J. Inher. Metab. Dis. 22 (1999) 883–890

Malonic aciduria in Maltese dogs: Normal methylmalonic acid concentrations and malonyl-CoA decarboxylase activity in fibroblasts

D. P. O'Brien[1]*, B. A. Barshop[3], K. K. Faunt[1], G. C. Johnson[2], K. M. Gibson[5] and G. D. Shelton[4]

[1] *Department of Veterinary Medicine and Surgery and* [2] *Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, Missouri;* [3] *Department of Pediatrics and* [4] *Comparative Neuromuscular Laboratory, School of Medicine, University of California, La Jolla, California;* [5] *Biochemical Genetics Laboratory, Oregon Health Sciences University, Portland, Oregon, USA*

* *Correspondence: Department of Veterinary Medicine and Surgery, University of Missouri, 379 E. Campus Dr., Columbia, MO 65211, USA. E-mail: OBrienD@missouri.edu*

MS received 9.03.99 Accepted 28.05.99

Summary: A family of Maltese dogs with malonic aciduria is reported. The propositus presented at 3 years of age with episodes of seizures and stupor with hypoglycaemia, acidosis, and ketonuria. Urinary organic acid assays showed elevated malonic acid without elevation of methylmalonic acid. Cultured fibroblasts had normal malonyl-CoA decarboxylase activity. Treatment with frequent feedings of a low-fat diet high in medium-chain triglycerides resulted in normalization of clinical signs and a resolution of the malonic aciduria. Two full siblings of the propositus had died at a young age of undiagnosed metabolic and neurological disease. Urine organic acid assays were performed on other family members. A half-sister showed mild malonic aciduria and other organic acid changes similar to the propositus, while the mother and half-brother showed mildly elevated ketone bodies. This family suggests further genetic and clinical heterogeneity in the malonic acidurias.

Malonic aciduria has been reported in 14 human patients and in all there was also an elevation in urine methylmalonic acid (MMA) (Brown et al 1984; Buyukgebiz et al 1998; Gao et al 1999; Gregg et al 1998; Haan et al 1986; Krawinkel et al 1994; MacPhee et al 1993; Matalon et al 1993; Ozand et al 1994; Yano et al 1997). Of the 11 patients in whom it was measured, 9 demonstrated deficient malonyl-CoA decarboxylase activity (McKusick 248360) (Brown et al 1984; Gao et al 1999; Gregg et al 1998; Haan et al 1986; Krawinkel et al 1994; MacPhee et al 1993; Matalon et al

1993; Ozand et al 1994; Yano et al 1997). Clinical features reported in these patients included short stature, developmental delay, dystonia, spastic paralysis, seizures, hypoglycaemia, ketoacidosis, cardiomyopathy, gastrointestinal disturbances and dysmorphic features (Brown et al 1984; Buyukgebiz et al 1998; Gao et al 1999; Gregg et al 1998; Haan et al 1986; Krawinkel et al 1994; MacPhee et al 1993; Matalon et al 1993; Ozand et al 1994; Yano et al 1997).

Combined malonic and methylmalonic aciduria has been reported in one Labrador retriever dog and, as in some human patients, MMA concentrations were greater than malonic acid (MA) concentrations (Podell et al 1996). That dog showed progressive, spastic quadriplegia and at necropsy had dramatic cerebral atrophy and dilation of the ventricular system. Malonyl-CoA decarboxylase activity was not measured. In this report, we describe a family of Maltese dogs with malonic aciduria but no methylmalonic aciduria and normal malonyl-CoA decarboxylase activity in cultured fibroblasts. We also report effects of dietary manipulations, which may be beneficial for evaluation in human patients with malonic aciduria. A brief abstract of the preliminary results was published earlier (Faunt et al 1998).

MATERIALS AND METHODS

The propositus was studied at the University of Missouri, Veterinary Medical Teaching Hospital. Urine from the propositus and family members was collected by cystocentesis and organic acids were assayed using gas chromatography–mass spectroscopy (Hoffman et al 1989). Fibroblast cultures were established from skin biopsy from the propositus and 5 normal control dogs. Malonyl-CoA decarboxylase activity was determined on the six canine samples and fibroblasts from a human patient with malonyl-CoA decarboxylase deficiency (Gao et al 1999) using previously described methods (Brown et al 1984).

