Thrombosis Research 177 (2019) 154–156

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Letter to the Editors-in-Chief

The influence of rivaroxaban on markers of fibrinolysis and endothelial cell activation/injury in patients with venous thrombosis

1. Introduction

Factor (F) Xa inhibitors are safe and effective alternatives to warfarin in the treatment of venous thrombosis and as stroke prophylaxis in patients with non-valvular atrial fibrillation. Even though the risk of major bleeding is lower for FXa inhibitors than warfarin in general, FXa inhibitors have been reported to cause a different bleeding profile. Inhibition of FXa by rivaroxaban is associated with more gastrointestinal bleeds and heavy uterine bleedings [1–3] than warfarin. Also, low-dose rivaroxaban has been shown to reduce major ischemic outcomes and death after acute coronary events [4]. It is unclear why rivaroxaban has a different bleeding pattern and a protective effect in vascular disease, and few studies exist on the effect of rivaroxaban on markers of fibrinolysis and endothelial cell activation/injury. We aimed to investigate whether rivaroxaban influences fibrinolysis or endothelial cell activation/injury.

2. Methods and results

The inclusion criteria were a first time venous thrombosis provoked by a transient risk factor, treatment with rivaroxaban 20 mg once daily for at least 4 weeks and no clinical or surgical comorbidities or drugs known to influence hemostasis, including platelet inhibitors, NSAIDs, steroids or oral contraceptives. We included seven men and five women, all of Caucasian ethnicity, with mean (SD) age of 53 (16) years. Blood cell counts, creatinine concentrations and liver function tests were all within normal reference ranges (Mean creatinine concentration 74 μmol/L). The samples were collected before drug intake (trough concentration), 2 h after drug intake (peak concentration), and four or more weeks after discontinuing rivaroxaban. Blood samples were collected from an antecubital vein after minimal stasis with a 21 G × 19 mm butterfly needle (Vacuette® GreinerBioOne GmbH, Kremsmünster, Austria) into 4.5 mL Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ, USA) containing 0.5 mL with 0.109 M buffered citrate. The test tubes were completely filled, and the first few mL of blood was discarded. The samples were collected at approximately the same time of the day (± 2 h) for all patients. Platelet poor plasma (PPP) was prepared by centrifugation of citrated whole blood for 15 min at 2000 ×*g* at 20 °C, and the top plasma supernatant was stored at −80 °C until analysis.

To investigate the levels of fibrinolytic markers and markers of endothelial activation/injury, we performed enzyme-linked immunosorbent assays. Thrombin generation (TG) was measured in platelet-poor plasma after initiation with the reagent PPP (tissue factor 5 pM, 4 μM phospholipids) and calcium, using a Calibrated Automated Thrombogram (CAT) (Diagnostica Stago, Asnières, France). All tests were run in doublets except for the thrombin generation assays where three parallels were run. The differences between the repeated measurements were calculated pairwise with the paired *t*-test. When data were not normally distributed, we used the Wilcoxon signed rank test. The tests were followed by post hoc tests to correct for multiple testing.

We found a significant reduction of the plasminogen activator inhibitor-1 (PAI-1) concentration and activity (Zymutest, Hyphen BioMed, Neuville-sur-Oise, France) in samples with rivaroxaban in peak concentrations, compared with samples drawn after withdrawal of the drug ($p = 0.01$ and 0.05 respectively) (Table 1). The other fibrinolytic markers tissue plasminogen activator antigen (tPA), thrombin activatable fibrinolysis inhibitor antigen (TAFI) (Diagnostica Stago, Asnières, France), plasminogen (Diapharma Inc., West Chester, Ohio, USA) and plasmin-antiplasmin complex (PAP) (DRG Instruments GmbH, Marburg, Germany) were not affected by rivaroxaban (Table 1).

