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ORIGINAL ARTICLE

Daclizumab induction and maintenance steroid-free immunosuppression with mycophenolate mofetil and tacrolimus to prevent acute rejection of hepatic allografts

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Summary

Steroid-free immunosuppressive regimens reduce corticosteroid-related side effects in liver transplant recipients although their efficacy is very variable. We evaluated the efficacy and safety of a steroid-free regimen in a 6-month, open-label, multicenter, pilot study, which involved 102 liver transplant patients treated with daclizumab (2 mg/kg within 6 h following transplant and 1 mg/kg on day 7), mycophenolate mofetil (MMF, 1 g b.i.d) and tacrolimus (trough levels of 5–15 ng/ml in the first month and 5–10 ng/ml thereafter). One intra-operative dose of methylprednisolone was administered. At 6 months, the acute rejection rate was 9.8%, and patient and graft survival rates were 96% and 95%, respectively. Acute rejection rates were similar for hepatitis C-positive patients (8.6%) and hepatitis C-negative patients (10.4%). Infections occurred in 22% of patients; most cases were considered mild or moderate. Post-transplantation hypertension and diabetes mellitus developed in 37% and 14% of patients, respectively, during the study period, but were markedly less frequent (8% and 6%, respectively) at 6 months. Hypercholesterolemia was observed in only 2% of patients. In conclusion, the steroid-free immunosuppressive regimen of daclizumab, MMF, and tacrolimus effectively prevents acute rejection after liver transplantation without decreasing safety.

Introduction

The optimal regimen for preventing rejection following liver transplantation remains to be defined. Corticosteroids are a cornerstone of most regimens due to their efficacy in the prevention and treatment of rejection. Long-term use of corticosteroids, however, is associated with significant side effects such as hypertension, diabetes, hypercholesterolemia, and osteoporosis [1]. In numerous retrospective studies the withdrawal of corticosteroids, even in early periods after liver transplantation, has been shown to improve steroid-related adverse events without any apparent negative impact on rejection rates [2–11] with few exceptions [12].

In an effort to reduce the long-term morbidity associated with corticosteroid therapy, the feasibility of corticosteroid-free regimens as initial immunosuppression has been evaluated in different studies involving adult liver transplant patients [13–20]. The immunosuppressive regimens used in these studies varied greatly, ranging from

monotherapy with a calcineurin inhibitor to a triple therapy with a calcineurin inhibitor, mycophenolate mofetil (MMF), and either anti-interleukin-2 receptor antibody or antithymocyte globulin induction. The efficacy of these regimens in terms of prevention of rejection also varied greatly, with a reported incidence of rejection of 20–65%. In most of these studies, steroid-free regimens were associated with a decrease in the incidence of hypertension, diabetes, hypercholesterolemia, and cytomegalovirus (CMV) infection.

Daclizumab, a humanized monoclonal antibody that targets the 55-kDa alpha chain of the interleukin-2 receptor, suppresses clonal expansion of antigen-activated T cells critical for causing acute allograft rejection [21,22]. Daclizumab has been widely evaluated in renal transplantation but has only recently been studied in liver transplant recipients, mainly as a calcineurin inhibitor-sparing agent in the setting of perioperative renal impairment [23–31]. In the vast majority of the studies, the efficacy and tolerability of regimens including daclizumab and reduced doses of calcineurin inhibitors were similar to those achieved with standard immunosuppressive regimens not including daclizumab. In two other studies, daclizumab was administered as part of steroid-free immunosuppressive regimens: daclizumab, tacrolimus, and MMF in one study, which included a small number of patients [14], and daclizumab and tacrolimus in the other, involving a large series of patients [20]. In both studies, the incidence of rejection was the same, 25%.

In the present study, we evaluated the effectiveness of a steroid-free regimen with daclizumab, tacrolimus, and MMF for the prevention of acute rejection in 102 patients receiving primary liver allografts. The safety of this immunosuppressive regimen was also investigated.

Patients and methods

Study population

Recipients of primary liver allografts were eligible if they received a single organ transplant and were between 18 and 65 years of age. Women of childbearing potential required a negative pregnancy test and used a reliable form of contraception. Patients were excluded if they had received a previous organ transplant or previous treatment with daclizumab or MMF, required anti-lymphocyte antibodies, or were expected to need oral corticosteroids after transplant for treatment of co-morbid conditions. Patients with severe gastrointestinal disorders, active peptic ulcer disease, a history of malignancy (other than localized, treated skin cancer or primary hepatic carcinoma without metastases), white blood cell count $<2.5 \times 10^9$/l, or renal insufficiency (serum creatinine ≥1.8 mg/dl or oliguria during the first postoperative hours) were also excluded, as were human immunodeficiency virus-positive patients.

