VASCULAR ENDOTHELIAL GROWTH FACTOR IS ASSOCIATED WITH BLOOD BRAIN BARRIER DYSFUNCTION IN EOSINOPHILIC MENINGITIS CAUSED BY ANGIOSTRONGYLUUS CANTONENSIS INFECTION

HUNG-CHIN TSAI, YUNG-CHING LIU, SUSAN SHIN-JUNG LEE, ENG-RIN CHEN, AND CHUAN-MIN YEN*

Section of Infectious Diseases, Department of Medicine, Kaohsiung Veterans General Hospital. Kaohsiung City, Taiwan and National Yang-Ming University, Taipei, Taiwan, Republic of China; Department of Parasitology and Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China

Abstract. Vascular endothelial growth factor (VEGF) is a potent vascular permeability factor and a mediator of brain edema. To assess the role of vascular endothelial growth factor in eosinophilic meningitis, vascular endothelial growth factor measurements were made in the cerebrospinal fluid (CSF) and blood of 9 patients with eosinophilic meningitis in a cohort study. VEGF<sub>CSF</sub> was detected in 8 (90%) of 9 eosinophilic meningitis patients (range, 45–2190 pg/mL) at presentation. The mean VEGF<sub>CSF</sub>, at presentation, 1 week, and 2 weeks after admission was 568 pg/mL, 751 pg/mL, and 1031 pg/mL, respectively. There was an association between VEGF<sub>CSF</sub>. CSF protein, white cell count, and eosinophil counts. The VEGF<sub>SERUM</sub> fluctuated during the 6-month follow-up period. These results indicate that vascular endothelial growth factor may be associated with blood-brain barrier disruption in patients with eosinophilic meningitis.

INTRODUCTION

Angiostrongylus cantonensis, also known as the rat lungworm, is the most common cause of eosinophilic meningitis in the Pacific Islands and Southeast Asia. Rats serve as the definitive host of the nematode. If an infection occurs in nonpermissive hosts, including humans and mice, the development of the parasites will terminate at the young–adult worm stage in the brain and cause eosinophilic meningitis or meningoencephalitis.1–4 Several indices have been used to assess the blood–brain barrier (BBB) integrity in an individual with a central nervous system infection. It was reported that most human immunodeficiency virus (HIV)-infected patients have pathologic cerebrospinal fluid (CSF) findings for at least one parameter, such as an elevated CSF total protein level, CSF white blood cell count, or CSF serum immunoglobulin G ratio.5 In a mice animal model of eosinophilic meningitis caused by A. cantonensis infection, researchers showed that dysfunction of the BBB occurred in mice infected with A. cantonensis, evidenced by the high concentrations of protein and albumin, high leukocyte counts in CSF, high ratio of CSF/serum protein and albumin, and high permeability of BBB.6 Infection of the CSF causes a severe inflammatory reaction, mediated by pathogen products and host cytokines. This inflammatory reaction compromises the function of BBB, resulting in the exudation of plasma proteins and development of vasogenic brain edema, which contributes to cerebral dysfunction and brain damage.7 Vascular endothelial growth factor (VEGF), a 46-kDa glycosylated homodimeric protein, is a regulator of angiogenesis and a potent inducer of vascular permeability.8 VEGF is implicated in the pathogenesis of brain edema related to ischemia, trauma, bacterial meningitis, tuberculous meningitis, and tumors.7,9,10 To assess the role of VEGF in the pathophysiology of eosinophilic meningitis, dynamic VEGF levels were measured in CSF and serum of patients with eosinophilic meningitis in our cohort study.

