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MAJOR ARTICLE

Factor Xa Inhibition Reduces Coagulation Activity but Not Inflammation Among People With HIV: A Randomized Clinical Trial

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Background. Coagulation activity among persons with HIV is associated with end-organ disease risk, but the pathogenesis is not well characterized. We tested a hypothesis that hypercoagulation contributes to disease risk, in part, via upregulation of inflammation.

Methods. Treatment effects of edoxaban (30 mg), a direct factor Xa inhibitor, vs placebo were investigated in a randomized, double-blind crossover trial among participants with HIV and viral suppression and D-dimer levels ≥100 ng/mL. During each 4-month crossover period, blood measures of coagulation, inflammation, and immune activation were assessed. Analyses of change on edoxaban vs change on placebo used linear mixed models.

Results. Forty-four participants were randomized, and 40 completed at least 1 visit during each study period. The mean age was 49 years, and the CD4+ count was 739 cells/mm^3. Edoxaban treatment led to declines in D-dimer (44%) and thrombin-antithrombin complex (26%) but did not lower inflammatory or immune activation measures. More bruising or bleeding events occurred during edoxaban (n = 28) than during placebo or no drug periods (n = 15).

Conclusions. The direct factor Xa inhibitor edoxaban led to a substantial reduction in coagulation but no effect on inflammation or immune activation. These results do not support that hypercoagulation contributes to ongoing inflammation during chronic antiretroviral therapy–treated HIV disease.

Keywords. coagulation; HIV; immune activation; inflammation.

Effective antiretroviral therapy (ART) has shifted the spectrum of disease among people with HIV (PWH) from AIDS events toward cardiovascular disease (CVD) and other non-AIDS-defining end-organ diseases [1]. In addition to important behavioral factors, a key contributor to this disease spectrum includes HIV-associated inflammation and coagulation activation [2–4].

Reasons for hypercoagulation during HIV disease include more prevalent traditional risk factors (eg, smoking tobacco), consequences of prior immune depletion and tissue injury, and the direct effects of viral replication [5–8]. We have previously shown that HIV viral replication increases procoagulant factors (eg, factor VIII) and decreases anticoagulant factors (eg, antithrombin and protein C), changes that at least partially reverse with ART treatment [6]. Aside from examples where HIV treatment is started during HIV seroconversion [9], D-dimer levels during viral suppression in chronic HIV disease typically remain ~50%–75% elevated when compared with uninfected persons [3, 10].

One question in this context is how low-level persistent hypercoagulation contributes to excess disease risk across a wide spectrum of vascular and other disorders, beyond vessel thrombosis. PWH are well known to be at excess risk for venous thromboembolism (VTE) [6, 11]. Atherosclerotic CVD events are now a leading cause of morbidity and mortality among PWH, and chronic hypercoagulation remains 1 aspect of this pathogenesis [12]. Still, beyond manifestations of macro-level thrombosis, elevations in D-dimer levels are also associated with increased risk for end-stage liver or renal disease, additional grade 4 adverse events, the frailty phenotype, and all-cause mortality among PWH [4, 13, 14]. These observations emphasize the need for novel treatment strategies and a better understanding of the underlying disease pathogenesis.

We studied a hypothesis that HIV-associated hypercoagulation may contribute to end-organ disease by amplifying

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inflammation-mediated injury. Clotting factors stimulate protease-activated receptors (PARs) expressed on leukocytes and other cells, and activated factor X (Xa) is a key driver in this context. Activation of PAR-1 and/or PAR-2, in part, drives immune activation and inflammation along vascular surfaces and within tissues [15, 16]. In murine models of sickle cell disease (SCD), circulating levels of soluble vascular cell adhesion molecule (sVCAM) and IL-6 are reduced by either pharmacologic inhibition of activated factor X (FXa) or deficiency in the gene for PAR-2, a key receptor for FXa on immune cells [15]. In these studies of SCD mice, neither direct thrombin inhibition (ie, with dabigatran) or PAR-1 deficiency influenced plasma levels of IL-6 [15]. Although mechanisms driving inflammation and coagulation differ between SCD and HIV disease, the interconnection supports that there is potential to downregulate both pathways.

In this study, we assessed the treatment effects from pharmacologic inhibition of FXa in PWH, exploring the influence of HIV-associated hypercoagulation on blood measures of inflammation and immune activation. Edoxaban is a direct-acting oral anticoagulant (DOAC) that inhibits FXa and is licensed in the United States for the treatment of VTE at a dose of 60 mg daily. To this end, we conducted a crossover placebo-controlled randomized clinical trial of edoxaban given at 30 mg once daily to PWH with viral suppression receiving ART. We studied a lower dose of 30 mg to reduce risk for bleeding among a population where anticoagulation treatment was not currently indicated.

