Cerebrospinal Fluid Tenascin-C Increases Preceding the Development of Chronic Shunt-Dependent Hydrocephalus After Subarachnoid Hemorrhage

Hidenori Suzuki, MD, PhD; Noriaki Kinoshita, PhD; Kyoko Imanaka-Yoshida, MD, PhD; Toshimichi Yoshida, MD, PhD; Waro Taki, MD, PhD

Background and Purpose—The possible cause of chronic hydrocephalus after subarachnoid hemorrhage (SAH) has been reported to be meningeal fibrosis. We examined whether the induction of tenascin-C (TN-C), an extracellular matrix glycoprotein known to promote tissue fibrosis, was associated with chronic hydrocephalus after SAH.

Methods—We prospectively measured cerebrospinal fluid TN-C levels in 7 control patients with unruptured cerebral aneurysms and in 29 consecutive patients with aneurysmal SAH on days 1 to 12.

Results—Cerebrospinal fluid TN-C levels were less than the diagnostic threshold level in control patients but markedly increased after SAH. Higher TN-C levels were observed in patients with more severe SAH on admission CT, ventricular drainage for acute obstructive hydrocephalus, and a worse outcome. Independent of these factors, however, cerebrospinal fluid TN-C levels were significantly higher in patients with than without subsequent chronic shunt-dependent hydrocephalus on days 1 to 9.

Conclusions—These findings suggest the possible involvement of TN-C in the development of chronic hydrocephalus after SAH and encourage further studies. (Stroke. 2008;39:000-000.)

Key Words: cerebrospinal fluid ■ extracellular matrix ■ hydrocephalus ■ subarachnoid hemorrhage ■ tenascin-C

Chronic communicating hydrocephalus that requires cerebrospinal fluid (CSF) shunting commonly occurs after aneurysmal subarachnoid hemorrhage (SAH). Although the cause is still uncertain, previous studies suggested that proliferation of arachnoid cells and leptomeningeal fibrosis, triggered by an inflammatory reaction or blood clotting products, may impair CSF flow through the arachnoid villi, resulting in hydrocephalus. Alternative pathophysiologies may also be involved in the development of chronic hydrocephalus after SAH, because it is frequently associated with poor neurological outcomes that are not completely reversed after CSF shunting.

Tenascin-C (TN-C), an extracellular matrix glycoprotein, appears in adult tissues undergoing tissue remodeling, for example, in inflammation and wound healing. TN-C is reported to be induced in the CSF after SAH and also to promote cell proliferation and fibrosis. We therefore speculate that TN-C may be involved in the occurrence of chronic hydrocephalus after SAH. This study evaluated whether CSF TN-C levels change in association with the occurrence of chronic shunt-dependent hydrocephalus after SAH.

Subjects and Methods
This prospective study included 29 consecutive patients with aneurysmal SAH who underwent CSF drainage concomitant with clipping or coiling for ruptured aneurysms within 24 hours of initial onset. The study was approved by the Institutional Ethics Committee, and all patients or their relatives gave written informed consent. Exclusion criteria were any angiographic or surgical complications, death within 14 days of onset irrespective of cause and concomitant inflammatory, and malignant or other diseases that can affect TN-C metabolism. The Fisher group 4 on admission CT was defined as SAH of Fisher CT group 3 associated with intraventricular hemorrhage in this study.

Ventricular drainage was established to control acute obstructive hydrocephalus attributable to hematoma. Cisternal (after clipping) and spinal (after coiling except for 1 clipped patient) drainage, 150 to 250 mL per day, was placed to promote SAH clearance for 7 to 12 days. A total of 99 CSF samples were obtained from cisternal (61 samples), spinal (26 samples), or ventricular (12 samples) drains on days 1 to 12 after onset. For the analyses, 4 sampling groups were chosen according to the time points at which the samples had been obtained: days 1 to 3 (26 samples), 4 to 6 (28 samples), 7 to 9 (26 samples), and 10 to 12 (19 samples). Two to 4 samples falling into different time groups were obtained from 27 patients. Control samples were obtained via lumbar tap from 7 patients with unruptured cerebral aneurysms. TN-C concentrations were determined using an enzyme-linked immunosorbent assay kit for human TN-C high molecular weight variants (IBL; Takasaki).

