Molecular imaging of thrombosis and thrombolysis using a new fibrin-binding PET probe

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Background: Thrombosis is the underlying cause of many major cardiovascular diseases, and the early detection of thrombus formation is critical for both diagnosis and intervention. Current thrombus imaging techniques (e.g., echocardiogram, ultrasound, CT, MR) are specific just for particular anatomical and vascular districts, and their sensitivity shows considerable degrees of variation depending on thrombus location. An imaging modality capable of whole-body thrombus detection could overcome these limitations. Here, we describe a molecular imaging approach to detect thrombosis using a new PET probe targeting fibrin, which is a major component of both arterial and venous thrombi but is absent in circulating blood.

Methods: The fibrin-binding probe FBP7 was synthesized by conjugation of the short cyclic peptide FHCHypY(3-Cl)DLCHIL-PXD (Hyp=L-4-hydroxyproline,Y(3-Cl)=L-3-chlorotyrosine, PXD=p-xylenediamine) to a cross-bridged chelator, followed by labeling with ^{64}Cu. Adult male Wistar rats underwent either carotid crush injury (mural thrombosis model) or embolic stroke (occlusive thrombosis model) followed by recombinant tissue plasminogen activator treatment (rtPA, 10 mg/kg, i.v.). Imaging properties and target uptake were assessed non-invasively with PET-CT imaging and then confirmed by ex vivo biodistribution and autoradiography.

Results: FBP7 detected the thrombus at the level of the common carotid artery after crush injury (*Figure 1*). PET quantification showed high target-to-background ratios that increased over time along with the reduction of off-target activity (*Figure 2*). Similar results were obtained from ex vivo biodistribution and autoradiography of the ipsilateral vs. contralateral carotid arteries. In embolic stroke animals, PET-CT imaging localized the clot in the internal carotid/middle cerebral artery segment of all rats (*Figure 3 A-B*), result confirmed by tissue inspection (*Figure 3 C-D*) and autoradiography (*Figure 3 E*). Time-dependent reduction of activity at the level of the internal carotid artery was clearly detected in rtPA-treated rats but not in vehicle-injected animals (*Figure 4*). Reduction of activity was also detected at the level of the middle cerebral artery after rtPA treatment (*Figure 5 A-B*). Visual inspection of the circle of Willis (*Figure 5 C*) and brain autoradiography (*Figure 5 D*) confirmed clot dissolution in rtPA-treated animals, but enduring high thrombus activity in control rats (*Figure 5 F*).

Conclusions: We demonstrated that FBP7 is suitable for molecular imaging of thrombosis and thrombolysis, and represents a promising candidate for bench-to-bedside translation.

