The anti-factor XIa antibody BAY 1213790 is a novel anticoagulant that shows strong antithrombotic efficacy without an increased risk of bleeding in rabbit models

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Introduction
• Coagulation factor XI (FXI); the precursor of the plasma coagulation protease activated FXI (FXIa) is triggered by contact activation and during the amplification phase of the coagulation cascade after formation of small amounts of fibrin. FXI contributes to clot formation, clot stabilization and amplification of clot growth.1,2
• Mice lacking FXI are resistant to experimentally induced thrombosis and have a low risk of bleeding complications.1,3
• Inhibition of FXIa therefore offers the potential to reduce the risk of thrombosis without increasing the risk of bleeding and is an attractive target for novel antithrombotic therapies.1

Methods
• In vitro properties of BAY 1213790 were analyzed using:
  a. a standard enzyme-linked immunosorbent assay (ELISA, Haemostatic Technologies Inc.)
  b. a biochemical assay using a FXIa-specific, fluorogenically labeled substrate (Bachem)
  c. standard clotting assays (activated partial thromboplastin time [aPTT; Diagnostica Stago] and prothrombin time [PT; Instrumentation Laboratory])
  d. a thrombin-generation assay (validated automated thrombogram; Thrombinscope) with 0.1 µM tissue factor in human citrated plasma (Octapharma).
• The antithrombotic efficacy of BAY 1213790 administered at doses of 0.1, 0.3 and 1.0 mg/kg was assessed in a rabbit model of arterial thrombosis induced by ferrous chloride. Ear and gum bleeding times were also evaluated in this model.
• The hemorheologic effects of BAY 1213790 were evaluated in a rabbit liver injury model and in a rabbit model of ear and gum bleeding induced by tissue plasminogen activator (tPA).
  a. In the rabbit liver injury model, the effects of BAY 1213790 10 mg/kg on liver bleeding time and blood loss were compared with the effects of vehicle (phosphate buffered saline). The direct thrombin inhibitor bivalirudin (6 mg/kg bolus followed by 4.2 mg/kg/h infusion and 15 mg/kg bolus followed by 12 mg/kg/h infusion) was administered as a positive control. The liver was isolated and a standardized incision made. A sponge (Merocel®) was placed in the wound and changed every 30 seconds; blood loss was determined by weighing the sponges (maximum observation time: 30 minutes).
• In the rabbit model of ear and gum bleeding induced by tPA, BAY 1213790 3.0 mg/kg or vehicle was administered in the absence and presence of tPA 0.1 mg/kg for 2 h. At 2 h, a 0.5 cm incision was made in the left ear in the gum, beneath the front teeth, and the time to complete cessation of bleeding was recorded.
  a. In both of these models, BAY 1213790 was administered by intravenous bolus 15 minutes before the injury/surgery.

Results
In vitro properties in human plasma
• BAY 1213790 bound specifically to FXIa (Figure 1) with high affinity (half-maximal effective concentration (EC50) 0.2 nM)(Table 1) and inhibited human FXIIIa activity with a half-maximal inhibitory concentration (IC50) of 3 nM (Table 1).
• At a concentration of 20 nM, BAY 1213790 was associated with a 1.5-fold prolongation of aPTT in human plasma (Figure 2; n = 18); there was no significant effect on PT (Table 1).
• BAY 1213790 was associated with a concentration-dependent inhibition of thrombin generation; all readout parameters of the assay were modulated (Figure 3). Thrombin formation was inhibited with an IC50 peak (of 35 nM (Table 1).

Table 1. In vitro binding and anticoagulant properties of BAY 1213790 in human plasma.

<table>
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<tr>
<th>Assay</th>
<th>ELISA (binding to FXIa EC50)</th>
<th>Biochemical/FXIa (FXIa activity) IC50</th>
<th>aPTT 1.5-fold prolongation from baseline</th>
<th>PT 1.5-fold prolongation from baseline</th>
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<tr>
<td>Thrombin generation (peak IC50)</td>
<td>35 nM</td>
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| aPTT, activated partial thromboplastin time; EC50, half-maximal effective concentration; ELISA, enzyme-linked immunosorbent assay; FXIa, activated factor XI; IC50, half-maximal inhibitory concentration; PT, prothrombin time.

Conclusions
• BAY 1213790 demonstrated potent anticoagulant activity in vitro in human plasma and striking antithrombotic efficacy in vivo in a rabbit model, with a broad therapeutic window.
  a. Prolongation of aPTT in human plasma was consistent with the observed antithrombotic effect of BAY 1213790 in rabbits.
  b. Administration of BAY 1213790 did not significantly increase bleeding time or amount of blood lost in the rabbit models studied.
• Taken together, these findings demonstrate that the anti-FXIa antibody BAY 1213790 shows strong antithrombotic efficacy without increasing the risk of bleeding in rabbits, therefore offering potential as an anticoagulant with a broad therapeutic window.

Acknowledgments
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References
11. Anja Buchmueller, Andreas Wilmen, Julia Strassburger, Martina Victoria Schmidt and Volker Laux are employees of Bayer AG, which provided funding for this study.