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Decreased Plasma and Cerebrospinal Fluid Ascorbate Levels in Patients With Septic Encephalopathy

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Septic encephalopathies rapidly affect brain function without the involvement of a specific area causing a broad range of reversible neurologic symptoms. Capillary leakage including dysfunction of the blood–brain barrier has been proposed as a potential pathogenic mechanism in this entity. We tested the hypothesis that oxidative stress measured in plasma and cerebrospinal fluid (CSF) of patients suffering from septic encephalopathy could be linked to the neurologic symptoms of the disease. The neurologic symptoms of eleven patients with septic encephalopathy were described semiquantitatively through a score system. The ascorbate levels were significantly lower in both plasma and CSF from patients with septic encephalopathy than controls, and in CSF but not plasma this decrease correlated with the severity of neurologic symptoms. No significant changes were found for α-tocopherol. Our findings suggest that the short-term oxidative stress may be an important factor in the development of septic encephalopathy, possibly through dysregulation of the blood–brain barrier.

Keywords: Sepsis syndrome; Blood–brain barrier; Oxidative stress; Cerebrospinal fluid; Plasma

INTRODUCTION

Septic encephalopathies occur in between 50 and 62% of septic patients and are associated with a high mortality ranging between 33 and 39%.[1] Some have even reported an incidence of more than seventy percent with a mortality of 47%.[2] They are characterized by a rapid but usually reversible symptomatology due to a diffuse affection of brain function without involvement of a specific area.[3] Alteration of mental status is the fundamental neurologic abnormality; paratonic rigidity, the most common motor sign, is present in about 30% of encephalopathic patients. Less frequent are static tremor, multifocal myoclonus, asterixis and focal signs such as hemiparesis, gaze palsy or oculomotor disturbance.[2] A quantification of this complicated clinical picture is desirable for the classification of the stage of the disease. Electroencephalographic (EEG) recordings may help to determine the class or general category of the disease process,[4] but they are not specific for its etiology and standard EEGs do not record brainstem activity.[5]

Several hypotheses have been proposed for the pathogenesis of the encephalopathy. Production of capillary leakage possibly mediated by cytokines is a central element in many of them and there is evidence that the blood–brain barrier becomes leaky at an early stage of sepsis[3] and amino acid transport across it may be disturbed.[6] Dysfunction of the blood–brain barrier could further alter the chemical milieu of central neuronal cells and evoke a functional disturbance of the brain.[3] However, none of these pathophysiologic concepts has been proven to be operative to date.

Excessive production of highly reactive free radicals derived from oxygen can lead to the damage

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TABLE I Hamburg encephalopathy score; range 0–13 (0, best; 13, worst)

Item	Score	
Vigilance level	Coma	4
	Sopor	3
	Somnolescene	2
	Altered sleep wake cycle	0
Intended or explorative movements	No	1
	Yes	0
Pupil width	Dilated or miotic	1
	Normal (2–5 mm)	0
Oculomotor findings, nystagm, oculo- cephalic reflex	Pathologic	1
	Normal	0
Vegetative signs (sweating, broncho-spasm, or hypertension)	Yes	1
	No	0
Hyperkinesia, rigor, myoclonus, muscle	Pathologic	1
tonus, or tremor	Normal	0
Epileptic fits	Yes	1
	No	0
Dysarthria	Yes	1
	No	0
Deep tendon reflexes	Pathologic	1
	Normal	0
Palmomental reflex	Positive	1
	Negative	0

of cell surface proteins, lipids, and signal transduction into the cell as well as disturbance of cytoskeletal organization.[7] Sepsis can lead to oxidative stress through activation of the immune system and subsequently to the production of reactive oxygen species.[8] Protein carbonyl measurements show evidence of early oxidation of plasma proteins in sepsis patients.[9] An inflammatory cascade is triggered by sepsis which also includes pro-inflammatory cytokines; these are known to cause increases in blood–brain barrier permeability.[10] Given the possibility of a leaky blood–brain barrier in septic encephalopathy and its clinical resemblance to a rapid but reversible neurodegenerative process it is reasonable to argue that reactive oxygen species from the blood of septic patients could get access to the brain and evoke neurotoxic effects there.

In order to test this hypothesis, we used methods for the assessment of the level of oxidative stress in both plasma[11] and CSF.[12] Using ascorbic acid[13] and α-tocopherol[14] as indirect indicators of oxidative stress we correlated the results with a semiquantitative score for the neurologic symptoms of septic encephalopathy.