The markers of endothelial cell activation/injury, thrombomodulin, ICAM-1, VCAM-1, E-selectin, CD163 and CD146 (R&D systems, Minneapolis, Minnesota, USA) and the anticoagulant protein tissue factor pathway inhibitor (TFPI) (Diagnostica Stago, Asnières, France) were not significantly different in rivaroxaban-containing samples, compared with after withdrawal of the drug (Table 1). TG was significantly reduced in rivaroxaban-containing samples (both trough- and peak concentration) compared with after withdrawal of the drug. Prothrombin fragment 1&2 (F1 + 2) (Abbexa, Cambridge, UK) was decreased in samples with rivaroxaban, compared with samples without rivaroxaban (Table 1).

3. Discussion and conclusion

In this study, we found reduced PAI-1 antigen/activity in samples with peak concentrations of rivaroxaban compared to samples collected after the withdrawal of the drug. Furthermore, TG and F1 + F2 in plasma were reduced in patients treated with rivaroxaban compared with samples where the drug had been withdrawn for > 4 weeks. Other fibrinolytic parameters, markers of endothelial cell activation/injury or the anticoagulant TFPI were not affected by rivaroxaban.

PAI-1 is an inhibitor of tPA and urokinase-type plasminogen inhibitor (uPA) and both convert plasminogen to plasmin. We speculate that the reduction of PAI-1 antigen/activity by rivaroxaban may lead to less inhibition of tPA and uPA. Even though the tPA antigen was similar in samples with and without rivaroxaban, we cannot exclude that a change in the function of tPA and uPA may increase the fibrinolysis. Resultant hyperfibrinolysis would increase the prevalence of heavy uterine bleeding and gastrointestinal hemorrhage in rivaroxaban-

https://doi.org/10.1016/j.thromres.2019.03.010
Received 29 October 2018; Received in revised form 10 March 2019; Accepted 13 March 2019
Available online 14 March 2019
0049-3848/

Table 1
TG parameters, TFPI, fibrinolytic markers and markers of endothelial activation/injury during rivaroxaban treatment (trough and peak concentrations) and after withdrawal of the drug. $N = 12$.

	Trough (just before rivaroxaban intake)	Peak (2 h after rivaroxaban intake)	Control (> 4 weeks after withdrawal of rivaroxaban)	*p*-Value (peak versus control after withdrawal of rivaroxaban)
Lag time (min)	3.7 (0.7)[a]	5.5 (1.0)[a]	2.8 (0.5)[a]	0.003
Peak (nM)	217 (70)[a]	79 (32)[a]	347 (84)[a]	0.002
Endogenous thrombin potential (nM × min)	1677 (309)[a]	1353 (312)[a]	1709 (415)[a]	0.008
F1 + 2 (pmol/L)	183 (44)[a]	174 (34)[a]	266 (83)[a]	0.002
TFPI total (ng/mL)	92.0 (30)[a]	92.2 (29)[a]	90.1 (24)[a]	0.70
TFPI free (ng/mL)	14.3 (5)[a]	14.3 (4)[a]	14.6 (4)[a]	0.88
Plasminogen (μg/mL)	117 (24)[b]	121 (15)[b]	112 (34)[b]	0.16
tPA (ng/mL)	11.2 (5)[a]	10.3 (5)[a]	11.0 (4)[a]	0.26
PAP (μg/L)	514 (88)[a]	525 (71)[a]	551 (97)[a]	0.27
TAFI (ng/mL)	14.0 (5)[a]	13.4 (4)[a]	13.7 (5)[a]	0.31
PAI-1 antigen (ng/mL)	12.5 (18.1)[b]	8.5 (11.2)[b]	15.6 (20.2)[b]	0.01
PAI-1 activity (ng/mL)	1.7 (4.3)[b]	0.7 (1.8)[b]	1.7 (2.8)[b]	0.05
ICAM 1 (ng/mL)	196 (52)[b]	182 (52)[b]	184 (43)[b]	0.17
VCAM 1 (ng/mL)	697 (109)[a]	692 (118)[a]	680 (98)[a]	0.79
E-selectin (ng/mL)	23.2 (9)[a]	24.0 (10)[a]	24.8 (9)[a]	0.39
CD163 (ng/mL)	501 (119)[a]	493 (112)[a]	510 (133)[a]	0.35
CD146 (ng/mL)	312 (67)[a]	309 (61)[a]	310 (63)[a]	0.78
Thrombomodulin (pg/mL)	3360 (513)[a]	3339 (418)[a]	3352 (436)[a]	0.76
Rivaroxaban (ng/mL)	32 (10)[b]	190 (79)[a]	–	–