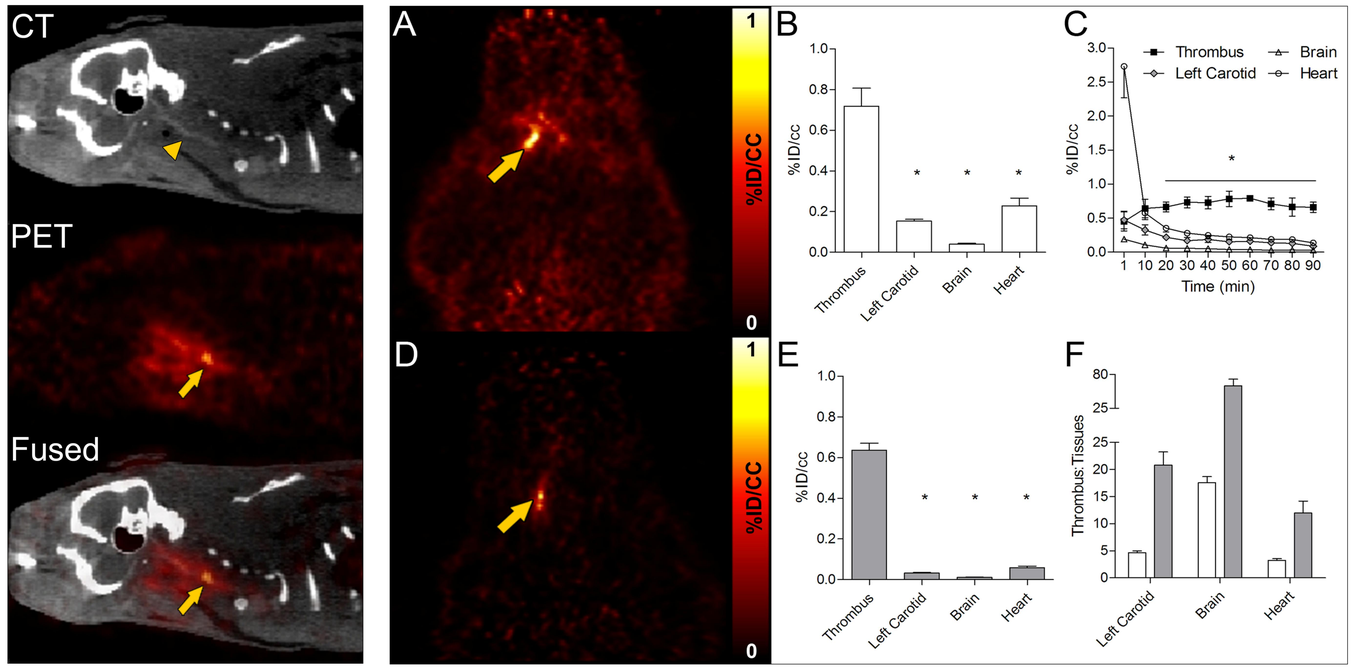

Figure 1
Sagittal views obtained from a representative rat injected with FBP7 after carotid crush injury. PET-CT imaging revealed the location of the thrombus (arrow) at the level of the carotid artery (arrowhead).

Figure 2
PET images obtained at 30-90 min (*A*, n=5) and 240-285 min (*D*, n=5) post-injection showed persistent thrombus signal and decreased background activity over time. Target uptake was significantly higher than background tissues at 30-90 min post-injection (*B*), a difference more pronounced at 240-285 min post-injection (*E*), consistent with the wash-out of non-target activity. Time-activity curves of the thrombus and background tissues (*C*). PET activity ratios over time (*F*, white bars: 30-90 min post-injection; gray bars: 240-285 min post-injection). Error bars are S.E.M. *P<0.0001.

High target affinity: 660 nM | **Fast blood clearance: 18 min half-life** | **High metabolic stability** | **Low off-target uptake and tissue retention**

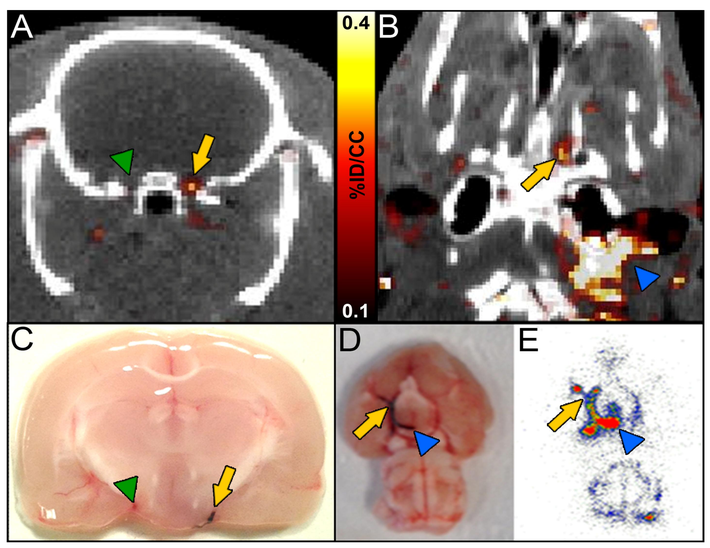

Figure 3
FBP7 detects the occlusive embolus in both middle cerebral (MCA, *A-B*) and internal carotid (ICA, *B*) arteries. The yellow arrows indicate the clot in the ipsilateral MCA; the green arrowheads indicate the patent contralateral MCA; the blue arrowheads indicate the clot in the ipsilateral ICA. The presence of thrombus was confirmed by postmortem visualization (*C-D*, note that the thrombus stained with Evans blue dye) and autoradiography (*E*, high activity levels overlap with the path of the thrombus).

