could potentially have an additional benefit by reducing immune activation. We searched PubMed for articles published between Jan 1, 2000, and July 1, 2015, using the terms "vorapaxar" and "HIV", reporting a "study" or "trial". We found no reports of vorapaxar in the context of HIV infection.

Added value of this study

To the best of our knowledge, this multicentre, double-blind, randomised, placebo-controlled trial is the first to study vorapaxar in people with HIV infection who are at risk of future cardiovascular disease and other poor clinical outcomes. This study found no effect of 12 weeks of treatment with vorapaxar on several biomarkers of cardiovascular risk, including no effect on D-dimer, IL-6, or hs-CRP concentrations.

Implications of all the available evidence

The results of our study suggest that vorapaxar should not be studied further as a treatment to reduce cardiovascular risk in people with HIV infection. Existing proven interventions in HIV-negative populations to modify cardiovascular disease risk remain the best available method to reduce cardiovascular disease in people with HIV infection.

overexpress proteinase activated receptor-1 (PAR-1).[14] PAR-1 is activated by thrombin, and CD8 cells expressing PAR-1 become activated (ie, express cytokines and chemokines) in a dose-dependent fashion by exogenous thrombin.

The sources of tissue injury, immune activation, and hypercoagulopathy in people with well controlled HIV replication are not known. Increased tissue factor expression is present in monocytes from people with HIV-1 infection.[15] Analysis of thrombin generation suggests the net effect of HIV replication is pro-coagulant, although the degree to which this persists after suppression of HIV replication is uncertain.[16] It is plausible that tissue injury in the setting of HIV replication promotes thrombin formation and PAR-1-dependent signalling, which in turn supports immune activation and inflammation.[17] PAR-1 could therefore be a potential target for therapeutic manipulation in the setting of well controlled HIV infection.

Vorapaxar is an oral, competitive PAR-1 antagonist that mediates anticoagulation by inhibiting thrombin-induced platelet aggregation. Vorapaxar has been studied in large clinical endpoint trials in cardiovascular disease and is licensed as secondary prophylaxis for patients with a history of myocardial infarction or peripheral arterial disease.[18,19] We hypothesised that vorapaxar could reduce markers of hypercoagulation and inflammation in patients with well treated HIV at risk of adverse clinical outcomes.

Methods

Study design and participants

The ADVICE study (Attenuation of D-dimer using Vorapaxar to target Inflammatory and Coagulation Endpoints) was a double-blind, randomised, placebo-controlled trial done at seven health centres in five hospital clinic or general practice sites in Australia (Melbourne and Sydney) and two hospital clinic sites in the USA (Minneapolis and Washington, DC). HIV-infected people older than 40 years with suppressed HIV viraemia (plasma HIV RNA <50 copies per mL) for at least 24 weeks and a D-dimer concentration of greater than 200 ng/mL were eligible. We excluded patients with antiretroviral regimens of HIV protease and non-nucleoside reverse transcriptase inhibitors (except rilpivirine) because of potential drug–drug interactions with vorapaxar. Patients taking other anticoagulants or who had a history of cardiovascular disease were also excluded. A complete list of inclusion and exclusion criteria is provided in the trial protocol (appendix pp 29, 30).

The trial was approved by the research ethics board for each trial centre and was done in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consent. The trial was monitored by an independent data and safety monitoring board. The results were collected and analysed by the writing committee. No interim analysis was specified in the protocol nor recommended by the data and safety monitoring board, which reviewed safety data at predefined intervals or in response to reported serious adverse events.

Randomisation and masking

We randomly assigned patients (1:1) using computer-generated block lists of size two, stratified by site, and they were allocated, double-blind, with a web-based database system. The randomisation sequence was generated by an independent statistician who had no involvement in enrolling participants or in the final analysis.

Procedures

Consenting participants were screened and within 14 days, they were randomly allocated to receive either vorapaxar sulphate (2·5 mg daily) or matched placebo for 12 weeks. Participants were reviewed and had a blood sample taken at weeks 1, 4, 8, and 12 during treatment. At the week 12 visit, the study treatment was stopped, and patients were reviewed and had a blood sample taken at the week 18 final visit.