Cerebrospinal fluid TN-C was measured in 39 cases using an enzyme-linked immunosorbent assay kit for human TN-C high molecular weight variants (IBL; Takasaki). Sequential samples were obtained after SAH in 39 of 47 patients. Sequential CSF samples were obtained: days 1 to 3 (26 samples), 4 to 6 (28 samples), 7 to 9 (26 samples), and 10 to 12 (19 samples). Two to 4 samples falling into different time groups were obtained from 27 patients. Control samples were obtained via lumbar tap from 7 patients with unruptured cerebral aneurysms. TN-C concentrations were determined using an enzyme-linked immunosorbent assay kit for human TN-C high molecular weight variants (IBL; Takasaki).

Cerebrospinal fluid TN-C was measured in 39 cases using an enzyme-linked immunosorbent assay kit for human TN-C high molecular weight variants (IBL; Takasaki). Sequential samples were obtained after SAH in 39 of 47 patients. Sequential CSF samples were obtained: days 1 to 3 (26 samples), 4 to 6 (28 samples), 7 to 9 (26 samples), and 10 to 12 (19 samples). Two to 4 samples falling into different time groups were obtained from 27 patients. Control samples were obtained via lumbar tap from 7 patients with unruptured cerebral aneurysms. TN-C concentrations were determined using an enzyme-linked immunosorbent assay kit for human TN-C high molecular weight variants (IBL; Takasaki).
Table. Clinical Features in Patients With and Without Chronic Shunt-Dependent Hydrocephalus After SAH

<table>
<thead>
<tr>
<th>Feature</th>
<th>Without Hydrocephalus</th>
<th>With Hydrocephalus</th>
</tr>
</thead>
<tbody>
<tr>
<td>N of patients</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Age yr, mean (SEM)</td>
<td>64.4 (3.7)</td>
<td>59.8 (3.4)</td>
</tr>
<tr>
<td>Man/woman</td>
<td>2/14</td>
<td>6/7</td>
</tr>
<tr>
<td>WFNS grade at admission, I–III/IV–V</td>
<td>12/4</td>
<td>6/7</td>
</tr>
<tr>
<td>Fisher group at admission, 3/4</td>
<td>12/4</td>
<td>9/4</td>
</tr>
<tr>
<td>Ventricular dilatation at admission, Presence/absence</td>
<td>4/12</td>
<td>9/4*</td>
</tr>
<tr>
<td>Aneurysm location, Anterior/posterior circulation</td>
<td>15/1</td>
<td>12/1</td>
</tr>
<tr>
<td>Coil/clip</td>
<td>3/13</td>
<td>4/9</td>
</tr>
<tr>
<td>Drainage, lumbar/cisternal/ventricular, Presence/absence</td>
<td>4/12/0</td>
<td>3/7/3*</td>
</tr>
<tr>
<td>Symptomatic vasospasm, Presence/absence</td>
<td>5/11</td>
<td>8/5</td>
</tr>
<tr>
<td>GOS at 3 mon, GR&amp;MD/SD&amp;PVS</td>
<td>15/1</td>
<td>5/8†</td>
</tr>
</tbody>
</table>

GOS indicates Glasgow outcome scale; GR, good recovery; MD, moderate disability; PVS, persistent vegetative state; SD, severe disability; WFNS, World Federation of Neurosurgical Societies. χ² test, *P<0.05, †P<0.005 vs patients without hydrocephalus.

Statistics
All values are expressed as mean (SEM). Unpaired t test and χ² test were used as appropriate, and comparisons among ≥3 groups were assessed using repeated-measures analysis of variance (ANOVA) with Scheffe correction. P<0.05 was considered statistically significant.

