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Methylmalonic and Malonic Aciduria in a Dog with Progressive Encephalomyelopathy

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A 12 week old female Labrador retriever dog with signs of progressive diffuse degeneration of the brain and spinal cord was found to have methlymalonic and malonic aciduria. Over a 5 month period, the dog developed neurologic signs compatible with disease of the central nervous system with predominant diffuse cerebral and right lateralizing brainstem deficits. Gross pathological examination of the brain showed that the lateral, third, and fourth ventricles of the brain were markedly enlarged and associated with white and grey matter atrophy. Syringomyelia and hydromyelia of the central canal into the dorsal funiculus of the spinal cord beginning at the level of the cervical intumescence and extending to the lumbar intumescence was also present. Significant biochemical abnormalities include methylmalonic and malonic aciduria, mild lactic and pyruvic aciduria. There was also accumulation of citric acid cycle intermediates including succinic, aconitic, and fumaric acids. Disordered fatty acid oxidation was suggested by increased excretion of adipic, ethylmalonic, suberic and sebacic acids. Neither ketoacidosis nor hyperammonemia were present, and serum cobalamin levels were normal. Overall, this dog demonstrates an inborn error of metabolism resulting in abnormal organic acid accumulation associated with a neurodegenerative disease.

Keywords: Methylmalonic aciduria; malonic aciduria; encephalomyelopathy; hydrocephalus; dog

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INTRODUCTION

Inborn errors of metabolism that produce organic-acid accumulation may result in severe encephalopathies (Haas and Nyhan, 1992; Marsden and Nyhan, 1992). Neuronal metabolism may be affected directly when the defect is located in a major metabolic pathway. Malonic and methylmalonic aciduria have recently been described in three human infants with severe and progressive encephalopathy (Ozand *et al.*, 1994). Clinical presentation was of dystonia, spasticity and episodic metabolic acidosis. Excretion of other metabolic intermediates included lactic acid, 3-hydroxybutyric acid, aconitic acid and 3-hydroxy-3-methylglutaric acid. In this paper the clinical presentation, pathological and metabolic abnormalities in a young dog with progressive encephalomyelopathy and severe hydrocephalus with malonic and methylmalonic aciduria are described.

MATERIALS AND METHODS

Clinical evaluation

A 12 week old, 8.7 kg male Labrador retriever presented to The Ohio State Veterinary Teaching Hospital because of progressive tetraparesis. The dog was obtained from a private breeder. The first series of vaccinations for canine distemper, adenovirus, parainfluenza, parvovirus and coronavirus were given at 8 weeks of age. A second series was administered at 12 weeks of age. At 10 weeks of age the dog developed intermittent, symmetric pelvic limb stiffness. One week later, stiffness progressed to the thoracic limbs. Within the next two days, the dog was markedly ataxic in all limbs. One day prior to presentation, the dog was non-ambulatory and anorexic. On physical examination the dog appeared alert and responsive but exhibited generalized muscle wasting. The dog was in a left lateral recumbent position and had marked extensor rigidity in all limbs, worse in the thoracic limbs. The neck was extended, and the dog attempted repeatedly to turn the neck and body to the right. Mental status and arousability appeared normal for this age of dog. The dog could neither sit nor stand and had absent proprioceptive placing of all limbs. Motor activity was present in all limbs when the dog was supported to walk. Spinal reflex testing elicited present but decreased withdrawal reflexes in the thoracic limbs which was attributed to the marked extensor tone. Hyperreflexive bilateral patellar and tibial deep tendon reflexes were present along with a crossed-extensor reflex. Nociceptive and funduscopic examination was normal. Diagnosis was of an encephalomyelopathy with possible peripheral nervous system involvement. Persistent turning of the neck and body to the right was indicative of right forebrain lesion (cerebral cortex, internal capsule or diencephalon). At this time, the dog was donated to one of the authors (AG) for further evaluation and potential therapy.

