

LIVER TRANSPLANTATION FOR TYPE I GLYCOGEN STORAGE DISEASE

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Summary A 16½-year-old girl with type I glycogen storage disease was treated by orthotopic liver transplantation under cyclosporin/steroid immunosuppression. All metabolic stigmata of the disease were relieved and 1 year postoperatively she follows a normal diet and lifestyle.

Introduction

STUDIES of haptoglobin,¹⁻³ group-specific component,^{3,4} and numerous other products of hepatic synthesis⁵⁻⁹ have shown that liver homografts permanently retain their original metabolic specificity after transplantation. Consequently, liver transplantation has been regarded for some years⁴ as a potentially decisive way to treat those inborn errors of metabolism which result partly or completely from defects in hepatic function. This objective has been realised in several different metabolic disorders.^{8,9} We report here the first use of liver transplantation for the treatment of type I glycogen storage disease, a disorder caused by glucose-6-phosphatase deficiency.

Case-report

The patient was a girl aged 16½ yr whose sibling had died in infancy of the same disease. At age 2½ yr she had had a prolonged hypoglycaemic seizure which resulted in transient blindness, hemiparesis, and a residual seizure disorder requiring therapy with anticonvulsants. She continued to have recurrent hypoglycaemia, acidosis, hyperuricaemia, hyperlipidaemia, abnormal coagulation, and epistaxes, and her growth was retarded. After an end-to-side portacaval shunt at age 8 yr,¹⁰ all these abnormalities were relieved except hypoglycaemia. The frequency of feedings necessary to avoid hypoglycaemic symptoms increased from every 4-5 h to every 2 h. Subsequently, overnight nutrient infusions were begun by nasogastric tube.¹¹

During the 2 yr before transplantation, multiple filling defects in her enlarged liver were seen on liver scans, with a dominant lesion in the right lobe. These were thought (and were later proved) to be slowly growing adenomas, but the possibility of a malignant change could not be ruled out. She had episodes of severe upper abdominal pain and liver swelling. In the year before transplantation, she had jaundice with a peak serum bilirubin concentration of 8 mg/dl (137

517
mmol/l) and serum alkaline phosphatase exceeding 3000 IU, encephalopathy with blood ammonia levels around 350 µg/dl (206 µmol/l), and persistent increases in serum transaminases. She was treated by protein restriction and intermittent intravenous alimentation. Her management was complicated by bacterial pseudomembranous colitis which was relieved by cholestyramine.

At the time of transplantation, she was small with normal vital signs, Tanner stage III pubertal changes, and the typical "potato face" of type I disease. She had bilateral right visual-field defects. The liver and spleen were palpable 10 and 6 cm below the right and left costal margins, respectively. There was no ascites. Orthotopic transplantation (liver replacement) was done with conventional techniques.¹² The donor was a 14-year-old boy of the same blood type. Cold ischaemia of the graft was 5 h. Detachment of the portacaval shunt, closure of the resulting defect in the inferior vena cava, and anastomosis of the portal vein to the graft portal vein were not difficult. Biliary reconstruction was with an end-to-end duct anastomosis over a T-tube stent. The T-tube was clamped at 4 wk and removed at 7 wk.

Postoperatively, a small bile leak and right subphrenic haematoma necessitated a minor drainage procedure. Immunosuppression was with cyclosporin and prednisone.⁸ After 5 wk she was discharged on daily doses of 12 mg/kg cyclosporin and 15 mg prednisone. There has been no evidence of rejection. After 1 yr, the daily cyclosporin dose is 9 mg/kg and prednisone has been reduced to 5 mg/day.

Results

The histopathological findings in the native liver, which weighed 3400 g, were consistent with type I glycogen storage disease. There were multiple hepatic adenomas. Biochemical studies of samples quick-frozen at -70°C and stored on dry ice were done by Dr Barbara Illingworth Brown, Washington University, St Louis. The findings, including absence of glucose-6-phosphatase activity, were typical of type I glycogen storage disease (table). The glycogen concentration in the liver was 8.7%.

