

## **DIFFERENT CHARACTERISTICS OF DIRECT FACTOR Xa INHIBITORS: *IN VITRO* COMPARATIVE STUDIES OF RIVAROXABAN AND APIXABAN**

Elisabeth Perzborn, Adrian Tersteegen, Michaela Harwardt, Uwe Lange

Pharma R&D Discovery Research, Bayer Schering Pharma AG, Wuppertal, Germany

**Background/Aims :** Rivaroxaban and apixaban are selective, reversible, structurally different, direct Factor Xa inhibitors in late-stage clinical development for the prevention and treatment of venous and arterial thrombosis. Animal studies have demonstrated venous and arterial antithrombotic efficacy with these agents. This study characterizes and compares these agents in *in vitro* functional assays. **Materials and Methods:** Factor Xa activity and rate constants ( $k_{on}/k_{off}$ ) were assessed by measuring the amidolytic activity of purified Factor Xa. Prothrombinase activity was measured in a reconstituted prothrombinase complex with prothrombin as substrate and measuring the amidolytic activity of the generated thrombin. Clotting times and thrombin generation (TG) were measured using commercially available kits. Tissue factor (TF)-mediated platelet aggregation was measured in defibrinated plasma. **Results:** Rivaroxaban and apixaban showed similar characteristics: affinity for free Factor Xa ( $K_i$  0.4 nM and 0.6 nM, respectively); association ( $k_{on}$   $1.7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  and  $0.88 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ , respectively) and dissociation ( $k_{off}$   $5 \times 10^{-3} \text{ s}^{-1}$  and  $2.4 \times 10^{-3} \text{ s}^{-1}$ , respectively) rate constants; and inhibition of prothrombinase-bound Factor Xa ( $IC_{50}$  2.1 nM and 2.7 nM, respectively). However, in human plasma-based systems, these agents showed different potencies. Although similar plasma protein binding has been reported for apixaban and rivaroxaban (87% and 92–95%, respectively), higher molar concentrations of apixaban were required, compared with rivaroxaban, to inhibit TG ( $IC_{50}$ , peak TG 0.20  $\mu\text{M}$  and 0.06  $\mu\text{M}$ ; endogenous thrombin potential 4.96  $\mu\text{M}$  and 1.48  $\mu\text{M}$ , respectively) and for TF-mediated platelet aggregation ( $IC_{50}$  0.51  $\mu\text{M}$  and 0.06  $\mu\text{M}$ , respectively). In addition, the concentrations needed to double clotting times in different assays were three- to eightfold higher for apixaban than for rivaroxaban. **Conclusions:** Although they have a similar affinity to Factor Xa, structurally different Factor Xa inhibitors may differ in their antithrombotic potency.

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