Novel Intravenous Anticoagulant: Development and Testing for Safe, Effective Thrombosis Prevention

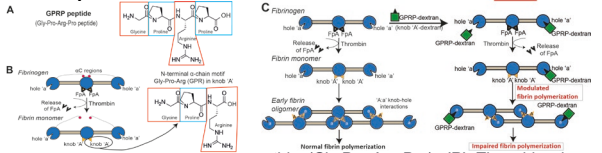
Alina D. Peshkova[1], Rustem I. Litvinov[2], John W. Weisel[2], Vladimir R. Muzykantov[1]

[1]Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania Perelman School of Medicine, PA, USA
[2]Department of Cell and Developmental Biology, University of Pennsylvania Perelman School of Medicine, PA, USA

INTRODUCTION

Fibrin polymerization is initiated by thrombin-catalyzed cleavage of fibrinopeptides A from fibrinogen followed by exposure of the Gly-Pro-Arg(GPR) motif, called knob 'A', which interacts with complementary holes 'a' in other fibrin molecules. The peptide GPRP mimics the knob 'A' and modulates competitively the knob-hole interactions, impeding fibrin polymerization. Disruption of fibrin formation represents a novel strategy for anticoagulation.

Competitive interactions of GPRP in fibrin polymerization



(A) The structure of the GPRP peptide (Gly-Pro-Arg-Pro). **(B)** Thrombin cleaves fibrinopeptide A from the N-terminal α-chain of fibrinogen, exposing knob 'A' (Gly–Pro–Arg), which initiates fibrin polymerization by enabling subsequent binding interactions. **(C)** GPRP-dextran blocks hole 'a', impeding fibrin polymerization.

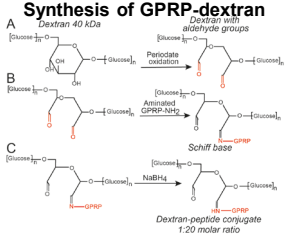
AIM

To synthesize the GPRP-dextran conjugate and investigate its anticoagulant activity *in vitro* and *in vivo*.

METHODS

GPRP-dextran was synthesized by covalently attaching the GPRP peptide to dextran (A-C). Fibrin polymerization was assessed by turbidimetry in human platelet-free plasma (PFP). Anticoagulant activity was measured by clottable fibrinogen, activated partial thromboplastin time (APTT) and rotational thromboelastometry (ROTEM). Clot structure was examined by scanning electron and confocal microscopy.

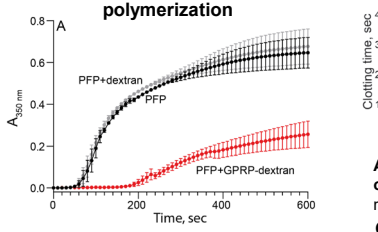
Synthesis of GPRP-dextran

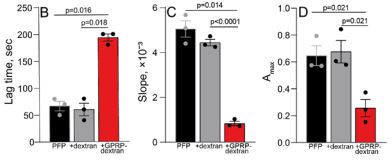


In vivo effects were evaluated in mice after intravenous (IV) injection via the tail bleeding time and clotting assays.

RESULTS

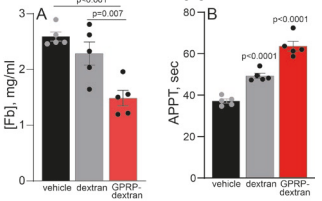
GPRP–dextran significantly impairs fibrin polymerization





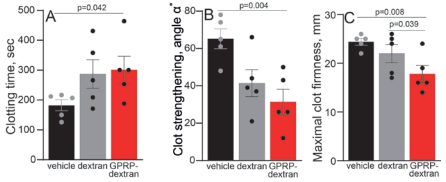
Partial inhibition of fibrin polymerization by GPRP–dextran: (**A**) Turbidity curves in PFP with/without GPRP–dextran; (**B**) Lag time; (**C**) Polymerization rate (slope); (**D**) Max OD (Amax) indicating fibrin amount and fiber thickness.

GPRP–dextran decreases the clottable fibrinogen level and prolongs APTT *in vitro*



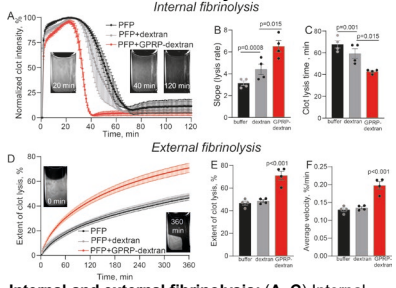
(A) Clottable fibrinogen after incubation with GPRP–dextran. **(B)** APTT after treatment with GPRP–dextran.

GPRP–dextran modulates ROTEM parameters



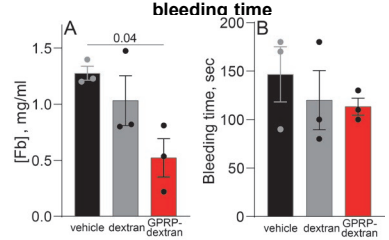
Average ROTEM parameters in the absence/presence of GPRP-dextran: (**A**) Clotting time, (**B**) Clot formation rate (clot strengthening), (**C**) Maximum clot firmness.

GPRP–dextran promotes internal and external fibrinolysis



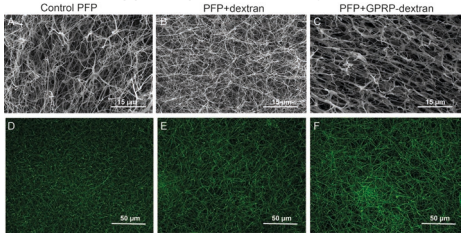
Internal and external fibrinolysis: (**A–C**) Internal fibrinolysis: t-PA before clotting. (**A**) Clot intensity, (**B**) Lysis rate, (**C**) Lysis time. (**D–F**) External fibrinolysis: t-PA after clotting. (**D**) Lysis curve, (**E**) Final extent, (**F**) Lysis velocity.

IV administration of GPRP–dextran reduces clottable fibrinogen without affecting tail bleeding time



(A) Plasma levels of clottable fibrinogen measured after infusion of GPRP-dextran. **(B)** Tail bleeding time, used as an indicator of primary hemostasis, remained unchanged across all groups.

GPRP-dextran impairs the structure of fibrin clots suggesting defective polymerization



Fibrin Clot Structure: (**A–C**) Scanning electron microscopy and (**D–F**) Confocal images of clots from control, dextran, and GPRP–dextran PFP. Scale: 15 μm (SEM), 50 μm (confocal).

CONCLUSION

GPRP–dextran exhibits consistent anti-fibrin polymerization effects *in vitro* and *in vivo*, highlighting its potential as a novel anticoagulant and antithrombotic agent with minimal bleeding risk.

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CONTACT INFORMATION

Dr. Alina Peshkova
alina.peshkova@pennmedicine.upenn.edu