Conversion of Ischemic Brain Tissue Into Infarction Increases With Age

Hakan Ay, MD; Walter J. Koroshetz, MD; Mark Vangel, PhD; Thomas Benner, PhD; Christopher Melinosky, BS; Mingwang Zhu, MD; Nina Menezes, PhD; Chloe J. Lopez, MA; A. Gregory Sorensen, MD

Background and Purpose—Brain regions normal on diffusion-weighted imaging (DWI) but abnormal on mean transit time (MTT) maps represent tissue at risk of infarction, yet the fate of these regions is quite variable. The imperfect correlation between tissue outcome and initial imaging parameters suggests that each patient’s brain may have different susceptibility to ischemic stress. We hypothesize that age is a marker for tissue susceptibility to ischemia and thus plays a role in determining tissue outcome in human stroke.

Methods—Sixty patients with acute ischemic stroke and a region of DWI/MTT mismatch that was \( >20\% \) of the DWI volume were included. All patients were scanned twice, within 12 hours of symptom onset and on day 5 or later. The percentage mismatch lost (PML) was calculated as percentage of initial DWI/MTT mismatch volume that was infarcted on the follow-up MRI. The statistical analysis explored relationships among the covariates age, Trial of Org 10172 in Acute Stroke Treatment (TOAST) subtypes, time-to-MRI, and initial DWI, MTT volume, mean arterial blood pressure and blood glucose level at admission, and previous history of hypertension and diabetes mellitus.

Results—Univariate comparisons showed that age (\( P=0.003 \)), hypertension (\( P=0.009 \)), and diabetes mellitus (\( P=0.0002 \)) were significantly associated with PML. Regression analyses showed age to be a significant covariate (\( P=0.02 \)). The regression model predicted a change in PML of \( 0.65\% \) per year. The adjusted proportion of variance (\( R^2 \)) in PML that could be explained by age alone was 14%.

Conclusion—Age-dependent increase in conversion of ischemic tissue into infarction suggests that age is a biological marker for the variability in tissue outcome in acute human stroke. (Stroke. 2005;36:2632-2636.)

Key Words: aging ■ cerebral ischemia ■ imaging, diffusion-weighted ■ magnetic resonance imaging ■ magnetic resonance imaging, perfusion-weighted ■ mitochondria ■ penumbra

Brain regions with compromised blood supply on perfusion-weighted imaging (PWI) but not yet abnormal on diffusion-weighted imaging (DWI) have been postulated to represent tissue at risk of infarction and therefore a potential therapeutic target. However, quantifying the risk of infarction in this region of DWI-PWI mismatch is quite difficult because the fate of tissue is highly variable; the extent of abnormality on maps of cerebral blood flow (CBF) and tracer mean transit time (MTT) can only explain 32% and 36% of the variability in final infarction volume, respectively. Even with improved mathematical approaches that combine initial apparent diffusion coefficient (ADC), DWI, T2, cerebral blood volume (CBV), CBF, and MTT information, tissue outcome could be predicted with only 65% sensitivity and 80% specificity as a voxel-by-voxel estimate of the risk of infarction. The inability of imaging to unambiguously differentiate between ischemic tissue destined to infarction and potentially salvageable tissue might, in part, be attributable to individual variability in the susceptibility of patient’s brain to ischemic stress (ie, there might be a biological component to variability in tissue outcome).

One of the factors responsible for this observed biological variability might be patient age. Age is the leading risk factor and the strongest predictor of clinical outcome after ischemic stroke. Even when adjusted for comorbid disorders that appear with age, age is still associated with poor outcome. Aging is characterized by compromised energy metabolism at the cellular level. Impaired energy metabolism, in turn, is the hallmark of tissue susceptibility to ischemia. Therefore, we hypothesized that the conversion of ischemic but viable tissue into infarction increases with aging in acute human ischemic stroke.