Study design

This open-label, single arm, 6 month, prospective pilot study was conducted at eight centers in Spain. The protocol was approved by the ethics committee at each participating center and all patients provided written informed consent before participating. Eligible patients received methylprednisolone (500 mg or 1 g intravenously) during surgery and were then treated with a steroid-free immunosuppressive regimen following transplantation that consisted of daclizumab, MMF, and tacrolimus. Daclizumab was administered within 6 h following transplant surgery at a dose of 2 mg/kg via an intravenous infusion given over 15 min (day 0). A second dose of daclizumab (1 mg/kg) was administered on day 7. MMF was administered at a dose of 1 g twice daily throughout the study period. The first dose was administered intravenously within 12 h following transplant surgery, and then subsequent doses were given intravenously until the patient was able to resume oral intake. Tacrolimus was also started within 12 h following transplant surgery at an initial dose of 0.05 mg/kg b.i.d, orally. The dose was adjusted to maintain trough blood levels of 5–15 ng/ml during the first month and then tapered to maintain levels of 5–10 ng/ml thereafter. The maximum allowable daily dose of tacrolimus was 0.4 mg/kg.

Patients with biopsy-proven rejection were treated with high-dose methylprednisolone (1 g/day for 3 days) if considered necessary, and, in cases without response to this treatment, with a second course of high-dose methylprednisolone. Anti-lymphocyte therapy (OKT3 or polyclonal antithymocyte globulin) was scheduled to be administered if acute rejection persisted after the second steroid course.

Efficacy parameters

The primary efficacy end point was the proportion of patients with a biopsy-proven acute rejection episode within 6 months following transplant. Liver biopsies were performed when clinical signs and symptoms suggested acute rejection. A diagnosis of acute rejection was established by a local pathologist from the participating centers according to the criteria of the Banff International Consensus Schema [32].

Safety assessments

Clinical adverse events, laboratory abnormalities, infectious episodes, and malignancies were monitored throughout the study.

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Statistical analysis

The sample size was determined according to Fleming's single-stage method based on acute rejection rates in previous clinical trials of daclizumab and MMF [14,23,24,26–29,31]. The undesirable low response rate was based on a p_0 of 66.6% (i.e. rejection rate ≥33.3%) and the desirable high-response rate on a p_1 of 80% (i.e. rejection rate ≤20%). A one-sided significance level was defined at 5%. Based on these assumptions, 85–100 evaluable patients would be needed in order to reject the null hypothesis of a response rate inferior to p_0 with statistical power of 87–92%.

Efficacy and safety parameters were evaluated on an intention-to-treat basis. Absolute frequencies and percentages were used to describe categorical variables, while mean and SD were used to describe numerical variables. In the comparison between hepatitis C virus (HCV)-positive patients and HCV-negative patients, the Fisher exact test was used to determine whether associations existed for the qualitative variables. The Kaplan–Meier method was used to analyze survival times and rejection. Safety parameters were evaluated descriptively.

Table 1. Patient characteristics.

Age, mean (±SD)	52.5 (±11.0)
Gender, *n* (%)	
Male	77 (75.5)
Female	25 (24.5)
HCV positive, *n* (%)	35 (34.3)
Primary indication for transplantation, *n* (%)	
Alcoholic cirrhosis	41 (40.2)
Hepatocellular carcinoma*	19 (18.6)
Posthepatitic C cirrhosis	18 (17.7)
Posthepatitic B cirrhosis	11 (10.8)
Primary biliary cirrhosis	4 (3.9)
Cryptogenic cirrhosis	3 (2.9)
Other	6 (5.9)
Liver function tests at inclusion	
Serum bilirubin (mg/dl), mean (±SD)	3.1 (±4.3)
Serum albumin (g/l), mean (±SD)	33.1 (±7.2)
Prothrombin (%), mean (±SD)	48 (±31)
MELD score, mean (±SD)	14 (±4)
Cold ischemia time (min), mean (±SD)	375 (±148)
Surgery duration (min), mean (±SD)	337 (±91)

HCV, Hepatitis C virus; MELD, model for end-stage liver disease.
*All hepatocellular carcinoma occurred in patients with cirrhosis.

