MR imaging of an arachidonic acid-induced mouse model of thrombosis using an activated platelets-targeted paramagnetic contrast agent.

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Introduction: Rupture of atherosclerotic plaques frequently leads to thrombus formation and may trigger clinical events like myocardial infarction or stroke. Furthermore, it is well documented that thrombi are responsible for promoting inflammation and plaque progression. Activated platelets play a critical role in thrombogenesis through both platelets aggregation and activation of the coagulation cascade. Therefore, imaging adherent or aggregated platelets may represent a unique opportunity to identify thrombi and characterize vulnerable atherosclerotic plaques. As of yet, most of the studies about experimental thrombosis have been using a ferric chloride model of thrombogenesis. This model does not involve the physiological mechanisms of thrombi formation but rather creates a severe injury that leads to thrombosis. Recently, an improved model of thrombosis where arachidonic acid (AA) is topically applied to mice carotid arteries has been introduced. This model more closely mimicks physiological thrombogenesis. In the current study, we aim to image thrombi formation in the AA model using an activated platelet targeted MRI contrast agent (P975, Guerbet, France) composed of a peptide targeting the glycoprotein GPIIb/IIIa conjugated to Gd-DOTA.

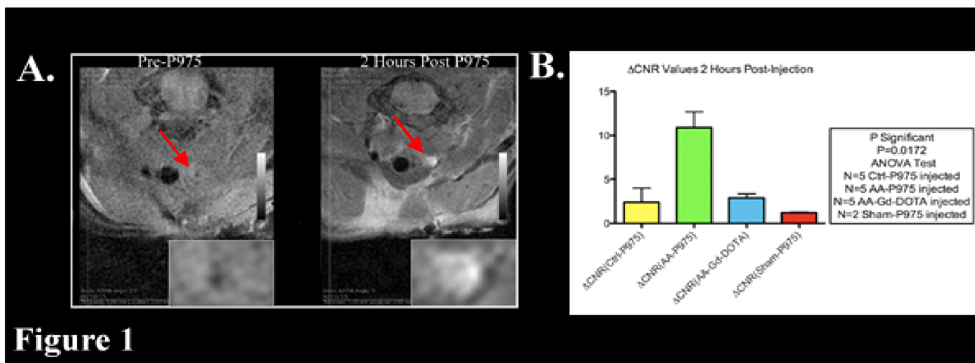

Figure 1

Methods: Specificity of the P975 to activated human platelets was first demonstrated through an in vitro competition experiment. The capacity of the P975 to inhibit the binding of a GPIIb/IIIa agonist (fibrinogen-FITC) on TRAP activated platelets was studied using increasing concentrations of P975. For the in vivo experiments, 17 wild type mice in an age range of 10 to 11 weeks were used. After surgically isolating and mildly ligating a segment of the right carotid artery, thrombosis was induced by continous, topical application of 4 ul of arachidonic acid at 200 mg/ml for 1 minute (AA Carotid). The mice were scanned at baseline and longitudinally over 2 hours after P975 or Gd-DOTA injection (Both groups: 100 µmol Gd/kg, N=5) on a Bruker 9.4 T using a 35mm whole body resonator. We applied a spin echo T1-weigthed sequence with a spatial resolution of 117 µm (1mm slice thickness, matrix size 256x256), a repetition time (TR) of 800 ms and an echo time (TE) of 10.2ms resulting in an imaging time of 13.2 minutes The contralateral carotid was used as a control. A sham surgery control group, where the right carotid was isolated and ligated without the application of AA, was also imaged after the administration of P975 (N=2). After imaging, all mice were sacrificed and the right carotid was excised for immunohistochemistry. MRI image analysis was performed at the site of thrombus formation. Contrast to noise ratios (CNRs) were calculated at baseline and at each time points up until 2 hours post-injection. Differences in CNRs at 2 hours - (ΔCNR=CNR(2h)-CNR(Baseline)) - were then determined and compared between groups. In addition, the evolution of mean CNR values over time was plotted and compared.

Results and Discussion: In vitro experiments showed an efficient inhibition of fibrinogen-FITC binding to platelets by P975. The affinity of the P975 for these GPIIb/IIIa-expressing cells was determined with an IC50 value of 2 to 4 µM. As for in vivo results, a typical image acquired before and 2 hours post administration is depicted in Figure 1A. The right carotid, where thrombosis was induced, showed a high signal enhancement compared to the contra-lateral control carotid. The mean values of ΔCNR at 2 hours post-injection were plotted (Figure 1B) for each of the 4 groups and revealed significant differences (P=0.0172 ANOVA test). In Figure 2, the evolution of the mean CNR values over time for the same four groups is depicted. In animals where thrombosis was induced, P975 administration produced an immediate increase in the mean CNR value that remained elevated over time. On the other hand, after Gd-DOTA injection there was only a weak increase in mean CNR values 30 minutes after injection, going back to baseline level within 1 hour. The Sham operated animals injected with P975 had similar enhancement patterns to the aforementioned Gd-DOTA control group. Finally, the control group remained constant over the 2 hours of monitoring.

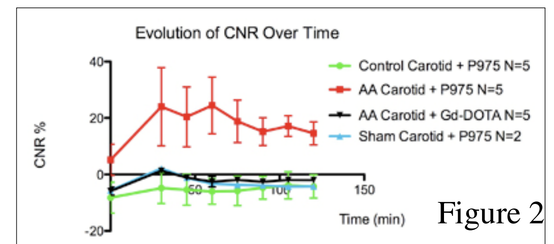

Figure 2

Conclusion: P975 allows in vivo target-specific MR imaging of activated platelets. In this study, we applied this agent to visualize thrombogenic activity was monitored in an arachidonic acid mouse model of thrombosis for the first time. This approach represents a novel non-invasive technique for early detection of platelet-containing thrombi.

References: 1. Gross S. J. Exp. Med. 2007