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Magnetic resonance imaging of perfusion–diffusion mismatch in rodent and non-human primate stroke models

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Abstract

Stroke is a leading cause of death and long-term disability. Non-invasive magnetic resonance imaging (MRI) has been widely used for the early detection of ischemic stroke and the longitudinal monitoring of novel treatment strategies. Recent advances in MRI techniques have enabled improved sensitivity and specificity to detecting ischemic brain injury and monitoring functional recovery. This review describes recent progresses in the development and application of multimodal MRI and image analysis techniques to study experimental stroke in rats and non-human primates.

Keywords

Magnetic resonance imaging; Perfusion–diffusion mismatch; Apparent diffusion coefficient; Cerebral blood flow; Diffusion-weighted imaging; Perfusion-weighted imaging; Experimental stroke model; Rodents; Oxygen challenge; Predictive mode; Rats; Hyperperfusion; Functional magnetic resonance imaging

Introduction

Stroke is a leading cause of death and a cause of long-term disability.¹ Ischemic stroke, the most prevalent form of stroke, is caused by a loss of blood flow to the brain, resulting in loss of brain functions. The prevalence of stroke and the financial burden of stroke care are steadily rising because the conditions that put people at risk for stroke (such as heart disease, diabetes, and obesity) are steadily on the rise. The ability to minimize neurological deficit in stroke patients remains extremely limited. The ability to reliably distinguish salvageable versus non-salvageable tissue and longitudinally monitor novel treatment strategies remain high priority for the treatment and management of acute stroke. This paper reviews recent progresses in the development and application of magnetic resonance imaging (MRI) and image analysis techniques to study ischemic tissue at risk in experimental stroke models using rodents and non-human primates (NHPs).

Conventional T₁ and T₂ MRI

Conventional T₁ and T₂ MRI are part of standard imaging protocols in stroke imaging² and, along with normal non-contrast computed tomography (CT), have been used in acute stroke

primarily to rule out hemorrhage.³ While both T1WI and T2WI identify vasogenic edema at later times during stroke,⁴ 90% of infarctions are visible on T2WI at 24 hours, relative to only 50% on T1WI, and thus T2WI is the 'gold standard' in clinical settings for imaging cerebral infarction.⁵ However, both normal non-contrast CT and conventional MRI have shown sensitivities of <50% in imaging ischemic stroke within six hours of onset,^{6,7} despite the fact that T2WI has signal changes as early as 30 minutes post-stroke in cats and primates.⁸ In addition, T1WI and T2WI have shown high false negative rates during the first day after stroke onset.⁵

Perfusion and Diffusion MRI

Diffusion-weighted imaging (DWI)⁹ in which contrast is based on water apparent diffusion coefficient (ADC) is known for its ability to detect stroke within minutes after onset, whereas CT and other imaging modalities fail to detect stroke injury for at least a few hours. Tissue ADC decline has been correlated with energy failure and breakdown of membrane potential in animal models.^{10–12} The reduction in cerebral blood flow (CBF) in the brain can be imaged using an exogenous intravascular contrast agent or by arterial spin labeling of the endogenous water in blood.^{13,14} The 'perfusion–diffusion mismatch' – defined as the difference in area between perfusion abnormality and diffusion abnormality (the region of interest outlined in Fig. 1) – approximates the 'ischemic penumbra.' The mismatch concept is being used in preclinical investigation and clinical decision-making in the management of acute stroke.

Perfusion and diffusion MRI has been applied to evaluate the spatiotemporal progression of middle cerebral artery occlusion in rats during the acute phase.^{15,16} Areas with ADC reduction grow with time after permanent occlusion, eventually reaching the CBF-defined lesion volume (Fig. 2, left). Reperfusion salvages substantial amount of mismatch tissue (Fig. 2, right). The ADC and CBF viability threshold in this rat stroke model was determined to be $0.53 \pm 0.02 \times 10^{-3} \text{ mm}^2/\text{s}$ ($30\% \pm 2\%$ reduction) and $0.30 \pm 0.09 \text{ ml/gram/minute}$ ($57\% \pm 11\%$ reduction), respectively, in rats.^{15,16} Although these thresholds are model dependent, they are potentially useful and easy to use. Reperfusion at 60 minutes post-occlusion showed that some 'perfusion–diffusion' mismatch defined by these thresholds was salvaged, with ADC lesion volume at 180 minutes reaching ~50% of the permanent occlusion group.^{16,17} The degree of salvaged mismatch tissue is dependent on occlusion durations as expected.¹⁸ Using this approach, a few drugs have been shown to be effective in reducing infarct volume in rats by salvaging the perfusion–diffusion mismatch defined by the viability thresholds.^{19,20}