Results

A total of 102 patients were enrolled from May 30, 2000 to March 6, 2001, which represents a mean number of 1.4 patients per center per month. Patient characteristics and indications for liver transplantation are shown in Table 1. Eighty-four patients (82.4%) completed the 6-month study. Eighteen patients (17.6%) were withdrawn from the study because of adverse events (11 patients), death (four patients), re-transplantation (one patient), and protocol violations (two patients). Both protocol violations were the administration of 2 mg/kg of daclizumab instead of 1 mg/kg for the second dose. The adverse events leading to withdrawal included CMV disease in four patients, leukopenia in two patients, recurrent hepatitis C in two patients, and abdominal pain, tacrolimus neurotoxicity, and non-Hodgkin lymphoma in one patient each. All patients were included in the intent-to-treat analysis of efficacy and safety.

The mean dose of MMF was 1.9 ± 0.3 g/day at 1 month of the study and 1.5 ± 0.5 g/day at 6 months (Fig. 1a). The mean trough blood level of tacrolimus was 11.6 ± 4.4 ng/ml at 1 month, which declined gradually to 8.5 ± 4.8 ng/ml by the end of the study (Fig. 1b).

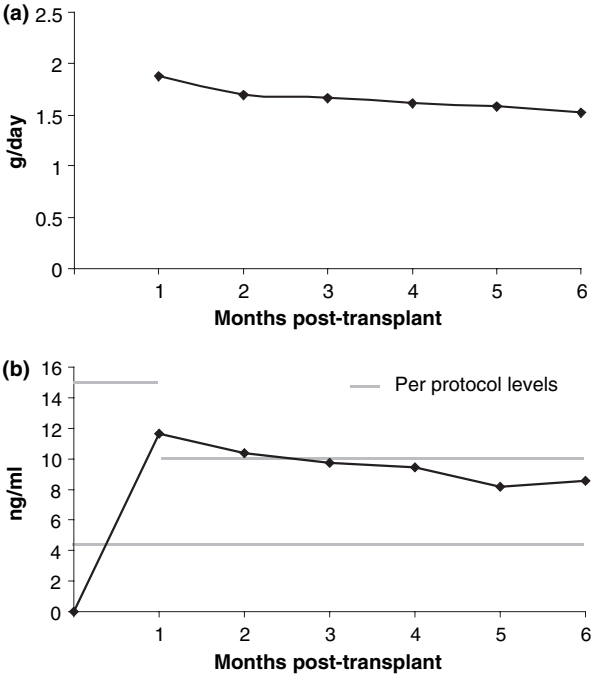

Figure 1 (a) Mean mycophenolate mofetil dosage. (b) Tacrolimus mean trough levels.

Rejection

Ten patients (9.8%) had biopsy-confirmed acute rejection episodes within the 6-month study period, with only one episode of rejection per patient. No acute rejection episode was graded as severe (five were graded as mild and the other five as moderate) (Table 2). Eight of the

Table 2. Incidence, severity, and onset of acute rejection.

Incidence	
Overall ($n = 102$)	10 (9.8%)
Hepatitis C-positive patients ($n = 35$)	3 (8.6%)
Hepatitis C-negative patients ($n = 67$)	7 (10.4%)
Severity (Banff criteria)	
Mild	5 (4.9%)
Moderate	5 (4.9%)
Severe	0
Time after transplantation (months)	
0–1	8 (7.8%)
2–3	0
4–6	2 (2.0%)

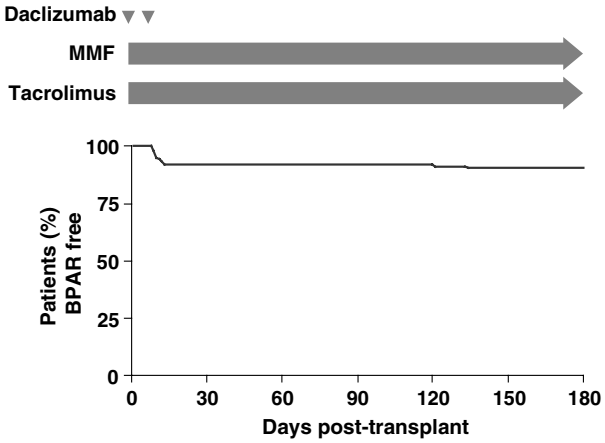

Figure 2 Actuarial rate of biopsy-proven acute rejection (BPAR). MMF, mycophenolate mofetil.

episodes of acute rejection were diagnosed from 9 to 13 days following transplantation. One mild acute rejection occurred at 121 days and one moderate acute rejection occurred at 134 days (Fig. 2). Four patients (3.9%) needed pulse corticosteroid treatment, but none required anti-lymphocyte therapy. Therefore, the rate of steroid-resistant rejection was 0%.