METHODS

Research Setting and Target Population

Participants were recruited at 2 HIV clinics within Minneapolis, Minnesota. The trial protocol was approved by the site institutional review board for conduct of human subjects research and was registered on ClinicalTrials.gov (NCT02339415). All study participants underwent a verbal and written informed consent process.

Eligibility criteria consisted of PWH aged ≥18 years who were receiving continuous ART and had maintained HIV RNA levels <200 copies/mL for at least 2 years. Participants also had a plasma D-dimer level ≥100 ng/mL (in fibrinogen equivalent units) at screening, as well as an estimated creatinine clearance ≥50 mL/min and weight ≥60 kg. Participants were excluded if they had a recent VTE or a clinical indication or contraindication to anticoagulant or antiplatelet therapy. Additional exclusion criteria included pregnancy, daily nonsteroidal anti-inflammatory drug use, history of gastrointestinal bleeding disorder in the past year, prior stroke or space-occupying lesion in the central nervous system (eg, toxoplasmosis), invasive cancer within the past year, rheumatologic disease or treatment with immune-modulatory drugs, current treatment for hepatitis C infection, or end-stage liver or renal disease.

Study Design and Intervention

We investigated the treatment effects of oral edoxaban (30 mg once daily) vs placebo in a randomized, double-blind, crossover clinical trial design. The dose of 30 mg was chosen over 60 mg to mitigate risk of bleeding complications. Study drug (edoxaban and matched placebo) was provided in tablet form by Daiichi-Sankyo Pharmaceuticals. The crossover design consisted of 2 treatment periods of 4 months' duration separated by a 4-month "washout" period (Figure 1). Each treatment period consisted of 4 monthly visits, with "period 1" including baseline and months 1, 2, 3, and 4 and "period 2" including months 8, 9, 10, 11, and 12. After oral and written informed consent procedures, participants were randomized to a drug sequence: either active edoxaban during period 1 and placebo during period 2 (E-P) or placebo during period 1 and edoxaban during period 2 (P-E).

The primary outcome was plasma levels of IL-6, with secondary outcomes including blood measures of inflammation, immune activation, and coagulation (Table 1; Supplementary Material). Power for n = 40 participants in this crossover design was 80%, at an alpha = .05, to detect a 23% relative difference in IL-6 levels between treatment conditions; this degree of IL-6 reduction is posited to correspond to a 25% reduction in non-AIDS conditions or death [4]. Adherence was assessed both subjectively and objectively via pill count. Safety was evaluated through ascertainment of any bleeding or bruising event and other (nonbleeding) adverse events of grade 3 or higher.

Laboratory Methods

Participants were fasting for all blood draws. Plasma and serum were processed within 30 minutes of collection. All samples were analyzed blinded to treatment group. Soluble (s) biomarker levels were measured from batched cryopreserved samples. Systemic inflammation was assessed via levels of high-sensitivity interleukin-6 (IL-6; electrochemiluminescence, Mesoscale), tumor necrosis factor receptor–1 (TNFr-1; enzyme-linked immunosorbent assay [ELISA], R&D Systems), and IL-1β (electrochemiluminescence, Mesoscale); monocyte activation via measures of sCD14 (ELISA, R&D Systems) and sCD163 (ELISA, R&D Systems); and vascular injury via sVCAM (ELISA, R&D systems). Plasma biomarkers of thrombin generation included D-dimer (a fibrin degradation product dependent on fibrin production by thrombin; Sta-R analyzer, Liatest D-DI, Diagnostic Stago), and thrombin antithrombin complexes (TAT; a direct measure of thrombin production and inhibition by antithrombin; ELISA, Siemens).

Circulating microparticle procoagulant activity was assessed in 3 contexts. Platelet-free plasma was used to measure microparticle-associated tissue factor procoagulant activity (MPTF) as previously described [17]. Whole-blood tissue factor procoagulant activity (WBTF) was assayed as previously described [18]. Procoagulant activity among the total pool of

