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Conventional and quantitative liver function tests after hepatic transplantation: a prospective long term follow-up

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Abstract In long-term survivors of liver transplantation, hepatic function is obviously of vital importance. Therefore, we prospectively performed conventional and quantitative liver function tests in patients who had survived a first transplantation for at least 4 years. Compared to 6 months after transplantation, serum bilirubin concentration and γGT activity were significantly lower after 3, 4, and 5 years (bilirubin 1.2 ± 0.2 mg/dl at 6 months vs 1.0 ± 0.1, 1.0 ± 0.2, and 0.8 ± 0.1 mg/dl respectively; γGT 106 ± 33 U/l at 6 months vs 56 ± 17, 67 ± 35, 39 ± 10 U/l respectively). At these points in time, blood levels of cyclosporin A were also significantly lower. Other parameters of liver cell function and liver cell integrity (AP, AST, ALT, GLDH, total protein, thromboplastin time, partial thromboplastin time) were unchanged over time. Serial quantitative liver function tests (indocyanine green half-life, galactose elimination capacity, lidocaine half-life, and MEGX formation) also remained stable. Thus, we conclude that hepatic function remains stable in long-term survivors of liver transplantation for at least several years.

Key words Liver transplantation · Liver function tests

Introduction

Over the last two decades, liver transplantation has become an established treatment option for patients with acute liver failure or end-stage chronic liver disease [3, 12, 13]. Early on in the transplantation era, centers were primarily concerned about morbidity and mortality in the early postoperative period. With considerable progress having been made in surgical techniques and immunosuppressive therapy, the vast majority of patients now survive liver transplantation well beyond the 1st year. For these patients, long-term graft function is very important. After all, in contrast to kidney transplant recipients, who can easily resume hemodialysis, there is no extracorporal means to replace inadequate liver function. Yet, long-term data on hepatic function after transplantation have rarely been analyzed [5]. We, therefore, prospectively studied long-term graft function in a series of patients who survived liver transplantation for a minimum of 4 years.

In addition to conventional function tests, serial quantitative liver function tests were performed. These tests have been advocated as useful in determining the prognosis of patients with liver cirrhosis [7] or primary biliary cirrhosis [10]. In addition, the MEGX formation test has been suggested as helpful in the selection of suitable donor organs for transplantation, although some groups have reported the test efficacy to be low [8, 14, 16].

Methods

Study design, data handling, and statistical analysis

Between February 1988 and December 1991, 114 donor livers were transplanted into 87 adult patients at our institution. All patients,

who were discharged from the hospital, were entered into a surveillance program, and we prospectively studied their liver function. Here we report on the follow-up of 29 patients who survived a first transplantation for a minimum of 4 years. Sixteen patients were followed for 4 years, ten patients for 5 years, one patient for 6 years, and two patients for 7 years. Indications for transplantation were alcoholic liver disease ($n = 8$), cirrhosis due to hepatitis B or C ($n = 8$), cirrhosis of unknown origin ($n = 4$), primary biliary cirrhosis ($n = 4$), primary sclerosing cholangitis ($n = 1$), hepatocellular carcinoma ($n = 2$), fulminant hepatitis A ($n = 1$), and HELLP syndrome ($n = 1$). Seventeen patients were male and 12 were female. The mean age was 48 years, with a range from 20 to 63 years.

During the 1st week after transplantation, patients were treated with quadruple therapy (cyclosporin A, azathioprine, prednisone, and ATG) and then with triple therapy for about 3 months (cyclosporin A, azathioprine, and prednisone). Twenty-eight patients were on long-term immunosuppression with cyclosporin A (serum concentration between 150 and 250 ng/ml) and one received FK 506 as rescue therapy because of chronic rejection (serum concentration between 5 and 10 ng/ml). In addition, patients were treated with prednisone (2.5 mg/day). After 1989, patients with hepatitis B infection were given passive immunoprophylaxis (Hepatect). Until the end of the recruitment in December 1991, no other antiviral therapy was given to patients with viral hepatitis.