Methods

Study Population

The current study was part of a prospective ongoing study evaluating the utility of DWI and PWI in predicting tissue risk of infarction (MRI Diffusion/Perfusion Mismatch in Human Acute Stroke). The
study covered a 5-year period, from 2000 to 2005. Patients with ischemic stroke admitted within the first 12 hours of symptom onset and who did not receive any thrombolytic treatment or investigational drugs were included. Each patient underwent 2 MRI studies: 1 obtained within 12 hours of symptom onset and the second on day 5 or later. In the first MRI, T2-weighted images, ADC maps, DWI, MTT maps, CBF, and CBV maps were obtained. The purpose of the second MRI was to evaluate the final infarction and included only T2-, and fluid-attenuated inversion recovery (FLAIR)-weighted sequences. In circumstances under which the assessment of final infarction volume was not possible, patients were excluded. These included motion artifacts, extensive hemorrhagic conversion or brain edema with mass effect causing anatomical distortion or limited life expectancy, and brain surgery (hemispherectomy). To depict the effect of age on lesion growth, only patients with DWI/MTT mismatch that was >20% of the DWI volume were studied. Hence, none of the included patients had complete spontaneous reperfusion. Age, gender, stroke risk factors, etiologic TOAST subtype, and time to MRI were recorded in each patient. The etiologic subgroups included cardiac embolism, large artery atherothrombosis, small artery occlusion, other rare causes, and undetermined causes. The study was conducted at a single academic center and the study protocol was approved by the local institutional review board.

**Image Acquisition**

MRI was performed on 1.5-T whole body scanners (GE Signa; GE Medical Systems; or Siemens Sonata; Siemens Medical Solutions). DWI was obtained using echo planar imaging (EPI) with a repetition time (TR) of 7500 ms, an echo time (TE) of 99.3 ms, a field of view (FOV) of 22×22 cm, image matrix of 128×128, slice thickness 5 mm with 1 mm gap, and b values of 0 s/mm and 1000 s/mm². Diffusion-weighted images were corrected for motion and eddy-current distortions using the functional MRI of the brain (FMRIB) Linear Image Registration Tool (FLIRT 5.0; Oxford Centre for Functional Magnetic Resonance Imaging of the Brain). Average DWI maps as well as ADC maps were computed from these images. Perfusion-weighted images were acquired using dynamic susceptibility contrast EPI. Imaging parameters were TR 1.4 to 1.5 s and TE 65 to 75 ms, with the same spatial resolution as for DWI. MTT and CBF maps were calculated using methods described previously. Fast spin-echo T2-weighted images were acquired with TR 4200 ms, TE 102 ms, FOV of 22×22 cm, acquisition matrix of 256×256 pixels, and slice thickness 5 mm with 1 mm gap. FLAIR images were acquired with TR 10 002 ms, TE 126 ms, FOV of 22×22 cm, acquisition matrix of 256×256 pixels, and slice thickness 5 mm with 1 mm gap.

**Image Analysis**

All images were coregistered to a T2 template using FLIRT. The lesion on DWI, MTT maps, and final T2- or FLAIR-weighted images were outlined manually using a commercial image display and analysis program (ALICE; Hayden Image Processing Solutions), and lesion volumes were computed. The amount of lesion growth was defined as the percentage of initial DWI/MTT mismatch that was infarcted on the follow-up MRI (percentage mismatch lost [PML]) and calculated as follows: (follow-up T2- or FLAIR volume-initial DWI volume)/(initial MTT volume-initial DWI volume)×100.

**Statistics**

In addition to age, the statistical models explored relationships between PML and the covariates that are known to be potential markers of infarction growth. These included TOAST subtypes, time from symptom onset to MRI, initial DWI volume, initial MTT volume, admission blood glucose, and admission mean arterial blood pressure. Previous history of diabetes mellitus and hypertension were also included as a covariate because their prevalence strongly correlates with age. Univariate relationships were tested using Pearson correlation, t test or F test from 1-way ANOVA and Fisher’s exact test for situations in which the covariates were both continuous, 1 continuous and 1 categorical, and both categorical, respectively. Variables with univariate P value <0.05 were entered into a linear regression model with PML as response. All numerical variables were expressed as mean±SD. A level of P<0.05 was considered statistically significant.

**Results**

A total of 1876 consecutive patients with ischemic stroke were admitted during the study period. During the last 19 months of the study, patients were entered regularly into a stroke log that listed reasons for exclusion. There were 585 consecutive admissions listed in this log. Patients were excluded because of admission >12 hours after symptom onset (51%), inability to obtain MRI (18%), no consent (7%), contraindication to MRI (7%), thrombolytic or experimental treatment (6%), lost to follow-up (3%), images of insufficient quality because of motion artifact or other reasons (1%), massive edema or hemorrhagic conversion on follow-up imaging (1%), or hemispherectomy (<1%). The remaining 35 of the 585 patients were included in the current study. From the 41-month period preceding the log, there were 54 additional eligible patients who were scanned per protocol. These 54 patients were identified using the same criteria as the latter 35 patients. Thus, the total study population consisted of 89 patients. Sixty of these 89 patients had DWI/MTT mismatch that was >20% of the DWI volume. Further analyses included these 60 patients.