In patients who developed rejection, tacrolimus levels at the time of this complication were 9.0 ± 3.1 ng/ml. Considering only the eight rejection episodes that occurred during the first postoperative month, tacrolimus levels at the time of rejection were 9.3 ± 3.3 ng/ml, which did not significantly differ from the values at the first month in nonrejecting patients (11.6 ± 4.4 ng/ml; $P = 0.151$).

The rate of acute rejection among HCV-positive patients (three of 35 patients; 8.6%) did not significantly differ from the rate among HCV-negative patients (seven of 67 patients; 10.4%). There was no significant difference in the severity of acute rejection between HCV-positive and HCV-negative patients.

Table 3. Adverse events.

Event	Number of patients (%)
Surgical complications	
Primary graft dysfunction	4 (3.9)
Hepatic artery thrombosis	1 (0.9)
Biliary complications	6 (5.9)
Abdominal hemorrhage*	2 (2.0)
Infections	22 (21.6)
Viral	14 (13.7)
Cytomegalovirus†	9 (8.8)
Herpes simplex	5 (4.9)
Bacterial	12 (11.8)
Fungal	7 (6.9)
Hypertension	38 (37.3)
Diabetes mellitus	14 (13.7)
Hypercholesterolemia	2 (2.0)
Renal dysfunction	31 (30.4)
Diarrhea‡	16 (15.7)
Anemia‡	6 (5.9)
Leukopenia‡	31 (30.4)
Tumors	1 (0.9)
Bone fractures	0
Hepatocellular carcinoma recurrence	0

*Requiring surgical re-intervention.
†Cytomegalovirus disease.
‡Requiring mycophenolate mofetil dose reduction.

Adverse events

A total of 22 patients (21.6%) developed 33 infection episodes. Twenty-one episodes corresponded to opportunistic infections: nine CMV disease, five herpes simplex and seven fungal infections (six *Candida* spp. and one *Aspergillus* spp.). Twelve episodes corresponded to bacterial infections: cholangitis in four cases (three related to biliary stenosis), bacteremia in three, pneumonia in two, urinary tract infection in two, and peritonitis in one. Most infections were mild (55.6%) or moderate (37.0%). Infectious complications caused the death of patients in only three cases.

During the study period, hypertension occurred in 38 patients (37.3%), but at the end of the study, at 6 months after transplantation, only seven of 84 patients (8.3%) remained hypertensive. Post-transplantation diabetes mellitus was observed in 14 patients (13.7%), but persisted in only five of 84 patients (6.0%) by the end of the study. Hypercholesterolemia was observed in only two patients (2.0%) during the study period and in one patient (1.2%) at the end of the study. Table 3 summarizes the adverse events observed in the study period.

There were no significant differences between HCV+ and HCV− patients with respect to the development of adverse events during the study period, with an incidence

of 99.0% and 98.5%, respectively, and a mean number of events per patient of 8.8 and 7.4. Nevertheless, severe adverse events occurred more frequently in HCV+ patients than in HCV− patients: incidence of 42.8% and 17.9% ($P = 0.007$), and the mean number of events per patient of 0.9 and 0.3, respectively ($P = 0.015$).

Survival

At 6 months after transplantation, the actuarial patient survival rate was 96.1%. In the four patients who died during the study, the causes of death were hepatitis C recurrence, pneumonia, sepsis associated with coagulopathy, and sepsis. The actuarial graft survival rate was 95%, with one patient retransplanted 1 day after the first transplant due to hepatic artery thrombosis.

