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HEPATOCYTE TRANSPLANTATION IN A 4-YEAR-OLD GIRL WITH PEROXISOMAL BIOGENESIS DISEASE: TECHNIQUE, SAFETY, AND METABOLIC FOLLOW-UP[1]

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Hepatocyte transplantation is an investigational alternative to orthotopic liver transplantation to treat liver based inborn errors of metabolism. We report successful hepatocyte transplantation in a 4-year-old girl with infantile Refsum disease. Hepatocytes were isolated from the left liver segment of two male donors using a classic two-step perfusion method. Fresh cells were transplanted first and then cryopreserved cells, for a total of 2 billion cells. Total bile acids and abnormal dihydroxycoprostanoïc acid markedly decreased in the patient's serum, indicating resolution of cholestasis and re-population of liver cells. Pipecholic acid decreased by 40% and c26:c22 fatty acid ratio by 36% after 18 months. Donor chromosomes sequences were detected on biopsy posttransplant, indicating engraftment. Hepatocyte transplantation is a safe and promising technique in the treatment of rare inborn errors of metabolism. Future improvements of cell viability and prevention of apoptosis may increase engraftment and subsequent re-population.

Hepatocyte transplantation has been successfully performed in a few children with an inborn error of liver metabolism (*1*,*2*). Infantile Refsum disease (IRD) is an autosomal recessive inborn error of peroxisome metabolism (IRD; OMIM 266510), with defects of very-long-chain fatty acid (VLCFA) beta-oxidation, bile acid and plasmalogen biosynthesis, and pipecolic acid catabolism. Symptoms include both central nervous system and digestive tract manifestations. Few patients survive beyond the first decade. Supportive treatment includes low phytanic acid diet and supplements of docosahexaenoic acid.

Orthotopic liver transplantation is successful to treat liver-based inborn errors of metabolism and may clear metabolites produced in excess from extrahepatic sites (*3*,*4*). Hypothesizing that neurologic disease may partially be because of toxic circulating metabolites, and because peroxisome number and activity are particularly high in the liver, we postulated that hepatocyte transplantation might lead to improvement of extrahepatic symptoms.

PATIENTS AND METHODS

A 1-year-old girl was referred with hypotonia and psychomotor retardation. She had poor visual contact, brachycephaly, and apparent hypoacusia. Abnormal plasmalogens, increased VLCFA with impaired C26:C22 ratio, and the presence of abnormal bile acids in serum (dihydroxycoprostanoïc acids [DHCA]) were combined with deficient activity of dihydroxyacetone (DHA) phosphate acyltransferase and an absence of catalase-containing particle peroxisomes on cultured fibroblasts. She was started on DHA supplementation at 0.5 mg per day and a low phytanic acid diet.

At 4 years, she had severe hearing and visual impairment, failure to thrive, and was unable to walk. She was presented to our team with the hypothesis that liver transplantation might improve her condition by normalizing the disease associated circulating metabolites. The uncertain long-term benefit of orthotopic liver transplantation for this condition and the child's already advanced neurologic disease outweighed the theoretic benefit so that orthotopic liver transplantation was not proposed. A project of hepatocyte transplantation was offered as an alternative. The procedure was indeed considered less aggressive. It could be attempted on an experimental basis, despite the uncertainty of potential benefit. The parents were fully informed and involved in all steps of the decision.

Liver cells were obtained from unused left liver segments of two compatible male donors (Children's Memorial Hospital, Northwestern University, Chicago, IL), as described (*5*,*6*). In donor 1, 4.1×10^9 cells with 85% viability were obtained and kept on ice in the wash medium for the trip from Chicago to Brussels. In donor 2, 3.4×10^9 cells with 76% viability were obtained and frozen (University of Wisconsin solution with 10% dimethyl sulfoxide and 10% fetal bovine serum, the latter now replaced by human albumin to avoid hazards related to xenogeneic products). After travelling in liquid nitrogen, the cells were rapidly thawed in a

