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Plasma Lactic Acid and Pyruvic Acid Levels in Patients with Chronic Primary Headaches during the Headache Free Period

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To study mitochondrial abnormalities in patients with chronic primary headaches, lactic acid (LA) and pyruvic acid (PA) levels in plasma were measured. Subjects were 14 patients with migraine [age, 38.0 ± 14.7 years (mean ± SD); male/female ratio, 6/8; 3 with aura and 11 without aura] and 17 patients with tension-type headache (TH) (age, 44.5 ± 19.5 years; male/female ratio, 8/9; 3, chronic and 14, episodic) during headache free periods; 12 healthy volunteers [age, 36.3 ± 8.4 years; male/female ratio, 6/6] served as controls. The plasma LA level was measured with the lactate oxidase-peroxidase method, and the plasma PA level, with the pyruvate oxidase peroxidase method. The migraine, TH and control groups showed 9.6 ± 5.0 mg/dL, 7.2 ± 5.8 mg/dL and 3.3 ± 1.9 mg/dL as the mean plasma LA, respectively; and 0.51 ± 0.30 mg/dL, 0.47 ± 0.45 mg/dL and 0.26 ± 0.20 mg/dL as the mean plasma PA, respectively. The migraine group showed significantly higher means for plasma LA and PA than the control group. The means in the TH group were lower than in the migraine group and higher than in the control group, without significant differences. These results may support the hypothesis that migraine patients have functional abnormalities in their mitochondrial energy metabolism.

Key words: energy metabolism; migraine; mitochondrial dysfunction; tension-type headache

Migraine and tension-type headache (TH) are common forms of chronic primary headaches. Approximately half of the patients visiting neurology clinics suffer from primary rather than non-organic headaches. The life prognosis of primary headache syndrome is essentially good; however, these headaches annoy patients and disturb their quality of life. The etiology of migraine and TH still remains uncertain. Some compounds are known to be useful in the management of headaches, but most therapeutic methods remain for symptomatic treatment. Studies aiming to reveal the pathophysiology and mechanism of headache and a search for relevant therapies should be carried out. Among the hypotheses on the etiology of migraine headache, we focused on one which describes mitochondrial dysfunction (Montagna et al., 1988, 1994; Bresolin et al., 1991; Sangiorgi et al., 1994; Watanabe et al., 1994). Plasma pyruvic acid (PA) and lactic acid (LA) levels can reflect anaerobic and aerobic glycolysis, and the energy metabolism in mitochondria. In this paper, to clarify whether or not patients with chronic primary headaches have mitochondrial abnormalities, we examined the PA and LA levels in the plasma of patients with migraine and TH during headache free periods.

Abbreviations: ANOVA, analysis of variance; 5-HIAA, 5-hydroxyindole acetic acid; IHS, International Headache Society; LA, lactic acid; MELAS, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes; PA, pyruvic acid; TH, tension-type headache

Table 1. Background of patients and healthy controls

	No. of subjects	Male/female ratio	Age*
Patients with migraine	14	6/8	38.0 ± 14.7
Patients with TH	17	8/9	44.5 ± 19.5
Healthy controls	12	6/6	36.3 ± 8.4

* Mean ± SD.
TH, tension-type headache.

Subjects and Methods

Subjects

Serial headache sufferers visiting the neurology clinic of Tottori University Hospital and fulfilling the diagnostic criteria were requested to participate in this study. Fourteen patients with migraine and 17 patients with TH gave their informed consent after they fully understood the nature and scope of this study. Twelve healthy persons volunteered for this study as healthy controls. The background of the participants are summarized in Table 1. The patients' headache characteristics and the accompanying symptoms are summarized in Table 2. No participants received any prophylactic medication for at least 1 month before blood sampling. All patients were free from the abortive use of drugs at least 5 days before blood sampling. Diagnostic criteria of migraine headache and TH were established in accordance with International Headache Society (IHS) criteria (Headache Classification Committee of the IHS, 1988). Thus, the participants were required to satisfy all of the following qualifications: i) fulfilling the IHS criteria for the diagnosis of migraine or TH; ii) giving informed consent; iii) free from daily basic medication for at least 1 month; and iv) free from temporary use of drugs at least 5 days before blood sampling.

