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LETTER

T2* Magnetic Resonance Imaging Sequences Reflect Brain Tissue Iron Deposition Following Intracerebral Hemorrhage

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Abstract The purpose of this study is to examine the utility of magnetic resonance imaging (MRI) T2* sequences as a measure of iron overload in the brain following intracerebral hemorrhage (ICH). We examined the time course of T2* changes in the brain around intracerebral hemorrhages in a series of patients. We also performed a series of experiments in an animal model of ICH, examining the time course of T2* changes along with correlation of these changes with histological markers of ferric iron deposition. We found that T2* changes in the brain occur with increasing intensity and spatial distribution over a 3-month period. Experimental ICH in the rat model induces similar changes, and these changes correlate tightly with histological markers of ferric iron deposition. MRI T2* changes after ICH can be used to measure the degree of iron overload in the brain. The T2* sequence may be useful as a measure of interventions aimed at reducing ICH-related brain injury by reducing iron deposition.

Keywords Cerebral hemorrhage · Iron · T2* magnetic resonance imaging

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Introduction

Intracerebral hemorrhage (ICH) occurs in 80,000 patients annually in the USA, and is the source of considerable morbidity and mortality [1, 2]. Recent studies have examined the role of tissue iron deposition in the pathogenesis of brain injury and poor neurological outcome related to ICH [3, 4]. However, despite the existence of pathological evidence of tissue iron deposition in experimental models of ICH, it is unclear whether magnetic resonance imaging (MRI) abnormalities correspond to these changes in the clinical setting [5]. We hypothesize that the MRI signal changes seen in gradient echo sequences (GRE, or T2*) correspond closely to tissue iron deposition. Given the potential toxicity related to iron deposition within brain tissue, the use of these MRI sequences is potentially important for the study of interventions aimed at reducing brain iron load.

Materials and Methods

MRI Appearance of Spontaneous ICH in a Clinical Series

Brain MRI scans obtained at the University of Michigan in patients with spontaneous ICH were examined. By utilizing a search on the ICD-9 code 431, a total of 260 scans obtained between 2004 and 2008 were collected. Of these scans, 53 included a T2* or GRE sequence. Most scans were obtained within 2 days of the ICH, although there were several scans performed late, over 3 months following ICH. In general, patients had only one T2* scan following ICH. Several representative MRI scans of patients who did not undergo surgical evacuation of their hematoma were selected at early and late

time points following hemorrhage, and the T1 and T2* images were compared. The lesion sizes on T1 and T2* images were measured using ImageJ software, and the ratio of T2*/T1 calculated.

Animal Preparation and Intracerebral Infusion

Male 12-week-old Wistar Kyoto rats (Charles River Laboratories) were used, and protocols were approved by the University of Michigan Committee on the Use and Care of Animals.

Animals were anesthetized with pentobarbital (45 mg/kg, i.p.). The femoral artery was catheterized for blood pressure monitoring and blood sampling. Rats were placed in a Kopf stereotactic head frame, and a 1 mm burr hole was drilled on the right coronal suture 3.5 mm lateral to midline. A 26-gage needle was inserted into the right basal ganglia (0.2 mm anterior, 5.5 mm ventral, and 3.5 mm lateral to bregma). This needle was used for infusions of 100 ul autologous blood at a rate of 10 ul/min with a microinfusion pump [3]. Body temperature was maintained at 37.5°C. Blood pH, PaO_2, PCO_2, hematocrit, and glucose levels were monitored. Animals were killed after 1, 3, or 14 days (n=6 each time point)

Magnetic Resonance Imaging

Serial MRI was performed with a 7.0 Tesla 18-cm Horizontal Bore (Unity Inova, Varian Inc.) imaging spectrometer on day 1, 3, and 14 after ICH. T2-weighted and T2* GRE were performed for evaluation of lesion volume. Fifteen 0.5 mm-thick slices around the injection tract were acquired (FOV=35×35mm^2, matrix=256×256, slice gap=0, flip angle=25, TR/TE=4,000 ms/60 ms and 200 ms/5 ms for T2 and T2* imaging, respectively). Total scan time was about 4.5 and 1.5 min for T2 and T2* imaging each. Images were analyzed with NIH Image.