RESULTS

Case report: The propositus is a spayed, female Maltese dog. She had a history of tonic-clonic seizures at 6 months of age and an episode of stupor at 1.5 years of age following a period of anorexia. Two full siblings had died of undiagnosed neurological or metabolic disease at less than 5 years of age, but all other family members were reported to be normal (Figure 1). The propositus was referred to the University of Missouri, Veterinary Teaching Hospital at 3 years of age for progressive alteration of consciousness. She had been anorectic and polydipsic for over a week. On presentation she was stuporous with pendular nystagmus and diminished muscle tone. She was small for her breed and age, weighing only 1.5 kg. Serum glucose was low (27 mg/dl) and there was a severe metabolic acidosis (pH 7.087). Her urine showed 3+ ketonuria and a pH of 5.0.

She was treated with intravenous dextrose and bicarbonate, and improved dramatically over the first few hours. She was started on oral electrolyte solution and force-feeding of an energy-dense diet (Table 1). Her blood glucose returned to normal range while being force-fed, but she became hypoglycaemic again when

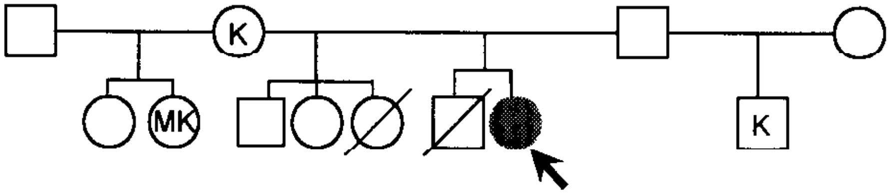

Figure 1 Pedigree of family of Maltese dogs with malonic aciduria. Mother and two half-siblings showed elevation of malonic aciduria (M) and/or ketonuria (K). See Table 2 for values. Two full siblings died at a young age of undiagnosed disease (cross slash)

allowed to eat on her own. She continued to show 3+ ketonuria. The persistent hypoglycaemia and ketonuria prompted investigation of inborn errors of metabolism.

Urine organic acids and therapy: Urine organic acid assays showed elevations in MA as well as lactic acid, ketone bodies and some intermediary metabolites (Table 2). MMA was not detected. Total, free and esterified carnitine were within normal ranges, but the ratio of ester to free carnitine was elevated at 6.92 (normal 0–3). Blood short-chain acylcarnitine concentrations were normal. The dog was placed on a low-fat diet (Table 1), and given a vitamin B complex injection (thiamin 50 mg, riboflavin 2.5 mg, pyridoxine 5 mg, niacinamide 50 mg, *d*-panthenol 5 mg, cyanocobalamin 50 μg). Within 2 weeks the blood glucose was normal and ketone bodies were no longer detectable in the urine. Urine organic acid assays were repeated one and two months after instituting the low-fat diet (Table 2). One month after changing the diet, all values had improved except lactic acid. By two months, all values were within normal limits except for mild elevations in suberic and acetoacetic acids.

Urine organic acid assays were also performed on the dam, sire and two half-siblings of the propositus (Figure 1). The half-sister had elevated urine organic acids similarly to the propositus, while the dam and half-brother had mild elevations in some organic acids but no malonic aciduria (Table 2). The sire had normal urine organic acids.

Table 1 Composition of fed diets (per cent dry matter) compared to a typical maintenance diet for the dog

Diet	*Fat*	*MCT*	*Carbohydrates*	*Protein*
Calorie-dense diet[a]	28.7	Low[d]	17.4	45.7
Low-fat diet[b]	13.75	3–4.7	48.87	30.52
Typical maintenance diet[c]	16	Low[d]	54	25

[a] A/D, Hills Prescription Diet, Topeka, KS, USA

[b] EN, Purina Clinical Nutrition Management, St Louis, MO, USA

[c] Canine Maintenance, Hill's Prescription Diet, Topeka, KS, USA

[d] Exact value not available

Table 2 **Urine organic acids in the propositus before and after instituting a low-fat, low-protein, high-carbohydrate diet, and in family members on a commercial dog food diet**