Blood sampling

To exclude the influence of probable diurnal variation and exercise, we obtained 1.0 mL blood between 09:00 and 11:00 in the sitting position after more than 15 min rest, by puncture of the cubital vein with a 21 gauge needle and a 2.5 mL plastic syringe. Blood samples of 0.5 mL were immediately transferred to 1.5 mL Eppendorf tubes containing 0.5 mL of 0.8 N

Table 2. Patient characteristics of headaches with associated symptoms

		Migraine patient		TH patient	
		With aura	Without aura	Episodic	Chronic
No. of patients		3	11	14	3
Male/female ratio		1/2	5/6	6/8	2/1
Duration of headache	(year)	18.7	18.0	5.5	15.0
		[6.0–30]	[1.0–40]	[0.5–25]	[5.0–20]
Frequency of headache attacks	(/month)	1.1	4.8	2.7	20.0
		[0.2– 2]	[1.0–14]	[0.5–10]	[20.0]
Duration of an attack	(h)	20.0	3.0	41.6	*
		[12.0–24]	[0.3–24]	[2.5–96]	*
Associated symptoms	(%)				
Nausea and/or vomiting		33.3	57.1	20.0	0
Dizziness		33.3	42.9	10.0	33.3
Photophobia and/or phonophobia		33.3	71.4	14.3	0
Muscle stiffness of neck and shoulder		33.3	36.4	55.6	100.0

[ ], range.
TH, tension-type headache.
*This disease is chronic TH.

cooled perchrolic acid (Wako Pure Chemicals, Osaka, Japan). The tubes containing the samples were vortexed and kept on ice for few hours. Deproteinized supernatant plasma by centrifugation (1200 × *g* 15 min) was stocked at –20˚C until use for assay.

Estimation of plasma LA levels

Plasma LA levels were measured with the lactate oxidase-peroxidase method. All chemicals were purchased as a Determiner LA kit (Kyowa Medics, Tokyo, Japan). In brief, 6 µL each of patient plasma samples and 40 mg/dL of the standard plasma were taken into a 1.5 mL eppendorf tube, and then the *N*-ethyl-*N*-(3-methylphenyl)-*N'*-acetylethylenediamine solution was added and incubated at 37°C in a water bath for 3 min. A solution of peroxidase was added, and the mixture was incubated at 37°C in a water bath for 4.5 min. The color intensity (l max = 555 nm) was immediately measured at 660/600 nm by a dual length spectrophotometer, UV 160 (Shimadzu, Tokyo, Japan). The LA concentration was calculated using the following formula:

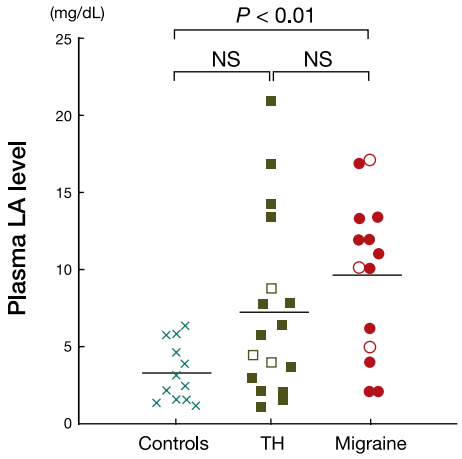

Fig. 1. Plasma lactic acid (LA) levels in groups of healthy controls (*n* = 12), patients with migraine (*n* = 14) and patients with tension-type headache (TH) (*n* = 17). The horizontal bar in each group represents the mean value. ×, healthy control; ○, migraine with aura; ●, migraine without aura; □, chronic TH; ■, episodic TH.

LA concentration (mg/dL) =
(Es/Estd) × standard plasma concentration,

where, Es = absorption of samples and Estd = absorption of the standard plasma. The assay coefficient variation was 5.0% and the measurement was linear up to 130 mg/dL. All samples were encoded and assayed in blind.

Estimation of plasma PA levels

The pyruvate oxidase-peroxidase method was used in measuring plasma PA levels. All chemicals were purchased as a Determiner PA kit (Kyowa Medics). All procedures and the calculation formula were essentially the same as the LA measurement except for the color intensity (l max=755 nm). All samples were encoded and assayed in blind.

Statistical analysis

Statistical analysis of data was performed with the one-way analysis of variance (ANOVA), the Student-Newmann-Kleus post hoc test and simple regression. *P* values less than 5% were considered to be significant throughout this study.

Results

Plasma LA levels

The migraine, TH and control groups showed 9.6 ± 5.0 mg/dL, 7.2 ± 5.8 mg/dL and 3.3 ± 1.9 mg/dL as the mean plasma LA, respectively. All values of patient samples and mean bars are illustrated in Fig. 1. The migraine group showed a significantly higher mean of plasma LA than the control group ($P < 0.01$ ANOVA, the Student-Newmann-Kleus post hoc test): the relation between the existence of aura symptoms and plasma LA levels was not significant. In the TH group, plasma LA levels varied from 1.04 mg/dL to 20.94 mg/dL, with the mean not significantly different from that in the migraine or control group. The plasma LA level showed no significant relation to the duration of headache or frequency of headache attacks.

