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Selective treatment of early acute rejection after liver transplantation: effects on liver, infection rate, and outcome

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Abstract To evaluate the results of selective treatment of biopsy-proven mild acute rejection episodes, we retrospectively studied 1-week liver biopsies of 103 patients with a primary liver graft in relation to liver function tests. The overall incidence of rejection was 35 %. In four patients the biopsy showed histological features consistent with rejection; in 27 patients it showed mild acute rejection (grade 1), and in 5 patients it showed moderate acute rejection (grade 2). Study group 1 consisted of 19 untreated patients with grade 1 rejection and group 2 of 8 treated patients with grade 1 rejection. At 30 and 90 days, no differences in liver function tests were found. The infection rate, histology after 1 year, and survival in the two groups did not differ. It may, therefore, be concluded that withholding treatment in histologically proven mild acute rejection is possible in selected patients based on histological, biochemical, and clinical criteria. This may reflect the functional diversity of morphologically similar lymphocytic infiltrates observed in graft biopsies showing features of mild acute rejection.

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

Introduction

After orthotopic liver transplantation (OLT), the incidence of acute rejection (AR) varies from 40 % to 80 % [2, 4, 6, 7, 9, 14, 19]. In most cases, AR is diagnosed early, with a peak incidence at the end of week 1 post-OLT [1]. Although it remains a serious problem, AR is not a major cause of graft loss [5, 19]. It has been suggested that not every AR requires treatment [1, 2, 6, 7, 20]. In these cases, there could be a discrepancy between the histological, biochemical, and clinical signs of rejection. Histology, however, is still regarded as the gold standard for the diagnosis of rejection [2, 4, 6, 7, 19, 20]. Over the years, our experience has shown that

Table 1 Characteristics of group 1 (untreated) and group 2 (treated) patients with grade 1 acute rejection confirmed by liver biopsy 1 week post-OLT (*M* male, *F* female, *CAC* chronic active cirrhosis, *PBC* primary biliary cirrhosis, *PSC* primary sclerosing cholangitis)

Variable		Group 1 ($n = 19$)	Group 2 ($n = 8$)	*P* value
Median age in years (range)		46 (23–59)	36 (25–43)	NS
Sex M/F		8/11	3/5	NS
Diagnosis:	CAC	5	2	
	PBC	4	1	
	PSC	4	1	
	Wilson' disease	–	2	
	Other	6	2	
Preservation solution: Euro-Collins/UW		2/17	2/6	
Median cold ischemia time in hours (range)		13 5–24	14 4–23	NS

some early, mild AR does, indeed, disappear spontaneously. Therefore, from a series of 112 consecutively transplanted adult patients, we retrospectively studied 27 with a biopsy-documented grade 1 AR at 1 week post-OLT. We focused on two issues. First, as management is variable in the case of a mild AR (grade 1 according to Snover et al. [15]), we evaluated whether the decision criteria to withhold treatment had been effective. Second, we assessed the influence of selective treatment of AR not only on the early post-transplant course but also on the long-term effects.

Patients and methods

Patients

The study group was drawn from 112 consecutively transplanted adult patients with first liver grafts. Nine patients were excluded, three of whom died within 7 days post-OLT and six of whom had no 1-week biopsies due to coagulation disorders. A total of 103 biopsies were, therefore, included in the study. For histological diagnosis of rejection, grading according to Snover et al. was applied [15]. In 36 of the 103 biopsies (35%), features of AR were observed, which is comparable to findings in an earlier report [10]. Four biopsies were diagnosed as consistent with, but not diagnostic of, rejection. Twenty-seven biopsies showed grade 1 and five biopsies grade 2 AR; severe AR (grade 3) was not observed. The four patients with features consistent with rejection were not treated for AR, while the five with grade 2 were. These nine patients were excluded from further analysis. Thus, our study group 1 (19 of the 27 patients with grade 1 AR) received no treatment, while group 2 (the other 8 patients) did. Patient characteristics are shown in Table 1.

Donor livers, preservation, and transplantation

All patients received ABO-identical or compatible grafts harvested from hemodynamically stable, brain-dead donors with near-normal liver function tests (LFT). In situ cooling was done with Euro-Collins or University of Wisconsin (UW) solution (Table 1). All livers were preserved and implanted following standard techniques [16, 17].

