Suitability of Chromogenic Anti-Factor Xa Methods to Measure Rivaroxaban in Human Plasma

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Introduction

- In recent years, new anticoagulants targeting individual components of the coagulation cascade have been developed[1]
- Rivaroxaban is a highly selective, reversible, oral, direct Factor Xa inhibitor that dose-dependently inhibits Factor Xa activity, thrombin generation, and thrombus formation[2]
- Unlike fondaparinux, which expresses an indirect anti-Factor Xa catalytic activity in the presence of antithrombin,[3] direct Factor Xa inhibitors bind directly to the catalytic site of the serine protease Factor Xa, independently from antithrombin[1]
- The chromogenic methods that are routinely implemented to measure the effects of heparins in human plasma are expressed in anti-Factor Xa units, and are, therefore, not appropriate for rivaroxaban

Objective

- To evaluate the suitability of two assay principles; one exogenous and two endogenous Factor Xa inhibition assays were conducted to measure the pharmacodynamics of rivaroxaban

Methods

- The first endogenous Factor Xa inhibition assay was used for the initial screening of chemical compounds.[2] In undiluted plasma, spiked with increasing concentrations of rivaroxaban, and supplemented with hirudin to prevent clot formation, endogenous Factor Xa was generated by adding Russell's viper venom in the presence of calcium. The amidolytic Factor Xa activity was measured using a specific chromogenic substrate (S-2765, Chromogenix, Milan, Italy)
- The second endogenous Factor Xa assay was similar to the one used in clinical trials.[4] In this method, a high plasma dilution (1:20) is used to prevent clot formation without using hirudin
- The exogenous Factor Xa inhibition assay consisted of a modified version of a two-stage chromogenic assay, using human rather than bovine Factor Xa and a buffer with higher ionic strength to reduce possible interference with antithrombin activity

Results

- The first endogenous method allowed reliable measurements at very low concentrations of rivaroxaban (the method is linear up to 0.03 µg/mL; Figure 1)
- The second endogenous Factor Xa inhibition assay allowed measurements of concentrations of rivaroxaban up to 0.4 µg/mL, with a dilution of 1:4 in normal plasma for concentrations over 0.1 µg/mL because the concentration–effect relationship is linear up to 0.1 µg/mL (Figure 2)

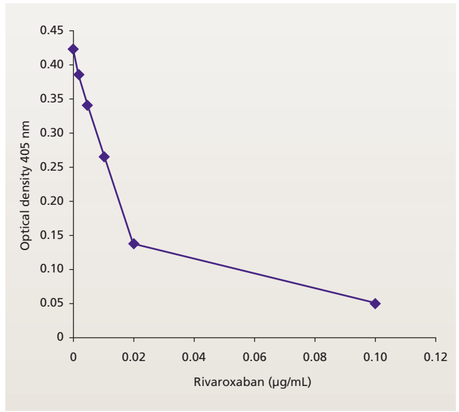

Figure 1. The influence of rivaroxaban on the first endogenous Factor Xa inhibition assay.

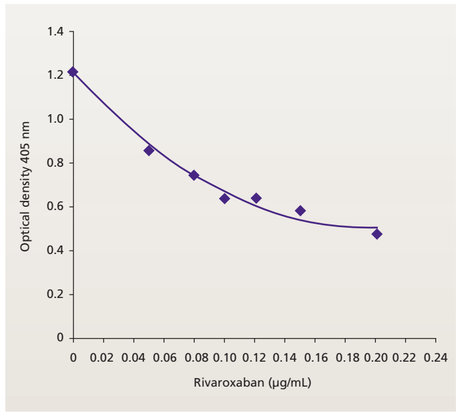

Figure 2. The influence of rivaroxaban on the second endogenous Factor Xa inhibition assay (dilution of 1:4 in normal plasma for concentrations over 0.1 µg/mL).

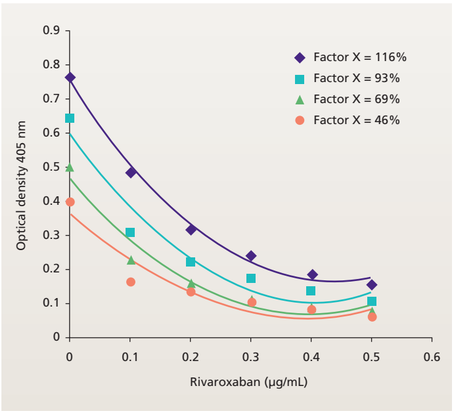

Figure 3. The influence of Factor X plasma concentration in the second endogenous method.

- Results were influenced by the plasma concentration of Factor X in both endogenous assays (Figure 3)
- The exogenous Factor Xa inhibition assay led to linear responses up to concentrations of 1.0 µg/mL (Figure 4). The limit of quantitation was calculated at 0.05 µg/mL
  - The coefficient of variation values in 20 plasma samples spiked with rivaroxaban 0.1 and 0.5 µg/mL were 1.75% and 2.48%, respectively, for the within-run and 7.12% and 15.18%, respectively, for the between-run, thus demonstrating the reliability of the test
- The C_{max} of rivaroxaban after 10 mg is between 0.1 and 0.2 µg/mL in thromboprophylaxis

Conclusion

- Modified commercial specific chromogenic assays could be used to measure the effects of rivaroxaban with drug concentrations being expressed in µg/mL of plasma

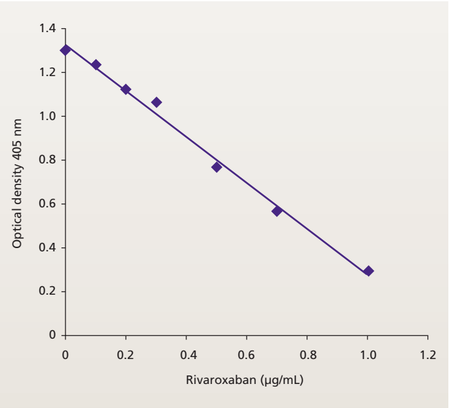

Figure 4. The influence of rivaroxaban on the exogenous Factor Xa inhibition assay.

References

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Disclosure of conflict of interest

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