Elevation of Transforming Growth Factor-β1 Level in Cerebrospinal Fluid of Patients With Communicating Hydrocephalus After Subarachnoid Hemorrhage

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Background and Purpose Transforming growth factor-β1 (TGF-β1) is a multifunctional polypeptide that controls the production of extracellular matrix protein. Platelets store a large quantity of TGF-β1, which is released at hemorrhage. We recently reported that human recombinant TGF-β1 induces communicating hydrocephalus in mice. The aim of this study was to determine whether TGF-β1 is related to the development of communicating hydrocephalus after subarachnoid hemorrhage (SAH).

Methods TGF-β1 in the cerebrospinal fluid of 24 patients with SAH was measured with enzyme-linked immunosorbent assay. The levels were compared between hydrocephalic and nonhydrocephalic groups. Western blot analysis was performed to determine active TGF-β1 in the cerebrospinal fluid.

Results TGF-β1 rapidly decreased from the onset of SAH. The level of TGF-β1 of 13 patients showing ventricular dilatation with periventricular low density on computed tomographic scan was 1.07±0.37 ng/mL on days 12 through 14, which was significantly higher than 0.52±0.21 ng/mL in patients without ventricular dilatation (P<.02). Furthermore, the TGF-β1 level of patients who had undergone ventriculoperitoneal shunt (n=11) was 1.11±0.09 ng/mL on days 12 through 14, which was also higher than the level of the nonshunt group (n=13) (0.56±0.22 ng/mL; P<.01). A 25-kD band was demonstrated by Western blot analysis in the cerebrospinal fluid of a patient with SAH.

Conclusions Our results strongly suggest that TGF-β1 plays an important role in generating communicating hydrocephalus after SAH. (Stroke. 1994;25:1400-1404.)

Key Words • cerebrospinal fluid • hydrocephalus • subarachnoid hemorrhage • transforming growth factors

Approximately one quarter of patients with subarachnoid hemorrhage (SAH) suffer dementia, gait disturbance, and urinary incontinence within a few months of the ictus. Although the computed tomographic (CT) cisternogram of such symptomatic patients reveals ventricular dilatation and reflux of contrast material, their cerebrospinal fluid pressure is usually normal. In evaluating these patients, the concept of normal pressure hydrocephalus was first introduced by Hakim and Adams.1 Despite the report that fibrosis and arachnoidal-pial adhesion in the subarachnoid space, especially around arachnoid villi, may disturb cerebrospinal fluid circulation, the generating mechanism has not been clarified.2,3

Transforming growth factor-β1 (TGF-β1) is a 25-kD multifunctional polypeptide that plays an important role in fibrosis of granulation tissue as seen in wound healing4,5 and in some fibrotic diseases such as liver cirrhosis,6 pulmonary fibrosis,7 and glomerulonephritis.8 Recently we found that human recombinant TGF-β1 induced communicating hydrocephalus in mice by intrathecal injection.9 It is important to determine whether the TGF-β1 is contained in active form in the cerebrospinal fluid and develops communicating hydrocephalus in patients with SAH.

In this study we measured the TGF-β1 levels in the cerebrospinal fluid of patients with SAH in relation to the CT findings and the clinical course. We also studied by Western blot analysis whether TGF-β1 exists in active form in cerebrospinal fluid.

Subjects and Methods

Patient Selection

The Table shows clinical data of 24 patients with SAH. Patients ranged in age from 39 to 78 years (mean, 61.2 years) and included 9 men and 15 women. SAH was caused by ruptured cerebral aneurysms in all patients except 1, in whom it was of unknown origin. Twenty-three patients were subjected to clipping surgery for the ruptured aneurysms and placement of cisternal drainage. The Hounsfield numbers of basal cisternal clots at the onset of SAH ranged from 31 to 66 (mean, 51.2±10.8).