Immunosuppression and rejection treatment

All patients received triple therapy immunosuppression based on azathioprine, prednisolone, and cyclosporin (CyA) [10]. During the 1st week post-OLT, 100 $mg.day^{-1}$ cyclophosphamide was added. No patient received OKT3 or antithymocyte globulin (ATG) for induction therapy. Azathioprine was started immediately after OLT in a continuous dose of 125 $mg.day^{-1}$. Prednisolone was given in a starting dose of 200 $mg.day^{-1}$ and tapered to 30 $mg.day^{-1}$ at 1 month. CyA was started parenterally on day 2. In the first 4 weeks post-OLT, whole blood CyA trough levels were maintained at 200–250 $ng.ml^{-1}$ (HPLC method [5]). The maintenance CyA dosage was lowered to achieve trough levels of 100–150 $ng.ml^{-1}$. When rejection was treated, methylprednisolone was given in dosages of 1000 $mg.day^{-1}$ on 3 consecutive days. If AR was steroid-resistant, ATG (4 $mg.kg^{-1}.day^{-1}$) was administered in five doses on alternate days. Rejection treatment was based both on histology and rapidly increasing LFT, combined with clinical signs, i.e. eosinophilia, a decreasing bile volume, and fever.

Laboratory methods

All biochemical determinations, except pseudocholinesterase, were carried out following standard techniques using a sequential multiple analyzer computer (SMAC). A positive immediate early antigen test was diagnostic of CMV infection [3].

Prophylaxis of bacterial and viral infection

Starting peroperatively, all patients received nonabsorbable antibiotics orally during the first 4 weeks for selective bowel decontamination [18]. These included amphotericin B, polymyxin, and tobramycin. To prevent herpes simplex virus infection, acyclovir was administered orally in a dose of four times 200 mg daily. Infection was diagnosed when clinical signs existed in combination with positive cultures.

Liver biopsy

At 1 week post-OLT, a standard percutaneous liver biopsy was taken with a Menghini needle. Liver specimens were fixed by immersion in 8% phosphate-buffered formaldehyde and embedded in paraffin. Sections were stained with hematoxylin-eosin, PAS after

Table 2 Median and ranges of liver function tests on day 7 after liver transplantation in group 1 (untreated) and group 2 (treated) patients (*APh* alkaline phosphatase, *GGT* gamma glutamyl transferase, *ASAT* aspartate aminotransferase, *ALAT* alanine aminotransferase, *LDH* lactate dehydrogenase, *N* upper limit of normal laboratory value)

Variable (normal value)	Group 1 (n = 19) Median (range)	Group 2 (n = 8) Median (range)	P value
APh (N < 120 U.l^{-1})	177 (60–454)	154 (97–192)	NS
GGT (N < 65 U.l^{-1})	271 (49–760)	194 (69–484)	NS
ASAT (N < 30 U.l^{-1})	54 (27–238)	156 (51–653)	0.007
ALAT (N < 30 U.l^{-1})	319 (55–940)	412 (151–1205)	NS
LDH (N < 235 U.l^{-1})	358 (251–903)	484 (396–1766)	0.02
Total bilirubin (N < 17 μmol.l^{-1})	99 (24–358)	247 (101–350)	0.01
Pseudocholinesterase (N = 1900–3600 U.l^{-1})	1235 (283–1769)	1235 (960–1410)	NS

Table 3 Median increase or decrease (–) in liver function tests measured on days 5 and 7 in group 1 (untreated) and group 2 (treated) patients with grade 1 acute rejection confirmed by liver biopsy 1 week post-OLT. Abbreviations as in Table 2

Liver function test (normal value)	Group 1 (n = 19) Median (range)	Group 2 (n = 8) Median (range)	P value
APh (N < 120 U.l^{-1})	271 (49–760)	411 (307–1185)	NS
GGT (N < 65 U.l^{-1})	378 (217–765)	513 (400–1464)	< 0.03
ASAT (N < 30 U.l^{-1})	–4 (–193–168)	64 (–82–583)	< 0.02
ALAT (N < 30 U.l^{-1})	–83 (–1235–457)	42 (–246–1002)	< 0.03
LDH (N < 235 U.l^{-1})	16 (–121–362)	113 (16–1331)	< 0.03
Total bilirubin (N < 17 μmol.l^{-1})	2 (–24–184)	83 (–13–186)	< 0.03

diastases digestion, azan, reticulin, Perls' iron, and rhodamine stains.

Statistical analysis

Data were analyzed with the Statistical Package for the Social Sciences (SPSS). Testing for differences between groups 1 and 2 was done on day 7 with a Mann-Whitney U-test, as were the differences in LFT between days 5 and 7. A two-factorial designed Manova was used to test differences in LFT in time between groups 1 and 2. Differences were considered significant when P was below 0.05.