MATERIALS AND METHODS

Subjects

Patients suffering from septic encephalopathy ($n = 11$) and control subjects ($n = 14$) were recruited from two intensive care units in the neurological clinic and the medical clinic of the University Hospital Hamburg-Eppendorf. Septic patients were diagnosed as encephalopathic by ruling out other organic causes for the encephalopathy through standard procedures including CSF and blood examinations as well as cranial computer tomographic (CCT) scanning and EEG recordings. We developed a clinical score system for the semiquantitative description of the neurologic symptoms in septic encephalopathy, which we termed Hamburg Encephalopathy Score (HES) (Table I). Scoring of neurologic symptoms using the HES was performed on a daily basis. The control subjects attended the neurological clinic and underwent lumbar puncture for diagnostic purpose, but no disease was diagnosed in any of them.

Sample Collection and Preservation

From each patient, 1 ml of CSF (obtained as a surplus of diagnostic lumbar puncture) and 10 ml of ethylenediaminetetraacetic acid—anticoagulated blood were sampled at the same visit. CSF was immediately placed on dry ice; only the last fraction of the punctuate, which did not show any visible traces of blood, was taken. Blood was placed on ice and centrifuged at 4°C for 10 min at 2500 rpm to obtain plasma which was freshly frozen at −80°C. For ascrobate measurements an equal volume of fresh 10% *m*-phosphoric acid was added to 800 µl of plasma for deproteinization and centrifuged for 10 min at 4700 rpm; the supernatant obtained was also freshly frozen at −80°C. All samples were stored at −80°C not later than 60 min after puncture. The samples were thawed at room temperature immediately before analysis.

TABLE II Antioxidants in Plasma and CSF of encephalopathic patients and control subjects

	Patients ($n = 11$)	Controls ($n = 14$)
Plasma		
Ascorbate (μM)	18.8 ± 11.4*†	75.5 ± 5.8
α-Tocopherol (μM)	27.2 ± 3.5‡	26 ± 1.4
CSF		
Ascorbate (μM)	65.8 ± 9.8***	218 ± 11.5
α-Tocopherol (nM)	44.2 ± 8.2	58.1 ± 7.6

*$p < .05$; ***$p < 0.001$ vs. control group. † $n = 4$. ‡ $n = 9$.

Antioxidants

CSF and plasma ascorbate was measured photometrically at 520 nm after its reaction with 2,6-dichlorophenolindophenol.[13] Alpha-tocopherol and ubiquinol-10 were measured as lipophilic antioxidants in plasma. For CSF, only the levels of α-tocopherol were determined. Determination of ubiquinol-10 was not possible in CSF due to insufficient sensitivity of the measurements. All lipophilic antioxidants were quantified by reversed-phase high-performance liquid chromatography (HPLC) with electrochemical detection as described elsewhere,[15] except that the system was calibrated using an external standard method.

Plasma Lipids

Plasma total cholesterol and triglycerides were quantified by commercially available enzymatic kits (Boehringer Mannheim, Mannheim, Germany).

Statistical Analysis

Differences between the patient and control groups were analyzed using ANOVA with age as a covariable. Pearson's moment-product correlation coefficients were calculated to evaluate relationships between variables. Spearman's rank correlation coefficient was calculated to elucidate the extent to which they were specifically influenced by the neurologic symptomatology. All results are expressed as means ± S.E.M. The quality of the assays was controlled by measuring the assay variability, which was not higher than 8% for all the parameters measured.[8,9]

RESULTS

Characterization of Patients

Eleven patients (five males and six females) were diagnosed as encephalopathic at between 5 and 32 (mean 14.5 ± 9) days after the onset of sepsis. Causes of sepsis varied from peritonitis, enteritis, endocarditis, and urogenital infection to pneumonia which was the most frequent (5/11). Sedation and other conditions potentially involved in the neurologic symptoms of septic encephalopathy were excluded using blood, CSF, cerebral computerized tomography, and EEG examinations. EEG findings were all pathologic according to the classification for metabolic and septic encephalopathies as has been established by others.[5] Neurologic symptomatology was assessed semiquantitatively using the Hamburg Encephalopathy Score (HES) on a daily basis up to discharge (4/11) or death (7/11).

The patients were significantly older than control subjects (63.1 ± 4.2 vs. 46.9 ± 3.9 years, $p < 0.01$). Therefore, all between-group-differences were calculated using ANOVA with age as a covariable as described above.

