Coagulation assays for assessing direct FXa inhibitor oral anticoagulant reversal effect

M. Nagy[1],T. Gomes[2], van Oerle R[1,3], T. Hacking[1,3], P. Reitsma[2,4], G. Short[2], H.M.H. Spronk[1,3], D. Verhoef[2,4]

[1]Coagulation Profile, Maastricht, the Netherlands; [2]VarmX, Leiden, the Netherlands, [3]Department of Biochemistry, School for Cardiovascular Diseases, Maastricht, the Netherlands; [4]Division of Thrombosis and Hemostasis, Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, the Netherlands

INTRODUCTION

Factor Xa direct oral anticoagulants (Fxa-DOACs) require rapid reversal in patients with serious bleeding or prior to urgent surgery. VMX-C001 is a recombinant variant of human FX under clinical development as a FXa-DOAC bypassing agent. However, standard laboratory coagulation tests lack sensitivity to assess the effects of FXa-DOAC. Two commercially-available coagulation assays, dilute Russell's viper venom time (dRVVT) and dilute prothrombin time (dPT), were modified to develop more sensitive assays.

AIM

To evaluate the modified dRVVT and dPT assays and their sensitivity to FXa-DOACs using *ex vivo* spiked plasma samples and samples from healthy volunteers receiving FXa-DOACs.

METHOD

Ex vivo assay validation: Citrated plasma samples were obtained from healthy volunteers. Assay was measured in 3x and 4x diluted plasma for dRVVT and in undiluted and 2.5x diluted plasma for dPT with(out) apixaban and/or VMX-C001. Plasma samples were then spiked *ex vivo* with apixaban, edoxaban, or rivaroxaban and with(out) VMX-C001 and dRVVT and dPT were measured.

In vivo assay validation: Citrated plasma samples were taken from healthy volunteers (n=13) who had received apixaban or rivaroxaban for 3.5 days before measuring dRVVT and dPT in samples taken before and after the FXa-DOAC dose on day 4.

RESULTS

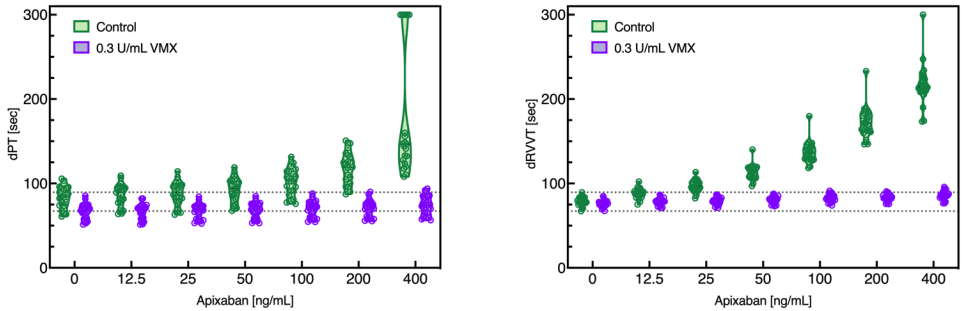

Figure 1. Validation of the dPT and dRVVT assays measured in 2.5x and 4x diluted plasma, respectively, in samples spiked ex vivo with apixaban 0–400 ng/mL without (control) and with VMX-C001 (0.3 U/mL). Outliers with clotting times above 300 sec have been removed. The dotted lines represent the min and max values for the control samples with 0 ng/mL apixaban. dRVVT was measured with a lupus anticoagulant assay kit using the confirmatory high phospholipid reagent (Instrumentation Laboratory, MA, USA) and dPT was measured with a lupus anticoagulant assay using the confirmatory high phospholipid reagent (BioMedica Diagnostics, Ontario, Canada); both assays were run on Siemens BCSxp autoanalyzers

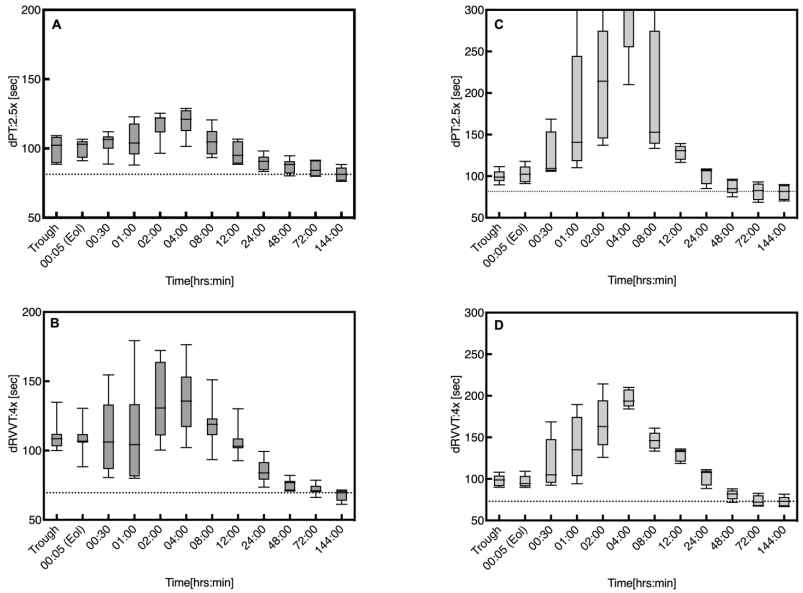

Figure 2. Time course of dPT assay results in subjects who received apixaban (A) or rivaroxaban (B) and time course of dRVVT assay results in subjects who received apixaban (C) or rivaroxaban (D). dPT and dRVVT were measured in 2.5x and 4x diluted plasma, respectively, in samples taken from healthy subjects (aged 18-49 years) who received apixaban (5 mg twice daily) or rivaroxaban (20 mg once daily) for 3.5 days followed by a single intravenous infusion of placebo for 5 min started 5 min after the last dose of FXa-DOAC on day 4. Citrated platelet-poor plasma samples were obtained immediately before the final dose of apixaban or rivaroxaban (trough), at the end of placebo infusion (00:05 (EOI)), and at various times up to 144 hours after the start of infusion. The dotted line represents the median level at 144 hours when the effects of apixaban or rivaroxaban have worn off.

CONCLUSIONS

dRVVT and dPT assays can be used to monitor FXa-DOAC anticoagulation with a high degree of sensitivity and can serve as surrogate endpoints in studies of the FXa-DOAC reversal agent VMX-C001.

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CONTACT INFORMATION

Magdolna Nagy, PhD
m.nagy@coagulationprofile.com

Daniël Verhoef, PhD
d.verhoef@VarmX.com