LIVER TRANSPLANTATION 12:544-549, 2006

ORIGINAL ARTICLE

Quantitative Liver Function Tests in Donors and Recipients of Living Donor Liver Transplantation

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The unique ability of the liver to regenerate quickly after resection makes living donor liver transplantation (LDLT) possible. This technique uses the unique ability of the liver to regenerate to full size after partial resection. However, the quality and course of this regeneration process in humans are still widely unexplored. In the present study we investigated the quantitative liver function tests galactose elimination capacity (GEC), indocyanine green half-life (ICG), and lidocaine half-life as markers for the quality of the liver regeneration in the first 3 months after LDLT. In this study, 22 consecutive living liver donors and their corresponding recipients were analyzed at baseline and at 10 and 90 days after LDLT. Six recipients lost their grafts during the study period. We compared donors and recipients at the different time points. After LDLT, GEC decreased (−42.6%) and ICG increased (+50.6%) significantly in donors. ICG and GEC remained significantly altered over 3 months in donors with an improvement between days 10 and 90 (GEC, +59.3%; ICG, −9.1%). ICG and GEC improved significantly in recipients between days 10 and 90 (ICG, −63.7%; GEC, +16.3%). The lidocaine half-life showed no significant changes. The donors had better test results and recovered faster than the recipients. In conclusion, after LDLT the parameters for liver capacity and flow remain altered in donors and recipients despite rapid volume growth. ***Liver Transpl 12:544-549, 2006.*** © **2006 AASLD.**

Received February 17, 2005; accepted October 3, 2005.

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Recently, living donor liver transplantation (LDLT) has become an alternative to cadaveric liver transplantation as a means to overcome the perpetual shortage of donor organs. The unique ability of the liver to regenerate completely after resection makes this approach possible. Remarkably, the liver volume restoration occurs within 4 weeks after LDLT in the vast majority of both donors and recipients.[1,2] Standard liver function parameters such as aspartate aminotransferase, alanine aminotransferase, bilirubin, factor VII, and prothrombin time all normalize within a few days.[2] However, more sophisticated quantitative liver function assays, such as galactose elimination capacity (GEC), a marker for the cytosolic capacity of the liver,[3] suggest a prolonged regeneration of liver function after LDLT.[4]

The aim of our study was to analyze the functional recovery after LDLT in donors and recipients using quantitative liver function tests.

PATIENTS AND METHODS

Patients

Twenty-two consecutive living donors of a right lobe of the liver and their recipients were included in this study. All patients provided informed consent. All donors were healthy and eligible for LDLT following the guidelines for selection of living liver donors at the University of Essen, Essen, Germany.[5] Table 1 shows the schedule of examinations for donors and recipients.

Abbreviations: LDLT, living donor liver transplantation; GEC, galactose elimination capacity; ICG, indocyanine green half-life; POD, postoperative day; bw, body weight; min, minutes.

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DOI 10.1002/lt.20627
Published online in Wiley InterScience (www.interscience.wiley.com).

TABLE 1. Patient/Investigation Characteristics

Parameter	Donors	Recipients
N =	22	22
Organ failure*	–	6
LFT before LDLT	22	–
LFT at 10 POD	22	18
LFT at 3 months	18	13

NOTE. This table shows the examinations that were performed in this study.
Abbreviation: LFT, quantitative liver function test.
*Organ failure within the first 4 weeks after transplantation.

Quantitative Liver Function Tests

The "quantitative liver function tests" consisted of 3 pharmacokinetic tests[6,7]:

(1) GEC as a marker for cytosolic capacity, (2) indocyanine green (Cardio Green) half-life (ICG), which acts as a marker for blood flow and intrahepatic bile excretion, and (3) lidocaine half-life as marker for the Cytochrome p450 system and quality of detoxification of the liver cells.