MATERIALS AND METHODS

Patients. A case of eosinophilic meningitis was clinically defined as presentation with an acute onset of headache, eosinophil pleocytosis in the blood/CSF, accompanied by at least one of the following: fever, ataxia, visual disturbances, photophobia, nuchal rigidity, neck pain, hyperesthesias, or paresthesias.11,12 Three outbreaks of eosinophilic meningitis, caused by A. cantonensis occurred in Kaohsiung, Taiwan, in 1998, 1999, and 2001.11–13 Most of the patients (77%) were adult, male. Thai laborers who had eaten raw golden apple snails within 3 weeks. Study subjects were derived from the second outbreak in 1999,12,13 All of the 9 Thai laborers received only analgesics or glycerol as treatment (or both). Each patient underwent a physical, neurologic, and ophthalmic examination. Laboratory tests were performed at the time of admission and spinal taps were performed on all patients. CSF analysis included cell count, glucose and protein levels, gram and acid-fast stains, India ink preparation, wet mount preparations for larvae, and measurement of cryptococcal antigen. The patients were observed during their hospital course. CSF was examined weekly until discharge. Blood was obtained weekly for the first 2 months, every other week for the next 2 months, and monthly thereafter for as long as 6 months. Blood and CSF samples were centrifuged (1700 g at 4°C) immediately, aliquoted, and stored at −70°C.

Antibodies to A. cantonensis were detected in serum and CSF by a microenzyme-linked immunosorbent assay (ELISA) using young-adult worm antigen, with a molecular weight 204KD, and purified by monoclonal antibody.14

Vascular endothelial growth factor measurements. Levels of VEGF in the serum and CSF (VEGF<sub>SERUM</sub> and VEGF<sub>CSF</sub>, respectively) were measured by enzyme immunoassay (Neogen Corporation, Lansing, MI). This Neogen’s Sandwich Human VEGF is a sandwich enzyme immunoassay (EIA), which measures the free forms of the cytokine VEGF<sub>lig</sub>. Mouse monoclonal antibodies generated against human VEGF were used to capture human VEGF in a sample. Simultaneously, biotinylated rabbit anti-human VEGF polyclonal antibodies detected VEGF in the sample. The assay was visualized using a streptavidin alkaline phosphatase conjugate and an ensuring chromogenic substrate reaction. The assay sensitivity was 26.6 pg/ml, the range of de-
The association between VEGF and CSF protein, white cell count, and eosinophil counts. White cell count, and eosinophil counts. For patients with undetectable VEGF level, an arbitrary level of 25 pg/ml was used for statistical purpose.

**Statistical analysis.** The association between VEGF and CSF laboratory abnormalities was analyzed with Pearson correlation test. A *P* value < 0.05 was considered statistically significant.

**RESULT**

All 9 patients in the cohort study were young Thai men. The source of epidemic was ingestion of raw snails seasoned with lemon juice and red pepper. Patients in this outbreak in 1999 only received supportive therapy. A total of 25 lumbar punctures were performed in these 9 patients. Nine lumbar punctures (5 and 4, respectively) were done in two patients due to recurrent headache. Headache, stiff neck, transient right facial palsy, ataxia, and diplopia recurred in 1 patient 24 days after treatment. A spinal tap revealed an elevated opening pressure of 250 mm H₂O, a white cell count of 578 × 10⁵ cells/µL with 24% eosinophils, a protein level of 165 mg/dL, and a glucose level of 43 mg/dL. (initial CSF white cell count 1270 × 10⁵ cells/µL at admission). He was treated with intravenous glycerol for 7 days and recovered in a week. The serial VEGF₉₅ levels in this patient were 2190 pg/mL, 2200 pg/mL, 1550 pg/mL, 3640 pg/mL, and 45 pg/mL. Vomiting and headache developed in a second patient 15 days after treatment. A spinal tap revealed an elevated opening pressure of 240 mm H₂O, a white cell count of 1110 × 10⁵ cells/µL with 74% eosinophils, a protein level of 107 mg/dL, and a glucose level of 46 mg/dL. (initial CSF white cell count 1390 × 10⁵ cells/µL at admission). He recovered without treatment in about a week. The serial changes of VEGF₉₅ were 595 pg/mL at presentation, 160 pg/mL and 3250 pg/mL one and two weeks after admission, and 3640 pg/mL at recurrence of headache. VEGF₉₅ was detected in 8 (90%) of 9 eosinophilic meningitis patients (range, 45–2190 pg/mL) at presentation. The mean VEGF₉₅ at presentation, 1 week, and 2 weeks after admission was 568 pg/mL, 751 pg/mL, and 1031 pg/mL, respectively. The VEGF₉₅ fluctuated during the 6-month follow-up. The mean CSF protein was 131 mg/dL at presentation and dropped to 81 mg/dL 2 weeks later. One patient had a rebound CSF protein level (141 mg/dL) on fifth lumbar puncture done 4 weeks later. CSF white cell count showed a time-dependent decrease, with mean levels of 618 cumm/ml, 320 cumm/ml, and 433 cumm/ml at presentation, 1 week, and 2 weeks after admission, respectively. The CSF white cell counts during the fourth and fifth lumbar puncture in the two patients with recurrent headache were 369 cumm/ml and 667 cumm/ml, respectively. There was an association between VEGF₉₅, CSF protein, white cell count, and eosinophil counts (Table 1).