Results

Liver tests after 1 week

The median and range of the LFT of group 1 (untreated) and group 2 (treated), measured on day 7, are listed in Table 2. Alanine aminotransferase (ASAT), total bilirubin, and lactate dehydrogenase (LDH) were all significantly higher in group 2 ($P < 0.05$). The median differences in LFT, determined on days 5 and 7, are indicated in Table 3. In group 2, all LFT except alkaline phosphatese (APh) showed a significantly less favorable course than in group 1.

Liver tests after 3 months

The LFT of groups 1 and 2, measured on days 30 and 90, showed no significant differences, though LFT in group 2 were consistently higher.

Histology and outcome after 3 months

In group 1, 2 of the 19 patients developed a grade 2 AR episode after 7 and 8 weeks, respectively. Both patients were treated successfully with i.v. methylprednisolone. One patient was treated with ATG 7 weeks post-OLT as the AR was steroid-resistant. No chronic rejection was diagnosed.

In group 2, four of the eight patients had no further AR. One of the remaining four patients developed chronic rejection after treatment with ATG 1 month post-OLT. Seven months later, a retransplantation was performed. The patient died 1 year after his first trans-

plant of recurrence of hepatocellular carcinoma. One of the other three patients died of cerebral bleeding 10 days post-OLT. The two remaining patients were treated with ATG as their AR was steroid-resistant. One of them died 8 weeks post-OLT of ongoing rejection, resulting in multiple organ failure and sepsis. The other patient died of a systemic CMV infection.

Bacterial, fungal, and viral infection rates

In group 1, 11 of the 19 patients had no infectious episodes of bacterial or fungal origin. Two patients had one infectious episode each, four patients had two episodes, and two patients had three. In group 2, four out of eight patients had no infectious episodes. Two patients had one episode each, one patient had two episodes, and another had three infectious episodes. In both groups, one patient died of sepsis. No difference in infection rate was found during the first 3 months. Fifteen of 17 patients with either a CMV-positive status before OLT or a CMV-positive donor liver developed CMV infection.

Outcome at 1 year after liver transplantation

Histology

Fourteen biopsies were available in group 1. No biopsy was available for two patients and three patients died within 1 year after OLT. Four biopsies were available in group 2. Three patients died within 1 year after OLT and one patient was retransplanted for chronic rejection. All available biopsies showed a normal liver histology.

Survival

Three patients died in group 1: one of multiple organ failure at 10 days, one of sepsis at 3 months, and one of recurrence of hemangiosarcoma at 8 months. In group 2 three patients also died: one of intracranial bleeding at 10 days, one of a systemic CMV infection at 6 weeks, and one of multiple organ failure and sepsis at 2 months.

Discussion

In the early period after liver transplantation, not all rejection episodes require treatment [1, 6, 7, 9, 14, 19]. However, there are no definite criteria to distinguish which mild AR needs treatment and which does not. In this study, the majority (19/27) of the patients was left untreated. Retrospectively, when AR was diagnosed, serum ASAT, LDH, and bilirubin were significantly higher in the treated group 2. Almost none of the means means of the LFT in group 2 were significantly higher than in group 1 at day 30 or day 90. In the untreated group 1, a slower increase, or even a decrease in LFT was observed. None of the untreated patients in group 1 developed chronic rejection, and liver histology was normal or without any significant changes at 1 year. Therefore, withholding treatment had no deleterious short- or long-term effects.

Could treatment have been withheld in group 2 as well? Indeed, the course of AR in this group seemed more aggressive than in group 1. One patient developed ongoing rejection. Three patients needed ATG, one of whom even developed chronic rejection. Moreover, on days 30 and 90, the means of the LFT in group 2 were still higher than in group 1, suggesting that treatment was justified. The rapid increase in all LFT except APh between days 5 and 7 reflects the severity of cellular damage due to AR. Apparently, this determines when mild AR should be treated.

A policy of selective AR treatment may also lower the chances of opportunistic infections. Treatment of AR with high-dose corticosteroids may have induced more severe infection, resulting in a proportionally higher mortality from infection in group 2. This study demonstrates that histologically similar AR may have variable courses and outcome, representing a variable graft-host interplay. During AR the portal tracts contain a mixed cellular infiltrate consisting of CD4 + and CD8 + cells and macrophages. The majority of these cells are probably nonspecific, activated by lymphokines [8, 11]. A part of this portal infiltrate is a normal response of the host, directed at newly presented antigens [8, 11, 19]. Based on "false-positive liver biopsies", Schlitt et al. hypothesize that the infiltrates observed in AR are morphologically similar but functionally different [14]. Thus, the lymphocytic infiltrates in our group 1 may have been different from those in group 2.

Discrimination (and quantification) of activated and nonactivated effector cells may be essential [12, 13]. This may predict the course of early, mild AR. Only then will treatment be based on exact criteria.

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