

Cerebrospinal fluid nerve growth factor and total protein concentration in the children with meningitis

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ARTICLE INFO

Article history:

Received 5 November 2010

Accepted 7 February 2011

Available online 11 April 2011

Keywords:

Meningitis,
cerebrospinal fluid,
nerve growth factor,
Total protein
Concentration
ELISA

ABSTRACT

Meningitis is one of the most common infectious cerebral nervous system (CNS), defined as an inflammation of the meninges. It is clinically categorized into a chronic and acute based on the acuity of symptoms. Vomiting, bulging fontanel and fever are the main symptoms in the patients with meningitis. Bacterial meningitis is a severe, potentially life-threatening infection that is associated with high rates of morbidity and disability in survivors. The examination of cerebrospinal fluid (CSF) is a keystone in diagnostic procedure for patients with suspected meningitis. CSF contains cytokines and changes in the levels of cytokines have been shown in some neurological diseases. In this study, the total protein contents (TPC) and NGF concentration in the CSF of normal subjects and patients with bacterial meningitis was measured using enzyme linked immunosorbent assay (ELISA). CSF was obtained by lumbar puncture. No significant change in the TPC has been seen between two groups. We have also shown that the concentration of NGF in the CSF of patients with meningitis is higher than in normal control. The data from this study indicate that NGF is a constant component of human CSF. It is also concluded that high concentration of CSF NGF may be partly related to the pathophysiology of meningitis.

1. Introduction

Meningitis, also termed arachnoiditis or leptomeningitis, is an inflammation of the membranes that surround the brain and spinal cord, thereby involving the arachnoid, the pia mater and the interposed (cerebrospinal fluid) CSF (Mace, 2008). Meningitis has been divided into bacterial and viral (aseptic) meningitis. Bacterial meningitis is a life threatening and infectious disease, especially for the children.

The leading pathogens are *Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae* (SP) and *Neisseria meningitidis*, all of which are now vaccine preventable. Worldwide Hib meningitis has the greatest incidence and is estimated to affect annually 400000 children aged under five years, 100000 of which die from the infection. Further neurological sequel, including hearing or vision loss, paralysis and cognitive deficits occur in 10-30% of cases (Peltola, 2000).

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For different reasons the diagnosis of bacterial meningitis in developing countries is difficult. Bacterial meningitis is an acute meningitis inflammation secondary to a bacterial infection that generally evokes a polymorphonuclear response in the CSF. Aseptic meningitis refers to a meningeal inflammation without evidence of pyogenic bacterial infection on Gram's stain or culture. Aseptic meningitis is subdivided into two categories: nonbacterial meningeal infections and noninfectious meningeal inflammation from systemic disease, neoplastic disease or neoplastic meningitis or drugs (Mace, 2008).

In the United States of America, epidemics of acute meningococcal meningitis are a common occurrence, while in parts of sub-Saharan Africa (meningitis belt) meningococcal meningitis is endemic. In the USA, the overall incidence of meningitis is about five per 100000 populations per year. The incidence is greatest in the children, with the frequency of about 400 per 100000 (Mace, 2008).

The examination of CSF samples may provide information about causative microorganism. The sensitivity of Gram-stained specimen of CSF ranges from 60% to 90% (Carpenter and Petersdorf, 1962; Geiseler et al., 1980). CSF is secreted by the choroids plexus and contains growth factors including transforming growth factor (TGF), nerve growth factor (NGF), brain derived growth factor (BDNF) and other neurotrophic factors which are present under specific pathological and physiological conditions (Salehi and Mashayekhi, 2009). Changes in the CSF growth factors has been shown in different neurological diseases including hydrocephalus, Alzheimer's disease, hydrocephalus and Parkinson's disease (Mashayekhi and salehi, 2005; Salehi and Mashayekhi, 2009; Salehi et al., 2008). As CSF is in close contact with the extracellular space of the brain, biochemical brain modifications could be reflected in the CSF and analysis of cytokines and growth factors might identify biomarkers of meningitis (Mashayekhi and Salehi, 2005). Thus, it is important to study the biochemistry of CSF in order to find a reliable biomarker.