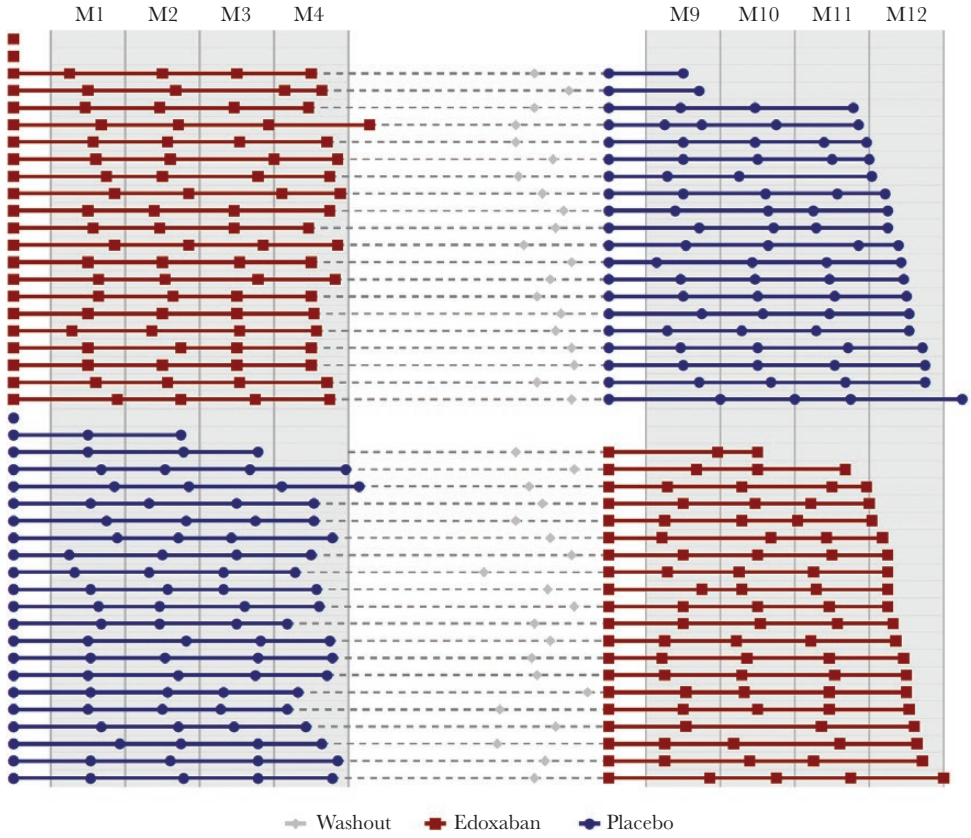

Figure 1. Study design and visit completeness flow diagram (n = 44). Visit attendance for each of the 44 randomized study participants is plotted. Participants (n = 22) randomized to receive active edoxaban (red plots/lines) followed by matched placebo (blue plots/lines) are shown on top ("EP" sequence), with participants (n = 22) randomized to the reverse study drug sequence shown below ("PE" sequence). Thirty-day visit windows are indicated by vertical gray lines and labeled as M1 for month 1, etc. Solid lines between visits represent time spent on the study drug. Horizontal gray dashed lines represent time not prescribed study drug during the washout period. Points are re-aligned at the month 8 visit. Seven participants were lost to follow-up, 4 during the study period 1 (3 of whom never followed up after randomization) and 3 during study period 2. Thus, 40 participants completed study period 1 and at least 1 visit on the study drug during period 2, such that they could be included in the analysis sample for primary comparisons (n = 20 from each EP and PE randomized sequence).

microparticles was also assessed using a functional phospholipid surface (PS) equivalents assay, performed per manufacturer instructions (Zympuhen MP-activity, Hyphen Biomed).

Immunophenotyping of cryopreserved peripheral blood mononuclear cells (PBMCs) was performed for a subset of participants (n = 12). Dead cells were identified and excluded from further analysis with the LIVE/DEAD Fixable Aqua Dead Cell Stain kit (Invitrogen). PBMCs were stained using fluorescent labeled monoclonal antibodies against extracellular and intracellular antigens. Samples were acquired on an LSRFortessa cytometer (BD Biosciences) and analyzed with FlowJo 10.4.2 (BD Biosciences). Lymphocytes and monocytes were identified based on their light-scattering properties.

Statistical Methods

Participant characteristics and laboratory measures were summarized by mean (SD) or median (interquartile range) for continuous variables and proportion (count) for categorical variables. Data were included in treatment analyses if the baseline visit (or month 8 visit for period 2) and at least 1 follow-up visit were completed in each of the study drug periods, such that within-person treatment comparisons could be performed. Primary analysis for the treatment effect was intent-to-treat using generalized linear mixed models with log-e-transformed biomarker values as outcomes, random subject-specific intercepts and fixed effects for assigned treatment, assigned treatment sequence (E-P or P-E), and pretreatment biomarker level. Exponentiated treatment coefficients then estimated the mean percent difference in biomarker levels on edoxaban vs on placebo during follow-up. Coefficients of the assigned treatment sequence were tested for evidence of carryover effects (ie, a treatment effect that lasted longer than the washout period of 4 months). Treatment effects on cellular phenotypes (percentages) were evaluated similarly to plasma biomarkers, except that percentages were not log-e-transformed. Proportions of participants with adverse events were tallied while on edoxaban and while on placebo or in washout. Between-group differences in number of events were tested by event type and overall using Poisson random effects

