APAC, A DUAL ANTIPLATELET AND ANTICOAGULANT HEPARIN PROTEOGLYCAN
MIMETIC TARGETS VON WILLEBRAND FACTOR IN INJURED VESSEL WALL

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Background of APAC

Mast cell heparin proteoglycans (HEP-PG) abolish collagen-induced platelet aggregation1, and Von Willebrand (VWF)-mediated thrombus growth, but not platelet tethering in vitro2 and in vivo3

APAC mimics HEP-PG structure and function:

• unfraccionated heparins (UFH) coupled to a protein core
• dual antiplatelet and anticoagulant activity
• can be tailored by modifying UFH conjugation4
• inhibits arterial thrombosis (Folts and AV-shunt model) and reduces fibrin formation in baboons
• maintains arterial integrity of femoral anastomosis in rats
• reduces ischemic reperfusion injury, acute kidney injury model in rats5
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METHODS

To assess important vascular properties we further characterized APAC’s role in

1. attenuating whole blood aggregation (Multiplate), and global coagulation (ROTEM)
2. local adhering to denuded porcine iliac artery, and femoral artery and vein

Methods

• APAC and UFH at equal heparin concentrations in PBS at pH 7.4
• Result are mean ± standard deviation (SD)
• n = number of individual donor blood samples or animals

In vitro spiking with APAC and UFH

• Agonist-induced platelet aggregation in citrated (3.2%) human blood with 3.2 µg/mL collagen, 0.77 mg/mL ristocetin (Multiplate®), and in platelet-rich plasma (PRP) with 0.5 µg/mL collagen (Born-method)
• Rotational thromboelastometry (ROTEM®) in citrated blood: INTEM, EXTEM, FIBTEM (cytochalasin D to inhibit platelets) or APTEM (fibrinolysis inhibited by aprotinin) and HEPTEM (in the presence of heparinase)

In vivo vascular injury model with biotinylated APAC (0.5 mg/mL) after 2 min administration and blood flow release

• Arterio-venous fistulae (AVF) of the femoral and iliac artery
• Tissue harvested at 30 min
• Balloon angioplasty denudation of iliac artery (IA)
• Tissue harvested at 10 min
• Tissues were fixed (10% formalin 4h), and processed for cryosectioning

Imaging

• Samples were immunostained for VWF, PECAM and laminin
• APAC-Biotin was detected with streptavidin conjugated to EFluor 660
• Pictures were taken with SP8 leica confocal microscope
• Mander’s colocalisation coefficients were calculated with ImageJ software

RESULTS

Colocalisation analysis of APAC, VWF and Laminin in balloon angioplasty (IA)

Colocalisation analysis of APAC, VWF and PECAM in AVF

APAC inhibits A) collagen (CIPA) - and B) ristocetin-induced platelet aggregation distinct from UFH (Multiplate) in whole blood

At 150 µg/mL of APAC, mean inhibition of aggregation:

• 58 ± 15 % (n=6) for collagen and 25 ± 2 % (n=4) for ristocetin, representative examples

APAC inhibits C) UFH unlike UFH in PRP

• PBS max aggregation (n=9): 90 ± 8 % and AUC 48 ± 8 %
• APAC (1-100 µg/mL) dose-dependently prolonged lag time and decreased slope and inhibited AUC reaching plateau at 30 µg/mL, 85 ± 12 % (Table)
• UFH (100 µg/mL) was without any effect

APAC broadly attenuates coagulation in ROTEM whole blood analysis while UFH acts mainly in INTEM

CONCLUSIONS

• APAC inhibits collagen- and ristocetin-induced aggregation unlike UFH
• APAC’s ~ED50 was 150 µg/mL in whole blood and 3 µg/mL in PRP CIPA
• APAC is a stronger and broader anticoagulant than UFH in ROTEM
• APAC adheres to injury site co-localizing with VWF, laminin and PECAM
• APAC powerfully protects from thrombosis at site of vascular damage with its binding avidity and dual anti-platelet and anticoagulant action