Characterization of Arterial Thrombus Composition by Magnetic Resonance Imaging in a Swine Stroke Model

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Background and Purpose—The aim of this study is to analyze the histological composition of acute arterial thrombi and their MRI signals.

Methods—Two different types of experimental thrombi, erythrocyte- and fibrin-rich thrombus, were created and injected into an experimentally formed stenotic common carotid artery in swine. MRI of the in vivo and in vitro samples was obtained immediately after the thrombus application.

Results—Erythrocyte component showed high on fluid-attenuated inversion recovery, and iso on T2-weighted signal in both in vitro and in vivo. Fibrin-rich thrombus was composed of fibrin/platelet and nucleated cell layers, which demonstrated iso- and low intensities, respectively, in both fluid-attenuated inversion recovery and T2-weighted images in vitro. Mixed signal was obtained in vivo.

Conclusions—We successfully characterized the only erythrocyte component of acute thrombus by MRI. (Stroke. 2013;44:1463-1465.)

Key Words: imaging ■ MRI ■ stroke ■ thrombus ■ swine

Thrombi retrieved from intracranial arteries can exhibit a diversity of histological patterns, with a nonuniform composition of fibrin/platelet bands, linear cellular deposits, and erythrocyte-rich accumulations. The morphological properties of thrombus may play an important role in the success of acute stroke treatments. Erythrocyte-rich thrombi have been reported to be responsive to thrombolysis. We also demonstrated that the fibrin-rich thrombi, which were histologically similar to retrieved human thrombi, were intractable for mechanical thrombectomy compared with erythrocyte-rich thrombi. In this report, we examine the correlation between MRI findings and histology of 2 different experimental thrombi of known composition, and characterize an acute arterial thrombus by MRI.

Methods

All animal experiments followed regulations set by the Chancellor’s Animal Research Committee of the University of California, Los Angeles. A total of 8 common carotid arteries in 4 healthy Yorkshire swine (age range, 3–4 months; weight range, 30–40 kg) were included in this study.

An erythrocyte-rich thrombus was prepared by a conventional thrombin-induced method as described previously. We also created a spontaneous-forming thrombus by allowing separation of whole blood components through 2.5 hours of precipitation, with serum on top, fibrin/platelet/nucleated cells in the middle, and erythrocytes at the bottom. The fibrin-rich thrombus (fibrin/platelet and nucleated cells) was manually resected from the solid component of spontaneous-forming thrombus excluding serum.

Two types of experimental thrombi (erythrocyte- and fibrin-rich thrombi) aged 3 hours were injected through a catheter into experimentally created stenotic common carotid arteries, as demonstrated previously. Some of erythrocyte- and fibrin-rich thrombi were randomly selected for in vitro imaging and embedded in small cryo-vials with 2.5% gelatin (G1890; Sigma, Fukushima, Japan). These in vitro samples were attached to swine’s neck and scanned at the same time.

A Siemens 3.0 T MAGNETON Trio system MRI (Munich, Germany) with a head/neck coil was used for imaging of thrombi aged around 3.5 hours. The MRI protocol included a T2-weighted gradient recalled echo (GRE: TR 1900, TE 2.24, TI 1100), fluid-attenuated inversion recovery (FLAIR: TR 9000, TE 88, TI 2500), and T2-weighted (T2W: TR 8450, TE 101) sequences with slice thickness of 3 mm and no gap. Five axial slices at 3 mm intervals, which were located just proximal to the arterial stenosis, were selected, and the mean signal intensity was quantified in the lumen and ipsilateral sternohyoid muscle using Image J software (National Institutes of Health, Bethesda, MD) by 2 raters (N.S., K.T.). The intensity of sternohyoid muscle was expressed as mean±SD per sequence. Because of the mixed thrombus components and low spatial resolution of MRI, we quantitatively compared the average of thrombus intensity between erythrocyte- and fibrin-rich thrombus for in vivo analysis.

Statistical comparisons were conducted using the Wilcoxon Mann Whitney test. P<0.05 were considered statistically significant.

