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Efficacy of a Novel Contact Pathway Inhibitor, Ir-CPI, on in vitro Clotting Induced by PCI Catheter Segment

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PB 2151 | Heparin Calibrated Anti-Xa Assays for the Measurement of Low Levels of Direct Factor Xa Inhibitors

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Background: Apixaban, edoxaban and rivaroxaban do not require frequent monitoring but an assessment of the intensity of anticoagulation may be required in emergent or elective surgery. Some experts reported that anti-Xa activity below 0.1 IU/mL using heparin calibrated chromogenic assays may assert the absence of clinically relevant (i.e. < 30 or < 50 ng/mL depending on the clinical situation) direct factor Xa levels. However, it is not clear if difference in response will depend on the anti-Xa agent and also on the chromogenic anti-Xa kit used to assess the anti-Xa activity.

Aims: To assess if a cut-off of 0.1 UI anti-Xa/mL is able to exclude apixaban, rivaroxaban or edoxaban concentration < 30 ng/mL or < 50 ng/mL using different heparin calibrated chromogenic anti-Xa kits.

Methods: Apixaban, edoxaban and rivaroxaban were added to normal pooled plasma at increasing concentrations ranging from 0 to 500 ng/mL. Anti-Xa activities were measured using

- (1) STA®-Liquid Anti-Xa (STA®LAX) on a STA-R Evolution Coagulometer,
- (2) Biophen®Heparin LRT (BP®LRT) on a STA-R Evolution coagulometer and
- (3) HemosIL®-Liquid Anti-Xa (IL®LAX) on a ACL-TOP 700 according to manufacturer recommendations.

Results: At 30 ng/mL of rivaroxaban, BP®LRT, STA®LAX and IL®LAX provided anti-Xa results >0.1 IU/mL. At 30 ng/mL of apixaban or edoxaban, BP®LRT and IL®LAX were below the cut-off but the STA®LAX was not. At a concentration of 50 ng/mL, only edoxaban with the BP®LRT kit showed an anti-Xa activity < 0.1 UI/mL.

Conclusions: Low (< 0.1 IU/mL) anti-Xa activity is not safe to exclude clinically relevant direct factor Xa levels and should be avoided. It can only inform if the drug is present or not. Chromogenic anti-Xa assays calibrated against the appropriate agent and using the appropriate

TABLE 1 Anti-Xa activities using STA®LAX on a STA-R Evolution Coagulometer, BP®LRT on a STA-R Evolution coagulometer and (3) IL®LAX on a ACL-TOP 700

concentration (ng/mL)	anticoagulant	STA®LAX (UI/mL)	BP®LRT (UI/mL)	IL®LAX (UI/mL)
30	Rivaroxaban	0,19	0,12	0,08
30	Apixaban	0,09	0,03	0,04
30	Endoxaban	0,07	0,02	0,02
50	Rivaroxaban	0,45	0,34	0,16
50	Apixaban	0,17	0,10	0,07
50	Endoxaban	0,13	0,06	0,04

procedure remains the more accurate method to assess accurately low levels of direct FXa inhibitors.

PB 2152 | Efficacy of a Novel Contact Pathway Inhibitor, Ir-CPI, on in vitro Clotting Induced by PCI Catheter Segment

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Background: Ir-CPI, a protein derived from the tick *Ixodes ricinus* salivary, is a serine protease inhibitor of both factor XIa (FXIa) and FXIIa. In patients undergoing percutaneous coronary intervention (PCI), catheter thrombosis may occur as catheters trigger activation of FXII/FXI.

Aims: The aim of this study was to evaluate the effect of Ir-CPI on in vitro clotting induced by PCI catheter segment.

Methods: Catheter segments were pressed flat, shaped into rings and placed around the perimeter of wells (96-well plate), leaving the center of the well unobstructed. To the wells were added serial dilution of Ir-CPI (until 10 µM) with normal pooled plasma (NPP) or plasmas deficient in FXI or FXII. After incubation at 37°C and addition of a CaCl₂ solution, clot formation was assessed by monitoring absorbance at 340nm. Time to reach one-half maximal absorbance (IC50) was defined as the clotting time. Thrombin generation test (TGT) was also assessed using catheter segment as trigger of the process. Positive inhibitory controls were used (fondaparinux, enoxaparin).

Results: Presence of the catheter reduced the clotting time of NPP; an effect reversed by the addition of Ir-CPI. At high concentrations (> 5µM), Ir-CPI allowed to overpass the clotting time without catheter. On TGT (Fig 1), catheter segments decreased lag time and time

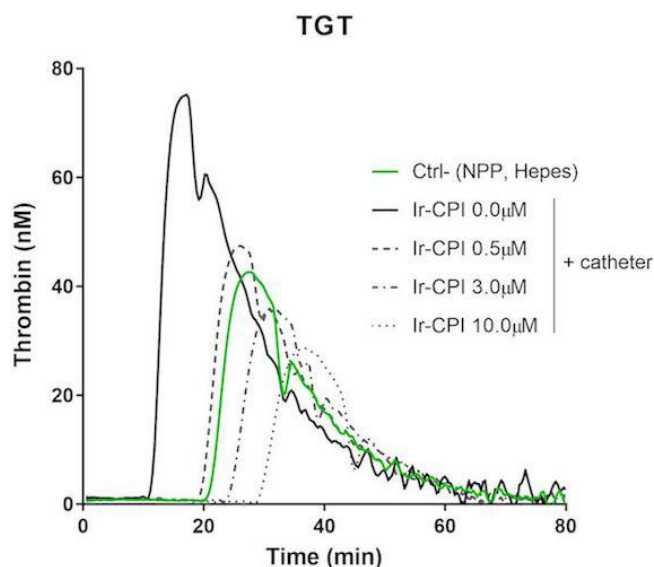


FIGURE 1 Effect of Ir-CPI on Thrombin Generation Time (TGT) in NPP exposed to PCI catheter segments

to peak while the endogenous thrombin potential (ETP) and the peak were increased. The presence of Ir-CPI allowed the restoration of baseline value, i.e. value of the NPP without exposition to catheter segments, in a concentration-dependent manner.

