Impact of betrixaban on chromogenic anti-Xa assays

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Impact of betrixaban on chromogenic anti-Xa assays – Methodologies improvements

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Background and aim

Betrixaban, a novel direct oral factor-Xa inhibitor, has received its market authorization on the 23th of June 2017 in the United States (US) for the prophylaxis of venous thromboembolisms (VTE) in adult patients. Although direct oral anticoagulants (DOACs) do not require routine monitoring, the assessment of their effect on coagulation may be useful in some clinical situations (e.g. detection of drug accumulation in acute renal or hepatic failure; planning the timing of urgent invasive procedure; …). Previous study mentioned that chromogenic anti-Xa assays seem to be the most appropriate tests to estimate the amount of betrixaban in plasma. Nevertheless, adapted methodologies should be evaluated to improve the sensitivity of these reagents covering the on-therapy range which is from ± 9ng/mL at Ctrough to ± 122ng/mL at Cmax for 40 and 120mg once daily dose. The aim of this study was to improve the sensitivity of chromogenic anti-Xa assays to betrixaban by adjusting sample dilution schemes of three commercial products.

Methods

Betrixaban was spiked at final concentrations ranging from 0 to 500ng/mL in normal pooled plasma. Three commercial tests were used (Biophen® DiXaI® (France), STA® Liquid Anti-Xa (France), HemosIL® Liquid Anti-Xa (USA)) and adaptation of their sample dilution scheme were performed. Interestingly, the Biophen® DiXaI® has been developed to be insensitive to the presence of unfractionated heparin (UNF) and low molecular weight heparin (LMWH) due to its TRIS-NaCl-EDTA buffer.

In parallel, lower dilution of the sample may render inefficient the buffer of the Biophen® DiXaI® assay and may make this test sensitive to the presence of heparin and derivatives. Thus, our newly proposed dilution schemes were tested on plasma spiked with UNF (Heparin Leo®, Denmark), with LMWH (tinzaparin sodium, Innohep®, Denmark) or with fondaparinux (Arixtra®, UK).

Results : *Improvements of sensitivity to betrixaban*

Results showed concentration-dependent decreases in OD/min inversely proportional to the dilutions of the sample (►Figure 1). While modifications improve the sensitivity of these tests to betrixaban (e.g. ½*OD/min of 502ng/mL for Biophen®DiXaI® (1:50) is reduced to 51ng/mL for improved Biophen®DiXaI® (1:5)), results also showed an increased sensitivity to indirect factor Xa inhibitors, except for Biophen®DiXaI® which remains insensitive to UFH and tinzaparin.

