Lactate and Pyruvate Levels in Blood and Cerebrospinal Fluid in Patients with Menkes Disease

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Objective To examine levels of lactate (LA) and pyruvate (PA) in both blood and cerebrospinal fluid (CSF) in patients with Menkes disease (MNK).

Study design A nationwide survey involving a retrospective review of medical records or medical record summaries of 42 male patients with MNK born between 1993 and 2008 were performed, and the genetic analysis of their *ATP7A* gene was reviewed.

Results In these patients, LA and PA levels and the lactate vs pyruvate ratio (L/P ratio) at diagnosis in both blood and CSF were abnormally high. There were no significant differences in LA levels, PA levels, and the L/P ratio between blood and CSF at diagnosis ($P > .05$). There was also no correlation between LA levels, PA levels, and the L/P ratio, and age at measurement ($P > .05$). There was no or slight metabolic acidosis, as analyzed by blood gas analysis, in 7 patients. During treatment with subcutaneous injections of copper-histidine, LA and PA levels and the L/P ratio in both the blood and CSF decreased.

Conclusion Our findings suggest that LA and PA levels, and in particular, the L/P ratio, and blood gas analysis can be used to guide the diagnosis and management of MNK. *(J Pediatr 2014;164:890-4)*.

Menkes disease (MNK; Online Mendelian Inheritance in Man #309400), an X-linked recessive metabolic disorder associated with copper deficiency, is caused by mutations in the *ATP7A* gene (OMIM#300011), expressed in a variety of cell types and tissues.[1,2] Between 1992 and 2002, the incidence of MNK at birth in Japan was 2.8 per million total live births and 4.9 per million male live births.[3]

In the human placenta, ATP7A delivers copper to the fetus, and ATP7B returns excess copper to the maternal circulation, with opposite responses to insulin.[4] After birth, the expression of the *Atp7a* gene in mice shows developmental changes with the loss of epigenetic control.[5] In mice with enterocyte-specific deletion of the *Atp7a* gene, copper supplementation leads to a marked decrease in early postnatal mortality, suggesting the presence of a gene-nutrient interaction.[6] Moreover, early copper-histidine injections after birth improve clinical outcomes, depending on the residual ATP7A activity.[7,8]

After birth, intestinal cells do not efflux copper into the blood, making all other tissues deficient in copper.[2] In affected cells, a reduction in copper efflux lowers the activities of copper-dependent enzymes, such as cytochrome c oxidase (COX), the last enzyme in the respiratory electron transport chain in the mitochondrial membrane. We previously reported that low COX activity in MNK caused hypothermia and abnormalities in lactate (LA) and pyruvate (PA) in blood and cerebrospinal fluid (CSF) in some patients with MNK at onset, which led to misdiagnosis (eg, mitochondrial diseases or disorders of organic acid metabolism).[9-11] However, little is known about LA and PA levels in blood and CSF at disease onset and during treatment in patients with MNK. Therefore, differentiating among these diseases can be challenging. Moreover, in patients with MNK, valproic acid is commonly used as an antiepileptic drug, which also causes abnormalities in the levels of LA and PA, further obscuring the relationship between MNK and LA and PA levels. In this study, we aimed to examine PA and LA levels in both blood and CSF and to determine whether LA and PA levels can serve as potential markers for guiding clinical diagnosis and clinical management of MNK.

Methods

This was an observational case series study. A nationwide retrospective survey on MNK and occipital horn syndrome was performed, which involved sending a questionnaire to pediatricians at the end of 2010. The first survey identified a total of

^{1}H-MRS	Proton magnetic resonance spectroscopy
COX	Cytochrome c oxidase
CSF	Cerebrospinal fluid
L/P ratio	Lactate vs pyruvate ratio
LA	Lactate
MNK	Menkes disease
MRS	Magnetic resonance spectroscopy
PA	Pyruvate

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134 patients by board-certified pediatric neurologists, departments of pediatrics of university and government hospitals, and departments of pediatrics at facilities for those with mental and physical disabilities. A second questionnaire survey was conducted for these 134 patients, which included the following items: status of birth, diagnosis, treatment with copper-histidine and anticonvulsants, nutritional intake, change of body weight and height, genetic analysis, and prenatal diagnosis. Data were obtained from medical records or summaries.