According to Fisher's classification,9 5 patients were group 2, 14 were group 3, and 3 were group 4 on the initial CT scan. Thirteen of the 24 patients developed ventricular dilatation with periventricular low density on the follow-up CT scan taken between 0 and 80 days after SAH; 11 of the 13 patients exhibited dementia, urinary incontinence, and gait disturbance. The 11 patients were diagnosed as having communicating hydrocephalus and underwent ventriculoperitoneal (VP) shunt between days 22 and 88. Our criteria for selection of VP shunt were basically the same as those commonly used, which include ventricular dilatation and periventricular low density on the CT scan and characteristic symptoms such as dementia, urinary incontinence, and gait disturbance.

Table

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Fisher's Classification</th>
<th>Follow-up CT Scan</th>
<th>VP Shunt</th>
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Clinical Summary of 24 Patients With Subarachnoid Hemorrhage

Pt | Age, y | Sex | Fisher | CT No. | PVL | VP Shunt | Result of Shunt
--- | --- | --- | --- | --- | --- | --- | ---
1 | 71 | F | 2 | ... | + | + | Effective
2 | 53 | M | 3 | ... | + | + | Effective
3 | 58 | M | 3 | 61 | + | + | Effective
4 | 62 | M | 3 | 58 | + | + | Effective
5 | 66 | M | 3 | 66 | + | + | Effective
6 | 70 | F | 3 | 56 | + | + | Effective
7 | 75 | F | 3 | 46 | + | + | Effective
8 | 75 | F | 3 | 63 | + | + | Effective
9 | 78 | F | 3 | ... | + | + | Effective
10 | 52 | F | 4 | ... | + | + | Effective
11 | 63 | F | 4 | ... | + | + | Effective
12 | 66 | F | 3 | 47 | + | - | ... 
13 | 73 | F | 4 | ... | + | - | ... 
14 | 42 | F | 2 | 45 | - | - | ... 
15 | 56 | M | 2 | 31 | - | - | ... 
16 | 59 | F | 2 | ... | - | - | ... 
17 | 66 | F | 2 | ... | - | - | ... 
18 | 76 | F | 2 | 32 | - | - | ... 
19 | 39 | M | 3 | 54 | - | - | ... 
20 | 45 | M | 3 | ... | - | - | ... 
21 | 47 | F | 3 | 55 | - | - | ... 
22 | 52 | M | 3 | 52 | - | - | ... 
23 | 61 | M | 3 | ... | - | - | ... 
24 | 63 | F | 4 | ... | - | - | ... 

Pt indicates patient; Fisher, Fisher's classification on initial computed tomographic (CT) scan; CT No., Hounsfield number of basal cisternal clots on initial CT scan; PVL, periventricular low density area on follow-up CT scan; and VP, ventriculoperitoneal.

Cerebrospinal Fluid

Eighty-three samples of cerebrospinal fluid from the 24 patients were obtained through cisternal drainage from day 0 to day 17 after onset. In patients without cisternal drainage or after removal of the drain, the fluid was obtained by lumbar puncture. The collected samples were centrifuged at 3000 rpm for 20 minutes. The supernatant was passed through a 0.22-μm filter and stored at −20°C.

TGF-β1 Assay

To activate TGF-β1, all the samples were dialyzed against 1N acetic acid and then against phosphate-buffered saline (pH 7.4) two times. The concentration of TGF-β1 was measured with a TGF-β1 enzyme-linked immunosorbent assay kit (King Jozo Corp).

Western Blot Analysis

Three samples of cerebrospinal fluid were collected from a patient at days 0, 1, and 16 after SAH. They were used for a Western blot analysis without acidification. Sample buffer including 2-mercaptoethanol was added to an equal volume of the sample and heated for 5 minutes with boiling water. The samples were run on a 10% sodium dodecyl sulfate–polyacrylamide gel for detection of TGF-β1. The gel was electroblotted onto a nitrocellulose membrane (Immobilon-P, Milipore). The membrane was incubated with tris(hydroxymethyl)aminomethane-HCl buffer (pH 7.2) containing bovine serum albumin, followed by incubation with polyclonal anti-TGF-β1 antibody (R&D). Then the membrane was incubated with biotin-labeled horse anti-rabbit IgG incubated with alkaline phosphatase–conjugated avidin. Finally, color reagents were added to the membrane to develop a colorimetric reaction. We used 2.5 ng of human recombinant TGF-β1 as a control.