Cryopreserved plasma samples were retrospectively batch-analysed at Leidos Biomedical Research Inc (Frederick, MD, USA). D-dimer was measured with an enzyme-linked fluorescent assay on a VIDAS instrument (bioMerieux, Marcy l'Etoile, France). Traditional ELISAs were used to measure soluble CD14 (R&D Systems, Minneapolis, MN, USA) and soluble CD163 (Aviscera Bioscience Inc, Santa Clara, CA, USA). hs-CRP and IL-6 were measured by electrochemiluminescence (Meso Scale Discovery, Rockville, MD, USA).

To measure PAR-1 concentrations, we used flow cytometry for PAR-1 expression on gated CD4 and CD8 T cells. This was done on fresh blood (<4 h from venepuncture) in the subset of enrolled patients in Australia with access to validated flow cytometry assays. Detailed methods and gating strategies of the flow cytometry assays are shown in the appendix (p 5).

All adverse events were collected and summarised by randomised treatment group, severity, and relation to study drug (appendix pp 40–44). Serious adverse events were summarised for all enrolled participants. A particular focus of safety analyses was bleeding events, given the anticoagulant nature of vorapaxar, and these were classified according to the Bleeding Academic Research Consortium (BARC) criteria (appendix pp 86–88).

Outcomes

The primary endpoint was the difference between treatment groups in mean change in D-dimer concentration from baseline to the average concentration of weeks 8 and 12. Secondary endpoints were change in D-dimer between week 12 and week 18 (after cessation of vorapaxar or placebo), changes in HIV RNA, changes from baseline in CD4 and CD8 T-cell counts, and changes in the inflammatory markers hs-CRP and IL-6. Prespecified exploratory endpoints included changes in activation markers of monocytes (soluble CD14 and CD163) and PAR-1 expression on CD4 and CD8 T cells. Full details of all endpoints are provided in the protocol (appendix pp 25–27). We did not pursue the additional exploratory endpoints of cell-associated HIV con-centrations, ultrasensitive viral load, T-cell activation markers, and natural killer cell functions, which are also listed in the protocol.

Statistical analysis

For the primary endpoint, we used a linear regression for change (log_{10}) of D-dimer from baseline to an average of weeks 8 and 12, modelled against the treatment and baseline outcome variable. The SD of D-dimer concentrations in a previous study[20] of patients treated with antiretroviral therapy, with D-dimer concentrations greater than 200 ng/mL, was 0·36 log_{10} ng/mL at week 12, and 0·41 log_{10} ng/mL at week 24. Using a repeated measures regression analysis at weeks 8 and 12, assuming variability in change in log_{10} D-dimer of 0·4, and correlation between these two timepoints as 0·57, a total sample size of 56 patients (28 in each group) gives 80% power to detect a mean difference of 0·26 logs. We planned to recruit 60 patients, allowing for some non-completion. To give an idea of the absolute magnitude of differences the study would be powered to detect, assuming no change in log_{10} D-dimer in the placebo group, this mean difference of 0·26 logs corresponds with a 45% decrease in D-dimer from baseline in the vorapaxar group. This would move most patients to at least one lower quartile of D-dimer concentrations. It has previously been calculated that a one quartile change in D-dimer concentrations is associated with an adjusted odds ratio of 5·3 for the risk of serious non-AIDS events (including cardiovascular events) or death.[4] We reasoned that such a change in D-dimer, as a marker, would be required to justify pursuing vorapaxar in larger clinical studies.

We prepared and finalised a detailed statistical analysis plan before the database was finalised

