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Original article

Cardiac dysfunction and peri-weaning mortality in malonyl-coenzyme A decarboxylase (MCD) knockout mice as a consequence of restricting substrate plasticity

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ABSTRACT

Inhibition of malonyl-coenzyme A decarboxylase (MCD) shifts metabolism from fatty acid towards glucose oxidation, which has therapeutic potential for obesity and myocardial ischemic injury. However, ~40% of patients with MCD deficiency are diagnosed with cardiomyopathy during infancy.

Aim: To clarify the link between MCD deficiency and cardiac dysfunction in early life and to determine the contributing systemic and cardiac metabolic perturbations.

Methods and results: MCD knockout mice ($^{-/-}$) exhibited non-Mendelian genotype ratios (31% fewer MCD$^{-/-}$) with deaths clustered around weaning. Immediately prior to weaning (18 days) MCD$^{-/-}$ mice had lower body weights, elevated body fat, hepatic steatosis and glycogen depletion compared to wild-type littermates. MCD$^{-/-}$ plasma was hyperketonemic, hyperlipidemic, had 60% lower lactate levels and markers of cellular damage were elevated. MCD$^{-/-}$ hearts exhibited hypertrophy, impaired ejection fraction and were energetically compromised (32% lower total adenine nucleotide pool). However differences between WT and MCD$^{-/-}$ converged with age, suggesting that, in surviving MCD$^{-/-}$ mice, early cardiac dysfunction resolves over time. These observations were corroborated by in silico modelling of cardiomyocyte metabolism, which indicated improvement of the MCD$^{-/-}$ metabolic phenotype and improved cardiac efficiency when switched from a high-fat diet (representative of suckling) to a standard post-weaning diet, independent of any developmental changes.

Conclusions: MCD$^{-/-}$ mice consistently exhibited cardiac dysfunction and severe metabolic perturbations while on a high-fat, low carbohydrate diet of maternal milk and these gradually resolved post-weaning. This suggests that dysfunction is a common feature of MCD deficiency during early development, but that severity is dependent on composition of dietary substrates.

1. Introduction

The heart has high energy demands that are primarily met by mitochondrial fatty acid oxidation (FAO) [1]. This is regulated at the level of the outer mitochondrial membrane by the activity of carnitine palmitoyl transferase 1 (CPT-1), which acts to shuttle cytosolic long chain acyl-CoA esters into the mitochondria [1]. Malonyl-CoA is a potent endogenous allosteric inhibitor of CPT-1 and thereby a key regulator of fatty acid catabolism. It has a rapid turnover in the heart ($t_{1/2}$ ~ 1.25 min) [2,3] resulting from the balance between synthesis from acetyl-CoA by acetyl-CoA carboxylase (ACC), and the reverse reaction catalysed by malonyl-CoA decarboxylase (MCD). By controlling

Abbreviations: α-SA, alpha skeletal actin; Atg3, autophagocytosis associated protein 3; β-MHC, beta myosin heavy chain; CPT-1, carnitine palmitoyl transferase 1; FAO, fatty acid oxidation; GLUT1, glucose transporter 1; GLUT4, glucose transporter 4; HDL, high density lipoprotein; LDH, lactate dehydrogenase; LDL, low density lipoprotein; MCD, malonyl-coenzyme A decarboxylase; MTE-1, mitochondrial thioesterase 1; PDK4, pyruvate dehydrogenase kinase 4; TAN, total adenine nucleotide pool; UCP3, uncoupling protein 3.

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malonyl-CoA levels, MCD determines the rate of myocardial fatty acid oxidation [1].

Acute pharmacological MCD inhibition has been shown to decrease fatty acid oxidation and accelerate glucose oxidation in both ex vivo rat and in vivo pig hearts [4–6]. Furthermore, significant reduction of infarct size has been reported in MCD deficient mice in which glucose oxidation rates were enhanced as a result of the inhibition of fatty acid oxidation [6,7]. Collectively these findings support the proposition that MCD inhibition can be targeted as an effective approach to treating myocardial ischemia.

While inhibition of MCD and decreasing fatty acid oxidation may have potential therapeutic benefit in the mature heart, it is not clear what consequences of MCD inhibition would be in the newborn period. While foetal hearts rely primarily on glycolysis and lactate oxidation as sources of energy, shortly after birth there is a rapid maturation of fatty acid oxidation [4,8]. In contrast, glucose oxidation does not mature until weaning [9], and in the newborn period the heart actually becomes more reliant on fatty acid oxidation as an energy source than the adult heart [8,9]. As a result, MCD inhibition has the potential to decrease cardiac energy production in the newborn period [1].

Concerns for the general safety of MCD inhibition arise from patients with in-born MCD deficiency, a rare autosomal recessive disorder characterized by severe metabolic perturbation in the form of malonic aciduria and variable presentation of developmental delay, seizures, and hypoglycaemia [10,11].

In particular, cardiomyopathy develops at an early age in up to 40% of MCD deficient patients contributing to morbidity and mortality [12–14]. However, the pathogenesis of cardiomyopathy remains unclear and may not be directly related to MCD deficiency since not all patients are affected.

