The Relationship between Hematoma Iron Content and Perihematoma Edema: An MRI Study

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Key Words

Iron · Edema · Hematoma · Intracerebral hemorrhage · Magnetic resonance imaging

Abstract

Background: Iron neurotoxicity has been linked to delayed neuronal injury and edema formation after intracerebral hemorrhage (ICH). We have previously shown that serum ferritin, an indicator of body iron load, correlates with the relative perihematoma edema volume (RPHEV) on days 3–4 after ICH. We undertook this study to directly examine the relationship between in vivo brain and hematoma iron content, measured by MRI, and RPHEV. **Methods:** We retrospectively reviewed prospectively collected clinical and laboratory data from 36 consecutive patients with acute spontaneous lobar ICH who had MRI performed within 2–4 days of ICH onset. We measured hematoma and edema volumes, and the signal intensity on T₂-weighted images (T₂SI), as an estimate of iron content, in the hematoma and contralateral globus pallidus (GP). We calculated the RPHEV and T₂SI in the hematoma and GP, relative to T₂SI in the frontal deep white matter which contains negligible iron, to estimate the hematoma and brain iron load. We used Spearman correlation coefficient to determine the association of relative T₂SI of the hematoma and GP with RPHEV. **Results:** We found a significant inverse correlation between the relative

T₂SI in the hematoma ($r = -0.75$, $p < 0.001$) and to a lesser extent in the GP ($r = -0.34$, $p = 0.04$) and the RPHEV. **Conclusions:** Our findings suggest that in vivo brain and hematoma iron content, as measured by MRI, is linked to perihematoma edema after ICH, and provide further support to existing preclinical evidence linking iron-mediated toxicity to delayed neuronal injury after ICH.

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Introduction

Studies in animal models of intracerebral hemorrhage (ICH) show that hemoglobin and its degradation products, particularly iron, are neurotoxic and are causally related to delayed neuronal injury and edema formation after ICH [1–4]. Iron can promote oxidative damage by catalyzing hydroxyl radical formation and lipid peroxidation [2, 4]. In a recent study, we observed a significant positive correlation between serum ferritin and relative perihematoma edema volume (RPHEV) on days 3–4 in patients with spontaneous ICH [5]. We used serum ferritin, which is a reliable indicator of body iron load [6], as an indirect measure of iron availability in the erythrocytes and the area of ICH since we could not directly assess iron content in the vicinity of the hemorrhagic brain tissue.

Fig. 1. Representative pictures of hematoma and perihematoma edema measurements. **a** ROIs drawn around the perimeters of the perihematoma edema and hematoma. **b** ROIs drawn on the frontal white matter and GP.

Iron deposition in the brain can be detected with magnetic resonance imaging (MRI) [7–11]. The iron atoms form ferric oxyhydroxide particles, which shorten the relaxation times on T_2 -weighted images resulting in darker (hypointense) signal in brain regions with higher iron content [7]. Signal intensity on T_2 -weighted images (T_2 SI) has been used to reflect the regional iron content of brain tissue in normal aging and neurodegenerative conditions associated with pathological disturbance of brain iron homeostasis [8–11]. Therefore, we employed MRI to assess in vivo brain iron in the current study to directly examine the relationship between brain and hematoma iron load and RPHEV in patients with acute spontaneous ICH.

Methods

Patient Selection and Data Collection

We retrospectively reviewed our prospectively collected stroke database for consecutive patients with ICH admitted to our service during an 18-month period from 2006 to 2008. We identified patients with spontaneous lobar ICH who had a brain MRI performed within 2–4 days of ICH symptom onset. Patients with secondary causes of ICH, such as anticoagulant use, underlying aneurysm vascular malformation, or tumor, head trauma, or hemorrhagic transformation of ischemic infarcts were excluded. In addition, we excluded patients with initial or subsequent intraventricular and infratentorial hemorrhages and hemorrhage in the basal ganglia to avoid technical flaws regarding accurate edema volume measurements and the effect of iron deposit in basal ganglia on signal intensity of deep hematoma in these situations. We also excluded patients who received antiedema treatments, or underwent surgical intervention during hospitalization before

having the MRI scan. We retrieved demographic, clinical, laboratory, and radiological data, and examined the admission CT scans of eligible patients to confirm that none had evidence of calcification in the globus pallidus (GP) which could result in T_2 SI shortening on MRI.