These tests[6,7] were performed preoperatively (donors only) and on postoperative day (POD) 10 and POD 90 for donors and recipients. Four out of 6 recipients with organ loss did not undergo postoperative testing due to the severity of their illness. Three months after transplantation, 18 donors and 13 recipients were tested. The patients who withdrew their consent and the recipients with graft lost were not tested. To obtain pharmacokinetic data, 23 blood samples were taken over 24 hours: before intravenous infusion of the test reagents, and 3, 6, 10, 15, 20, 25, 30, 35, 40, 50, 60, 80, 100, 120, 180, 240, 300, and 360 minutes, and 10, 14 and 24 hours afterward. The reagents were administered in the following order and dosage:

Galactose (25% D-Galactose, Pharmaceutical Institute, University of Essen, Essen, Germany) was infused as a short infusion with a dose of 0.5 g/kg/body weight (bw) for the initial and POD 90 tests and 0.33 g/kg/bw for testing at POD 10. The smaller dose was employed postoperatively to prevent galactose overload within the reduced size of the liver (unpublished observation).

Lidocaine (Xylocaine 2%, Firma Astra, Wedel, Germany) was given as a single bolus injection of 1 mg/kg/bw [8] over 2 minutes.

Cardio Green ICG (Firma Paesel, Hamburg, Germany) was given as single bolus injection of 0.5 mg/kg/bw.[9]

Galactose Measurement and Calculation

Serum samples were treated with 1 ml of 0.33 M perchloric acid (or 0.33 mol/L percholic acid) per 200 μl serum and centrifuged at 2,400*g*. The supernatant was analysed using the Enzymatic Test Kit Galactose (Firma Roche Diagnostics GmbH, Mannheim, Germany). Urine was collected over a period of 5 hours after injection of galactose. The volume of the collected urine was measured and the galactose concentration was determined in an aliquot using the same procedure as for the serum samples.

This test kit is based on the following chemical reaction: galactose + NAD^+ + galactose dehydrogenase = Galactono-lactose + NADH + H^+ . NADH content was photometrically measured using a spectral photometer at λ 340 nm (PM6, Fa. Zeiss, Germany). The extinctions were calculated to mg/dL.

After plotting galactose concentrations in the serum (mg/dL) vs. time of blood draws (minute) (time point 0 = end of infusion) the linear part of the curve, beginning at about 15-20 minutes, was used to extrapolate the time when the concentration was 0. This time point was marked by the intersection of the resulting straight line with the X-axis. The GEC was calculated using the following formula: (infusion dose [g] galactose − galactose in urine [gl])/(time at concentration 0 + 7 [i.e., infusion time, corrected if longer]) × 1,000/kg bw. The results were expressed as mg/kg/minute.[3,10]

Cardio Green ICG Measurement and Calculation

Cardio Green ICG was measured within 2 hours after the blood draws in all native serum samples spectrophotometrically at λ 805 nm (PM6, Fa. Zeiss, Oberkochem, Germany). Extinctions were plotted vs. time on log-lin system paper after subtraction of the serum extinction at time point 0. The initial linear part of the curve over the double extinction value at the time 0 was calculated by approximation by regression analysis and expressed as half-life in minutes.

Lidocaine Measurement and Calculation

Lidocaine was detected in serum using the Fluorescence Polarization Immunoassay method by the ABBOTT TDX-System (Firma ABBOTT, Germany, Wiesbaden, Germany).[11] Lidocaine concentrations were expressed in μg/mL and were calculated against an internal calibration curve. Background concentration measured before infusion of lidocaine was subtracted from all results. The concentrations were plotted against time on log-lin system paper. Lidocaine half-life was calculated by regression analysis of the linear part of curve (starting at about 60 minutes after infusion) until the end of the testing period or concentration 0. Lidocaine half-life was expressed in minutes.

Normal values in young, healthy individuals for these tests are given in Table 2.

For statistical analyses, the Mann-Whitney *U* test for unrelated groups was used to compare donors and recipients at the different time points. For comparison within donors and recipient groups between the different time points, we used the Wilcoxon test for connected samples. Statistical significance was assumed for $P < 0.05$. Calculations were made using Microsoft Excel software and publicly available Internet sources.

