

In vivo correlation of T1 and methemoglobin in a mouse model of deep vein thrombosis

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INTRODUCTION

Magnetic resonance imaging (MRI) has been proposed as a way of detecting venous thrombosis as it is relatively quick, non-invasive and can provide different information about anatomy, flow and stage of the thrombus. MRI has high sensitivity and specificity compared with the gold standard, contrast venography, in diagnosing DVT [1] and longitudinal relaxation T_1 -weighted images are able to clearly delineate acute venous thrombi. [2] This direct thrombus imaging technique (MR-DTI) is based on the hypothesis that methemoglobin (methHb), which contains Fe^{3+} , accumulates in thrombi and causes T_1 shortening compared with the Fe^{2+} found in the hemoglobin of normal blood. The quantitative measurement of T_1 relaxation time for a given pixel (T_1 -mapping) has been suggested as a method to provide information regarding tissue characteristics of the thrombus in DVT. [3] However, the direct relationship between methHb and T_1 relaxation times has not been studied in-vivo to date. The purpose of this study was to investigate the correlation between T_1 , methHb and organization in greater detail by comparing T_1 times with both Fe^{3+} concentration and histology using an animal model of resolving thrombus.

METHODS

Venous thrombosis was induced in the inferior vena cava of 8-10wk male BALB/C mice in a surgical procedure that involved a combination of reduced blood flow and endothelial disturbance. A partial stenosis reduced blood flow by approximately 90%. [4] The T_1 relaxation times of in vivo thrombi were measured at days 1, 4, 7, 10, 14, 21 and 28 after thrombus induction (N=6 per group). Mice were imaged using a clinical 3T MR-scanner (Achieva, Philips, Netherlands) using a dedicated small animal solenoid coil for signal reception. Arterial and venous time-of-flight (TOF) and quantitative flow sequences were obtained to find the location and dimension of the thrombus. A 3D T_1 -mapping sequence was performed over the entire thrombus. The fast T_1 -mapping sequence consisted of a modified Look-Locker sequence. [5] T_1 maps were generated using a customised programme (MATLAB software, MathWorks). Mice were divided into two groups. In one group, thrombi were excised and processed for histology. Sections were stained using a hematoxylin and eosin (H&E) for anatomical detail; Martius Scarlet Blue (MSB) for red cell and collagen content; and Prussian Blue for haemosiderin content. In a second group of mice, the thrombi were immediately processed after imaging to determine Fe^{3+} concentration using a QuantiChrom™ Iron Assay Kit. Briefly, thrombi were weighed and homogenized immediately after imaging in kit buffer. The color intensity of the formed chromagen was measured at 590 nm using a spectrophotometer. Fe^{2+} content of the thrombus was subtracted from the total iron content to provide measurement for Fe^{3+} relative to an iron standard and thrombus weight.

RESULTS

Thrombus was most occlusive at day 7 and almost completely recanalized by day 28. Thrombus stained using MSB, appears to both decrease in size and show increased organization as demonstrated by the blue staining collagen. T_1 maps taken of corresponding sections show that T_1 times appear to increase between day 7 and 28. (Figure 1). The mean T_1 values in the thrombus decreased during the first 7 days then increased with time ($P<0.0001$) towards the relaxation time of blood. In Figure 2, T_1 times are shown for each group (blue triangles) over time with the lowest value occurring at 7 days post thrombus induction. This correlates with the buildup of methHb in response to inflammation and hypoxia in this region as expressed by Fe^{3+} (Figure 2, red squares).

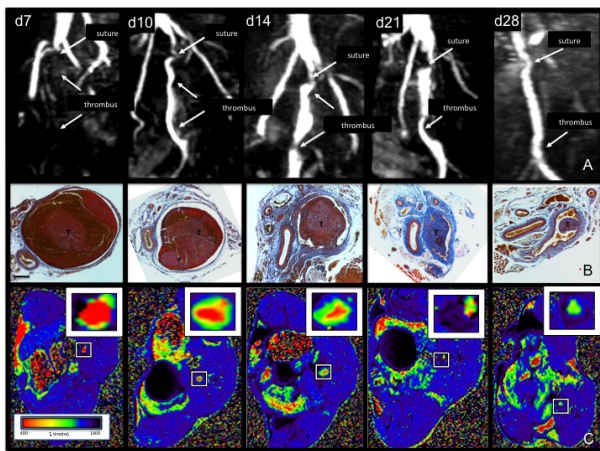


Figure 1. TOF venography (row A), MSB stained thrombus (B) and the corresponding T_1 maps (C) show the resolution of thrombus from day 7 to day 28.

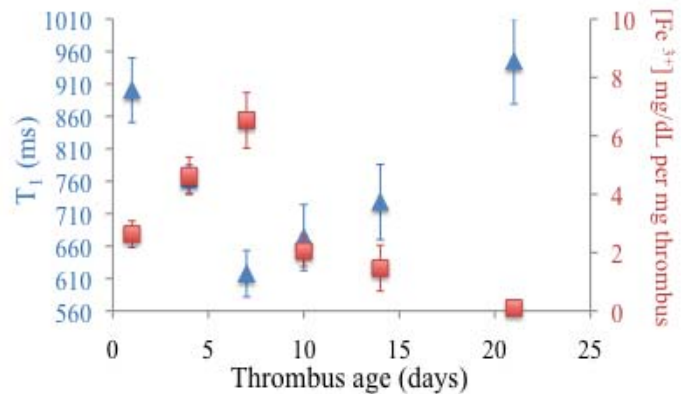


Figure 2. T_1 times and Fe^{3+} concentration versus thrombus age.

DISCUSSION AND CONCLUSION

In the murine model of thrombosis used in this study, two distinct stages can be observed. The first includes propagation of the thrombus (days 0-7) followed by its resolution (days 7-28). The T_1 of a thrombus shortens during the propagation phase and returns to that of blood as its resolution proceeds. The accumulation of Fe^{3+} in the form of methHb correlates directly with the T_1 of the thrombus.

REFERENCES

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