Functional Imaging of Thromboembolic Stroke in Rats Using PET, Ultrasound and MRI

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1 INTRODUCTION

Embolic stroke is a significant cause of mortality and neurological deficits e.g. paralysis, throughout the world. It is a devastating complication resulting from blood clots forming elsewhere in the body and traveling through the blood stream to the brain where it enters a blood vessel that is too small to allow it to pass and blocks the flow of blood to the brain area. It is associated with multiple risk factors including atrial fibrillation, atherosclerosis and hypertension. Fast diagnosis and early start of thrombolytic treatment is critical for limiting the ischemic damage and for the patient's well-being. The objective of this study was to visualize ischemic area with multiple imaging techniques and to see effect of clot dissolving treatment using PET scanning in a thromboembolic stroke (TBE) model in SHR rats. Additionally, the ischemic area was assessed with functional ultrasound imaging (fUS) and magnetic resonance imaging (MRI).

TBE stroke was induced to rats by injection of autologous blood clots in suspension into the internal carotid artery (ICA). The animals were transferred to acute MRI followed by 90 minute dynamic PET scan 60-180 minutes after the stroke with hypoxia radiotracer ^{18}F-FMISO. Intravenous infusion with vehicle or tissue plasminogen activator (tPA, Actilyse) was performed during the PET scanning, 90-150 minutes after the stroke. On the following day animals were scanned with fUS and MRI for vascular map and lesion volume, respectively (**Figure 1**).

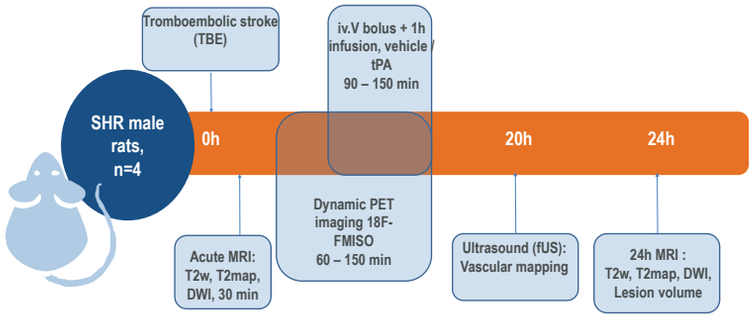


Figure 1. Outline of TBE imaging study.

2 METHODS

All animal experiments were carried out according to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals, and approved by National Animal Experiment Board. The animal facility has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), International. The animals were imaged under isoflurane anesthesia and their ventilation rate and temperature were monitored during the scanning.

Surgery – Venous blood from target male SHR rats weighing 180-220 g was collected into PE-50 catheter and retained for 24 hours to complete clot formation before being rinsed with PBS and cut into 2 mm-long pieces. The rats were anesthetized with isoflurane and right common carotid artery (CCA), internal (ICA) and external carotid artery (ECA) were carefully exposed. Inferior thyroid and occipital arteries, branching from ECA, were visualized and cauterized; the distal portion of ECA was ligated and cut along with the terminal lingual and maxillary artery branches, and the carotid bifurcation identified. ICA was dissected cranially up to the origin of pterygopalatin branch, which was ligated using a suture. The CCA and ICA were temporarily clamped. The blood clots were infused via catheter inserted into the ECA stump advanced 1–2 mm beyond carotid bifurcation. The clip around ICA was removed and 3 clots injected while CCA was still occluded. Finally, the ECA stump was ligated and the CCA clip removed.

MRI – MRI was performed 30 min post TBE stroke and on the following day. Acquisitions were performed using a horizontal 7.0 T (Bruker Biospin GmbH, Ettlingen, Germany). A volume coil (Bruker Biospin GmbH, Ettlingen, Germany) was be used for transmission and a two-element surface array coil for receiving (Rapid Biomedical GmbH, Rimpar, Germany). Imaging protocol consisted of EPI-based Diffusion Weighted Imaging (DWI), T2 relaxometry with multi-slice multi-echo sequence (MSME, TR = 2.5 s, 12 different echo times 10-120 ms in 10 ms steps and 4 averages), T2-weighted imaging suing Fast Spin Echo with eighteen (18) coronal slices of 1 mm thickness, field-of-view 30x30 mm2 and 256x128 imaging matrix.

