Adenosine and Neopterin Levels in Cerebrospinal Fluid of Patients with Neurological Disorders


We determined the cerebrospinal fluid (CSF) levels of adenosine, a mediator of cerebral blood flow regulation, and neopterin, a macrophage-producing compound, in patients with neurological disorders. Compared to control subjects, the adenosine levels were significantly increased in the patients with acute-stage cerebral infarction (n=12, p<0.0001), acute meningitis (n=10, p<0.0001), or amyotrophic lateral sclerosis (ALS, n=12, p<0.05) (Mann-Whitney U-test). The neopterin levels were significantly increased in the 41 patients with human T-lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis (HAM/TSP, p<0.0001), acute meningitis (p<0.0001), ALS (p<0.05) (Mann-Whitney U-test), or acute-stage cerebral infarction (p<0.005, Student’s t-test). In the analysis of 41 HAM/TSP patients, the neopterin levels were significantly correlated with the cell number and glucose levels in the CSF, and were a sensitive marker of inflammation. Several of the HAM/TSP patients with increased adenosine levels were probably complicated with other diseases. The increased neopterin levels in the HAM/TSP group persisted, suggesting that the mononuclear cellular infiltration remained for a long time.

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Key words: determination, cerebral infarction, human T-lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), meningitis, amyotrophic lateral sclerosis, neuropathy

Introduction

Adenosine is a mediator of the metabolic regulation of cerebral blood flow (CBF) and may be a critical factor in this regulation (1-3). Brain adenosine levels have been shown to rise in the brain interstitial fluid or cerebrospinal fluid (CSF) soon after the onset of total ischemia and hypoxia (4-13). Adenosine in CSF is thought to be responsible for the cerebral vasodilation which increases CBF, because a ventriculocisternal perfusion of adenosine causes an increase in CBF (14), and because the instillation of adenosine deaminase or a potent adenosine receptor blocker, 8-phenyltheophylline (8-PT) or theophylline into CSF reduces the hypoxic and hypercapnic dilatation or rat pial arterioles or CBF, respectively (3, 15).

Adenosine is also a potent neuromodulator of synaptic transmission that may function as an endogenous anticonvulsant (16). Adenosine triphosphate (ATP) is co-released with neurotransmitters from synaptic terminals (17, 18) and is hydrolyzed to adenosine in the synaptic cleft (19). Therefore, adenosine may be released in abundant amounts with the release of neurotransmitters in brain ischemia (20, 21). High levels of adenosine in the CSF of patients with status epilepticus have been reported (22). In the brains of patients with meningitis, ATP depletion takes place, and subsequently the degradation products including adenosine increase (23). In pathological conditions such as brain ischemia, hypoxia, and injury, the CSF adenosine levels are presumed to increase. There are a few reports which measured CSF adenosine levels in patients with cerebral thrombosis. In the present study, to determine whether CSF adenosine levels are increased in neurological disorders and whether the CSF adenosine level contributes to the neurological disorders, we examined the adenosine levels in CSF samples from patients with varying neurological disorders. We also measured the inosine, xanthine and hypoxanthine levels in the CSF, quantified together with adenosine levels by the same high-performance liquid chromatography (HPLC) to determine whether these levels are increased like those of adenosine.
Neopterin, a pyrazino-pyrimidine compound biosynthesized from guanosine triphosphate in macrophages, is an in vitro indicator of immune system activation (24–26). Increased neopterin levels in the CSF indicate a mononuclear cellular infiltration of the central nervous system (CNS). We determined the CSF levels of neopterin in neurological disorders including human T-lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (27), amyotrophic lateral sclerosis (ALS) and cerebral infarction. Increased CSF neopterin levels in patients with HAM/TSP have been reported (28). HAM/TSP is a chronic progressive HTLV-I infectious myelopathy that presents clinically as paraplegia with neurogenic bladder and mild sensory disturbances, and is characterized by an activated T-lymphocyte infiltration of the spinal cord (29). Herein, we examined the sensitivity of CSF neopterin levels as a marker of CNS inflammation by comparing the neopterin levels with the cell numbers, protein levels, and glucose levels in the CSF of patients with HAM/TSP. We also investigated whether the adenosine and neopterin levels in the same CSF samples to contribute the diagnosis of HAM/TSP.

