PB1295

Recombinant Factor IX fused with albumin (rFIX-FP) is underassigned by one-stage methods with silica as contact activator.

S Rosén[1], P Bryngelhed[2]
[1]Private Consultant, Kallered, Sweden, [2]Rossix AB, Mölndal, Sweden

INTRODUCTION

One-stage (OS) methods show in general considerably lower assigned FIX potency for Factor IX fused with albumin, (albutrepenonakog alfa, rFIX-FP) than chromogenic substrate (CS) methods. The cause(s) of the discrepancy is not known.
Aim: Investigation of causes of discrepancy on rFIX-FP potency assignment by commercial and new variant OS methods and a CS method.

MATERIALS AND METHODS

FIX sources: rFIX-FP (CSL Behring, Germany), plasma derived (pd) FIX concentrate (BPL, UK) and purified human FIX (Enzyme Research, USA), denoted pure pdFIX. The 4th and 5th IS FIX Concentrate (NIBSC, UK) were used as calibrators. Colloidal Silica Bindzil 309/220 (AkzoNobel, Sweden).

OS methods using A) APTT reagents Pathromtin SL (Siemens, Germany) and SynthAFax (IL, USA); B) purified phospholipids (PL) Phospholipid-TGT (Rossix, Mölndal, Sweden) and a platelet membrane like PL composition PL-PF3 (in-house), both without any contact activator and instead including FXIa with $CaCl_2$; C) APTT reagents comprising Phospholipid-TGT (PL-TGT) and PL-PF3 with 1.8 µL colloidal silica (CSi) / mL PL emulsion. CS method: Rox Factor IX (Rossix) applied on ACL TOP 500 and manually in microplates.

RESULTS

Fig. 1 shows close to 80% higher assigned FIX potencies for rFIX-FP with Rox Factor IX and SynthAFax as compared to Pathromtin SL. No difference was obtained for pdFIX.

Fig. 2 shows that OS methods with PL-TGT and PL-PF3 and including FXIa as replacement of contact activation yield rFIX-FP potency similar to Rox Factor IX and SynthAFax. Addition of colloidal silica to both phospholipids drastically reduces rFIX-HSA potency, yielding results similar to Pathromtin SL.

Fig. 3 shows a gradual decrease of rFIX-FP potency with increasing concentration of colloidal silica in the phospholipid emulsion.

No anomaly was observed with Rox Factor IX for the activation kinetics of rFIX-FP vs the 5th IS (data not shown).

Submitted for publication

CONCLUSIONS

1. rFIX-FP potency is underassigned in OS methods using APTT reagents with colloidal silica as contact activator, most likely due to attenuated activation and hence deviation from like-vs-like behavior compared to a pdFIX concentrate standard.
2. OS methods with Phospholipid-TGT and a platelet membrane like PL composition, both with FXIa included with $CaCl_2$ (no contact activation), give results in line with Rox Factor IX and SynthAFax.
3. pdFIX potency is similar with all OS methods and with Rox Factor IX, thus displaying like-vs-like behavior to a pdFIX concentrate standard.

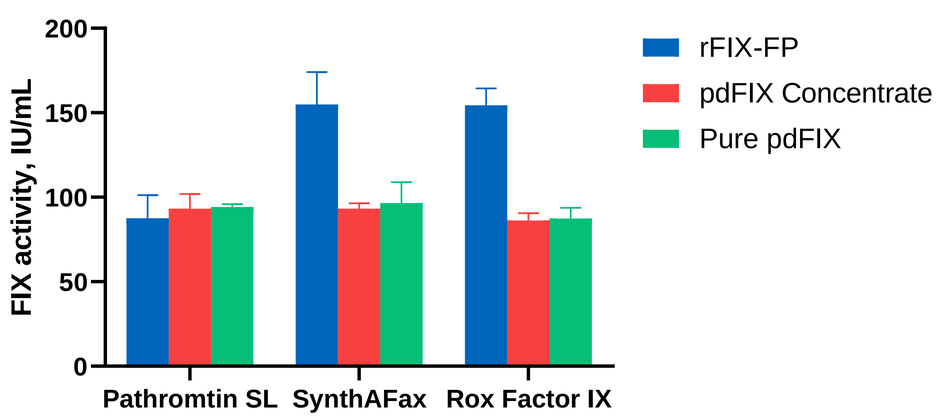

Fig. 1 Mean FIX potencies obtained with Pathromtin SL, SynthAFax and Rox Factor IX. At least two dilutions were assayed of each sample with each method.
rFIX-FP (n = 6-7), pdFIX Conc (n = 3-7), pure pdFIX (n = 3-4).