Alternative to the threshold method, automated cluster analysis based on ISODATA (self-organizing data analysis algorithm) technique²¹ has been used to characterize the spatiotemporal dynamic evolution of ischemic brain injury based on high-resolution, quantitative perfusion, and diffusion measurements. Multiple clusters were automatically resolved after stroke, with pixels corresponding to the 'normal', 'at risk' ('perfusion–diffusion' mismatch), and 'ischemic core' tissue. The unique advantage of the ISODATA is that the number clusters in the data set can be statistically determined. Tissue volumes, ADC, and CBF of each ISODATA cluster were quantified. Pixels of different ISODATA clusters can be color-coded and mapped onto the image and ADC-CBF spaces. In permanent stroke, the ADC regions decreased precipitously as ischemia progressed. In transient stroke, reperfusion salvaged the majority of the mismatch pixels and some core pixels. This approach allows objective classification of different tissue types, which can be mapped back onto the image spaces, providing a powerful and objective means for pixel-by-pixel visualization of different tissue fate.

Prediction of Ischemic Tissue Fate

The ultimate goal of acute stroke imaging is to predict tissue fate based on acute MRI data. Sophisticated algorithms have been developed to predict ischemic tissue fate on a pixel-by-pixel basis. They included predictive models based on generalized linear model,^{22,23} probability-of-infarct,^{24,25} and artificial neural network (ANN)²⁶ and support vector machines (SVM).²⁷ These predictive models provide statistical or probabilistic maps of infarct likelihood on a pixel-by-pixel basis utilizing only the acute MRI data. Performance analysis showed accurate prediction when compared with endpoint T2 MRI and/or histology. In addition, the effects of neighboring pixels and infarct incidence on prediction accuracy were also evaluated. Other potential *a priori* information can be incorporated in these predictive models.^{24,25} Prediction accuracy can be quantified using receiver-operating characteristic (ROC) analysis.

BOLD fMRI of Perfusion–Diffusion Mismatch

In addition to anatomical MRI techniques based on tissue perfusion and diffusion, functional MRI of stroke animals can also be performed to evaluate the functional status of the 'perfusion–diffusion mismatch.' fMRI is a non-invasive imaging modality and has been widely exploited for mapping brain processes, ranging from perceptions to cognitive functions.²⁸ fMRI applications to neurological diseases in animal models are emerging. We and others have previously demonstrated that bilateral forepaw somatosensory stimulation activated the somatosensory cortices of both hemispheres in a normal rat using isoflurane as the anesthetics^{29,30} instead of the more common α -chloralose (which is not compatible with survival study).^{31,32} Recent development²⁹ also allows the addition of oxygen-consumption imaging to map oxidative metabolism and neural-vascular coupling in stroke rats. In the stroke rat 30 minutes after occlusion, we demonstrated that activations in the somatosensory cortices were not detected in the ischemic hemisphere.¹⁸ Functional MRI in stroke should be useful in determining whether risky therapeutic intervention should be performed if the 'perfusion–diffusion' mismatch is already non-functional. More recently, T2*-weighted MRI of transient oxygen challenge^{33,34} has been proposed to improve delineation of reversible from irreversibly damaged ischemic brain tissue in ischemic stroke. The at-risk regions surrounding the infarct core showed an exaggerated increase in OC T2*-weighted signal intensity. OC brings in oxygenated blood displacing the high deoxyhemoglobin concentration in the at-risk region where CBF is partially compromised but its metabolic activity remains significant. Thus, tissue with exaggerated increase in T2*-weighted signal intensity during OC is potentially salvageable.