Urine organic acids[a]	*Propositus before dietary therapy*	*1 month after dietary therapy*	*2 months after dietary therapy*	*Dam*	*Sire*	*Half-brother*	*Half-sister*	*Normal canine range*
Malonic	**97**	**17**	0	0	0	0	**3**	0–1
Methylmalonic	0	0	0	0	0	0	0	0–2
Lactic	**389**	**802**	138	**286**	141	129	**1083**	0–200
Pyruvic	4	23	16	16	18	9	**63**	0–26
3-Hydroxybutyric	**1196**	**464**	0	**70**	7	**61**	**104**	0–11
Acetoacetic	**410**	**130**	**6**	**5**	0	**9**	**9**	0–1
2-Ethyl-3-hydroxypropionic	**72**	**17**	1	**27**	0	2	**34**	0–14
Succinic	**21**	8	3	6	2	10	14	0–17
2-Hydroxyglutaric	2	0	0	0	**4**	1	**5**	0–3
Suberic	**12**	**10**	**7**	**6**	4	1	**27**	0–4
Citric	**365**	118	89	**191**	28	14	163	0–190
2-Oxoglutaric	**34**	18	10	20	17	**28**	**65**	0–22

[a] Values reported in mmol/mol creatinine; values outside reference range shown in **bold**. Normal ranges are derived from studies of 25 healthy canines of various breeds

Table 3 Malonyl-CoA decarboxylase activity

Subject	*Activity*[a]
Propositus	8.7
Control dogs	10.2 ± 4.9 ($n = 5$, ± 1 SD)
Control humans	11.9 ± 4.0 ($n = 10$, ± 1 SD)
Affected human	1.7 ± 0.8 ($n = 4$, ± 1 SD)

[a] Values reported in nmol/h per mg protein

Malonyl-CoA decarboxylase: Malonyl-CoA decarboxylase activity in cultured fibroblasts from the propositus was 8.7 nmol/h per mg protein (Table 3). This was consistent with the canine controls and within normal range in humans. Fibroblasts assayed concurrently from a human case of malonyl-CoA decarboxylase deficiency showed a very low value (Gao et al 1999).

DISCUSSION

Malonic acid derives from malonyl-CoA, an intermediate in the synthesis of free fatty acids from acetyl-CoA (Figure 2). In the cytosol, acetyl-CoA carboxylase converts acetyl-CoA to malonyl-CoA, which in turn is metabolized to free fatty acids by the fatty acid synthetase complex. In the mitochondria, propionyl-CoA carboxylase can act on acetyl-CoA to produce malonyl-CoA, although its normal substrate is

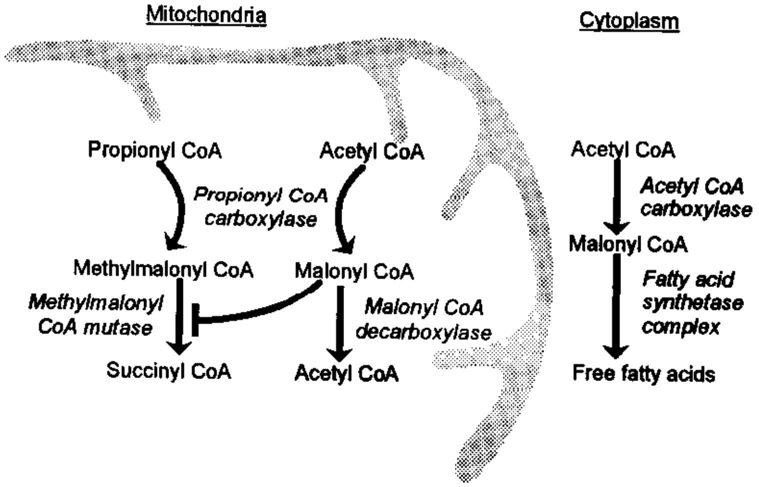

Figure 2 Mitochondrial and cytoplasmic pathways of malonyl-CoA metabolism

propionyl-CoA and the rate with acetyl-CoA is low (Brown et al 1984). Malonyl-CoA decarboxylase prevents accumulation of malonyl-CoA in the mitochondria by converting it back into acetyl-CoA. Malonyl-CoA levels regulate the activity of carnitine palmitoyltransferase I, which transports acyl-CoA into the mitochondria (McGarry and Foster 1980). During the fed state, high levels of malonyl-CoA in the cytosol inhibit carnitine palmitoyltransferase I activity, thus favouring the synthesis of triglycerides and fats over oxidation of fatty acids. During the fasting state, malonyl-CoA levels decrease, allowing free fatty acids to be transported into the mitochondria for β-oxidation (McGarry and Foster 1980).