The Table summarizes the baseline characteristics and clinical and imaging features. The mean age of the study population was 65.3 years (median 69 years). Of the 60, there were 7 patients ≥45 years, 21 patients ≥60 years, 35 patients ≥70 years, and 54 patients ≥80 years. Infarctions were caused by occlusion of the middle cerebral artery in 44, anterior cerebral artery in 5, posterior cerebral artery in 4, internal carotid artery in 3, posterior inferior cerebellar artery in 2, and basilar artery and superior cerebellar artery in 1 patient each. The second MRI was obtained 5 to 56 days after stroke onset (mean±SD; 17.2±16.0 days). There was previous history of hypertension in 39 patients and diabetes mellitus in 8 patients. The mean arterial blood pressure and blood glucose levels were determined on admission to the

<table>
<thead>
<tr>
<th>Baseline Characteristics and Clinical and Imaging Features</th>
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<tbody>
<tr>
<td>Mean age±SD (range)</td>
<td>65.3±15.1 y (26–96)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>43/17 patients</td>
</tr>
<tr>
<td>Mean time from symptom onset to MRI±SD</td>
<td>6.1±3.0 h</td>
</tr>
<tr>
<td>Mean initial DWI volume±SD</td>
<td>38.1±47.9 mL</td>
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<tr>
<td>Mean initial MTT volume±SD</td>
<td>142.2±117.9 mL</td>
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<tr>
<td>Mean admission blood glucose±SD</td>
<td>128.4±45.4 mg/dL</td>
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<tr>
<td>Mean arterial blood pressure at admission (mean±SD)</td>
<td>87.7±47.9 mm Hg</td>
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<tr>
<td>Etiologic stroke subtype (TOAST)</td>
<td></td>
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<tr>
<td>Large artery atherosclerosis</td>
<td>17 patients</td>
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<tr>
<td>Cardiac embolism</td>
<td>23 patients</td>
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<tr>
<td>Other causes</td>
<td>9 patients</td>
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<tr>
<td>Undetermined</td>
<td>11 patients</td>
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emergency room (mean±SD; 4.2±2.7 hours after symptom onset).

The PML varied from 0% to 100% (Figure). The mean (±SD) and median PML were 24.8±30.9% and 12.3%, respectively. There was no mismatch loss in 14 patients (PML=0), whereas the whole region of DWI/MTT mismatch underwent infarction in 4 patients.

Univariate comparisons with PML showed a significant association with age (R=0.38; P=0.003), previous history of hypertension (P=0.009), and diabetes mellitus (P=0.0002). Within the 12-hour window, there was no correlation between PML and time to MRI (P=0.86), DWI volume (P=0.67), MTT volume (P=0.87), TOAST subtype (P=0.46), and mean arterial pressure (P=0.34) and blood glucose levels at admission (P=0.95). The association between age and PML as assessed by linear regression taking other factors with univariate P value <0.05 (history of hypertension and diabetes mellitus) into account as additional covariates showed that the association with age was statistically significant (P=0.02). In addition to age, history of diabetes mellitus was also significant in this model (P=0.001). The adjusted proportion of variance (R²) in PML that could be explained by age alone was 14%. The regression model predicted a change in PML of 0.65±0.26% per year.

The linear regression analysis was repeated after including patients who had been initially excluded from the study because of severe brain edema. There were 9 such patients. The DWI/MTT mismatch was >20% of the DWI volume in 8 of them (ages 53, 60, 60, 67, 74, 80, 85, and 89). The PML was presumed to be 100% in these 8 patients. The influence of age on PML adjusted for other covariates remained statistically significant in the cohort including these 8 patients (P=0.007).