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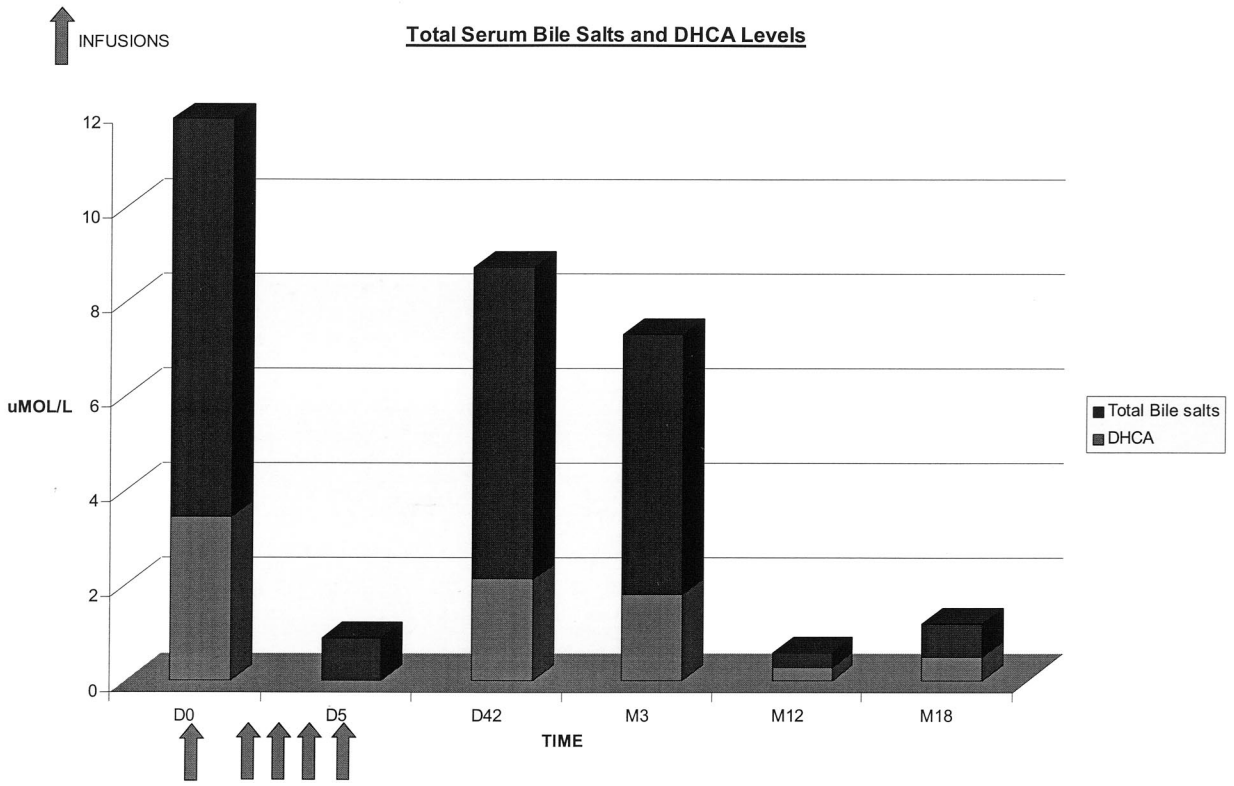

FIGURE 1. Total bile acids and the abnormal DHCA decreased markedly in the serum, showing the potential cure of the patient's cholestasis. In addition, complete disappearance of the abnormal bile acids DHCA was noticed shortly after transplantation, possibly related to the mass of infused cells. Total bile acids, normally undetected; DHCA, normal not found.

