Thrombin-Inhibiting Perfluorocarbon Nanoparticles Manifest Versatile Inhibition and Contrast for Thrombosis

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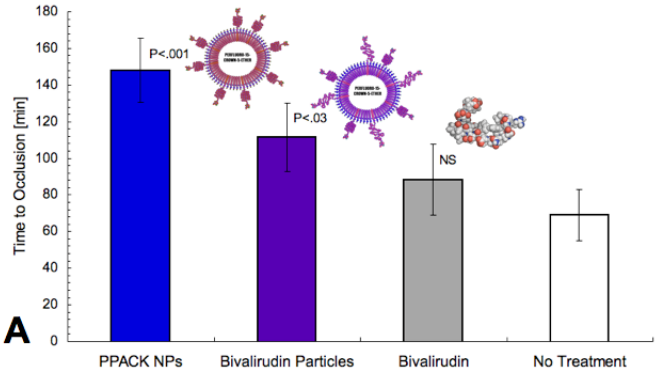

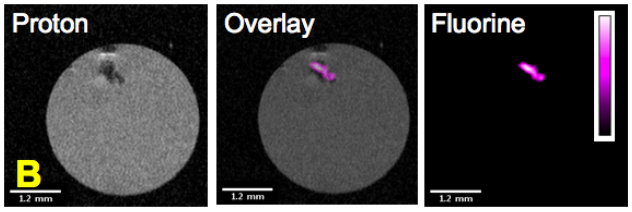

Acute thrombosis is currently addressed with a cocktail of anticoagulants, antiplatelet agents, and thrombolytics. Optimization of antithrombotics for emergency treatment of acute arterial or venous occlusions remains a significant research challenge. Direct thrombin inhibitors represent a promising class of targeted anticoagulant. Imaging contrast agents specific to thrombi would represent a boon to clinical intervention in thrombosis, enabling monitoring of progress in treatment of thrombotic events. In recent work, a thrombin-inhibiting perfluorocarbon nanoparticle (PFC NP), functionalized by the direct thrombin inhibitor, PPACK (Phe(D)-Pro-Arg-Chloromethylketone), was presented as a prototype for novel class of targeted antithrombotic. Here, an NP functionalized with Bivalirudin (BVR), was compared to its component drug and the PPACK NP. Both particles were examined as agents for inhibition and specific imaging of acute thrombosis.

PPACK or BVR were covalently attached to PFC NPs. Attachment was verified by chromatography and optical absorbance from the inhibitors. Optical assay verified that PPACK and BVR selectivity and activity against thrombin was not diminished on the NPs. In vivo activity was assessed for PPACK NPs, PPACK, BVR, BVR NPs, heparin, non-functionalized NPs, or saline in C57BL6 mice subjected to laser injury of the carotid artery. Time to thrombotic occlusion of the injured artery was assessed via Doppler flow measurement. Selected arteries were excised to assess NP retention via ^{19}F MR at 12T. In additional in vivo work, the laser injury arterial thrombosis model was applied in cholesterol-fed rabbits to demonstrate high-resolution 3T MRI of thrombosis following administration of PPACK NPs. ^{19}F MRI of the affected femoral artery segment at 12T enabled mapping of the thrombus ex vivo.

Greater than 10,000 PPACK or BVR were attached to each NP. PPACK NPs exceeded PPACK's activity against thrombin. BVR activity on NPs was insignificantly diminished versus free BVR. Previously, PPACK NPs outperformed both heparin (p=.001) and PPACK (p=.0006) in delaying occlusion of the carotid artery. PPACK or non-functionalized NPs failed to delay occlusion of the carotid artery. BVR NPs significantly delayed occlusion (p=.02) whereas an equivalent dose of free BVR (120 mg/kg) did not (figure a). ^{19}F MR captured specific PFC NP retention in occluded arteries (figure b), with ^{19}F imaging demonstrating colocalization of particles with the arterial thrombus and quantitative ^{19}F spectroscopy demonstrating that BVR or PPACK NPs retain in injured arteries in greater quantities than do non-targeted particles. Ultrasound imaging of the laser-injured rabbit femoral artery showed development of thrombi (figure c) and MRA showed a formed thrombus (figure d). Ex vivo ^{19}F MRI at 12T mapped the presence of PPACK NPs in the affected artery.

Anticoagulant PFC NPs were designed as new antithrombotics with intrinsic magnetic resonance contrast, concentrated therapeutic impact conferred by a thrombin-specific particle surface, and well-defined pharmacokinetics controlled by the particle itself. As potent agents that can enhance the therapeutic performance of different thrombin inhibitors, these NPs are promising new antithrombotics. Further clinical potential is conferred on the thrombin-inhibiting PFC NP by its ability to guide further intervention by specifically localizing magnetic resonance contrast to sites of thrombosis.