Thrombosis Research 135 (2015) 198–204

Contents lists available at ScienceDirect

Thrombosis Research

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

Regular Article

Consequences for the APTT due to direct action of factor XIa on factor X, resulting in bypassing factors VIII-IX

C. Kluft *, C.J.M. van Leuven

Good Biomarker Sciences, Zernikedreef 8, 2333CL Leiden, The Netherlands

ARTICLE INFO

Article history:
Received 25 August 2014
Received in revised form 15 October 2014
Accepted 7 November 2014
Available online 13 November 2014

Keywords:
APTT
Haemophilia
Factor XI
Ellagic acid
Silica

ABSTRACT

Back ground: It has recently been reported that factor XIa can activate factor X directly and can bypass factors VIII-IX. We evaluated the consequences for factor analysis with the one-stage APTT.
Methods: APTT was performed with the Actin FS reagent with ellagic acid as the standard. Silica, high lipid (PTT-A) or low lipid (PTT-LA) were also tested. Factor depleted and deficient plasma's were obtained from commercial sources.
Results: The APTT clotting times in factor XII, XI, High Molecular Weight Kininogen, factor X and factor V deficient plasma's were all significantly longer (>100 s) than the clotting times of factor VIII- and IX-depleted or deficient plasma's (<100 s). That the shorter times for factor VIII and IX deficient plasmas were due to contact activation was supported by biphasic inhibition of the clotting times with addition of Corn Trypsin Inhibitor and Trasylol. The role of factor XI and the by-passing of factor VIII/IX was shown by the use of quenching antibodies towards factor XI and VIII. Enriching factor VIII or IX depleted plasma with purified factor XI and addition of factor XIa showed a strong dependence on factor XI level. Calibration curves for factor analysis were steeper for factors FXII, HMWK, FX and FV, compared to those of both factors VIII and IX. Curves for VIII/IX were found steeper by the use of APTT-A/silica-based, 50% diluted substrate plasma and low factor XI in the substrate plasma.
Conclusions: In factors VIII and IX deficient plasmas, the APTT shows an activity which can be attributed to contact activation of factor X by factor XIa.
This direct activity is lower with silica reagent compared to ellagic acid, dilution of plasma and low factor XI in substrate plasma.

Introduction

Recently, basic studies on the actions of factor XIa revealed that direct activation of factor X is possible [1]. It was shown that the effect could express in the APTT. Notably, the inhibition of the factor XIa effect resulted in further prolongation of the APTT in factor IX deficient plasma.

We were interested in the magnitude of the effect and the consequences for the factor assays of factors VIII and IX using the one-stage APPT test.

Materials and Methods

APTT standard procedure : activated thromboplastin time test on the STA Compact Analyser (Roche) for the measurement of intrinsic pathway, using 50 µl plasma, 50 µl ellagic acid reagents (Actin FS) and after 4 minutes at 37 °C, addition of 50 µl CaCl2 (STA CaCl2, 0.025 M, Stago) to induce clotting.

Two methods are used to determine the concentration of or the effect on a specific coagulation factor: (A) The undiluted method uses calibration by mixing a standard sample direct in a factor deficient plasma; (B) The diluted method uses calibration by diluting a standard sample in STA diluent buffer STA Diluent Buffer (Roche, lot:642170) followed by a 1:1 mixing with factor deficient / depleted substrate plasma.

The activation of the intrinsic pathway of the APTT was done by three different reagents, Actin FS, (Soy phosphatide and ellagic acid, Roche), PTT-A (high Cephalin and silica, Stago) and PTT-LA (low Cephalin and silica, Stago) followed by a recalcification step (STA $CaCl_2$, 0.025 M, ref:03138089: lot:677678).

Factor XI was measured using Hemosil XI depleted plasma and Actin FS, and calibration with Preciclot I labelled in IU/ml by comparison with the WHO International Standard for F XI.

In addition to clotting times also 100/clotting time (sec) was used to represent activity directly and not reciprocal. Margolis and Bruce [2] and Hemker [3] asked attention for the reciprocal relationship between concentration of factors or activity and clotting times suggesting the use of the reciprocal clotting time as a direct representation of activity.