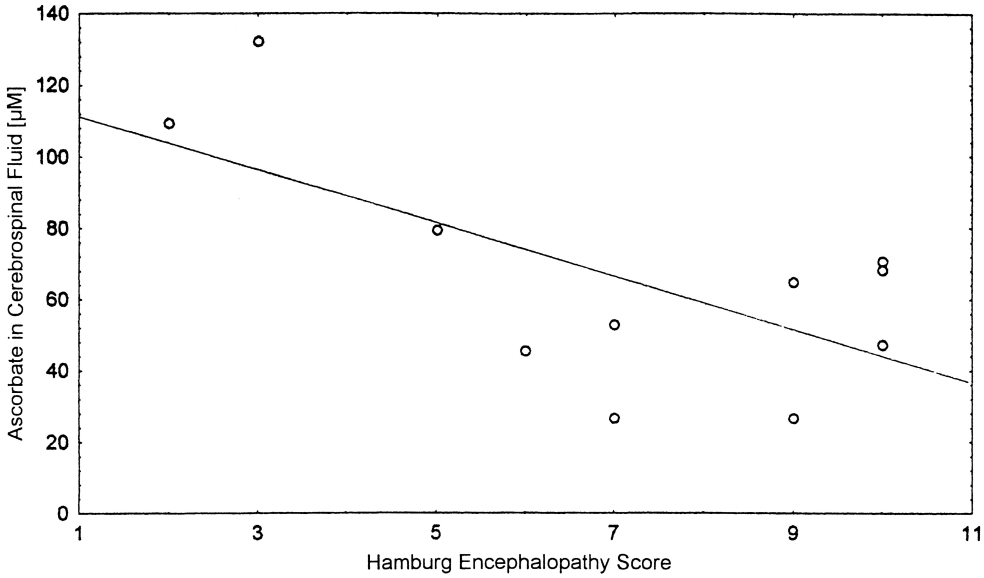

FIGURE 1 Correlation of CSF ascorbate with Hamburg Encephalopathy Score $p < 0.05$, $r = -0.65$.

Oxidation Parameters in Plasma

The levels of hydrophilic and lipophilic antioxidants were measured in the same samples (Table II). Ascorbate was significantly ($p < 0.05$) lower in the patients with septic encephalopathy compared to controls. In contrast, α-tocopherol was similar in encephalopathic patients and controls. Lipid-normalized antioxidants showed similar between-group differences (data not shown). Total cholesterol was significantly lower (112 ± 13.6 vs. 207.9 ± 10.6 mg/dl) and triglycerides tended to be higher (175.1 ± 23.3 vs. 147.8 ± 24 mg/dl) in patients than controls. Neither ascorbate nor α-tocopherol correlated significantly with neurologic symptomatology as determined by the HES.

Oxidation Parameters in CSF

The levels of ascorbate and α-tocopherol were determined in the same samples (Table II). Here, ascorbate was again significantly lower in the encephalopathic patients compared to controls ($p < 0.001$). Alpha-tocopherol concentration was not significantly lowered. There was a significant negative correlation of ascorbate level with the HES ($r = -0.65$, $p < 0.05$) linking ascorbate consumption in CSF with more severe neurologic symptomatology (Fig. 1). In contrast, CSF α-tocopherol concentration did not correlate with the HES.

DISCUSSION

The hydrophilic antioxidant ascorbate was found to be significantly lower in both plasma and CSF of patients suffering from septic encephalopathy compared to control subjects. In CSF this decrease correlated with the severity of neurologic symptoms. In contrast, in plasma no decrease of the lipophilic antioxidant α-tocopherol was detected in the patient group. The same held true for α-tocopherol in CSF. However, the difference in the average age between the patients and the controls is a limitation of this study.

Ascorbate seems to be of special relevance for the protection from oxidation of lipoproteins in CSF, as its concentration is three to five times higher in CSF than in plasma.[16] It is the antioxidant consumed first during *in vitro* CSF oxidation.[12] Oxidative stress is generally increased during sepsis.[8] Electron paramagnetic resonance studies following spin trapping gave evidence for high free-radical concentrations in the blood of septic patients.[17] Serum taken within the first ten days of sepsis has been shown to have a higher level of lipid peroxidation and a lower antioxidative capacity.[9,18] This may explain why ascorbate but not α-tocopherol levels were low in the plasma of patients who became diagnosed at a relatively early stage (mean 14.5 ± 9 days) of sepsis.

The interaction between pro-inflammatory cytokines, oxygen free radicals and other mediators of the inflammatory cascade triggered by sepsis leads to a loss of endothelial integrity.[10] The blood–brain barrier can be damaged by highly reactive free radicals.[19] Dysfunction of the blood–brain barrier during sepsis in general has been shown by others;[20] however, evidence for its role specifically in septic encephalopathy came only from rats.[6,21] Our findings support such a model in humans where oxidative stress compromises the blood–brain barrier. Dysregulation of the blood–brain barrier may allow neurotoxic agents to get access directly to central neuronal cells.[3]

The modulation of protein structure by reactive oxygen species has been found to be reversible in some cases depending on the modifying agent;[22] this could also contribute to the reversibility of clinical symptoms. However, we cannot discriminate between cytotoxicity which might also involve excitotoxicity[23]—and dysfunction of the blood–brain barrier in this work; both may be reversible. Testing for the cytotoxicity of the CSF from our patients is presently underway.

The present study suggests that the oxidative stress may be an important factor in the development of septic encephalopathy. This not only gives new insights into the pathophsiology but might also allow for therapeutic options using antioxidants in a condition which otherwise may take several months to reverse.

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