The major change in the neurological examination over the next two months was the development of a positional, vertical downbeat, nystagmus that changed in direction over time. At five months of age, the dog appeared disoriented and demented, although he was arousable and semi-responsive to visual and auditory stimuli. There was a marked, persistent right torticollis and right tilt of the head and neck. Coordination and strength of

the head and neck was poor. Cranial nerve examination revealed a decreased menace response; direct and consensual pupillary light reflex activity was present in both eyes. The right eye had a resting dilated pupil, decreased oculocephalic reflex, ventral-lateral strabismus, and decreased retractor oculi reflex. The gag reflex was diminished, and the dog did not bark. The dog was still non-ambulatory and had severe extensor ridgidity of the thoracic and pelvic limbs that was worse on the right side. Withdrawal reflexes of all limbs were diminished. Deep tendon reflexes of all limbs were increased, with cross-extensor reflexes. At this time, it was evident that there was a multifocal disease involving the central nervous system, with predominant diffuse cerebral and right lateralizing brainstem deficits. Cranial nerves III, V, VI, and VIII were affected with right lateralization. In view of the progression of the disease, severity of lesions and no identifiable treatable disease, the dog was euthanized with an intravenous overdose of pentobarbital and a necropsy performed.

Clinical diagnostic testing

Needle electromyography of appendicular and epaxial muscles was performed at 3 and 5 months of age with the dog under general anesthesia (isoflurane) using a computerized electrodiagnostic system (Cadwell Quantum 84, Cadwell Labs, Kennewick, WA). Motor nerve conduction of the peroneal nerve was performed as previously described (Bowen, 1987). Values were compared to a reference range for this laboratory derived from normal dogs recorded under similar conditions. Cerebrospinal fluid (CSF) was collected sterilely from the cerebellomedullary cisterna with a 20 gauge x 1.5 inch spinal needle at the same time and characterized by cytology and total protein. For the cytologic evaluation, total white and red blood cell counts were determined by hemocytometry followed by light microscopic evaluation of cytocentrifuged preparations. Total protein was analyzed with a microprotein determination kit (Microprotein Rapid Stat Kit, Pierce, Rockford, IL).

The initial metabolic diagnostic evaluation consisted of a complete blood count (Coulter counter Model S-Senior, Coulter Electronic, Hialeah, FLA), serum biochemical profile (Technicon 18/60, Technicon Industrial Systems, Inc, Tarrytown, NY), and urinalysis (Chemstrip 7 urine test strips, Boehringer Mannheim, Indianapolis, IN). Serum IgG antibodies for protozoal infection with Toxoplasma gondii and Neospora caninum were detected with ELISA methodology at Auburn University (Auburn, Alabama). Serum viral neutralizing antibodies to canine distemper virus were detected with an indirect immunofluorescence antibody technique at Cornell University (Ithaca, NY). Plasma ammonia was analyzed with a Kodak Ektachem DT60 analyzer (Rochester, NY). Venous blood gas was analyzed for pH, carbon dioxide, and oxygen content with a Radiometer blood gas analyzer (ABL500, Westlake, OH). Serum folic acid and cobalamin were measured by a radiobinding commercial assay (Quantiphase B12/Folate Radioassay, Hercules, CA).

Biochemical analysis

Urinary organic acids were quantified by gas chromatography-mass spectrometry (GC/MS) as described by Hoffman *et al.* (1989). Amino acids in plasma and urine were quantified by automated column chromatography by the method of Spackman *et al.* (1958).

Urine, plasma and muscle concentrations of carnitine were determined by a radioisotopic enzyme assay (Bieber and Lewin, 1981). Urinary organic acids were tested again following 8 weeks of treatment with L-carnitine (1000 mg/day), vitamin B_{12} (0.5 mg /day)) and a formulated protein restricted diet. Ten normal dogs equally divided by gender ranging in age from 6 months to 2 years of age were used as a control population.

Histopathology

Fresh muscle biopsies were taken from the cranial tibial and vastus lateralis muscles, snap frozen in isopentane precooled in liquid nitrogen and evaluated by a standard panel of histological stains and enzyme reactions at five months of age. Histopathologic evaluation of fresh frozen muscle biopsy samples was by a standard panel of histological and histochemical reactions (Dubovitz, 1985). At necropsy, the brain and spinal cord was removed in toto for immersion fixation in 10% neutral buffered formalin. Serial sections of brain and spinal cord were embedded in paraffin and 5-μm sections stained with hematoxylin and eosin for histopathologic evaluation.