* Corresponding author.
E-mail addresses: kluft@euronet.nl (C. Kluft), vanleuven@gbsleiden.nl (C.J.M. van Leuven).

http://dx.doi.org/10.1016/j.thromres.2014.11.004
0049-3848/

Diluted PT: prothrombin time test on the STA compact analyser for measurement of the extrinsic pathway .The activation of the pathway is done by the prothromboplastin reagent Innovin (Dade Innovin, Siemens, ref:B4242-40, lot:539295) diluted in 0.025 M CaCl2 to 0.8% of its original concentration.

Reagents

Actin FS, Siemens, Ellagic acid based, ref:B4218-010, lot: 538462. PTT-A, Stago, silica based, ref: 01281, lot:109864. PTT-LA, Stago, silica based, low lipids, ref: 02818, lot:111029.

Trasylol, Bayer, 84000 KIU/ml, lot:L4/82; Trypsin Inhibitor Corn Kernels (CTI), Sigma, ref:93622, lot:BCBC9823; Purified XI, Enzyme Research Laboratories, ref:hfXI-1111, lot: HFXI 2401; 185 U/mg: purified XIa, Enzyme Research Laboratories, lot HFXIa 278, 183.5 U/mg; Anti factor VII antibody, CLB VII-1, ref:MW1899, lot:8000120517: anti factor XI CLBXI lot 8000122017, 2 mg/ml; anti factor VIII antibody sheep, lot IG 1503 F12, 420 BU/ml.

Plasma's

STA Preciclot Plus I, Roche, ref:11776886, lot:677673: Homemade pooled normal plasma (NPP, 19 donors), lot: sept-2013. F VIII depleted plasma, Hemosil, IL lab., ref:0020011800, lot:N1137527; F IX depleted plasma, Hemosil, IL lab., ref:0020011900, lot:N0534541; Immunodef VIII, Stago, ref:00728, lot:111229; Immunodef IX, Stago, ref:00734, lot:110046; F IX depleted plasma, Cryoprep, ref:6-1900-ID, lot:1900-0013. FXI depleted plasma, IL lab, ref:0020011300, lot:N0333624; Factor X depleted plasma, Cryocheck, ref:FDP10-10, lot:D10-18; Factor V depleted plasma, Cryocheck, ref:FDP05-10, lot:D5-26; FXI depleted plasma, Affinity Biol., lot:DP11-0053R2; FXI depleted plasma, Cryocheck, ref:FDP11-10, lot:D11-22; FXI depleted plasma, HTI, ref: FXI-ID, lot: BB1003, Factor IX depleted plasma (nr 155) from the ECAT Foundation with F VIII = 41%, factor XI = 57 IU/ml and factor XII = 67%.

FVIII deficient plasma, Technoclone, ref:5154016, lot:4831B00; FIX deficient plasma, Technoclone, ref:5164016, lot:4926B00; FVIII:C depleted plasma, Hyphen Biomed, ref:DP040K, lot:24202-1; FIX depleted plasma, Hyphen Biomed, ref:DP050K, lot:34503-1; FVIII deficient plasma, Cryoprep, ref:7-0800, lot:892-2658; FXII deficient plasma, George King, ref:800-255-5100, lot:1214-2864; FXII deficient plasma, Technoclone, ref:5194008, lot:4Z21B01.01; FXI deficient plasma, Technoclone, ref:5184004, lot:4E21B00; FXI deficient plasma, Helena Biosc. ref:5196, lot:21094387; FXI deficient plasma, George King, ref:1100, lot:1122-2651; HMWK deficient plasma, Technoclone, ref:5204006, lot 4K31B0D. WHO International Standard. 1st International Standard for factor XI, Plasma, Human, NIBSC code 04/102.

Results

APTT in Deficient and Depleted Plasma's

The APTT results using our standard Actin FS based assay, for various factor deficient or depleted plasma's were tested and are shown in Fig. 1.

