Focus on Stroke: determining the outcome/prognosis through experimental work.

ISMRM 08 syllabus contribution:

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Introduction

Stroke is the third leading cause of death and the leading cause of disability in developed countries globally. Ischemic stroke is the most prevalent type, and occurs when a blood vessel that supplies the brain becomes blocked with an embolic or thrombotic blood clot. There is only one approved treatment for ischemic stroke patients, thrombolysis with recombinant tissue plasminogen activator (rtPA). The therapeutic window for rtPA is only three hours after symptom onset; this highlights the importance of investigating the potential of acute neuroimaging strategies to improve diagnosis and outcome prediction. In addition to being the preferred modality for preclinical stroke imaging, magnetic resonance imaging (MRI) offers improved soft tissue contrast, when compared to computed tomography (CT), as well the potential to be completely noninvasive.

Animal models of ischemia, primarily rodents, have been essential in furthering our knowledge of the pathophysiological mechanisms of stroke, reviewed in [1]; the details of which will follow in the context of relevant imaging techniques. The most common rodent model of ischemic stroke is a middle cerebral artery occlusion (MCAO). This model is similar in etiology to large vessel thrombosis in humans, which has been fairly well characterized using acute imaging.

Considerations for animal experiments

Unlike humans, animal experiments require anesthesia to minimize movement as well as stress to the subjects. Anesthesia has a profound effect on physiology, for example, the suppression of neuronal activity, or influence on hemodynamics and blood gases, which can potentially affect stroke evolution/outcome as well as the measurements
obtained with imaging techniques. The anesthetic requirements mean that it is necessary to monitor the subjects’ physiological parameters remotely, with MRI compatible equipment, in order to prevent hypothermia, hypotension, or blood acidosis. Furthermore, the rodent brain is much smaller with less white/gray matter contrast when compared to the human brain; this means higher resolution images are required, and thus longer scan times, and possibly higher field strengths to achieve this.

**Acute imaging of MCAO in rodents**

Time of flight (TOF) angiography can rapidly provide three dimensional images of the Circle of Willis and large cerebral vessels in rats. It can be easily used to identify the blocked vessel. However, the scope of this type of imaging is relatively limited, primarily because the experimenter is typically aware of the location of the vessel occlusion, and the resolution required to visualize microvessels is still lacking. Angiography is typically used to confirm success of thrombolysis treatment [2].

Perfusion weighted imaging (PWI) is sensitive to blood flow and therefore used to image ischemia in the acute stages. One method to acquire perfusion images is dynamic susceptibility contrast (DSC) bolus tracking. After baseline acquisition a contrast agent with T₂ susceptibility is administered intravenously, which causes signal loss in the vessels and surrounding tissue. The signal loss can be used to quantify cerebral blood flow (CBF) or cerebral blood volume (CBV). This technique, by nature of administration is invasive, requires high temporal resolution, and the number of measurements is restricted by wash out time, which makes it less common in pre-clinical research. An alternative method is continuous or pulsed arterial spin labelling (CASL and PASL respectively), which is completely noninvasive because it uses the blood protons as an endogenous contrast agent. Proton spins are both non-selectively and then selectively inverted and the resulting T₁ signal difference is proportional to the amount of inflowing protons (CBF). Some of the first experiments to make use of this technique in a thrombotic MCAO model were able to distinguish regions with severe and moderate perfusion deficits, which were correlated with invasive measurements of tissue metabolism [3]. Generally, thresholds are used to define the degree of deficit based on a comparison to the intact hemisphere, or a separate cohort of animals. However, quantification of CBF values using this technique remains difficult. It is complex, and highly dependent on the defined algorithm. Furthermore, the images often exhibit susceptibility artifact, and the areas with perfusion deficits exhibit lower signal intensities; repetitive measurements over time must be interpreted with caution because T₁ will gradually increase during the acute period.

Diffusion weighted imaging (DWI), first described in cats [4], is sensitive to intercompartmental water shift, and can detect ischemia within minutes of onset. An
initial gradient pulse dephases, and a second rephases, proton spins if no net
movement has occurred, so signal is attenuated in intact tissue. The energy depletion
produced during ischemia causes failure of ion pumps, which induces cell
depolarization and water influx, cytotoxic edema. Thus, proton motion is restricted in
this area and corresponding diffusion signal is hyperintense. The apparent diffusion
coefficient (ADC) in ischemic tissue will decrease. The degree of reduction has been
shown to be dependent on lesion severity [5], and is also typically defined as a
threshold when compared to intact tissue. A reduction of approximately 77% has been
shown to represent a reliable indicator of breakdown of energy metabolism, and
decreases of 86%, tissue acidosis [5, 6]. If reperfusion occurs, the region of DWI
abnormalities will transiently decrease followed by a gradual expansion, the eventual
size of which is dependent on ischemic duration [7]. Despite this dependence on
severity, often a secondary expansion around 12 hours has also been observed
following short durations of transient MCAO (35 minutes) [8].

Observations in clinical patients that the region of diffusion abnormality was smaller
than the region of perfusion abnormality, and that the region of diffusion abnormality can
continue to expand in the days following initial symptom onset, lead to the development
of the idea that the perfusion/diffusion ‘mismatch’ could be used to identify tissue at risk
[9]. Essentially the diffusion information provides an estimation of the tissue with
irreversible damage (the infarct core) and the tissue with intact diffusion but impaired
CBF (the penumbra) might still retain enough metabolic activity to be salvaged if
treatment is administered quickly. PWI and DWI have subsequently been used in the
clinic to identify patients who might benefit from rtPA treatment beyond 6 hours.