Statistical Analysis

The Welch t test was used to determine statistical significance; P<.05 was considered significant.

Results

TGF-β1 in Cerebrospinal Fluid

Relation to Total Protein

Total protein levels in cerebrospinal fluid within 17 days after SAH ranged from 36 to 337 mg/dL. Although there was no relation between total protein concentration and days after SAH, TGF-β1 and total protein levels were well related (r=.60, P<.001) (Fig 1).

Relation to Initial CT Findings

To determine whether the volume of SAH is related to TGF-β1 content in the cerebrospinal fluid, we divided the patients into two groups according to the Hounsfield number of the basal cistern on the initial CT scan and compared the time course of TGF-β1 content between the two groups. As a result, the TGF-β1 concentration of patients with higher density (Hounsfield number ≥55) of the basal cisternal clots on the
Fra 1. Line graph shows relation between total protein (TP) and transforming growth factor-β1 (TGF-β1 [TGF-β]) concentration in cerebrospinal fluid of patients with subarachnoid hemorrhage. Twenty-six cerebrospinal fluid samples were obtained from 13 patients. Shown are 95% confidence bands for the true mean of y. TGF-β1 and total protein were well related (r=.60, P<.001).

initial CT scan was not different from that of those with lower density (Hounsfield number <55) (Fig 2).

We also compared the TGF-β1 content between different combinations of Fisher's groups. TGF-β1 level was not related to Fisher's classification in any combination. Fig 3 shows the comparison of TGF-β1 levels between the patients of Fisher's group 2 and group 3.

Relation to Ventricular Dilatation

Thirteen of 24 patients in our series developed ventricular dilatation with periventricular low density. TGF-β1 content in the cerebrospinal fluid of the 13 patients was compared with TGF-β1 content in those without ventricular dilatation. The mean±SD values of TGF-β1 of the two groups on days 3 through 5 were 1.93±0.63 ng/mL and 1.61±1.03 ng/mL, respectively. These values rapidly decreased to 0.88±0.27 ng/mL and 0.83±0.32 ng/mL, respectively, on days 6 through 9. During this period there was no statistical difference between the two groups. TGF-β1 levels in patients with ventricular dilatation on days 9 through 11, 12 through 14, and 15 through 17 were 1.06±0.41 ng/mL, 1.07±0.37 ng/mL, and 1.05±0.44 ng/mL, respectively; TGF-β1 levels in patients without ventricular dilatation were 0.67±0.24 ng/mL, 0.52±0.21 ng/mL, and 0.68±0.20 ng/mL, respectively. The TGF-β1 level on days 9 through 17 of the patients with ventricular dilatation stayed higher than that of the group without ventricular dilatation; the difference was significant on days 12 through 14 (P<.02) (Fig 4).

Relation to Clinical Symptoms

To determine whether TGF-β1 in the cerebrospinal fluid of patients with communicating hydrocephalus is higher than that of those without hydrocephalus, TGF-β1 levels in cerebrospinal fluid of patients who received VP shunt operation and those who did not were compared. In the VP shunt group, improvements in clinical symptoms were seen in all patients. TGF-β1 levels of the VP shunt group versus the non-VP shunt group were 1.96±0.68 ng/mL versus 1.64±0.94 ng/mL on days 3 through 5 and 0.88±0.29 ng/mL versus 0.83±0.31 ng/mL on days 6 through 8, respectively. The patterns of rapidly decreasing TGF-β1 of both groups were similar, and there was no statistical difference between them during this period. However, the TGF-β1 levels of the VP shunt group on days 9 through 17 were significantly higher than those of the non-VP shunt group.
Active Form of TGF-β1 in Cerebrospinal Fluid

Western blot analysis indicated that a 25-kD band of the active form of TGF-β1 was recognized in the day 0 sample (Fig 6).

Discussion

Communicating hydrocephalus is now a well-recognized complication of SAH. Many authors suspect that fibrosis and arachnoid-pial adhesion in the subarachnoid space, especially arachnoid villi, may disturb cerebrospinal fluid absorption.2,3,11 Thickened arachnoid and proliferative fibrosis in the subarachnoid space were also demonstrated by scanning electron microscopic observation.3 However, the exact generating mechanism has not been clarified.

