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Zinc, Copper, and Manganese Concentrations in Cerebrospinal Fluid of Patients with Viral Meningitis

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ABSTRACT

We investigated whether information on concentrations of the trace metals in cerebrospinal fluid of patients with viral meningitis could be of value in diagnosis or prognosis. Samples from ten patients and 11 control subjects were analysed for zinc, copper, and manganese by atomic absorption spectrophotometry method. Protein concentrations in cerebrospinal fluid were also determined. The mean pretreatment values of zinc and copper were significantly lower ($p<0.05$ and $p<0.01$, respectively) than those of control subjects and returned to the control value after treatment. The mean pretreatment value of manganese was significantly lower ($p<0.001$) than the control value and became still lower ($p<0.01$) after treatment. The estimation of the trace metals in cerebrospinal fluid of patients with viral meningitis is very helpful to determine the diagnosis and prognosis.

There is a substantial published literature on concentrations of trace metals in the blood of patients with various diseases, but little information is available on concentrations of metals in cerebrospinal fluid (CSF) of patients with viral meningitis. In an attempt to determine if CSF trace metal concentrations are of value in diagnosis and prognosis, we measured concentrations of zinc, copper, and manganese in CSF of patients with viral meningitis before and after treatment.

MATERIALS AND METHODS

Patients

Ten patients who required a spinal tap on the Paediatric Service at Chugoku Rosai Hospital, Kure city, Hiroshima, were studied from June through August, 1983. Five male and five female patients with viral meningitis aged $4^{7}/_{12}$ to $12^{8}/_{12}$ years were studied with consent of their parents. The diagnosis of viral meningitis was established on the basis of benign clinical course, negative results of cultures of CSF, and predominantly lymphocytic pleocytosis in CSF. CSF findings became normal after treatment. We obtained 11 specimens of spinal fluid from age-matched control subjects.

Sample collection and analysis

Two ml of CSF were obtained and collected in acid-rinsed and metal-free glass tubes. All glassware was tested for contamination. CSF samples containing blood were discarded. After centrifugation at 1,300g for 5 min, the supernatant was obtained and stored at −20°C until the analysis. All determination was made with a model AA-8500 Atomic Absorption Spectrophotometer equipped with FLA-100 Flamless Atomizer (Nippon Jarrell-Ash, Kyoto, Japan). Analytical conditions were shown in Table. The background absorption was automatically cor-

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Table Atomic absorption spectrophotometer instrument parameters for zinc, copper, and manganese analyses.

Material	Zinc	Copper	Manganese
Absorption wave length	213.9 nm	324.8 nm	279.5 nm
Lamp current	8 mA		
Heating program			
Dry ramp mode	22A,30s	20A,10s	20A,30s
Ash ramp mode	30A,30s	40A,20s	40A,35s
Atomize step mode	150A,10s	250A,10s	240A,10s
Argon gas flow	3.0L/m		
Cuvette	tube type		
Sampling volume	10 μl		

rected by the hollow cathode D_2 lamp. One thousand μg/ml standard solutions of zinc, copper, and manganese (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used to prepare the standard curves. Average absorbances of duplicate pipettings were compared with the standard curve. Zinc, copper, and manganese concentrations were calculated by linear regression lines. The recovery was approximately 93.2% for zinc, 92.5% for copper, and 95.8% for manganese. The coefficient of variation (CV) for the assay was approximately 2.3% for zinc, 2.2% for copper, and 2.7% for manganese. The protein concentration in CSF was determined by the Lowry method[5].

RESULTS

Figure shows the concentrations of zinc, copper, and manganese in CSF of patients with viral meningitis. There was no significant correlation between each metal concentration and the cell number, protein or sugar concentrations in CSF. This is in agreement with the previous report that there is no significant correlation between CSF protein and CSF calcium or zinc[1]. Normal values (per mg protein) were as follows: CSF zinc, 60.5 ± 22.1 ng (mean ± SD); copper, 81.4 ± 30.0 ng; and manganese, 5.39 ± 1.53 ng. The mean metal concentrations (per mg protein) in the viral meningitis group were compared between before and after treatment and were as follows: CSF zinc, 38.8 ± 17.4 and 54.1 ± 16.9 ng; copper, 44.7 ± 25.1 and 71.8 ± 46.6

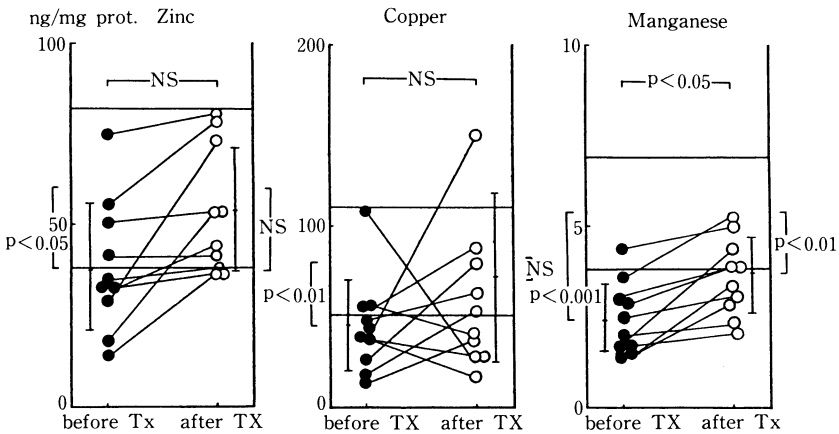

Fig. Zinc, copper, and manganese concentrations in cerebrospinal fluid of patients with viral meningitis before and after treatment.
a) Values are the mean ± 1 SD. b) NS : not significant

ng; and manganese, 2.47 ± 0.93 and 3.67 ± 1.10 ng. The mean pretreatment values of CSF zinc and copper were significantly lower ($p<0.05$ and $p<0.01$, respectively) than the value in control subjects and returned to the control value after treatment. The mean pretreatment value of CSF manganese was significantly lower ($p<0.001$) than the control value and became still lower ($p<0.01$) after treatment.