The concept of the mismatch in the preclinical literature is somewhat more ambiguous,
particularly because lesion severity is usually defined by thresholds, and therefore, the
region of diffusion abnormality is not always considered to be equivalent to complete
tissue loss. Nonetheless, significant mismatches have been consistently reported within
the first 60 minutes of permanent MCAO; there is relatively little change in perfusion,
and the region of ADC abnormality gradually expands until it is no longer distinguishable
from the region of perfusion deficit within the first 2-3 hours of the initial insult [10-12],
which highlights the importance for early reperfusion. Following reperfusion the situation
becomes even more ambiguous because the size of both regions (diffusion and
perfusion abnormalities) shrinks substantially and will subsequently begin to expand as
the stroke evolves. CBF and ADC impaired regions decreased to almost 30% of their
original size following reperfusion in the rat, but gradually increased to 60% of the
original size within the first 24 hours [10]. The degree of recovery within the infarct core
and the mismatched tissue, as defined prior to reperfusion using thresholds, is radically
different. Recovery of the ‘core’ pixels is highly dependent on the duration of the
occlusion; 35, 60, and 95 minutes of MCAO result in ultimate recovery of 46, 28, and
9% of the pixels, whereas approximately 85% of all mismatched pixels recovered
regardless of occlusion duration [11]. This implies again that early reperfusion is critical
for adequate tissue salvage both in the core, and to prevent lesion expansion of the
mismatched tissue that would occur if occlusion was permanent. Some differences in the evolution of the diffusion and perfusion deficits have also been reported to differ with rat strain [13] and model [12].

It is likely that a combination of imaging techniques could ultimately provide the best information regarding the characterization of tissue at risk. Nuclear magnetic resonance (NMR) spectroscopy can detect region specific metabolite changes, such as a reduction in the neuronal marker N-acetyl-aspartate (NAA). $^{13}$C glucose metabolization was found in tissue with reduced DWI, which would imply that the tissue was metabolically active [14]. More recently, pH-weighted MRI was able to detect abnormalities in tissue with impaired CBF, but intact diffusion [15]. Finally, the use of paramagnetic manganese ions as a bio-analogue of calcium ions to enhance image intensity, manganese enhanced magnetic resonance imaging, which has been suggested as a better technique to define the ‘true’ ischemic core of anoxic depolarization in animal models [16].

**Chronic imaging of MCAO in rodents**

The most common type of anatomical imaging to observe stroke in the chronic stages is T$_2$ weighted imaging, which is dependent on transverse relaxation for contrast. T$_2$ becomes hyperintense within a few hours of stroke onset and the signal is maximal by 24 hours; it is thought to reflect vasogenic edema [17]. T$_2$ signal intensity does not decline with time, it either stabilizes or slightly increases as the infarcted tissue is gradually removed by macrophages and replaced by cerebral spinal fluid (CSF). T$_2$ can be used within the first few days to observe edema through ventricle compression and midline shift, as well as to observe ventricular enlargement in the chronic phase. The excellent tissue contrast means this modality is often used to obtain the primary outcome measure in neuroprotective stroke studies, infarct volume. Recent studies have indicated that T$_1$ and T$_2$ relaxation times might provide more information towards understanding the pathology of ischemia than previously thought. T$_1$ and T$_2$ values returned to normal within 2-10 weeks in animals with small subcortical lesions, despite selective neuronal death, astrocytic reactivity, and inflammatory cell infiltration that was evident in histology [18].

T$_2^*$ imaging can also play an important role in imaging cerebral ischemia. T$_2^*$ effects are observed when field inhomogeneity decreases transverse relaxation by dephasing the spins faster. The susceptibility effects produced by iron accumulation, for example, from a hemorrhage, will produce a strong hypointense T$_2^*$ signal. T$_2^*$ imaging has also been used to image the inflammatory response by systemically administering ultra small super paramagnetic iron oxide (USPIO) nanoparticles (for example, Sinerem®, 10-40nm diameter) for phagocytosis by blood borne macrophages. Animals treated with
contrast 24 hours prior to imaging exhibit a hypointense $T_2^*$ signal in the periphery of a photothermophotic lesion around 5-6 days post insult, that has been correlated with macrophage accumulation (ED-1 immunohistochemistry) and iron containing macrophage accumulation (Prussian blue staining) [19]. Similar results have also been obtained within 24 hours following USPIO administration at five hours after permanent MCAO in rats [20] and mice [21]. However, this technique should be interpreted with caution, particularly since it has also been used in a clinical Phase II pilot trial to observe inflammation. Contrast can accumulate non-specifically through a disrupted blood brain barrier (BBB) or via disruption of the CSF especially when administered near to the time of the insult. Erythrocyte accumulation will also produce $T_2^*$ effects, so control images should be acquired and signal changes should be interpreted within the known time course of the inflammatory response.

The $T_2$ and $T_2^*$ susceptibility of iron has also been exploited to ‘track’ iron-oxide labelled stem cell migration in rodent models of stroke [22-24], which has substantial implications for research into regeneration.

**Future Considerations**

Clearly, work is required in order to improve the quality of preclinical stroke research in order to make an impact in the clinic. Low statistical power, flawed statistical interpretation, reproducibility, blinding randomization, quality control, and publication bias towards positive data have all been suggested to contribute to the lack of effective translational stroke research [25]. The same holds true for imaging studies. A recent review on the impact of the literature with diffusion imaging in animals indicated there were only 6 publications (out of a possible 141) that achieved quality scores sufficient to draw conclusions from [26]. Future research might have greater impact if more rigorous experiments were performed with consideration to the heterogeneity of the existing research.

**References**


