Enzymological Characterization of a Feline Analogue of Primary Hyperoxaluria Type 2: a Model for the Human Disease

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Summary: This paper concerns an enzymological investigation into a putative feline analogue of the human autosomal recessive disease primary hyperoxaluria type 2. The hepatic activities of d-glycerate dehydrogenase, using both d-glycerate and hydroxypyruvate as substrates, and glyoxylate reductase, which are the deficient enzyme activities in human primary hyperoxaluria type 2, were markedly depleted in four affected cats (0–6% of controls). The activities of a number of other enzymes, lactate dehydrogenase, glutamate dehydrogenase, d-amino acid oxidase, aspartate:2-oxoglutarate aminotransferase, glutamate:glyoxylate aminotransferase and alanine:glyoxylate aminotransferase (the deficient enzyme in primary hyperoxaluria type 1) were unaltered. The intracellular distribution of d-glycerate dehydrogenase and glyoxylate reductase in cat liver was shown to be cytosolic, as they are in human liver. The activities of d-glycerate dehydrogenase and glyoxylate reductase were determined in unaffected related cats and putative heterozygotes were identified. The correlation between d-glycerate dehydrogenase and glyoxylate reductase activities in the related cats and their combined deficiency in the affected cats confirmed previous suggestions that they are identical gene products.

INTRODUCTION

The human autosomal recessive disease primary hyperoxaluria type 2 (McKusick 26000) is characterized by recurrent calcium oxalate nephrolithiasis and nephrocalcinosis, hyperoxaluria and hyper-L-glyceric aciduria (Williams and Smith, 1983). Peripheral blood leukocytes from patients with primary hyperoxaluria type 2 have been shown to be deficient in d-glycerate dehydrogenase activity (Williams and Smith, 1968; Chalmers et al., 1984). D-Glycerate dehydrogenase also possesses...
glyoxylate reductase activity (Willis and Sallach, 1962; Dawkins and Dickens, 1965), and recently a deficiency of both D-glycerate dehydrogenase and glyoxylate reductase has been demonstrated in the liver of a patient with primary hyperoxaluria type 2 (Mistry et al., 1988).

Useful animal models for human genetic diseases are rare. A colony of cats has recently been described in which there was a high incidence of a condition that was superficially similar to human primary hyperoxaluria type 2 (Blakemore et al., 1988; McKerrell et al., 1989). The disease, which appeared to be transmitted through the colony in an autosomal recessive manner, was characterized by oxalate deposits in the kidney, hyperoxaluria and hyper-L-glyceric aciduria.

In the present study we have determined the activities of a variety of enzymes, including D-glycerate dehydrogenase and glyoxylate reductase, in livers from affected cats and compared them with unaffected related and unrelated cats. The value of this cat model for human primary hyperoxaluria type 2 is discussed.

METHODS

Cats: The cats were maintained in a colony at the Department of Clinical Veterinary Medicine, University of Cambridge. The four affected cats examined all had hyperoxaluria and hyper-L-glyceric aciduria. Two were killed in acute renal failure by barbiturate overdose. They had renal tubular depositions of calcium oxalate crystals (Blakemore et al., 1988; McKerrell et al., 1989). One was killed before the onset of renal failure and one was biopsied before the onset of renal failure. The 16 unaffected related cats were either obligate heterozygotes, offspring of obligate heterozygotes or offspring of litter mates of obligate heterozygotes (see Figure 1). Obligate heterozygotes are defined as those cats who have produced litters containing affected animals. Livers were also obtained from five control unrelated cats. These cats were housed outside the colony. Two had been subjected to prolonged anaesthesia; three had been killed by barbiturate overdose, one by intravenous administration of KCl and one had died of a haemorrhage.

Livers: Samples of cat liver were obtained either at autopsy (three of the affected cats, all five unaffected unrelated cats and two unaffected related cats) or by open biopsy while under general anaesthesia (one affected cat and all the other 14 unaffected related cats). They were frozen immediately and stored at -20°C until required.

For the D-glycerate dehydrogenase and glyoxylate reductase assays, thawed specimens of liver were homogenized in a ground glass mortar and pestle by hand in ice-cold 154 mmol/L KCl, containing 10 mmol/L mercaptoethanol and 100 mmol/L MnCl₂ (Mistry et al., 1988). The homogenate was dialysed overnight at 4°C against 50 mmol/L sodium acetate buffer pH 6.0, containing 10 mmol/L mercaptoethanol. Any insoluble material was spun off and the supernatant used for the assays.

For the other enzyme assays, thawed samples of liver were sonicated in 100 mmol/L potassium phosphate buffer pH 7.4, containing 100 μmol/L pyridoxal