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Original article

Profiles of blood biomarkers in alternating hemiplegia of childhood – Increased MMP-9 and decreased substance P indicates its pathophysiology

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Abstract

Alternating hemiplegia of childhood (AHC) is a rare disorder characterized by repeated plegic attacks, movement disorders, autonomic phenomena, and developmental delay. To obtain insights into the pathophysiology of AHC, we determined the concentrations of matrix metalloproteinase-9 (MMP-9), tissue inhibitor of MMP-1 (TIMP-1), calcitonin gene-related peptide (CGRP), and substance P (SP) in the serum/plasma of AHC patients ($n = 6$) and control subjects ($n = 11$) by performing enzyme-linked immunosorbent assay (ELISA).

Decreased levels of serum SP (382 ± 161 pg/ml), increased levels of plasma MMP-9 (111.0 ± 99.3 ng/mL) and increased MMP-9/TIMP-1 ratio (0.65 ± 0.44) were revealed, compared to those in control subjects (SP: 620 ± 223 pg/mL, $p < 0.05$; MMP-9: 33.5 ± 20.3 ng/mL, $p < 0.05$; MMP-9/TIMP-1 ratio 0.21 ± 0.09, $p < 0.005$). Serum CGRP levels in AHC patients (32.6 ± 14.4 pg/mL) were comparable to those in control subjects (37.0 ± 17.0 pg/mL). Increased MMP-9 levels may be linked to the vascular insult and is common in migraineurs. However, because AHC patients showed different changes in SP and CGRP levels compared to those shown by migraineurs, these results suggest that AHC has a pathomechanism different from the hypothesis of trigeminovascular theory. Decreased SP may represent the autonomic dysfunction in AHC, for which an etiology with progressive neuronal damage can be hypothesized.

Keywords: AHC; Autonomic dysregulation; Blood vessel; Cortical spread depression; ELISA; MMP-9; Neuropeptide; Substance P; TIMP-1

1. Introduction

Alternating hemiplegia of childhood (AHC) is a rare disorder characterized by repeated plegic attacks on one or both sides of the body, involuntary movements, cognitive impairment, and dysautonomic phenomena [1]. Since this episodic hemiplegia is reminiscent of hemiplegic migraine, AHC was initially considered a complicated migraine. The precipitating factors for hemiplegic attacks in AHC (e.g., emotional stress, cold temperature), flunarizine-induced effects, and the disappearance of symptoms upon sleeping are similar to those seen in migraine [2]. However, the additional static neurological symptoms, including cognitive decline and paretic

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fixation, are far more severe in AHC [3–5] than those that can accompany some cases with migraine [6,7]. Examination of the genes causing familial hemiplegic migraine has failed to identify causal genes for AHC [8,9].

The understanding of the pathophysiology of migraine has recently advanced based on the identification of biological markers. Cortical spreading depression, assumed to play a role in migrainous aura, may induce the release of matrix metalloproteinase-9 (MMP-9). MMP-9 then degrades the endothelial basal lamina and disrupts the integrity of the blood–brain barrier [10]. Further, unknown triggers for headache may activate perivascular trigeminal nerve terminals, which then release vasoactive neuropeptides including calcitonin gene-related peptide (CGRP) and substance P (SP). These peptides produce vasodilatation and plasma extravasation, eventually causing headache [11]. They are elevated in the blood of migraineurs even in headache-free periods [12–14]. Thus, examination of these substances in AHC patients would determine if there are common pathomechanisms for AHC and migraine, thereby explaining both common symptoms and the differences that are indicative of distinct pathomechanisms.

2. Materials and methods

2.1. Study subjects

Six AHC patients (age range, 4–31 years; mean ± SD, 13.3 ± 9.4; male:female, 5:1) were included in the study. Clinical data for 5 patients have been described previously [5]. All patients fulfilled the diagnostic criteria [1] of classic AHC. All patients had developed symptoms of episodic autonomic dysfunction such as facial pallor, flushed skin, and breathing abnormality. The conduction velocities of peripheral nerves had been examined in 5 patients and were normal. Four patients had experienced motor and cognitive deterioration (Table 1). No episodic plegic or autonomic attacks had emerged at least 72 h prior to blood collection. Eleven control subjects (5–32 years, 14.2 ± 9.0; male:female, 7:4) were also enrolled in the study. Among these, 5 had epilepsy, 3 had mental retardation, and the remaining 3 were healthy. No patient or control subject had ever taken drugs that elevate blood SP levels [15] or decrease blood MMP-9 levels [16]. No control had experienced a headache at least 72 h before blood samples were collected. The study methods were conducted in accordance with the Declaration of

Table 1
Characteristics of patients with alternating hemiplegia of childhood (AHC).

	Age	Sex	Motor skill	Speech	Progressive impairment	Autonomic impairment	NCV	MRI
1	29	F	3 years walked with support 14 years suffered paretic fixation 16 years became bedridden	3 years spoke words 16 years did not speak any words	Regression after SE at 16 years	16 years experienced flushed skin 16 years experienced apnea	4 years normal	10 years vermis atrophy 18 years high intensity area in the hippocampus 16 years small high intensity area in frontal white matter
2	11	M	2 years stood with support 10 years became bedridden	2 years spoke words 10 years did not speak any words	Regression after SE at 10 years	1 month experienced flushed skin 6 years experienced apnea	5 years normal	5 years mild cerebral atrophy
3	9	M	6 years walked with help 9 years was unable to stand	6 years spoke words 9 years did not speak any words	Regression after SE at 9 years	9 years experienced facial pallor 9 years experienced apnea	3 years normal	8 years high intensity area in the hippocampus
4	9	M	6 years stood with support 7 years became bedridden	1 years spoke words 7 years spoke few words	Regression after SE at 7 years	3 months experienced facial pallor 3 months experienced apnea	NE	3 years 2 months normal
5	15	M	3 years walked	3 years spoke words 5 years spoke sentences	(–)	15 years experienced facial pallor 15 years experienced apnea	3 years normal	15 years normal
6	4	M	3 years sat	Did not speak any words	(–)	4 months experienced apnea	9 months normal	9 months normal

NCV, nerve conduction velocity; NE, not examined; SE, status epileptics.