We analyzed results from conventional and quantitative liver function tests performed 1, 6 and 12 months after transplantation and then at yearly intervals. When data were not available for a particular point in time, the only data accepted for our study were those generated either in the previous or the subsequent month. Thus, the following number of data sets were missing: 3/29 for month 1, 2/29 for month 6, 3/29 for month 12, 1/29 for month 24, 5/29 for month 36, 6/29 for month 48, and 3/13 for month 60.

Data obtained during long-term follow-up were compared to data from 6 months post-transplantation. After 6 months, patients have usually fully recovered from the operation and stable medical conditions have usually been established; confounding medical events, such as infectious complications, renal dysfunction, or episodes of rejection, are rare at this point.

Analysis was performed using the paired Student's t-test, and data are either given as mean ± SEM or presented as individual data.

Biochemical tests

Conventional laboratory tests were performed using routine clinical methods.

Quantitative liver function tests were performed as described previously [11, 16]. Briefly, galactose elimination capacity (GEC) was determined after a single intravenous bolus of 0.5 g galactose per kilogram body weight, as described by Tygstrup [15]. Galactose is extracted by the liver at a relatively low rate, and it is metabolized independently from the cytochrome system. The rate-limiting step is the initial phosphorylation by the cytoplasmic galactokinase. When the enzyme system is saturated, the zero-order elimination of galactose is a function of liver cell mass [4].

The half-life of indocyanine green (Cardio Green, Paesel, Frankfurt, Germany) was determined from the initial linear phase of the elimination curve after an intravenous bolus of 0.5 mg/kg body weight. Indocyanine green (ICG) is extracted by the liver at a high rate and excreted into the bile without being metabolized [6]. Its elimination is a parameter for liver blood flow and biliary excretion.

The half-life of lidocaine was measured after intravenous administration of 1 mg lidocaine per kilogram body weight, using an immunofluorescence polarization assay (Abbott TDx System, Abbott, Wiesbaden, Germany). Lidocaine is almost completely metabolized by the hepatic cytochrome P-450 system [2]. The half-life of lidocaine was calculated using the initial elimination phase and the terminal elimination phase, starting about 1 h after the administration of lidocaine.

MEGX is generated in the liver from lidocaine, and MEGX serum concentrations 15 min after the administration of lidocaine were measured with an immunofluorescence polarization assay (Abbott TDx System, Abbott, Wiesbaden, Germany).

Before and after the injection of galactose, indocyanine green, and lidocaine, blood samples were drawn at 0, 3, 6, 10, 15, 20, 25, 30, 35, 40, 50, 60, 80, 100, 120, 180, 240, 300, and 360 min.

Table 1 Characteristics of patients, who died later than 4 years after a first liver transplantation (LTX)

Patient's initials	Age at LTX	Age at death	Indication for LTX	Cause of death
PE	56	64	Alcoholic cirrhosis	Carcinoma of the pancreas
KG	49	54	Alcoholic cirrhosis	Carcinoma of the pharynx
OJ	33	37	Alcoholic cirrhosis	Chronic rejection, renal insufficency

Results

Follow-up

Of the 29 patients who survived liver transplantation for a minimum of 4 years, 3 later died, 2 from malignant tumors (Table 1). Another patient developed a malignant skin tumor that could be resected. Two patients developed end-stage renal insufficiency and had to undergo maintenance hemodialysis. One of these two patients also suffered from recurrent attacks of alcohol-induced chronic pancreatitis, which was complicated by pancreatic pseudocysts. Fourteen patients required antihypertensive therapy. Viral hepatitis recurred in only one out of eight cases.

Eighteen patients had returned to full-time jobs or were seeking full-time employment. A 26-year-old patient chose to become pregnant and delivered a premature, but otherwise healthy, baby.

Conventional liver and kidney function tests

Results are summarized in Table 2. After transplantation, serum bilirubin concentration and serum activity of γGT decreased over time. For bilirubin, the decrease was small but statistically significant when data from 36, 48, and 60 months after the operation were compared to values obtained 6 months post-transplantation

Table 2 Conventional liver function tests in patients who survived liver transplantation for a minimum of 4 years. * $P < 0.05$ compared to values obtained 6 months after transplantation).