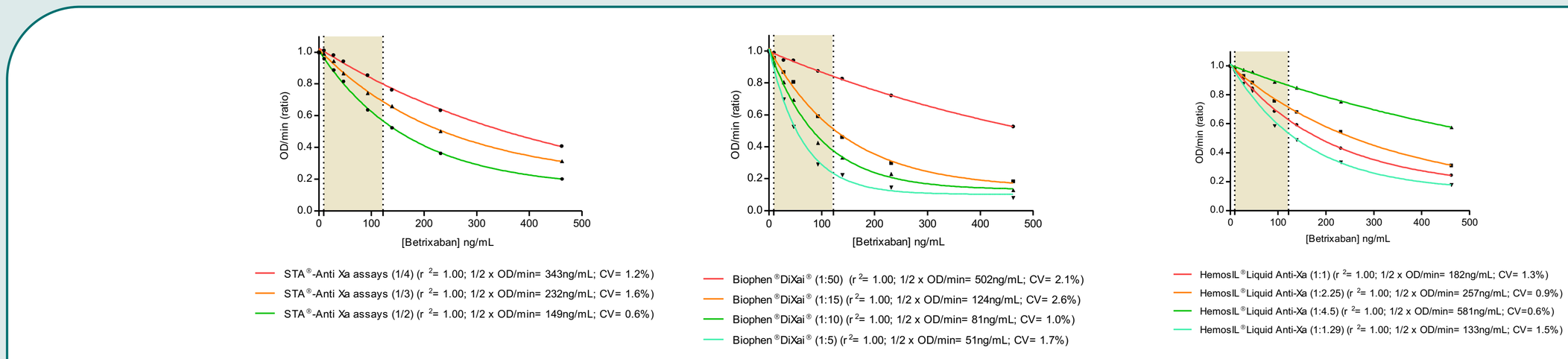

Figure 1: Impact of betrixaban on different anti-Xa assays. There was a concentration-dependent decrease of the OD/min. (r^2: Correlation Coefficient; ½xOD/min: Halve in optical density by minute expressed in ng/mL; CV: Coefficient of variation expressed in percentage [%]). The grayed area represents the on-therapy range for venous thromboembolism prophylaxis (from ± 9ng/mL at C_{trough} to ± 122ng/mL at C_{max} for the 40 and 120mg once daily doses according to the clinical pharmacology and biopharmaceutics reviews of the Center for Drug Evaluation and Research).

Results on UFH, tinzaparin and fondaparinux showed a concentration-dependent decrease in OD/min inversely proportional of the dilution of the sample. The reactions were fitted by a linear or an exponential model and the ½*OD/min were calculated (►Figure 2-4).