NGF is important in cellular development, maintenance and regeneration in the brain. It is

produced in the hippocampus and cerebral cortex, and is retrogradely transported to support basal forebrain cholinergic neurons (Whitemore et al., 1988). A variety of brain injuries and infections including treatment with neurotoxins can upregulate NGF production, which plays an important role in neuronal repair of axonal damage (Scott and Crutcher, 1994). In normal brain, neurons play a major role in the synthesis of NGF, while in injured brain, glial cells may produce NGF (Lu et al., 1991). NGF supports neuronal survival in the brain. Two distinct types of receptors, the high affinity neurotrophin receptor tyrosine kinase A (TrkA) and the common low affinity neurotrophin receptor p75 (p75NTR) have been shown to mediate NGF signaling (Chao et al., 1998). The p75NTR has been found to have a widespread distribution within the CNS, including the cortical neurons (Lee et al., 1998). p75NTR is a member of the tumor necrosis factor (TNF)-receptor superfamily and contains intracellular death domains that have been linked to apoptotic signaling pathways in neurons (Rabizadeh and Bredesen 1994). It has been shown that p75NTR enhances the ability of TrkA to respond to NGF in neurons (Hantzopoulos et al., 1994). The aim of this study was to analyze the concentration of NGF and total protein concentration in the CSF of the children with bacterial meningitis using ELISA.

2. Material and methods

2.1. Patients

After ethic committee's approval and informed consent the total of 48 samples of CSF from normal subjects and patients with bacterial meningitis were collected by lumbar puncture performed routinely on the basis of the clinical suspicion of neurological disease. Samples were aged matched between the two groups, analysed and ranged in age between 4 and 11 years. None of the patients suffered from known diabetes mellitus, earlier diagnosed tumors of the nervous system or infection. Samples were taken from both male and female patients. For the lumbar puncture the skin were cleaned with 70% alcohol. 0.5 mls of CSF were collected and used

for this study. The samples that we used for analysis had no visible sign of contaminating neuroepithelium cells or red blood cells detectable under the microscope. When the tap was bloody samples were discarded. The samples were centrifuged at 10000 rpm for 7 minutes, the supernatant frozen immediately and stored at 70°C until used. 22 samples from normal and patients with meningitis (n=22 for each group), were used for analysis of protein and NGF concentration. Three independent repeats of each analysis were carried out on each sample.

All data presented are expressed as mean \pm standard error of the mean (SEM). Statistical analysis was performed using Student's *t*-test and only values with $P \leq 0.05$ were considered as significant.

2.2. Protein analysis: Western blot

The total concentration of proteins in CSF was determined by the Bio-Rad protein assay based on the Bradford dye procedure.

NGF in CSF was measured using the sensitive two sited ELISA and antiserum against human NGF. Microtiter plates (Dynatech, Chantilly, VA) were first coated with 60 ng primary anti-NGF antibody per well in 0.1 M Tris buffer. After overnight incubation, the plates were blocked with EIA buffer (50 mM Tris, pH 7.5, 0.3 M NaCl, 0.1% Triton X-100, 1% BSA and 1% Gelatine). The samples and standards were placed in triplicate wells and incubated overnight at room temperature. After washing a biotinylated secondary antibody (5 ng/ml) was added to each well and the incubation was carried out overnight at room temperature. b-Galactosidase coupled to avidin was then added and after 2 hours was followed by washing. Finally 200 μ M 4-methylumbelliferyl-b-galactoside (Sigma, Poole, UK) in 50 mM sodium phosphate and 10 mM MgCl₂ buffer were added and the amount of fluorescence was measured after 40 min incubation at 37 °C using a fluorimeter (Dynatech).

3. Results

3.1. Total protein concentration

The total concentration of proteins in CSF from patients with meningitis and normal subjects was determined by the Bio-Rad protein assay. The total protein concentration of CSF samples from patients with meningitis and the controls were 0.42 ± 0.07 and 0.43 ± 0.06 g/l, respectively. No significant difference has been seen in total protein concentration between two groups ($P = 0.40$).

3.2. NGF concentration

Using ELISA, it was shown that the concentration of NGF in the CSF samples with meningitis was higher than in normal CSF. The mean NGF concentration in CSF of patients with meningitis amounted to 23.14 ± 6.07 pg/ml, which was significantly higher than that of normal, i.e. 6.02 ± 1.657 pg/ml (Figure 1) ($P < 0.001$). This study has shown that NGF is present in human cerebrospinal fluid. The level of CSF NGF in patients with meningitis is more than that in normal CSF.

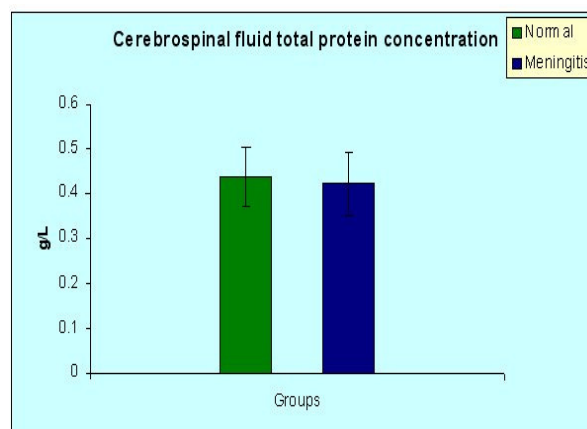


Figure 1. Total protein concentration (g/L) in the CSF (b) of normal and patients with meningitis. No significant difference in CSF total protein concentrations was seen between the two groups ($P = 0.40$).