Radiological Measurements

All MRI studies were performed on a 1.5-tesla MR whole-body scanner with echo-planar imaging capability, using a standardized imaging protocol including T_2 -weighted images. We measured the hematoma and edema volumes, and the signal intensity in the region of the hematoma and the contralateral GP and frontal lobe white matter on T_2 images off-line using the National Institute of Health imaging processing software. We measured the signal intensity in the contralateral GP and frontal white matter in order to avoid the confounding effects of ICH-associated edema in the ipsilateral hemisphere on T_2 SI. A single evaluator (M.L.) experienced in the interpretation of MRI images analyzed all scans to conduct all measurements. Definition of the hematoma and perihematoma edema was assisted by adjustment of the contrast and threshold value until the hematoma consisting of a dark rim and bulk on T_2 images could be well separated from the surrounding hyperintense edematous brain tissue in terms of appropriate high- and low-attenuation zones [12]. The examiner then manually drew regions of interest (ROIs) by tracing the perimeters of the hematoma and perihematoma edema in each slice throughout the hemorrhagic lesion (fig. 1a). The traced ROIs in contiguous voxels were then summed up after adjusting for the slice thickness to yield a hematoma volume and an absolute edema volume (the volume of the hematoma and surrounding edema) (fig. 1a). To control for hematoma volume, we subtracted the hematoma volume from that of the absolute edema, and divided the product by the hematoma volume, to express the perihematoma edema volume as a ratio of the associated hematoma volume (RPHEV).

The observer (M.L.) drew the ROIs on an initial subset of 10 patients twice, at an interval of 6 months apart. The intraobserv-

Table 1. Summary of hematoma and edema (absolute and relative) volumes

Volume	Hematoma cm ³	Absolute edema, cm ³	Relative edema
Median	16.90	20.57	1.43
Range	0.77–96.42	1.49–89.48	0.53–3.02
Mean $\pm$ SD	23.79 $\pm$ 22.43	26.92 $\pm$ 20.56	1.49 $\pm$ 0.72

Table 2. Summary of relative T₂SI in the hematoma and contralateral GP

	T ₂ SI of hematoma	T ₂ SI of GP
Median (range)	1.90 (0.80–2.70)	0.61 (0.49–1.10)
Mean $\pm$ SD	1.42 $\pm$ 0.47	0.91 $\pm$ 0.13

er test/retest agreement was $r = 0.95$ for absolute edema volume, $r = 0.98$ for hematoma volume measurements. Another investigator (K.L.) independently drew the ROIs on the same subset of 10 patients. The interobserver agreement was $r = 0.91$ for absolute edema volume and $r = 0.98$ for hematoma volume measurements. Because the intra- and interobserver reliability measures were high, only one measurement by 1 observer (M.L.) was used for the remainder of the ROIs.

ROIs were also manually drawn in the GP and frontal white matter, contralateral to the hematoma (fig. 1b). The GP was chosen because it has the highest iron content in the brain and is easily visible on T₂ images [13–15]. The frontal white matter was selected, as a control, because it contains negligible amounts of iron [13–15]. The T₂SI of hematoma and GP ROIs in each voxel was automatically measured as a gray scale and summed up to calculate the average T₂SI in each of these regions. A lower T₂SI value represents more T₂ shortening (darker signal), and presumably reflects higher iron content. Three measurements were made on the frontal white matter with a 3-mm² square ROI and then averaged to obtain a final value, which was used as an internal reference. We then calculated the relative T₂SI of the hematoma and GP after dividing the mean T₂SI within each region by that of the frontal white matter to derive MRI estimates of relative iron content in the hematoma and GP. A lower ratio between mean regional hematoma or GP and white matter T₂SI indicated a lower T₂SI, presumably related to higher iron content within these regions.

