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Chapter 10

Treatment of organic acidurias

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Summary

The major recent advances in disorders of amino acids and organic acids have been in their management. Treatment regimens for many disorders have been improved and standardized. Treatment options commonly used in organic aciduria include: (a) substitution, elimination or restriction of a substrate(s) (dietary treatment); (b) cofactor replacement; (c) enhancing alternative pathways or increasing excretion of toxic metabolites; (d) gene therapy (organ transplant). It is important to realize that real efforts do have to be made not only initially at diagnosis but also in long-term management, to reach the goal of preventing mental and physical handicap.

Introduction

Organic acidurias are inherited disorders of the catabolism of amino acids, carbohydrates and fatty acid oxidation characterized by the accumulation and excretion of non-amino organic acids in the urine. Disorders of fatty acid β-oxidation are dealt with in the chapter by Uziel (Ch. 2) while defects in the metabolism of carbohydrates are covered by De Vivo's (Ch. 4).

Organic acidurias have the reputation of being associated with the urinary excretion of huge amounts of metabolites; for example, in propionic acidaemia, patients typically excrete large amounts of 3-hydroxypropionic acid and methylcitric acid; those with isovaleric acidaemia excrete large amounts of 3-hydroxyisovaleric acid and isovalerylglycine.

Many of the organic acids that accumulate in this type of disorder are relatively strong and will accordingly give rise to changes in the acid–base status of the blood plasma. The extent of the acid–base changes is highly dependent on the concentration of their organic acids, but on the other hand, it cannot be overlooked that some organic acidurias are characterized by moderate (or even low) excretions of abnormal organic acids (i.e. 4-hydroxybutyric aciduria, tyrosinaemia type I, and 3 methylglutaconic aciduria type II) and for this reason many patients with organic acidaemia do not have a metabolic acidosis. For all these reasons, the diagnosis is still difficult (Duran *et al.*, 1991). Moreover, specific reagents for the detection of organic acids do not exist and diagnosis in infants and children is performed by demonstrating specific abnormal organic acids in urine, usually by combined gas chromatography-mass spectrometry (GC-MS) (Burlina, 1986).

The most frequent organic acidurias susceptible to treatment are reported in Table 1. Some of these disorders have more than one cause. For example, multiple carboxylase deficiency may be due to defects of biotinidase or holocarboxylase synthetase; methylmalonic acidaemia may be due to

defects of methylmalony-CoA mutase or of any of several enzymes involved in the synthesis of the mutase coenzyme, such as adenosyl-B_{12}.

Table I. Organic acidurias capable of treatment

MSUD
Propionic aciduria
Methylmalonic aciduria
Glutaric aciduria type I
3-Methylglutaconyl-CoA lyase
Tyrosinaemia type I
Isovaleric acidaemia
β-ketothiolase deficiency
Multiple carboxylase deficiency

Therapeutic protocols are therefore influenced by the pathophysiology and disease mechanism and good therapy has to be the result of a true symbiosis between the fields of human biochemical genetics and nutrition.

Therapeutic strategies for organic acidurias

The management of infants with organic acidurias is demanding (Nyhan, 1991). Treatment options commonly used include:

- substitution, elemination or restriction of a substrate(s) (dietary treatment);
- cofactor replacement;
- enhancing alternative pathway or increasing excretion of toxic metabolites;
- gene therapy (organ transplant).

Dietary treatment

The mainstay of treatment for most organic acidurias is dietary restriction of amino acid(s) that are not metabolized properly. The substrate must be provided, however, in adequate amounts to promote normal growth while avoiding excesses that are toxic (Ogier *et al.*, 1990).

In many organic acidurias we are confronted with the problem that products of the catabolism of essential amino acids are highly toxic. Their accumulation leads to serious clinical illness and death. It has seemed intuitively reasonable to approach this problem with the rigid restriction of the intake of protein in all organic acidurias, except for maple syrup urine disease (MSUD) in which the natural protein is partially replaced with a synthetic amino acid mixture.

