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Full Length Article

Simple method for removing DOACs from plasma samples

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ABSTRACT

Aim: To evaluate a simple method using an adsorbent product (DOAC Stop) for extracting direct oral anticoagulants (DOACs) from plasmas.
Method: DOAC Stop was tested on normal and a range of abnormal plasmas initially using activated partial thromboplastin time (APTT) tests and a more DOAC-sensitive Russells viper venom-based clotting test (DOAC Test). Further tests for prothrombin time/International Normalized Ratio (PT/INR), lupus anticoagulants, activated protein C (APC) resistance, antithrombin, plasminogen, protein C and S were carried out on various patient samples.
Results: DOAC Stop was found to remove all types of DOACs including dabigatran, apixaban, rivaroxaban and edoxaban from test plasmas with minimal effect on any of the (mainly clotting) tests considered in this study.
Summary: DOAC Stop can be used to identify plasmas containing DOACs using simple clotting tests. It reduces the false positivity for lupus anticoagulants observed in dilute Russells viper venom time (dRVVT) tests on DOAC-containing plasmas and could be useful for eliminating unwanted effects of DOACs on routine coagulation testing.

1. Introduction

Direct Oral Anti-Coagulants (DOACs) are increasingly used in therapeutic practice [1] and are known to interfere with almost all clotting tests to varying degrees [2]. Thus, in the coagulation testing laboratory, plasmas giving unexpectedly prolonged clotting time or abnormal chromogenic test results may need to be screened for the presence of DOACs if this is clinically relevant. Current chromogenic assays for DOACs are designed for individual agents, using either anti-thrombin activity or anti-factor Xa activity and each test kit relies on specific calibrators. Thus, such procedures can be quite expensive and reversal techniques such as that recently described for dabigatran [3] may be preferable in screening for DOACs.

Reversing agents for individual DOACs are being developed for therapeutic use but are not yet widely available for lab tests. Idarucizumab (Boehringer-Ingelheim, Germany) has been shown to be effective in neutralizing the effect of dabigatran in citrated plasma samples [3]. Andaxanet alpha (Portola Pharmaceuticals, USA) has been shown to work in vivo for the factor Xa inhibitors [4] and Ciraparantag (PER977, Perosphere, USA) may be effective against DOACs as well as various other anticoagulants [5]. However, the latter 2 antidotes may need to be titrated into test plasmas to match the exact amount of DOAC present because any excess might interfere with subsequent tests. This may not be convenient for general laboratory practice.

Therefore, we screened various candidate materials for agents which might be able to inactivate or bind DOACs in plasma samples in vitro, considering also that such agents should not affect clotting tests in the absence of DOACs. Initial studies with various dyes, organic compounds and a range of ion exchange resins were unsuccessful. However, we did find a hydrophobic binding agent which seemed to work quite well and this, after optimization, is the basis of DOAC Stop.

The aim of the following work was to investigate the use of a simple new product (DOAC Stop, Haematex Research, Sydney) based on this selected adsorbent mainly on routine coagulation tests. The testing was carried out initially at Haematex on known DOAC-containing plasmas looking at the effectiveness of the procedure for removing DOACs. Then also looking for any problems or clotting abnormalities that the procedure might cause, especially on in vitro-generated abnormal test plasmas and lupus anticoagulant samples. Finally, some limited testing at 2 external sites on various “real” patient plasmas on DOACs and with other defects before and after treatment with DOAC Stop is reported.

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2. Materials and methods

Pooled normal plasma (PNP) was prepared from selected fresh frozen plasmas provided by the Australian Red Cross Blood Transfusion Service, Sydney. Alumina adsorbed plasma was prepared by extracting PNP with 2–3% of alumina gel (Haematex, Sydney) so to achieve 50–80 s APTT result. Heparin plasma was prepared by mixing PNP with unfractionated heparin (Pharmacia-Upjohn, USA) to achieve 50–80 s APTT result. Lupus anti-coagulant (LAC) positive and factor deficient plasmas were purchased from Hyphen BioMed (Paris, France). Plasmas containing 10% of individual factors were prepared by mixing 10% PNP with 90% by volume of each deficient plasma.

Rivaroxaban, dabigatran-base and edoxaban-base powders were purchased from Chemieliva (China). Apixaban was kindly donated by BMS Pfizer (USA). These DOACs were dissolved initially in dimethyl sulfoxide (DMSO), then diluted to 10,000 ng/ml in a stock "in house" stabilizing solution. This concentrate was usually added at 0.02–0.05 ml per 1 ml into the various parent plasmas to achieve final concentrations of 200-500 ng/ml (or used at higher dosage levels). DOAC calibrator plasmas were purchased from Hyphen BioMed.

Citrated patient samples collected routinely into Vacutainers (Becton Dickenson, USA) at various clinics and sent for testing at laboratories at Queensland Medical Laboratories (QML), Brisbane and Queen Elizabeth II Medical Centre (QEII/Pathwest), Perth were used in some of these studies. Use of leftover patient plasmas was authorized in consideration that these studies were simply evaluations intended to improve the requested and already completed test procedures. None of the experimental test results from our study were used for patient diagnosis.

