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Early acute cellular rejection: no effect on late hepatic allograft function in man

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Abstract Whereas early acute cellular rejection, even if successfully treated, seems to have an impact on late function and survival of kidney and heart transplants, little quantitative data are available on its effect(s) on liver transplants. Routine liver function tests, the functioning liver cell mass (galactose elimination capacity) and microsomal metabolic capacity (aminopyrine breath test) were determined prospectively in 37 consecutive patients 1 year after liver transplantation. Of these, 19 (7 females and 12 males, 32–69 years of age) had previously required treatment for at least one biopsy proven acute cellular rejection episode occuring a median 7 days after grafting, while 18 (6 females and 12 males, 30–67 years of age) had not. The functioning liver cell mass and microsomal metabolic capacity were both within normal limits for the majority of patients and did not differ significantly between patients with and without previous acute cellular rejection episodes. In contrast to other solid organ transplants, early acute cellular rejection episodes do not affect late function of liver allografts in man.

Key words Liver transplantation, acute rejection, liver function tests · Liver function tests, acute rejection, liver transplantation · Acute rejection, liver function tests, liver transplantation

Introduction

Despite currently available, powerful immunosuppressive regimens, rejection continues to be a major cause of morbidity and graft loss after transplantation of all solid organs. Thus, an episode of acute cellular rejection occurs in at least 50 % of liver transplants [5, 30, 34], and chronic rejection is an important, if not the most important, cause of late liver graft dysfunction, graft loss and retransplantation [12, 33].

During acute cellular rejection, the function of transplanted solid organs declines, the impaired graft function thereby serving as a corner stone in the diagnosis of rejection. This impairment of graft function is often only partially reversible upon successful antirejection treatment. Thus, early acute cellular rejection seems to have an impact on late function of kidney [6, 24, 25, 31] and heart transplants [20, 22]. Moreover, the number and severity of acute cellular rejection episodes have been shown to represent an important, if not the single most important, predictor of late graft function and graft survival after kidney transplantation [6, 24, 25, 31]. While clinical experience holds that early acute rejection has less of an impact on the survival and long-term histology of liver allografts, little quantitative data exist on the effect(s) of early acute rejection on late function of liver transplants.

Whereas other terminally differentiated organs lack the ability to regenerate destroyed functional units, the liver has a unique regeneration potential (for a review cf. [13]). Thus, the liver is able to fully recover both structurally and functionally from massive damage, such as that inflicted by fulminant hepatitis [14], and a normal liver volume is regained within weeks after large liver resections in man [7]. It might therefore be anticipated that a transplanted liver, unlike other solid organ grafts, will recover fully from the functional impairment associated with acute cellular rejection. To test this hypothesis, graft function was determined prospectively at 1 year after orthotopic liver transplantation (OLT) performed on a consecutive series of patients, of whom 18 had experienced at least one acute cellular rejection episode a median 7 days after transplantation, whereas 19 had not.

Patients and Methods

Patients

Between 1 June 1991 and 31 October 1995, 51 OLT operations were performed on 49 patients at the Inselspital Berne, Switzerland. This includes 2 retransplantations after 10 days and 6 months because of primary nonfunction and chronic rejection, respectively. In addition to these 2 subjects on whom retransplantation was performed, 6 patients (12%) died within the 1st postoperative year. Death was attributable in 1 patient each to fulminant HBV reinfection, herpes simplex sepsis, central nervous Aspergillus infection, a granulocytosis with sepsis and multiorgan failure recurrent chronic rejection and recurrent malignant hemangioendothelioma, respectively. Of the 41 patients who had survived the 1st year, 4 were felt to be too ill to undergo complete liver function testing because of the terminal recurrence of hepatocellular carcinoma (2 patients) or cholangiocarcinoma (1 patient), or due to severe brain damage following transplantation because of disulfiram-induced fulminant hepatitis (1 patient), respectively. Further more, the last-mentioned patient refused to undergo liver function testing on his own behalf. Thus, 37 of all 41 (88%) consecutive transplant recipients that had survived the 1st year were studied prospectively and are included in this analysis.

OLT had been performed on all patients by means of veno-venous bypass and end-to-end choledocho-choledochostomy with T-tube drainage for 3 months. All patients were started on triple immunosuppression with steroids, azathioprine and cyclosporin A. Steroids were rapidly tapered and discontinued for the majority of patients at 4 months after OLT, azathioprine was continued at 1.5 mg/kg per day in as much as the blood count permitted, and cyclosporin A trough levels were targeted to 180–250 ng/ml during the 1st year after OLT. Details of the perioperative management and the immunosuppressive regimen have been reported recently [28].

