Is White Matter Involved in Patients Entered into Typical Trials of Neuroprotection?

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Background and Purpose—One of the reasons for the failure of trials of neuroprotection in stroke may be the lack of white matter (WM) protection. However, whether patients entered into typical neuroprotection trials have WM involved in the ischemic process is unknown. We studied patients who were enrolled in neuroprotection trials at our center and used a neuroimaging coregistration approach to determine whether final infarcts involved WM and, if so, in what proportion. We also aimed to provide the first in vivo volume distribution of gray matter (GM) and WM in normal stroke-aged brains. These were then superimposed on a probabilistic map of GM and WM, which was developed from age-matched normal controls in whom GM and WM volumes were assessed.

Methods—Patients enrolled in trials of neuroprotection had late computed tomography or magnetic resonance scans coregistered in standard stereotaxic coordinate space after segmentation of symptomatic cerebral infarcts. These were then superimposed on a probabilistic map of GM and WM, which was developed from age-matched normal controls in whom GM and WM volumes were assessed.

Results—Forty-two patients (mean age, 73.7 ± 10.5 years) were studied from 6 trials of neuroprotection. WM formed 41.7% of the brain volume in 37 control subjects (mean age, 73.5 ± 8.4 years). In the segmented infarcts, WM comprised a median of 49% (interquartile range, 36.5 to 77.9) of the infarct volume. Ninety-five percent of infarcts had some involvement of WM tracts.

Conclusions—WM occupies ≈42% by volume of the normal stroke-aged brain. Patients entered into typical trials of neuroprotection may have significant WM volumes involved in the ischemic process, thus providing a rationale for the development of neuroprotectants for this compartment. (Stroke. 2005;36:000-000.)

Key Words: imaging ■ neuroprotective agents ■ ischemia

There have been numerous trials of neuroprotectants in patients with ischemic stroke, but none have shown a definite benefit.1 Interestingly, in the recently concluded Intravenous Magnesium Efficacy in Stroke (IMAGES) trial using magnesium, an a priori subanalysis revealed a possible benefit for subcortical lacunar strokes.2 This raises the question as to whether neuroprotectants may have a differential effect on central white matter (WM) compared with gray matter (GM). There are inherent differences between these brain compartments; the ischemic cascade is dominated by glutamate toxicity in GM,3 whereas, in WM, ion exchange channels and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors are more important.4 The majority of neuroprotectants have been developed in small animal models, particularly the rat, in which ≈10% of brain volume is WM. In contrast, the proportion in human brain is ≈50%, although this is less certain in stroke-aged brains.5 Hence, the efficacy of most neuroprotectants is GM based with scant attention paid to WM effects.

This issue may not be important if neuroprotection trial patient populations have ischemic lesions involving mainly GM; this is possible, because most of the trial entry criteria favor patients with hemispheric stroke and cortical signs.2,6–8 We thought it important to establish this more certainly in a small sample of patients who were entered into trials of neuroprotection in our center by using neuroimaging coregistration techniques. Specifically, we wished to test the hypothesis that significant proportions of WM are involved in the ischemic process in patients entered into trials of neuroprotection, thus providing a rational for developing compounds, which may protect this compartment. We also wished to establish with more certainty the in vivo volume distribution of GM and WM in the human brain, particularly in stroke-aged patients.

Methods

All of the patients with ischemic stroke entered into neuroprotection trials at the Austin Hospital between April 1997 and June 2001 in whom cerebral infarcts were evident on computed tomography (CT) or MRI were considered. To provide a magnetic resonance (MR) template to allow GM and WM segmentation, age-matched, neurologically normal volunteers were recruited.
Imaging Protocol
All of the patients had a CT scan before therapy and repeated at 7 to 10 days. MRI scans were performed on a 1.5-T SIGNA GE or Siemens Magnetom 63. A MR probabilistic map was generated from controls using a 3D T1-weighted, radiofrequency-spoiled gradient echo sequence (echo time, 2.2 ms; repetition time, 10.5 ms; inversion time, 350 ms; flip angle, 20°; matrix size, 256×256; number of excitations, 1; field of view, 25 cm).

Image Registration, Segmentation, and Analysis
All of the patient and control images were register into standard stereotaxic coordinate space using automated image registration (AIR 3.0).9 All of the control standard space images were then classified into GM, WM, and cerebrospinal fluid using SPM 99 software. Each image was smoothed using a Gaussian kernel of 8 mm. These images were averaged to create a probabilistic map with each voxel classified using a threshold probability of 50%.

Symptomatic infarct areas (hypodense areas on CT and hyperintense areas on T2 with signal intensity obviously different to background for both white and gray compartments as agreed by 2 blinded independent observers) were manually segmented and registered with the probabilistic map (Figure 1).