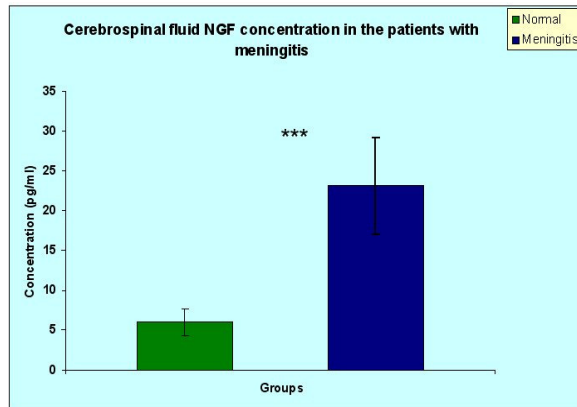


Figure 2. NGF concentrations in the CSF samples from controls and patients with meningitis (pg/ml). Significant increase in CSF NGF level has been seen in the CSF of patients with meningitis samples when compared with normal ($P < 0.001$). Significance values are shown as stars: 3 stars $P < 0.001$.

4. Discussion

Despite improved antibiotic treatment, bacterial meningitis remains a devastating disease, with mortality of up to 30% (Bedford et al., 2001). Since, the discovery of the potent survival-promoting effects of neurotrophic factors, there has been the hope that they can be used successfully in treating diseases of the CNS. NGF is of particular interest in this regard, as its receptors (Trk A and P75NTR) have been found to have a widespread distribution within the CNS (Lee et al., 1998), which is a prerequisite for a beneficial effect in disease affecting multiple regions of the brain, as in the case in bacterial meningitis.

Bacterial meningitis causes wedge-shaped lesions in the cortex, defined by neuronal loss and morphological features of cellular necrosis (Pfister et al., 2000). The present study showed that the concentration of CSF NGF is increased in the children with meningitis. NGF is important in cellular development, maintenance and regeneration in the brain. It is produced by neurons and glial cells in the cerebral cortex and hippocampus (Wittemore et al., 1988). Since NGF is known to be involved in the regulation of survival and differentiation of neurons, it may play a role in the recovery of damaged nerve cells in the patients with bacterial meningitis.

In normal brain, neurons play a major role in the synthesis of NGF, while in injured brain, glial cells may produce NGF (Lu et al., 1991). It is possible that the elevation of NGF level in the CSF in the patients with meningitis was caused by increased of glial cells that resulted from brain damage. It has been shown that if there is extensive damage of cortical neurons, reactive glial cells rather than neurons, may be the major source of NGF in the CSF (Suzuki et al., 1997). Production of NGF by glial cells in the CSF of patients with bacterial meningitis represents an active response to neurodegenerative changes.

Another possible explanation for the beneficial effects of NGF in patients with meningitis may be related to its ability to increase antioxidant enzyme activities and thus attenuate the harmful effects of reactive oxygen species (Galvin and Oorschot, 2003). Adjuvant antioxidants reduce cortical injury in the patients with bacterial meningitis (Auer et al., 1000).

In the CNS bacterial meningitis causes caspase-3 dependent apoptosis of neurons (Gianinazzi et al., 2003). NGF has been shown to be protective against cell death by blocking activation of caspase-3 through the trk receptor (Han and Holtzman, 2000). Interestingly, this receptor, which mediates progenitor cell survival and neurogenesis, is expressed in the same region where caspase-3-dependent cell death effects progenitor cell in meningitis (Bifrare et al., 2003). The inhibitory effects of NGF on caspase-3-mediated proapoptotic pathways is likely responsible for its beneficial effects on cell death.

In summary, NGF is neuroprotective against neuronal cell injury in bacterial meningitis. It is concluded that CSF NGF concentration is increased in the children with bacterial meningitis, which suggests that it could be involved in the pathophysiology of meningitis. Our data strengthen the association between NGF expression and meningitis. The results of our study indicate that NGF may have neuroprotective properties in meningitis and provide a basis for future studies related to neuroprotective mechanisms exerted by NGF in bacterial meningitis.

Acknowledgements

I would like to thank all the people in the genetic laboratory, Faculty of Sciences, the University of Guilan for their technical supports. I would also like to thank Dr.P.Dolati, Neurosugeon, for providing CSF samples.

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