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Human hepatocyte transplantation corrects the inherited metabolic liver disorder arginase deficiency in mice

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ABSTRACT

The transplantation, engraftment, and expansion of primary hepatocytes have the potential to be an effective therapy for metabolic disorders of the liver including those of nitrogen metabolism. To date, such methods for the treatment of urea cycle disorders in murine models has only been minimally explored. Arginase deficiency, an inherited disorder of nitrogen metabolism that presents in the first two years of life, has the potential to be treated by such methods. To explore the potential of this approach, we mated the conditional arginase deficient mouse with a mouse model deficient in fumarylacetoacetate hydrolase (FAH) and with Rag2 and IL2-Rγ mutations to give a selective advantage to transplanted (normal) human hepatocytes. On day −1, a uroplasminogen-expressing adenoviral vector was administered intravenously followed the next day with the transplantation of 1×10^6 human hepatocytes (or vehicle alone) by intrasplenic injection. As the initial number of administered hepatocytes would be too low to prevent hepatotoxicity-induced mortality, NTBC cycling was performed to allow for hepatocyte expansion and repopulation. While all control mice died, all except one human hepatocyte transplanted mice survived. Four months after hepatocyte transplantation, 2×10^{11} genome copies of AAV-TBG-Cre recombinase was administered IV to disrupt endogenous hepatic arginase expression. While all control mice died within the first month, human hepatocyte transplanted mice did well. Ammonia and amino acids, analyzed in both groups before and after disruption of endogenous arginase expression, while well-controlled in the transplanted group, were markedly abnormal in the controls. Ammonium challenging further demonstrated the durability and functionality of the human repopulated liver. In conclusion, these studies demonstrate that human hepatocyte repopulation in the murine liver can result in effective treatment of arginase deficiency.

1. Introduction

Arginase deficiency is a rare metabolic disorder resulting from a loss of arginase 1 (*ARG1*), the final enzyme in the urea cycle, which is the major pathway for the detoxification of ammonia in terrestrial mammals. *ARG1* is expressed most prevalently in hepatocytes and red blood cells. Through *ARG1* in the liver, in coordination with the other enzymes of the cycle, nitrogen is sequestered as urea [1]. Arginine undergoes hydrolysis by *ARG1* to produce ornithine which then re-enters the cycle as urea and is excreted as waste in the urine.

Abbreviations: FAH, fumarylacetoacetate hydrolase; FRG, Fah$^{-/-}$/Rag2$^{-/-}$/Il2rγ$^{-/-}$ mouse; CKO, conditional knockout; NTBC, 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione

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ARG1 deficiency usually presents later in life beginning in late infancy to the second year with microcephaly, spasticity, seizures, clonus, loss of ambulation (often manifesting as spastic diplegia that may be indistinguishable from cerebral palsy), and failure to thrive associated with hyperargininemia [2]. The neurologic manifestations seen in arginase deficiency may arise from the accumulation of arginine metabolites or may result from hyperargininemia, in which multiple guanidino compounds (putative neurotoxins) increase, though the exact cause is not known. Patients typically avoid the catastrophic hyperammonemic crises characteristic of the other urea cycle disorders and thus tend to survive much longer [1]. At present, long-term therapy rests on the provision of a restricted protein diet and administration of nitrogen scavengers. While these treatments together can partially alleviate *ARG1* deficiency, there is no completely effective therapy available today.

Because of the marginally effective therapies that are available for this disorder, new strategies, including preclinical application of gene therapy to treat this disorder, have been applied and proven successful, albeit with limitations [3–7]. The extensive episomal loss of adeno-associated viral vectors (AAV) in rapidly dividing tissues such as the neonatal liver, results in minimal residual hepatic AAV expression of arginase in adulthood and thus the treated animals remain quite vulnerable to ammonia and at risk for hyperammonemia and death [7].

Because of these limitations, other approaches, including that of cellular therapy, deserve attention and an evaluation of their potential efficacy in treating arginase deficiency and other disorders of the liver that present early in life. In this report, we describe the therapeutic efficacy of hepatocyte transplantation to treat the biochemical defect of arginase 1 deficiency in an adult conditional murine knockout model and demonstrate the marked improvement in ammonia-mediated vulnerability with exogenous challenging by this method of treatment.

2. Materials and methods

2.1. FRG-CKO arginase mouse

Animals received humane care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86–23 revised 1985). FRG ($Fah^{-/-}$/$Rag2^{-/-}$/$Il2r\gamma^{-/-}$) mice on the C57BL/6 background were obtained from Yecuris (Tualatin, OR) and after a breeding colony was established, mice were mated with a conditional arginase knockout mouse (Stock number 8817) on the C57BL/6 background obtained from The Jackson Laboratory (Bar Harbor, ME). Previous studies by our group [8] and others [9] have demonstrated the utility of this model to replicate human arginase deficiency (except for the frequency of hyperammonemia) and its mortality in the floxed model ~3–4 weeks after initiation of cre-lox recombination. Mating was performed until all 4 genes were homozygous and these mice were used for the studies conducted herein and were designated as FRG-CKO Arginase.