Histological and Immunohistochemical Study

Rats were reanesthetized with pentobarbital (60 mg/kg, i.p.), followed by intracardiac perfusion with 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline, pH 7.4. Brains were removed, kept in 4% paraformaldehyde for 6 h, and immersed in 25% sucrose for 3-4 days at 4°C. They were then embedded and sectioned (18 μm slices) on a cryostat. Hematoxylin and eosin staining was performed. Immunohistochemistry was done by avidin-biotin complex technique. Primary antibodies were polyclonal rabbit anti-rat heme oxygenase-1 (HO-1) IgG (StressGene; 1:400 dilution), polyclonal rabbit anti-human ferritin IgG (DACO; 1:200 dilution). Normal rabbit IgG or mouse IgG was used as negative control [3].

Enhanced Perls' Reaction

Brain sections were incubated in Perl's solution (1:1, 5% potassium ferrocyanide and 5% HCl) for 45 min, washed in distilled water, and incubated in 0.5% diamine benzidine tetrahydrochloride with nickel for 60 min [3].

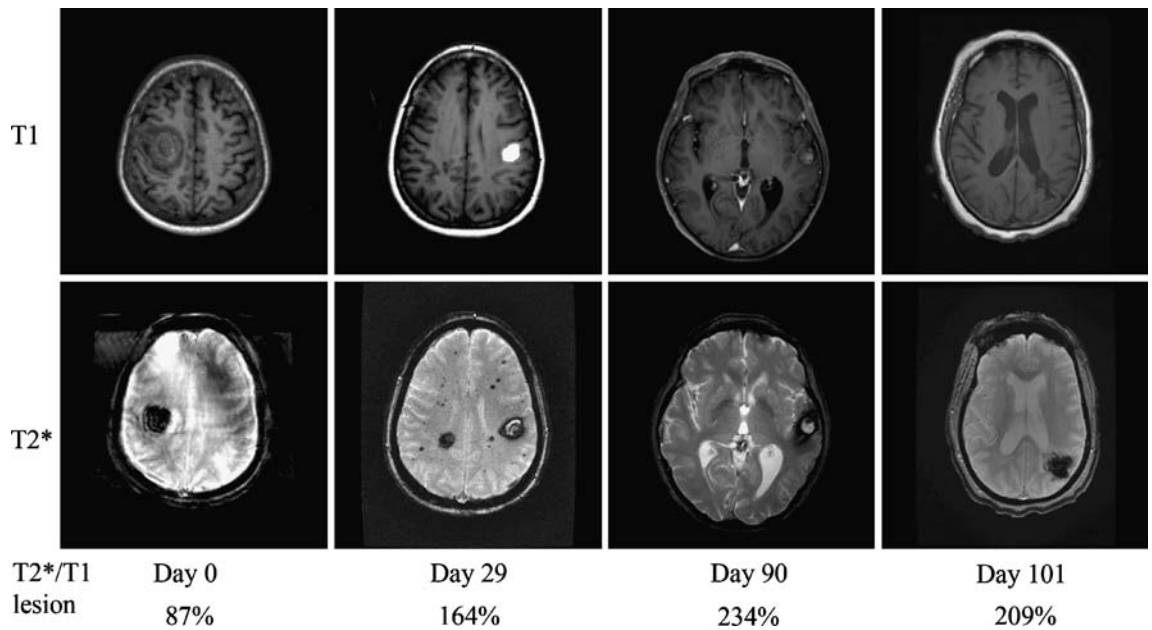

Fig. 1 MRI scans obtained at 0, 29, 90, and 101 days following ICH in four patients. T1 and T2* of corresponding regions are shown and the ratios of the abnormalities on T2* and T1 scans are shown