TABLE 2. Quantitative Liver Function Tests (Norm Study)

	Age	Sex	ICG t/2 (minutes)	GEC (mg/kg/minutes)	Lidocaine t/2 (minutes)
N = 14		7 male, 7 female			
Average	25.86		3.895	6.764	83.26
Median			3.63	6.695	82.6
Min	22		2.45	5.22	39.7
Max	29		6.11	8.12	130.6
sx			1	0.867	27.97
SEM			0.267	0.232	7.476
Norm values (±2 sx)			<5.9	5.0-8.5	<140

NOTE. This table summarizes the normal values of the quantitative liver function tests according to a study performed at the University Hospital of Essen, Essen, Germany, in 1990. The normal values were calculated as average value ±2 SD.
Abbreviations: t,/2, half-life; Min, minimal value in the study; Max, maximal value in the study; sx, standard deviation; SEM, standard error of the mean

TABLE 3. Patient Characteristics

Parameter	Donors	Recipients
Age	19-58 years; median, 29.5 years	26-65 years; median, 52 years
Male	13	12
Female	9	10
Ethanol related Cirrhosis	–	7
Hepatitis C	–	6
Hepatitis B	–	5
HCC	–	8
Secondary biliary Cirrhosis	–	1
Autoimmune Hepatitis	–	3
Cholangiocellular Carcinoma	–	1

NOTE. One recipient with ethanol cirrhosis of the liver was also infected with hepatitis C virus. Eight recipients had hepatocellular carcinoma in the cirrhotic liver.
Abbrevation: HCC, hepatocellular carcinoma.

RESULTS

Graft Survival and Patients Dropout

Demographics of the donors and the recipients and the indications for LDLT are summarized in Table 3. Six recipients lost their graft in the early posttransplant period. Four patients had vascular complications, 1 patient developed a sepsis, and 1 patient showed signs of rejection. Three of these remaining recipients and 4 donors withdrew their consent 3 months after transplantation due to failure to comply. Two of the 6 recipients, who lost their graft, were able to undergo a liver function test 10 days after LDLT.

The recipients received between 54.7 and 66.4% of the donor's liver volume (median, 60.6%; mean, 60.4%). All recipients received immunosuppressive therapy (prednisone and cyclosporin A) after transplantation. Patients with higher risk of rejection also received additional mycophenolate mofetil. Six recipients required retransplantation due to organ failure within the study period. There were no differences regarding age and gender distribution between the 2 recipient groups. United Network for Organ Sharing stages at the time of transplantation and organ failure after transplantation were not correlated. There were more patients with autoimmune hepatitis and fewer patients with toxic cirrhosis in the group with organ loss. Viral hepatitis and hepatocellular carcinoma were present in both groups at similar rates.

The results of the liver function tests are summarized in Figure 1 and Table 4.

GEC

All preoperative GEC values were within the normal range. After transplantation, the GEC decreased significantly in donors ($P < 0.01$, Wilcoxon test). At POD 10, GEC loss averaged 42.61% in donors. Between POD 10 and POD 90, the GEC values increased significantly in donors to 91.4% of preoperative values (range, 65.9 to 120.4%; $P < 0.001$ Wilcoxon test). In donors the differ-

