

Thrombus development and fragmentation in rats using non-enhanced MRI

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Introduction

Terminal measurements of thrombus size are the most common method for evaluation of antithrombotic treatment, however they do not provide any insight on formation, development, fragmentation or dissolution of the thrombus. Therefore a non-invasive imaging technology such as MRI is essential to follow the fate of thrombi in individual animals. Further, repeated scanning of individual animals delivers a drastic sparing in animal usage and the ability to use the same methods in subsequent clinical trials. Such imaging methods can be used to test novel anti-thrombotic agents.

Material and Methods

Two series of experiments were conducted with 30 Sprague Dawley rats; (a) 10 rats imaged every 20 min post thrombus induction for 3 hours, and (b) 20 rats imaged at 50 min, and again day 2 and day 4 post thrombus induction. Rats were anaesthetised and thrombosis induced in the abdominal caval vein with ferric chloride as described previously [1]. In experiment (b) rats were allowed to recover between imaging sessions. Images were acquired on a 9.4T/20 USR Bruker BioSpec with the selfgated sequence IntraGateFLASH [2], TR/TE 200/2 ms, FA 25°, NEX 20, FOV 50x50 mm, matrix 256x256 and 30 axial 1 mm thick slices from the inferior caval vein bifurcation to the left renal vein. A 10 mm navigator slice with 3° flip angle was placed over the liver to provide respiratory motion data for retrospective gating. Thrombus was segmented in each slice using an in-house semi-manual segmentation procedure. Immediately after the last imaging session in each study the rats were sacrificed and the wet weight of the thrombi assessed.

Results

Figure 1 shows image examples of four different time points acquired at the same slice positions in one animal. The MR volume measured at the last time point (3 hours or 4 days) revealed a very significant correlation of $r = 0.92$ ($P < 0.0001$) with wet weight (Fig. 2). Figure 3 shows the development of the thrombus volume as measured by MRI in the early phase (30 – 180 min) and a later phase (2 and 4 days). There is no significant difference between the groups at the 50 minutes time point (Mann-Whitney test). In general thrombus volume increases over the first hour post surgery followed by only a slight decrease over the next 4 days. However, thrombus development varies greatly between the individual animals. Figure 4 shows thrombus timecourse in three example rats from the 3 hours group where in 2 rats the thrombus appears to fragment.

Discussion

To our knowledge this is the first study to show the time course of acute thrombus formation over the first 4 days. Maximal average thrombus size was obtained 50 minutes after thrombus induction with no marked decrease over the following 4 days. The observation of thrombus fragmentation in several rats was surprising but potentially explains some of the variability seen in thrombosis models. An excellent correlation was found for MRI volume measurement and wet weight. MRI can follow thrombus formation, growth, fragmentation and dissolution in the same animal and thus drastically reduce the number of animals needed. This is a suitable technique for investigating new anti-thrombotic drugs.

References

[1] Carlsson S, Elg M (2005) Blood Coagulation & Fibrinolysis 16:245-249. [2] Van Echteld, et al, Proc. Intl. Soc. Mag. Reson. Med. 14, 17 (2006).

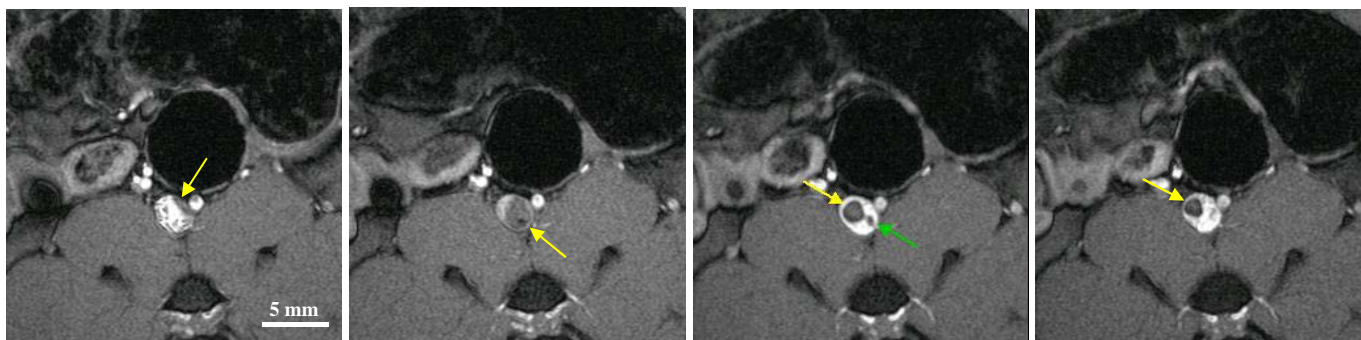


Figure 1. Example images of a thrombus (yellow arrows) in a representative slice acquired at 30 min, 70 min, 110 min and 150 min (left to right) after surgery demonstrating the initial thrombus growth followed by a size reduction. The thrombus appears dark and blood bright in the images. Note signs of thrombus fragmentation at 110 min (green arrow).

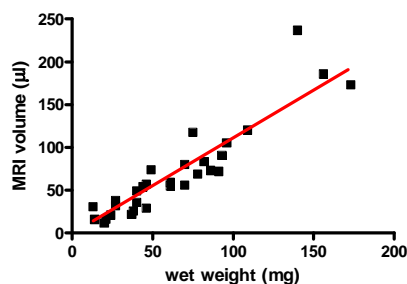


Figure 2. Correlation ($r = 0.92$, $P < 0.0001$) between thrombus volumes measured by MRI and thrombus wet weight.

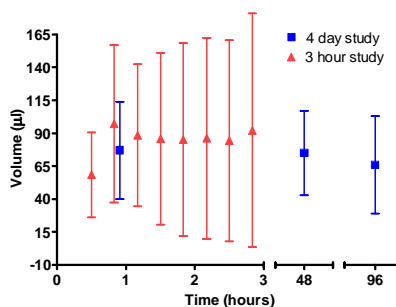


Figure 3. Mean thrombus volumes (\pm SD) of the 3 hour ($n = 10$) and the 4 day ($n = 20$) groups measured by MRI. Note the gaps in the time axis.

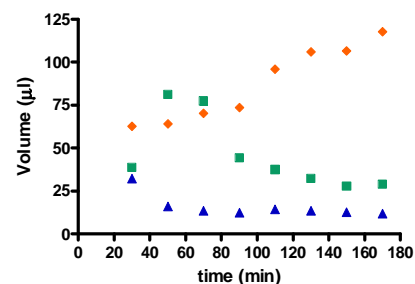


Figure 4. Examples of thrombus development in the acute phase demonstrating the large biological variability of thrombus growth.