Medium-chain acyl-CoA dehydrogenase deficiency: A $^1$H-n.m.r. spectroscopic study

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Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency which causes an impairment of the mitochondrial β-oxidation of medium-chain fatty acids, is one of the more commonly diagnosed inborn errors of metabolism [1]. The disorder presents as a non-ketotic hypoglycaemia associated with reduced plasma carnitine levels resembling Reye’s syndrome [2]. The disorder is characterized by the detection of urinary dicarboxylic and hydroxyacids derived from ω-oxidation and ω-1-oxidation, respectively, of the accumulating parent monocarboxylic acids [3] and by the excretion of acylglycine (suberylglycine or hexanoylglycine) and acylcarnitine (octanoylcarnitine or hexanoylcarnitine) conjugates which appear to be exclusive to this disorder and could serve as specific diagnostic markers [4].

Previous work has shown the value of utilizing $^1$H-n.m.r. for diagnosis and therapeutic monitoring in several inherited disorders [5, 6]. The present study involved following the urinary metabolite pattern from an MCAD-deficient patient, both during a fat challenge in the form of long-chain triacylglycerol and after L-carnitine administration.

Fig. 1. Detection of urinary metabolites indicative of perturbed metabolism in MCAD deficiency

(a) $^1$H-n.m.r. spectrum of urine from an acutely ill child with MCAD deficiency showing the characteristic metabolites excreted in this disorder. The spectrum, showing the aliphatic region (0.50-4.50 p.p.m.) is the result of 100 accumulations. Legend: Adp, adipic acid; Cit, citrate; Cr, creatine; Crn, creatinine; DMA, dimethylamine; Gly, glycine; HG, hexanoylglycine; 3OH, 3 hydroxybutyrate; 5OH, 5 hydroxyhexanoic acid; OC, octanoylcarnitine; SEB, sebacic acid; Sub, suberic acid; SG, suberylglycine; Suc, succinate. (b) Time course of urinary carnitine recovery (expressed as mol/mol creatinine) after dosing the patient with 200 mg of L-carnitine/kg body weight as an oral dose at time $T=0$. Legend: ⃝, free carnitine; ×, acetylcarnitine; ○, octanoylcarnitine.
This in turn drains the hepatic regulatory cholesterol pool from the body. The rate-limiting step in bile acid synthesis is catalysed by microsomal cholesterol 7α-hydroxylase [1]. This enzyme is believed to be regulated by negative feedback by bile acids returning to the liver using insoluble bile acid-conjugates (Fig. 1b). Use of carnitine supplements during metabolic perturbation would result in the liberation of free CoA for other key metabolic reactions; however, under basal conditions, when the patient is metabolically well, carnitine administration could be contraindicated, since this could increase the transfer of long-chain fatty acyl-CoAs into the mitochondria exerting stress on the defective medium-chain β-oxidation pathway.

An additional novel finding was the detection of a large increase in urinary trimethylamine-N-oxide (TMAO) concentration subsequent to carnitine administration. These findings have been substantiated in two other cases of MCAD deficiency, where TMAO levels were elevated above normal after carnitine treatment. We believe this may be a result of carnitine degradation by intestinal micro-organisms [7] and may be a major route of carnitine loss during therapy in some metabolic disorders.

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Study on the effect of adrenergic agents on bile acid synthesis in vitro

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Bile acid synthesis from cholesterol takes place exclusively in the liver and represents the major pathway for elimination of cholesterol from the body. The rate-limiting step in bile acid synthesis is catalysed by microsomal cholesterol 7α-hydroxylase [1]. This enzyme is believed to be regulated by negative feedback by bile acids returning to the liver via the portal circulation. A pharmacological approach to the modulation of plasma cholesterol levels is the interruption of the return of bile acids to the liver using insoluble bile acid-binding drugs which activate cholesterol 7α-hydroxylase. This in turn drains the hepatic regulatory cholesterol pool resulting in low-density lipoprotein receptor activation. Hyper tension, a known factor for coronary heart disease markedly affects plasma lipid profiles. The full extent and importance of adrenergic and other unknown factors on the regulation of bile acid synthesis has not yet been adequately evaluated. The availability of primary cultures of non-proliferating adult rat hepatocytes affords an opportunity to study the effect of a range of pharmacological agents on bile acid synthesis. Thus, the aims of this study were to determine the normal range of cholesterol 7α-hydroxylase activity in rat hepatocytes, and to examine the effect of an α-adrenergic antagonist, prazosin, and a β-adrenergic antagonist, propranolol, on this enzyme.

Rat hepatocytes and microsomes were prepared as described by Devery [2]. Isolated hepatocytes were incubated with either physiological saline (0.9%, w/v), prazosin (5.5 x 10^-3 m) or propranolol (5.5 x 10^-3 m). Rat liver microsomes were resuspended in 0.1 m-potassium phosphate buffer, pH 7.4 and 2 mM-diithiothreitol, and freeze-dried as described previously [3]. Freeze-dried microsomal powder was extracted with 50 vols n-butanol and the suspension centrifuged at 15,000 rev./min for 5 min in an IEC Centra-M bench centrifuge. The pellet was dried under vacuum and resuspended in buffer containing 20% (w/v) glycerol. Microsomal cholesterol 7α-hydroxylase activity was measured as described by Nimmanit & Porter [3]. A portion (100 μl) of the microsomal pellet suspension containing 0.5–3 mg of protein was preincubated at 37°C for 5 min with 900 μl of cofactor solution in buffer containing 16.7 mM-glucose 6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase and 2 mM-NADP+. The reaction was initiated by the addition of 100 nmol of [4-14C]cholesterol (1 Ci/mmol). The reaction was terminated after 25 min at 37°C.

![Fig. 1. The range of cholesterol 7α-hydroxylase activity observed in control hepatocytes incubated with physiological saline](image)

(a) Range of microsomal cholesterol 7α-hydroxylase activity (pmol min^-1 mg^-1) in rat hepatocytes (means ± S.E.M. = 4.36 ± 1.6). (b) Relative specific activity of cholesterol 7α-hydroxylase in control, (●) (100%) prazosin (○) (120.2%) and propranolol-treated (□) hepatocytes (53.9%). Results are expressed as the means of three experiments conducted in triplicate.

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