Metabolic investigations were done 6 wk postoperatively. The oral glucose tolerance test was normal after transplantation, with no hypoglycaemia at 3 h—in contrast to the grossly aberrant findings at age 8 yr, just before the portacaval shunt (fig. 1). At age 8 yr, fasting for a brief period caused hypoglycaemia, whereas after transplantation, euglycaemia was maintained through a full day of fasting (fig. 2). Postoperatively, in either the fasting or the postprandial state, intramuscular injection of glucagon caused plasma glucose increments greater than 100% (fig. 3).

Standard liver function tests have been normal for 1 yr. Serum or plasma concentrations of lactates, lipids, and uric acid have been normal. The patient's lifestyle has been transformed. She eats three meals a day. For the first time in her life, she can participate in the educational and social overnight activities that are part of normal teenage development.

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LIVER ENZYME ACTIVITY OF EXCISED LIVER*

Enzyme	Patient (µmol/min/g liver)	Controls (µmol/min/g liver)
Glucose-6-phosphatase	0.08	3.7-14.4
Debranching enzyme	0.58	0.25-0.75
Phosphorylase	18.5	12.6-32.4
Fructose diphosphatase	9.1	4.2-15.5
Lysosomal α-glucosidase	2.32	0.63-2.85

*Analyses by Dr Barbara Illingworth Brown. The liver specimen contained 8.7% glycogen (normal < 5%). Control patients had liver biopsies because of metabolic disorders, hepatomegaly, or failure to thrive. The control ranges include at least 10 different samples.

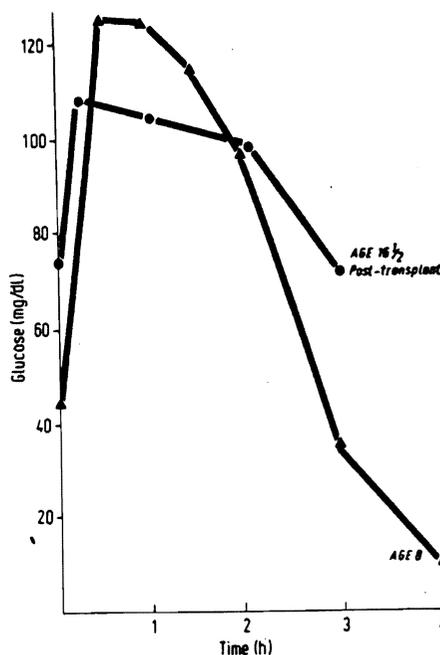


Fig. 1—Oral glucose tolerance test at age 8 yr (before portacaval shunt) and 6 wk after transplantation.

1 mg/dl = 0.055 mmol/l.

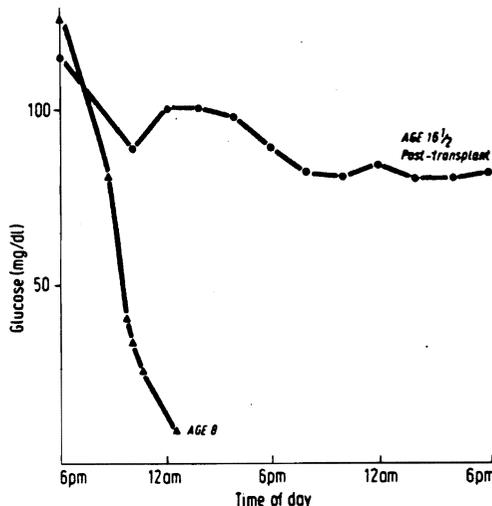


Fig. 2—Response of blood glucose concentrations during fasting at age 8 yr (before portacaval shunt), and 6 wk after liver transplantation.