Liver function tests

Conventional liver function tests determining the activities of liver enzymes [aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase, γ-glutamyltransferase], bilirubin and albumin levels in serum, and prothrombin time were conducted according to routine methods in the central hospital laboratory. Total serum bile acids were determined using a commercially available radioimmunoassay (Becton Dickinson Co., Orangeburg, N.Y.). Cyclosporin A levels were measured in the drug monitoring lab of the Department of Clinical Pharmacology using whole blood, a monoclonal fluorescence polarisation immunoassay (Cyclosporine Monoclonal Whole Blood, Abbott Laboratories, Abbott Park, Ill.) and an automated analyzer (TDX-FLX™, Abbott Laboratories, Abbott Park, Ill.).

Quantitative liver function tests [galactose elimination capacity (GEC) and aminopyrine breath test (ABT)] were performed on patients fasted overnight and immobilized in bed for 30 min prior to and during testing. The GEC, which provides a measure of the functioning liver cell mass [8, 18, 21], was determined according to Tygstrup after i.v. injection of 0.5 g/kg body weight galactose [32]. The ABT which, measures the hepatic microsomal function [11, 16, 17], was determined after i.v. injection of 1.5 μCi 14C-aminopyrine (with a specific activity of 0.4 μCi/μmol, Du Pont de Numours International SA, Regensdorf, Switzerland), as described [23].

Statistics

The results of this study are presented as median values (with pertinent ranges in parentheses). The Mann-Whitney U-test or the χ^2-test were used for group comparisons, as appropriate, and a P value of less than 0.05 was considered statistically significant.

Results

Patients' characteristics

Of the 37 liver transplant recipients, 18 patients had experienced at least one episode of biopsy proven acute cellular rejection prior to liver function testing (ACR +), whereas 19 had not (ACR −). The characteristics of both patient groups are given in Table 1. The ACR + and ACR − patient groups were similar with respect to sex, age, underlying disease, and interval between OLT and liver function testing, respectively.

Table 1 Characteristics of patients showing no significant differences between the two groups (Mann Whitney U-test or χ^2-test as appropriate). The quality of donor organs, whether assessed as peak postoperative transaminase levels or as transaminase levels at postoperative day 1, was similar in patients with and without acute cellular rejection episodes (*OLT* orthotopic liver transplantation, *PBC* primary biliary cirrhosis)

	Acute cellular rejection episode(s)	
	With	Without
Number of patients	18	19
Female/male	6/12	7/12
Median age at OLT in years (range)	52 (32–69)	52 (30–67)
Underlying disease		
– PBC	5	4
– Postviral	5[a]	6[b]
– Postalcoholic	5	2
– Miscellaneous	3[c]	7[d]
Liver function testing		
Median months after OLT (range)	13 (12–21)	12 (11–16)

[a] Two HCV-, one HBV-, one HBV/HDV-, one nonA/nonB/nonC-related
[b] Two HBV-, three HBV/HDV-, one HCV-related
[c] All three kryptogenic cirrhosis
[d] Two kryptogenic cirrhosis, one alpha-1 antitrypsine deficiency, one autoimmune CAH, one primary sclerosing cholangitis, one hemochromatosis, one familial amyloid-polyneuropathy type I

Table 2 Immunosuppression at the time of liver function testing in patients with and without prior acute cellular rejection episode(s). All values given as median (range). There are no significant differences between the two groups (Mann Whitney U-test or χ^2-test, as appropriate; *n* number of patients, *Cl* clearance)

	Acute cellular rejection episode(s)	
	With (n = 18)	Without (n = 19)
Prednisolone		
n [%]	5 [28]*	1 [5]
Daily dose, in mg	5 (5–10)	5
Azathioprine		
n [%]	8 [44]	11 [58]
Daily dose, in mg	100 (25–150)	50 (25–150)
Cyclosporin A		
n [%]	18 [100]	19 [100]
Daily dose, in mg	288 (150–350)	250 (175–500)
Trough level, in ng/ml	221 (139–284)	204 (86–287)
Arterial hypertension, n [%]	10 (56)	10 (53)
Serum creatinine, in μmol/l (Norm 45–100 μmol/l)	114 (93–158)	106 (70–160)
Creatinine Cl, in ml/min · 1.73 m^2 (Norm 75–125 ml/min · 1.73 m^2)	59 (29–136)	70 (43–100)

* P = 0.06 vs without acute cellular rejection

Except for 1 patient with HBV/HDV-related cirrhosis in the ACR + group who suffered a self-limiting reinfection, followed by viral elimination, a few months after OLT, all patients with HBV- or HBV/HDV-related cirrhosis were treated with long-term passive anti-HB_s immunoglobulin prophylaxis [9, 27]. At the time of liver function testing, none of these patients showed any clinical or laboratory evidence of recurrent HBV infection; 1 patient in the ACR + and 2 in the ACR – group suffered from mild recurrent chronic hepatitis C, while liver biopsy showed in 1 patient per group a fatty liver attributable to recurrent alcohol abuse.