Post-trial follow-up extended to month 12

From month 6 to 12 after transplantation, no patient died but one patient was lost to follow-up. Therefore, at 1 year after transplantation, 97 patients continued to be followed. After month 6, immunosuppressive treatment could be changed at the discretion of the investigators. Two additional episodes of biopsy confirmed acute rejection occurred in the period between 6 and 12 months. One episode of mild acute rejection occurred in a patient who had discontinued MMF, but continued to receive tacrolimus, and did not require treatment with corticosteroids. Another episode of severe, steroid-resistant acute rejection occurred in a patient still being treated with MMF and tacrolimus. Therefore, the 1-year rates for different features regarding to rejection were the following: overall acute rejection of 11.8%, severe rejection of 1%, rejection requiring corticosteroid therapy of 4.9%, and steroid-resistant rejection of 1%. Three cases of herpes zoster were observed during this extended follow-up period. At month 12, histological data on hepatitis C recurrence were available in 30 patients with pretransplantation HCV infection. Hepatitis C recurrence was found in 21 of these patients (70%). No *de novo* tumors or hepatocellular carcinoma recurrence was observed in any patient during this extended follow-up period.

Discussion

This pilot study has shown that daclizumab induction and maintenance immunosuppression with MMF and tacrolimus is an effective and safe strategy for the prevention of acute rejection in primary hepatic allograft recipients. Our observed biopsy-proven acute rejection rate of 9.8% at 6 months and 11.8% at 1 year (extended post-trial period) compares favorably with the results of other trials published during the last years involving large series of patients whose characteristics did not substantially differ from those of our patients [9,33–38]. In these studies, the immunosuppressive regimens were based on the administration of either tacrolimus or cyclosporine (CsA) combined with the administration of steroids and, in some trials, with azathioprine, MMF, or basiliximab. Within a few months after liver transplantation (3 or 6 months), the rate of biopsy-proven rejection ranged from 24% to 39% in the studies using tacrolimus-based regimens and from 26% to 43% in the studies using CsA-based regimens. Interestingly, the rates of histologically severe rejection and steroid-resistant rejection among our patients during the first six postoperative months were nonexistent, while rates of 1–13% and 3–25%, respectively, were reported in the aforementioned studies [9,33–38]. Furthermore, at 1 year after transplantation, only 4.9% of patients in our series had rejection requiring corticosteroid treatment, and there was no graft loss due to rejection, whereas in a large trial comparing a microemulsified CsA-based regimen versus a tacrolimus-based regimen (both immunosuppressive regimens also included steroids and azathioprine) the 1-year rates of acute rejection requiring specific therapy in the two treatment arms were 18% vs. 19%, respectively, and the overall rate of graft loss due to rejection was 2% [39].

Our low rejection rate and severity also compares favorably with the results of other steroid-free immunosuppressive regimens. In one study of 64 patients randomized to CsA-microemulsion or tacrolimus monotherapy, 65% and 66% of patients in the respective groups showed biopsy evidence of rejection [13]. Due to the unacceptably high-rejection rate with the calcineurin inhibitor monotherapy in this pioneering study, the efficacy of regimens with a calcineurin inhibitor and other nonsteroidal immunosuppressive agents has since been investigated by several authors. The addition of MMF or azathioprine to tacrolimus was associated with a reduced rejection rate of around 25% [15,16,18]. Similarly, the rejection rate with the addition of anti-interleukin-2 receptor antibody induction to tacrolimus or CsA was 25% and 38%, respectively, in two studies [12,20]. Finally, in two other trials using triple immunosuppressive therapy that included tacrolimus, MMF, and rabbit anti-thymocyte globulin induction, and CsA, azathioprine, and basiliximab induction, the rejection rate was 25% and 39%, respectively [17,19]. All these figures are higher than the rates of rejection in our study, 9.8% at 6 months and 11.8% at 12 months, thus suggesting that substituting steroids with the combination of MMF and daclizumab in our protocol may explain the low incidence of rejection observed in the present study compared with others.

In one study by Washburn *et al.*, in which the immunosuppressive regimen was the same as that used in our investigation, four (26.6%) of 15 patients developed rejection [14], which represents a rejection rate higher than that in our series. However, it is possible that the small number of patients enrolled in the Washburn study was inadequate for the assessment of the true efficacy of the regimen.

Late-onset rejection episodes were infrequent in our patients. There were only two rejection episodes beyond postoperative month 6. This argues against the possibility that daclizumab induction prevented early rejection by merely delaying its development. It is likely that the maintenance immunosuppression with MMF combined with tacrolimus was associated with this favorable outcome.

Studies investigating the efficacy of steroid-free regimens reported rates of histologically severe rejection and steroid-resistant rejection of 0–10% and 0–9%, respectively [12,14–16,18,20]. In our study, no patient had severe rejection or steroid-resistant rejection during the study period (6 months after transplantation), and only one patient developed one episode of rejection with these characteristics during the extended follow-up period (from 6 to 12 months).