Subjects and Methods

Our subjects were inpatients at the Kagoshima University Hospital or Kikuno Hospital in Kagoshima Prefecture, Japan. Control CSF samples were obtained from patients with a bone fracture of the leg and without neurological disorders \[n=39, 55±18 \text{ years (mean±SD), 15–87 \text{ years (12 men and 27 women)}}\] when they received spinal anesthesia for surgery. All patients and control individuals gave their informed consent to all procedures. The patient CSF samples which were collected to check the anti-HTLV-1 antibody titer for the diagnosis of HAM/TSP or HTLV-I-related diseases were used for this study. The CSF was collected from patients with HAM/TSP \(n=41, 8 \text{ men, 33 women, 61±11 \text{ years, 40–76 \text{ years}})\} \) who were ambulatory (walking with or without a cane); patients with acute-stage cerebral infarction \(n=12, 64±9 \text{ years, 52–79 \text{ years}}) \) who were diagnosed as having a cardiogenic cerebral embolism or atherothrombotic cerebral infarction and had hemiplegia and consciousness loss (somnolence or more serious loss) 2 or 3 days after the onset; patients with chronic-stage cerebral infarction \(n=9, 69±13 \text{ years, 53–88 \text{ years}}) \) who had had hemiplegia due to a cardiogenic cerebral embolism or atherothrombotic cerebral infarction more than one month after the onset; patients with acute meningitis \(n=10; 3 \text{ tuberculous, one cryptococcal, } 4 \text{ viral, and } 2 \text{ bacterial causes; 43±20 \text{ years, 14–72 \text{ years}})\} \) CSF cell number, 299±399/μl, 7.3–918/μl; CSF protein levels, 203±247 mg/dl, 21–850 mg/dl; CSF glucose levels, 71±39 mg/dl, 39–162 mg/dl); patients with ALS \(n=12, 60±8 \text{ years, 45–69 \text{ years}}) \) who were diagnosed by neurological status and the findings of electromyography, and patients with polyneuropathy \(n=9, 54±15 \text{ years, 16–70 \text{ years}}) \) consisting of hereditary motosensory neuropathy and toxic neuropathy. To determine the adenosine level, 20 μl of concentrated perchloric acid was added to one ml of CSF to inactivate adenosine deaminase soon after the CSF was drawn. The first 2 to 3 ml of CSF was discarded and the second 2 to 3 ml was used to avoid the effect of artificial bleeding. The cell count, protein and glucose assays were performed in the clinical laboratory of Kagoshima University Hospital. The CSF samples for adenosine determination were neutralized with 100 μl of 2 M KHCO₃ and centrifuged at 3,000 rpm for 10 minutes. The supernatant was kept at -20°C until the assay. Within 2 weeks, the samples were thawed and subjected to HPLC (JASCO880-PU with 820-FP and 875-UV, Japan Spectroscopic Co., Tokyo) in an apparatus equipped with an isocratic cation-exchange column, SP-25W 4.6×250 mm (Toyo Co., Tokyo) using an elution buffer of 185 mM Na acetate, pH4.2, according to the method of Morimoto et al (30). The inosine and hypoxanthine-xanthine levels in the CSF were determined together with the adenosine levels by HPLC (hypoxanthine and xanthine could not be separated in this HPLC system).

The neopterin level in the CSF was determined by the method of Fukushima and Nixon (31). For this assay system, CSF samples were kept at -20°C without the addition of the concentrated perchloric acid, and determinations were carried out within 2 weeks of the sample collection. Chemicals were obtained from Wako Pure Chemical Industry (Osaka). Statistical analyses were performed with a Macintosh computer (Apple) using Statview version 4.5 software (Abacus Concepts, Inc., Berkeley, CA). Student's t-test was used when a significant F ratio was not found in the analysis of variance (ANOVA). Otherwise, Mann-Whitney U-test was performed for each variable. The significance level was set at \(p<0.05\).