Table 1. Baseline Participant Characteristics (n = 44)

	Mean (SD) or % (No.)		
	Sequence E-P (n = 22)	Sequence P-E (n = 22)	Overall (n = 44)
Demographic characteristics			
Age, y	47 (10)	51 (8)	49 (9)
Male sex at birth	100% (22)	82% (18)	91% (40)
Race/ethnicity	–	–	–
White	82% (18)	59% (13)	70% (31)
Black	9% (2)	36% (8)	23% (10)
Asian	5% (1)	0% (0)	2% (1)
Hispanic or Latino	5% (1)	5% (1)	5% (2)
CVD-related clinical characteristics			
Smoking	–	–	–
Current	50% (11)	23% (5)	36% (16)
Ever	36% (8)	32% (7)	34% (15)
Hypertension diagnosis	36% (8)	32% (7)	34% (15)
Body mass index, kg/m^2	26.9 (4.4)	29.3 (7.1)	28.1 (5.9)
Systolic blood pressure, mmHg	126 (13)	122 (17)	124 (15)
Diastolic blood pressure, mmHg	79 (10)	81 (11)	80 (11)
Prescribed lipid lowering therapy	36% (8)	27% (6)	32% (14)
Total cholesterol, mg/dL	186 (29)	193 (32)	189 (30)
LDL cholesterol, mg/dL	107 (23)	117 (29)	112 (26)
HDL cholesterol, mg/dL	48 (14)	47 (14)	48 (14)
HIV-related history and clinical characteristics			
CD4 count, nadir, cells/μL	329 (243)	336 (245)	332 (241)
CD4 count, current, cells/μL	789 (318)	687 (294)	739 (308)
CD4:CD8	1.2 (0.6)	1.2 (0.5)	1.2 (0.6)
Undetectable HIV viral load	91% (20)	100% (22)	95% (42)
Current antiretroviral regimen	–	–	–
Includes NNRTI	14% (3)	27% (6)	20% (9)
Includes protease inhibitor	23% (5)	18% (4)	20% (9)
Includes integrase inhibitor	73% (16)	68% (15)	70% (31)
Prior AIDS	–	–	–
Opportunistic illness	0% (0)	0% (0)	0% (0)
CD4 count <200 cells/μL	32% (7)	45% (10)	39% (17)
Hepatitis C antibody positive	5% (1)	32% (7)	18% (8)

Abbreviations: CVD, cardiovascular disease; E, edoxaban; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NNRTI, non-nucleoside reverse transcriptase inhibitor; P, placebo.

models. All analyses were conducted using SAS, version 9.4, with a 2-sided type I error probability of .05.

RESULTS

Study Participants

Eighty-three participants were screened, of whom 44 were randomized into the study. The most common reason for exclusion included a screening D-dimer level <100 ng/mL (n = 14). Figure 1 presents the study design and flow diagram for all randomized participants through follow-up, including study visit attendance. Forty participants completed study period 1 and at least 1 follow-up visit on study drug during period 2 and were included in analyses for the primary treatment comparisons.

Participant characteristics are presented in Table 1. The mean age was 49 years, with 90% male sex at birth and 70% white non-Hispanic. The prevalence of CVD risk factors included 36% current smokers, 34% with hypertension, and 32% prescribed lipid-lowering therapy. The median (interquartile range [IQR]) CD4+ count was 675 (613–823) cells/mm^3, the median number of years since ART initiation (IQR) was 11 (5–18), and the ART regimen consisted of an integrase strand transfer inhibitor (INSTI) in 70% and a pharmacologic booster in 45% (36% receiving cobicistat and 9% receiving ritonavir).

Inflammation and Immune Activation

Median (IQR) levels of inflammatory and immunologic measurements are reported at baseline and then after washout at month 8 (Supplementary Table A). Figure 2 presents the primary treatment comparisons showing that edoxaban treatment did not reduce IL-6 or any of the other plasma biomarkers of inflammation, monocyte activation, or vascular injury. Monocyte and T-cell phenotypes were characterized in a subset of study participants (n = 12), and the treatment effects are presented in Figure 3. Edoxaban did not influence the frequency of individual