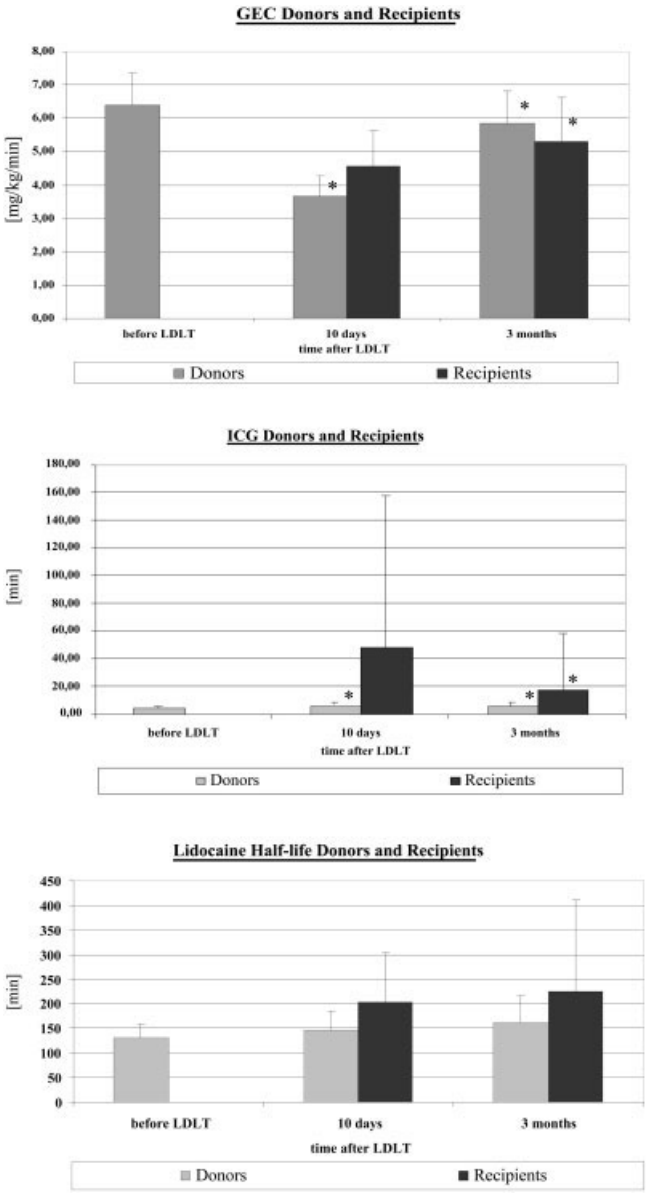

Figure 1. Average values of the quantitative liver function tests, before, on POD 10, and on POD 90 after LDLT in donors and recipients. The tests before LDLT were performed only in donors and represent the values of the graft. The error bars show the standard deviation. *Significant changes (P < 0.05).

ence between preoperative values and POD 90 was still statistical significant ($P < 0.001$ Wilcoxon test).

The GEC in recipients increased significantly between POD 10 and POD 90 ($P = 0.0015$, Wilcoxon test). Recipients had GEC values at POD 10 significantly higher ($P = 0.004$, Mann-Whitney *U* test) than the donors had at POD 10. At 3 months after LDLT, the difference between donors and recipients was no longer statistically significant ($P = 0.31$, Mann-Whitney *U* test).

ICG

Before transplantation, only 1 of 22 donors showed a prolonged ICG half-life. This result did not exclude this donor from donation.

At POD 10, the ICG half-life increased significantly in donors ($P < 0.001$, Wilcoxon test). Despite a significant decrease ($P < 0.001$, Wilcoxon test), the ICG values of the donors at POD 90 were still significantly higher than before transplantation ($P < 0.001$, Wilcoxon test). At POD 90, ICG half-life of the recipients decreased significantly compared to levels at POD 10 ($P < 0.001$, Wilcoxon test). The differences in ICG levels between donors and recipients at POD 10 and POD 90 showed no statistical significance ($P = 0.286$ and $P = 0.352$, respectively, Mann-Whitney *U* test).

Lidocaine

Nine out of 22 donors had a prolonged lidocaine half-life according to normal values summarized in Table 2. These donors were also not excluded from donation.

The lidocaine half-life showed an increased trend in donors at POD 10 compared to the values before transplantation, though this increase was not significant ($P = 0.13$, Wilcoxon Test). At POD 90, the lidocaine half-life showed no significant differences compared to POD 10 in donors and recipients ($P = 0.45$ in donors, $P = 0.49$ in recipients, Wilcoxon test). The average lidocaine half-life in donors at POD 90 showed a strong increasing trend in donors compared to pretransplant values ($P = 0.09$, Wilcoxon test). At POD 10 the recipients had a significantly higher lidocaine half-life than the donors ($P = 0.042$ Mann-Whitney *U* test). The comparison of donors and recipients showed no statistical difference at POD 90.