Often the best that can be done is to reduce the total protein intake to the minimum needed to maintain satisfactory growth whilst maintaining normal energy intake. Essential amino acids and other nutrients have to be maintained at the optimum level at which the offending amino acid or acids can be provided that will meet the anabolic needs for growth while keeping the accumulation of toxic intermediates at minimal levels. It requires careful monitoring of the levels of the accumulating amino acid or organic acid, rates of growth in weight, height and head circumference, and nitrogen balance.

In normal conditions it is expected that if the precursors are not provided in excess of requirements, the toxic metabolite will not accumulate. However, it is not as simple as this because during anabolism in the presence of minimal quantities of amino acids, even at quantities below requirements, these substrates are available to catabolic enzymes and therefore there is always a certain level of accumulation. This certainly is the case in disorders of propionate metabolism: in fact below a certain amino acid intake we would expect the catabolism of tissue proteins and an

increased of accumulation of metabolites (Nyhan *et al.*, 1991). Similarly an appropriate intake of vitamins and minerals must be maintained.

During illness and fasting various metabolic adaptations occur and the diet may need to be altered. Metabolic stress precipitated by minor infections or surgery may lead to serious biochemical derangement (the rate of protein breakdown exceeds that of synthesis with a net production of amino acids and an increase in their irreversible catabolism) and to prevent such serious complications the diet is changed to an 'emergency regimen' (Dixon & Leonard, 1992). This consists of a high carbohydrate intake (a solution of glucose polymer), sometimes with the addition of a fat emulsion, to reduce endogenous protein metabolism and hence the accumulation of toxic intermediates.

The use of special metabolic formulas is still controversial. Those formulas which are free of the precursor amino acids are important factors in the treatment of these disorders because they provide a buffer of nitrogen, which is beneficial in the promotion of visceral and somatic protein synthesis. A low-protein diet that supplies only whole protein sources may not have sufficient nitrogen for synthesis. In addition, the use of special metabolic formulas allows the flexibility to decrease toxic amino acids, if clinically indicated, while supplying enough amino acids to promote growth and present catabolism (Queen *et al.*, 1981). Anyway, it is important to point out that the use of metabolic formulas is not accepted worldwide (Poggi *et al.*, 1993).

At the meeting of European Metabolic Group in Lausanne, 1992 (Milupa Scientific Information, 1994) a workshop was held in order to establish proposals for the follow up of patients with metabolic disorders. Biochemical indicators of overtreatment and insufficient treatment in organic acidurias are summarized in Table 2.

Table 2. Biochemical indicators of overtreatment and insufficient treatment in propionic and methylmalonic acidurias

Overtreatment	Insufficient treatment
Plasma protein ↓	Ketoacidosis (Acetest +)
Albumin ↓	Anaemia/thrombopenia
Prealbumin ↓	Lactate ↑
Trace elements ↓	Ammonia ↑
Essential amino acids ↓	OLCFA ↑
Transaminases ↑	Urinary metabolites ↑
Urinary metabolites ↑	Free carnitine ↓
	Acyl/free carnitine ↑

Clinically it is difficult to differentiate signs of overtreatment from insufficient treatment. Failure to thrive, oedemas, loss of appetite, vomiting, cardiomyopathy (Massaud & Leonard, 1993), pancreatitis (Burlina *et al.*, 1993) and in particular skin and hair abnormalities (De Raeve *et al.*, 1994) are common in both situations.

The frequency at which the treatment should be controlled will depend on the severity of the enzyme defect; it further depends on the compliance and on the extent of understanding of the disease shown by the parents, and the experience of the paediatricians with management of the specific disorder (Poggi *et al.*, 1993).

Cofactor replacement

In some organic acidurias there is altered binding of a vitamin cofactor to the mutant apoenzyme and it is often possible to provide a large excess of the cofactor and overcome this altered binding affinity.

The cofactor-responsive organic aciduria can be classified according to the mechanism by which the holoenzyme activity is increased. The mechanisms include defective processing of a vitamin or

cofactor (cobalamin for methylmalonic aciduria, biotin for multiple carboxylase deficiency), defective binding of a cofactor (thiamine for MSUD), and undetermined mechanisms (riboflavine for β-oxidation defects) (Sweetman, 1991).