PET – PET scan was performed 60 minutes post TBE stroke. ^{18}F-FMISO (26.4 ± 1.9 MBq) was injected intravenously followed by 90 minute dynamic list mode PET scan and CT using BioPET/CT scanner (Sedecal), reconstructed with 3D-OSEM (1 iteration, 25 subsets) with attenuation correction and analysed using PMOD software (v3.7).

fUS – Functional ultrasound imaging was performed using a prototype functional ultrasound system (Iconeus, Paris, France), consisting of ultra-fast ultrasound imaging system, miniature probe with 128 ultrasonic transducers, acoustic lens and motorized positioning system allowing precise positioning of the probe in 3 orthogonal planes. Plane-wave illumination of the brain at high frame rate (500 Hz compounded sequence with three tilted plane waves, PRF = 1500Hz with a 15 MHz linear transducer), yields highly-resolved neurovascular maps.

For the TBE lesion visualization, scalp was dissected and periosteum removed, to reduce the signal contribution from extracranial tissues. Intravenous infusion of the echogenic contrast agent (SonoVue, Bracco Imaging France SAS) was started just before the acquisition to overcome the attenuation of the ultrasound signal by the scull.

4 SUMMARY

In this study multiple imaging approaches were utilized to monitor pathological changes in thromboembolic stroke rat model. The lesion size, ischemic area, vasculature and perfusion were evaluated using MRI, PET and fUS, respectively. Additionally, the functional PET imaging approach was used to estimate effects of acute thrombolytic treatment during PET scan. All of the used readouts can be applied similarly to other stroke models.

Using hypoxia tracer and PET imaging ischemic core and penumbra could be clearly distinguished. For treatment efficacy studies this is valuable as the penumbra area may be affected in treatment experiments due to reasonable blood perfusion. In the core of the lesion minimal perfusion exists and hence minimal bioavailability of dosed compounds can enter the area. Furthermore, ^{18}F-FMISO could be used in the longitudinal quantification of therapeutic effect in stroke with non-invasive and translational imaging technique.

With acute MRI imaging 30 minutes after TBE stroke, not all conventional MRI imaging modalities could reveal full pathological changes of stroke. Only DWI was able to detect ischemic lesion due to early diffusion changes induced by cytotoxic edema. On the following day the multimodal MRI has the best resolution and sensitivity to accurately estimate the lesions size. All three modalities, T2-weighted, T2-relaxation map and DWI can be used to estimate the lesion volume. Furthermore, the readouts can be used in the estimation of tissue viability and obtaining more accurate anatomical and structural information about the ischemic area.

Ultrasound 3D reconstruction of the brain vasculature highlights the changes in vasculature after TBE stoke. Ischemic area had smaller blood flow signal levels and vessel density compared to hemisphere contralateral to the lesion.

Stroke is a significant cause of mortality and neurological deficits throughout the world and there is a growing need for diagnostic tools and treatment approaches. This combination of functional and structural imaging platforms provides the tools to study novel treatments of ischemic stroke allowing accurate measurement of lesion size, ischemic area and vasculature.

3 RESULTS

^{18}F-FMISO localization to hypoxic areas could be visualized from the PET images. The tracer accumulation was highest on the ischemic penumbra, which has adequate vascular perfusion compared to the ischemic core where the tracer accumulation was limited (**Figure 2A, B**). The effect of thrombolytic treatment with tPA was not quantified in acute model due to variability in lesion size between individuals (**Figure 2C**).

Acute MRI was performed 30 minutes post TBE stroke. A panel of conventional MRI modalities was evaluated for their ability to visualize acute lesion (**Figure 3A, Figure 4**) and its evolution at 24h (**Figure 3B, Figure 4**). At acute timepoint, only DWI was able to robustly detect ischemic lesion, due to its sensitivity to early changes induced by cytotoxic edema (**Figure 3A, 4C**). At 24h timepoint T2-weighted imaging and relaxometry was showing similar sensitivity and performance in lesion detection as DWI, also providing additional useful quantitative readout (T2 relaxation time) correlating with tissue viability as well as more detailed anatomical and structural information about the ischemic area.

Ultrasound 3D reconstruction of the brain vasculature revealed clear reduction of the vessel density and blood flow in ischemic area, with significantly ($p<0.01$) smaller flow signal levels compared to unaffected contralateral side, as well as strikingly different pattern of the deeper vasculature (**Figure 6**).