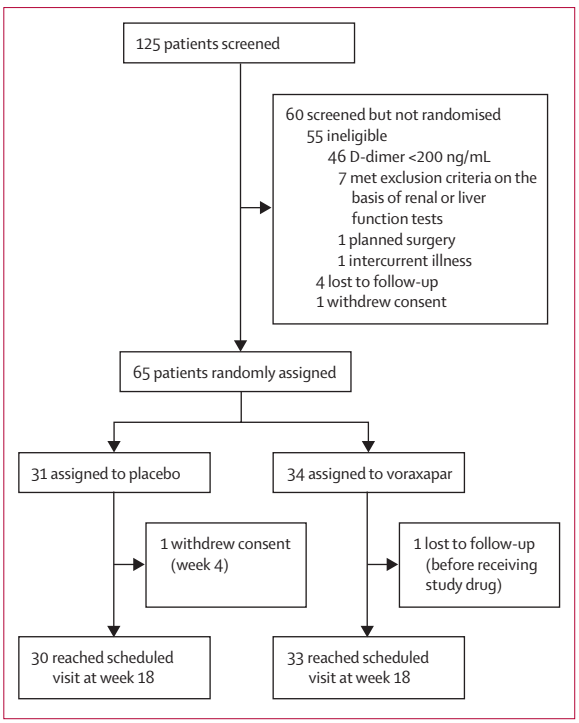

Figure 1: **Trial profile**
The modified intention-to-treat group (placebo n=31, vorapaxar n=34) excluded the one patient randomly assigned to vorapaxar, who was lost to follow-up before receiving any study drug.

	Placebo (n=31)	Vorapaxar (n=33)	Total (n=64)
Age (years)	52 (48–60)	53 (48–58)	52 (48–60)
Sex			
Male	28 (90%)	31 (94%)	59 (92%)
Female	3 (10%)	2 (6%)	5 (8%)
Total cholesterol (mmol/L)	4·7 (3·9–5·4)	4·6 (4–5·5)	4·7 (4–5·4)
HDL cholesterol (mmol/L)	1·3 (0·9–1·6)	1·2 (1–1·4)	1·2 (1–1·5)
Systolic blood pressure (mm Hg)	127 (120–136)	125 (115–133)	126·5 (117·5–135)
Diastolic blood pressure (mm Hg)	80 (70–86)	77 (70–85)	78·5 (70–85·5)
Current smoker	9 (29%)	9 (27%)	18 (28%)
Framingham heart score: 10-year coronary heart disease risk score*	12·1% (8·3–19·4)	10·6% (7·3–21·1)	11·4% (7·9–19·8)
D-dimer (ng/mL)	391·6 (302·3–813·7)	432·5 (298·0–531·1)	421·9 (299·0–687·6)
hs-CRP (µg/mL)	1·97 (0·61–4·81)	1·53 (0·50–3·01)	1·58 (0·50–3·86)
IL-6 (pg/mL)	0·99 (0·69–1·54)	0·93 (0·61–1·39)	0·94 (0·62–1·52)
Estimated duration of HIV infection (years)	12·2 (8·6–22·4)	12·8 (9·2–24·3)	12·5 (8·8–23·2)
Plasma HIV RNA (copies per mL)	20 (20–48)	20 (20–48)	20 (20–48)
CD4 count (cells per µL)	698 (490–869)	639 (504–768)	643 (497–829)
Time on current antiretroviral therapy (years)	2·0 (0·7–4·0)	1·3 (0·7–3·0)	1·5 (0·7–3·9)
ART regimens			
2×NRTI plus dolutegravir or raltegravir	28 (90%)	27 (82%)	55 (86%)
2×NRTI plus rilvipirine	3 (10%)	6 (18%)	9 (14%)
HCV RNA positive *vs* HCV seropositive (%)	0 *vs* 3 (10%)	0 *vs* 5 (15%)	0 *vs* 8 (12%)

Data are median (IQR) or n (%), unless otherwise indicated. hs-CRP=high-sensitivity C-reactive protein. IL-6=interleukin 6. ART=antiretroviral therapy. HCV=hepatitis C virus. NRTI=nucleotide or nucleoside reverse transcriptase inhibitors. *As calculated by D'Agnostino and colleagues.[21]

Table 1: **Baseline characteristics (modified intention-to-treat population)**

(appendix pp 112–17). We used a modified intention-to-treat approach for primary analyses, including all randomly assigned participants who received study drug and had any follow-up data. We included all available follow-up data regardless of whether participants ceased study drug. We analysed changes in continuous endpoints using regression models adjusted for baseline values. We analysed binary endpoints using logistic regression. We compared proportions of participants with adverse events with Fisher's exact test. All analyses were done with Stata (version 14.2). The study protocol was registered at ClinicalTrials.gov, number NCT02394730.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

125 participants were screened; 65 were eligible. Between Oct 14, 2015, and July 14, 2017, these patients were randomly assigned to receive vorapaxar (n=34) or placebo (n=31) at five centres in Australia and two in the USA. Reasons for screening failure were primarily related to D-dimer concentrations less than 200 ng/mL (figure 1). One participant assigned to vorapaxar was lost to follow-up immediately after randomisation, before receiving any study drug, leaving 31 patients in the placebo group and 33 in the vorapaxar group in the modified intention-to-treat population. One participant in the placebo group withdrew consent at week 4. All remaining participants reached the end of the study at week 18 (figure 1).