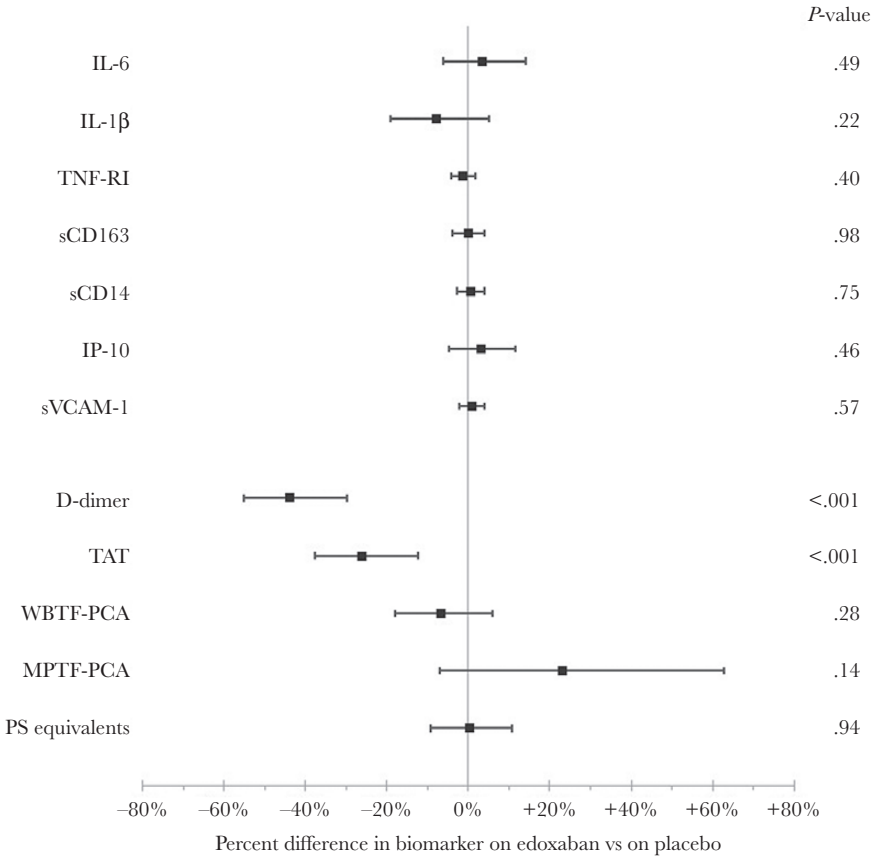

Figure 2. Treatment effect of edoxaban on inflammation and coagulation. Treatment effects of edoxaban (E) vs placebo (P) are plotted for inflammation (upper) and coagulation (lower) blood biomarkers (n = 40). Point estimates and *P* values reflect the E vs P mean percent differences in biomarker levels. Significant declines in D-dimer and thrombin antithrombin complex (TAT) levels were observed. Point estimates and 95% confidence intervals can be found in Supplementary Table B.

monocyte or T-cell activation phenotypes but was associated with decreases in effector memory T cells when compared with placebo (CD4+CD27-CD45RO+, –3.0% different; 95% confidence interval [CI], –5.7% to –0.3%; CD8+CD27-CD45RO+, –4.7% different; 95% CI, –8.7% to –0.7%). There was no evidence of a significant carryover effect from study drug exposure during period 1, through the washout phase and into period 2. In sensitivity analyses restricted to data from period 1, results were similar and showed no consistent treatment effect on inflammatory or immune activation markers.

Coagulation Activity

Median [IQR] levels of coagulation activity biomarkers, functional tissue factor (TF) procoagulant activities, and clotting potential are reported at baseline and then after washout at month 8 (Supplementary Table A). Figure 2 presents the treatment effect of edoxaban, characterized by reductions in D-dimer (–44%; 95% CI, –55% to –30%) and TAT (–26%; 95% CI, –38% to –12%), but no evidence of changes in TF (microparticle or whole-blood) procoagulant activity or PS equivalents. We also assessed the treatment effect of edoxaban on clinical coagulation parameters. When compared with placebo, edoxaban treatment was associated with a 0.08-unit increase (95% CI, 0.06 to 0.11) in the international normalized ratio for prothrombin time and 2.4-second increase (95% CI, 1.7 to 3.1) in activated partial thromboplastin time. There was no evidence of a significant carryover effect from period 1 into period 2 in any of the coagulation parameters assessed, and results were similar in sensitivity analyses of comparisons that were restricted to data from period 1. Additionally, due to the high proportion of 0 values in MPTF-PCA (ranging from 26% to 50% of measures at each visit), a sensitivity analysis was conducted to evaluate if edoxaban treatment influenced the time spent in a state of detectable MPTF-PCA (ie, values of >0 thrombin units/second), and this did not influence results.

Adherence and Adverse Events

The percentage of participants reporting that they were taking study drug during the week before a study visit ranged from 86% to 100% during follow-up and did not differ between randomized groups or study drug periods. Of 160 and 159 dispensed medication bottles containing edoxaban and placebo periods, respectively, 89% and 94% were returned. Among this subset, objective adherence by pill count was calculated as 94% for edoxaban and 97% for placebo (*P* for difference = .24).