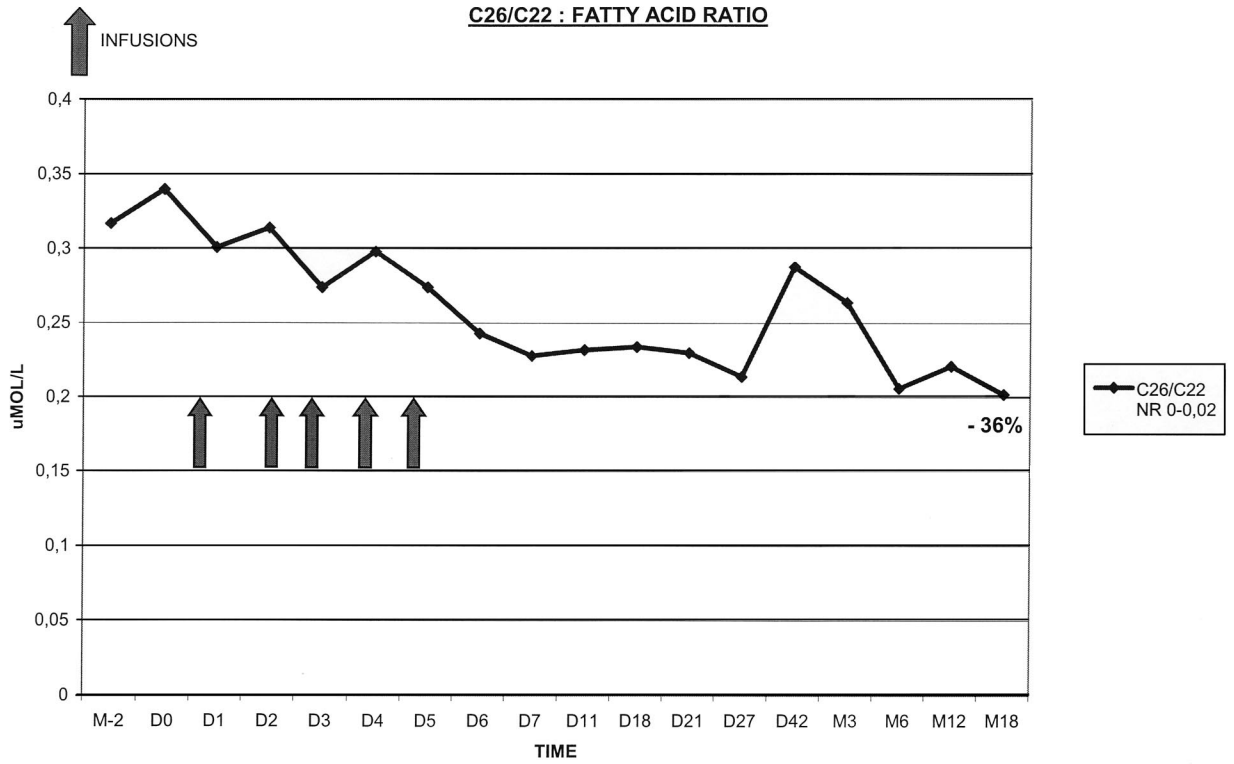

FIGURE 2. The C26:C22 ratio (nl<0.02) displayed a continuous decreasing trend on follow-up as compared with pretransplant values, reaching 64% of pretransplant values at 1 year.

37°C water bath, filtered, and washed in a stable solution of plasmatic proteins (85% albumin).

A 6F Broviac (Bard, Salt Lake City, UT) catheter was placed surgically in the spleno-mesenteric confluent and perfused with normal saline (3 mL/hr) containing 1 IU/mL of heparin (Heparin Leo, Brussels, Belgium).

Day 1: Fresh liver cells immediately before infusion had a viability of 50%. They were transplanted in two successive 30-min infusions for a total of 1.1×10^9 cells diluted in 50 mL of buffer. Portal pressure during infusion increased from 12 mm Hg to 28 mm Hg and decreased to 17 mm Hg 30 min later. No infusion occurred on day 2.

Days 3 to 6: Cryopreserved cells were used for the following infusions: over 3 days, she received a total of 6 cryopreserved cell infusions (doses in millions were 140 and 90 on day 3, 184 and 243 on day 4, and 196 on day 5). Viability of the cells was more than 90% before infusion.

During infusions, the portal-pressure gradient increased on average from 12 to 26 mm Hg, returning to basal within 30 min. Doppler ultrasound showed no significant decrease in portal flow velocity, remaining at approximately 25 to 30 cm/sec.

Blood saturation decreased temporarily to 95% after the first two infusions but not subsequently with reduced infusion rates.

Liver enzymes increased to 1733 IU/L for aspartate aminotransferase (nl, 6–33 IU/L), 2,260 IU/Lfor alanine aminotransferase (nl, 14–63 IU/L), and 792 IU/L (nl, 7–50 IU/L). Liver enzymes had normalized 10 days later.