RESULTS

Clinical evaluation

No significant abnormalities were detected on a complete blood count and serum biochemistry panel. Serum antibody titers for protozoal infection with Toxoplasma gondii and Neospora caninum were negative. Serum viral neutralizing antibody titer to canine distemper virus was 1:16.

At three months of age, the CSF analysis yielded a total protein of 17 mg/dl (Reference <25 mg/dl) with no white or red blood cells detected. Similar data were observed at 5 months of age, and the blood ammonia and venous blood gas analysis were normal. Electromyographic evaluation demonstrated diffusely increased insertional activity in all muscles tested with multifocal areas of spontaneous activity in appendicular and epaxial muscles consisting primarily of positive sharp and fibrillation waves. A second electromyographic study at 5 months of age demonstrated a consistently increased insertional activity in all muscles tested with multifocal areas of spontaneous activity. This activity consisted primarily of fibrillation and positive sharp waves and was more pronounced at that time in the distal appendicular muscles. Motor nerve conduction testing of the left peroneal nerve exhibited a normal velocity (76.7 m/s; normal: >70 m/s) with a temporal dispersion of the evoked compound muscle action potentials (4.2 ms; normal: <3.5 ms).

Biochemical analysis

The excretion of urinary organic acids in the canine patient, an unaffected male littermate and reference values averaged from ten normal dogs are shown in Table 1. Notable abnormalities include methylmalonic and malonic aciduria, mild lactic and pyruvic aciduria and increased excretion of ketones as shown by the elevated 3-hydroxybutyric acid. There

was also accumulation of the citric acid cycle intermediates succinic and aconitic acids along with increased excretion of adipic, ethylmalonic, suberic and sebacic acids. Serum folic acid and vitamin B_{12} (cobalamin) concentrations were within established reference ranges (Fyfe *et al.*, 1991). Following an 8 week course of treatment including L-carnitine (1000 mg/day), vitamin B_{12} (0.5 mg/day) and a formulated protein restricted diet, repeat evaluation of urinary organic acids showed a marked improvement in all values (Table 1). The dog, however, showed no clinical improvement.

Table 1. Urinary organic acids in a dog with methylmalonic and malonic aciduria. (mmol/mol creatinine)

Organic acid	Pre carnitine	Post carnitine	Control littermate	Upper limit normal x=10
Methylmalonic	449	15	0	9
Malonic	101	0	0	0
Lactic	783	95	178	200
Pyruvic	71	25	9	26
3-0H propionic	37	3	2	6
2-ethyl,3-0H propionic	181	5	0	18
3-0H butyric	112	2	4	8
3-0H isobutryic	329	13	14	19
Acetoacetic	5	0	1	1
3-0H isovaleric	31	1	0	7
Succinic	61	7	7	9
2-oxoglutaric	14	31	3	22
Aconitic	577	71	22	58
Malic	6	3	1	6
Fumaric	17	1	2	2
3-0H,3-ME glutaric	183	0	0	0
3-ME glucaconic	25	0	0	5
Adipic	23	1	2	6
3-0H adipic	0	1	1	3
Glutaric	4	1	0	0
2-0H glutaric	14	1	2	3
3-0H glutaric	1	4	0	0
Suberic	68	4	6	6
Sebacic	19	0	0	0
Ethymalonic	65	1	1	3

Histopathology

There was a mild variation in myofiber size with scattered singular atrophic fibers having an angular shape and of both fiber types. These changes were consistent with mild denervation. No abnormalites of the peripheral nerve were found.

Gross examination of the brain showed that the lateral, third, and fourth ventricles of the brain were markedly enlarged and associated with white and grey matter atrophy (Figure 1). Dorsal expansion of the fourth ventricle was pronounced as a result of marked atrophy of

the cerebellar white matter and associated roof nuclei. Cerebellar morphology was otherwise normal, and the lateral apertures of the fourth ventricle were patent.

