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Neuroglobin Expression in Human Arteriovenous Malformation and Intracerebral Hemorrhage

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Abstract

We reported previously that Notch signaling is activated in human arteriovenous malformations (AVMs) and that intracerebral hemorrhage (ICH) in humans is accompanied by increased neurogenesis. The former phenomenon may be involved in AVM pathogenesis and the latter in the brain's response to ICH-induced injury. Here we describe increased expression of the hypoxia-inducible neuroprotective protein, neuroglobin (Ngb), in neurons surrounding unruptured AVMs and in the perihematomal region adjacent to ICH. In these disorders, as in other clinical settings, such as ischemic stroke, AVM- and ICH-induced overexpression of Ngb may be stimulated by ischemic hypoxia and may help to constrain brain injury.

Keywords

Neuroglobin; Arteriovenous malformation; Intracerebral hemorrhage; Hypoxia; Ischemia

Introduction

Intracerebral hemorrhage (ICH) accounts for approximately 10–15% of strokes and is associated with high early mortality. Causes include head trauma, chronic hypertension, vascular malformations, amyloid angiopathy, bleeding into primary or metastatic tumors, coagulopathy and therapeutic anticoagulation [1]. Despite extensive clinical experience with ICH, its pathophysiology [2, 3] and optimal treatment [4, 5] are poorly defined.

One important class of lesions associated with ICH – vascular malformations of the brain, including arteriovenous malformations (AVMs) – are thought to arise during embryonic development, but typically present in middle age and appear to grow over time [3]. This suggests that the brain may have the opportunity to mount postnatal adaptive responses that limit tissue dysfunction, as is observed in other pathological processes, such as ischemia or tumor. By extension, it may be possible to intervene medically to check the growth of vascular malformations or mitigate their clinical sequelae by targeting these responses.

ICH has been considered to produce symptoms – typically altered consciousness and focal neurological deficits – through a combination of tissue destruction, mass effect and perhaps ischemia in the surrounding brain tissue. However, the role of perihematomal ischemia has been questioned by recent studies [2]. This may have important clinical implications, because the desire to avoid exacerbating ischemia has limited enthusiasm for reducing blood

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Conflict of interest statement

We declare that we have no conflict of interest.

pressure early in the course of ICH, despite the fact that some studies show a beneficial effect of blood pressure reduction [4].

In previous work related to AVM and ICH, we investigated mechanisms that could be involved in the pathophysiology and in the brain's response to these disorders. In one study, we examined the role of Notch signaling in human AVMs [6]. Notch1 signaling was activated in smooth muscle and endothelial cells of AVMs compared to normal cerebral blood vessels. Activated Notch1 (Notch intracellular domain, or NICD) was detected immunohistochemically in brain AVMs but not control vessels. Notch1 ligands (Jagged1 and Delta-like 4) and a downstream target (Hes1) were also overexpressed in AVMs. A functional role for Notch1 signaling in AVMs was supported by the finding that Notch1 activation in rats promoted angiogenesis, which could contribute to the development of AVMs.

In another study, we reported that neurogenesis, which is stimulated by a variety of experimental brain lesions and by cerebral ischemia in humans [7], was increased in the brains of patients with ICH [8]. This was manifested by the expression of neural stem or progenitor cell markers together with markers of cell proliferation in regions of the basal ganglia and parietal lobe surrounding brain hematomas. Thus, the brain may respond to ICH, as it does to infarction, with an increased capacity for generating new neurons. As in infarction, this may be a mechanism for moderating the extent of injury [9].

Here we report findings regarding the expression of neuroglobin (Ngb), a hypoxia-inducible neuroprotective protein, in the brains of patients with AVM or ICH. Ngb is expressed, predominantly in neurons, at low basal levels [10]. In response to hypoxia, ischemia or related insults, Ngb is transcriptionally activated [11]. Evidence for a neuroprotective role of Ngb includes the observations that infarct size and associated neurobehavioral abnormalities following occlusion of the middle cerebral artery are reduced in rodents that overexpress Ngb [12, 13], whereas knockdown of Ngb expression exacerbates these deficits [13]. Ngb expression is also increased in the vicinity of cerebral infarcts in humans [14]. However, the role of Ngb in cerebral responses to other vascular lesions has not been reported.