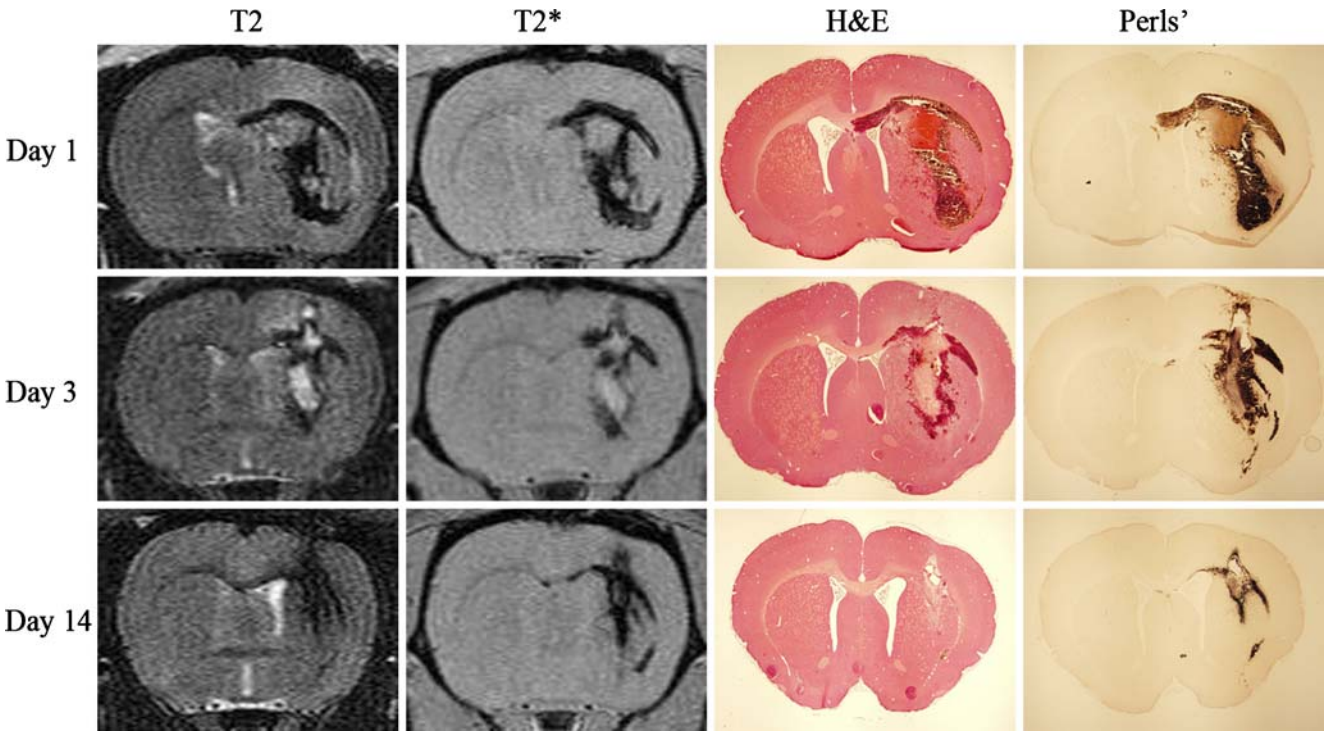

Fig. 2 T2, T2*, H&E, and Perls' staining 1, 3, and 14 days after ICH

Results and Discussion

Approximately 20% of MRI scans obtained on patients following ICH included T2* sequences. T2* sequences were usually obtained to look for small cavernous malformations as a source of the ICH. The majority of these sequences were therefore obtained in the acute post-hemorrhage period. However, several scans obtained between 1 week and 3 months after the ICH demonstrated changes in T2* signal in the surrounding brain. The region affected by the T2* changes, initially limited to the clot itself, increased to involve roughly twice the region affected by the ICH cavity in the 3-month period (Fig. 1). While the clinical significance of this T2* abnormality is not yet clear,

