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Treatment of Crigler-Najjar Syndrome Type 1 by Hepatic Progenitor Cell Transplantation: A Simple Procedure for Management of Hyperbilirubinemia

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ABSTRACT

Crigler-Najjar Syndrome (CNS) is characterized by mild, chronic unconjugated hyperbilirubinemia resulting from an autosomal-recessive inherited deficiency of hepatic uridine/diphosphoglucuronate-glucuronosyl transferace 1Al since birth. Herein we have reported a confirmed case of CNS type 1 in a 2-year-old girl with an unconjugated hyperbilirubinemia (>30 mg/dL) treated by hepatic progenitor cell infusion through the hepatic artery. No procedure-related complications were encountered. No kernicterus was observed. The total bilirubin started falling at 10 days after cell infusion. Two months after cell infusion the bilirubin fell from 29.0 to 16 mg/dL, with the conjugated bilirubin increasing approximately fivefold, the unconjugated bilirubin decreasing nearly twofold, and the SGPT also decreasing from 210 U/L to 64 U/L. This study demonstrated the efficacy of hepatic progenitor cells to manage hyperbilirubinemia in these patients. As the procedure is simple and the patient has tolerated the cell therapy, infusion can be repeated as required to manage hyperbilirubinemia, which often causes lethal kernicterus. This study was developed to assess the safety, feasibility, and efficacy of hepatic progenitor cell transplantation in a child with CNS type 1.

CRIGLER-NAJJAR SYNDROME type 1 (CNS) is an inherited disorder characterized by severe unconjugated hyperbilirubinemia since birth.[1] The syndrome results from absence of hepatic uridine diphosphoglucuronate (UDP) glucuronosyltransferase activity, which is essential for the conjugation and excretion of bilirubin. Accumulation of unconjugated bilirubin in plasma leads to kernicterus.[2] Although phototherapy successfully reduces serum bilirubin levels, patients are again at risk for kernicterus around the time of puberty, when phototherapy becomes less effective.[3] Except for the defect in UDP glucuronosyltransferase, hepatic architecture and function are normal in CNS patients. Isolated hepatocyte transplantation has been shown to be effective to restore biochemical functions in an animal model of a liver-based metabolic disease. Over two decades age, this technique was performed in Gunn rats[4] by reconstituting bilirubin glucuronidase activity deficiency, which is a model of CNS. Since that report, other animal models have confirmed this efficacy.[5–8] Muraca et al[9] published their experience with isolated hepatocyte transplantation in an elderly woman with glucogen storage disease type Ia. Fox et al[10] also reported isolated hepatocyte transplanta-

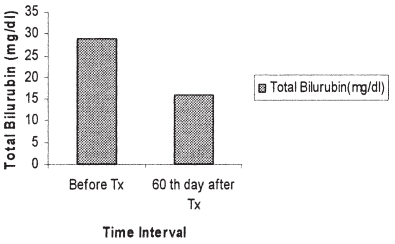

Fig 1. Bilirubin estimation (mg/dL).

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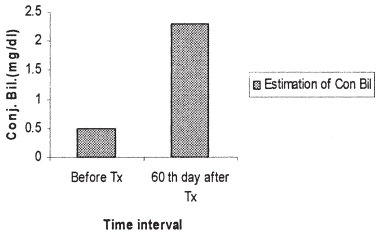

Fig 2. Estimation of conjugated bilirubin (mg/dL).

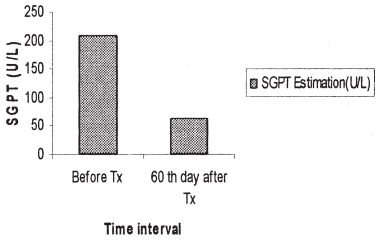

Fig 4. SGPT estimation (UL).

tion in the treatment of a child with CNS. Biochemical and clinical evidence of transplanted hepatocyte engraftment and function were present for >2 years following the procedure. Simon et al[11] also published their experience in isolated hepatocyte transplantation, which was performed to attempt metabolic reconstitution in a male infant with severe ornithine transcaramylase deficiency. In principle, this technique is applicable to any metabolic disease in which the deficient enzyme is primarily expressed in hepatocytes and where a small increase in enzyme activity can be expected to have a significant effect on the outcome of the clinical disease. In the present study, we have reported a confirmed case of CNS in 2-year-old girl with an increased bilirubin (>30 mg/dL), who was treated with hepatic progenitor cell infusion through the hepatic artery.

METHODS

Human hepatic progenitor cells were isolated using a standard procedure. Briefly, liver cells were isolated by a two-step collagenase digestion method[12] from an aborted fetus of 18 weeks gestation. Total liver cells were subjected to magnetic activated cell sorting (MACS) with CD326 antibody directly labeled to microbeads (MACS, Miltenyi Biotec) and collected hepatic progenitor cells. Cell viability and cell activity were assessed by the trypan blue dye exclusion method and an MTT assay, respectively. The reported viability was 90% with high cellular activity. Microbiologically, the isolated cells were checked for sterility; they were negative for all pathogens. Using a polymerase chain reaction (PCR), with a sense primer 5′-GAGGTTCTGGAAGTACTTTGC-3′ and antisense primer 5′-CCAAGCATGCTCAGCCAG-3′, genomic DNA of isolated cells was taken to diagnose the gene bilirubin UDP-glucuronosyltransferase 1: five exons constituting the coding region of the and their flanking intron–exon junctions from nucleotide 227 to nucleotide 132. PCR was performed for 30 cycles consisting of denaturation at 95°C for 60 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 60 seconds, and final extension at 72°C for 5 minutes.

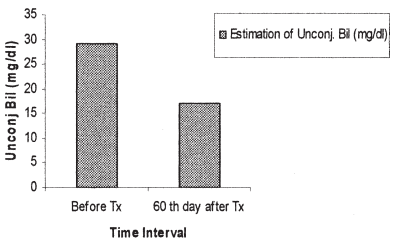

Fig 3. Estimation of unconjugated bilirubin (mg/dL).

Transplantation of Hepatic Progenitor Cells

Under general anaesthesia, standard transfemoral catheterization technique was used to selectively cannulate the hepatic artery with a 4-French catheter. Fifteen million isolated hepatic progeniter cells in 3 mL of dextrose normal saline solution were infused at 1 mL/min. The function of the transplanted hepatic progenitor cells was evaluated clinically by analyzing bile pigments and serum bilirubin levels. No procedure-related complications were encountered.

RESULTS

All procedures were safely performed without any specific side effects or complications except mild pain at the site of infusion. There was no hypersensitive fever reaction; nausea and vomiting were seen following cell infusion. Liver function tests were performed at each follow-up visit. No kernicterus was observed. The patient was discharged after 7 days of cell infusion. Total bilirubin started falling 10 days after cell infusion. At 2 months after cell infusion, the bilirubin decreased from 29.0 to 16 mg/dL (Fig 1); conjugated bilirubin increased approximately fivefold (Fig 2); unconjugated bilirubin decreased nearly twofold (Fig 3); and SGPT from 210 to 64 U/L (Fig 4).

In conclusion this study demonstrated the efficacy of hepatic progenitor cell to manage hyperbilirubinemia. As the procedure is simple and the patient tolerated the cell therapy, infusion can be repeated as required to manage hyperbilirubinemia, which often causes lethal kernicterus.

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