Due to the triple immunosuppressive therapy used in our study, one concern might be the possibility of an increase of general, unspecific adverse effects related to excessive immunosuppression such as infections, development of *de novo* tumors, and recurrence of hepatocellular carcinoma and hepatitis C. However, the immunosuppressive regimen used in our study was well tolerated by most patients with respect to these events. Compared with the results obtained in other studies in which theoretically less intense immunosuppression was used [9,35,20,18,37,38], our total 22% incidence of infection is acceptable. Interestingly, most infectious episodes in our series were mild or moderate. In contrast, in one study in which similar immunosuppression was used with the exception of the administration of rabbit anti-thymocyte globulin instead of daclizumab, there was a 22% rate of bacterial and fungal infections requiring hospitalization or intensive care unit management [17], thus suggesting that daclizumab induction may be associated with a lower risk of infection than polyclonal anti-lymphocyte antibody induction. The incidence of CMV disease, the commonest opportunistic infection in our series, was 9%, while the incidence reported for liver transplant recipients in whom CMV prophylaxis was not administered (as in our patients) was approximately 15–30% [40,41]. Our 1% incidence of *de novo* tumors at 12 months is also comparable with that recently found in other studies [12,33,34,38,39]. Recurrence of hepatocellular carcinoma did not occur in any of our 19 patients with this tumor prior to transplantation. Finally, our incidence of histological recurrence of hepatitis C at 12 months after transplantation was 70%, a figure within the range recently reported by other authors (56–76%) [12,17,34,42]. Nevertheless, the real impact of the steroid-free immunosuppressive regimen on hepatitis C recurrence in our patients, as well as in patients included in other short-term studies, cannot be adequately evaluated because the course and outcome of this complication require a more prolonged period of time, habitually several years, to be assessed. HCV viral load was not determined in our study, thus precluding any conclusion about the possible influence of our steroid-free regimen on this parameter.

As expected from the results obtained in most studies investigating the efficacy and safety of steroid withdrawal or avoidance in liver transplant recipients [1–11,13–20,43], the rate of adverse events typically associated with the administration of corticosteroids was, in general, reduced in our patients. Arterial hypertension occurred in 37% of the patients, within the range of the reported rates of hypertension in studies using steroids, which is approximately 25–50% [20,33,39]. However, few of our patients (8%) remained hypertensive at the end of the study (month 6 after transplantation). By comparison, in a study including a large number of liver transplant patients treated with CsA or tacrolimus combined with corticosteroids, hypertension was observed in around 40% of patients at month 6 following transplantation, with no significant difference between the two treatment arms [33]. A 14% incidence of *de novo* post-transplantation diabetes mellitus was observed in our series, a figure within the 12–33% range observed in patients treated with immunosuppressive regimens including corticosteroids [12,17,20,33,39], although only 6% of our patients remained diabetic at postoperative month 6. Compared with the incidence of hypercholesterolemia of 15–45% reported in other studies using immunosuppressive regimens with steroids [44], the incidence of hypercholesterolemia in our series was markedly low throughout the study period (about 2%). The low incidence and severity of rejection in our study and, consequently, the low necessity for administration of steroids during the study period could also have contributed to the reduced incidence of hypertension, diabetes mellitus, and hypercholesterolemia observed at the end of the study. Because these complications are known risk factors for cardiovascular disease and the risk of death due to cardiovascular events in transplant patients has been found to be increased by approximately threefold compared with age- and sex-matched, nontransplanted controls [45], the decrease in hypertension, diabetes mellitus, and hypercholesterolemia seen during the course of our study may

have important implications for improving long-term patient survival. Interestingly, no patient in our series developed bone fractures. This result contrasts with the 20–35% incidence of bone fractures observed in studies using classic immunosuppressive regimens [46,47]. Other adverse events in our patients were within the expected incidence in liver transplant recipients.

In summary, a steroid-free immunosuppressive regimen including daclizumab, MMF, and tacrolimus appears to be very effective in preventing acute rejection without decreasing safety in liver transplant recipients. Furthermore, adverse events potentially related to corticosteroids were less frequently observed than in other trials. Nevertheless, prospective, long-term trials comparing this regimen with standard immunosuppression protocols are needed to confirm the promising results of this pilot study.

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Potential conflict of interest

No author has any commercial association that might represent a conflict of interest.

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