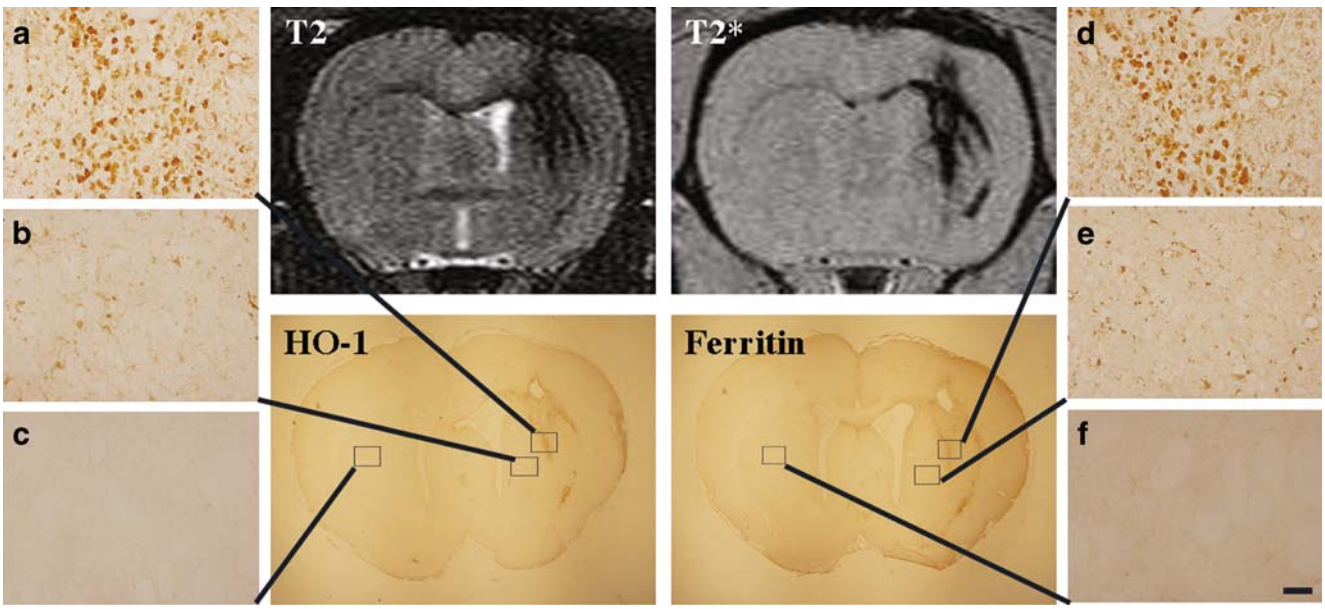

Fig. 3 Heme oxygenase-1 (HO-1) and ferritin immunoreactivity in the brain 14 days after ICH. *Scale bar* (**a-f**)=50 μm

it likely represents ferric iron deposition within the brain parenchyma. As brain iron deposition has been shown to be toxic, the region of T2* abnormality may represent a useful measure of ICH-related toxicity and a likely target for therapeutic interventions aimed at iron chelation [4, 6]. Since the patient MRI scans in this study were chosen based upon the existence of follow-up MRI imaging in the absence of surgical intervention, the sample of patients analyzed includes several etiologies of the intracerebral hemorrhage, including hypertension, cavernoma, and hemorrhagic metastasis. Because of the small number of patients in this series, it is not possible to compare the temporal evolution between the various hemorrhagic etiologies. However, the pairwise comparisons undertaken in this study suggest that iron deposition into brain parenchyma occurs in the setting of various hemorrhagic etiologies. Similarly, it is not possible for us to make any observations on the spatial patterns of distribution given the small sample of patients analyzed in a retrospective fashion.

Our studies of ICH in a rodent model corroborate a number of the observations in the clinical study and demonstrate the histopathological changes that correlate with MRI T2* changes. The evolution of T2* changes closely mimics that seen in the clinical study with acute changes seen within the hematoma only and evolving T2* changes outside the cavity over a 14-day period. The Perls' staining pattern in these animals demonstrates that the region of T2* change correlates closely with ferric iron deposition (Fig. 2). Furthermore, immunostaining also showed that the T2*-change regions associate with both HO-1, a key enzyme of heme degradation, and ferritin, an iron-storage protein (Fig. 3).

Much of the recent attention T2*-weighted MRI scans have garnered centers around their ability to detect small areas of heme deposition (so called "microbleeds") as well as their use as a marker of secondary damage after stroke [7–9]. These sequences have not been studied as a tool to measure changes in the brain surrounding ICH, however. The present study demonstrates that MRI T2* changes in the brain correlate closely with brain iron overload following ICH. Imaging with T2* sequences represents a non-invasive way to document the evolution of iron deposition in brain parenchyma. Moreover, T2* imaging may allow for the study of interventions aimed at reducing iron deposition, such as deferoxamine[4, 6]. While the impact of T2* changes per se is not yet well-delineated, our study suggests that these regions correlate with parenchymal iron overload, a marker of chronic brain injury. Further studies are required to correlate the extent of such changes with neurological outcome.

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