Baseline demographic and clinical characteristics of the two trial groups were balanced (table 1). Participants were primarily male (n=59, 91%), had a median age of 52 years (IQR 48–60), and had a baseline risk of cardiovascular disease within 10 years of 11·4% (IQR 7·9–19·8), using a Framingham Heart Study calculator. Participants had been diagnosed with HIV infection for a median of 12·5 years (8·8–23·2). Participants had controlled HIV RNA (<50 copies per mL) and an average CD4 count of 643 cells per µL. The most common current antiretroviral therapy regimen was two nucleoside or nucleotide reverse transcriptase inhibitors in combination with either dolutegravir or raltegravir (n=55, 86%), with the remaining participants taking two nucleoside reverse-transcriptase inhibitors in combination with rilpivirine. The trial successfully recruited participants at risk of future adverse outcomes (ie, increased cardiovascular events and other serious non-AIDS events and death),[1,2,4] on the basis of the median D-dimer concentration (ng/mL) at baseline being 421·9 ng/mL.

12 weeks' treatment with vorapaxar did not reduce concentrations of D-dimer, which were essentially unchanged throughout the trial (figure 2). There was no significant difference between groups in the mean change in $\log_{10}$ D-dimer from baseline to the average concentration of weeks 8 and 12 (–0·02 $\log_{10}$ ng/mL, 95% CI –0·10 to 0·05, p=0·56, using a regression model adjusted for baseline D-dimer concentration; table 2). The mean percentage change in D-dimer concentration from baseline to the average of weeks 8 and 12 was –10·8% (95% CI –23·1 to 3·4) for vorapaxar and –8·5% (–18·4 to 2·5) for placebo. There was no difference in D-dimer concentrations after either 8 weeks or 12 weeks, when analysed separately, and there was no increase in D-dimer concentration between 12 and 18 weeks after vorapaxar was ceased (table 2). There was no significant difference in the proportion of patients in each group who achieved a low D-dimer concentration (<165 ng/mL) at week 12: two (7%) of 30 patients in the placebo group versus one (3%) of 33 patients in the vorapaxar group (p=0·60).

Vorapaxar treatment had no significant effect on concentrations of plasma hs-CRP or IL-6 during the study (figure 2; table 2). After treatment with vorapaxar, the change in hs-CRP from baseline to the average concentration of weeks 8 and 12 was 0·02 $\log_{10}$ ng/mL

(95% CI –0·20 to 0·24; p=0·84), compared with placebo. The change in IL-6 from baseline to the average concentration of weeks 8 and 12, after treatment with vorapaxar, was 0·08 $\log_{10}$ ng/mL (–0·06 to 0·22; p=0·25) compared with placebo.

Vorapaxar had no adverse effect on control of HIV viraemia or maintenance of CD4 T cells. Plasma HIV RNA of less than 50 copies per mL was maintained at week 18 in 29 (97%) of 30 patients in the placebo group and 31 (94%) of 33 patients in the vorapaxar group (p=0·40). There was no significant difference in total CD4 or CD8 T-cell counts between the placebo and vorapaxar groups during the course of the trial (appendix p 7).

Vorapaxar was generally well tolerated, with one participant ceasing vorapaxar because of an adverse event. Since vorapaxar is an anticoagulant, we were particularly interested in bleeding events. There were 25 bleeding events (n=13 in the placebo group, n=12 in the vorapaxar group) in 18 patients (table 3). 23 events were mild, such as easy bruising or bleeding at the venepuncture site (BARC grade 1) with no treatment required. One event (in a participant taking vorapaxar) was graded as moderate (BARC grade 2); this was related to a cut from a kitchen instrument and the patient continued taking vorapaxar. One event (in a participant taking vorapaxar) was graded as severe (BARC grade 3); this was related to a spinal haematoma developing after an emergency operation for spinal canal stenosis, which required surgical treatment, and the participant ceased taking vorapaxar. There was no difference between proportions of participants with adverse events (p=0·99) or bleeding events by treatment group (p=0·58).

