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REVIEW ARTICLE

Neuroimaging of Stroke and Ischemia in Animal Models

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Abstract Magnetic resonance imaging (MRI) has dramatically changed our ability to diagnose and treat stroke as well as follow its evolution and response to treatment. Early stroke and ischemia can be visualized using diffusion-weighted imaging that utilizes water diffusion within tissues as a reporter for evolving neuropathology that reflects cytotoxic edema, particularly during the first several days after injury. T2-weighted imaging is used for evaluation of vasogenic edema but also is a reliable indicator of the volume and regional distribution of injured tissues. Perfusion-weighted imaging can be used to assess vascular function and also to evaluate potential tissues that might be rescued using therapeutic interventions (core vs. penumbra). Other imaging modalities such as magnetic resonance spectroscopy, diffusion tensor imaging, and susceptibility-weighted imaging are also being used to assist in rapid diagnosis of injured tissues following stroke. While visual analysis of MR data can provide some information about the evolution of injury, quantitative analyses allow definitive and objective evaluations of the injury and could be used to assess novel therapeutic strategies. We review here the basic uses of neuroimaging, focusing on MR approaches to assess stroke and ischemic injury in animal models.

Keywords Magnetic resonance imaging · T2 · Diffusion-weighted imaging · Spectroscopy · Diffusion–perfusion mismatch · Rodents

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Introduction

Magnetic resonance imaging (MRI) of acute ischemic stroke, clinically, is an important part of decision making in the evaluation of patient status and also guides the appropriate therapeutic intervention [1, 2]. Given MRI's ability to visualize non-invasively the ongoing evolution of injury, its ability to "time" the stroke and use for monitoring treatment, it is an ideal clinical modality for assessing acute stroke. Clinically, structural imaging such as T1-, T2-weighted and fluid-attenuated inversion recovery are used to assess vasogenic edema that occurs relatively late temporally. However, diffusion-weighted imaging (DWI) and its quantitative indices, apparent diffusion coefficient maps (ADC), are routinely used to evaluate the ongoing acute (hours to days) evolution of stroke. More recently, a combination of DWI combined with perfusion-weighted imaging (PWI) has been used to identify diffusion–perfusion mismatch as a basis for tissue that might be salvageable from cell death [3], although alternate methods may be equally effective [4].

Neuroimaging of small animal models of stroke is becoming more commonplace and gaining importance as a prelude to future clinical trials of potential therapeutic compounds. This review will briefly acquaint the reader with current imaging modalities that have been used in neuroimaging of

animal models of stroke and ischemia. Novel emerging MRI methods will also be discussed.

Standard MR Imaging for Stroke

As noted above, DWI is now considered the standard of care for acute clinical assessment of stroke. Similarly, DWI is also used extensively in animal models of stroke/ischemia. DWI is thought to reflect the ability of water molecules to diffuse through tissues and can report alterations such as cell swelling (restricted diffusion) or death (increased diffusion) (see [5]). Alterations in cellular swelling or death can be visualized following acute cerebrovascular infarction [6, 7]. Increases or decreases in diffusion can often be seen within the same tissues, but is time-dependent. This is true in stroke/ischemia where early (<3 days) after injury, there is a decrease in the ADC, but at later time points (>3 days), there is a prolonged increase. Questions of the source of these signal changes (intracellular vs. extracellular) are still largely unresolved [8, 9]. We have recently suggested that water channels (aquaporins, particularly aquaporin 4) within the brain may provide a cellular basis for many of the tissue alterations following stroke/ischemia [5]. The quantitative measure of DWI is the computed ADC value, where a change in ADC implies that the water diffusion is altered as a result of tissue level biophysical changes; these changes report maximal sensitivity to injury rather than isolated individual contributions. A recent study in an MCAO rat model followed neuroimaging changes for over a year using MRI [10]. They reported that the ADC along with T2 values continuously increased in the core for the first 6 months post-injury and then plateaued for the remaining 1 year observation period. The lesion area was fairly constant after the first month in this adult model. This is in contrast to our own report where neonatal lesion volume continues to expand over the first 4 months when it reached the final volume that remained unchanged for the rest of the year [11]. Quantitative DWI (ADC values) can be used separately to identify tissues that are altered by therapeutic compounds and provide a non-invasive measure of neurorepair [18, 19].

Perfusion-weighted imaging has been utilized to detect regions of brain tissue that are undergoing a reduction in cerebral perfusion following stroke [3, 12]. Identification of salvageable/non-salvageable (i.e., core/penumbra) tissues over time is crucial in assisting clinicians in patient selection for approved clinical trials. The core/penumbra is a complex interplay of timing, cellular and molecular changes, vascular perfusion, and anatomical susceptibility [13, 14]. The importance of identification of the core/penumbra in animal studies is now getting more attention due to increasing assessment of potential therapeutic compounds [15].

While PWI by itself is an important physiological reporter of tissue injury, a combination of DWI–PWI or diffusion–perfusion mismatch has been utilized as a key reporter of salvageable tissues in human stroke studies [2]. Interestingly, diffusion–perfusion mismatch in the rodent literature has not been used extensively for evaluation of neurological stroke but numerous publications report its use for assessment of myocardial function [16]. A recent report using emobolic stroke in a canine model demonstrated excellent identification of tissues that may become infarcted using diffusion–perfusion mismatch [17]. Furthermore, a multimodal imaging study in rats using a permanent focal model of stroke revealed that the regions of diffusion–perfusion mismatch also showed amplified responses to an oxygen challenge [14]. This approach could be used as an alternative method for assessment of tissue at risk.