Discussion

Type I glycogen storage disease was the first inborn error of metabolism to be classified on the basis of deficiency of a specific enzyme, glucose-6-phosphatase.¹³ This enzyme is found in the liver, kidneys, and intestine.¹⁴ Deficiency of glucose-6-phosphatase activity precludes normal breakdown of glycogen to glucose and inhibits gluconeogenesis. The consequent metabolic disturbances include growth retardation, acidosis, lactic acidemia, hyperlipidaemia, and hyperuricaemia.¹⁵ Effective treatment for the disease has been developed only within the past two decades, and our patient has participated in each step of this therapeutic evolution. The first step was the demonstration that portal-systemic diversion ameliorated all of the disease

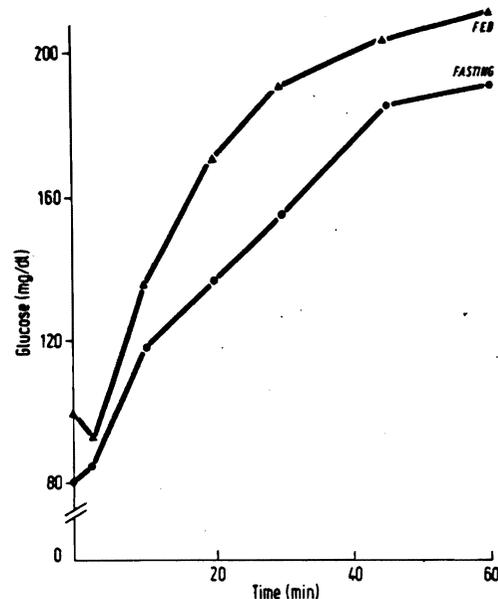


Fig. 3—Glucose response to 1 mg intramuscular glucagon in fasting and fed state, 6 wk after liver transplantation.

manifestations except hypoglycaemia.^{10,16} Folkman et al¹⁷ showed that the same benefits could be achieved acutely by continuous parenteral alimentation. In an extension of this observation, Greene et al¹¹ reported such good palliation, including control of hypoglycaemia, with round-the-clock enteral alimentation that this has become the treatment of choice. The overnight feedings have usually been by gastric tube.

Liver transplantation is a more specific option, but one which has been too dangerous to consider except for life-threatening complications such as those in our patient. However, the criteria for candidacy for this procedure will probably become less restrictive in view of the improved results with liver replacement that are being reported.⁸ Although liver transplantation does not provide glucose-6-phosphatase directly for the kidneys or intestine, there has been no evidence of incompleteness of metabolic correction by the normally functioning transplanted liver.

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Addendum

About 1 yr after transplantation, the patient had an episode of melaena. Oesophageal varices were the site of the haemorrhage. Superior mesenteric artery angiography and transhepatic portography revealed extrahepatic portal vein thrombosis. Excellent collateral filling of the intrahepatic portal vein and its branches was seen, indicating that the homograft still had good hepatopetal splanchnic flow. Sclerotherapy controlled the bleeding and liver functions and carbohydrate metabolism continue normal.

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THROMBOXANE A₂ IN SKIN-BLEEDING-TIME BLOOD AND IN CLOTTED VENOUS BLOOD BEFORE AND AFTER ADMINISTRATION OF ACETYSALICYLIC ACID

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Summary The 'Simplate' technique for measuring skin bleeding time was adapted to quantify thromboxane A₂ in the emerging blood as the stable degradation product thromboxane B₂ in twelve Swedish and ten English volunteers. During the bleeding time thromboxane B₂ concentrations increased, but as the rate of blood loss fell the rate of production of thromboxane A₂ was constant. The English subjects had shorter bleeding times and produced more thromboxane A₂ than the Swedish subjects. When the Swedish subjects were grouped according to bleeding times those with the shortest had more thromboxane A₂ than those with longer bleeding times. Clotting venous blood in vitro produced much more thromboxane A₂ than bleeding-time blood and there was no correlation with bleeding time. Determination of the capacity of clotting blood to form thromboxane A₂ is therefore irrelevant to in-vivo haemostasis. Acetylsalicylic acid greatly diminished the appearance of thromboxane A₂ in the bleeding time and prevented the increase of thromboxane A₂ concentration with time.