The aim of this study was to examine the early cardiac and metabolic phenotypes in MCD knockout ($MCD^{-/-}$) mice to determine whether functional and metabolic alterations are intrinsic features of chronic MCD perturbation during infancy. Here we show that $MCD^{-/-}$ mice universally develop cardiac dysfunction during the peri-weaning period associated with significantly increased mortality and a severe metabolic phenotype of lipid accumulation and carbohydrate depletion, which gradually improves with age. This suggests that restricting substrate plasticity at times of high energy demand and major dietary change is capable of triggering conditions of energy starvation that are detrimental to the young heart.

2. Methods

2.1. Mouse colonies

MCD knockout mice ($MCD^{-/-}$) were imported from the originating colony at the University of Alberta and backcrossed with C57BL/6J mice for >6 generations. All mice were generated by heterozygous pair mating thus littermates could be used as the appropriate wild-type controls ($MCD^{+/+}$). Mice were kept under pathogen-free conditions, 12 h light–dark cycle, controlled temperature (20–22 °C), and fed chow and water ad libitum. A breeding diet was used for all dams, LabDiet PicoLab® Mouse Diet 5058, while weaned mice were gradually introduced to PicoLab® Rodent diet 5053 over a 6 week period. This protocol is identical to the originating colony. This investigation conforms to UK Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act, 1986.

2.2. In vivo assessment

Mice were imaged and left ventricular (LV) hemodynamic measurements (adult mice) performed as previously described [15,16]. Body composition was analysed by magnetic resonance relaxometry in conscious restrained mice [15].

2.3. Tissue and plasma collection

All mice, knockout ($MCD^{-/-}$), wild type ($MCD^{+/+}$) and heterozygous ($MCD^{+/-}$) were non-fasted, since fasting would have adverse effects at 18 days of age. Mice were killed by cervical dislocation; the heart, skeletal muscle (mixed soleus and gastrocnemius) and liver were excised, snap frozen in liquid nitrogen and stored at −80 °C for biochemical analyses. Owing to limited tissue availability, body weight and age matched stock C57BL/6J male mice (Harlan, UK) were used as controls for the malonyl-CoA level assessment only. Blood was collected from terminally anaesthetized mice into EDTA microcapillary tubes (Sarstedt, UK) and centrifuged (3000 rpm, 10 min) to obtain plasma. Concentrations of free fatty acids, triglycerides, cholesterol, high-density lipoprotein, low-density lipoprotein, total creatine kinase (CK), lactate, lactate dehydrogenase, and 3-hydroxybutyric acid (ketone bodies) were measured by the Mouse Biochemistry Laboratory, Addenbrooke's Hospital, Cambridge University Hospitals NHS Trust.

2.4. Biochemical and gene expression analysis

The activity of the pyruvate dehydrogenase complex (active and total portion of the enzyme), citrate synthase, triglyceride and glycogen content in LV, skeletal muscle and liver were determined spectrophotometrically (Online Supplement Methods). Total creatine, total adenine nucleotide pool (ATP + ADP + AMP) and malonyl-CoA content were measured by HPLC [4,16]. Total RNA was extracted from heart tissue of $MCD^{+/+}$, $^{+/-}$, $^{-/-}$ (n = 4 per group), survivor $MCD^{-/-}$, $^{+/+}$ (n = 9 per group) and messenger RNA levels analysed using qRT-PCR for a panel of hypertrophic markers and metabolic genes [16] (Online Supplement Methods).

Liver lipid content was assessed in frozen tissue sections (12 μm), fixed in 4% PFA, rinsed with 60% triethyl phosphate, stained with oil red O (0.5%) and counterstained with haematoxylin.

2.5. CardioNet metabolic network reconstruction

In silico simulations were performed using the metabolic network of the cardiomyocyte CardioNet (Online Supplement Methods) [17].

2.6. Data analysis and statistics

All data was analysed blind to genotype. Comparison between two groups was by Student's t-test (Gaussian data distribution), Mann–Whitney U test (non-Gaussian data distribution) and between 3 groups by one-way analysis of variance (ANOVA) using Bonferroni's correction for multiple comparisons. Kaplan–Meier survival curves were compared by log-rank test. Pearson's correlation coefficient was used to assess the relationship between the variables. Data are presented as mean ± S.E.M. Statistical analysis was carried out using GraphPad Prism software, v. 5.04. Differences were considered significant when $P < 0.05$.

3. Results

3.1. MCD knockout mice exhibit significantly higher early mortality

After establishing the mouse colony using heterozygote pair breeding, non-Mendelian offspring ratios were noted at time of ear-notch sampling for genotype analysis (18 days of age): from 503 pups observed, the frequency at 18 days was $MCD^{+/-}$ 276: $MCD^{+/+}$ 173: $MCD^{-/-}$ 54 (Chi squared = 61; $P < 0.0001$; Fig. 1A). A further five $MCD^{-/-}$ mice were identified from cadaver remains at between 1 and 16 days of age. Since the expected Mendelian outcome was for 126 $MCD^{-/-}$, this suggests that approximately 53% of $MCD^{-/-}$ pups are unaccounted for, presumably dying either in utero or in post-partum and cannibalised.