Results

In Vitro Imaging

Erythrocyte-rich thrombus demonstrated a homogeneously high intensity in FLAIR and iso-intensity in T2W imaging.
(Figure 1A). Histological analysis indicated that the thrombus was composed of a cluster of erythrocytes. Fibrin-rich thrombus exhibited a double-layered structure, with nucleated cell and fibrin/platelet layers (Figure 1B). These layers demonstrated low and iso-intensities, respectively, in both FLAIR and T2W images. The 3 components of experimental thrombi (erythrocyte, nucleated cell, and fibrin/platelet layers) could be clearly distinguished using the combination of FLAIR and T2W imaging. Specifically, the erythrocyte and other components were discriminated in FLAIR, whereas the difference between nucleated cell and fibrin/platelet layers was more apparent in T2W imaging than in FLAIR. GRE could not detect an obvious difference between the compositions.

In Vivo Analysis
In vivo imaging of erythrocyte-rich thrombus showed a high intensity in FLAIR and an iso- to high intensity in T2W images (Figure 2A). In contrast to in vitro studies, the intraarterial fibrin-rich thrombi showed lower intensities in FLAIR and T2W imaging (Figure 2B). The signal intensity of the sternohyoid muscle (n=8) did not show the distinct difference (FLAIR; 39.6±3.6, T2W imaging; 11.7±0.8, GRE; 60.6±5.5). The compositions of fibrin/platelet and nucleated cell layers within fibrin-rich thrombus were indistinguishable. Fibrin-rich thrombus showed diversity in composition, including an erythrocyte layer. There was a significant difference in the relative thrombus intensities between erythrocyte- and fibrin-rich thrombi in both sequences (Figure 3, both; P<0.0001). There was no significant difference in GRE (P=0.06).

Discussion
Arterial thrombus has been demonstrated as hyperdense middle cerebral artery sign on CT, FLAIR vascular hyperintensities, and blooming artifact on GRE sequences. Of these signs, FLAIR vascular hyperintensities exhibit a higher sensitivity (65%–100%) and specificity (75%–100%). Arterial occlusion on FLAIR sequence causes the flow void in vessels to disappear, and superacute hemoglobin, oxyhemoglobin can be detected with T2 prolongation owing to the high protein content. We previously reported the possibility that blooming artifact and hyperdense middle cerebral artery sign may reflect erythrocyte predominance by the analysis of retrieved thrombi. Nevertheless, the retrieved thrombi may not correspond to the whole thrombosis within arteries before treatments. The lower sensitivity of both hyperdense middle cerebral artery sign and blooming artifact to detect the thrombosis remains problematic. The variable thrombus characteristics and technical challenges associated with the small size of the arterial lumen have limited our understanding of arterial thrombus.

In the current study, we used 2 types of experimental thrombi that were similar to human thrombi, and evaluated them by MRI after injection into the stenotic common carotid arteries of the swine. The swine common carotid artery has a diameter of 4 to 5 mm, and our stroke model can simulate an internal carotid artery occlusion in a human. The distinction between fibrin/platelet and nucleated cell layers could not be identified owing to the mixture with erythrocytes. This is probably because erythrocyte-rich fresh thrombus can form around the fibrin-rich thrombus in this model. The reason why
GRE could not detect these obvious differences in thrombus composition might be because of its reduced sensitivity for oxyhemoglobin.

Thrombus structure ranges widely and changes dramatically at an acute phase, which can contribute to the signal intensity of MRI. Unfortunately, it takes 2.5 hours to form the fibrin-rich thrombus. We could not present the thrombus images within <3 hours and their variation. This preliminary study analyzed the MRI finding for 2 types of experimental thrombus only at a single time point, corresponding to the time limit of intravenous tissue plasminogen activator. Further imaging analysis for acute thrombus over time is necessary for optimal therapeutic strategies.

Conclusions
We compared MRI and histopathology to examine thrombus characteristics in a swine stroke model. FLAIR and T2W were able to identify the erythrocyte from other components for both in vivo and in vitro thrombi. Precise interpretation of thrombus composition before treatment is potentially useful for selection of therapeutic strategies and assessment of cause and secondary prevention of stroke.

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Disclosures
None.

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