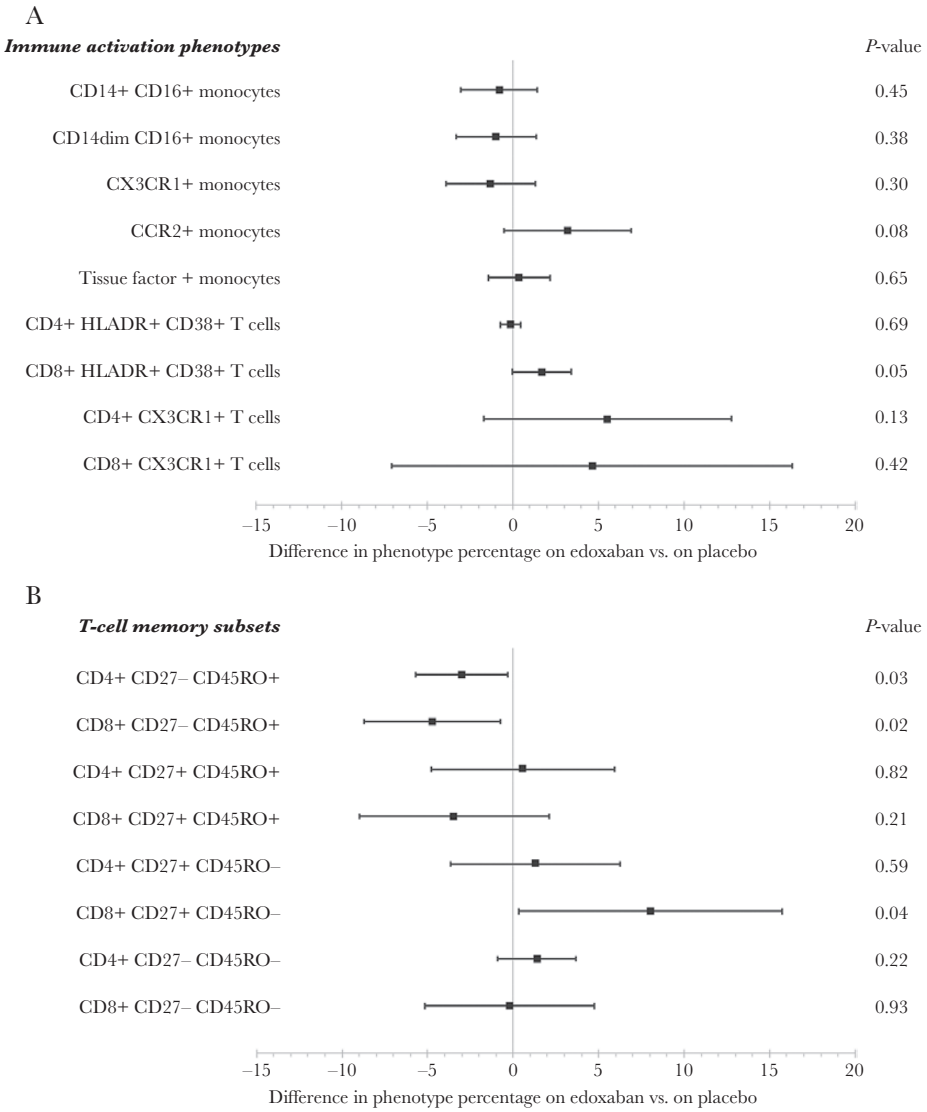

Figure 3. Treatment effect of edoxaban on (A) immune activation cell phenotypes and (B) T-cell memory subsets. Treatment effects of edoxaban (E) vs placebo (P) are plotted for (A) immune activation cell phenotypes (n = 8–11) and (B) T-cell memory subsets (n = 12). Point estimates and *P* values reflect E vs P mean differences in phenotype percentages. There was a significant decline in effector memory T cells (CD27-CD45RO+), but no other significant differences were observed in cellular phenotypes. Point estimates and 95% confidence intervals can be found in Supplementary Table B.

Table 2 presents a summary of grade 3 or 4 adverse events and any bleeding or bruising event. There were 70 adverse events overall, 43 occurring while participants were receiving edoxaban and 27 while receiving placebo or during the washout period. Of the 70 adverse events, 20 were grade 3 or 4, equally divided between the edoxaban (n = 10) and placebo (n = 10) periods. All of the 50 bleeding or bruising events that occurred among 27 participants were less than grade 3 or 4; 7 were attributed to a laceration, 12 were bruising without bleeding, and the most common spontaneous bleeding events were bleeding gums (n = 13), epistaxis (n = 10), and blood in stool (n = 7). Ultimately, the frequency of nonlaceration minor bleeding or bruising events was approximately twice as common when receiving edoxaban (n = 28) vs placebo or during the washout period (n = 15; $P = .03$).