An example of defective processing of a vitamin-related organic aciduria is represented by biotinidase deficiency. The treatment of biotinidase deficiency is essentially the administration of pharmacological doses of biotin. The customary dose is 10 mg of biotin/day given orally, but doses as high as 30–60 mg/day have been given either orally or intravenously. The optimum amount has not yet been established. For most patients the response is immediate and dramatic, with correction of biochemical markers (lactic acidosis) and clinical signs (skin rash, alopecia, neurological symptoms), and development becomes normal. Some patients have developed nerve deafness and optic atrophy while being treated with biotin. These problems have not occurred in any patients with holocarboxylase synthetase deficiency (the other defect in which biotin can be used) while being treated with biotin, and may be due to the toxicity of biocytin in the patients with biotinidase deficiency (Wolf & Heard, 1990).

Several important points must be emphasized in vitamin-responsive organic acidurias (Leonard & Daish, 1985).

(a) The requirement of pharmacological doses of the cofactor to produce a therapeutic response is very high (1000 μg/day) compared with the recommended daily allowance (25 μg/day);
(b) it is desirable to demonstrate a reproducible improvement in specifically aberrant clinical chemical values whether or not the precise defect in the reaction mechanism is known, so as to identify candidates for such therapeutic approaches;
(c) The ultimate designation of 'responsiveness' must be derived from observations of the clinical efficacy of therapy rather than from measurements of reaction precursors or products.

Increasing excretion of toxic metabolites or enhancing alternative pathways

Carnitine

Treatment of organic acidurias by means of conjugation of potentially hazardous substances are nowadays frequently used. Originally it was thought that only glycine conjugates were formed (i.e. isovalerylglycine in isovaleric acidaemia) but now other conjugating substrates such as carnitine and glucuronic acid are well recognized.

A secondary deficiency of carnitine occurs widely in patients with organic acidaemias. These include propionic acidaemia, methylmalonic acidaemia, isovaleric acidaemia, glutaric acidaemia type I, 3-hydroxy-3-methyl-glutaryl CoAHMG-CoA lyase deficiency and the fatty acyl-CoA dehydrogenase deficiencies (Roe *et al.*, 1991). In these patients, acyl coenzyme A compounds accumulate in the mitochondria and cause inhibition of many of the respiratory enzymes. They are removed by forming an acyl–carnitine complex that is excreted in the urine. The use of carnitine for 'buffering' acyl coenzyme A compounds leads to loss of tissue carnitine and the development of a secondary carnitine deficiency. The clinical complications can be prevented by carnitine supplementation (Winter *et al.*, 1992), treating patients with these disorders are persuaded that this is a real advance in management, above all when the patients are in catabolic state or are faced with intercurrent infections or surgery. Anyway, it is still difficult to document the physiological effects of treatment because therapy in these patients is not only by administering carnitine. Theoretically, the maximum benefit should accrue not simply when the free carnitine level in the blood is normal, but when urinary esters are maximal; however, it is difficult to achieve this status despite progressive increases in carnitine dosage.

The customary dose is 100 mg/kg/b.w. by mouth but doses as high as 400 mg/kg/b.w. have been suggested. This compound is virtually non-toxic, but the amount that can be tolerated is limited by the production of diarrhoea at higher doses. The effectiveness of replenishing deficient stores can be readily assessed by measuring the concentration of acyl and free carnitine in the blood and urine.

Metronidazole

Different antibiotics have a markedly greater effect on fecal propionate concentrations but they also have a high incidence of side-effects. Metronidazole has recently been chosen because there is a low risk of complications and because the antibacterial mechanism of action is specific for anaerobes, the bacterial subgroup to which propionate production capacity is most closely linked (Brain *et al.*, 1988).

