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International Journal of Biochemistry & Physiology
ISSN: 2577-4360

Current Status and Limitation of Direct Oral Anticoagulants Testing

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Research Article
Volume 7 Issue 1
Received Date: May 31, 2022
Published Date: June 27, 2022
DOI: 10.23880/ijbp-16000204

Abstract

Direct oral anticoagulants (DOACs) are a class of drugs that are prescribed for preventing and treating thromboembolism. The DOACs have better efficacy and do not require regular monitoring. However, in emergency scenarios where patients suffer from impaired renal function, haemorrhage, and ischemic stroke, it is critical to know that the drug concentration is ≤30 ng mL^{-1} before making any life-saving decisions. Traditional laboratory tests are insensitive to low concentrations of DOACs. Unlike traditional laboratory-based coagulation assays, point-of-care (POC) testing is better suited for this application. This review summarises the recent advancements in DOACs testing, focusing on the need for a whole blood-based electrochemical POC assay to quantify DOACs in an emergency care setting.

Keywords: Direct Oral Anticoagulants; FXa; Dabigatran; Rivaroxaban; Apixaban

Abbreviations: DOACs: Direct Oral Anticoagulants; FXa: Direct Factor Xa; POC: Point-of-Care; aPTT: Activated Partial Thromboplastin Time; PT: Prothrombin Time; INR: International Normalised Ratio; LC-MS/MS: Liquid Chromatography-Mass Spectrometry/Mass Spectrometry; PNA: P-Nitroaniline.

Introduction

Thromboembolism-related complications such as myocardial infarction, stroke, and deep vein thrombosis are frequent causes of death and disability worldwide [1,2]. Traditionally, heparin and warfarin have been used to prevent and treat these diseases. However, warfarin, the only conventional oral anticoagulant, has several complications, including delayed action, interaction with other medications, complex dosing adjustments, and risk of bleeding [3,4]. These challenges led to the discoveries of newer agents known as direct oral anticoagulants (DOACs). DOACs are mainly divided into two categories based on inhibiting two different coagulation factors. These two classes are direct factor Xa (FXa) inhibitors (i.e., rivaroxaban, edoxaban, and apixaban) and direct thrombin inhibitors (i.e., dabigatran).

This review focuses on the various qualitative and quantitative assays for DOACs and their shortcomings. The need to quantify DOACs in the emergency point-of-care setting when the test turnaround time is less than 30 minutes. In addition, the current status of electrochemical POC systems to monitor DOACs are also discussed.

DOACs Testing

Experts believed regular coagulation monitoring was unnecessary when the first DOACs were introduced in anticoagulation therapy. Despite this, it would be crucial

to know the concentration of DOACs in the blood in circumstances such as severe bleeding, reduced kidney function, emergency surgery, the need for thrombolytic therapy in acute stroke, administration of an antidote for anticoagulant overdose, or the requirement to use other forms of anticoagulation [5-8]. In addition, small quantities of DOACs can interfere with thrombophilia testing [9]. For example, screening lupus anticoagulant by activated partial thromboplastin time (aPTT) or Russel viper venom time assay may inaccurately estimate test results. Dabigatran affects aPTT assay values, while dabigatran and direct FXa inhibitors affect Russel viper venom time assays [9]. Therefore, care must be taken to quantify residual DOAC concentrations present in the blood before thrombophilia testing [9].

Similarly, if patients are already under DOAC therapy and require emergency surgery, any concentration <30 ng mL^{-1} is considered safe to operate without any complications associated with severe bleeding. Likewise, in the case of patients suffering from acute ischemic stroke, DOAC concentrations <50 ng mL^{-1} may permit the initiation of thrombolytic treatment [10-12]. In some scenarios, when a patient admitted to an emergency centre is unconscious or unaware of DOAC therapy, it is vital to know anticoagulation status immediately before any clinical decisions are made [13]. A sensor that accurately quantifies DOACs <30 ng mL^{-1} in a point of care setting at trauma and acute care surgery is desirable. However, it is not yet available.

