Fibrin targeted MRI allows staging of deep venous thrombosis and identifies thrombi amenable for thrombolysis

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INTRODUCTION: Deep venous thrombosis (DVT) remains a major health problem. Although thrombolytic therapies are effective in recanalising the veins, restoring blood flow and preventing post-thrombotic complications there is still no consensus on the selection criteria for this invasive treatment. Thus, there is a need for a diagnostic technique that identifies patients amenable for venous thrombolysis. The aim of this study was to investigate the merits of a fibrin-specific MRI contrast agent (EP-2104R; EPIX Pharmaceuticals, MA) for the accurate staging of thrombus organization and to identify thrombus suitable for lysis in an experimental animal model of venous thrombosis. **METHODS:** Venous thrombosis was induced in the inferior cava vein (IVC) of 8-10 weeks old male BALB/C mice using an established technique [1]. MRI was performed at day 2, 4, 7, 10, 14 and 21 following thrombus inductions. Twelve mice were scanned at each time point pre and 2 hours post injection of a fibrin binding MRI contrast agent (8.0 µmol/kg EP-2104R [2]). 6 of 12 mice at each time point were used to correlate fibrin contrast uptake with the histological stage of thrombus. The remaining 6 mice were subjected to intravascular thrombolysis therapy (10 mg/kg of tissue plasminogen activator (Actilyse, Boehringer Ingelheim, Germany)) at each time point. 24 hours after thrombolytic treatment mice were scanned again post EP-2104R injection. Two additional mice where scanned with a non-specific Gd-DTPA contrast at each time point for control purposes. All scans were performed on a 3T Philips Achieva Gyroscan scanner (Philips Healthcare, Best, The Netherlands) equipped with a dedicated small animal surface coil. An inversion recovery (IR) 3D segmented gradient echo (TFE) sequence was performed for selective visualisation of the thrombus and the inferior cava vein (IVC). In addition, T1 mapping of thrombus was performed using a Look-Locker based sequence [3]. T1 maps of 20 slices were calculated using custom-made software implemented in Matlab. To evaluate the success of the thrombolytic therapy a phase contrast sequence was performed to measure blood flow in the infrarenal IVC pre and post thrombolysis. Thrombolysis was considered successful if an increase of IVC blood flow of greater than 50% was observed on 24 hours post lysis scans compared to pre lysis scans. Thrombi were harvested after each scan. Sections were stained with hematoxylin and eosin (H&E) for anatomical detail and Martius Scarlet Blue (MSB) trichrome stain for mature fibrin visualization and quantification **RESULTS and DISCUSSION:** Thrombus was successfully visualized in all mice. High signal intensity on pre contrast IR images and short T1 relaxation times were observed in the central but not in the distal part of thrombus primarily on day 4, 7 and 10 (Figure 1). This is presumably related to the formation of methemoglobin in the hypoxic core of the thrombus as has been previously described [4][5]. After injection of EP-2104R larger areas with high signal intensity and short T1 relaxation times were observed. These new areas lead to a larger visualized thrombus enhancement volume in the post-contrast images. The average T1 value in the middle segment of the thrombus decreased during the first phase of thrombus organisation both on pre and post contrast images reaching a minimum of 734 and 489 ms at day 10 respectively (Figure 1). In the distal segment, the T1 value pre-contrast has not shown a significant change during thrombus organisation, but the post-contrast T1 value showed a similar pattern as the central portion, with a minimum T1 value of 498 ms between day 7 and 10. There was good spatial correspondence between high signal intensity areas on LGE-IR images, low T1 relaxation times on T1maps, and the fibrin distribution on MSB stained histological sections (Figure 2). Contrast uptake was positively correlated with the percentage of fibrin in thrombus as measured with histology (R2=0.97, P<0.01). Flow changes in the IVC pre and post thrombolysis in relation with T1 values pre and post contrast and visualized thrombus enhancement are shown in Figure 3. ROC curve analysis demonstrated that visualized enhanced thrombus volume on pre contrast IR-LGE images is not a good predictor for successful thrombolysis result (AUC of 0.748; CI95%: 0.58–0.92). Better results were obtained for the average pre contrast thrombus T1 relaxation time resulting in an AUC of 0.893 (CI95% 0.79–0.99). A cut-off point of pre contrast T1 relaxation time of 774 ms resulted in a sensitivity of 72% and specificity of 93% to predict successful thrombolysis. The difference between the pre and post EP-2104R visualized thrombus enhanced volume before treatment had an AUC of 0.992 (CI95%: 0.98–1.00) to predict successful thrombolysis. The thrombus T1 relaxation time post EP-2104R injection was found to have an AUC of 0.993 (CI95%: 0.98–1.00). For a thrombus T1 relaxation time less than 630 ms on post contrast images, the sensitivity was 94% and specificity 99% to predict successful thrombolysis.