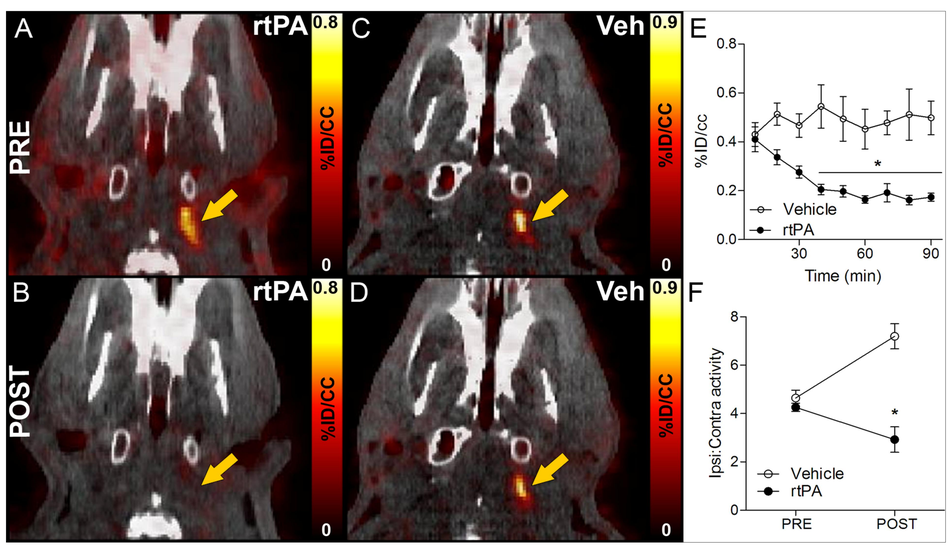

Figure 4
FBP7 enabled in vivo visualization of thrombolysis at the level of the internal carotid artery (ICA). *A-D*: Representative pre and post PET-CT images of the ICA (arrow) from rtPA- (n=7) and vehicle-treated (n=6) animals; the hyperintense signal at the level of the ICA disappeared after thrombolytic treatment, suggesting clot resolution. *E*: time-activity curves showed reduction of the signal after rtPA treatment (filled symbols), consistent with thrombolysis, but a steady signal in vehicle-injected rats (empty symbols). *F*: ipsilateral:contralateral activity ratios before and after rtPA (filled symbols) and vehicle (empty symbols) administration. Error bars are S.E.M. *P<0.001.

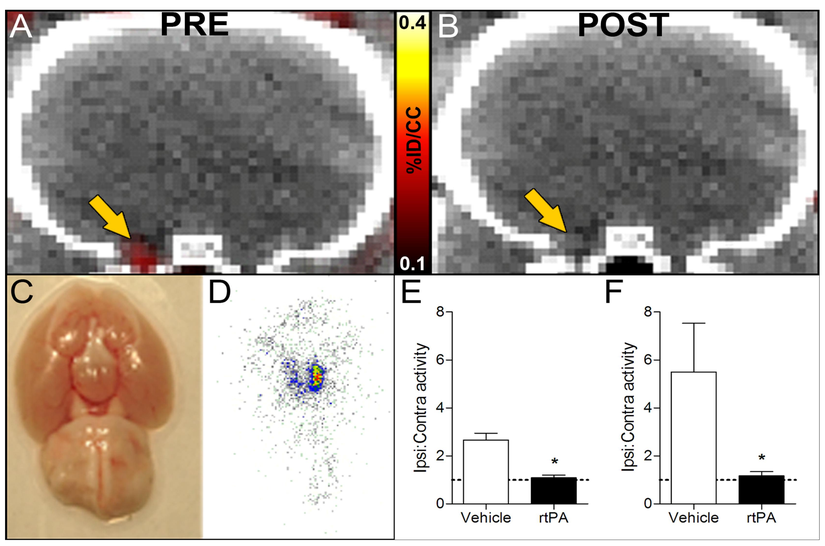

Figure 5
FBP7 detected intracranial thrombolysis. *A-B*: Representative pre and post PET-CT images at the level of the middle cerebral artery (MCA, arrow); the hyperintense signal at the level of the ICA disappeared after rtPA administration. Thrombolysis was confirmed by post-mortem visual inspection of the circle of Willis (*C*) and autoradiography (*D*). Results of ipsilateral:contralateral activity ratios in rtPA- (black bars) and vehicle-treated (white bars) animals obtained from PET (*E*) and autoradiography (*F*) experiments. Dashed lines show ratio=1. Error bars are S.E.M. *P<0.001.

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