Months	1	6	12	24	36	48	60
Bilirubin (< 1.0 mg/dl)	2.2 ± 0.3*	1.2 ± 0.2	1.1 ± 0.1	1.3 ± 0.3	1.0 ± 0.1*	1.0 ± 0.2*	0.8 ± 0.1*
AP (< 180 U/l)	336 ± 167	231 ± 42	231 ± 40	284 ± 74	187 ± 28	300 ± 156	140 ± 15
γGT (< 28 U/l)	150 ± 38	106 ± 33	98 ± 34*	86 ± 27	56 ± 17*	67 ± 35*	39 ± 10*
AST (< 18 U/l)	15 ± 3	16 ± 2	15 ± 2	16 ± 2	13 ± 1	14 ± 2	15 ± 3
ALT (< 22 U/l)	21 ± 6	23 ± 4	22 ± 4	23 ± 5	17 ± 3	17 ± 3	14 ± 2
Total protein (> 6.0 g/dl)	6.8 ± 0.2	7.4 ± 0.1	7.3 ± 0.2	7.1 ± 0.2	7.0 ± 0.1	6.8 ± 0.2	7.0 ± 0.2
Quick (> 65 %)	85 ± 4	91 ± 2	93 ± 2	91 ± 2	90 ± 4	89 ± 5	95 ± 2
aPTT (< 47 s)	42 ± 3	34 ± 1	36 ± 1	37 ± 1	39 ± 2	37 ± 1	38 ± 2

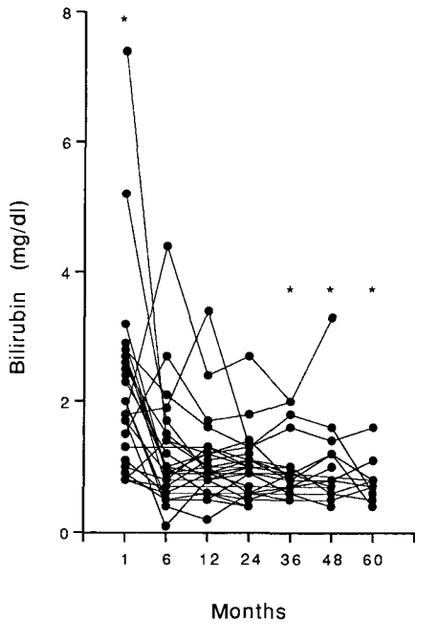

Fig. 1 Bilirubin serum concentration in long-term survivors of a first liver transplantation. *$P < 0.05$ compared to values obtained 6 months after transplantation

(Fig. 1). Another parameter of excretory liver function, serum activity of alkaline phosphatase, however, did not change over time. Likewise, serum activities of AST, ALT, and GLDH, reflecting liver cell integrity, were not affected by time. Parameters of synthetic liver function, protein serum concentration, thromboplastin time (Quick), and activated partial thromboplastin time were also stable over time.

Serum concentrations of sodium, potassium, and calcium were normal and did not change over time (data not shown). Mean serum creatinine concentration, however, was slightly higher than normal in the patient group; this did not increase further over time (1.8 ± 0.4, 1.5 ± 0.1, 2.0 ± 0.4, 1.6 ± 0.1, 1.6 ± 0.1, 1.7 ± 0.1, and 1.6 ± 0.1 mg/dl at 1, 6, 12, 24, 36, 48, and 60 months after liver transplantation). Impairment of renal function was probably related to immunosuppressive therapy with cyclosporin A.

Cyclosporin A blood levels were 295 ± 36, 279 ± 22, 255 ± 10, 235 ± 13, 223 ± 13, 234 ± 26, and 231 ± 22 ng/ml at 1, 6, 12, 24, 36, 48, and 60 months after liver transplantation.

Quantitative liver function tests

Quantitative liver function tests were performed in 11 patients 6–12 months before they received a liver transplant. Results of these tests were compared to values obtained 6 months after transplantation, thus comparing the function of the recipients' own liver to the function of the transplanted liver. After transplantation the half-life of indocyanine green was significantly shorter (18.0 ± 3.0 vs 5.7 ± 1.1 min; normal < 6.5 min), galactose elimination capacity was significantly greater (4.3 ± 0.2 vs 6.9 ± 0.3 mg/kg per minute; normal > 6.6 mg/kg per minute) and the lidocaine half-life was significantly decreased (191 ± 67 vs 74 ± 40 min; normal < 80 min). The MEGX serum concentration was not routinely measured in potential candidates for liver transplantation.

