Uptake of a fibrin-targeted contrast agent could direct therapy following deep vein thrombosis

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INTRODUCTION: Deep venous thrombosis (DVT) can lead to death by pulmonary embolism and is associated with chronic complications as part of the post-thrombotic syndrome. This is characterised by persistent limb pain, swelling and ulceration. Current treatments for DVT are mainly based on the use of anticoagulation, which prevents thrombus propagation, but has little effect on resolution. Studies have, however, shown that thrombi that recanalise more quickly are associated with fewer post-thrombotic complications, leading to some advocating the use of targeted thrombolytic therapy. The optimal time for these treatments, however, remains unknown [1]. Venous thrombi resolve by a process of organisation and recanalisation that is similar to the formation of granulation tissue in healing wounds [2]. Degradation of the fibrin matrix, recruitment of inflammatory cells and neovascularisation are key events in the resolution process [3]. There is not currently an imaging method that can accurately quantify thrombus organisation and resolution. In this study we investigate the merits of a fibrin-specific MR contrast agent (EP-2104R; EPIX Pharmaceuticals, MA) in an experimental animal model of venous thrombosis that could be used to accurately stage DVT *in vivo*.

METHODS: Venous thrombosis was induced in the inferior vena cava (IVC) of BALB/C mice using an established technique [4]. MR imaging was performed at day 2, 4 and 7 following thrombus inductions to demonstrate thrombus organisation. Six mice were scanned at each time point pre and 3 hours post injection of 8 µmol/kg of EP-2104R, a fibrin-specific contrast agent [5][6]. 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 vena cava vessel wall using EP-2104R. 30 slices were acquired starting above the renal vessels and extending below the confluence of the iliac veins. Imaging parameters included matrix=448x448, spatial resolution of 100x100x500µm, TR/TE=27.4/8.2ms, flip angle=30°, TI=450 ms, and 2 signal averages. In addition, T1 mapping of the thrombus and vessel wall was performed using a Look-Locker based sequence [7]. T1 maps of 20 slices were calculated using custom-made software implemented in Matlab (Mathworks, Natick, MA, USA). Imaging parameters included spatial resolution 100x100µm, slice thickness of 500µm, TR/TE=9.0/4.6 ms, flip angle=10°. Thrombi were harvested after each scan and processed for histological sections that co-registered with images taken. **RESULTS and DISCUSSION:** Thrombus was successfully visualized in all mice. Pre- and post-contrast IR TFE images taken of the middle segment of thrombus (slice 9) and its lower limits (slice 18) are shown in Figure 1. High thrombus signal intensities are observed on pre-contrast images in the middle segment, but not in the lower segment of thrombi. This is presumably related to the presence of methemoglobin, as has previously been described [8]. Images without contrast may therefore lead to an underestimation of thrombus size and its extent. In contrast enhanced IR TFE images, high signal intensity was observed at all segments of thrombus allowing for more accurate thrombus size estimation (confirmed by histology). T1 quantification at different levels of the thrombus is shown in Figure 2. The dotted lines represent the mean T1 value of the entire thrombus (slices 6 to 20). The use of a fibrin-binding contrast agent allowed complete thrombus visualization throughout its evolution. T1 maps made at different levels of thrombus are shown in Figure 3. They reveal a temporal change to contrast uptake, with increased uptake correlating with thrombus size. Spatial changes in uptake were also observed. At day 2, contrast was located around the peripheries of the thrombus, in the vessel wall and when thrombus appeared histologically less organised. By day 7, however, contrast was observed in the centre of thrombus when thrombus appeared more organised. EP-2104R could potentially therefore identify early thrombi that would be amenable to thrombolytic therapy.

Figure 1- Inversion Recovery images pre and post contrast injection at the level of middle segment of thrombus (slice 9) and its lower limits (slice 18)

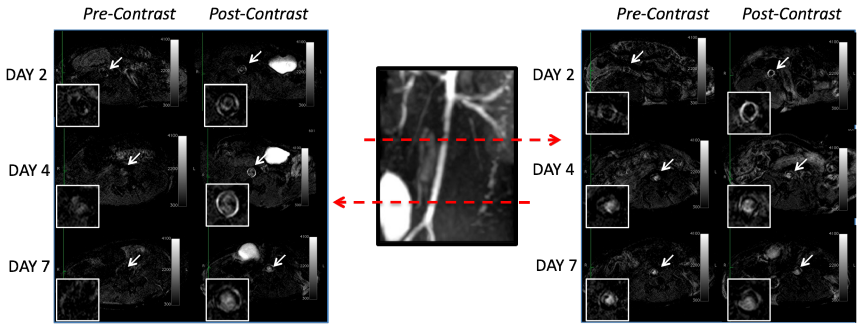


Figure 2- Average T1 value of the thrombus pre and post injection of EP-2104R between slices 1 (renal vessels) and 20 (confluence of the iliac veins). The head of the thrombus was at level of slice 5-6

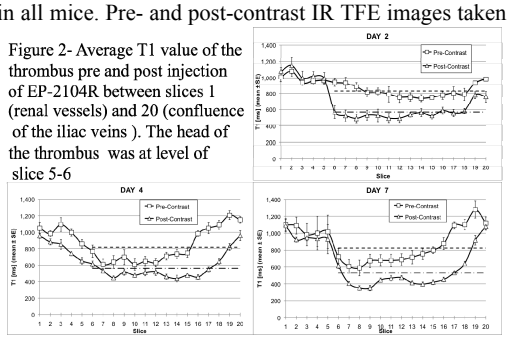
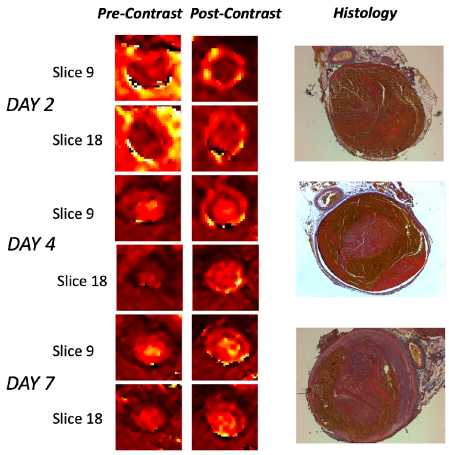


Figure 3: T1 mapping of thrombus pre and post injection of EP-2104R at the level of the middle and lower segments of the thrombus. Pre contrast images of the middle segment (slice 9) at day 4 and 7 demonstrate low T1 values suggesting methemoglobin formation. Post contrast T1 maps show high contrast uptake in the external layer of the thrombus in the early days of evolution and within the thrombus when the neovascularisation process is observed



CONCLUSIONS: The use of a fibrin specific contrast agent provides information on the presence of an intravascular thrombus and allows assessment of thrombus size and its stage of organisation and resolution. This information may help guide clinical decision-making at the time of diagnosis, direct therapy and help monitor its response.

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