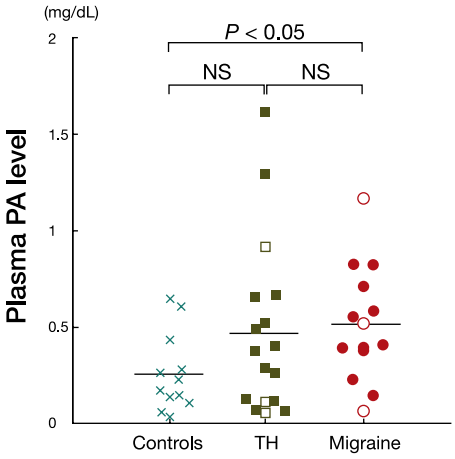

Fig. 2. Plasma pyruvic acid (PA) levels in groups of healthy controls (n = 12), patients with migraine (n = 14) and patients with tension-type headache (TH) (n = 17). The horizontal bar in each group represents the mean value. ×, healthy control; ○, migraine with aura; ●, migraine without aura; □, chronic TH; ■, episodic TH.

Plasma PA levels

The migraine, TH and control groups showed 0.51 ± 0.30 mg/dL, 0.47 ± 0.45 mg/dL and 0.26 ± 0.20 mg/dL as the mean plasma PA, respectively. Figure 2 shows all data of plasma PA in headache patients and controls with mean bars. The mean PA was significantly higher in the migraine group than in the control group ($P < 0.05$ ANOVA, the Student-Newmann-Kleus post hoc test).

Correlation between plasma PA and plasma PA

The plasma LA concentration was significantly correlated with the plasma PA concentration (simple regression: $r = 0.489$, $P < 0.01$) (Fig. 3).

Discussion

PA is synthesized in an anaerobic process of glycolysis from glucose or glycogen, which is known as the Embden-Myerhof pathway (Harper et al., 1939). Glucose enters into a glycolytic pathway by phosphorylation into glucose 6-phosphate, which is accomplished by the enzyme hexokinase and by an additional enzyme in the liver, glucokinase. ATP is required as a phosphate donor, and reacts as the Mg-ATP complex. Finally 1 mol of glucose produces 2 mol of PA and 2 mol of ATP following enzymatic steps. LA dehydrogenase converts PA to LA. PA enters a tricarboxylic acid cycle, which is an aerobic process of glycolysis, in the mitochondria. PA and LA levels in plasma increase in some pathological conditions such as hard exercise, hepatic and renal dysfunction, diabetes mellitus and mitochondrial diseases (Cohen and Simpson, 1975). Plasma LA and PA levels at rest can reflect congenital or acquired mitochondrial dysfunction.

Montagna and others (1988) first reported that during effort, blood lactate rises more significantly in patients with migraine than in controls and suggested impaired mitochondrial energy metabolism in migraine. They also reported that by using magnetic resonance spectroscopy (MRS) technique (Montagna et al., 1994), brain energy metabolism in migraine appears abnormal. Watanabe and others (1994) reported that MRS was performed in a woman with migraine attacks accompanied by right hemiplegia and responsive lesion of the brain who showed elevated LA levels in the left lobe. Our data presented here agree with earlier reports which suggest dysfunction of energy metabolism in migraine.

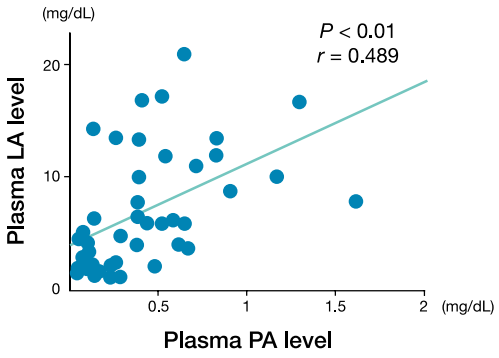

Fig. 3. Correlation between plasma lactic acid (LA) levels and plasma pyruvic acid (PA) levels in all subjects (n = 43).