[a] Results shown in mean (± SD).
[b] Results shown in median (interquartile range).

treated patients. A previous study has shown that women with heavy menstrual bleeding have increased fibrinolytic activity in the menstrual fluid, measured as plasmin activity, and the increased fibrinolysis was proposed to be a contributory factor in the etiology of menorrhagia [5]. Menorrhagia associated with PAI-1 deficiency has also been reported [6], but whether the influence of rivaroxaban on PAI-1 has a clinical impact is not known. Increased risk of heavy uterine bleedings has not been shown in studies investigating the safety of another FXa inhibitor, apixaban [7], and if the effect on PAI-1 is only present for rivaroxaban is not known. Fibrinolytic properties of gastric juices has also been demonstrated [8] which may support the hypothesis that increased risk of bleeding in GI tract in rivaroxaban-treated patients is due to hyperfibrinolysis.

To our knowledge, the rivaroxaban-induced reduction of PAI-1 has not previously been demonstrated. Horinaka and co-authors did not find any differences in PAI-1 antigen in rivaroxaban-treated patients compared with before initiation of rivaroxaban, possibly because in that study PAI-1 antigen was measured in samples with rivaroxaban in trough, not in peak concentrations [9].

The reduction of thrombin generation and F1 + F2 in samples with peak concentration of rivaroxaban compared to samples with trough concentration and samples with no treatment reflects the rivaroxaban effect with impaired prothrombin activation hence reduced thrombin generation.

Endothelial dysfunction and activation are features associated with acute coronary syndrome [10], and rivaroxaban has previously been shown to have a beneficial effect in this condition [4]. However, in this study, we did not find any differences in markers of endothelial function between samples with rivaroxaban in trough and peak concentrations, and samples drawn after withdrawal of the drug. We postulate that the protective effect of rivaroxaban in acute coronary syndrome would not involve improvement of endothelial dysfunction, but reduction of PAI-1 levels/activity or the reduction of TG.

The main limitation of this study is the low number of participants. Moreover, as the variability of pharmacokinetics of rivaroxaban is considerable, one must be careful in interpreting the results.

The strength of our study is that in contrast to other comparable studies, we have included samples > 4 weeks after withdrawal of the drug from every patient, in addition to the rivaroxaban-containing samples in trough- and peak concentrations. Furthermore, we included several fibrinolytic markers, both activators and inhibitors in the study.

In conclusion, this study has demonstrated that rivaroxaban in peak concentrations reduces the level and activity of PAI-1. Reduced PAI-1 concentration and activity may contribute to less inhibition of tPA and uPA. Resultant hyperfibrinolysis may increase the prevalence of heavy uterine bleeding and gastrointestinal hemorrhage in rivaroxaban-treated patients.