Acute rejection episodes and overall immunosuppression

The 18 ACR + patients experienced a total of 19 biopsy proven acute rejection episodes. Rejection was histologically classified as mild (corresponding to ≤ points by the Banff classification) in 7 (39%), moderate (corresponding to 4–6 points by the Banff classification) in 6 (33%), and severe (corresponding to ≥ 7 points by the Banff classification) in 5 patients (28%), respectively [1]. Acute rejection episodes occurred a median 7 days (within a 3- to 188-day range) after OLT and required 3–5 i.v. bolus doses of 1000 mg methylprednisolone each. Two of the 19 acute rejection episodes (11%) were steroid-resistant, i.e., had not resolved upon biopsy after a maximum i.v. dosage of 5 × 1 g methylprednisolone, and required OKT3 therapy (5 mg i.v. per day for 10–14 days), which in both cases lead to a resolution of the acute rejection. While the proportion of patients on steroids (and, thus, triple therapy) tended to be slightly higher in ACR + than ACR – patients at the time of liver function testing, this failed to reach statistical significance. Thus, immunosuppressive regimens were comparable in both groups (Table 2). Moreover, a similar proportion of patients in both groups was treated for cyclosporin A-related arterial hypertension, and renal function was similarly impaired – presumably due to cyclosporin A nephrotoxicity – in ACR + and ACR – patients. This further attests to an overall comparable immunosuppression in both groups.

Routine liver function tests

Serum bilirubin concentrations, and biochemical indicators of cytolytic activity (ASAT, ALAT) and cholestasis (alkaline phosphatase, γ-glutamyltransferase) were similar and normal for the majority of ACR + and ACR – patients (Table 3). Moreover, prothrombin time and serum albumin concentrations were comparable and with-

Table 3 Routine liver function tests in patients with and without prior acute cellular rejection episode(s). All values given as median (range). Except for one parameter (serum bile acids), there are no significant differences between the two groups (*n* number of patients, *ASAT* aspartate aminotransferase, *ALAT* alanine aminotransferase)

	Acute cellular rejection episode(s)	
	With (n = 18)	Without (n = 19)
Bilirubin, in μmol/l (Norm 3.5–25.5 μmol/l)	14.5 (9.0–41.0)	14.0 (5.0–25.0)
ASAT, in U/l (Norm 10–35 U/l)	22 (16–222)	20 (11–102)
ALAT, in U/l (Norm 10–40 U/l)	18 (9–125)	22 (9–183)
Alkaline phosphatase, in U/l (Norm 35–120 U/l)	117 (56–285)	93 (63–196)
γ-Glutamyltransferase, in U/l (Norm 10–45 U/l)	33 (8–528)	18 (8–318)
Prothrombin time, in % (Norm 70–100%)	96 (77–100)	96 (83–100)
Serum albumin, in g/l (Norm 30–50 g/l)	38 (33–43)	38 (31–44)
Serum bile acids, in μmol/l (Norm ≤ 6 μmol/l)	2.3 (1.1–26.5)*	1.5 (1.1–6.9)

* P = 0.02, Mann Whitney U-Test; significantly different from corresponding value in patients without acute cellular rejection episode(s)

in normal limits in all ACR + and ACR – patients, attesting to the maintenance of a normal synthetic function of the liver graft, irrespective of previous acute rejection episodes. Solely fasting serum bile acid concentrations were significantly higher in ACR + than in ACR – patients; however, they remained, within normal limits for the vast majority of ACR + patients as well.

Quantitative liver function tests

In order to quantitate liver function, the GEC was determined and the ABT performed. Both were within normal limits for the majority of patients (Fig. 1), and did not significantly differ between ACR + and ACR – subjects [GEC: 432 (240–666) mg/min or 6.1 (4.2–7.7) mg/min · kg body weight vs 463 (312–623) mg/min or 6.8 (3.7–8.1) mg/min · kg body weight, respectively; ABT: 0.73 (0.07–1.26) %dose · kg/mmolCO_2 vs 0.67 (0.33–1.03) %dose · kg/mmolCoO_2, respectively). Moreover, the GEC and ABT were not significantly different in subjects with either histologically mild, moderate or severe early acute rejection episodes (data not shown).