**DISCUSSION**

In this study, we showed that VEGF₉₅ was detected in 8 (90%) of 9 eosinophilic meningitis patients (range, 45–2190 pg/mL) at presentation. There was an association between VEGF₉₅, CSF protein, white cell count, and eosinophil counts. These results indicate that VEGF may play a role in the pathophysiology of eosinophilic meningitis. An increase in leukocyte count can be observed in the CSF of patients with central nervous system infection due to BBB damage. Outbreaks of eosinophilic meningitis occurred in the southern region of Thailand during the dry season. Factors included geographical factors, exposure to certain foods, and local climate. The source of epidemic was ingestion of raw snails seasoned with lemon juice and red pepper. Patients in this outbreak in 1999 only received supportive therapy. A total of 25 lumbar punctures were performed in these 9 patients. Nine lumbar punctures (5 and 4, respectively) were done in two patients due to recurrent headache. Headache, stiff neck, transient right facial palsy, ataxia, and diplopia recurred in 1 patient 24 days after treatment. A spinal tap revealed an elevated opening pressure of 250 mm H₂O, a white cell count of 578 × 10⁵ cells/µL with 24% eosinophils, a protein level of 165 mg/dL, and a glucose level of 43 mg/dL. (initial CSF white cell count 1270 × 10⁵ cells/µL at admission). He was treated with intravenous glycerol for 7 days and recovered in a week. The serial changes of VEGF₉₅ were 595 pg/mL at presentation, 160 pg/mL and 3250 pg/mL one and two weeks after admission, and 3640 pg/mL at recurrence of headache. VEGF₉₅ was detected in 8 (90%) of 9 eosinophilic meningitis patients (range, 45–2190 pg/mL) at presentation. The mean VEGF₉₅ at presentation, 1 week, and 2 weeks after admission was 568 pg/mL, 751 pg/mL, and 1031 pg/mL, respectively. The VEGF₉₅ fluctuated during the 6-month follow-up. The mean CSF protein was 131 mg/dL at presentation and dropped to 81 mg/dL 2 weeks later. One patient had a rebound CSF protein level (141 mg/dL) on fifth lumbar puncture done 4 weeks later. CSF white cell count showed a time-dependent decrease, with mean levels of 618 cumm/ml, 320 cumm/ml, and 433 cumm/ml at presentation, 1 week, and 2 weeks after admission, respectively. The CSF white cell counts during the fourth and fifth lumbar puncture in the two patients with recurrent headache were 369 cumm/ml and 667 cumm/ml, respectively. There was an association between VEGF₉₅, CSF protein, white cell count, and eosinophil counts (Table 1).