Studies by Walter *et al.* (1988) and Thompson *et al.* (1990) have shown that a dose of metronidazole varying from 10 to 20 mg/kg resulted in a fall in the excretion of all metabolites in methylmalonic (MMA) and propionic (PA) patients, and in clinical improvement (increased alertness, increased activity and improved appetite).

The optimal method of administering metronidazole in MMA and PA has not yet been determined. At the moment such therapy should be approached with some caution and with careful monitoring for side-effects (including gastrointestinal disturbances, leucopoenia and peripheral neuropathy) until safety has been reached in these disorders. It is possible that continuing metronidazole treatment at a dose of 10 mg/kg per day may lead to the appearance of resistant strains of anaerobic bacteria, which are the major bacterial source of propionate. Alternatively, the use of metronidazole intermittently, or by rotation with other antibiotics, may decrease the likelihood of developing resistant strains . Our preliminary results (unpublished observations) in five patients with MMA have shown that the breath hydrogen test remains negative after 6 months' treatment with metronidazole and clinically an apparent improvement in appetite was present. This effect may be attributable to lowering of the plasma propionate concentration, particularly in the portal vein. In sheep, infusion of propionate into the portal vein markedly inhibits feeding, an effect apparently mediated through hepatic receptors (Amil & Forbers, 1980), and it would implicate increased propionate concentrations as a cause of poor appetite in disorders of propionate metabolism.

While current studies underline the importance of bacterial propionate production in the gut in disorders of propionate metabolism, the optimal long-term therapeutic approach remains to be determined. A large study aimed at better delineation of the best approach to therapy with metronidazole in PA and MMA patients has recently been done and will be soon published (Thompson, in press).

NTBC

Treatment with a diet restricted in phenylalanine and tyrosine may prevent or alleviate the kidney damage, but does not prevent a fatal outcome in patients affected with tyrosinaemia type I. At present, liver transplantation is the only effective therapy but one of the major problems of follow-up studies of tyrosinaemia is predicting the moment at which hepatocellular carcinoma will develop and the time of the liver transplantation (Kvittingen, 1991). Today there are no reliable biochemical parameters able to predict the correct time. Recently Lindstedt *et al.* (1992) reported treating one acute and four subacute-chronic cases with 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cyclohexanedione (NTBC), a potent inhibitor of 4-hydroxylphenylpyruvate dioxygenase, to prevent the formation of maleylacetoacetate and fumarylacetoacetate and their saturated derivatives. The oral daily dose was 0.1–0.6 mg/kg and the biochemical data showed decreased excretion of succynilacetoacetate succynilactone and α-fetoprotein. Improved liver function was reflected by normal concentrations of prothrombin complex and in decreased activities of alkaline phosphatase and γ-glutamintransferase in serum. Computed tomography revealed regression of hepatic abnormalities in three patients. No side-effects were encountered.

A large trial, including more than 10 centres, is currently in progress to confirm if this type of treatment may thus offer an alternative to liver transplantation in hereditary tryosinaemia type I.

Gene therapy (organ transplant)

The ultimate goal of treatment of organic aciduria, as of many genetic diseases, is repair of the genetic defect, i.e. gene therapy. Recent advances in the technology of gene transfer, and in particular the use of modified viruses to carry genetic material, have turned what was previously a distant prospect into an immediate reality. There are currently more than 40 approved human gene therapy protocols, including for organic acidurias, with more being approved constantly (Levine & Friedman, 1993).

Liver transplantation has become a realistic alternative for the treatment of some organic acidurias such as tyrosinaemia type I. The question is whether we have to restrict this type of therapy to organic acidurias that are mainly or completely confined to the liver or whether we can extend it to those affecting not only the liver but other tissues as well. For example in propionic aciduria and methylmalonic aciduria the enzyme is ubiquitous, but the main site of production of the toxic compound is muscles while for the catabolism it is the liver. For this reason, patients who present with propionic or methylmalonic acidurias that are difficult to control with conventional therapy could benefit because a transplanted liver may well be able to clear all toxic metabolites.

At the moment little information is available (J.-M. Saudubray, personal observations) but this problem will be one of the main topics for the next future on treatment of organic acidurias.

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