In humans with malonyl-CoA decarboxylase deficiency, malonyl-CoA cannot be converted back to acetyl-CoA. Some will be hydrolysed to free MA while some will be esterified to malonylcarnitine. The elevated malonyl-CoA inhibits carnitine palmitoyltransferase I, preventing utilization of free fatty acids as an energy source during fasting (McGarry and Foster 1980). It also inhibits pyruvate carboxylase, reducing the supply of oxaloacetic acid, an essential step in the citric acid cycle. In addition, elevations of MA will inhibit succinate dehydrogenase, further disrupting the citric acid cycle (Ozand et al 1994). Although most human cases of malonic aciduria had decreased malonyl-CoA decarboxylase activity in fibroblasts, two published cases did not (Gregg et al 1998; Ozand et al 1994), and we are aware of other cases with elevated MMA and MA with normal activities. This suggests that other enzymes may be responsible for regulating malonyl-CoA levels or that the enzyme functions normally in the fibroblast assay but not somatically *in vivo*.

The dog in this report showed clinical signs of stupor and seizures. These improved rapidly with correction of her hypoglycaemia and acidosis. In addition to the presence of lactic acid and ketone bodies in the urine, there was a dramatic elevation in MA as well as mild elevation of the citric acid cycle intermediates (citric, oxoglutaric, and succinic acid) and dicarboxylic acids (suberic acid). The hypoglycaemia in response to fasting would be expected if elevated malonyl-CoA levels inhibited the use of free fatty acids as an energy source. Initially, the dog was being force-fed a commercial diet formulated to provide calorie-dense nutrition for compromised dogs (high in fats and protein and relatively low in carbohydrates). The dog continued to be hypoglycaemic and ketoacidotic while on the high-fat diet. When the dog was switched to a highly digestible commercial diet that was low in total fat with a high percentage of fat as medium-chain triglycerides (MCT), the dog's clinical signs improved and the malonic aciduria resolved. Protein content of the second diet was lower than that of the calorie-dense diet, but still higher than that of a typical maintenance dog food (Table 1).

The effects of diet on humans with malonyl-CoA decarboxylase deficiency have been mixed, with reports that high-fat (Haan et al 1986), high-carbohydrate (Brown et al 1984) or high-protein (Gregg et al 1998) diets exacerbated the condition. Gregg and colleagues (1998) also showed increases in MA and MMA in the urine with a diet high in MCT, although they attributed this to carryover from the prior high-protein diet. Other reports suggest that a high-carbohydrate diet was beneficial (Haan et al 1986; Krawinkel et al 1994). The urinary organic acid profile in the previously reported dog with malonic and methylmalonic aciduria improved with carnitine supplementation and protein restriction, but the dog did not improve clinically (Podell et al 1996). Although the dog in this report appears to have a different defect from those cases, our results would support the idea that a high-carbohydrate, high-MCT diet is beneficial in malonic aciduria. They do not, however, resolve whether the high fat content or the high protein content of the original diet was detrimental.

The dog in this report had elevated concentrations of MA in the urine, but not of MMA. In all prior reports of malonic aciduria in both humans and the dog, there has been an elevation of both MA and MMA (Brown et al 1984; Buyukgebiz et al 1998; Gregg et al 1998; Haan et al 1986; Krawinkel et al 1994; MacPhee et al 1993; Matalon et al 1993; Ozand et al 1994; Podell et al 1996; Yano et al 1997), although in some of these MMA was not elevated in all samples evaluated (Yano et al 1997). The MMA elevation in these patients is attributed to malonyl-CoA inhibition of methylmalonyl-CoA racemase/mutase. One of the two human patients reported with normal malonyl-CoA decarboxylase activity had elevation of MMA greater than MA and other such cases are known. The situation in the dogs described in this report is unusual in that they had only an elevation of MA and normal malonyl-CoA decarboxylase activity. If the malonyl-CoA increase in the Maltese dogs were limited to the cytosol pool, methylmalonyl-CoA mutase within the mitochondria might not be affected. Alternatively, active malonyl-CoA decarboxylase in the mitochondria could have prevented inhibition of methylmalonyl-CoA mutase. However, the elevated citric acid cycle intermediates, though not as dramatic as seen in the human cases (Haan et al 1986; Ozand et al 1994), suggest a disturbance of mitochondrial metabolism. It is conceivable that the normal activity of malonyl-CoA decarboxylase seen in fibroblasts is the result of tissue-specific isoforms in the dog, or that there is a kinetic variant with abnormal K_m but normal V_{max}. The absence of MMA in this case could be due to decreased sensitivity and/or increased activity of canine methylmalonyl-CoA mutase. However, another case of malonic aciduria in a dog was accompanied by methylmalonic aciduria (Podell et al 1996). It is also possible that the primary problem involves hyperactivity of cytoplasmic acetyl-CoA carboxylase or a defect in the fatty acid synthetase complex.