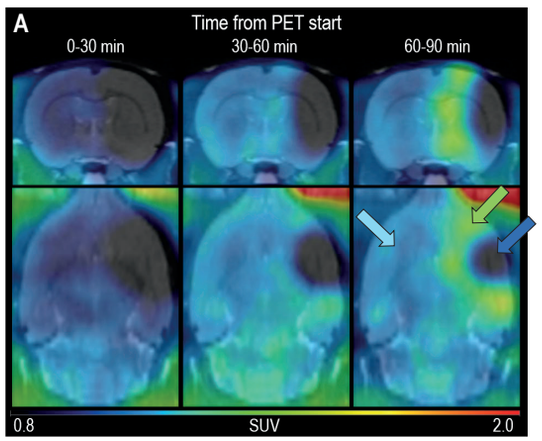
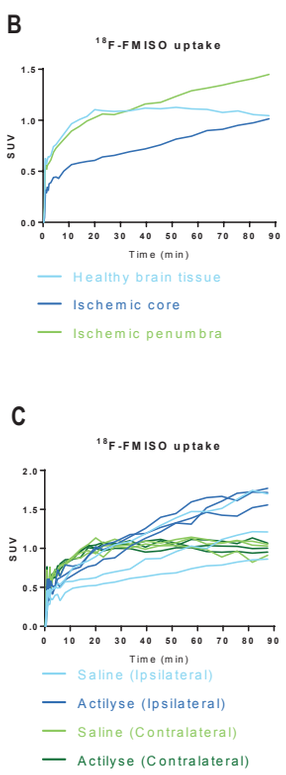


Figure 2. PET imaging results 1h after lesion. Representative PET images in coronal and horizontal plane from a single rat with TBE stroke on the right hemisphere (A) and corresponding time activity curves from ischemic core, ischemic penumbra and healthy brain tissue (B) showed reduced blood flow in the ischemic brain area (right hemisphere). Ischemic penumbra was visualized with accumulation of ^{18}F-FMISO 60 – 90 min post tracer infusion. Tracer accumulation in the ischemic core showed similar trend and was visualized from the time activity curve. Due to minimal blood flow and hence bioavailability in the core lesion area, no tracer uptake was observed. Acute treatment effect with thrombolytic tPA was not be quantified during the PET scan due to the variation in the lesion size. Individual time activity curves showed ^{18}F-FMISO uptake on ipsilateral side (C). On the contralateral side the tracer uptake was similar in both dosing groups.



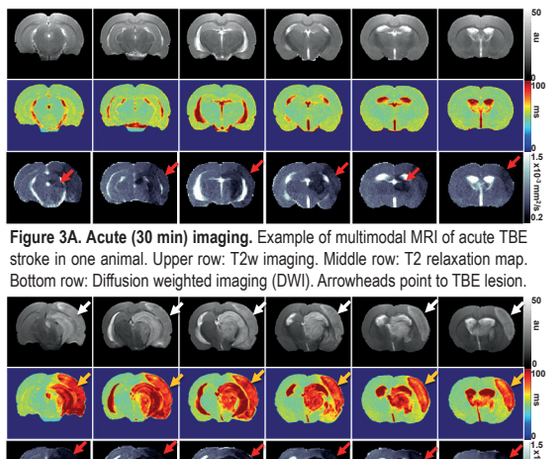


Figure 3A. Acute (30 min) imaging. Example of multimodal MRI of acute TBE stroke in one animal. Upper row: T2w imaging. Middle row: T2 relaxation map. Bottom row: Diffusion weighted imaging (DWI). Arrowheads point to TBE lesion.

Figure 3B. 24h imaging. TBE lesion evolution in multimodal MRI. Upper row: T2w imaging. Middle row: T2 relaxation map. Bottom row: Diffusion weighted imaging (DWI). Note the similarity in lesion area and contrast difference accross different modalities. Same animal as in **Figure 3A**.

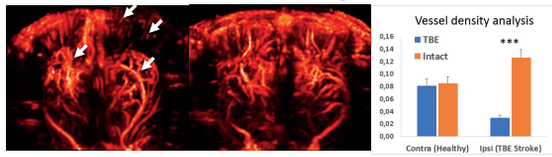
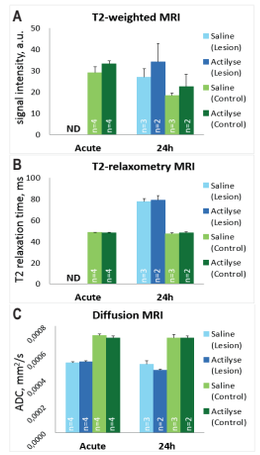


Figure 5. fUS imaging. Left: TBE animal, 24h timepoint. Middle: Sham animal. Right: Vessel density analysis of the 3D fUS maximum intensity projection data.



▲Figure 4. Comparison of MRI modalities in TBE stroke.

A – T2 weighted MRI
B – Diffusion MRI (DWI)
C – Transverse relaxation time

Acute TBE lesion was not detectable in T2-weighted imaging and T2-mapping, while DWI provided good detection and window between TBE and control groups. However at 24h timepoint T2w-MRI and T2-mapping provided similar results to DWI. Group sizes provided within the bars in white. **ND** – Not determined.

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