The Turkish Journal of Pediatrics 35: 267-270, 1993

IS L – CARNITINE PROTECTIVE IN HYPOXIC CEREBRAL EDEMA IN NEWBORN MICE?*

*Murat Yurdakök MD***, Rıfkı Hazıroğlu DVM***, Turgay Coşkun MD***

SUMMARY: Yurdakök M, Hazıroğlu R, Coşkun T. (Department of Pediatrics, Hacettepe University Faculty of Medicine, and Department of Pathology, Ankara University Faculty of Veterinary Medicine, Ankara, Turkey). Is carnitine protective in hypoxic cerebral edema in newborn mice? Turk J Pediatr 1993; 35: 267-270.

The value of carnitine in the prevention of cerebral edema due to acute severe hypoxia in 38 newborn mice was studied. Twenty-nine animals received normal saline (0.25 ml) or carnitine (16 mmol / kg) intraperitoneally, thirty minutes before exposure to 0% oxygen in inspired air for two minutes. Nine mice received no drug and were exposed to room air. After hypoxic insult, brain water content was measured and was found to be similar in all groups. These findings suggest that carnitine pretreatment does not prevent brain edema associated with cerebral hypoxia in newborn mice. *Key words: carnitine, hypoxia, cerebral edema, mice.*

L-carnitine is necessary for the transformation of acyl-compounds (free long-chain fatty acids) to acyl-carnitines, and for their transport from the cytosol into the mitochondrial matrix. Beta-oxidation of these compounds precedes their entry into the Krebs cycle, where energy production occurs[1]. When beta-oxidation is impaired in various conditions including cellular hypoxic-ischemic damage, L-carnitine may relieve the pathological acumulation of acyl-CoA esters by extracting the acyl-compounds from coenzyme A. Excess acyl-carnitine esters may then be transported out of the mitochondrion and the cell, and excreted in the urine as acyl-carnitine esters. This peculiar detoxifying activity of L-carnitine may cause a relative deficiency of carnitine in cells. In the absence of L-carnitine, the accumulation of free fatty acids in the cytoplasm produces a toxic effect on the cell, and an energy deficit arises from the unavailability of fatty acids within the mitochondria[2].

Asphyxia represents a major cause of brain injury in the newborn. In the premature infant, asphyxia is often complicated by cerebral hemorrhage and death, but neonatal death or later neurological disability is also seen in the term infant[3]. Studies have shown that hypoxic brain injury is seen in nearly all infants dying within the first days of life[4].

---

* From the Department of Pediatrics, Hacettepe University Faculty of Medicine and Department of Pathology, Ankara University Faculty of Veterinary Medicine, Ankara.

** Associate Professor of Pediatrics, Hacettepe University Faculty of Medicine.

*** Associate Professor of Veterinary Pathology, Ankara University Faculty of Veterinary Medicine.

Fatty acid oxidation is virtually non-existent in the adult brain, but it can be detected in the fetal and neonatal brain where it supplies 25% of the metabolites required in the Krebs cycle. Since fatty acid oxidation and ketogenesis are critical steps for neonatal survival and development, an adequate supply of carnitine at this stage of life is essential[5].

The present study was carried out to assess the value of carnitine in the prevention of cerebral edema due to acute severe hypoxia in newborn mice, although reduced carnitine or increased acyl-Coa levels has not been reported in hypoxic cerebral damage.

Material and Methods

The maturation level of a newborn mouse's brain is similar to that of a newborn infant's brain at 36 weeks gestation. One-day-old newborn mice (n = 38) weighing 25.4 ± 3.9 g (mean ± SD) were therefore studied. Throughout the study, the animals were kept in three plastic chambers with an air temperature of 37 °C.

The animals were randomly divided into three groups. Nine mice were separated as the control group, received no drug and were exposed to room air. Twenty-nine animals in the study group received either normal saline or carnitine intraperitoneally (i.p.) prior to hypoxia. Fourteen mice were injected (i.p.) with 0.25 ml of saline (injectable form, pH 7.0). Fifteen mice in the study group were injected (i.p.) with 16 mmol / kg of carnitine as a 20% (wt / vol) solution in water. L-carnitine (injectable form, pH 7.0) was purchased from Sigma Co. Thirty minutes after the injections, all animals in the study group were exposed to hypoxia for two minutes. Hypoxia was established with 0% oxygen in inspired air by substituting O_2 with N_2.

After hypoxic insult, all animals were sacrified by cervical dislocation. The brains (including brainstem and cerebellum) were quickly dissected and placed in pre-weighed 5 ml glass vials and weighed on a microanalytical balance. Subsequently, the tissue specimens were desiccated at + 70 °C for 48 hours. Vials were re-weighed to ascertain the brain dry weight (DW), and by subtraction from the total brain weight (TW), the wet weight of the tissue was obtained. Brain water content (BWC) was determined as a percentage of total brain weight according to the following formula:

$$\text{Water content } (\%) = [(TW - DW) / TW] \times 100$$

Values were expressed as means ± SD. Differences were analyzed by Student's *t* test and considered statistically significant when $p < 0.05$.