Statistical Analysis

We used the Spearman correlation coefficient to determine the presence or absence of a correlation between the relative T₂SI of the hematoma, as a measure of hematoma iron load, and GP, as a measure of brain iron load, and RPHEV. Statistical significance was set at a probability value of ≤ 0.05 .

Results

A total of 36 subjects who met all of our inclusion and none of our exclusion criteria were included in the present analysis. Sixty-one percent were men, and 39% women. Mean age was 74.6 ± 16.1 years (range: 28–93). Mean onset-to-imaging time for the MRI scan was 2.0 ± 1.0 days. Thirteen patients (36%) were discharged home, 22 (61%) to extended-care facilities or rehabilitation, and 1 (3%) died during hospitalization. None of the patients had conditions that could affect the T₂SI on MRI, such as severe anemia, Parkinson's disease, Huntington's disease, schizophrenia, demyelinating disease, or visible calcification of the basal ganglia on admission CT scan.

Table 1 lists the hematoma and perihematoma edema volumes on T₂-weighted MRI. The mean T₂SI in the subcortical frontal white matter was 61 ± 18 . Table 2 lists the T₂SI in the hematoma region and contralateral GP relative to the frontal subcortical white matter. The ratio for T₂SI between the GP and white matter ranged from 0.49 to 1.1 and that between the hematoma and white matter from 0.80 to 2.7, reflecting the large variability in brain and hematoma iron content between the subjects. Figure 2 depicts the correlation between the relative T₂SI in the hematoma region and the RPHEV. As it shows, there was a significant inverse correlation between the relative T₂SI of the hematoma and the RPHEV ($r = -0.75$, $p < 0.001$). Similarly, figure 3 shows that there was also an inverse correlation, albeit less robust, between the relative T₂SI of the GP and the RPHEV ($r = -0.34$, $p = 0.04$). We found no correlation between the T₂SI in the frontal white matter and RPHEV ($r = 0.219$, $p = 0.199$), or between the hematoma volume and T₂SI within it ($r = 0.303$, $p = 0.073$).

Discussion

We found significant inverse correlations between the relative T₂SI in the hematoma region and GP, and the RPHEV in this cohort of patients with acute spontaneous lobar ICH. Our results indicate that higher iron content in the hematoma region and to a lesser extent in the GP correlate with perihematoma edema volume after ICH. These findings lend further support to the important role of iron-mediated neurotoxicity after ICH.

Evidence linking iron to neuronal injury and perihematoma edema formation derives mostly from studies in experimental models of ICH [1–4]. Several studies have shown that hemoglobin and its degradation products, in

Fig. 2. Scatter plot showing the correlation between RPHEV and relative T_2 SI of hematoma ($r = -0.75$, $p < 0.001$, $n = 36$).

Fig. 3. Scatter plot showing the correlation between RPHEV and T_2 SI of contralateral GP ($r = -0.34$, $p = 0.04$, $n = 36$).

particular iron, resulting from erythrocyte lysis after ICH exert neurotoxic effects and contribute to the development and progression of brain edema following ICH and that treatment with iron chelators can reduce these effects [1–4, 16, 17]. There have not been many clinical studies examining the status and role of iron in the brain. We recently observed a positive correlation between serum ferritin levels on admission and the RPHEV on days 3–4 after ICH onset, suggesting that increased body iron load is associated with edema progression after ICH [5]. We undertook the current study in order to further examine the relationship between in vivo iron load in the brain and the hematoma and RPHEV after ICH.

