Letters to the Editor

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Evaluating the potential benefits of the extravascular pool of factor IX

David Lillicrap

Richardson Laboratory, Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada

Correspondence to David Lillicrap, MD, Richardson Laboratory, Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada K7L 3N6
Tel: +001 613 533 6342; e-mail: david.lillicrap@queensu.ca; ORCID ID: 0000-0003-2410-6312

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To the Editor,

In practice, we evaluate the efficacy of haemostatic therapies through a combination of clinical observation (the acute response to bleeding and annualized bleed rates) and the application of laboratory tests that inform us of the activity (or antigen) levels of individual clotting factors or provide a global assessment of haemostatic activity (thromboelastography and rotational thromboelastography or thrombin generation assays). While the correlation between the clinical outcomes and laboratory test results is usually positive and consistent, the recent development of novel haemostatic agents with unconventional modes of action has challenged the paradigm of laboratory testing for clinical assessment.

Nevertheless, in haemophilia care we have been accustomed for a long time to consider the plasma activity levels of factor VIII and factor IX (FIX) as being representative of the haemostatic competency of the patient – that is levels more than 50% would provide robust haemostatic support and at levels progressively lower than this, an inadequate haemostatic response is more likely. However, recent studies concerning the haemostatic benefit of FIX have called this assumption into question [1,2].

The central theme of this question concerns the ability of FIX to bind reversibly to extravascular type IV collagen [3,4], and the proposal from a series of preclinical studies is that this extravascular FIX has the potential to support haemostasis in the absence of measurable plasma FIX activity. Thus, FIX has been shown to bind to type IV collagen in the endothelial basement membrane and provides haemostatic protection, that lasts much longer than predicted by the FIX plasma half-life, and correlates with the binding affinity of FIX for type IV collagen [5,6]. Since the endothelial basement membrane is exposed at the time of vascular injury, FIX binding to type IV collagen at this site enables an immediate response to vessel injury, and as the binding of FIX is reversible, FIX can also be released to support haemostasis at distant sites.

The preclinical studies evaluating this proposal can be summarized as follows. When haemophilia B mice are infused with 150 IU/kg of recombinant FIX (rFIX; BeneFIX, Pfizer, New York, New York, USA) or the FIXFc fusion protein (rFIXFc; Alprolix, Bioverativ, Waltham, Massachusetts, USA), and their haemostatic efficacy is assessed at 7 days postinfusion with the saphenous vein bleeding model, the products are equivalent, even though the terminal half-life of rFIXFc is significantly longer than that of rFIX [2]. This equivalence of haemostatic efficacy is proposed to be due to the contribution of the extravascular pool of FIX that is saturated at this level of FIX infusion as doses higher than 150 IU/kg provided no additional haemostatic benefit [2].

The generation of a knock-in mouse model in which a variant FIX with reduced collagen IV binding was investigated demonstrated a mild bleeding phenotype despite documentation of normal in-vitro clotting activity [3]. Additional studies of this variant FIX confirmed that the lack of collagen IV binding altered the haemostatic properties of FIX.

In a presentation by Matino [7], haemophilia B mice were treated with rFIX, rFIXFc or a rFIX with enhanced collagen IV binding (K5R variant). The haemophilic mice underwent 5 days of moderate treadmill exercise, were then sacrificed and the number of muscle bleeds were evaluated. rFIXFc was detected in the plasma at day 5, while the K5R mutant FIX was not; however, bleed protection was equally good for both rFIXFc and the K5R variant. These studies suggest that an extravascular source of FIX, likely the collagen IV bound protein, was still contributing to the correction of haemostasis at the 5-day time point.

Further indirect support for a haemostatic role of the extravascular pool of FIX comes from the study of a mouse model in which a cross-reactive material positive (CRM+) haemophilia B mouse model was generated and compared with haemophilia B mice with a CRM− mutation [4]. These studies showed that the infusion of rFIX was less efficacious in the CRM+ as compared with the CRM− mice. CRM+ FIX may occupy extravascular space binding sites, and the inference of these studies is that competitive binding to extravascular collagen IV by dysfunctional FIX reduced the haemostatic efficacy of the infused rFIX.

The introduction of extended half-life FIX products has produced a significant benefit to the prophylactic management of haemophilia B patients. These concentrates result in three-fold to five-fold extensions to the plasma

half-lives of FIX activity and the annualized bleed rates with these products is similarly reduced [8–10]. However, while preliminary studies document a normal interaction with the extravascular tissues with the albumin and Fc fusion proteins, there is some evidence that PEGylation may interfere with this pattern of biodistribution [2,11,12].

While the evidence that FIX binds reversibly to extracellular collagen IV is strong, a question that remains unanswered is how this source of FIX can achieve sufficient access to local and distant anatomic sites to achieve persistent haemostatic protection.

Against the background of these interesting and novel biological observations, the importance of extravascular FIX in the treatment of patients with haemophilia B is unknown. We have no easy way of determining the levels of extravascular FIX in patients, and assumptions based upon the preclinical studies that have been reported should be viewed with caution. Similarly, we cannot currently demonstrate that the transfer of extravascular FIX from a 'depot' bound to type IV collagen contributes to local or distant haemostasis. Furthermore, as stated above, we have several decades of evidence that the association of plasma FIX activity levels correlates well with clinical outcomes, and thus any proposal that low plasma levels of FIX may be rescued by extravascular FIX is speculative. Thus, in clinical practice, any assumption that haemostatic efficacy can be sustained beyond the time FIX is measurable through pharmacokinetic analysis in plasma may result in an increased risk of breakthrough bleeds and joint damage.

Recent studies indicate that further research into the physiological relevance of extravascular FIX is warranted, and attempts should be made to identify novel strategies that could incorporate objective evaluation of this FIX pool into the optimization of clinical care for haemophilia B patients. In the meantime, the haemostatic control of these patients should be managed with routine plasma FIX activity assays and by clinical evaluation.

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Conflicts of interest

There are no conflicts of interest.

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The impact of atorvastatin on dabigatran plasma levels in patients with atrial fibrillation

Tomáš Bolek[a], Matej Samoš[a], Lucia Stančiaková[b], Ingrid Škorňová[b], Kristína Grilusová[a], Peter Galajda[a], Ján Staško[b], Peter Kubisz[b] and Marián Mokáň[a]

[a]Department of Internal Medicine I and [b]National Centre of Hemostasis and Thrombosis, Department of Hematology and Blood Transfusion, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic

Correspondence to Matej Samoš, MD, PhD, Department of Internal Medicine I, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Kollarova 2, 036 59 Martin, Slovak republic
Tel: +421 907 612 943; fax: +421 43 4203 820;
e-mail: matej.samos@gmail.com

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To the Editor,

Long-term direct oral anticoagulants (DOACs), including direct thrombin inhibitor dabigatran, should be preferred