Discussion

The aim of the present study was to determine the effect of early acute cellular rejection episodes on late function of liver allografts in man. Our observations indicate that late function of liver allografts (1) was entirely normal in most patients and (2) was similar, irrespective of whether an acute cellular rejection episode(s) had been experienced previously or not. Thus, in contrast to other transplanted solid organs such as the kidney [6, 24, 25, 31] and heart [20, 22], early acute cellular rejection does not affect late function of liver allografts in man. This may be attributable to the liver's unique regeneration potential.

Of the 49 liver allograft recipients that consecutively underwent transplantation at our clinic from 1 June 1991 to 31 October 1995, 41 survived the 1st year, of which 37 (88%) were studied prospectively. Four subjects could not be studied: 1 patient refused to participate, and with 3 patients it was felt unethical to perform quantitative liver function tests due to their severely compromised general condition attributable to the terminal recurrence of either hepatocellular or cholangiocellular carcinoma. There was no significant difference between the ACR + and ACR – groups with respect to sex, age at OLT, interval between OLT and liver function testing, and underlying disease. Moreover, both groups showed a similarly small proportion of patients with recurrent mild chronic hepatitis C or alcoholic fatty liver. In all, a relevant selection bias seems therefore unlikely.

The results of routine liver function tests, including enzyme markers for cytolysis and cholestasis, and indicators of the liver's synthetic function (prothrombin time, serum albumin), were within normal limits for the majority of patients, irrespective of previous acute cellular rejection episodes. Solely fasting serum bile acids were elevated in ACR + as opposed to ACR – subjects, but remained also within normal limits for the majority of ACR + patients. Altogether, this seems to indicate that late function of liver allografts is not appreciably impaired after the successful treatment of early acute rejection episodes.

In the present study, liver function was quantitatively assessed by the GEC and the ABT. Since no single quantitative liver function test allows an assessment of overall liver function, but rather, each test measures a particular partial function of the liver, it is important to recognize which partial functions are assessed by the GEC and ABT. For the determination of the GEC according to Tygstrup et al. [32], a large amount of galactose is administered i. v. and its disappearance from plasma monitored. Under these conditions, the total activity of the enzyme galactokinase is rate-limiting for the plasma disappearance of the sugar [15]. The majority of galactokinase is found in the cytosole of hepatocytes. Thus, the GEC depends on the total volume of hepato-