Materials and Methods

The provenance of brain specimens from and clinical features of patients with cerebral AVM and presumed hypertensive ICH have been described previously [6, 8]. All studies were conducted under protocols approved by local Institutional Research Review Boards. Single-label Ngb immunohistochemistry was conducted using 6-mm paraffin-embedded sections, which were deparaffinized with xylene and rehydrated with ethanol, following antigen retrieval with antigen unmasking solution (Vector Laboratories) according to the manufacturer's instructions. Rabbit polyclonal anti-Ngb (1:500; Sigma) was added in blocking buffer and incubated with sections at 4°C overnight. Sections were then washed with PBS and incubated with biotinylated anti-rabbit IgG for 1 h at room temperature. Avidin-biotin complex (Vector Elite; Vector Laboratories) and Ni solution (Vector Laboratories) were used to obtain a visible reaction product. A Nikon microscope and Nikon digital color camera were used to examine and photograph the slides, respectively.

For double-label immunohistochemistry, the primary antibodies used were rabbit polyclonal anti-Ngb (1:200; Sigma), mouse monoclonal anti-NeuN (1:200; Chemicon) and mouse monoclonal anti-GFAP (1:150; Sigma), and the secondary antibodies were FITC-conjugated anti-rabbit and rhodamine-conjugated anti-mouse IgG (1:200; Jackson ImmunoResearch). DAPI (Molecular Probes) was used to counterstain nuclei. Fluorescence signals were detected using an LSM 510 NLO Confocal Scanning System mounted on an Axiovert 200

inverted microscope equipped with a two-photon Chameleon laser. Controls included omitting primary or secondary antibody or preabsorbing primary antibody.

Results

Basal expression of Ngb immunoreactivity in normal human brain was low (Fig. 1), as noted previously [14]. In contrast, immunoreactivity was higher in brain parenchyma adjacent to AVM and ICH. Within these regions, Ngb was co-localized with the neuronal marker NeuN, but not the astroglial marker GFAP, consistent with its preferential expression in neurons [10].

Discussion

These results indicate that neuronal Ngb expression is induced in brain tissue surrounding human AVM and ICH, as reported before for ischemic stroke [14]. In the case of ischemic stroke, increased Ngb expression in penumbral brain tissue has been attributed to ischemic hypoxia, but how AVM and ICH induce Ngb is less obvious.

Brain parenchyma adjacent to AVMs may show reactive gliosis, necrosis and hemosiderin deposition, which are thought to result from some combination of increased pressure, thrombosis, venous congestion, ischemia and bleeding [15]. Astrocytic expression of vascular endothelial growth factor (VEGF), a hypoxia-inducible angiogenic factor, is increased in surgical specimens from some patients with AVM [16], suggesting that hypoxia in the surrounding tissue might be responsible for inducing Ngb expression as well.

The role of perihematoma ischemia in the pathophysiology of ICH is controversial [2]. Neuronal expression of hypoxia-inducible factor-1 α is increased in the perihematoma region after experimental ICH in rats [17], consistent with ischemic hypoxia. Magnetic resonance imaging in patients with ICH suggests the existence of an ischemic penumbra around hematomas in some [18] but not other [19] studies, perhaps reflecting clinical heterogeneity [20]. Alternative explanations for perihematoma tissue dysfunction include impaired mitochondrial respiration [21] and iron-induced oxidative injury [22], but whether these processes rather than ischemic hypoxia could account for induction of Ngb is unclear.

We conclude that both unruptured AVM and presumed hypertensive ICH induce expression of Ngb in neurons located in the surrounding tissue, but the mechanism through which this occurs is unknown. It is also uncertain whether, as appears to be the case for ischemia stroke [12, 13], AVM- or ICH-induced overexpression of Ngb helps to mitigate the resulting tissue injury and dysfunction.

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Fig. 1.

Ngf expression in normal human brain, AVM and ICH. Low basal expression of Ngf immunoreactivity in normal human brain (a) is increased in brain parenchyma adjacent to AVM (b) and ICH (c), each shown at low (left) and high (right) magnification. Double-label immunohistochemistry shows co-localization of Ngf with the neuronal marker NeuN (d), but not the astroglial marker GFAP (e).