INTRODUCTION

PROPIONIC ACIDEMIA: DISEASE BURDEN AND CHALLENGES IN DRUG DISCOVERY

The prevalence of propionic acidemia (PA), an inborn error of metabolism, is 1 in 100,000 in the US, but can be as high as 1 in 2000 in specific high-risk populations.

In PA, a defect of the propionyl-CoA carboxylase (PCC) enzyme (Fig. 1), impacts metabolism of branched chain amino acids and odd chained fatty acids, blocking the conversion of propionyl-CoA to methylmalonyl-CoA, and causing the accumulation of metabolic intermediates in the urine and the blood.

It is suggested that inhibition of the urea cycle by these intermediates results in clinically significant elevations in blood ammonia, contributing to both morbidity and mortality.

Additionally, it is suggested that the accumulation of organic acid metabolites induces upregulating of TCA cycle intermediates resulting in mitochondrial dysfunction that could underlie some of the long-term complications of PA such as cardiomyopathy.

Discovery and development of new therapies for rare diseases like PA is hampered by limited patient populations, and challenges of species representation and the complete disease phenotype in animal models.

Cell-based models, e.g. fibroblasts, often lack the complete metabolic machinery and functional specific high-risk populations.

The prevalence of propionic metabolic responses receptors (E-cadherin, CD26 and CD81), differentiated maturation markers (HNF-4, Hemoxygen), and transport conditions restore liver specific differentiated markers (HNF-4, Albumin, transporters) associated with viability and response to amino acid challenge (MTT, Ammonia and Urea).

To mimic a clinical metabolic decompensation event, the medium was supplemented with branched chain amino acids, isoleucine (5 mM) and valine (5 mM).

Hepatocytes from the PA patient produced a significantly higher amount of ammonia (measured in supernatant) when pre-conditioned with supplemented media (p<0.0086), relative to normal cell culture media (Fig. 8A).

However, a similar response to supplemented media was not observed in corresponding experiments using healthy donor hepatocytes (Fig. 8A).

RESTORING PHYSIOLOGICAL LIVER PARAMETERS PRESERVES HEPATOCYTE MORPHOLOGY, FUNCTION AND RESPONSE IN VITRO

The previously described Hemoshear liver system technology, based on the microarchitectural liver sinusoids, creates a flow-based culture system that recapitulates transmural perfusion, nutrient gradients, and interstitial fluid movement (Fig. 2).

The patient’s homogenous PCCA gene mutation (Fig. 5A, c.932C > T), in the PA patient exhibited normal morphology compared to healthy livers.

Over 10 days of culture in the system, the hepatocytes maintained a normal differentiated and polarized morphology seen in the peripheral actin staining and lack of fibroblastic transformation (Fig. 6A, Actin-Phalloidin – green, nuclear Draq staining – blue).

Albunin levels in the effluent (404.4 ± 53.4 mg/mL) and MTT assay readings were within the range we note with healthy hepatocytes.

Hematoxylin and eosin staining of paraffin embedded sections of the explanted liver (Fig. 6A) from the PA patient exhibited normal morphology comparable to healthy livers.

Hemodynamics and transport conditions restore liver specific differentiated function relative to static cultures (Fig. 28) as evidenced by urea and albumin secretion rates, CYP450 activity with simultaneous restoration of transport function.

Primary human hepatocytes in the Hemoshear technology retain polarized morphology and cell surface receptors (E-cadherin, C26 and CD81), differentiated maturation markers (HNF-4,xy), and caspase expression of efflux transporters (MDR-2) stably over 10 days (Fig. 2A).

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PMR PRIMARY HEPATOCYTES FROM THE PA PATIENT STABLY RETAIN DIFFERENTIATED MORPHOLOGY AND LIVER FUNCTION UNDER HEMODYNAMICS AND TRANSPORT

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PMR PRIMARY HEPATOCYTES FROM THE PA PATIENT CULTURED IN THE SYSTEM EXHIBIT DECREASED EXPRESSION OF PCCA AND PCCB AT THE GENE AND PROTEIN LEVEL

The expression of PCCA and PCCB was assessed at the gene and protein level and compared to hepatocytes obtained from healthy donors cultured in a similar manner.

There was a decreased level of both PCCA RNA (9-fold) and PCCB RNA (2-fold) relative to normal healthy controls (Fig. 7A).

At the protein level, PCCB expression was completely absent while PCCB expression was reduced by 50% in the PA patient derived hepatocytes relative to healthy donors (Fig. 7B and 7C).

Detection of PCCA by immunofluorescence microscopy showed punctate intracellular staining in normal healthy hepatocytes (Fig. 7D), whereas it could not be detected in hepatocytes from the PA patient (Fig. 7E).

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RESULTS:

PRIMARY HEPATOCYTES FROM THE PA PATIENT PRODUCE INCREASED AMMONIA AND DECREASED UREA UNDER AMINO ACID SUPPLEMENTED CONDITIONS

Urea levels in the supernatants of hepatocytes from the PA patient were significantly decreased (p=0.017) in the presence of supplemented media relative to normal media conditions while healthy donor hepatocytes did not show a significant change in response to media supplementation (Fig. 8B).

Supernatants of PA patient derived dernal fibroblasts demonstrated overall lower levels of ammonia that was not significantly changed by media supplementation, and urea levels were undetectable (Fig 8A and B).

DISCUSSION:

Critical to the success of the described approach to create a propionic acidemia model are: 1) access to patient tissue, 2) timely isolation of hepatocytes and 3) the ability to maintain the hepatocytes in a functionally differentiated state in culture to preserve the altered functional phenotype.

In our model, the low levels of detectable RNA and absence of PCCA protein in our system reflect and confirm the predicted nonsense-mediated decay of messenger RNA before translation, due to the patient’s hemogygous non-sense mutations in the alpha subunit of PCCB.

To validate the functional relevance of the model, we supplemented the culture medium with increased levels of propionenic branched-chain amino acids, reproducing a clinical situation where PA patients experience increased circulating nitrogen load (e.g. catabolism or dietary increases in protein and propionic amino acids) that precipitates acute hyperammonemic crises requiring hospitalization.

Inhibition of early enzymes that aid the entry of ammonia into the urea cycle is hypothesized to cause the hyperammonemia with decreased urea production in patients with PA (Figure 9).

The finding of increased ammonia, with decreased urea levels in our PA model under supplemented amino acid conditions supports this hypothesis.

Taken together, these results highlight the necessity to use metabolically relevant tissues in this case, hepatocytes, while creating a PA disease model.

CONCLUSIONS:

We have presented direct evidence that the propionic acidemia disease phenotype can be retained in primary human hepatocytes from a patient with PA when placed into the appropriate physiological context.

Further use of this model system as in the present studies has tremendous utility for elucidating candidate factors and regulatory mechanisms that contribute to metabolic decomposition in PA.

The system as described has great potential for identifying new therapeutic options.

The paradigm deployed herein directly applies to other rare diseases with primary defects in hepatocytes and where mouse models are deficient.

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