Discussion

Early signal changes on DWI after brain ischemia often represent tissue that has already undergone irreversible injury unless there is immediate reperfusion. The hyperintense lesion on DWI exhibits potential for growth over time. The amount of growth depends on the degree of blood flow compromise within the region of mismatch as well as the volume of mismatch between DWI and PWI. There is also a time dependency of infarction growth, during which the most substantial loss of the mismatch tissue occurs within hours after onset. The current study is the first to show that in addition to imaging factors, age is also important in determining the fate of ischemic brain regions. The conversion of ischemic but viable tissue into infarction increased with age (Figure). The relationship between age and volume of tissue converted into infarction remained significant when adjusted for comorbid disorders that appear with age, and clinical and imaging parameters associated with the growth of acute DWI lesion. Every 1-year increase in age resulted in 0.65% more mismatch tissue to turn into infarction.

PML as a function of age. The graph shows individual patient data points (○), the mean PML for each 10-year interval (●), and corresponding SE bars. The curve represents locally weighted least square fit (LOWESS), which forms a prediction of PML at each age by weighting the data in such a way that the nearby points are weighted more heavily. The sharp incline in the slope of the curve as the age increases suggests that there are mechanisms that compensate for the age-related increase in PML, until a point (≈70 years) beyond which this compensation starts to fail.
The effect of age on tissue outcome is well established in animal models of focal cerebral ischemia. In a study of middle cerebral artery occlusion in rats, 30-month-old animals developed significantly larger volume of infarction as opposed to <17-month-old animals (41% versus 31% of the hemispheric volume). In another study in rats, there was a statistically significant 23% increase in infarct volume in older animals (20 to 27 versus 4 months old) when measured 7 days after stroke. In humans, the only study that a priori examined the effect of age on evolution of ischemic brain injury showed a trend toward earlier transition from decreasing to increasing ADC in older compared with younger patients. The ADC transition occurred at 13.4 hours after stroke onset in patients >66 years old and at 20.2 hours in patients <66 years old. In addition to earlier ADC transition, older patients had a slower rate of ADC pseudonormalization than younger patients. Given that ADC transition and pseudonormalization coincide with changes in the integrity of tissue architecture, these results suggest that the ischemic process including a variety of protective mechanisms and compensatory circuits differs according to age.

The mechanism by which aging is associated with increased tissue loss in human cerebral ischemia is currently not known. Aging causes a number of changes at the level of macrocirculation and microcirculation. Changes in macrocirculation include age-related cardiac dysfunction as well as increased burden of atherosclerosis in cerebral arteries. Age-related changes in the microcirculation are reduced capillary lumen diameter, increased tortuosity, vascular thickening, and impairment in cerebral autoregulation. Changes in macrocirculation and microcirculation can alter the tissue perfusion. Whether the influence of age on tissue outcome is dependent on age-related changes in tissue perfusion could be studied using MRI algorithms that predict tissue outcome as a voxel-by-voxel estimate of the risk of infarction. However, such algorithms require more patients to isolate the effect of age on tissue outcome as a continuous variable.

Age-dependent impairment in tissue handling of ischemic stress is supported by various lines of evidence. The aged brain is more susceptible to the excitotoxic damage. The sensitivity to excitotoxic amino acids increases with age, whereas their disposal from the synaptic cleft decreases. Aging is also associated with a slow accumulation of mitochondrial DNA mutations. There is a threshold of mutated mitochondrial DNA beyond which an age-related decrease of respiration and ATP–synthase activity could occur. Age-related respiratory chain dysfunction, in turn, leads to increased production of reactive oxygen species. Impaired cellular energetics and increased availability of oxygen free radicals might explain the link between impaired tolerance to ischemia and aging. The negative correlation between energy metabolism and life span in mammalian cells further support this concept. A multivoxel proton magnetic resonance spectroscopy study of 90 normal human brains in subjects between 4 and 88 years of age showed statistically significant decline in N-acetylaspartate/choline ratio with age in both gray and white matter. The reduction was in the range of 20% to 25% from 18.5-year-old to 88-year-old subjects, suggesting that mitochondrial metabolism declines with aging.

Experiments in animal models of ischemic stroke demonstrate that the development of infarction is dependent on degree and duration of ischemia. However, these experiments used animals of the same age, same gender, and similar risk factors (ie, spontaneously hypertensive rats). Our exploratory analyses in the current study suggest that age, as a means of “tissue factor” or “tissue capacity to handle ischemia,” is also important in determining the tissue outcome in humans. Further studies are needed to elucidate other biological factors and the interactions among them to find out why exactly each patient’s brain has different susceptibility to ischemic stress. Until that time, our data suggest that age needs to be carefully integrated into models to predict tissue outcome.

References


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