After transplantation, quantitative liver function tests remained unchanged over time. This was true for the indocyanine green half-life and galactose elimination capacity (Fig. 2), as well as for the lidocaine half-life and MEGX serum concentration measured 15 min after the administration of lidocaine (Fig. 3).

Discussion

Today, about 70 %–80 % of patients are alive 1 year after liver transplantation, and the chance of dying subsequently is low. Iwatsuki et al. reported that only 13 % of 1-year survivors die between 1 and 5 years after transplantation [3]. Therefore, the majority of patients now survive liver transplantation for many years. There are two main medical concerns about this group. For one, these patients are at increased risk of developing malignant tumors. These are often lymphomas, internal tu-

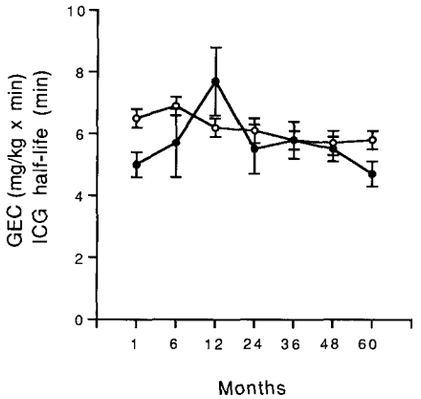

Fig. 2 Serial measurements of indocyanine green (*ICG*) half-life (○) and galactose elimination capacity (*GEC*; ●) in long-term survivors of a first liver transplantation. There were no statistically significant changes over time

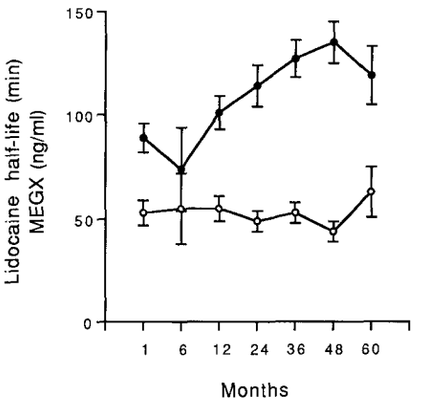

Fig. 3 Serial measurements of lidocaine half-life (●) and serum concentration of MEGX (○) 15 min after administration of lidocaine in long-term survivors of a first liver transplantation. There were no statistically significant changes over time

mors, or skin malignancies, and the tumor risk is related to the immunosuppressive therapy that transplant recipients must receive [9]. In fact, 2 of the 29 patients in this study died from de novo pharyngeal and pancreatic carcinoma 5 and 7 years after transplantation, respectively. A third patient had a malignant skin tumor that could be resected. None of the patients, however, developed a lymphoma.

There is also some concern about long-term function of the allograft [1], and hepatic function is obviously of vital importance to the patients. The question of long-term graft function after liver transplantation, however, has not been addressed in detail thus far. In this prospective study, we therefore analyzed allograft function in 29 patients who survived trans-plantation for a minimum of 4 years. The study, comprising a total of 134 patient years, demonstrated that both excretory and synthetic function of the transplanted liver remain remarkably stable over time. This is indicated both by conventional and quantitative liver function tests. In fact, serum bilirubin concentration and serum activity of γGT improved as late as 2 years after the operation; however, it seems unlikely that this reflects a true improvement in hepatic function. Rather, this was probably related to a less vigorous immunosuppressive therapy with cyclosporin A. Two years after transplantation, we aim at slightly lower cyclosporin A blood levels. The well-known hepatotoxic nature of the compound could, in itself, explain the decrease in serum bilirubin and γGT occuring late after transplantation. This, in turn, could also explain why the improvement in serum bilirubin and γGT was not reflected in the indocyanine green half-life, a parameter of liver blood flow and biliary excretion.

Thus, in long-term survivors of liver transplantation, allograft function remains stable for at least several years.

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