The child tolerated the procedure well, and left the intensive care unit the day after the last infusion.

Immunosuppression included 10 mg of Basiliximab (Simulect, Novartis SA, Quebec, Canada) at day 0 and tacrolimus (Prograf, Fujisawa, Deerfield, IL) to reach through blood levels of 6 to 8 ng/mL.

Figure 2 reports the evolution of the C26:C22 ratio, Figure 1 total bile acids and DHCA, and Figure 3 pipecholic acid (gas chromatography mass spectrophotometry analysis) before and up to 18 months posttransplant. Infusions were followed by an immediate improvement of metabolites and disappearance of abnormal bile acids, attributed to the mass of infused cells. A transient increase was then noticed at day 42, probably related to partial loss of infused cells. After that, total bile acids and DHCA gradually decreased in serum, showing potential cure of cholestasis, and this is attributed to repopulation of the recipient's liver. Pipecholic acid also decreased to 60% of pretransplant values at 18 months. The C26:C22 ratio displayed a continuous decreasing trend, reaching 64% of pretransplant values at 18 months. Donor Y chromosome sequences were detected

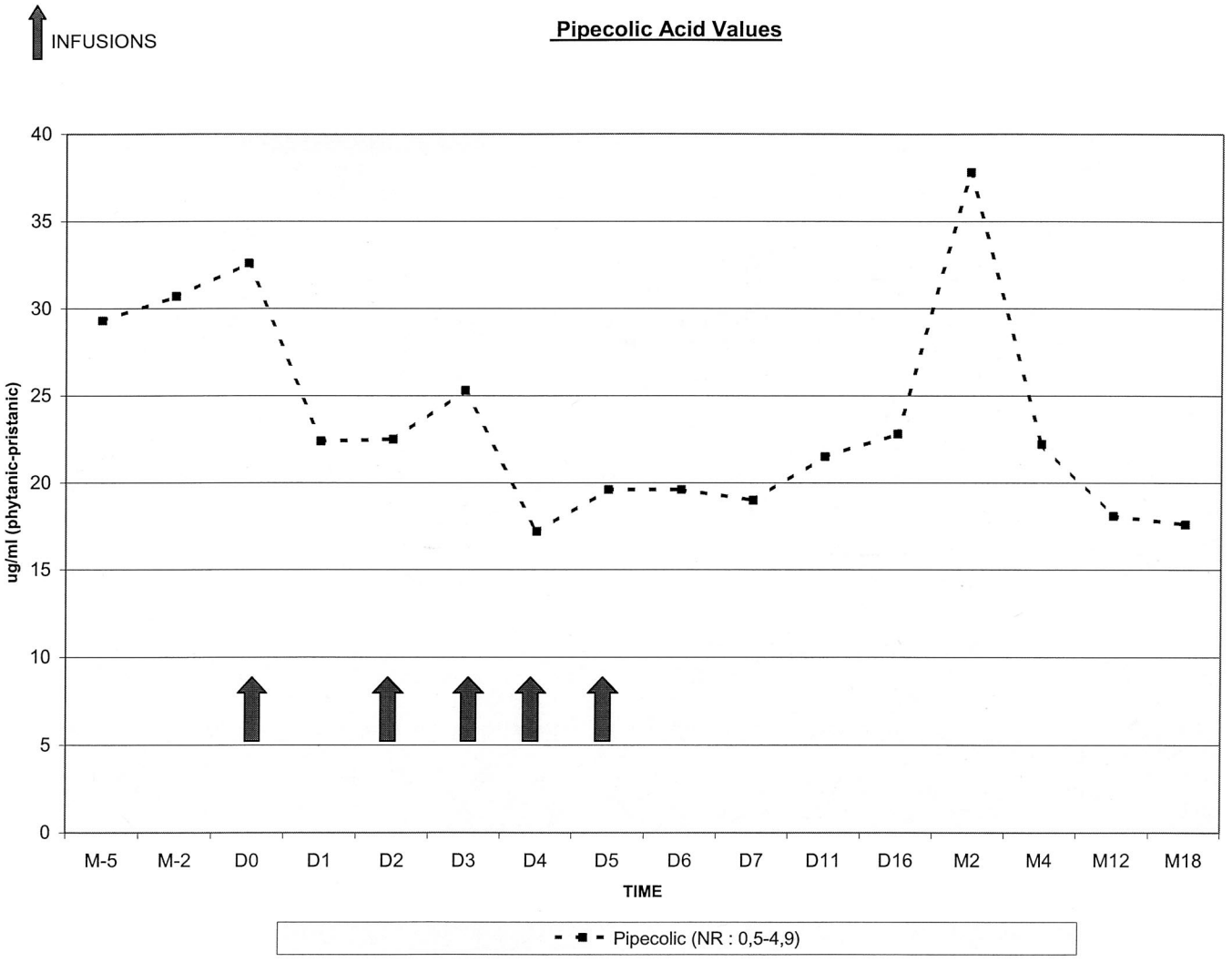

FIGURE 3. Pipecholic acid also showed a progressive decrease to 61% of pretransplant value 1 year after transplantation (normal value <4.9 μmol/L).

by real-time quantitative polymerase chain reaction in biopsy from day 7 but not in biopsy performed at month 4 (sampling phenomenon?).

Visual- and auditory-evoked potentials showed no improvement after the procedure, with a similar threshold of auditory potentials at approximately 80 dB.

Surprisingly, the girl's parents noticed an improved general condition and weight gain from 10,500 kg to 11,400 kg within 3 months. She started to stand up and walk alone within 6 months of the procedure.

DISCUSSION

Hepatocyte transplantation can partially correct liver-based inborn errors of metabolism, with best success obtained in Crigler Najjar syndrome (*2*). Other success has been obtained in an adult with type I glycogen storage disease (*7*). These successes are an argument to extend the procedure to other liver-based metabolic disorders.

The child received eight separate infusions for a total of 2 billion cells, i.e., 5% of her supposed hepatocyte mass (4 billion/kg). This cell mass is comparable to that used by Fox et al. (*2*), but in the present case, only half of the transplanted cells were fresh cells, whereas cryopreserved cells were used for the subsequent infusions. Viability of fresh cells decreased from 85% to 50% after the transatlantic transportation.