Prothrombin Time and aPTT Assay for DOACs

Direct thrombin and direct FXa inhibitors commonly affect traditional clot-based assays like prothrombin time (PT) and aPTT [14]. When studies were conducted using a range of spiked plasma samples, they have shown that the effect of DOACs on other clot-based assays depends on reagents used to perform the tests. As a result, when commercial calibrators specific for dabigatran and rivaroxaban were tested against PT and aPTT reagents, clot-based assays became unreliable for the DOACs measurement since they tend to misrepresent drug quantities [15,16].

The early laboratory assessments of dabigatran and rivaroxaban showed a direct relationship between PT values and DOAC drug concentrations. When tested using PT reagents, rivaroxaban showed good sensitivity compared to dabigatran. However, this trend is a function of the PT reagent used [16]. For example, 120 ng mL^{-1} rivaroxaban showed a PT ratio between 1.15-1.56, whereas a PT ratio of 1.31-1.88 was reported for 200 ng mL^{-1} of dabigatran [16-20]. This trend was also observed with apixaban. The concentration required to double the PT values varied from 480 ng mL^{-1}(low sensitive reagent) to 1000 ng mL^{-1}(high sensitive reagent) [16,21-23]. On the other hand, already existing PT/international normalised ratio (INR) reporting, which is calibrated initially for warfarin, becomes ineffective for DOACs measurement [24].

Mass Spectrometry

Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) is a sophisticated analytical technique used in various fields of synthetic chemistry, analytical chemistry, and clinical laboratories. Highly reliable, sensitive to ultra-low concentrations, and quantifying DOACs with high specificity make the LC-MS/MS technique a gold standard in clinical chemistry [25-27]. Several research groups adapted this technique and reported 5-500 ng mL^{-1} DOACs with a lower limit of detection of 0.025 ng mL^{-1} and 3 ng mL^{-1} as the lower limit of quantification depending on the type of DOACs tested [16,28-31].

Despite being the most sensitive technique to quantify DOACs, LC-MS/MS is not used in a routine clinical setup. They are more limited to the central research laboratory, which requires trained operators to perform assays. Their longer turnaround time makes them unsuitable when time-critical measurements are needed during emergency surgery [32]. Coupled with other issues such as target molecule coelution, running regular internal calibrators, and lack of globally harmonised protocols makes the adoption of LC-MS/MS difficult [16].

Chromogenic Assay

Chromogenic assays are commonly used for the quantification of DOACs. The chromogenic assay is commercially available to quantify direct thrombin and FXa inhibitors. Chromogenic assay for a direct antithrombin drug such as dabigatran can be carried out by following a simple set of procedures; at first, a thrombin-specific chromogenic substrate is incubated with plasma spiked with dabigatran for approximately 2-3 minutes, then the addition of thrombin initiates the substrate cleavage, which can be monitored. This assay showed an impressive lower limit of detection of approx. 15 ng mL^{-1} for dabigatran and returned a good correlation with LC-MS/MS (R^2=0.96, dabigatran <150 ng mL^{-1}) [16].

Similarly, chromogenic anti-FXa assay kits are commercially available and widely used for heparin quantification. First, excess FXa enzyme is mixed with plasma spiked FXa inhibitor sample. This initiates FXa inhibition, and then subsequent residual free FXa enzyme cleaves chromogenic substrate, releasing p-nitroaniline (PNA) chromophore. The optical density measured at a

specific wavelength is inversely proportional to the inhibitor under investigation. An individual drug-specific calibration plot is used to calculate the unknown drug concentration in the sample.

Chromogenic anti-FXa assays have been widely used to quantify rivaroxaban, edoxaban, and apixaban. For example, they showed an excellent lower limit of quantification of 15 ng mL^{-1} and 10 ng mL^{-1} for apixaban and edoxaban, respectively [17,18,33]. Nevertheless, this assay showed to be less sensitive to rivaroxaban, which could be attributed to the reagents used to perform the assay. This further emphasises the role of the reagents and methods used in controlling the DOACs lower limit of detection and lower limit of quantification observed [16,29,31,34,35].

