Hepatocyte Growth Factor Levels in Cerebrospinal Fluid: A Comparison between Acute Bacterial and Nonbacterial Meningitis

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The organotrophic functions of the hepatocyte growth factor (HGF) have been the subject of several studies. In the present study, we have measured the levels of HGF in cerebrospinal fluid (CSF) and sera from 78 patients divided into 6 different groups according to central nervous system (CNS) infection and control. Quantitative measurements of HGF in the CSF and serum were performed by an enzyme-linked immunosorbent assay. Elevated values of CSF HGF were found in the patients with acute bacterial/probable bacterial meningitis (P < 0.001), compared with nonbacterial CNS infections and facial palsy, as well as with a control group without signs of CNS involvement. The values of CSF HGF were not correlated to blood-brain-barrier disruption in the groups. These observations might indicate an intrathecal production of HGF in acute bacterial/probable bacterial meningitis.

Materials and Methods

Patients. As a routine in our wards, the CSF and serum specimens collected after lumbar and vein punctures are handled promptly, centrifuged (1000 g for 15 min), and stored at −70°C. In this retrospective study, the paired CSF and serum from 78 patients (collected between 1992 and 1998) were analyzed. All of the samples were collected at the acute stage of disease and were centrifuged again after they were thawed. The samples were divided into 6 groups according to the following criteria: group 1 (n = 20) was examined by lumbar puncture because of an idiopathic peripheral facial palsy. Patients in this group had normal c-reactive protein (CRP), normal CSF white-blood-cell counts, and, in 5 cases, slightly elevated CSF protein. Patients in group 2 (n = 12) had meningitis caused by Borellia burgdorferi with a verified intrathecal synthesis of specific antibodies against B. burgdorferi. They had normal CRP, CSF pleocytosis with lymphocytic predominance, and elevated CSF protein. Group 3 (n = 11) consisted of patients with viral meningitis. The serological tests showed herpes zoster in 3, enterovirus in 1, herpes simplex type II in 1, and unknown etiology in the others. The CSF white-blood-cell count and CRP were slightly elevated, and all of the patients in this group had a benign course of disease. The patients in group 4 (n = 6) suffered from encephalitis caused by herpes simplex type I (HSV-1). The diagnosis was verified by positive HSV-1 DNA in the CSF. The major population in group 5 (n = 19), acute bacterial meningitis, had high CRP (>150), CSF polynuclear pleocytosis, and elevated CSF protein. The cultures were positive in both CSF and blood in 9 patients. The cultures revealed Streptococcus pneumoniae in 4 cases and 1 case of each Staphylococcus aureus, coagulate-negative staphylococci, Escherichia coli, Neisseria meningitidis, and Listeria monocytogenes. In 3 patients, there were positive culture tests merely from blood (S. pneumoniae in 2 cases and S. oralis in

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Determination of HGF. Immunoreactive HGF was determined by an ELISA, using a commercially available kit (Quantikine HGF immunoassay; R&D Systems, Minneapolis). The CSF samples, stored at −70°C, were centrifuged at 1000 g for 15 min prior to analysis. The method was modified according to the manufacturer’s specifications to determine HGF in CSF (i.e., the calibrator diluent used was RD5P designed by R&D for cell culture supernate samples, and the incubation time for conjugating was 1.75 h). The calibrator consisted of recombinant human HGF with the following concentrations: 0.125, 0.250, 0.500, 1.00, 2.00, 4.00, and 8.00 ng/mL. The blank value at 450 nm was subtracted from the standards at the sample values. The lowest detectable amount by this assay was 0.04 ng/mL.

Routine biochemistry. Determinations of the liver enzyme activities and CRP, serum, and CSF analysis were performed with routine methods used at the biochemical laboratory at the University Hospital in Linköping and Göteborg.

Statistical analysis. Group differences were analyzed with analysis of variance followed by Duncan’s test in case of significance. A probability level of \( P < 0.05 \) was considered statistically significant. Double measurements of HGF in CSF and serum were made in all samples to assess the methodological error. The methodological error was calculated by Dahlberg’s equation and was found to be 15% (98 double measurements; coefficient of variation).

Results

There were no statistically significant age or sex differences between the groups, apart from the patients in group 3, who were substantially younger \( (P < 0.01) \).

