Short Report: Dynamic Changes of Hepatocyte Growth Factor in Eosinophilic Meningitis Caused by *Angiostrongylus cantonensis* Infection

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**Abstract.** Hepatocyte growth factor (HGF) is a member of the angiogenic growth factor family, which exerts a variety of effects on epithelial, endothelial, and neuronal cells by binding to the c-MET receptor tyrosine kinase. It was reported that HGF attenuates cerebral ischemia-induced increase in permeability of the blood-brain barrier (BBB) and decreases in expression of tight junction proteins in cerebral vessels of rats. Studies on the localization of the c-Met/HGF receptor in the rat brain and the interaction with HGF after brain injuries show that HGF plays an important role as a neurotrophic factor in the brain. To assess the role of HGF in patients with eosinophilic meningitis, a retrospective, cohort study was conducted to measure the dynamic changes of HGF in the cerebrospinal fluid (CSF) and blood of nine patients with eosinophilic meningitis. The mean HGF CSF at presentation, 1 week, 2 weeks, and 3 weeks after admission was 539 pg/mL, 540 pg/mL, 376 pg/mL, and 279 pg/mL, respectively. The mean level of HGF CSF at presentation (539 ± 242 pg/mL) and 1 week after admission (540 ± 213 pg/mL) was significantly higher than in controls (162 ± 207 pg/mL) (*P* = 0.02 and *P* = 0.01, respectively). The CSF/blood ratio of HGF at presentation (0.61) was higher when compared with physiologic situations in uninfected individuals (0.51). The levels of HGF in CSF were not correlated with the amount of CSF cells or proteins. All patients recovered without neurologic sequelae. These results indicate that high concentrations of HGF in the CSF occur in eosinophilic meningitis, and may have a role in protecting against endothelial injury and reducing BBB dysfunction.

*Angiostrongylus cantonensis* is the most common cause of eosinophilic meningitis in the Pacific Islands and Southeast Asia. Human infection occurs after ingestion of the worms in raw snails or fish that serve as intermediate hosts. Rats serve as the definitive host of the nematode. If an infection occurs in non-permissive 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 Lee and others7 showed that dysfunction of the blood-brain barrier (BBB) occurred in mice infected with *A. cantonensis*, evident by the high concentrations of protein and albumin, high leukocyte counts in the cerebrospinal fluid (CSF), a high ratio of CSF/serum protein and albumin, and high permeability of the BBB. Infection in 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.8,9

Hepatocyte growth factor (HGF) is a potent mitogen for hepatocytes and is a member of the angiogenic growth factor family.10 This secreted protein, originally purified from rat platelets and plasma from a patient with fulminant hepatic failure,11,12 exerts a variety of effects on many cell types by binding to the c-MET receptor tyrosine kinase.13,14 It can also improve learning and memory dysfunction15,16 and exert anxiolytic effects in rats.17 The HGF not only preserves from neuronal cells after cerebral ischemia induced by middle cerebral artery occlusion or microsphere embolism,18 but also protects against endothelial injury and reduces BBB disruption in rats.19 From studies on the localization of the c-Met/HGF receptor in the rat brain and the interaction with HGF after brain injuries, which HGF plays an important role as a neurotrophic factor in the brain.19 So far, large increases of HGF concentrations in the CSF have only been described in acute bacterial meningitis20,21 and tuberculous meningitis.22 We surmise that HGF may act as a neurotrophic factor in eosinophilic meningitis. To research the role of HGF in eosinophilic meningitis, a retrospective cohort study was conducted to measure dynamic changes in HGF levels in the CSF and serum of patients with eosinophilic meningitis.

A case of eosinophilic meningitis was defined as clinical presentation with an acute onset of headache, eosinophilic pleocytosis in the blood/CSF, and accompanied by at least one of the following: fever, ataxia, visual disturbances, photophobia, nuchal rigidity, neck pain, hyperesthesias, or paresthesias.5 Three outbreaks of eosinophilic meningitis, caused by *A. cantonensis* occurred in Kaohsiung, Taiwan, in 1998, 1999, and 2001.5,6,23 Most of the patients (77%23) were adult, male, Thai laborers who had eaten raw golden apple snails (*Ampullarium canaliculatum*) within 3 weeks of presentation. Study subjects were derived from the second outbreak in 1999.5,6 Each patient underwent a physical, neurologic, and ophthalmic examination. Laboratory tests were performed at the time of admission and lumbar spinal taps were performed on all patients. All CSF samples were obtained immediately at admission and before treatment. The 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 daily during their hospital course. The CSF and blood were examined weekly until discharge. The common source of the outbreak was raw snails that were eaten as a delicacy, seasoned with lemon juice and red pepper. The mean incubation
period was 13 days. Antibodies to *A. cantonensis* were detected at admission in either the serum (8, 90%) and/or the CSF (3, 33%) of the patients. Details of the clinical manifestations and laboratory findings have been described in detail elsewhere. All of the nine Thai laborers received only analgesics and/or glycerol as treatment. No patients died of this infection and neurologic sequelae were not observed after 6 months follow-up. The CSF control group (*N* = 12) consisted of patients presenting with headache or disturbance of consciousness, who underwent a diagnostic lumbar spinal tap that resulted in normal CSF parameters. All of the CSF/serum samples were centrifuged and the supernatants were frozen at −80°C until assayed. 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 204 kD, and purified by monoclonal antibody. The study protocol was reviewed and approved by the Commission on Medical Ethics of the Kaohsiung Veterans General Hospital (KVGH).

