Plasma-Type Gelsolin Is Decreased in Human Blood and Cerebrospinal Fluid After Subarachnoid Hemorrhage

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Background and Purpose—Subarachnoid hemorrhage (SAH) pathophysiology involves neurovascular proteolysis and inflammation. How these 2 phenomena are related remains unclear. We hypothesize that matrix metalloproteinases (MMPs) mediate the depletion of anti-inflammatory plasma-type gelsolin (pGSN).

Methods—We enrolled 42 consecutive SAH subjects and sampled cerebrospinal fluid (CSF) and blood on post-SAH Days 2 to 3, 4 to 5, 6 to 7, and 10 to 14. Control subjects were 20 consecutive non-SAH hydrocephalus patients with lumbar drains. Enzyme-linked immunosorbent assay, Western blotting, and zymography were used to quantify pGSN and MMP-9.

Results—In CSF, pGSN was lower in SAH compared with control subjects on post-SAH Days 2 to 3 ($P = 0.0007$), 4 to 5 ($P = 0.041$), and 10 to 14 ($P = 0.007$). In blood, pGSN decreased over time ($P = 0.001$) and was lower in SAH compared with control subjects on post-SAH Days 4 to 5 ($P = 0.037$), 6 to 7 ($P = 0.006$), and 10 to 14 ($P = 0.006$). Western blots demonstrated that SAH CSF had novel bands at 52 and 46 kDa, representing cleaved pGSN fragments. Gelatin zymography showed that CSF MMP-9 was elevated in SAH compared with control subjects. Higher CSF MMP-9 correlated with lower CSF pGSN on post-SAH Day 7 ($r = -0.38; P = 0.05$).

Conclusions—SAH is associated with decreased CSF and blood pGSN and elevated CSF MMP-9. Novel cleaved pGSN fragments are present in CSF of SAH subjects, consistent with pGSN cleavage by MMPs. Because pGSN is known to inhibit inflammatory mediators, these findings suggest that MMPs may reduce pGSN and exacerbate inflammation after SAH. Further studies are warranted to investigate the mechanisms underlying MMP–pGSN signaling in SAH. (Stroke. 2011;42:3624-3627.)

Key Words: biomarker □ gelsolin □ matrix metalloproteinases □ subarachnoid hemorrhage

Cerebrovascular inflammation after subarachnoid hemorrhage (SAH) may mediate vasospasm and poor outcomes.¹ Plasma-type-gelsolin (pGSN) scavenges circulating actin and inhibits peptide and lipid mediators of inflammation.² Total gelsolin depletion increases neuronal death after focal cerebral ischemia in mice.³,⁴ Clinically, blood pGSN is decreased in critical illness,⁵ and lower blood pGSN predicts trauma and critical illness mortality.⁶ Cerebrospinal fluid (CSF) pGSN is reduced in neuroinflammatory diseases such as multiple sclerosis.⁷ Matrix metalloproteinases (MMPs),⁸ which are elevated in CSF of patients with SAH,⁹ cleave pGSN. We hypothesize that blood and CSF pGSN depletion occurs in critically ill patients with SAH and that this may in part be caused by elevated MMPs.

Methods

Consecutive consenting patients with SAH (n=42) and patients with hydrocephalus without brain injury (n=20) were recruited as case and control subjects. SAH subjects are included if they are ≥18 years, within 96 hours of spontaneous SAH, and have an external ventricular drain placed for clinical indication. Blood and CSF were collected on post-SAH Days 2 to 3, 4 to 5, 6 to 7, and 10 to 14. Patients with secondary SAH, pregnancy, end-stage renal or hepatic disease, intracranial malignancies, or infectious meningitis were excluded. CSF was obtained from existing external ventricular drain or lumbar drains. All subjects were consented following Institutional Review Board-approved protocols.