Some congenital or hereditary mitochondrial diseases, including chronic progressive external ophtalmoplegia (Tome and Fardeau, 1986), mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) (Pavlakis et al., 1984; Montagna et al., 1988) and mitochondrial encephalopathy with ragged red fibers (Fukuhara et al., 1980) are identified mutations of mitochondrial DNA (Holt et al., 1988; Moraes et al., 1989; Goto et al., 1990; Kobayashi et al., 1990; Shoffner et al., 1990; Hammans et al., 1993). Patients suffering from MELAS, who bears a mutation of mitochondrial DNA at 3243 or 3271, frequently have migraines with severe headaches and vomiting (Hirano et al., 1992). Some members of MELAS families have only migraine-like headaches, but neither encephalopathy nor myopathy. One possibility is that migraine is one of the monosymptomatic forms of MELAS. On the basis of this possibility, there have been some efforts to identify mitochondrial DNA mutation in migraine. Bresolin and others (1991) reported a mitochondrial DNA deletion in one of 6 patients examined who suffered from migrainous stroke. Klopstock and others (1996) reported that they found no mitochondrial mutations in 23 patients with migraine. According to their observation, it is unlikely that migraine is a congenital mitochondrial disorder. They also concluded that their data were strongly against a hypothesis that some cases of migraine may be monosymptomatic forms of a MELAS syndrome.

If dysfunction of the mitochondria is not congenital, we should consider the acquired dysfunction of mitochondria and the possible relation to earlier observations of migraine pathogenesis. As we mentioned in the introduction, the etiology of migraine remains unknown. However, some clues and working hypotheses have been submitted. Three major hypotheses of migraine are: i) the neural theory, ii) the vascular theory and iii) the trigemino-vascular theory. The neural theory claims that hyperexcitability of the central nervous system causes aura symptoms and cortical spreading depression, with subsequent vascular throbbing headaches (Leao, 1944). The vascular theory claims that vasoconstriction causes local ischemia of the brain (Wolff, 1963), which is responsible for aura symptoms, such as scintillating scotoma, hemiplegia and/or aphasia. The following vasodilatation causes throbbing headaches. Abnormal platelet serotonin metabolism plays a crucial role in the alteration of vascular tone in migraine headaches (Hanington, 1978). The released serotonin from one platelet stimulates other platelets to release serotonin. The chain reaction of serotonin release causes high serotonin levels in the blood and vasoconstriction. Afterwards, the serotonin in the blood is metabolized into 5-hydoxyindole acetic acid (5-HIAA). 5-HIAA is secreted into the urine and blood and the platelet serotonin level decreases, which causes vasodilatation and throbbing headaches. The trigemino-vascular theory claims that an unknown neurochemical noxious trigger stimulates the trigeminal nerve and causes hyperactivity of trigeminal nerves (Moskowitz, 1984). Substance P and other neurotransmitters are released from small-diameter trigeminal sensory afferents around the meningeal and dural blood vessels, initiating vasodilatation, plasma extravasation, and release of histamine from mast cells and serotonin from platelets. Serotonin causes vasoconstriction and local ischemia of the brain.

The brain metabolism depends mostly on a mitochondrial energy supply. Ninety-five percent of the molecular oxygen is metabolized within the mitochondria by the electron transport system so that the mitochondria are highly exposed to oxidative stress. Oxygen radicals created during respiration can induce mitochondrial dysfunction which accelerates the production of a more deleterious species of oxygen. Such a mal-cycle due to ischemia-mitochondrial damage might produce a migrainous state. As mentioned above, the trigemino-vascular theory of migraine hypothesizes an unknown noxious stimulus. Metabolic acidosis resulting from chronic ischemia and increasing LA and PA may be one of the hypothesized trigger stimuli in the trigemino-vascular theory.

According to the IHS criteria, migraine and TH are different entities. However, some researchers suspect that migraine and TH are

similar and may have a common etiology (Bakal and Kaganov, 1977; Cohen, 1978; Featherstone, 1985). Actually, many patients with migraine, whose diagnoses have been established by the IHS criteria, frequently have TH. Featherstone (1985) stated that quantification of symptoms and characteristics showed unipolar distribution, and that therefore, migraine and muscle contraction (tension-type) headache were a continuum. Some researchers reported that some evidence supports this hypothesis from the viewpoint of pupillary function evaluated by video-pupillometry (Takeshima et al., 1987), plasma norepinephrine response to cold stimulus (Takeshima et al., 1989), cardiovascular reflex response by orthostastic test (Mikamo et al., 1989), platelet function (Rolf et al., 1981), prevalence of muscle tenderness (Tfelt-Hansen et al., 1981) and exteroceptive suppression (ES2) of muscle activity (Nakashima and Takahashi, 1991). In TH patients, LA and PA levels showed varying degrees of concentration. Some patients showed high levels in both the LA and PA concentration in the plasma. In this regard, continuous muscle contraction in TH patients may elevate the LA and PA levels. The mean plasma LA and PA levels in the TH group were between those in the migraine and control groups. Another possibility is that TH patients have milder mitochondrial abnormalities than migraine patients have.

We observed significantly increased plasma PA and LA levels in patients with migraine during headache free periods. These observations support the hypothesis that patients with migraine have some abnormalities in mitochondrial energy metabolism and mitochondrial function in an aerobic process of glycolysis.

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