References

[1] M.W. Sherwood, C.C. Nessel, A.S. Hellkamp, K.W. Mahaffey, J.P. Piccini, E.Y. Suh, et al., Gastrointestinal bleeding in patients with atrial fibrillation treated with rivaroxaban or warfarin ROCKET AF trial, J. Am. Coll. Cardiol. 66 (21) (2015) 2271–2281.
[2] I. Martinelli, A.W. Lensing, S. Middeldorp, M. Levi, J. Beyer-Westendorf, B. van Bellen, et al., Recurrent venous thromboembolism and abnormal uterine bleeding with anticoagulant and hormone therapy use, Blood 127 (11) (2016) 1417–1425.
[3] M. Ferreira, S. Barsam, J.P. Patel, J. Czuprynska, L.N. Roberts, R.K. Patel, et al., Heavy menstrual bleeding on rivaroxaban, Br. J. Haematol. 173 (2) (2016) 314–315.
[4] J.L. Mega, E. Braunwald, S. Mohanavelu, P. Burton, R. Poulter, F. Misselwitz, et al., Rivaroxaban versus placebo in patients with acute coronary syndromes (ATLAS ACS-TIMI 46): a randomised, double-blind, phase II trial, Lancet 374 (9683) (2009) 29–38.
[5] M. Edlund, M. Blomback, S. He, On the correlation between local fibrinolytic activity in menstrual fluid and total blood loss during menstruation and effects of desmopressin, Blood Coagul. Fibrinolysis 14 (6) (2003) 593–598.
[6] T. Repine, M. Osswald, Menorrhagia due to a qualitative deficiency of plasminogen activator inhibitor-1: case report and literature review, Clin. Appl. Thromb. Hemost. 10 (3) (2004) 293–296.
[7] M.P. Brekelmans, L.J. Scheres, S.M. Bleker, B.A. Hutten, A. Timmermans, H.R. Buller, et al., Abnormal vaginal bleeding in women with venous thromboembolism treated with apixaban or warfarin, Thromb. Haemost. 117 (4) (2017) 809–815.
[8] S.E. Patchett, H. Enright, N. Afdhal, W. O'Connell, D.P. O'Donoghue, Clot lysis by gastric juice: an in vitro study, Gut 30 (12) (1989) 1704–1707.
[9] S. Horinaka, R. Sugawara, Y. Yonezawa, T. Ishimitsu, Factor Xa inhibition by rivaroxaban in the trough steady state can significantly reduce thrombin generation, Br. J. Clin. Pharmacol. 84 (1) (2018) 79–87.
[10] G.K. Hansson, Inflammation, atherosclerosis, and coronary artery disease, N. Engl. J. Med. 352 (16) (2005) 1685–1695.

Nina Haagenrud Schultz[a,b,c,d,*], Pål Andre Holme[a,b,d], Carola Elisabeth Henriksson[d,e], Marie-Christine Mowinckel[a], Per Morten Sandset[a,b,d], Vibeke Bratseth[f], Eva-Marie Jacobsen[b]

[a] *Research Institute of Internal Medicine, Oslo University Hospital, Box 4950, Nydalen, N-0424 Oslo, Norway*

[b] *Department of Haematology, Oslo University Hospital, Box 4950, Nydalen, N-0424 Oslo, Norway*

[c] *Department of Haematology, Akershus University Hospital, N-1478 Lørenskog, Norway*

[d] *Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Box 1171, Blindern, N-0318 Oslo, Norway*

[e] *Department of Medical Biochemistry, Oslo University Hospital, Norway*

[f] *Center for Clinical Heart Research, Department of Cardiology, Oslo University Hospital, Box 4950, Nydalen, N-0424 Oslo, Norway*

E-mail addresses: n.h.schultz@medisin.uio.no (N.H. Schultz), pholme@ous-hf.no (P.A. Holme), carola.henriksson@medisin.uio.no (C.E. Henriksson), UXMAOW@ous-hf.no (M.-C. Mowinckel), p.m.sandset@medisin.uio.no (P.M. Sandset), vibeke.bratset@studmed.uio.no (V. Bratseth), UXEMJA@ous-hf.no (E.-M. Jacobsen).

[*] Corresponding author at: Research Institute of Internal Medicine, Oslo University Hospital, Box 4950, Nydalen, N-0424 Oslo, Norway.