Genotype screening was performed by PCR. Genomic DNA was prepared from an ear tip by standard methods (5-PRIME ArchivePure™ DNA Purification Kit, 5 Prime Inc., Gaithersburg, MD) and subjected to PCR. Screening for the conditional arginase allele was performed per the Jackson Lab strain technical support documents (https://www2.jax.org/protocolsdb/f?p=116:5:0::NO:5:P5_MASTER_PROTOCOL_ID,P5_JRS_CODE:23468,008817).

FAH is screened for by three primers: FAH Common primer: 5′ TTG CCT CTG AAC ATA ATG CCA AC 3′; FAH Mutant reverse primer: 5′ GGA TTG GGA AGA CAA TAG CAG GC 3′; FAH WT reverse primer: 5′ TGA GAG GAG GGT ACT GGC AGC TAC 3′ with 35 cycles: 95°C for 30 s, 58 °C for 30 s, and 72 °C for 1 min followed at completion by 72 °C for 5 mins with a mutant band present at 150 bp and wild type at 250 bp.

RAG2 is screened for by three primers: Rag2 Mut forward 2: 5′ CGG CCG GAG AAC CTG CGT GCAA 3′; Rag2 WT forward 2: 5′ GGG AGG ACA CTC ACT TGC CAG TA 3′; Rag2 common reverse 2 5′ AGT CAG GAG TCT CCA TCT CAC TGA 3′ with 35 cycles: 95 °C for 30 s, 62 °C for 45 s, and 72 °C for 1 min with 72 °C for 5 mins at completion. The mutant band is 300 bp while the wild type is 225 bp.

IL2rγ is screened for by three primers: Il2rg common forward 5′ CTG CTC AGA ATG CCT CCA ATT CC 3′; Il2rγ mutant reverse 5′ GGT CGC TCG GTG TTC GAG GCC AC 3′; Il2rγ wt reverse 5′ ACC GTT CAC TGT AGT CTG GCT GC 3′ with 35 cycles of 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 30 s with a 5 mins 72 °C extension at completion. The mutant band is 309 bp while the wild type is 473 bp.

Mice were maintained on 5% dextrose water (Fisher Scientific) with 16 mg/L 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) (Yecuris) with alternating Bactrim (Yecuris) added for *Pneumocystis carinii pneumoniae* prophylaxis.

2.2. Cellular transplantation

All animal procedures were performed according to institutional guidelines. Adult FRG-CKO arginase mice were administered 1.25×10^9 pfu/25 g body weight of Ad:uPA liver transplant enhancer (Yecuris, #20-0029) via retro-orbital injection 24 h prior to hepatocyte transplantation. Mice were given a Carprofen MediGel (Clear H_2O, Westbrook, ME) one day prior to surgery for pain control. Fresh human hepatocytes (Yecuris) were received in a transport buffer on ice and prepared according to the distributor's manual. The hepatocytes were washed by centrifugation at 140*g* for 5 mins at 4 °C followed by resuspension in HCM media (Lonza, CC-1398). Hepatocytes were counted using a cell counter (Nexcelom, Lawrence, MA) and resuspended in HCM media for a concentration of 1×10^6 cells/300 μL. Hepatocytes were kept on ice until transplantation.

The FRG mouse to be transplanted was anesthetized in an isoflurane chamber during the am and were not fasted prior. The area of the incision in the left subcostal region was clipped of hair and prepped with 70% EtOH and Betadine. The mouse was placed on a sterile surgery pad on a heated mat for the cellular transplantation procedure. A 0.5 cm transverse left subcostal incision was made through the skin, muscle and peritoneum. The spleen was identified and grasped gently with forceps to pull it out of the incision onto sterile gauze. A small part of the spleen was tied gently using a 4–0 silk tie taking care not to avulse the nub of spleen. 1×10^6 hepatocytes were slowly injected into the spleen using a 28.5G insulin syringe through the tied off splenic nub and loop of suture, so as to minimize bleeding from the puncture site. The suture was cut and the spleen was placed back into the abdomen. The peritoneum and muscle layers and subsequently the skin were closed using a 4–0 Vicryl suture. The mouse was recovered on a heated mat in a recovery cage. Controls, receiving saline, were sibling littermates and all mice were 3–4 months of age at transplantation with both male and female mice represented in these studies.

2.3. NTBC cycling

Prior to transplantation, FRG-CKO Arginase mice were maintained on 16 mg/L NTBC (Yecuris) water. 24 h prior to transplantation, the drinking water was switched to water with no NTBC for seven days, after which mice were given water with 8 mg/L NTBC for three days. This was followed by cessation of NTBC for 3 weeks. If mice lost > 20% of body weight, they were administered water with 8 mg/L NTBC for 1–3 days. This cycle was repeated for a total of about 120 days, after which mice were kept indefinitely on water with no NTBC.

2.4. Adeno-associated viral vectors

AAV8-TBG-Cre, a serotype 8 adeno-associated viral vector expressing cre recombinase under the thyroxine binding globulin promoter (hepatocyte-specific), was purchased from the University of Pennsylvania Vector Core (Philadelphia, PA).