We then explored factors that might identify participants at excess risk for bleeding or bruising on edoxaban by comparing those who had more nonlaceration bleeding or bruising events on edoxaban than on placebo (n = 15) with the remaining study participants (n = 29). These groups did not differ in demographic or clinical characteristics, including presence of comorbidities or class of antiretroviral medication used. Although D-dimer levels at entry did not differ between groups, those with more edoxaban-associated bleeding or bruising events had significantly lower soluble factor X antigen levels (91.3% of normal healthy mean; 95% CI, 83.4% to 99.2%; $P = .03$) compared with the remaining participants.

Table 2. Adverse Events

	Grade 1 or 2, No.	Grade 3 or 4, No.	Receiving Edoxaban, No.	Receiving Placebo or During Washout, No.	Edoxaban vs Placebo *P* Value
Bleeding/bruising events	50	0	33	17	.03
Bruising	12	0	7	5	.57
Bleeding gums	13	0	10	3	.07
Blood in stool	7	0	4	3	.71
Epistaxis	10	0	8	2	.09
Hematuria	1	0	1	0	–
Laceration	7	0	5	2	.28
Nonbleeding/bruising events	0	16	6	10	.33
Clinical laboratory abnormalities	–	4	4	0	–
Total adverse events	50	20	43	27	.06
Serious adverse events	–	2	1	1	–
Deaths	–	–	0	0	–

P values computed for between-group difference in number of events using a Poisson random effects model. Grade 1 or 2 clinical laboratory abnormalities (eg, CD4 count) were not captured. Grade 3 or 4 adverse drug reactions were not within a bleeding/bruising category and were assessed as not related to study drug or study participation.

DISCUSSION

In this randomized placebo-controlled trial, we tested the hypothesis that edoxaban given at a low dose of 30 mg once daily in addition to ART would reduce systemic inflammation and coagulation activity among PWH. In our population, edoxaban treatment did not reduce blood measures of inflammation, immune activation, or vascular injury despite a substantial reduction in coagulation activity (ie, 44% reduction in D-dimer levels). This anticoagulant effect was only apparent when assessing markers of downstream coagulation activity (eg, D-dimer), but not upstream (eg, TF activity), from FXa. Finally, edoxaban was associated with approximately twice the rate of nonserious bleeding or bruising events compared with placebo.

Inhibition of FXa has been demonstrated to reduce vascular inflammation and cytokine release (eg, IL-6), in part via reductions in PAR-2-dependent signaling [15]. Studies also suggest that HIV infection may increase PAR-1 expression on effector memory CD8+ T cells, which in turn release cytokines in response to thrombin signaling [19]. We did observe that edoxaban reduced circulating effector memory T cells (CD27-CD45RO+) but not T-cell activation or senescence per se. Still, we found no evidence that direct FXa inhibition with edoxaban reduces levels of IL-6, sVCAM-1, or other circulating blood markers of inflammation or immune activation. These findings are consistent with another randomized placebo-controlled trial of the direct PAR-1 inhibitor vorapaxar among PWH, which also failed to demonstrate reductions in plasma IL-6, hsCRP, or markers of monocyte or T-cell activation [20]. In contrast, nonhuman primate data demonstrated that a direct tissue factor inhibitor ("ixolaris") decreased factor Xa activation, D-dimer levels, and both T-cell and monocyte activation [21]. This novel study involved a different host context with acute simian immunodeficiency virus (SIV) infection characterized by untreated disease with high levels of viremia and much higher levels of immune activation. Still, the striking findings suggest that tissue factor inhibition may mitigate SIV-associated coagulopathy and concurrently reduce immune activation [21].

PAR signaling may also have less influence on inflammation than other drivers of immune activation during HIV disease, or when compared with other disease states (eg, sickle cell disease). Immunologic depletion at effector sites in the gastrointestinal and other secondary lymphatic tissues occurs early after HIV infection [22]. Unfortunately, this immunologic injury and loss of mucosal integrity largely persists within tissues despite effective viral suppression [22]. ART initiation during acute HIV seroconversion in the Thai RV254 study led to declines in levels of sCD14, a biomarker of lipopolysaccharide-induced monocyte activation, but sCD14 still remained higher than uninfected controls and there was no concurrent decline in IL-6 levels [9]. ART-treated HIV disease is then characterized by ongoing immune activation and low-level hypercoagulation, due, in part, to microbial antigens translocating across mucosal surfaces and a loss of immunologic control over other chronic co-pathogens (eg, cytomegalovirus) [3, 7, 22, 23]. In this context, any potential anti-inflammatory treatment effects modulated via PAR-signaling may simply not have a meaningful effect during chronic HIV disease when considered in the context of other factors driving immune activation.