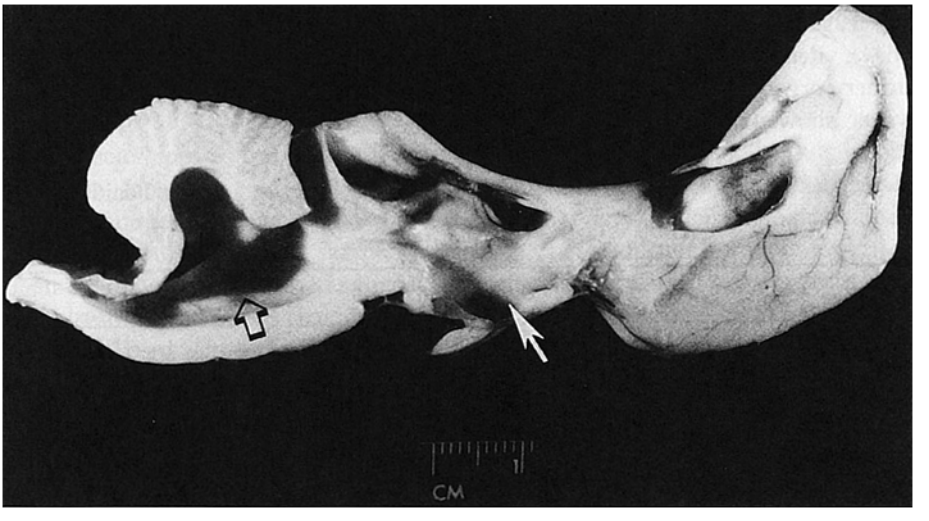

Figure 1. Mid-sagittal section of formalin fixed brain with overlying cerebral cortex removed. There is marked dilatation of the third (closed arrow) and fourth (open arrow) ventricles and mesencephalic aqueduct without evidence of any obstruction of spinal fluid outflow. This dilation extended caudal into the central canal of the spinal cord.

The thickness of the cerebral cortex (i.e., from the ependymal lining of the lateral ventricle to the leptomeninges) was uniformly reduced to 0.5 cm (Figure 2). There was bilateral fracturing of periventricular white matter, beginning at the apex of the ventral horn of the lateral ventricle and the caudal-lateral margin of the caudate nuclei. Progressive tearing on the left side resulted in coalescence of these fissures with complete separation of the caudate nucleus from the underlying internal capsule. The magnitude of hemorrhage, fibrous astrogliosis, and inflammation was pronounced in portions of tissue most isolated by the progressive fissure formation. Here, the white matter degenerative changes typifying the periventricular atrophy progressed to overt malacia. Lymphocytes and plasma cells infiltrated choroid plexuses in the vicinity of the more severe periventricular lesions. The mesencephalic aqueduct was enlarged, although ependymal and subependymal lesions were not observed within this compartment.

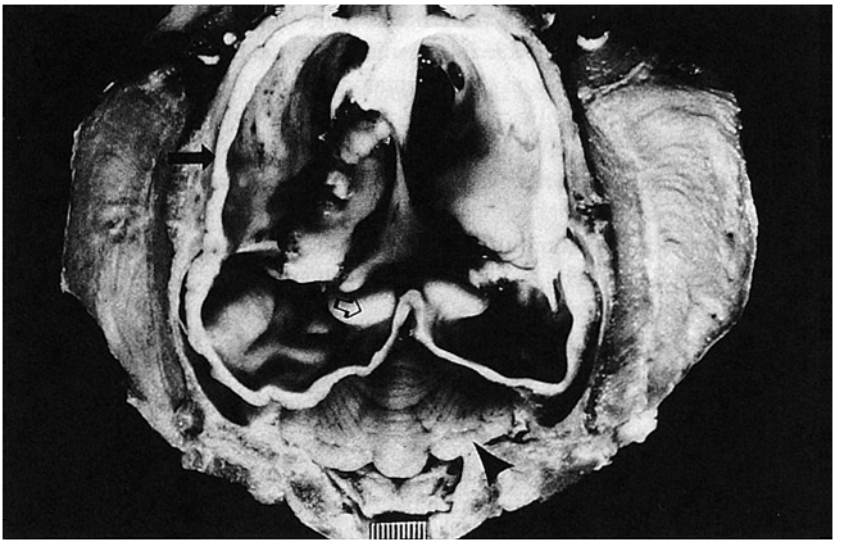

Figure 2. Coronal view of the formalin fixed brain *in situ* within the calvarium. Extreme dilatation with communication of the lateral ventricles is present that resulted in diffuse, severe cortical atrophy (arrow). The underlying hippocampus can be seen (open arrow) along with cortical cerebellar structures in the posterior fossa (arrowhead).

