Effect of dabigatran and rivaroxaban treatment on a prothrombinase-based assay for assessment of activated C protein resistance

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ABSTRACT

We present a report about the interference due to factor IIa and factor Xa direct oral inhibitors on activated C protein resistance ratio (APCr), evaluated with a prothrombinase-based assay, in a patient heterozygous for factor V Leiden treated first with dabigatran and then with rivaroxaban. In this patient dabigatran increased the APCr ratio to a degree compatible with values observed in homozygous wild-type carriers, thus causing a potential misdiagnosis. We also found that rivaroxaban therapy was effective in lowering the APCr ratio.

INTRODUCTION

Oral direct inhibitors of activated factor II (FIIa), such as dabigatran, and of activated factor X (FXa), such as rivaroxaban, may interfere with the majority of clot-based coagulation assays and some chromogenic assays, whereas these drugs are not expected to interfere with antigen-based assays (1-3).

Data concerning the influence of anti-FIIa and anti-FXa on functional assays for assessment of activated protein C resistance (APCr) are still scarce. Moreover, the available studies have been mainly performed *in vitro*, with drugs spiked at increasing concentrations in pooled citrated plasma obtained from ostensibly healthy subjects. From a technical perspective, these studies have been performed by using activated partial prothrombin time (aPTT)-based assays. With regard to dabigatran, Adcock et al. (1) reported an over-estimation of the APCr ratio using aPTT-based assays for APCr. This may lead to misclassification of heterozygous factor V Leiden (FVL) patients as being normal (1). With regard to rivaroxaban, Hillarp et al. (2) reported that APCr was underestimated using aPTT-based assays.

As previously reported, in our laboratory we use a prothrombinase-based method for assessing APCr (4). As data concerning the effect of dabigatran and rivaroxaban therapy on this assay are still scarce, we describe here the case of a subjects heterozygous for FVL, who was treated first with dabigatran and later with rivaroxaban.

CASE REPORT

A white male 77 years old, with non-valvular atrial fibrillation, was evaluated before treatment with direct inhibitors of FIIa and FXa by using the standard protocol used in our institution: complete blood cells count, liver damage tests and kidney function tests. A study of the following coagulation parameters was performed by using a Sysmex CA7000 analyzer: thrombin time (TT), prothrombin time (PT), aPTT and fibrinogen. Quantification of dabigatran was performed by using a TT diluted assay (5, 6) and quantification of rivaroxaban was performed by using a chromogenic assay (6, 7). Both tests were supplied by Hyphen. Detection of APCr was carried out by using Sysmex CA7000 analyzer and a prothrombinase-based assay (Pefakit APC-R). The following interpretation criteria were used for this assay: APCr ratio >2.2: normal subjects, from 1.2 to 2.2: heterozygous, <1.2: homozygous (4). Genetic investigation for factor V G1691A and factor II G20210A were performed using a Cepheid GeneXpert analyzer with GeneXpert HemosIL Factor II and Factor V cartridges (8).

Our protocol for monitoring patients treated with direct inhibitors of FIIa and FXa requires to perform a patient's basal evaluation and a follow-up after 30, 90, 180, 360 days of treatment (9). In our institution, blood samples for monitoring of dabigatran or rivaroxaban are obtained in the morning, before drug assumption.

The subject started treatment with dabigatran 220

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mg/die. The results obtained at basal evaluation (sample A) and after 30 (sample B), 90 (sample C) and 180 (sample D) days of treatment are reported in Table 1. We observed a modest prolongation of PT and aPTT and a marked prolongation of TT. Fibrinogen concentrations were unaffected by dabigatran intake. APCr increased from 2.13 (sample A) to 2.85 (sample B), 2.91 (sample C) and 2.99 (sample D). These values of were compatible with a homozygous status for wild-type factor V.

Due to gastroenteric intolerance, the subject discontinued dabigatran and therapy with rivaroxaban 15 mg/die was initiated. The Regional control protocol was applied. Results obtained after 30 (sample E), 90 (sample F), 180 (sample G) and 360 (sample H) days of treatment are reported in Table 1. We observed a substantial prolongation of PT and a modest prolongation of aPTT. Fibrinogen and TT were unaffected by rivaroxaban therapy. We also observed a decrease in APCr ratio from 2.13 (basal evaluation, sample A) to 1.35 (sample E), 1.41 (sample F), 1.32 (sample G) and 1.28 (sample H). These APCr ratio values were compatible with a FVL heterozygous carrier pattern, even if a significant decrease in APCr ratio was obtained.