DISCUSSION

The analysis of the quantitative liver function tests data showed a significant decrease in GEC and a significant increase of ICG half-life in donors after LDLT. Despite improvement, ICG and GEC levels in donors remained still significantly altered at POD 90 compared to baseline values. The recovery of the GEC was slower in recipients than in donors. The recipients showed a wider range in ICG half-life than donors. The recipients' results are consistent with findings in cadaveric liver transplantation.[12]

In both donors and recipients, lidocaine half-life did not change significantly over the posttransplant period. However, donors showed a strong trend toward a longer lidocaine half-life at POD 90.

Quantitative liver function tests after LDLT expressed not only a consistent change in the liver function but also a qualitative change during the postoperative regeneration period, despite an apparent rapid volume growth of the liver in donors.[4] At POD 90, ICG and GEC and lidocaine half-life levels were still altered in donors compared with their corresponding preoperative values. This shows consistent changes in the hepatic flow and in the overall cellular capacity and performance of the regenerating liver. We examined the correlation between ICG and GEC and found no significant correla-

TABLE 4. Results of the Quantitative Liver Function Tests

		Donors				Recipients			
Test	Time	Median	Mean	Range	SD	Median	Mean	Range	SD
GEC	Baseline	6.55	6.39	4.6-8.25	0.981				
	POD 10	3.6*	3.67	2.83-4.8	0.609	4.25	4.567	2.9-7.25	1.059
	POD 90	5.4*	5.84	4.7-7.8	0.959	5.6*	5.312	3.5-8.3	1.299
ICG	Baseline	4.05	4.309	2.3-7.9	1.2				
	POD 10	6.1*	6.491	3.2-11.8	2.362	8.05	48.128	3.2-462.9	109.3
	POD 90	5.4*	5.9	3.4-11.4	2.069	6.2*	17.477	3.5-155.1	41.374
Lido	Baseline	130	132.09	69-173	27.05				
	POD 10	155	145.59	77-243	39.64	183	202.5	77-505	100.32
	POD 90	144.5	161.28	90-304	54.7	181	224.5	89-811	186.83

This table 4 summarizes the results of the liver function tests.
Abbreviation: Lido: lidocaine half-life.
*Significant changes ($P < 0.05$)

tion between the 2 parameters. This indicates the capacity of the liver is reduced in addition to the altered hepatic flow after surgery. Altogether, these results indicate a longer functional recovery after LDLT in donors and recipients despite rapid volume growth.

Unlike the values of lidocaine half-life in cadaveric donors, the values were in general higher in the living donors, which may be due to an induction of the cytochrome p450 system in these patients at the time before organ donation.[13] The higher lidocaine half-life is consistent with findings in animal studies, which showed a decrease in cytochrome p450 activity after liver resection.[14,15] This may be due to indication that cytochrome p450, involved in the metabolism of most drugs and toxins, is downregulated after hepatectomy.[16] Remarkably, at POD 90 the lidocaine half-life remained high, which also indicates a prolonged reduction of the quality of the liver after LDLT. The lidocaine half-life showed no correlation to ICG values, except in recipients at POD 90. This shows that lidocaine half-life is generally independent of the altered hepatic flow after surgery, especially in the early postoperative period. The results show changes in liver cell quality early after LDLT. Later in the posttransplantation course, the changes in hepatic flow can dominate the lidocaine half-life. Whether the lidocaine half-life remains altered should be determined in a follow-up study. This can be important for donors who may need medication in the future that is metabolized by cytochrome p450.

In this study we evaluated only a small group of patients. However, the results indicate a longer and more profound change in the quality of the liver of donors and recipients after LDLT than conventional liver function tests indicate. Therefore, we think it is important to evaluate more patients and for a longer period of time. Since we evaluate only a small number of donor-recipient pairs, it is difficult to make statements about the value of quantitative liver function tests in the selection of suitable donors, even if a preliminary analysis (data not shown) indicates that the lidocaine half-life may be a selective marker. More patients and more experience with the technical difficulties of living related right-lobe transplantation are needed to make a valuable statement.

ACKNOWLEDGMENT

The authors thank Benedikt Reichert for technical assistance.

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