Results

One, four, or seven nmol of adenosine was added to each 1ml of CSF containing 0.49 nmol/ml of adenosine, and the mean recovery rates were 93.5±10.2% 100.1±2.0%, and 95.2±4.5% in 5 determinations, respectively. The coefficients of variability were 10.9%, 3.4%, and 4.1% in the five consecutive determinations of CSF containing 1.2 nmol/ml, 3.6 nmol/ml, 6.8 nmol/ml each of adenosine, respectively.

The adenosine levels were 0.55±0.22 nmol/ml in the control subjects (there was no significant difference between the levels of the men and those of the women), 1.02±1.25 nmol/ml in the patients with HAM/TSP, 2.71±1.09 nmol/ml in the patients with acute-stage cerebral infarction, 0.71±0.22 nmol/ml in the patients with chronic-stage cerebral infarction, 1.58±0.58 nmol/ml in the patients with acute meningitis, 0.95±0.51 nmol/ml in the patients with ALS, and 0.47±0.20 nmol/ml in the patients with polyneuropathy (Fig. 1).

There were significant differences in adenosine levels between the control subjects and the patients with acute-stage cerebral infarction \(p<0.0001\), those with acute meningitis \(p<0.0001\), and those with ALS \(p<0.05\) as shown by the Mann-Whitney U-test; however, no significant difference was recognized between the control subjects and the patients with HAM/TSP by this test, or between the controls and the patients with chronic-stage cerebral infarction or polyneuropa-
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Adenosine (nmol/ml)

<table>
<thead>
<tr>
<th>Controls</th>
<th>HAM/TSP</th>
<th>Acute Cl</th>
<th>Chronic Cl</th>
<th>Meningitis</th>
<th>ALS</th>
<th>Neuropathy</th>
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Controls indicates the group of patients with a leg bone fracture and without neurological disorders (n=39); Chronic Cl, patients with chronic-stage cerebral infarction (n=9); Neuropathy, patients with polyneuropathy (n=9).

Figure 1. Cerebrospinal fluid (CSF) adenosine levels in the controls and patients with various neurological disorders. The adenosine levels were increased in some patients with HAM/TSP (n=41), in those with acute-stage cerebral infarction (Acute Cl, n=12, **p<0.0001), in those with acute meningitis (Meningitis, n=10, **p<0.0001), and in those with amyotrophic lateral sclerosis (ALS, n=12, *p<0.05) as shown by the Mann-Whitney U-test. Controls indicates the group of patients with a leg bone fracture and without neurological disorders (n=39); Chronic Cl, patients with chronic-stage cerebral infarction (n=9); Neuropathy, patients with polyneuropathy (n=9).

Neopterin (pmol/ml)

<table>
<thead>
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Among the patients with meningitis, Patient No. 1, 16 years old (y/o) had bacterial meningitis and the CSF value of cell number 249/jL/l and the protein level 152 mg/dl; No. 2, 72 y/o, viral, cell 36/jL/l, protein 159 mg/dl; No. 3, 43 y/o, tuberculous meningitis, cell 918/jL/l, protein 342 mg/dl; No. 4, 52 y/o, viral, cell 7.3/jL/l, protein 36 mg/dl; No. 5, 63 y/o, tuberculous meningitis, cell 1,143/jL/l, protein 850.4 mg/dl; No. 6, 33 y/o, tuberculous meningitis, cell 45/jL/l, protein 124 mg/dl; No. 7, 62 y/o, viral, cell 166.7/jL/l, protein 210 mg/dl; No. 8, 14 y/o, bacterial, cell 249/jL/l, protein 45 mg/dl; No. 9, 46 y/o, cryptococcal, cell 124.7/jL/l, protein 88.3 mg/dl; No. 10, 24 y/o, viral, cell 48/jL/l, protein 21 mg/dl. Chronic Cl, n=9; Neuropathy, n=9.