Results

Brain water contents of animals are displayed in Table I. BWC was higher in hypoxic animals than in controls (86.60 ± 1.90% and 85.03 ± 1.58% respectively, $p < 0.02$). But in animals with pretreated carnitine before hypoxic insult, BWC was the same as in animals that did not receive any drug (86.49 ± 1.13% and 86.60 ± 1.90% respectively, $p > 0.05$). According to these findings, carnitine pretreatment does not prevent brain edema associated with cerebral hypoxia in newborn mice.

Table I: Effect of L-carnitine on Brain Water content (BWC) in Newborn Mice Challenged with Hypoxia

Group	Treatment	BWC (%)
Control	None (n = 9)	85.03 ± 1.58[a]
Hypoxic	Saline (n = 14)	86.60 ± 1.90[b]
Hypoxic	Carnitine (n = 15)	86.49 ± 1.13[c]

a vs b: $p < 0.02$, b vs c: $p > 0.05$, a vs c: $p < 0.05$

Discussion

Carnitine deficiency secondary to defects of intermediary metabolism, systemic disease, drug therapy or ischemia (especially of the heart and skeletal muscle) is common. Ischemia causes carnitine depletion in affected tissues and is particularly evident in heart and skeletal muscle, where energy production depends mainly on the utilization of fats and is therefore most likely to suffer from deficient mitochondrial transport of long-chain fatty acids. Although the plasma carnitine level may be in the normal range in the presence of severe tissue deficiency, increased acyl-carnitine in the urine is also an indication of possible tissue carnitine deficiency[6-8]. Abnormally high concentrations of acyl-Coa esters also occur in the myocardial cells during ischemia, the consequence of reduced fatty acid oxidation due to lack of oxygen. Myocardial fatty acid accumulation has been presented as a cause of both conduction disturbances and ventricular arrhythmias. The potential beneficial effects of L-carnitine therapy in these conditions have been demonstrated in careful experiments[2 6].

We made such an experimental study by giving L-carnitine in the prophylaxis of cerebral edema in newborn mice exposed to acute severe hypoxia. Although cerebral acyl-Coa and L-carnitine levels could not be determined in this study, our findings indicate that L-carnitine pretreatment was ineffective in protecting cerebral edema induced by hypoxia.

It has been reported that i.p.-administered L-carnitine accumulates preferentially in the brain[9]. Controversy exists as to regulation of the ability of L-carnitine to cross the blood-brain barrier. According to an earlier report, the passage of L-carnitine to the brain from blood in vivo is slow[10] indicating that L-carnitine may not cross the brain-blood barrier readily. Hearn et al[11] also reported that L-carnitine crosses the blood-brain barrier at a slow rate. So it is not clear whether the increase in L-carnitine content of brain observed in this study is sufficient to protect animals against cerebral edema due to hypoxia.

REFERENCES

1. Annonation. Carnitine deficiency (editorial). *Lancet* 1990; 335: 631-3.
2. Rizzon P, Biasco G, DiBiase M, et al. High doses of L-carnitine in acute myocardial infarction: metabolic and antiarrhythmic effects. *Eur Heart J* 1989; 10: 502-8.
3. DeSouza S. Richards B. Neurological sequelae in newborn babies after perinatal asphyxia. *Arch Dis Child* 1978; 53: 564-9.
4. Skullerud K, Westre B. Frequency and prognostic significance of germinal matrix hemorrhage, periventricular leukomalacia, and pontosubicular necrosis in preterm neonates. *Acta Neuropathol* 1986; 70: 257-61.
5. Giovannini M, Agostoni C, Salari PC. Is carnitine essential in children? *J Int Med Res* 1991; 19: 88-102.
6. Dipalma JR. Carnitine deficiency. *Clin Pharmacol* 1988; 38: 243-51.
7. Duran M, Loof NE, Ketting D, Dorland L. Secondary carnitine deficiency. *J Clin Chem Clin Biochem* 1990; 28: 359-63.
8. Breningstall GN. Carnitine deficiency syndromes. *Pediatr Neurol* 1990; 6: 75-81.
9. O'Connor JE, Costell M, Grisolia S. Protective effect of L-carnitine on hyperammonemia. *FEBS Lett* 1984; 166: 331-4.
10. Brooks DE, McIntosh JEA. Turnover of carnitine by rat tissues. *Biochem J* 1975; 148: 439-45.
11. Hearn TJ, Coleman AE, Lai JCK, et al. Effect of orally administered L-carnitine on blood ammonia and L-carnitine concentrations in postcaval-shunted rats. *Hepatology* 1989; 10: 822-28.