A summary of tests and reagents used is shown in Table 1. APTT tests at Haematex were carried out using Cephen LS (LAC sensitive) and Cephen (LAC resistant) both from Hyphen BioMed. Test plasmas (0.05 ml) were preincubated at 37 °C for 3 min with 0.05 ml of these reagents in ST4 cuvettes. Then 0.05 ml of prewarmed 0.025 M calcium chloride was added and timed to a clotting endpoint in a ST4 mechanical clot-timing instrument. Lupus anticoagulant sensitive dRVT-LS tests were carried out at Haematex using Hemoclot LA-S reagent and results usually compared with those from the paired dRVT-LR Hemoclot LA-R reagent; (both from Hyphen BioMed, France).

DOAC Test reagent (Haematex Research) was used as described previously [6] to carry out dRVT-DCT tests. This reagent is similar to dRVVT "Confirm" type high phospholipid reagents in being resistant to LAC and heparin, but it is approximately twice as sensitive to DOACs. For the DCT, test plasmas (0.05 ml) were prewarmed at 37 °C, then mixed with prewarmed 0.05 ml DOAC Test reagent and timed to a mechanical clotting endpoint in a ST4 instrument. All tests were carried out in duplicate.

Tests carried out at the PathWest laboratory (QEII Medical Centre) used dRVVT reagents LA Screen and LA Confirm from Diagnostica Stago (France). Factor Xa activity tests were carried out with a STA-Liquid anti Xa kit from Stago on a STA Evolution instrument (Stago). Thrombin clotting time (TCT) reagent and Neoplastin for prothrombin time/International Normalized Ratios (PT/INR) were also from Stago.

Tests at Queensland Medical Laboratory (QML, Brisbane) were automated on a Sysmex CS-5100 machine using all reagents and kits from Siemens (Germany). Thus, Thromborel S was used for PT/INR tests, Actin FSL for APTT tests, dRVVT- LA1 (Screen) and LA2 (Confirm) for LAC tests. Innovance (Siemens) kits were used for APC Resistance tests, antithrombin, free protein S, protein C, and plasminogen assays.

Plasmas were usually treated with DOAC Stop (Haematex Research, Australia) by mixing 1 ml volumes with one 18 mg "minitab for 5 min in a small plastic tube, then centrifuging down the adsorbent for 1 min at 7000*g* (10,000 rpm) in an Eppendorf (Germany) microfuge. External labs used 5 min centrifugation at 2000*g*. Centrifuging conditions were not critical. Sedimentation of the adsorbent was easily visible. Then supernatant plasmas were used directly for the various tests.

3. Results

3.1. Preliminary studies

To optimize the amount of DOAC Stop for effective use, dilutions of DOAC Stop suspension in PNP spiked with 500 ng/ml of all available DOACs were prepared and mixed for 5 min at 20 °C. They were then centrifuged and each supernatant plasmas tested with APTT (Cephen) and dRVT-DOAC Tests. The starting (highest) concentration of DOAC Stop used corresponded to 2 minitabs per ml of test plasma (double the recommended dose) and serial dilutions from this concentration provided the results shown in Fig. 1A and B.

As shown in Fig.1A, the initial APTT result on PNP containing 500 ng/ml of dabigatran (116 s) was significantly longer than those on plasmas containing the 3 factor Xa inhibitors (66–70 s). DOAC Stop was more effective at high dilution in shortening the APTT results of dabigatran and edoxaban plasmas than on apixaban and rivaroxaban-containing plasmas. Normalization of all APTTS to 42 ± 2 s was achieved with 0.25–1 minitabs of DOAC Stop per ml of plasma. This means that 1 minitab of DOAC Stop has the capacity to be effective with a range of 1–4 ml of the plasma to be treated.

In Fig. 1B, it is apparent that initial DOAC Test results on all the DOACs at 500 ng/ml in PNP were prolonged close to 140 s but were shortened by the presence of even quite high dilutions of DOAC Stop. Dabigatran and edoxaban were removed more readily than apixaban and rivaroxaban. However, after treatment with 0.5–2 minitabs of DOAC Stop, all the plasmas gave results close to those of the starting PNP (35 ± 2 s), indicating complete removal of all DOACs. It seems that one DOAC Stop minitab can fully extract at least 1000 ng/ml of any

Table 1
Summary of main tests and reagents used by the 3 participating labs in this study.

Test	Haematex research	Queen Elizabeth Hospital/Pathwest (QEII)	Queensland Medical Laboratory (QML)
Instrument	ST4 (Stago)	STA Evolution (Stago)	CS-5100 (Sysmex)
Activated partial thromboplastin time (APTT)	Cephen Cephen LS (Hyphen)		Actin FSL (Siemens)
Dilute Russells viper venom time (dRVVT)	Hemoclot LA-S Hemoclot LA-R (Hyphen)	LA Screen LA Confirm (Stago)	LA-1 LA-2 (Siemens)
Tests for DOACs	DOAC Test (Haematex)		
Prothrombin Time/International Normalized Ratio (PT/INR)		Neoplastin (Stago)	Thromborel S (Siemens)
Thrombin Clotting Time (TCT)		STA thrombin (Stago)	
Anti Factor Xa		STA liquid antiXa (Stago)	
Antithrombin III			Innovance (Siemens)
APC resistance			Innovance (Siemens)
Protein C			Innovance (Siemens)
Protein S			Innovance (Siemens)
Plasminogen			Innovance (Siemens)