Syringomyelia and hydromyelia of the central canal into the dorsal funiculus of the spinal cord was present beginning at the level of the cervical intumescence and extending to the lumbar intumescence. Direct communication between the central canal and the cystic cavity was variable, with frequent separation by residual spinal cord parenchyma. The communication between the central canal and the cavity was lined by severely attenuated ependymal cells whereas the latter was lined by fibrous astrocytes. No evidence of inflammation was detected.

DISCUSSION

Inborn errors of metabolism may result in a wide spectrum of neurological diseases (Hass and Nyhan, 1992; Marsden and Nyhan, 1992; Ozand and Gascon, 1991a; Ozand and Gascon, 1991b). The diagnosis of organic acidemias requires sophisticated testing in specialized laboratories. This report documents a neurodegenerative disorder in a young dog with methylmalonic and malonic acidemia.

The organic acid abnormalities described in this case are very similar to those previously described in three infants (Ozand *et al.*, 1994). All children had severe and progressive encephalopathy. Clinically the patients displayed dystonia and spastic tetraplegia. The metabolic pattern of organic acid excretion in the three children was quite similar to that of

the dog. In each of the children, the excretion of malonic acid was greater than that of methlymalonic acid, but otherwise the pattern was quite similar with increased excretion of lactic and pyruvic acids, citric acid cycle intermediates and dicarboxylic acid products of fatty acid oxidation. Malonic aciduria and methylymalonic aciduria have also been observed in patients with episodic metabolic acidosis and defective activity of malonyl-CoA decarboxylase (Brown *et al.*, 1984; McPhee *et al.*, 1993). These patients did not have neurological disease, and the activity of this enzyme was normal in the three patients reported by Ozand *et al.* (1994) in whom the pattern of excretion was similar to that of the dog. The biochemical localization of the metabolic defect in these children has not been established but is postulated to be an abnormality of flavoproteins linked to the metabolism of malonyl-CoA in mitochondria.

Methylmalonic aciduria (MMA) is the result of abnormalities of cobalamin metabolism or dietary deficiency of cobalamin because deoxyadenosylacobalamin is the cofactor for the mitochondrial methylmalonyl-CoA mutase enzyme. The other important pathway involving cobalamin in mammalian species involves methylcobalamin, the cofactor for the conversion of homocysteine to cystathionine. Human patients with mutations in the conversion of cobalamin to both cofactors have homocystinuria as well as methylmalonic aciduria and severe neurologic disease. Serum levels of cobalamin are normal and episodic ketoacidosis and hyperammonemia are absent (Ozand and Gascon, 1991a). In the dog, it appears likely that the methylmalonic aciduria resulted from inhibition of methlymalonyl CoA mutase by malonic acid (Brown *et al.*, 1984).

The pathogenesis of brain damage in organic acidemias may be due to many factors. In methylmalonic acidemia resulting from deficiency of methylmalonyl CoA mutase, most damage is believed to result from a combination of hyperammonemia, acidosis and hypoxic/ischemic insults. Incomplete development of the brain has also been described in a newborn with MMA suggesting a possible toxic effect of MMA in utero (Ostergaard *et al.*, 1991). Methylmalonic acid causes in young rats a reduction in ganglioside N-acetylneuraminic acid, which is an important component of synaptogenesis (Wajner *et al.*, 1988).

The treatment regimen instituted in the dog of this report was similar to that prescribed for children with MMA (Ozand and Gascon, 1991a; Ozand and Gascon, 1991b; Haas and Nyhan, 1992). This included a protein restricted diet and supplementary cobalamin. Supplementation with L-carnitine was also instituted since carnitine plays an important role in buffering accumulated organic acids and intracellular and extracellular stores of free carnitine may become depleted. Post-treatment quantitation of organic acids indicated a marked improvement, but the severe neurological damage was not amenable to treatment.

This inborn error of metabolism was associated with a severe neurologic disease. A heightened awareness of these entities in animals may serve to advance our knowledge of metabolic brain disease.

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