One unanticipated feature of our study population was the low level of systemic inflammation at entry, as reflected by plasma levels of IL-6. When comparing PWH of similar age with viral suppression in the Strategic Management of AntiRetroviral Therapy (SMART) study, median IL-6 levels measured at the same core lab among participants in our study were less than half those reported in our study (1.6 pg/mL vs 0.6 pg/mL, respectively) [3, 4]. This relatively low level of inflammation could be influenced by the current practice of starting ART earlier regardless of CD4+ count [24]. Minimizing the duration of untreated HIV infection before ART initiation may mitigate the degree of persistent immune damage and systemic

inflammation during chronic ART-treated HIV disease [9]. In addition, our study had a high frequency of INSTI use, which has less toxicity and has been associated with lower levels of inflammatory markers than older classes of antiretrovirals [25]. Finally, the current emphasis on traditional risk factor modification among PWH corresponded to lower smoking rates and higher prevalence of statin use in our study when compared with historic comparisons [3, 4, 26]. Additional research is needed to better identify the subset of PWH with higher levels of ongoing inflammation during viral suppression who may benefit most from adjunct treatment strategies.

Quite apart from any potential treatment effects on inflammation, establishing that a low dose (30 mg) of edoxaban decreases D-dimer levels by >40% among PWH has important implications. Among 249 PWH in the US Military HIV Natural History Study, D-dimer levels were reported to be 75% higher when on ART with viral suppression compared with levels measured within the same individuals before HIV seroconversion [10]. Our observation of nearly twice the rates of nonserious bleeding or bruising after a 4-month exposure to edoxaban raises legitimate questions about the long-term safety of DOACs for this purpose. Still, epidemiologic analyses suggest that the degree of D-dimer reduction we observed in this study could be associated with a 25% reduction in risk for a composite of non-AIDS end-organ diseases [4].

A central question is then whether hypercoagulation is a mediating factor within the causal pathway for non-AIDS-defining end-organ diseases, including those not attributable to large-vessel thrombotic occlusions (eg, myocardial infarction). One posited explanation is offered by nonhuman primate data showing that SIV-associated hypercoagulation may cause end-organ tissue damage via (microvascular) thrombotic microangiopathy [27]. Still, the robust epidemiologic associations between D-dimer levels and nonvascular end-organ disease events among PWH have inherent limitations related to reverse causality and confounding [4, 13, 14]. One approach to control for this confounding entails applying Mendelian randomization methods, leveraging the variability in genes to generate evidence for whether a candidate biomarker has a causal influence on an outcome. Such methods have provided causal evidence for coagulation biomarkers on ischemic heart disease [28], but not for outcomes less directly related to thrombosis such as neurocognitive decline [29]. In this vane, in a randomized trial of persons with heart failure, the FXa inhibitor rivaroxaban failed to reduce risk for myocardial infarction, likely because risk for these events in the context of heart failure is not driven by thrombosis per se [30]. Future research is needed to clarify whether thrombin-mediated injury or tissue damage influences risk for non-CVD-related outcomes among PWH.

Our study has several limitations. The small sample size is unable to fully rule out the presence of a small but potentially meaningful treatment effect of edoxaban on our study measures, or within specific immunologic pathways not assessed. Results may also not be generalizable across sex given the low numbers of women. We did not have the ability to specifically characterize PAR expression or function on circulating leukocytes in this study. The anticoagulation, and potentially anti-inflammatory, effects from edoxaban are also dependent on exposure, and we studied a lower dose (30 mg) than is approved for treatment (60 mg). It is also known that p-gp inhibitors such as atazanavir and cobicistat can increase exposure to edoxaban to a varying degree, though we did not see any indication of a greater treatment effect among participants receiving these HIV medications. Finally, our study population had low levels of IL-6 and only modestly elevated levels of D-dimer, which prevents characterization of a potential anti-inflammatory effect among persons with high levels of inflammation and coagulation. Despite these limitations, our findings suggest that edoxaban is unlikely to have a clinically meaningful impact on circulating inflammatory markers that associate with end-organ disease risk among the target population of PWH.

In summary, edoxaban treatment given in addition to ART among PWH did not reduce inflammation or immune activation, but was associated with reduction in coagulation activity and increased risk for nonserious bleeding or bruising events. These results suggest that hypercoagulation is not a meaningful contributor to ongoing systemic inflammation during chronic ART-treated HIV disease. Additional strategies to reduce inflammation and safely modulate coagulation activity are needed to improve the health of people with HIV.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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