DISCUSSION

The normal range of protein concentrations (per liter) in CSF is 200-450 mg[1]. The normal values of trace metals (per liter) in CSF are as follows: zinc, 30 μg; copper, 40 μg: and manganese, 0.83-1.50 μg[4,6]. Therefore, the normal values per mg protein are as follows: zinc, 67-150 ng; copper, 89-200 ng; and manganese, 1.84-7.50 ng. The mean metal concentrations in CSF we found are within these limits but are relatively low.

Among several trace metals, zinc plays an essential role in many vital enzymes, including DNA polymerase[15], DNA dependent RNA polymerase[14], and thymidine kinase[12]. Inasmuch as these enzymes are important for nucleic acid and protein synthesis and cell division, zinc appears to be essential for the integrity of host defense mechanisms.

Acrodermatitis enteropathica, a well-known disorder of zinc metabolism, has a high incidence of candida and bacterial infections and defects of cellular immunity such as chemotaxis, lymphocyte transformation with mitogens and a depression in T cell numbers which can be corrected by the addition of zinc[9,20]. Moreover, patients with Down's syndrome turn out to have a low serum zinc level and an immune deficiency characterized by depressed neutrophil chemotaxis, skin hypersensitivity and lymphocyte responsiveness to PHA, which are all corrected by the administration of zinc[2].

The changes of zinc metabolism have been observed in various infections. The serum zinc concentrations in patients with systemic bacterial or parasitic infections are low. Whereas in relatively localized bacterial infections such as cellulitis or pyelonephritis, the zinc concentrations are low but not so low as that observed with systemic bacterial infections. In contrast, viral infections produce only a slight decrease in the serum zinc concentrations[19]. These observations support the assumption that the change of serum zinc concentrations is the earliest indicators of the systemic infections. Furthermore, in an acute phase the serum zinc concentrations of patients with mucocutaneous lymph node syndrome (MCLS) decrease but increase in a subsiding phase[16]. This change seems to be under the influence of low serum albumin. The mechanism by which zinc influences immunity is not clear. A number of factors including blockage of cell membrane receptors, changes in fluidity of membrane components, interference with the cell microskeleton such as microtubules and microfilaments, and antagonizing cations may play a pathogenetic role[3].

Patients with Menke's disease, an inherited defect of copper metabolism resulting in copper deficiency, may suffer from increased susceptibility to infections[10]. The serum copper concentrations in patients with Hodgkin's disease fall dramatically in the period prior to the herpes zoster attacks and increase afterwards[17]. It is probable that the drop in serum copper parallels a process of defense mechanisms. In volunteers with typhoid fever, the serum copper concentrations rise to extremely high values in the course of infection[11]. The importance of trace-metal monitoring during infection is proposed. In acute phase the serum copper concentrations of patients with MCLS increase but return to normal in the subsiding phase[16]. Alterations in serum ceruloplasmin may play an important role in the serum copper concentrations. A decreased number of antibody-producing cells has been observed in mice with copper deficiency[8]. These findings suggest that copper may be necessary for immunocompetence.

During the active phase of acute hepatitis, serum manganese concentrations invariably increase but become normal during the subsiding phase[18]. An increased value of the serum manganese can be an index to liver cell damage. Experimental animals fed with a manganese-deficient diet show defective antibody formations[8]. This seems to support the view that adequate manganese nutriture is necessary for normal antibody productions. Although there is now some understanding of the role of trace metals in infectious diseases, very little is known about their role in CSF.

Under normal circumstances, it is generally accepted that the brain extracellular fluid and CSF are formed by both simple diffusion and active transport. The rate at which various substances penetrate the blood-brain barrier depends on their molecular weight, polarity, and metabolic demand. Under pathological circumstances, selective changes in trace element concentrations in CSF may occur independently of their concentrations in blood.

It is necessary to explain decreased concentrations of CSF zinc, copper, and manganese of patients with viral meningitis.

Superoxide dismutase (SOD), containing these three metals, is a biologically important enzyme whose function is to protect the cells against the free radical superoxide anion[7]. The polymorphonuclear leucocytes (PMNs) of children with virus pneumonia exhibit a decreased SOD activity[13]. It is possible that viral infections inhibit the SOD activity in PMNs by a nonspecific effect so far unknown. Since SOD generates bactericidal hydrogen peroxide and regulates the release of the toxic superoxide radical into the surrounding tissues, the alteration of blood and/or CSF SOD may be involved under the acute inflammatory conditions. Trace metal concentrations in CSF could also change if metalloenzyme such as SOD or cations leaked into CSF from damaged brain tissue. This may be the cause of the decreased CSF zinc, copper, and manganese concentrations that we found in viral meningitis patients.

The estimation of the CSF trace metals of patients with viral meningitis is very important and helpful to determine the diagnosis and prognosis.

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