Neuroglobin Expression in Ischemic Stroke

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Background and Purpose—We investigated whether neuroglobin, a neuronal protein that protects neurons from hypoxic–ischemic injury, is upregulated in ischemic stroke.

Methods—Neuroglobin immunoreactivity was measured in brain tissue from control subjects and patients with ischemic stroke.

Results—Neuroglobin was detected in several brain areas, and its expression was increased in the cortical peri-infarct region after stroke.

Conclusions—Ischemic stroke increases expression of the neuroprotective protein neuroglobin, suggesting neuroglobin may represent a novel target for stroke therapy. (Stroke. 2010;41:557-559.)

Key Words: neuroglobin ■ ischemia ■ neuroprotection

Neuroglobin (Ngb) is an oxygen-binding heme protein found in the brains of vertebrates, including humans.\(^1\) Ngb expression is increased in rodent brain neurons after hypoxia in vitro and focal ischemia in vivo,\(^2\) suggesting that Ngb may have a role in the response of the brain to hypoxic–ischemic injury. Additional evidence for such a role includes the observation that forced overexpression of Ngb reduces, whereas knockdown of Ngb expression exacerbates, hypoxic and ischemic neuronal damage in rodents.\(^2\)–\(^5\) Although human epidemiological studies have suggested a relationship between Ngb polymorphisms and risk for cerebrovascular disease,\(^6\) little is known about the relationship between acute stroke and Ngb expression. To investigate this question, we examined the expression of Ngb in normal human brain and in brain tissue from patients with a histopathologic diagnosis of ischemic cerebral infarction.

Materials and Methods

Control brain tissue was obtained at autopsy from patients (25 to 48 years of age) without neurological disease. Ischemic and adjacent nonischemic brain tissue was obtained from 6 patients (34 to 74 years of age) undergoing biopsy for possible ischemic stroke in whom the diagnosis was confirmed by histopathology. Additional data regarding the age, sex, nature and duration of symptoms, and location of infarcts have been published as part of another study.\(^7\) Informed consent was obtained, and the study was approved by the institutional research review board at Huashan Hospital of Fudan University, China. Western blotting with rabbit polyclonal anti-Ngb (1:500; Sigma) and mouse monoclonal anti-actin (1:2000; Sigma), single-label immunohistochemistry with rabbit polyclonal anti-Ngb (1:500; Sigma), and dual-label fluorescence immunohistochemistry with rabbit polyclonal anti-Ngb (1:200; Sigma), mouse monoclonal anti-neuronal nuclei (1:200; Chemicon) and mouse monoclonal anti–glial fibrillary acidic protein (1:150; Sigma) primary and fluorescein isothiocyanate–conjugated rat-absorbed donkey anti-rabbit polyclonal and rhodamine-conjugated donkey anti-goat (Jackson Immunoresearch; 1:200) secondary antibodies were performed as described previously.\(^2\)–\(^4\) In some cases, nuclei were counterstained with 4′,6-diamidino-2-phenylindole. Controls included omitting or preabsorbing primary or omitting secondary antibody. Fluorescence signals were detected with a Nikon E800 microscope, and results were recorded with a Magnifire digital camera (ChipCoolers). Slides were examined using an LSM 510 NLO Confocal Scanning System mounted on an Axiowert 200 inverted microscope (Carl Zeiss Ltd) equipped with a 2-photon Chameleon laser (Coherent Inc). Images were acquired using LSM 510 Imaging Software (Carl Zeiss Ltd).

Results

Ngb immunoreactivity, assessed by Western blotting, was detectable in normal human brain, with regional variations. Of 7 regions studied, Ngb levels were highest in cerebral cortex and caudatoputamen, intermediate in cerebellum, substantia nigra and medulla, and lowest in hippocampus and subventricular zone (Figure 1A). This resembles the distribution of human Ngb mRNA reported previously.\(^8\) Single-label immunohistochemistry with an anti-Ngb antibody showed that Ngb was expressed in cells with neuronal morphology (Figure 1B). This was confirmed by double-label fluorescence immunohistochemistry, which demonstrated a strong correlation between Ngb immunoreactivity and cellular expression of the neuronal marker neuronal nuclei (Figure 1C) but not the astroglial marker glial fibrillary acidic protein (Figure 1D).

Hematoxylin and eosin staining of biopsy specimens from patients with stroke showed regions of neuronal shrinkage and cell loss consistent with ischemic injury, surrounded by zones of hypercellularity reflecting infiltration of inflammatory cells, and adjacent normal tissue (Figure 2A). Ngb immunoreactivity in 6 of 6 patients was highest in the
intermediate, peri-infarct region (Figure 2B and 2C) and essentially absent from the infarct core, which is similar to the distribution of increased Ngb immunoreactivity after focal cerebral ischemia in rats. As observed in normal human brain, Ngb in brains of 6 of 6 patients with stroke was localized primarily to neurons, identified by staining for neuronal nuclei (Figure 2D) rather than glial fibrillary acidic protein–expressing astrocytes (Figure 2E). Thus, induction of neuronal Ngb expression appears to be one component of the response of the brain to ischemia in human stroke.

Discussion
The major finding of this study is that ischemic stroke leads to an increase in the expression of Ngb in the peri-infarct cerebral cortex compared with the adjacent normal brain and ischemic core. Ngb was discovered recently based on its homology to hemoglobin and myoglobin. Ngb is localized primarily to neurons and binds O₂, CO, and NO, but its physiological function is uncertain. However, the observation that Ngb expression is induced by hypoxia and ischemia is consistent with a role in neurocellular responses to these insults. The significance of increased Ngb expression in clinical stroke is unclear, but in rodents, overexpression of Ngb is associated with reduction of infarct size and improved functional outcome. Accordingly, Ngb might serve a similar neuroprotective role in humans. Although direct evidence for this is lacking, one study has shown an association between Ngb polymorphisms and susceptibility to stroke in the Southern Chinese Han population. Ngb can be detected in human cerebrospinal fluid, which might facilitate future clinical studies on the relationship between Ngb levels and stroke severity or outcome. Given the neuroprotective effect of Ngb in animal models of stroke and other neurological diseases, drugs that stimulate Ngb expression could be therapeutically useful in these disorders.
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Disclosures
None.

References
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