Portal vein thrombosis, although never reported so far with this procedure, remains one of the main feared complications. We demonstrated by monitoring portal pressure a temporary increase, with return to preinfusion values shortly after the infusion, whereas Doppler ultrasound of the portal vein did not show any flow-velocity impairment.

Blood oxygen saturation was transiently decreased after the second infusion, possibly because of increased blood viscosity or partial pulmonary embolization of the infused hepatocytes, as described in animal studies (*8*). This finding stresses the importance of limiting the number of cells infused and carefully monitoring blood oxygen saturation. Heparin was added in the suspension to further prevent the risk of vein thrombosis. Characteristic Refsum metabolites decreased, more significantly for total bile acids and DHCA. The complete disappearance of DHCA at day 7 could be caused by metabolic efficiency of the infused cell mass but without subsequent engraftment of all infused cells. Late metabolic improvement can possibly be attributed to re-population from engrafted cells. The effect observed on cholestasis may represent an important argument of possible efficiency in familial cholestatic diseases.

Male DNA was detected in one biopsy sample, within the reported percentage range after hepatocyte transplantation (*1*,*9*). In the second biopsy, no male DNA was found, which was attributed to sampling phenomenon (*10*). Previous animal studies have shown the re-population may start from as little as 0.3% of transplanted cells (*10*). The patient's metabolite profile was indeed maintained and even improved, which would not have been the case if the absence of male DNA was because of disappearance of the transplanted cell population. The limited percentage of transplanted donor cells that eventually engrafted contrasts with the high viability of transplanted cells after isolation and thawing, and may be related to an ongoing apoptotic process caused by loss of cell anchorage (*11*).

We conclude that hepatocyte transplantation is feasible, safe, and able to bring metabolic competent cells into a deficient liver. Improvement of therapeutic efficiency may come in the long term thanks to progressive re-population from transplanted cells.

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