**TABLE 1**

Pearson correlation test showed an association between VEGF₉₅, CSF protein, CSF white cell counts and CSF eosinophil counts

<table>
<thead>
<tr>
<th>Variable</th>
<th>VEGF₉₅₁</th>
<th>VEGF₉₅₂</th>
<th>VEGF₉₅₃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>r</em></td>
<td><em>p</em></td>
<td><em>r</em></td>
</tr>
<tr>
<td>CSF protein 1</td>
<td>0.334</td>
<td>0.465</td>
<td>0.788</td>
</tr>
<tr>
<td>CSF protein 2</td>
<td>0.128</td>
<td>0.742</td>
<td>0.829</td>
</tr>
<tr>
<td>CSF protein 3</td>
<td>0.237</td>
<td>0.701</td>
<td>0.860</td>
</tr>
<tr>
<td>CSF wbc 1</td>
<td>0.337</td>
<td>0.460</td>
<td>0.795</td>
</tr>
<tr>
<td>CSF wbc 2</td>
<td>0.505</td>
<td>0.166</td>
<td>0.817</td>
</tr>
<tr>
<td>CSF wbc 3</td>
<td>0.754</td>
<td>0.084</td>
<td>0.577</td>
</tr>
<tr>
<td>CSF cosin</td>
<td>0.15</td>
<td>0.001*</td>
<td>0.806</td>
</tr>
</tbody>
</table>

*VGEF₉₅₁, VGEF₉₅₂, VGEF₉₅₃ represent the CSF VEGF level at presentation (n = 7), 1 week (n = 9), and 2 weeks later (n = 6). CSF protein 1, CSF protein 2, CSF protein 3 are the protein concentration in CSF at presentation, 1 week, and 2 weeks after admission. CSF wbc1, CSF wbc2, CSF wbc3 show the white cell count in CSF at presentation, 1 week, and 2 weeks later. CSF cosin represented CSF eosinophil counts at presentation. * showed *P* < 0.05.
of patients who felt free from headache. In our study, VEGF
CSF measured 1 and 2 weeks after presentation was
significantly correlated with CSF abnormalities. The time
course is consistent to the clinical treatment duration for 1 or
2 weeks, although we did not use any steroids in our patients.
One of our patients with recurrent headache received gly-
cerol as an adjuvant therapy. Osmotherapy with mannitol,
glycerol, or hypertonic saline is often used in patients with
severe brain edema after stroke or neurotrauma. It can re-
sult in massive osmotic diuresis and fluid and electrolytes
imbalance. As a result of a disrupted blood–brain barrier, the
osmotic agents may leak into the brain parenchyma, with
further disturbance of the osmotic gradient as a result. In-
terestingly, osmotherapy may even enhance disturbance of the
blood–brain barrier. Mannitol cause the shrinkage of ce-
rebral capillaries resulting in the opening of endothelial tight
junction. This osmotic stress can involve in the calcium in-
flux, nitric oxide, and cytoskeletal changes and probably influence the measurement of VEGF in CSF.
The finding that VEGF CSF could not be detected in the
CSF of all our patients with eosinophilic meningitis may be
explained in several ways. First, locally secreted VEGF may
induce endothelial permeability via the luminal receptors of
endothelial cells, and may not be reflected in the CSF level.
Disruption of the BBB may be further aggravated by VEGF
secreted in the CSF via the abluminal receptors of endothelial
cells. Second, the timing of sampling may affect VEGF
CSF detection because the in vivo half-life of VEGF CSF is
unknown. Third, not every patient in our study had CSF ab-
normalities. Finally, BBB disruption during eosinophilic men-
ingitis involves a complex interaction between eosinophils
and mediators, such as interleukin-4, interleukin-5, platelet-
activating factor, nitric oxide, and matrix metalloproteinases,
which might induce BBB permeability, independent of VEGF.
We did not measure the serum/CSF albumin for VEGF
index. VEGF levels were higher in the serum compared with
the CSF, therefore the possibility of VEGF accumulation in
CSF caused by passive influx instead of intrathecal produc-
tion can not be excluded. However, it remains to be deter-
mined whether this is due to a fairly low production of VEGF
by inflammatory cells, rapid degradation of immunoreactive
VEGF, or binding of VEGF to soluble or cell-bound VEGF
receptors.
Based on our small cases series, we found that patients with
eosinophilic meningitis and presence of VEGF proteins in
CSF could be associated with disruption of BBB. There was
an association between VEGF CSF, CSF protein, white cell
count, and eosinophil counts. The VEGF CSF fluctuated
during the 6-month follow-up period. However, larger cases
studies are needed to justify this conclusion.