Blood and CSF samples were immediately centrifuged (3900 rpm, 15 minutes), aliquoted, and stored at −80°C. pGSN was quantified by enzyme-linked immunosorbent assay (KSB BioTechnology). Western blots were performed using antibodies specific to carboxyl and amino terminals of human pGSN. MMP-9 was assessed by

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Results

Study subject demographics are summarized in the Table. Blood pGSN levels in SAH (2236–3737 U/L; SD = 1395–2459 U/L) were significantly lower than control subjects (5792 U/L; SD = 4535 U/L; Figure 1A). Furthermore, blood pGSN levels on post-SAH Days 5 to 14 were significantly lower than initial levels on Days 2 to 3 ($P = 0.002$), and blood pGSN levels decreased over time ($P = 0.001$).

pGSN was detectable in CSF from both SAH and control subjects with CSF pGSN approximately 10-fold lower than blood pGSN. CSF pGSN in SAH (92–125 U/L) was significantly lower than control subjects (148 U/L) on all post-SAH days except for post-SAH Days 6 to 7 (Figure 1B). CSF pGSN levels were not associated with angiographic vasospasm.

Western blot of SAH CSF showed cleaved pGSN bands at 52 and 46 kDa that were not seen in controls (Figure 2A). The molecular weights of these cleaved bands are similar to those from in vitro MMP digestion of pGSN. Gel zymography of CSF from the same subjects showed elevation of MMP-9 but not MMP-2 after SAH (Figure 2B). Higher CSF MMP-9 correlated with lower CSF pGSN on post-SAH Day 7 ($r = -0.38; P = 0.05$; Figure 3).

Discussion

In this study, we explored blood and CSF pGSN as potential biomarkers linking neurovascular proteolysis with inflammation after SAH. We report for the first time that blood pGSN is reduced in SAH, like in sepsis and trauma. We also found CSF pGSN to be reduced in SAH compared with control subjects. Interestingly, novel CSF pGSN fragments were detected in SAH but not in control subjects, suggesting possible disease-specific pGSN proteolytic cleavage in CSF space. Elevated MMP-9 after SAH and the inverse correlation between MMP-9 and pGSN levels in CSF suggest that
MMP-9 may be 1 of the neurovascular proteases responsible for pGSN cleavage.

Taken together, our findings may provide a mechanistic link between neurovascular proteolysis and inflammation after SAH. However, this proof-of-concept study has several caveats. First, our small sample size limits power to detect small subgroup differences. Because critical illness in general may lower pGSN, we cannot rule out the possibility that changes in blood pGSN here may reflect nonspecific reaction to systemic illness in SAH. Second, our control subjects have no acute brain injuries, but we cannot unequivocally exclude other brain pathology (eg, Alzheimer) that might influence CSF pGSN. In addition, control CSFs were sampled through a lumbar drain, whereas SAH CSFs were sampled from an external ventricular drain. Recent CSF proteomic studies suggest that there is no clear ventriculolumbar gradient in low-molecular-weight CSF proteins, but we cannot exclude the possibility that different CSF sampling routes may affect our results. Third, we only explored MMP-2 and MMP-9. Our finding of elevated MMP-9 is consistent with experimental data, in which MMP-9 contributes to SAH pathophysiology. However, other MMPs and their inhibitors may also play a role. Finally, although this is the first report of pGSN cleavage in CSF, future effort is required to identify and quantify these novel pGSN fragments in patients with SAH.

SAH is a challenging problem involving parallel mechanisms of neurovascular proteolysis and inflammation. Our study points to a novel link between elevations in MMP-9 and reduction of the putative anti-inflammatory mediator pGSN. Blood and CSF pGSN and MMP-9 may be potential biomarkers and therapeutic targets for SAH. Larger studies with simultaneous quantification of pGSN, pGSN fragments, and other MMPs are warranted.

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Disclosures

P.S.L. is coinventor on patents filed by Brigham and Women’s Hospital involving therapeutic use of pGSN.

References

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