Alteration of urine organic acids in family members suggests a familial condition but no firm conclusions can be drawn from the pedigree regarding the mechanism of inheritance. One of the half-siblings of the propositus had malonic aciduria, while another half-sibling and the mother showed only mild ketonuria. Since the propositus showed a normal urine organic acid profile while on the low-fat diet, it is difficult to know whether the changes in related dogs reflect subclinical homozygous or heterozygous states. The diet the family members were on when tested is not known.

In conclusion, this report suggests that malonic aciduria occurs as a familial trait in Maltese dogs. This differs from prior reports of malonic aciduria in humans and the dog in that MMA concentrations in the urine and malonyl-CoA decarboxylase activity in fibroblasts are normal. It thus suggests further heterogeneity in the malonic acidurias or a significant difference between the canine and human conditions.

ACKNOWLEDGEMENT

The authors thank Dr M. Rashed from King Faisal Specialist Hospital, Riyad, Saudi Arabia for acylcarnitine assays and Dr David Thorburn from The Murdoch Institute, Melbourne, Australia for his advice on the malonyl-CoA decarboxylase assays.

REFERENCES

Brown GK, Scholem RD, Bankier A, Danks DM (1984) Malonyl coenzyme A decarboxylase deficiency. *J Inher Metab Dis* **7**: 21–26.

Buyukgebiz B, Jakobs C, Scholte HR, Huijmans JG, Kleijer WJ (1998) Fatal neonatal malonic aciduria. *J Inher Metab Dis* **21**: 76–77.

Faunt KK, O'Brien DP, Barshop B, Thorburn DR, Shelton GD (1998) Malonic aciduria in Maltese dogs. *J Vet Intern Med* **12**: 236.

Gao J, Waber L, Bennett MJ, Gibson KM, Cohen JC (1999) Cloning and mutational analysis of human malonyl-coenzyme A decarboxylase. *J Lipid Res* **40**: 178–182.

Gregg AR, Warman AW, Thorburn DR, O'Brien WE (1998) Combined malonic and methylmalonic aciduria with normal malonyl-coenzyme A decarboxylase activity—a case supporting multiple aetiologies. *J Inher Metab Dis* **21**: 382–390.

Haan EA, Scholem RD, Croll HB, Brown GK (1986) Malonyl coenzyme A decarboxylase deficiency. Clinical and biochemical findings in a second child with a more severe enzyme defect. *Eur J Pediatr* **144**: 567–570.

Hoffman G, Aramaki S, Blum-Hoffman E, Nyhan WL, Sweetman L (1989) Quantitative analysis for organic acids in biological samples: batch isolation followed by gas chromatographic–mass spectroscopy analysis. *Clin Chem* **38**: 587–595.

Krawinkel MB, Oldigs HD, Santer R, Lehnert W, Wendel U, Schaub J (1994) Association of malonyl-CoA decarboxylase deficiency and heterozygote state for haemoglobin *c* disease. *J Inher Metab Dis* **17**: 636–637.

MacPhee GB, Logan RW, Mitchell JS, Howells DW, Tsotsis E, Thorburn DR (1993) Malonyl coenzyme A decarboxylase deficiency. *Arch Dis Child* **69**: 433–436.

Matalon R, Michaels K, Kaul R, et al (1993) Malonic aciduria and cardiomyopathy. *J Inher Metab Dis* **16**: 571–573.

McGarry JD, Foster DW (1980) Regulation of hepatic fatty acid oxidation and ketone body production. *Annu Rev Biochem* **49**: 395–420.

Ozand PT, Nyhan WL, al Aqeel A, Christodoulou J (1994) Malonic aciduria. *Brain Dev* **16**(supplement): 7–11.

Podell M, Shelton GD, Nyhan WL, et al (1996) Methylmalonic and malonic aciduria in a dog with progressive encephalomyelopathy. *Metab Brain Dis* **11**: 239–247.

Yano S, Sweetman L, Thorburn DR, Mofidi S, Williams JC (1997) A new case of malonyl coenzyme a decarboxylase deficiency presenting with cardiomyopathy. *Eur J Pediatr* **156**(5): 382–383.