Received July 29, 2006. Accepted for publication December 5, 2006.

Acknowledgment: This work is supported by Grant VGHKS94-020
and VGHKS95-014 from Kaohsiung Veterans General Hospital, Tai-
wan.

Authors’ addresses: Hung-Chin Tsai, Yung-Ching Liu, and Susan
Shin-Jung Lee, Section of Infectious Diseases and Department of
Medicine, Kaohsiung Veterans General Hospital, 386 Ta-Chung 1st
Road, Kaohsiung, 813, Taiwan, Telephone: 886-7-3468299, Fax: 886-
7-3468292, Eng-Rin Chen and Chuan-Min Yen, Department of Para-
sitology, Kaohsiung Medical University, No. 100, Shih-Chuan 1st
Road, Kaohsiung 80708, Taiwan, Telephone: 886-7-3121101 ext. 2169.

REFERENCES

1. Beaver PC, Rosen L. 1945. Memorandum on the first report of
2. Rosen L, Chappell R, Laquerre GL. 1962. Eosinophilic meningo-
encephalitis caused by a metastrongylid lung worm of rats.
JAMA 179: 620–624.
gitis in Thailand: clinical studies of 484 typical cases probably
and meningoencephalitis caused by Angiostrongylus cantonensis
5. Marshall DW, Brey RL, Cahill WT, Houk RW, Zajac RA, Boswell
RN, 1988. Spectrum of cerebrospinal fluid findings in
various stages of human immunodeficiency virus infection.
Arch Neurol 45: 954–958.
Blood-brain barrier dysfunction occurring in mice infected
7. van der Flier M, Stockhammer G, Vonk GJ, Nikkels PG, van
Diemen-Steenvoorde RA, van der Vlist GJ, Rupert SW, Schelle-
ed E, Guiraud E, Guttman E, Hooftman AI, Kimpen JL, Geelen
SP, 2001. Vascular endothelial growth factor in bacterial
meningitis: detection in cerebrospinal fluid and localization
9. van Bruggen N, Thibodeaux H, Palmer JT, Lee WP, Fu L, Cairns
B, Tumas D, Gerlat R, Williams SP, van Lookeren Campagne
M, Ferrara N, 1999. VEGF antagonism reduces edema forma-
tion and tissue damage after ischemia/reperfusion injury in the
10. van der Flier M, Hoppener RJ, van Rensburg AJ, Ruyken M,
Kolk AH, Springer P, Hoepelman AI, Geelen SP, Kimpen JL,
Schoeman JF, 2004. Vascular endothelial growth factor and
blood-brain barrier disruption in tuberculous meningitis. Pedi-
Outbreak of eosinophilic meningitis associated with drinking
raw vegetable juice in southern Taiwan. Am J Trop Med Hyg
71: 222–226.
12. Tsai HC, Liu YC, Kunin CM, Lee SS, Chen YS, Lin HH, Tsai
TH, Lin WR, Huang CK, Yen MY, Yen CM, 2001. Eosino-
philic meningitis caused by Angiostrongylus cantonensis: re-
13. Tsai HC, Liu YC, Kunin CM, Lai PH, Lee SS, Chen YS, Wann
Eosinophilic meningitis caused by Angiostrongylus cantonensis
associated with eating raw snails: correlation of brain magnetic
resonance Imaging scans with clinical findings. Am J Trop Med
eosinophilic meningitis using an antigen of Angiostrongylus
cantonensis L5 with molecular weight 204KD. Acta Trop 75:
9–17.
15. Bisser S, Lejon V, Preux PM, Bouteille B, Stanghellini A, Jaub-
fluid barrier and intrathecal immunoglobulins compared to
field diagnosis of central nervous system involvement in sleep-
pathogenesis, pathophysiology, and progress. N Engl J Med
327: 864–872.
immunoglobulins in eosinophilic meningoencephalitis due to An-
455.
concept common to normal blood–CSF barrier function and to
Vogelbaum M, Kinter M, Rasmussen P, Mayberg MR, Janigro