Non-human primate stroke

Although rodent stroke models are necessary to develop imaging protocols and test novel treatment strategies, large NHPs are also important animal models for studying stroke because one can take advantage of brain organization and vascular circuitry that is more homologous with humans than the widely used rodents for stroke modeling. Non-human primates allow for the use of well-established behavioral test batteries that assess a wide range of cognitive functions in much the same way we do for clinical patients. Imaging technologies developed on the clinical scanners can be seamlessly translated to humans and vice versa. As such a few laboratories have investigated clinically relevant NHP stroke models paired with innovative high-resolution MRI. NHP stroke imaging studies are, however, challenging and sparse because of (i) high cost, (ii) NHP resources and trained staff are not widely available, and (iii) *acute* stroke imaging studies necessitate an expensive imaging facility to be located in very close proximity.

Liu *et al.*, measured ADC and fractional anisotropy (FA) changes in permanent and transient MCAO in macaques, and compared with T2 and histology to determine endpoint lesion size.³⁵ This study established the temporal profile of diffusion changes in NHP stroke. They found that reperfusion accelerated the changes of diffusion parameters when compared with permanent stroke. Decreases in ADC within lesion territory were apparent in the acute phase, and it pseudo-normalized ~10 days in macaques.³⁵ Wey *et al.* implemented perfusion MRI using arterial spin labeling technique, which is ideally suited for serial MRI within one imaging session, and demonstrated the spatial-temporal characteristics of perfusion–diffusion mismatch in baboon stroke.^{36,37} An advantage of using baboon is that it offers a larger brain compared to macaques making them more amenable to MRI studies. Reperfusion salvaged substantial amount of the perfusion–diffusion mismatch tissue.³⁷ The mismatch volume progressively decreased for up to six hours post-occlusion, in contrast to mismatch volumes in rodent models that lasted up to three hours post-occlusion.^{15,16,17,38} In humans, the perfusion–diffusion mismatch is often detected up to 6–12 hours after stroke onset, and the frequency of detection decreased with time.³⁹ Consistent with previous serial diffusion MRI studies in NHP stroke which reported temporal profiles of diffusion parameters concord more with humans than rodents, the spatial-temporal dynamics of perfusion–diffusion mismatch appear to be more similar to humans than rodents as well.

Perfusion–diffusion mismatch in human stroke

Perfusion–diffusion mismatch in stroke patients has also been widely observed.^{40–42} Clinical trials have demonstrated the benefits of thrombolytic therapy using recombinant tissue plasminogen activator (t-PA) within three hours after the onset of ischemia and the clinical benefit was associated with smaller late infarcts on CT.⁴³ More recently, t-PA treatment window has been extended to 4.5 hours after the onset of stroke with the help of perfusion and diffusion imaging.⁴⁴ Diffusion-weighted imaging and PWI are now widely used to help identify potential candidate patients for intravenous thrombolysis, intra-arterial thrombolysis or mechanical clot retrieval,⁴⁵ thus enhancing patient selection that could lead to a greater chance for therapeutic benefit. Stroke clinical trials have now routinely included perfusion and diffusion MRI.

While there is apparent clinical utility of perfusion–diffusion mismatch, there are also studies that suggest that diffusion–perfusion mismatch does not optimally define the penumbra, early diffusion lesions are in part reversible and often include both irreversibly infarcted tissue and penumbra.⁴⁶ The visible zone of perfusion abnormality could also overestimate the penumbra by including regions of benign oligemia. Despite these shortcomings, perfusion and diffusion MRI remains to be a very practical method for stroke imaging and some perfusion–diffusion mismatch is salvageable in humans.

Conclusions

Magnetic resonance imaging provides powerful tools to improve characterization of cerebral ischemia, to longitudinally monitor ischemic progression, statistically predict ischemic tissue fate, as well as the potential to evaluate of drug efficacy. Animal stroke models in which the 'perfusion–diffusion mismatch' can be reproducibly studied under controlled conditions are highly valuable for establishing novel MRI modalities and to characterize ischemic tissue fate. Non-invasive MRI can be applied to study stroke in high-order animals, such as NHPs, as well as in humans.

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Figure 1. Cerebral blood flow (CBF) and apparent diffusion coefficient (ADC) of a stroke rat obtained at 30 minutes after middle cerebral artery occlusion. The green regions of interest indicate the perfusion–diffusion mismatch.

Figure 2.

Temporal evolution of lesion volumes defined by cerebral blood flow (CBF) and apparent diffusion coefficient (ADC) thresholds for permanent and 60-minute middle cerebral arterial occlusion.