Introduction

THE extent to which physiological haemostasis depends on prostaglandins and related endogenous agents is still uncertain. Inhibition of prostaglandin biosynthesis by acetylsalicylic acid is associated with increases in bleeding time.¹⁻³ Some familial bleeding tendencies are associated with deficiencies in the synthesis of thromboxane A₂ by platelets.⁴ Neither of these raise bleeding time by more than about twice. Studies in human beings given thromboxane-A₂-synthetase inhibitors indicate that haemostasis depends only to a minor extent on thromboxane A₂.⁵

We have already shown that the impairment of human haemostasis by acetylsalicylic acid or by a diet enriched with fish, both of which diminish prostaglandin formation, occurs by different mechanisms.^{6,7} To determine the contribution of thromboxane A₂ to primary haemostasis of healthy human beings in vivo, we measured thromboxane A₂ (as its stable

metabolite thromboxane B₂) in blood coming from standard incisions made for determining skin bleeding time and the thromboxane A₂ produced during in-vitro clotting of venous blood from the same individuals, determined before and after they ingested one dose of acetylsalicylic acid.

Subjects and Methods

The subjects were twelve apparently healthy Swedish male volunteers (mean age 29±1 SEM years) who had taken no drugs for at least 2 weeks before these investigations. After an overnight fast for 12 h the bleeding time was determined on one arm. From a similar incision on the other arm blood was collected for thromboxane B₂ determinations. Thromboxane B₂ was also determined in a blood sample from the antecubital vein of that arm. A fortnight later the determinations were repeated before and 2 h after oral administration of a single dose of acetylsalicylic acid (5 mg/kg body-weight). In ten similar English subjects, bleeding times and thromboxane B₂ levels in bleeding-time blood were determined once in the same way. Bleeding times were determined by the same observer in all the subjects.

Bleeding times were determined with the standard 'Simplate I' device (General Diagnostics Inc).^{8,9} The blood emerging from the skin incision was removed quantitatively every 30 s with a disc of filter paper (Whatman no 1) until bleeding stopped. The bleeding-time-blood volumes were measured by eluting the spots of blood from the filter paper into Drabkin's solution as previously described.⁷ In each eluate the haemoglobin concentration was determined spectrophotometrically and the corresponding blood volume obtained from standard curves. The results were expressed as µl of blood emerging in each 30 s period.

Thromboxane A₂ concentrations in the bleeding-time blood were determined by radioimmunoassay of the stable degradation product thromboxane B₂. The blood emerging from skin incisions made by the simplate I device was collected in 1 min periods into capillary tubes containing known volumes of a mixture of 100 mmol/l ethylenediamine tetra-acetic acid and 5.59 mmol/l indomethacin to inhibit in-vitro formation of thromboxane B₂. After mixing, the tubes were centrifuged at 15 000 g for 2 min. The clear supernatants were removed, frozen, stored at -20°C, and assayed later for thromboxane B₂. The thromboxane B₂ standard (U51541 lot 0071-CLM-059C) and the antibodies against thromboxane B₂ (21) were kindly supplied by Dr John Pike of the Upjohn Company, Kalamazoo, USA, and Dr John Vane of the Wellcome Foundation Ltd, Beckenham, UK, respectively. The total amounts of thromboxane B₂ formed in the bleeding-time blood were calculated for each individual by summing the products of the concentrations and the volumes of successive blood samples. Thromboxane B₂ formation was determined also in the serum from blood collected by venepuncture into glass tubes in which it was made to clot by incubation at 37°C for 1 h. The tubes were centrifuged at 2000 g for 10 min, the supernatant sera separated, frozen, stored at -20°C, and assayed for thromboxane B₂ by the same procedure.

The results were evaluated statistically by Student's *t* test.

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