The regression analyses of the relation of adenosine or neopterin levels with the CSF cell numbers, protein levels, or glucose levels in the HAM/TSP patients were performed and the coefficients of correlation (r) of the adenosine levels to the CSF cell numbers, protein levels and glucose levels in the CSF samples were 0.024 (p=0.88, ANOVA), 0.099 (p=0.054), and 0.443 (p=0.053), respectively. Those of the neopterin levels to the CSF cell numbers, protein levels and glucose levels were 0.314 (p=0.046, Fig. 3), 0.297 (p=0.059, Fig. 4), and -0.470 (p=0.001).
Figure 3. The relation of CSF neopterin levels to CSF cell number in the 41 patients with HAM/TSP. The regression equation and coefficient of correlation (r) are shown; significance was found by the analysis of variance. The CSF neopterin levels were extremely increased and sensitive to the cerebrospinal cellular infiltration of HAM/TSP compared to the CSF cell number (normal, less than 5/μl).

Figure 4. The relation of CSF neopterin levels to CSF protein concentration in the 41 patients with HAM/TSP. The coefficient of correlation (r) was insignificant; however, the CSF neopterin levels were extremely increased and sensitive to the inflammation of HAM/TSP compared to the protein levels (normal, less than 40 mg/dl).

Figure 5. The relation of CSF neopterin levels to CSF glucose concentrations in the 41 patients with HAM/TSP. The coefficient of correlation (r) was significant. The CSF neopterin levels were more sensitive to the inflammation of HAM/TSP compared to the glucose levels.
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Figure 6. CSF adenosine levels compared to CSF neopterin levels. Open circles, control subjects (n=39); closed circles, patients with HAM/TSP (n=43); open triangles, those with acute-stage cerebral infarction (n=12); open rectangles, those with acute meningitis (n=10). Of the eight HAM/TSP patients, Nos. 1 to 8 with increased adenosine levels are described in the Results section.

Despite her normal serum calcium, phosphorus, and parathyroid hormone levels (32). Patients No. 4, 6, and 8 had adult-onset HAM/TSP. The case of Patient No. 7 was complicated with Sjögren’s syndrome.

In patients with HAM/TSP, the relationship between the duration of illness and the neopterin level was as follows: let the neopterin levels denote Y and years passed X; the regression equation is \( Y = -2.3X + 167.9 \) and \( r = 0.137 \) (p>0.1, n=60; in this analysis, the sample number of the patients with HAM/TSP was increased). The results indicated that the levels of neopterin in HAM/TSP patients are diverse and the monocytic infiltration remains for a long time after the onset.

Discussion

Adenosine levels in the CSF were intensely increased in the patients with acute-stage cerebral infarction and were significantly increased in the patients with acute meningitis or ALS, which might reflect the rapid ATP degradation induced by the destruction of the cortex or spinal cord. We determined the CSF adenosine levels in patients with acute-stage cerebral infarction with a serious disturbance of consciousness, because we speculated that the consciousness loss was correlated with the volume of the infarct and surrounding edematous area and with the decrease of cerebral blood flow. The adenosine level appears to be a marker of brain hypoxia or ischemia as well as cerebrospinal cellular injury. In the present patients with acute-stage cerebral infarction, the CSF levels of inosine, hypoxan-
count, the protein level, or the glucose levels in the CSF samples. Increased adenosine levels were found in the patients with HAM/TSP who had complications, while the other patients without increased adenosine levels had no complications. It is unknown whether the CSF adenosine levels are increased in the complications of Parkinson's disease, arterio-venous malformation, OYL, and Sjögren's syndrome, among others. A possibility that the adenosine levels were increased in the CSF samples of the non-complication patients with HAM/TSP remains; however, in light of the not very high adenosine levels in the patients with acute meningitis in whom severe inflammation was breaking out, such complications might cause the increased adenosine levels observed here in HAM/TSP patients.

In addition, the persistent increase of neopterin levels verified that HAM/TSP was a chronic type of progressive myelitis. The mononuclear cellular infiltration remained for a long time in patients with HAM/TSP, although it has been reported that the inflammation in the spinal cord is ameliorated naturally over time based on a study of CD4 and CD8 lymphocytic infiltration, in a histochemical study (29).

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References

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