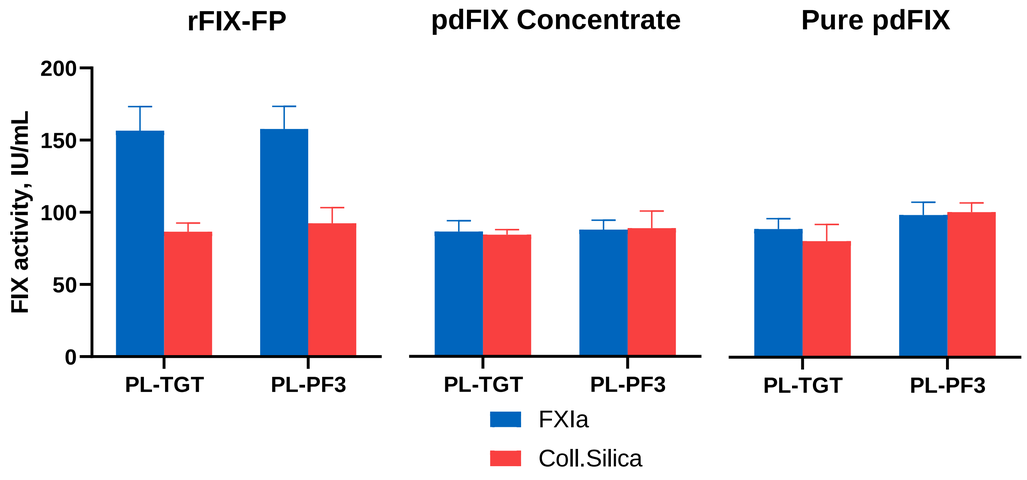

Fig. 2 Mean FIX potencies obtained with OS methods using two phospholipid emulsions, PL-TGT and PL-PF3, either with added colloidal silica (1.8 µL/mL) or with FXIa (1.5 - 6 IU/mL) included in the calcium chloride solution, thus omitting contact activation.

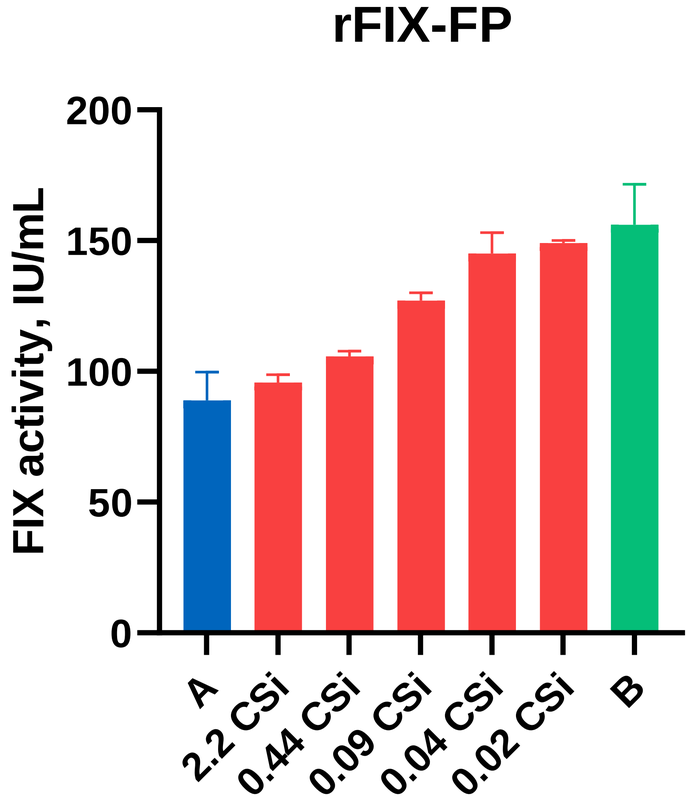

Fig. 3 Assigned mean FIX potencies of rFIX-FP in OS method with PL-TGT and with a range of colloidal silica concentrations (0.02-2.2 µL/mL). Contact activation time 5 min. Final phospholipid concentration 35 µM. Each bar represents the mean result from 3 determinations of 2-3 dilutions. For comparison, pooled mean potencies for different methods are inserted:
A) Pooled mean potencies obtained with Pathromtin SL and colloidal silica containing PL-TGT and PL-PF3; n = 15.
B) Pooled mean potencies obtained with SynthAFax, Rox Factor IX and PL-TGT, PL-PF3 with FXIa; n = 34.

ISTH 2019 MELBOURNE JULY6-10 ISTH2019.ORG RESEARCH DISCOVERY OUTCOMES

Kitchen S, Gray E, Mertens K. *Haemophilia* 2014; **20** (Suppl 4): 36-42.
Committee for Medicinal Products for Human (CHMP). European Medical Agency. Points to consider for assessors. *EMA/CHMP/BPWP/231587* Nov 2015: 1-4.
Rosén S, Bryngelhed P, Siegert G, Tiebel O. *Res Pract Thromb Haemost* 2018; **2** (Suppl. 1): 117
Horn C, Négrier C, Kalina U, Seifert W, Friedman KD.. *J Thromb Haemost* 2019; **17**: 138-148.
Young GA, Perry DJ for the Int. Prophylaxis Study Group. *J Thromb Haemost* 2019; **17**: 1-7.

✉ Steffen.rosen@rossix.com