There were five protocol-defined serious adverse events requiring hospital admission for 24 h or longer, two in the placebo group (n=1 pneumonia and n=1 colitis) and three in the vorapaxar group (n=1 spinal canal stenosis requiring surgery, n=1 spinal canal haematoma after surgery in the same patient, and n=1 gout). No patient had a serious non-AIDS-related event, AIDS, pregnancy, or death. There were 161 adverse events, 84 in the placebo group and 77 in the vorapaxar group. There was no difference in the proportion of participants in each group experiencing adverse events of any grade, and no individual adverse event was markedly more common in one group (table 3; appendix pp 8–10). There was no difference in standard laboratory measures between the vorapaxar and placebo groups (data not shown).

We studied additional exploratory outcomes to further investigate the effects of vorapaxar. Soluble plasma CD14 and CD163 concentrations, which are markers of inflammation (soluble CD14) and microbial translocation and monocyte activation (soluble CD163), were not changed by vorapaxar treatment (appendix p 6).

Vorapaxar is a PAR-1 antagonist and one rationale for studying vorapaxar in the context of HIV was the observation that surface PAR-1 concentrations are increased on CD4 and CD8 T cells in treated HIV infection.[14] A subset of 39 participants (19 in the placebo group, 20 in the vorapaxar group) had flow cytometric analysis of PAR-1 concentrations on CD4 and CD8 T cells from fresh blood samples. PAR-1 expression on the total populations of CD4 or CD8 T cells was not changed by vorapaxar treatment (appendix p 6).

In a post-hoc subgroup analysis of patients with higher than the median coronary heart disease risk (median 10-year risk of 19·8%), there remained no evidence of a

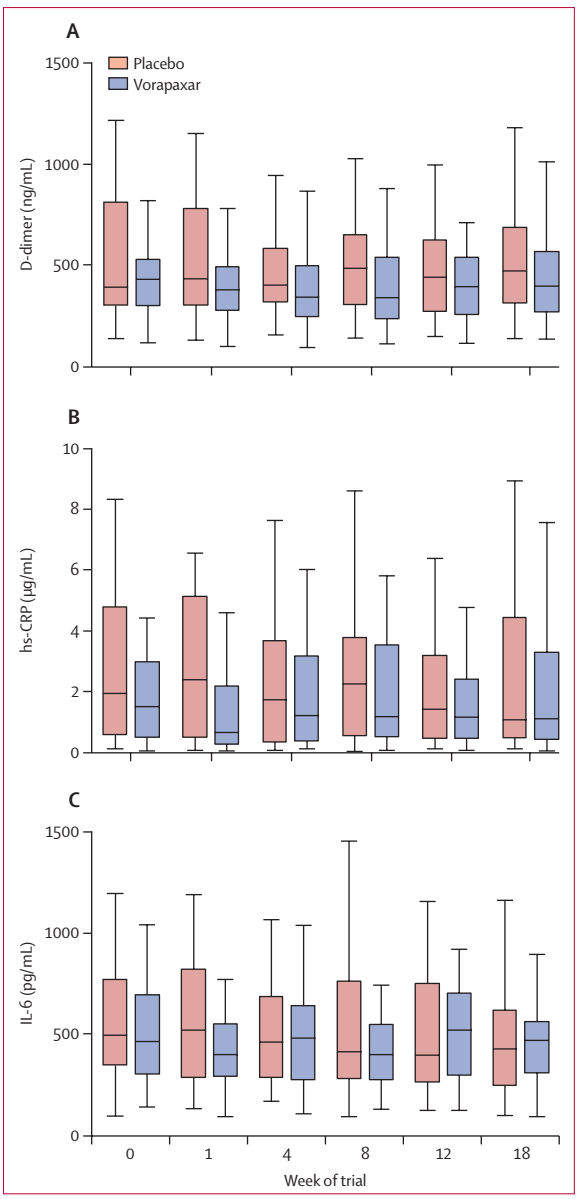

Figure 2: **Changes in D-dimer, hs-CRP, and IL-6 concentrations during the trial**
(A) Plasma D-dimer (ng/mL). (B) Plasma hs-CRP (µg/mL). (C) Plasma IL-6 (pg/mL). Box and whiskers plots show median (line), IQR (box), and whiskers (defined as upper quartile plus 1·5 × IQR and lower quartile minus 1·5 × IQR) for the two groups. Potential outliers are not shown. Vorapaxar or placebo was given for weeks 0–12 and then stopped. hs-CRP=high-sensitivity C-reactive protein. IL-6=interleukin 6.