Results
Clinical Features
Thirteen patients underwent ventriculoperitoneal shunting at 16 to 135 days after SAH. Shunted patients were associated with more frequent ventricular dilatation at admission, need of ventricular drainage, and a worse outcome at 3 months after SAH in comparison with nonshunted patients (Table). A worse outcome was the only significant factor (P<0.005), except for patients with ventricular drainage.

TN-C
CSF TN-C levels of control patients were less than the diagnostic threshold level of the method (<1.5 ng/mL). After SAH, CSF TN-C levels increased markedly and then reduced. No correlation was observed between age and TN-C levels (r=0.08; P=0.42), and TN-C levels were not different between men and women (P=0.58, unpaired t test). The CSF TN-C levels from ventricular drains, with which all patients eventually underwent shunting, were significantly higher than from cisternal or spinal drains. The TN-C levels from cisternal and spinal drains were not different (Figure 1A). Because patients with ventricular drains had acutely developed hydrocephalus and our aim was to assess the role of TN-C in the development of chronic hydrocephalus, for the sake of clarity, we did not take those data into account in further analysis.

The CSF TN-C levels from cisternal and spinal drains were significantly higher in patients with than without subsequent chronic hydrocephalus on days 1 to 9 (Figure 1B). The TN-C levels were not different among the clinical grades at admission (P=0.057, ANOVA), whereas higher values were associated with a worse outcome (Figure 2A), especially in shunted patients with severe disability and a persistent vegetative state (P<0.0001 vs the remaining patients, unpaired t test). The TN-C levels were also higher in patients of Fisher CT group 4 than of Fisher CT group 3 (P<0.01, unpaired t test), and were significantly higher in patients with than without chronic hydrocephalus, irrespective of the Fisher CT group at admission (Figure 2B). Patients with higher CSF TN-C levels underwent shunting earlier (r=0.48; P<0.05).

Discussion
This study showed that CSF TN-C levels became elevated in patients with SAH as compared to unruptured aneurysm patients. The highest increase in concentration occurred in the first 3 days, followed by a decrease over time, but on day 12,
the CSF concentration was still above the normal limit. Notably, patients who eventually had chronic shunt-dependent hydrocephalus had significantly higher CSF TN-C levels than nonshunted patients in the acute stage of SAH, irrespective of severity of SAH on CT, although there was a relationship between severity of SAH on admission CT and TN-C levels.

Compromised CSF reabsorption by cell proliferation and fibrosis in the arachnoid villi is the most plausible cause of chronic hydrocephalus after SAH. A previous study showed that meningeal collagen synthesis was induced within the first 2 to 3 days after SAH, when TN-C was prominently induced in the CSF in this study. Because TN-C is reported to regulate cell phenotype and promote the migration and proliferation of myofibroblasts and tissue fibrosis, TN-C induction may cause the proliferation of leptomeningeal cells and fibrosis observed after SAH.

Another finding of this study was that the more severe the disability present 3 months after SAH, the higher the CSF TN-C levels were on days 1 to 12, especially in eventually shunted patients. Although one possible explanation is that severe fibrosis along CSF pathways may interfere with brain function, it will be useful to examine whether TN-C contributes to a poor neurological status by directly altering the brain parenchymal and extracellular matrix properties.

This study does not show that TN-C has a causal relation to impaired CSF circulation and a worse outcome. However, the findings encourage further investigations to elucidate the role of TN-C in the pathophysiology of chronic hydrocephalus after SAH.

Sources of Funding
This work was supported in part by a grant-in-aid for Scientific Research (C) from the Japanese Ministry of Education, Culture, Sports, Science, and Technology to Dr Suzuki.

Disclosures
None.

References
Cerebrospinal Fluid Tenascin-C Increases Preceding the Development of Chronic Shunt-Dependent Hydrocephalus After Subarachnoid Hemorrhage
Hidenori Suzuki, Noriaki Kinoshita, Kyoko Imanaka-Yoshida, Toshimichi Yoshida and Waro Taki

Stroke. published online March 6, 2008;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/early/2008/03/06/STROKEAHA.107.505735.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/