Figure 1- LGE-IR and T1 relaxation time images pre- and 2 hours post-injection of EP-2104R of the middle and distal segments of thrombus at different time points post thrombus induction.

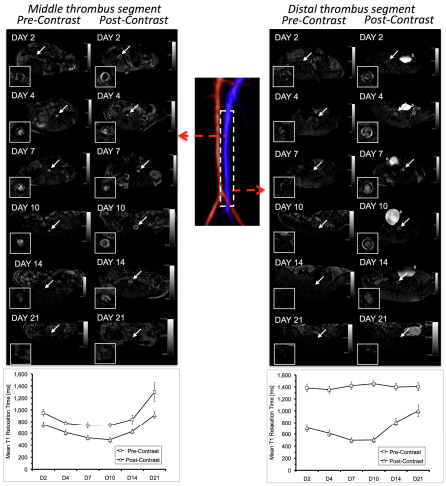


Figure 2- LGE-IR images pre and post injection of EP-2104R and T1 map images post contrast injection at the distal segment of the thrombus. MSB histology sections and fibrin segmentation at the corresponding levels.

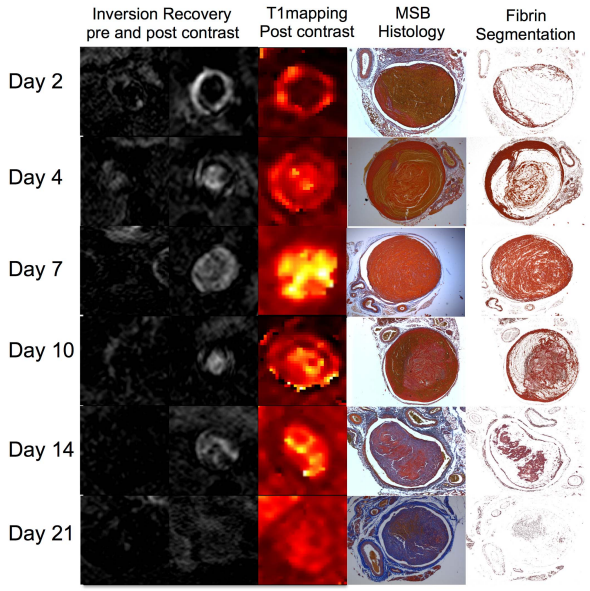
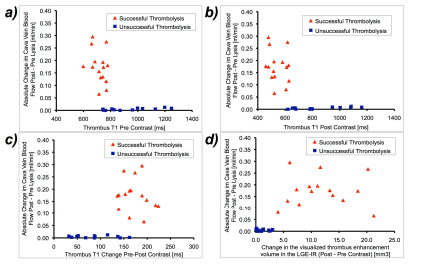


Figure 3- Average T1 relaxation time pre and post EP-2104R injection and the change in visualized enhanced thrombus volume measured on pre and post contrast IR-LGE images for successfully (red) and unsuccessfully (blue) lysed thrombi



CONCLUSIONS: The use of a fibrin specific contrast agent provides information not only on the presence of an intravascular thrombus but also allows assessment of thrombus age and organization. In addition, the presence of fibrin allows identification of thrombus amenable for lysis. Clinical studies are now warranted to validate these findings in humans. **REFERENCES:** [1] McGuinness CL *Thromb Haemost* 2001, [2] Overoye-Chan K *J Am Chem Soc* 2008, [3] Blume U MAGMA 2009, [4] Bradley WG *Radiology* 1993 [5] Moody J Thromb Haemost 2003