	Mean percent change (95% CI)		Log_{10} transformed data (95% CI)	p value
	Placebo	Vorapaxar	Difference between treatment groups*	
Baseline to average of weeks 8 and 12				
D-dimer (ng/mL)†	−8·5% (−18·4 to 2·5)	−10·8% (−23·1 to 3·4)	−0·02 (−0·10 to 0·05)	0·56
IL-6 (pg/mL)	−11·6% (−29·1 to 10·3)	12·6% (−15·6 to 50·4)	0·08 (−0·06 to 0·22)	0·25
hs-CRP (μg/mL)	−15·7% (−40·9 to 20·2)	−0·02% (−41·3 to 70·2)	0·02 (−0·20 to 0·24)	0·84
Baseline to week 8				
D-dimer (ng/mL)	−10·7% (−19·5 to 2·0)	−12·5% (−25·0 to 2·1)	−0·03 (−0·10 to 0·05)	0·52
IL-6 (pg/mL)	−14·3% (−33·4 to 10·2)	−1·1% (−27·2 to 34·4)	0·04 (−0·11 to 0·19)	0·63
hs-CRP (μg/mL)	−14·2% (−41·9 to 26·9)	−0·5% (−44·8 to 79·4)	0·02 (−0·24 to 0·27)	0·91
Baseline to week 12				
D-dimer (ng/mL)	−8·8% (−19·4 to 3·2)	−10·2% (−22·9 to 4·5)	−0·02 (−0·09 to 0·06)	0·64
IL-6 (pg/mL)	−13·3% (−29·6 to 6·9)	10·4% (−16·0 to 45·1)	0·08 (−0·05 to 0·21)	0·22
hs-CRP (μg/mL)	−28·2% (−50·5 to 4·2)	−24·3% (−50·9 to 16·5)	−0·02 (−0·21 to 0·16)	0·79
Between weeks 12 and 18				
D-dimer (ng/mL)	8·0% (−6·9 to 25·2)	8·9% (−5·0 to 24·9)	0·003 (−0·08 to 0·09)	0·95
IL-6 (pg/mL)	−8·2% (−21·5 to 7·5)	−1·9% (−29·1 to 35·7)	0·03 (−0·12 to 0·18)	0·70
hs-CRP (μg/mL)	11·6% (−14·4 to 45·3)	24·6% (−14·7 to 82·1)	0·07 (−0·13 to 0·25)	0·53

IL-6=interleukin 6. hs-CRP=high-sensitivity C-reactive protein. *Linear regression for change (log_{10}) modelled against treatment and baseline outcome variable. †Primary endpoint.

Table 2: **Mean change in concentrations of D-dimer, IL-6, and hs-CRP**

	Placebo group (n=31)		Vorapaxar group (n=33)	
	Number of events	Number of patients (%)†	Number of events	Number of patients (%)†
Grade 1 (mild)	52	14 (45%)	37	13 (39%)
Grade 2 (moderate)	16	10 (32%)	24	12 (36%)
Grade 3 (severe)	3	2 (6%)	3	2 (6%)
Grade 4 (potentially life threatening)	0	0	1	1 (3%)
BARC bleeding events	13	10 (32%)	12	8 (24%)
Total adverse events (including BARC)‡	84	28	77	28

*Defined by the NIH Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Paediatric Adverse Events (version 2.0, November, 2014) and the Bleeding Academic Research Consortium (BARC) Definitions for Bleeding Events (2011). †Patients were classified according to the highest severity adverse event and for these patients any BARC event (yes or no) was also compared. ‡Patients can have both a BARC event and a DAIDS event and therefore these are not mutually exclusive. Three patients in the placebo group and five in the vorapaxar group had no adverse events.

Table 3: **Adverse events (DAIDS) and bleeding events (BARC)***

change in D-dimer concentration in patients randomly assigned to vorapaxar (appendix p 11).