While the sensitivity of DWI to early ongoing pathology makes it the imaging modality of choice for evolving injury (early), for established chronic pathology T2WI is utilized. After the injury has been established and tissue characteristics have stabilized, it is often more difficult to visualize altered water movement as tissues can suffer from "T2-shine through." T2WI can, in these situations, assist in delineation of the region(s) of injury. T2WI is frequently used to determine the presence or absence of edema in both animal models and in human injury. After stroke has occurred, early appearance (<3 days) and late hyperintensities on images (>7 days) are often used to respectively signify cytotoxic and vasogenic edema. Quantitative evaluation of T2 values requires additional processing but can provide definitive T2 values for evaluation of neonatal stroke in humans and animals and report if a therapeutic treatment is effective in rescuing tissue [18, 19].

Emerging MR Approaches for Visualizing Injured Tissues in Stroke

A relatively new extension of diffusion-weighted imaging is the development of diffusion tensor imaging (DTI). The microstructure of the white matter fibers within the brain preferentially limits the directional diffusion of water molecules, where diffusion is restricted perpendicularly by myelin, but diffusion along the axon (parallel) tends to flow relatively unrestricted [20, 21]. This privileged water motion is termed anisotropy. Conversely, water motion is considered isotropic when unrestricted by physical boundaries. Once the MR DTI data have been collected and diffusion tensor elements derived, rotationally invariant parameters can be obtained. The mean diffusivity (or ADC) is a summary parameter that independently reports generalized changes in brain water diffusivity, while the trace is the sum of all the diffusivities in all directions. Fractional anisotropy (FA) constitutes a measure of the anisotropy present within the diffusion tensor, while relative anisotropy is a

mathematically derived ratio of anisotropic and isotropic components within the diffusion tensor. In addition, radial diffusivity is thought to report changes in myelin injury or loss, while axial diffusivity is used to assess axonal injury and/or loss. Taken together, these measures can provide insight into dynamic changes in white and gray matter following stroke. An exciting new extension of DTI is diffusion basis spectrum imaging that has been reported to be sensitive to imaging inflammatory events within white matter [22].

While there are numerous clinical studies investigating DTI in stroke patients [23], there are only a handful of reports in animal stroke models. In a study of neonatal hypoxic-ischemic injury, axial and radial diffusion along with reduced FA appeared to predict the type of injury (cystic vs. non-cystic) and severity of injury [24]. Two reports of DTI measures in mouse models of stroke, where decrements in FA were correlated to axonal loss, showed that at early time points, increased axial and radial diffusivities correlated with swelling of the white matter tracts [25, 26]. It is clear that more research is needed to correlate these findings to those seen in patients with stroke.

An extension of DTI is tractography, which utilizes the directionality of the eigenvectors to depict the underlying white matter tracts. While tractography is useful for presurgical evaluation of tumor location and other diseases [27–29], there are virtually no reports in animal models of stroke. A single report of tractography in a transient focal model of cerebral ischemia suggested that there was white matter remodeling that correlated with histological and functional measures [26]. Again additional research is needed to demonstrate the utility for underlying acute as well as chronic cellular changes following ischemia.

MR spectroscopy (MRS) is another powerful MR technique that utilizes frequency shifts of various metabolites within brain regions to determine regional concentrations. Depending on the field strengths (high field strengths enable better resolution of metabolites), one can detect common metabolites such as *N*-acetyl-aspartate—an amino acid present in neurons; choline—a lipid membrane component; creatine—a measure of energy metabolism; glutamate/glutamine—that at higher field strengths can be resolved into glutamate, glutamine and gamma-amino-butyric acid; and lactate—reflecting anaerobic glycolysis. MRS has been used clinically for the evaluation of human stroke [30] but fewer studies have used it the setting of animal studies. A recent investigation of MCAO in adult rats reported that lactate levels correlated and predicted neuronal damage up to 7 days after infarction, but at later time points was not a good predictor of final ischemic injury volume [31]. Other studies have reported the use of MRS in animal models of stroke [32, 33].

Functional MRI (fMRI) or blood oxygen level dependent imaging uses local blood flow to regions of high neuronal activity as a functional measure of cerebral activity. In stroke model studies, there are relatively few reports that describe the use of this imaging modality. fMRI revealed that delayed albumin treatment resulted in improved neuronal activation compared to sham treated animals, suggesting that fMRI can be used to assess stroke recovery [34]. In other studies, fMRI is used to map the response of tissues to oxygen [14], assess tissue viability [35], and to evaluate blood flow following stroke [36].

Susceptibility-weighted imaging (SWI) is a new imaging technique that has been used to evaluate extravascular blood within the brain [37–39]. Given SWI's sensitivity to blood and blood degradation products, it has been used to assess hemorrhagic transformation, microbleeds and response to anti-thrombolytic agents [40–42]. SWI's sensitivity to iron compounds can be used in animal models to image the location of iron-labeled macrophages [10] and angiogenesis [43]. Thus, SWI shows some experimental and clinical utility in evaluating stroke in experimental models and in human patients but it is not used routinely.

Summary

Use of MR imaging methods in experimental stroke are now being routinely used, particularly T2WI and DWI. Most often, these MR modalities are used to assess stroke/ischemic lesion volumes but additional information about the status of the injured tissue can be derived from quantitative T2 relaxation values and ADC values. Furthermore, a significant literature exists in using PWI for cardiac ischemia and only recently has there been an interest in using PWI in assessing stroke in rodent models. Diffusion–perfusion mismatch is a clinical standard for determining the salvageability of tissues that might be rescued by therapeutic intervention, but again, little experimental work has been reported. Other emerging MR modalities can be used to provide additional information on brain function and the effectiveness of potential therapeutics.

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