It is clear that clotting times of the deficient/depleted plasma's for factor VIII and IX are shorter than of any of the other deficient plasma's for factors XII, XI, HMWK, X and V. The data for five different plasma sources are homogeneous with CV% of 7 and 14% for depleted factors IX and VIII, respectively.

Characterisation of Short APTT in Factor VIII and IX Deficient/Depleted Plasma/s

Matafonov et al [1] reported that quenching factor XI by neutralising antibodies prolonged the APTT in factor IX deficient/depleted plasma.

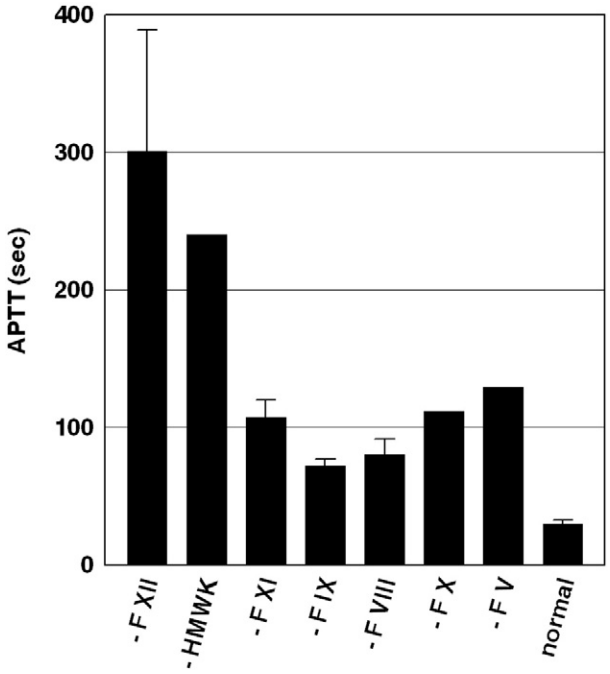

Fig. 1. APTT (mean, SD) with Actin FS for plasma's with the several factor deficiencies. For Factor VIII or IX deficiency or depletion it concerns 5 different sources of plasma for VIII and XI and 7 different sources of factor XI deficiency or depletion, for factor XII it concerned two sources. Other plasma's are single source; normal values are from 39 healthy individuals.

We confirmed this by adding quenching antibodies towards factor XI found to reduce activity (100/clotting time or prolonging of the APTT) in both factor IX and VIII depleted plasma (Fig. 2A).

To exclude residual factor IX or VIII to mediate this effect we added quenching antibodies to factor VIII, showing no effect in both factor VIII and IX depleted plasma (Fig. 2B).

In Fig. 2B the reduction in activity (100/clotting time) for 33 AU/ml VIII inhibitory antibody is 5 and 7% for the used plasma's. For two more factor IX depleted plasma's this was found 16% and 16% and for factor VIII depleted plasma's 10 and 4%.

We conclude that factor VIII/IX bypassing activity of factor XI occurs in the APTT and is the cause of short APTT values for factor VIII and XI deficient/depleted plasma's, and not related to residual VIII/IX in the deficient/depleted plasma's within limits of 5-15% uncertainty.

Effect of Pre-incubation of Plasma in the APTT

The incubation period of 4 minutes in the standard ellagic acid APTT (Actin FS) illustrates in normal plasma the activation process of the contact system. In factor VIII and IX depleted plasma we observed a similar effect (Fig. 3), supporting that contact activation was involved. It showed also that 4 minutes was adequate for the deficient plasma's and activity was not biased by a different time profile of activation in the different plasma's.

Factor VII Participation

Possible contribution of the tissue factor-VII route was evaluated using a factor VII neutralising antibody. Addition of this antibody to the prothrombin time with diluted Innovin (0.8%) showed in normal pooled plasma inhibition from 34 ± 1.1 sec to 150.1 ± 0 sec for both 3.3 and 10 μg/ml antibody. The use of this neutralising antibody at 3.3 μg/ml in the APTT (Actin FS), did not show an appreciable effect in factor VIII deficient (without 81.8 ± 1.0 sec ; with 83.0 ± 0.4 sec) or in factor IX deficient plasma (without 67.0 ± 1.3 sec ; with 69.1 ± 0.8 sec).