TGF-β was originally discovered as a factor to support the anchorage-independent growth of normal rat kidney cell.12 Several studies revealed that it is a multifunctional factor that not only facilitates but also suppresses many functions of various cells.13-17 TGF-β1, which is chemotactic to monocytes and fibroblasts, enhances angiogenesis and the formation of extracellular matrix protein, such as collagen and fibronectin.13,17

Because we thought that it would be possible to explain the fibrosis of subarachnoid space after SAH by this TGF-β1 function, we injected human recombinant TGF-β1 into the subarachnoid space of mice and found that it induced a slowly progressive communicating hydrocephalus.9 TGF-β1 indicates a marked conservation across species, and human TGF-β1 is a high-sequence homologue to murine TGF-β1.18

In this clinical study we measured the total TGF-β1 levels of the cerebrospinal fluid obtained from patients with SAH and compared them in relation to CT findings and clinical courses. The TGF-β1 levels of the patients who developed ventricular dilatation with periventricular low density on the follow-up CT scan were higher than those of the patients without ventricular dilatation. Furthermore, the TGF-β1 concentration of patients who underwent VP shunt surgery was also statistically higher than that of those without VP shunt. Thus, not only the CT findings but also the clinical findings were well related to the TGF-β1 levels of the cerebrospinal fluid. These results indicate that TGF-β1 concentration in the cerebrospinal fluid of patients with communicating hydrocephalus is higher than that of patients without hydrocephalus.

Source of TGF-β1 in Cerebrospinal Fluid

We previously demonstrated that TGF-β1 was absent in the cerebrospinal fluid under normal conditions.19 In the present clinical study the level of TGF-β1 in the cerebrospinal fluid was highest in the early stage of SAH and rapidly decreased; it correlated with the total protein level of the cerebrospinal fluid. These findings suggest the hypothesis that TGF-β1 is mainly derived from platelets that spread into the subarachnoid space at the time of SAH. However, TGF-β1 is produced by many kinds of cells, including fibroblasts. It is also reported that many cytokines would be released into the cerebrospinal fluid from inflammatory cells.20 It seems likely that not only platelets but also fibroblasts and inflammatory cells may release TGF-β1 into the cerebrospinal fluid of patients with SAH.

Active Form of TGF-β1 in Cerebrospinal Fluid

Platelets release a latent form of TGF-β1 at hemorrhage.21 The activation mechanism of the latent form of TGF-β1 derived from platelets has been gradually resolved,22 but the activation mechanism in cerebrospinal fluid has not been clarified. In this study we measured the total TGF-β1 level in cerebrospinal fluid that had been pretreated with acidification and found that the concentration of TGF-β1 in the hydrocephalus group...
was higher than that in the group without hydrocephalus. Because the latent form of TGF-β1 cannot bind its receptor until the binding protein is removed, it was necessary to demonstrate the active form of TGF-β1 in the cerebrospinal fluid of SAH patients. TGF-β1 is known to be active as a 12.5-kD monomer or a 25-kD dimer that is linked by disulfide bonds of 12.5-kD monomers. Western blot analysis of the cerebrospinal fluid of a patient with SAH, which had not been pretreated with acid, revealed a 25-kD band on day 0. Positive demonstration of the active form in a sample of cerebrospinal fluid is significant. Although active TGF-β1 could not be detected in the samples on days 1 and 16 by Western blot analysis, it is possible that a small amount of active TGF-β1 exists in the cerebrospinal fluid.

Conclusions

We compared the TGF-β1 concentration in the cerebrospinal fluid of 24 patients with SAH between hydrocephalic and nonhydrocephalic groups. The TGF-β1 level of the former (n=11) was statistically higher than that of the latter (n=13). We also demonstrated the active form of TGF-β1 in the cerebrospinal fluid by Western blot analysis. These results provide additional support to the theory that TGF-β1 plays an important role in generating communicating hydrocephalus after SAH.

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References

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