At the end, we collected data on 62 patients. After we excluded female patients, patients with occipital horn syndrome, patients with late-onset MNK (>1 year of age), repeated cases, and patients born before 1993 when treatment with copper-histidine was not established, there were a total 45 patients with classical MNK. Of these 45 patients, who were born between 1993 and 2008, 3 were diagnosed by prenatal diagnosis and were excluded. Accordingly, the final study population included 42 patients who were diagnosed by clinical examination, laboratory data including catecholamine tests, measurement of copper concentrations in cultured cells, and/or genetic analysis.[3,12-15] Of the 42 patients, 40 had been diagnosed by genetic analysis of the *ATP7A* gene and had been referred at least once to the Department of Pediatrics of Teikyo University School of Medicine from throughout Japan for counseling, copper-histidine therapy, and biochemical (including catecholamine associated data) and/or molecular pre- and postnatal diagnosis of MNK.[3,12-15] In Japan, pediatric patients with any 1 of 514 intractable diseases, such as MNK, are supported by medical aid programs.[16]

In Japan, copper-histidine is prepared via use of the protocol described by Sarker et al.[17] The dose of parenterally administered copper-histidine was adjusted to maintain serum copper and ceruloplasmin levels within a normal range, which were monitored regularly.

The study protocol was reviewed and approved by the Ethics Board of the Teikyo University School of Medicine (Tei-I-Rin 07-095 and 08-114). Parents or guardians of patients provided signed written informed consent. Clinical data for all patients were obtained from medical records or summaries written retrospectively by pediatricians. Measurements of LA and PA in both blood and CSF were performed by LA oxidase and PA oxidase-based enzymatic assays, respectively.[18]

The Wilcoxon matched pairs signed-rank test was used to compare differences in LA or PA levels between time of disease onset and after copper treatment. The Mann-Whitney *U* test was used to compare LA, PA, and LA vs PA ratio (L/P ratio) differences between blood and CSF at disease onset. Two-tailed Spearman correlation coefficients were used to evaluate whether age at examination correlated with LA or PA levels or the L/P ratio. IBM SPSS Statistics 21 (IBM Corporation, Armonk, New York) was used for statistical analysis. $P < .05$ was considered statistically significant.

Results

The age range at diagnosis and first measurement of blood and CSF LA and PA levels are shown in **Table I**. LA and PA levels were abnormally high in blood and CSF at diagnosis in most patients. At diagnosis, the proportion of those with an L/P ratio >20 in blood and CSF was 66.7% and 50.0%, respectively. Moreover, there was no significant difference in levels of LA or PA or the L/P ratio between blood and CSF at diagnosis (**Table I**). The details of blood gas analysis in 7 patients with MNK are shown in **Table II**. Although LA and PA levels and the L/P ratio in blood and CSF were abnormally high at diagnosis, 6 patients had no metabolic acidosis, and 1 had slight metabolic acidosis (**Table II**).

The administration of copper-histidine significantly reduced LA and PA levels in both blood and CSF (**Table I** and **Figure**). Moreover, statistical analysis of paired data confirmed that both LA and PA levels decreased significantly in blood after treatment with copper-histidine (**Figure**).

Of 42 patients with MNK, 5 patients who were recorded to have undergone magnetic resonance spectroscopy (MRS) examination exhibited a transient LA peak on proton magnetic resonance spectroscopy (^{1}H-MRS), with a decreased *N*-acetyl aspartate/choline or *N*-acetyl aspartate/creatine ratio at diagnosis (2 patients in **Table II**). Furthermore, another 2 patients had been reported previously.[9,11] Patient OK (with no mutation) only had records of MRS analysis and LA and PA data at diagnosis[11]; the other patient, YR (with a mutation R547X), had an LA signal that disappeared during treatment with copper-histidine; his tonic spasms also disappeared, along with an improvement in electroencephalogram findings, compared with those at diagnosis.[19]

There was no correlation between LA levels and age at LA measurement, between PA levels and age at PA measurement, or between the L/P ratio and age at LA and PA measurements in blood or CSF at diagnosis or during treatment (Spearman correlation, $P > .05$; **Table I**).

Of the 42 patients with MNK, 21 exhibited convulsions at onset, 17 exhibited other symptoms, and the remaining 4 were unknown. Moreover, convulsions also were observed in 16 patients during treatment. Thus, there were a total of 37 patients with MNK who exhibited convulsions. The age of convulsion onset in those patients was 4.0 ± 1.9 months (mean ± SD, n = 34), with a median age (range) of 4.0 (0.0-9.0, n = 34) months. Anticonvulsant drugs were administered to 22 patients, and valproic acid was used in 14 patients (63.6%). However, there was a description about period of administration of valproic acid accompanied with the information of LA and PA in 2 patients only. One patient, SY, exhibited respiratory distress immediately after birth. He had a R986X mutation in the *ATP7A* gene. Copper-histidine and valproic acid were administrated at age of 6 months when his β2-microgloblin level reached 13 900 μg/L, but