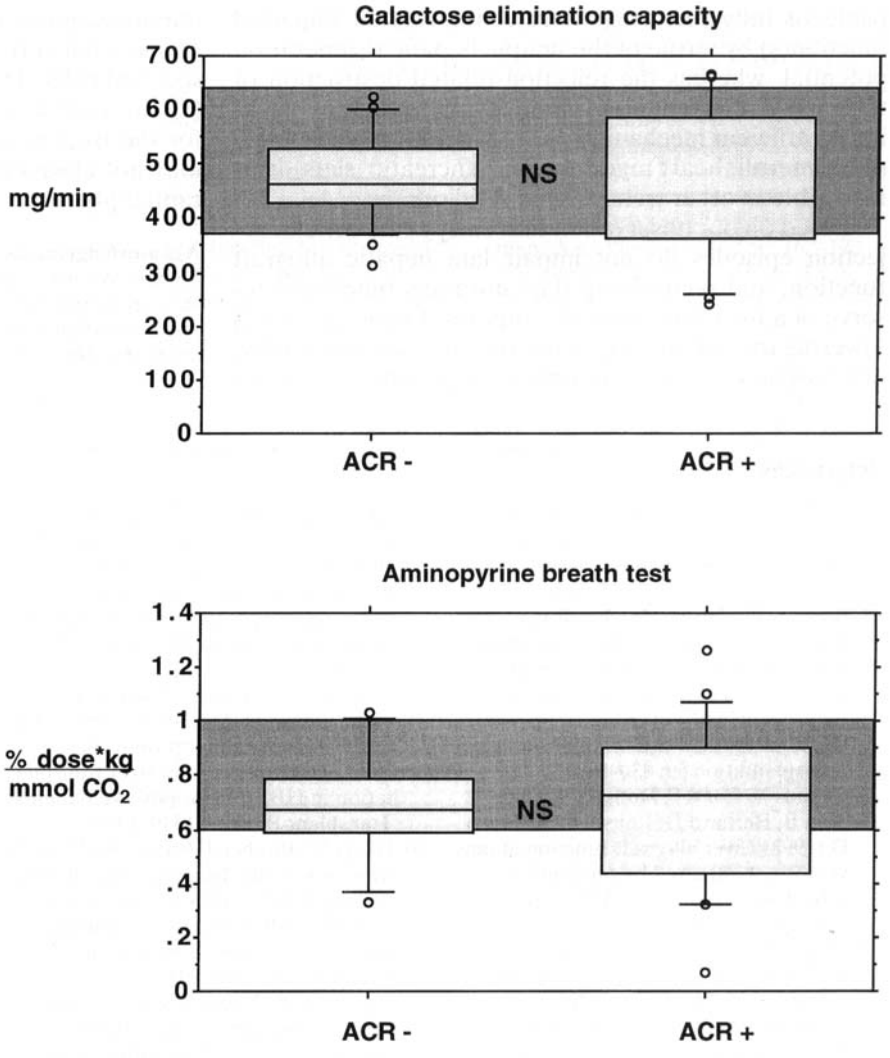

Fig. 1 Functioning liver cell mass (galactose elimination capacity, GEC; upper panel) and microsomal metabolic capacity (aminopyrine breath test, ABT; lower panel) in patients with (ACR +) and without (ACR –) previous acute cellular rejection episode(s). The data are presented as a box plot in which 50% of the values lay within the box, the median value is depicted by the horizontal line, the whiskers indicate the 10th and 90th percentile of all values, and outlying values are individually depicted by open circles. The shaded area indicates the normal range. Neither the GEC nor ABT were significantly different in ACR + and ACR – patients (Mann Whitney U-test)

cytes within the liver and measures the functioning liver cell mass [8, 18, 21]. The ABT is performed by injecting i.v. a tracer dose of the 14C-labelled drug aminopyrine [23]. Aminopyrine is demethylated in the liver by microsomal drug metabolizing enzymes, i.e., the P-450 system, and the methyl groups are further oxidized to $14CO_2$, which is exhaled in breath. Microsomal N-demethylation is rate-limiting for this metabolism of 14C-aminopyrine to $14CO_2$ and can be quantitated by measuring the amount of $14CO_2$ in breath [11, 16, 17, 23]. The ABT thus measures the microsomal metabolic capacity, which depends on the mass of normally functioning liver cells and the induction state of the P-450 system. Collectively, our observations therefore indicate that successfully treated early acute cellular rejection episodes do not affect the later functioning hepatocyte mass of a transplanted liver.

Calmus et al. have reported recently in preliminary form that previous acute rejection episodes do not have an impact on routine liver function tests and on the plasma disappearance of the anionic dyes indocyanine green (ICG) and bromosulphophthalein (BSP) 1 year after OLT [3]. Both ICG and BSP are extracted very efficiently from blood by hepatocytes, and their plasma disappearance is therefore proportionate to the liver blood flow [10, 19, 26]. Our study confirms and extends these preliminary findings by demonstrating that not only the liver blood flow, but also the functioning cell mass of the liver allograft is not affected by previous acute cellular rejection episodes.

Our study does not allow a drawing of conclusions as to the mechanism(s) responsible for this functional recovery of liver allografts from acute cellular rejection episodes, which is at variance with the situation pertaining to other transplanted solid organs, such as the kidney and heart, in which acute cellular rejection seems to decrease late graft function [6, 20, 22, 24, 25, 31]. It is, however, tempting to speculate that a liver graft is ca-

pable of fully restoring its rejection-related impaired function(s) by virtue of the unique hepatic regeneration potential, whereas the rejection-related destruction of terminally differentiated parenchymal structures lacking an efficient mechanism for regeneration (such as renal gomeruli, heart myocytes, or pancreatic islets) is irreversible in other transplanted solid organs.

Based on our observation that early acute cellular rejection episodes do not impair late hepatic allograft function, and recognizing the enormous functional reserve of a liver, two clinically important issues arise: (1) lowering the risk of early acute rejection episodes in liver transplantation by routinely using prophylactic heavy immunosuppression, e.g., by induction regimens using monoclonal anti-T cell antibodies, may not justify its associated risks of infection [2] and tumorigenesis [29] and (2) for similar reasons, additional immunosuppression for the treatment of low grade acute cellular rejection may not always be immediately necessary, if at all warranted [4].

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