MRI is a valuable assessment tool with its unique specificity to hemoglobin degradation products and iron. The specific appearance of hemoglobin and blood degradation products on MRI sequences has been extensively described in the literature [12, 18, 19]. Rapid deoxygenation of blood occurs at the border of the hematoma, which leads to a transition from the diamagnetic oxyhemoglobin to the paramagnetic deoxyhemoglobin. This creates a ‘boundary effect’ leading to signal loss (hypointensity) at the periphery of the hematoma and separating the hematoma well from the surrounding hyperintense edematous tissue [12]. The hypointensity of the hematoma on T_2 images reflects the paramagnetic iron-containing hemoglobin degradation products, which can be released from the erythrocytes in the area of ICH at the early stage [2, 3]. The iron molecules in the brain tissue and in the hemoglobin-degradation products within the hematoma region have unpaired elec-

trons, i.e. paramagnetic properties, which shorten the T_2 relaxation time and produce visible hypointensity on T_2 -weighted images caused by dephasing of water protons as they diffuse through the local magnetic field inhomogeneities created by the presence of the iron particles [15, 18–20]. T_2 SI is influenced by regional iron content. Several investigators have reported a correlation between T_2 shortening and regional brain tissue iron concentration, using the mean iron concentration of various brain regions and structures from postmortem and autopsy studies as a reference to validate their findings [13, 15, 21–23]. Accordingly, we used MRI T_2 SI as a surrogate measure to estimate in vivo the regional iron content of the hematoma in this study. Postmortem studies indicate that the basal ganglia, in particular GP, contain the highest levels of iron in the brain [13–15]. Therefore, we also examined the T_2 SI of the GP as an in vivo indicator of the total brain iron load.

The iron content in the brain increases gradually with age, up to about 50 years [8, 13, 23]. We found no significant correlations between age and relative T_2 SI in the GP, or age and RPHEV in the current study (unreported data). This is likely attributed to the fact that the vast majority of our patients were older than 60 years of age. Our finding of a modest correlation between relative T_2 SI in the GP, as an indicator of brain iron load [13–15], and RPHEV implies that brain iron status may be an important determinant of secondary neuronal injury and edema formation after ICH. However, it is possible that increased iron load in the GP could be secondary to ICH itself. Further studies are required to exam-

ine the relationship between baseline brain iron status and RPHEV.

Our findings indicate that the use of MRI to estimate in vivo regional iron content is feasible in ICH patients and that MRI can be potentially used as a tool to assess or guide interventions aimed at decreasing brain iron levels in future studies.

There are limitations to our study that are largely imposed by its retrospective nature. First, independent histopathological confirmation of T₂SI estimates of tissue iron was not feasible. However, previous histological and postmortem studies have reported a correlation between T₂SI and regional brain tissue iron concentration [13, 15, 21–23]. Second, the T₂SI of MR images depends on a variety of factors other than the presence of paramagnetic iron particles, such as regional tissue characteristics [14]. Other techniques, such as MRI field-dependent R₂ increase, to quantify brain iron levels may be more specific and superior to the semiquantitative methodology using T₂SI employed in this study [24]. However, this technique requires the use of two different field-strength MRI instruments. Third, we could not separately analyze the specific contribution of various forms of iron-containing degradation products to the T₂SI. It is unlikely that methemoglobin or hemosiderin significantly

contributed to the T₂SI of the hematoma in the current study since MRI was performed during the acute phase within 2–4 days of ICH onset [18]. Lastly, we could not measure the temporal evolution of T₂SI and perihematoma edema at different time intervals after ICH onset. It is therefore unknown if the T₂SI of the hematoma in this early period bears any relationship to the evolution of perihematoma edema beyond the acute phase of ICH. The above limitations need to be considered when interpreting our findings.

In conclusion, we found that the relative degree of T₂ shortening (hypointensity) in the hematoma region and GP correlates with RPHEV, suggesting that iron content in the brain and hematoma influences the extent of the surrounding edema. Future prospective studies using more advanced MRI techniques are warranted to confirm our findings.

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