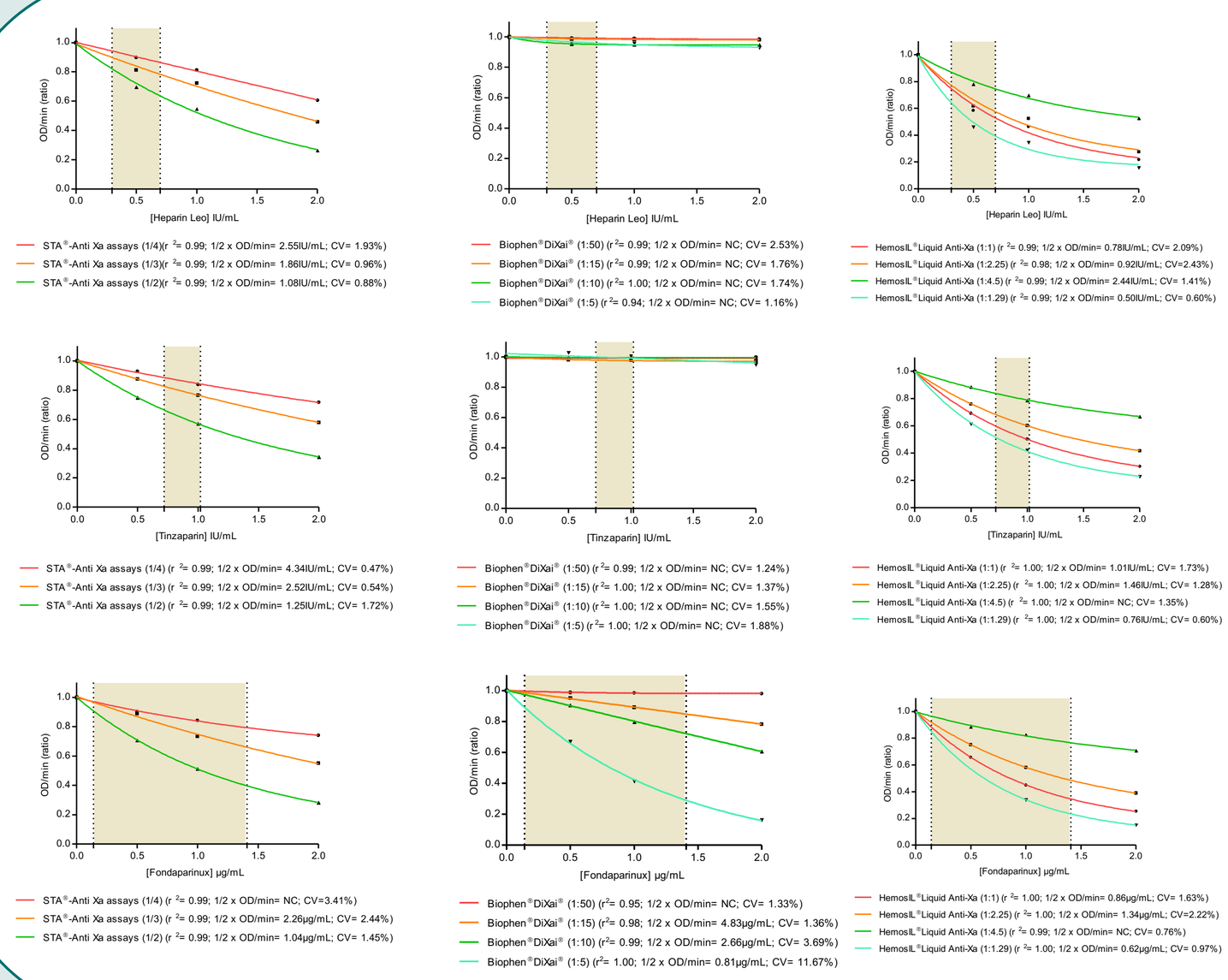

Figure 2: Impact of heparin on adapted methodologies. There was a concentration-dependent decrease of the OD/min. Tests with higher baseline OD/min had a wider range of quantitation compared to those starting at lower OD/min at baseline. (r^2: Correlation Coefficient; ½xOD/min: Halve in optical density by minute expressed in ng/mL; CV: Coefficient of variation expressed in percentage [%]). The grayed area represents the on-therapy range for thromboembolism treatment (from 0.3 to 0.7IU/mL).

Figure 3: Impact of tinzaparin on adapted methodologies. There was a concentration-dependent decrease of the OD/min. Tests with higher baseline OD/min had a wider range of quantitation compared to those starting at lower OD/min at baseline. (r^2: Correlation Coefficient; ½xOD/min: Halve in optical density by minute expressed in ng/mL; CV: Coefficient of variation expressed in percentage [%]). The grayed area represents the on-therapy range for constituted deep venous thromboembolism (DVT) and pulmonary embolism treatment (0.72 to 1.02IU/mL) once daily.

Figure 4: Impact of fondaparinux on adapted methodologies. There was a concentration-dependent decrease of the OD/min. Tests with higher baseline OD/min had a wider range of quantitation compared to those starting at lower OD/min at baseline. (r^2: Correlation Coefficient; ½xOD/min: Halve in optical density by minute expressed in ng/mL; CV: Coefficient of variation expressed in percentage [%]). The grayed area represents the on-therapy range for hip replacement surgery (0.14 to 0.39µg/mL), hip fracture (0.19 to 0.5µg/mL) and deep venous thrombosis and pulmonary embolism (0.52 to 1.41µg/mL).

Conclusion

The sensitivity of available tests for betrixaban is limited and improvement were needed to encompass the on-therapy range. Adapted methodologies are required but the possible increase of sensitivity towards UFH and LMWH must be considered when improving these methodologies for patients with bridging. Our results show that the improvement of current methodologies make the chromogenic anti-Xa assays more sensitive to betrixaban but also to heparins and derivatives, except for Biophen®DiXaI®. In conclusion, Biophen®DiXaI® with improved methodologies (1:10 or 1:5) should be considered for measurement of the on-therapy range of betrixaban for patients with or without potential bridging therapy with heparins. In regard to the dynamic range of quantification, Biophen®DiXaI® (1:15) should be preferred for higher concentration up to 450ng/mL of betrixaban. Concerning STA®-Liquid Anti-Xa and HemosIL®Liquid Anti-Xa, despite improvement of methodologies the increased sensitivity to heparins and derivatives may alter the measurements of betrixaban concentrations and the interpretation of the results. These reagents should be avoided to prevent the cross-interference between direct FXa inhibitors and heparin and/or LMWH. One possibility for these reagents could be the conception and the optimization of a new buffer equivalent to the TRIS-NaCl-EDTA buffer from Hyphen, adaptable for all chromogenic assays.

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