Statistical Analyses
Nonparametric statistical analyses (Wilcoxon signed-rank and Kruskal-Wallis tests) were used to compare the compartmental distribution of infarct volumes among patients and between trials, respectively.

Results
Seventy-seven patients were entered into 5 neuroprotection trials during the study period. The trials were as follows: (1) Glycine Antagonist In Neuroprotection (GAIN), time window 6 hours, 8 patients, 19.0%; (2) IMAGES, time window 12 hours, 7 patients, 16.7%; (3) European-Australian Fiblast Acute Stroke Trial, time window 6 hours, 1 patient, 2.4%; (4) Potassium-Channel Opening Stroke Trial, time window 6 hours, 18 patients 42.9%; and (5) Acute Stroke Therapy by Inhibition of Neutrophils, time window 6 hours, 8 patients, 19.0%. Nine patients were excluded because no lesion was evident on imaging, and 16 were excluded because of corrupted images. The mean age of the 42 patients was 73.7±10.5 (±SD) years and the range was 42 to 88 years, 26 of whom were men and 16 of whom were women. Scan times were 2 to 95 days after stroke (mean, 25 days).

Thirty-seven control subjects (17 men and 20 women) of mean age 73.5±8.4 years (range, 60 to 90 years) had MR scans to generate the probabilistic map. The total brain volume was 1473±8.5 mL, GM volume was 724.5±43.8 mL (58.2±2.6%), and WM volume was 519.6±34.1 mL (41.8±2.6%) excluding cerebrospinal fluid, cerebellum, and brain stem (Figure 2).

The median total infarct volume was 19.6 mL (interquartile range [IQR] 3.2 to 103.6 mL) with 8.8 mL (IQR, 0.4 to 48.1 mL) in GM (51%; IQR, 22.1% to 63.4%) and 10.5 mL (2.0 to 37.5 mL) in WM (49%; IQR, 36.5% to 77.9%). By increasing the control map probability to 60%, WM infarct proportion changed to 64% and did not alter our conclusions. There was no significant difference in this distribution (P=0.392; Figure 2). If the 9 negative scans were considered to be WM, the median WM proportion was 60.2% (IQR, 42.5% to 97.1%); if all were considered GM, the proportion was 45.2% (IQR, 15.5% to 73.2%). Ninety-five percent of all patients had at least some WM involvement (Figure 3) or 96% if all 9 of the negative scans were WM infarcts and 78% if all were GM. Between trials, there was no significant difference in the median WM proportions, although the highest was seen in the GAIN trial (89.6%; P=0.138).

Figure 1. Comparison of GM and WM proportions in the brains of normal subjects of stroke-age brain, as well as of the infarcts of neuroprotection trial patients.

Figure 2. Distribution by rank of the proportion of infarcts in WM of each patient entered into trials of neuroprotection. BMS indicates Bristol-Meyer Squibb. Trial of potassium channel opening compound 204352 in which entry criteria included cortical signs. Others refers to all other trials of neuroprotection studied.
To assess the generalizability of our data, demographic details were compared with the published trials and found to be similar: mean age (current study, 73.7 years versus GAIN, 69.7 years; Fiblast, 69.5 years; and IMAGES, 70.3 years), hypertension (current study 56.1% versus GAIN, 65.7%; and IMAGES, 55%). However, in the current study, fewer had diabetes (9.7% versus GAIN, 20.4%; and IMAGES, 17.5%), but more had atrial fibrillation (39.0% versus GAIN, 29.7%; and IMAGES, 19.5%).

Discussion

We have shown that, in this small sample of ischemic stroke patients who have been entered into trials of neuroprotection, \( \approx 50\% \) of the ischemic process involves WM of the brain, and \( \approx 95\% \) have at least some involvement of WM tracts. This reinforces the view that neuroprotection for this compartment needs to be taken into consideration when clinical trials are being planned, because a lack of WM protection may be a contributing factor to the numerous failures so far. Although this small sample from 5 major (negative) trials of neuroprotection may not be truly representative of the total patients studied and needs to be confirmed using larger samples, the demographic data were not greatly different from the published trial results. The sample was also too small to detect real differences in the compartmental distribution of infarcts between trials, but needs to be examined in a larger study.

Although there have been a number of attempts to estimate the WM components of the normal human brain, these were either autopsy studies or imaging studies, which did not explicitly generate GM and WM proportions in stroke-aged individuals. Our finding, that 42% of brain tissue is WM, is the first in both GM and WM. Second, we need to establish efficacy in small animal models to test these in animal models where both compartments are adequately represented. Third, we need to consider phase II testing in humans with imaging outcome measures involving WM and GM. This may help eliminate compounds that are less likely to improve clinical outcomes.

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