DISCUSSION

The available information about the interference of direct inhibitors of FIIa and FXa with functional assays for the assessment of APCr ratio are still scarce.

For the effect of dabigatran on coagulation assays, comprising the determination of APCr ratio, data obtained from multicentre *in vitro* studies are only available. In these studies, pooled citrated plasma obtained from normal subjects were spiked with dabigatran and then shipped to participant laboratories for test performance (1, 5, 7, 10, 11). Dabigatran increased the APCr ratio in 3 separate *in vitro* studies using aPTT-based functional assays (1, 8, 11). However, only subjects homozygous for the wild-type factor V were included in these trials. Moreover, no studies evaluated the effect of inhibitors of FIIa on APCr ratio performed using prothrombinase-based functional assays.

Data obtained from *in vitro* studies are only available for the effect of rivaroxaban on coagulation assays, including the assessment of APCr ratio (2, 7). Rivaroxaban was able to slightly decrease the APCr ratio using aPTT-based functional assays. Hillarp et al. (2) also evaluated the influence of rivaroxaban on the APCr ratio using a prothrombinase-based functional assay and concluded that this drug did not seemingly interfere with the APCr ratio as assessed with this type of assay.

Our case report has some strengths. We studied a FVL heterozygous patient and the APCr ratio was determined using a prothrombinase-based functional test. The results were therefore obtained *in vivo* and not *in vitro* by spiking drugs to normal plasma. It is also noteworthy that the same patient was first treated with dabigatran and then with rivaroxaban. So, this is probably the first study that has assessed the *in vivo* effect of dabigatran and rivaroxaban on the APCr ratio obtained using a prothrombinase-based assay in a subject heterozygous for FVL. According to our data, dabigatran increased the APCr ratio. During dabigatran treatment, APCr values were consistent with those observed in homozygous wild-type subjects, thus leading to a potential misdiagnosis. Moreover, the increase of the APCr ratio appeared to correlate with the dabigatran concentrations (R^2 = 0.918). Rivaroxaban tended to reduce the APCr ratio, which always remained

Table 1
Results of coagulation tests in our case

Sample ID[a]	PT (s)	aPTT (s)	TT (s)	Fibrinogen (g/L)	APCr	Dabigatran (μg/L)	Rivaroxaban (μg/L)
A	13.9	28.5	17.8	3.96	2.13	ND	-
Dabigatran							
B	22.1	44.3	354	3.27	2.85	97	-
C	22.5	36.4	526	3.78	2.91	126	-
D	23.4	37.4	721	3.22	2.99	158	-
Rivaroxaban							
E	36.4	32.6	18.3	3.46	1.35	-	181
F	34.4	34.1	18.7	3.54	1.41	-	152
G	35.2	36.4	19.1	3.48	1.32	-	165
H	36.1	37.1	20.2	3.75	1.28	-	208

PT, prothrombin time; aPTT, activated partial thromboplastin time; TT, thrombin time; APCr, activated protein C resistance; ND, undetectable.
[a]Basal sample obtained before starting treatment (A), samples obtained after 30 (B), 90 (C), 180 (D) days of treatment with dabigatran; samples obtained after 30 (E), 90 (F), 180 (G) 360 (H) days of treatment with rivaroxaban. All samples were obtained in the morning before drug assumption.
Reference ranges: PT, 10.7-14.6; aPTT, 23.2-30.9; TT, 12-20.5; fibrinogen, 2,00-4,50; APCr, >2.2 (normal subjects), from 1.2 to 2.2 (heterozygous), <1.2 (homozygous).

within the typical values of the patients heterozygous for FVL, but becoming closer to the cut-off value (<1.2) used to discriminate between FVL homozygous and heterozygous patients. Also, the effect on the APCr ratio appeared to correlate with the concentrations of rivaroxaban (R^2 = 0.933).

It is finally worthwhile mentioning that this is just a single case report and larger studies will be needed to assess the *in vivo* influence of direct inhibitors of FIIa and FXa on APCr assessed with different functional assays in homozygous wild-type factor V subjects and FVL heterozygous patients.

CONFLICTS OF INTEREST

None.

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