When clotting was triggered with FXII deficient plasma, we confirmed that catheter thrombosis is linked to FXI activation and that clotting can be abrogated with 3 μ M of Ir-CPI.

Conclusions: Ir-CPI can be used to inhibit the clotting induced by catheter segments and achieve antithrombotic effect. Ir-CPI is a promising agent with a better safety profile than heparins to face the problem of catheter thrombosis during PCI procedures.

PB 2153 | New Parenteral Poly (2-(acrylamide)-2-methylpropanesulfonic Acid) - Based Anticoagulants (NPACs): Efficacy and Safety Studies in Rats

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Background: Unfractionated heparin (UFH) remains an indispensable parenteral drug inhibiting blood coagulation. The biologic variability, immunogenicity, unpredictable anticoagulation, and narrow therapeutic range limit its utility. Other parenteral anticoagulants lack of an efficient antidote. We recently presented a novel agent neutralizing all parental anticoagulants (Trans Res 2016). There is still a need for synthetic UFH alternative with the available safe antidote and without unacceptable adverse effects.

Aims: The aim of the present study was to develop a novel, safe, and easily synthesized poly (2-(acrylamide)-2-methylpropanesulfonic acid) (PAMPS)-based parenteral anticoagulants.

Methods: We synthesized, purified and characterized 4 novel PAMPS-based polymers named by us as new parenteral anticoagulants (NPAC1, NPAC2, NPAC3, and NPAC4) and PAMPS. Then, we screened polymers for potential anticoagulant and antiplatelet activity

using the *in vitro* assays. Finally, we examined efficacy and safety of the most active polymers in male Wistar rats. We assessed aPTT, PT, anti-factor Xa activity, calcium concentration, and platelet aggregation as efficacy endpoints and cardiorespiratory and hematological parameters as safety measures.

Results: We found that all synthesized PAMPS-based polymers and PAMPS dose-dependently prolonged aPTT and PT and decreased platelet aggregation *in vitro*. The effect of NPAC1 on aPTT and PT was the weakest. Thus, we discontinued studying of this polymer. NPAC2 and NPAC4 significantly increased the anti-fXa activity. *In vivo*, all polymers significantly prolonged aPTT and PT. NPAC4 and PAMPS decreased, NPAC3 increased, whereas NPAC2 did not alter platelet aggregation. Unlike NPAC2 and NPAC3, NPAC4 and PAMPS caused unacceptable cardiorespiratory and/or hematological complications in rats (Table 1).

Conclusions: Documented efficacy and safety of NPAC2 in rats makes this polymer a promising candidate for a novel parenteral anticoagulant.

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PB 2154 | INR Might Be a Useful Predictor for Upcoming Minor to Major Bleedings in Patients Treated with NOAC

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Background: Patients treated with Novel Oral Anticoagulants (NOAC) are not required to be monitored laboratory, as no qualitative tests or therapeutic intervals are recommended today. Despite favorable pharmacokinetic properties, bleeding events are some of the few complications to the treatment. As there are no recommended global anticoagulation tests, prediction of these complications is today not possible.

TABLE 1 In vivo efficacy and safety of new parenteral anticoagulants (NPACs)

	Vehicle	NPAC2	NPAC3	NPAC4	PAMPS
aPTT (seconds)	25.7 \pm 4.9	94.1 \pm 21.9***	82.9 \pm 27.8***	113.3 \pm 24.8***	109.9 \pm 23.4***
PT (seconds)	10.8 \pm 0.5	13.5 \pm 0.9***	12.6 \pm 1.0***	114.5 \pm 1.2***	14.6 \pm 1.5***
Platelet aggregation - maximal extension (Ω)	12 (8.5-13)	11 (5.5-13.5)	14 (13-19)**	4 (2.5-6)**	4 (0.5-6.5)**
Platelet aggregation - slope	6 (4.5-7)	6 (3-8)	8 (6-10)*	3 (2-4)***	3 (2-4.5)**
Platelet aggregation - lag time (seconds)	72 (64-89)	71 (69-89)	67 (35-74)	119 (74-196)**	106 (79-297)**
Platelet aggregation - area under the curve	39 (26-41)	34 (19-43)	49 (41-63)**	11 (5-20)**	12 (1-19)**
Blood platelets (10 ³ /mm ³)	666 \pm 65	654 \pm 68	666 \pm 46	562 \pm 103*	497 \pm 79***
Cardiorespiratory complications	-	-	-	Cardiac and respiratory arrest	Cardiac and respiratory arrest

Results are shown as mean \pm SD or as median with lower and upper limits.*P<0.05, **P<0.01, ***P<0.001 vs. vehicle, unpaired Student t test or Mann-Whitney test, n=6-11.