The HGF levels were determined by a commercial kit (Hu HGF ELISA Kit, BioSource Europe S.A, Belgium) using a solid phase sandwich ELISA. The assay was performed following the manufacturer’s instructions. Results in the CSF concentrations of HGF between patient and control group were compared by using the Mann-Whitney *U* test. Correlations were quantified by using the Pearson correlation test. All results were presented as mean ± SD. A *P* value of < 0.05 was considered statistically significant.

A total of 23 lumbar spinal taps were performed in these nine patients. The HGF in CSF can be detected in all of the patients at presentation. The mean HGF in CSF at presentation, 1 week, 2 weeks, and 3 weeks after admission was 539 ± 242 pg/mL (*N* = 9), 540 ± 213 pg/mL (*N* = 9), 376 ± 216 pg/mL (*N* = 3), and 279 ± 137 pg/mL (*N* = 2), respectively. The HGF serum levels were higher than the HGF in our patients. The mean HGF in serum at presentation, 1 week, 2 weeks, and 3 weeks after admission was 883 ± 252 pg/mL, 1311 ± 310 pg/mL, 1426 ± 387 pg/mL, and 1240 ± 502 pg/mL, respectively. The CSF/blood ratio of HGF at presentation (0.61) was higher when compared with the physiologic situations in uninfected patients (0.51). The CSF/blood ratio of HGF at the convalescent stage of meningitis was much lower than the normal ratio. The HGF in CSF levels at presentation and 1 week after admission were significantly increased when compared with the controls (*N* = 12, 162 ± 207 pg/mL) (*P* = 0.02 and *P* = 0.01, respectively, Mann-Whitney *U* test). There was no association between HGF in serum total CSF protein concentrations (*P* = 0.36), white cell counts (*P* = 0.56), and eosinophil counts (*P* = 0.84) at presentation and 1 week after admission (*P* = 0.80, 0.93, 0.29, respectively).

In this study, we showed that HGF in CSF levels were significantly increased in patients with eosinophilic meningitis at presentation and 1 week after admission, when compared with the controls (*P* = 0.02 and *P* = 0.01, respectively). The localization and function of HGF in brain diseases have been reported by several authors. Fenton and others have reported a widespread HGF-like immunoreactivity in both the cerebral cortex and the white matter in patients with Alzheimer’s disease. Miyazawa and others showed that HGF successfully prevented the postchemically delayed death of hippocampal neurons in rats. Date and others found that HGF can protect against endothelial injury and reduce BBB dysfunction in animals. These results indicated that HGF may act as a neurotrophic factor in eosinophilic meningitis, with possible roles of preventing from endothelial damage and BBB dysfunction.

The time-dependent decrease in the CSF/blood ratio of HGF found in our study that was not correlated with the amount of CSF cells or proteins, suggests that this rapid decline at 1 week after admission may be explained by clinical recovery rather than the merely passive transfer from the serum. This postulation is supported with a study by Dorta-Contreras and others, which showed that during the first 3 days of acute phase of eosinophilic meningoencephalitis, a blood-CSF barrier dysfunction occurred, usually resulting from a reduced CSF flow rate. However, at the time of early clinical recovery the blood-CSF barrier dysfunction was normalized in 75% of the patients. Moreover, Nayeri and others also showed that the HGF levels were very low at 1 week after treatment in patients with bacterial meningitis.

Previous reports showed that HGF in CSF was increased significantly in patients with bacterial meningitis and some patients with tuberculous meningitis. The objective of the present study was to research the serial changes of HGF levels in patients with eosinophilic meningitis. We found that HGF in CSF levels were significantly higher in the acute stage of eosinophilic meningitis, compared with the controls. This is the first report of elevated levels of HGF found in CSF of patients with eosinophilic meningitis.

The finding that HGF in CSF was not associated with CSF parameters may be explained in several ways. First, locally HGF secretion from the cortex and white matter may not be reflected in the CSF level. Second, the timing of sampling may affect HGF in CSF detection, because the *in vivo* half-life of HGF in blood is unknown. Third, not every patient in our study had CSF abnormalities and our case numbers were small. Finally, BBB disruption during eosinophilic meningitis 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 HGF.

In conclusion, we found that patients with eosinophilic meningitis had elevated HGF levels in the CSF. The clinical significance needs to be clarified further.

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