Discussion

Vorapaxar was safe in people with well treated HIV infection, but did not influence D-dimer concentrations or a series of other inflammatory biomarkers associated with adverse outcomes. Our multisite, double-blind, randomised, placebo-controlled study was powered to detect a clinically meaningful change in D-dimer concentrations, but no effect was observed across a series of timepoints during the trial and there was no rebound change after vorapaxar was ceased.

The participants studied were at risk of future cardiovascular events, with median 10-year cardiovascular disease risk of 11·4%. The high baseline concentrations of D-dimer in this cohort suggest that their cardiovascular risk was higher than that calculated by standard algorithms.[4] The lack of effect of vorapaxar on biomarkers associated with cardiovascular risk emphasises the importance of standard cardiovascular risk reduction measures (including reducing cholesterol, controlling hypertension, stopping smoking) in this high-risk group. Our study excluded patients with known cardiovascular disease. We cannot exclude that vorapaxar might have influenced D-dimer in patients with known cardiovascular disease. However, vorapaxar is already licensed for prophylaxis of secondary cardiovascular disease. Furthermore, the post-hoc subgroup analysis did not show evidence of difference between groups in D-dimer changes in patients with higher risk of cardiovascular disease (appendix p 11).

We studied vorapaxar because it acts via PAR-1 and this molecule was upregulated on T cells in the setting of HIV infection.[14] The effect of vorapaxar on PAR-1 expression in T-cell subsets was not known before this study. We found no effect of vorapaxar on PAR-1 expression in T cells in the subset of 39 patients, for whom we studied this repeatedly using fresh blood samples. This finding suggests PAR-1 expression in T cells is not central to increased D-dimer concentrations, at least in the context of HIV infection. We did not study PAR-1 concentrations in platelets, which might have been influenced by vorapaxar. Better targets are needed that are susceptible to pharmacological interventions along the pathway of D-dimer and IL-6 production and cardiovascular disease.

Vorapaxar had an acceptable safety profile in this HIV-infected population. Two participants taking vorapaxar had moderate or severe bleeding episodes provoked by injury (one after a cut with a kitchen appliance and one after emergency back surgery). Future clinical trials of anticoagulant therapies in HIV infection are justified given the high rates of cardiovascular disease and expected safety profile of this level of anticoagulation in this population of patients.

We acknowledge several limitations of our study. We did not use a loading dose of vorapaxar as some cardiovascular studies have done,[19] but we observed no trend over time in D-dimer changes. The dose studied, 2·5 mg daily, is a relatively safe dose that is used in current practice for secondary prophylaxis.[18] Although larger doses for a longer duration could have been studied, this would have placed patients at higher risk of bleeding complications. Our use of biomarkers as primary and secondary endpoints might miss biological effects with the potential for clinical importance, but it is much more efficient than devoting the resources for a clinical endpoint study at this stage of investigation. The size of our study also means we cannot exclude a modest effect of vorapaxar on D-dimer concentrations. There was a mean 8·5% reduction in the average of week 8 and 12 D-dimer concentrations compared with baseline in the placebo group, and a 10·8% reduction in the vorapaxar group. In formal

adjusted analyses, this reduction corresponded with a difference in $\log_{10}$ D-dimer of –0·2 (95% CI –0·10 to 0·05) between the treatment groups. The 95% CI for the mean percentage reduction in week 8–12 D-dimer concentration, given our sample size, rules out a reduction of greater than 23% in D-dimer concentration in the vorapaxar group. This is much smaller than the 45% reduction in D-dimer as a marker endpoint that our study was powered to detect. We reasoned that a large effect on this marker would be required to justify future larger studies.[4] Because vorapaxar had no significant effect on D-dimer or the other surrogate markers of cardiovascular risk studied, including patients at risk of future cardiovascular disease, we believe clinical endpoint trials with vorapaxar are not justified in this patient population.

In conclusion, vorapaxar had no significant effect on D-dimer or markers of inflammation in the 64 people we studied with treated HIV infection, who were at risk of future cardiovascular disease. Improved therapies and targets are needed to reduce cardiovascular disease in this susceptible population.

Contributors

SJK wrote the first draft of the manuscript. DvB, MC, ADK, and SJK participated in laboratory analyses of samples. MGL and JH did the statistical analyses. SJK, JVB, and ADK enrolled participants. All members of the writing committee played a substantial part in design and execution of the study, analysed data, independently interpreted the results, and edited and approved the final Article.

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Declaration of interests

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