Values of CSF-HGF and serum HGF were log-normally distributed. In the following analysis, therefore, the logarithm of these parameters was used.

Values of CSF-HGF were significantly higher (figure 1; table 1) in group 5 than in all other groups \( (P < .001) \). In addition, group 4 exhibited slightly higher values, compared with both group 6 and group 3 \( (P < .04) \).

Discussion

The role of HGF in infectious diseases has recently been investigated. Some studies have shown the prognostic values of HGF in inflammatory diseases [9–10]. As an agent that promotes regeneration of epithelial cells, the potential therapeutic value of HGF has been investigated in severe infectious diseases [11].

In addition, the localization and function of HGF in brain diseases have been reported by several authors. Fenton et al. [12] have reported a widespread HGF-like immunoreactivity in both the cerebral cortex and the white matter in the brain. The neuroprotective action of HGF against the death of neurons was studied by continuous intrastriatal administration of human recombinant HGF after 5-min transient forebrain ischemia in Mongolian gerbils. It was shown that HGF successfully prevented the postischemically delayed death of hippocampal neurons [13]. Several studies have reported the high CSF levels of cytokines in bacterial meningitis [14]. To our knowledge, data of HGF values in CSF have not been published before.

The object of the present study was to investigate the HGF levels in different CNS infections. Therefore, the HGF levels were determined in CSF and paired serum in the acute phase of diseases in bacterial/probable bacterial and nonbacterial meningitis. We found that acute HGF levels in CSF were significantly higher \( (P < .001) \) in acute bacterial/probable bacterial meningitis, compared with all other groups.

Among the patients with acute bacterial/probable bacterial meningitis, 2 died, one of them during the first week after admittance to the hospital and the other nearly 1 month after the debut of disease. The first patient, but not the second, was included among those few patients who had relatively low acute HGF levels in CSF, despite much higher levels in serum at admittance.

In 5 patients in whom HGF levels were determined in serum

### Table 1. Measured parameters and differences between groups.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Mean age, in years (range)</th>
<th>Median/mean no. of cerebrospinal fluid (CSF) cells ( (&lt; 5 \times 10^9/L) ) (range)</th>
<th>Median value CSF protein (270 mg/L)* (range)</th>
<th>Median hepatocyte growth factor, ng/mL (no defined reference value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>55 (21–82)</td>
<td>0.8/1.3 (0.1–3.4)</td>
<td>257 (130–670)</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>54 (18–85)</td>
<td>40/91.7 (5.4–301)</td>
<td>432 (151–1690)</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>33 (18–71)</td>
<td>75/165 (8.5–752)</td>
<td>622.5 (124–1551)</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>68 (59–80)</td>
<td>122/124 (17–237)</td>
<td>361 (188–1549)</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>56 (19–85)</td>
<td>2600/4015 (317–10,000)</td>
<td>2020 (362–4590)</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>56 (31–90)</td>
<td>0.78/0.9 (0.3–2.6)</td>
<td>222 (171–490)</td>
</tr>
</tbody>
</table>

* Normal reference values.
and CSF 1 week after treatment, the levels were low, as were CSF cell count and protein (data not shown).

Three patients with meningitis caused by *B. burgdorferi* had blood samples taken and CSF analysis done several times in the course of treatment and during follow-up. The HGF levels in serum and CSF did not change during this period, but CSF cell counts and protein decreased (data not shown).

The blood-brain-barrier disruption is considered to be responsible for elevated protein levels in CSF [15]. Most patients with acute bacterial/probable bacterial meningitis and meningitis caused by *B. burgdorferi*, as well as encephalitis (HSV-1), had elevated protein levels in CSF. It was only the patients with acute bacterial/probable bacterial meningitis who had significantly higher HGF levels in CSF, compared with all other groups. In addition, high serum HGF levels were observed in the patients with encephalitis (HSV-1), despite low CSF HGF levels. It is therefore unlikely that high HGF levels in CSF were merely passive transfer from the serum (i.e., an intrathecal production of this cytokine may be responsible for this phenomenon).

Hence, we conclude that the high HGF levels that were observed in acute bacterial/probable bacterial meningitis might indicate an intrathecal production of this cytokine. This might be beneficial for the patient, as suggested by other studies in other patient groups [10] and in experimental studies [11]. More patient studies are needed to confirm this hypothesis in meningitis.

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References