Point-of-Care Assays

Undoubtedly, DOACs testing by standard methods can provide critical information to clinicians. However, if hospitals lack the infrastructure to report this DOACs concentration in less than 30 minutes during an emergency, the patient's life may be at risk. Therefore, an alternative, point-of-care testing using whole blood samples is highly required. Currently, only a few point-of-care (POC) assays are available in the market. One such sensor uses patients' urine samples treated with dabigatran, rivaroxaban, or apixaban for the qualitative and semiquantitative measurements [36]. Unfortunately, although this assay is a leap forward in POC testing, the urine sample of patients suffering from renal failure may underreport DOACs concentration. Furthermore, a microfluidic device for POC testing of heparin based on FXa activity using fluorescence technique has been developed. Nevertheless, its validation to DOACs quantification is unknown [37]. These devices required a calibration plot per drug tested, making the analysis tedious when the drug's identity is unknown.

Electrochemical Testing

Electrochemical methods are particularly well suited to POC systems since they can be simple and easy to miniaturise devices. The Coagucheck system (Roche, Switzerland) is a commercially available POC platform used in clot-based assays that works on an electrochemical detection platform. Blood collected from a finger prick is loaded into strips connected to a handheld device that analyses PT/INR values. Ebner et al. used the Coagucheck XS pro system to analyse DOACs concentration of patients samples and found that rivaroxaban showed an almost linear relationship (R^2=0.82) with PT values [38]. However, this approach failed to detect apixaban and dabigatran accurately [38]. They recommended that this assay be used only if alternative anti-FXa measurements are not available to quantify low concentrations of DOACs [38]. As mentioned above, the tests designed explicitly for traditional coagulation assays such as PT/INR and aPTT are unsuitable for the sensitive quantification of DOACs (<30ng mL^{-1}) [38]. To date, very little success has been achieved in providing a cheap, easy to operate, and highly sensitive alternative for quantifying DOACs at <30 ng mL^{-1} in POC setup. This further highlights the need for DOACs specific POC assays, especially at low concentrations of DOACs.

DOACs exhibit a mechanism of action by directly inhibiting thrombin or FXa, thereby regulating blood clots. Consequently, these enzymes become a common target for measuring the activity to predict and quantify drug effect on coagulation. Thrombin and FXa, two important coagulation factors, are protease enzymes. To date, the various analytical techniques used to quantify protease activity are colourimetric, Lou X, et al. [39] fluorescent Li J and Zhao Q, et al. [40,41], and electrochemical Zhang JJ, et al. [42-45] From these, the electrochemical method is the more studied due to its potential to create low cost, simplicity, reduced size, and high sensitivity devices. The different surface-confined electrochemical approaches used until now to determine protease activity are the following: Park S, et al. [46].

- Protease catalysed breaking of a thin film made up of gelatine or charged oligopeptide monolayer on the surface of the electrode.
- Protease catalysed breaking of chemically attached electroactive moieties such as 4-aminodiphenylamine, ferrocene, or methylene blue from the top layer of the electrode.
- Release of electroactive molecules such as 4-nitroaniline and 4-amino-2-chlorophenol from the substrate because of protease activity.
- Protease-induced fragmentation of polyionic polypeptide into shorter amino acids.

A clear drawback of these methods is that the sensing electrodes need to be modified with a film or a monolayer. Park and Yang adapted option 3 to produce a solution-based trypsin sensing method, with a detection limit of 1 and 100 ng mL^{-1} when an incubation time of 120 and 30 min was used, respectively [46]. Although this work opened an exciting research avenue, it was not used to detect DOACs activity.

Conclusion

DOACs are at the forefront of managing anticoagulation therapy. Despite their broad advantages, they require monitoring in a few scenarios such as severe bleeding, reduced renal function, anticoagulation reversal before emergency surgery, or another form of anticoagulation therapy. Many traditional anticoagulation assays such as PT and aPTT have been used to monitor DOACs. However, they

fail to address the need for whole blood-based POCs that can quantify drug at <30 ng mL^{-1} within 30 minutes in the emergency care setting. In addition, a POC assay for DOACs must use inexpensive reagents and should be able to run in a routine setting with minimum intervention by a trained medical professional. POC assays that use electrochemical detection techniques are poised to fit the above requirements very well. Electrochemical-based POC assay for DOACs is a nascent but challenging research field that requires the full attention of analytical chemists. The assays and challenges discussed in this review provide the current status of a poorly explored research field. Further development is highly required to support better clinical decisions.

Acknowledgement

AKVM acknowledge Universal Biosensors Inc and Deakin University for providing funding for graduate research scholarship.

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