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Tacrolimus-based immunosuppression after liver transplantation: a randomised study comparing dual versus triple low-dose oral regimens

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Abstract To evaluate the efficacy and safety of oral tacrolimus-based immunosuppressive induction therapy, 130 primary orthotopic liver transplant (OLT) recipients were randomised to treatment in an open, parallel-group, European multicentre trial. Following OLT, patients were immediately administered either tacrolimus (0.10 mg/kg) and prednisolone (dual therapy group) or tacrolimus (0.06 mg/kg) in conjunction with prednisolone and azathioprine (triple therapy group) both orally. Patient survival at 1 year was 79.4 % for the dual therapy group and 88.7 % for the triple therapy group ($P = 0.194$); 1-year graft survival rates were 76.5 % in the dual therapy group and 80.6 % in the group receiving triple therapy ($P = 0.615$). The frequencies of rejection (dual therapy 42.6 %, triple therapy 50.0 %; $P = 0.482$), infection, and other complications (renal, neurological and glucose metabolic disorders) were similar in both groups. Tacrolimus whole blood trough concentrations were detectable on days 1 and 2, respectively, in the dual and triple therapy treatment groups whilst median tacrolimus blood concentrations in the triple therapy group reached levels similar to those in the dual therapy group on postoperative day 11 following a steady increase in dose. After 1 year, 54.4 % of the patients randomised to dual therapy were receiving tacrolimus monotherapy and only 56.4 % of the patients randomised to triple therapy continued to receive azathioprine. In conclusion, oral tacrolimus-based immunosuppression is both potent and safe when administered as induction therapy after OLT. Treatment may commence at either 0.06 or 0.10 mg/kg per day, but doses may need to be increased to the latter value within the first 10 days to maintain effective immunosuppression.

Key words Tacrolimus, liver transplantation, dual versus triple therapy

Introduction

In addition to the well-established immunosuppressant cyclosporin A, the introduction of tacrolimus (FK 506) as primary immunosuppression following orthotopic liver transplantation (OLT) by Starzl et al. in 1989 provided the clinician with a second powerful therapeutic drug for the prevention of rejection and the maintenance of long-term immunosuppression [13]. Experimental studies had previously shown that tacrolimus had potent immunosuppressive qualities in various animal transplant models [7, 9, 10, 15–17]. Subsequent clinical studies investigated the efficacy and safety of tacrolimus as 'rescue' therapy in liver allograft recipients failing treatment with cyclosporin [4] and, in 1991, the results of the first randomised trial comparing primary immunosuppression with tacrolimus and cyclosporin were reported [5]. Further single-centre studies, mostly

performed at the University of Pittsburgh, showed favourable results in kidney, heart, and small bowel transplantation [2, 6, 12, 18], for paediatric as well as adult transplant recipients [1, 19].

On the basis of these results, two prospectively randomised multicentre studies were initiated, one in Europe and the second in the United States, to investigate the efficacy and safety of tacrolimus as primary immunosuppression following OLT in comparison with optimal, site-specific, cyclosporin-based therapy [3, 14]. In both of these studies, tacrolimus was initially administered intravenously in the immediate postoperative period with subsequent conversion to oral therapy after 2–7 days. Intravenous administration resulted in high peak blood concentrations which, in turn, were considered to be responsible for an increased incidence of serious adverse events (such as nephrotoxicity and neurotoxicity) in the tacrolimus treatment groups. In the European trial, an amendment to the study protocol lowering both the intravenous and oral tacrolimus doses was introduced and resulted in a marked reduction in the number of adverse effects reported [3]. However, since the absorption of tacrolimus is bile-independent [21], investigators from the European multicentre study felt that a further reduction in dose and the sole use of oral tacrolimus therapy might prove to be beneficial in terms of improving the safety profile of the drug.

These arguments formed the rationale for the design of a subsequent multicentre trial in which patients were to be treated with oral tacrolimus therapy at only half the dose administered previously. In an attempt to define the lowest effective initial dose and to minimise further the degree of early toxicity, a second group of patients was to receive an even lower tacrolimus dose, with azathioprine added to the treatment regimen. The present communication reports the 1-year data from this controlled, randomised, multicentre trial and investigates the efficacy and safety of oral tacrolimus therapy when administered as primary immunosuppression following OLT.

Materials and methods

Patients and study design

This open, randomised, parallel-group study was conducted between November 1992 and June 1993 at five centres in the United Kingdom and Germany, following approval from the appropriate ethics committees and the receipt of witnessed informed consent from each patient. The trial was performed in accordance with the Declaration of Helsinki. Two immunosuppressive regimens, both based upon oral tacrolimus therapy, were compared. Patients in the dual therapy group received tacrolimus and prednisolone (PRED) whilst patients randomised to receive triple therapy were administered azathioprine (AZA) in addition. Patients undergoing retransplantation or multiple organ transplantation were excluded, as were patients under 18 years of age, patients suffering from HIV or any active neoplastic disease, and patients receiving an ABO-incompatible graft. However, patients with fulminant liver failure were included.

Safety was assessed on the basis of spontaneously reported adverse events, whether of new onset or increased severity of an existing condition, and/or significant changes in laboratory parameters. Adverse events were classified by means of the COSTART coding system and graded for severity and causal relationship to the respective treatment regimens.

Immunosuppressive protocol

For patients randomised to receive dual therapy, tacrolimus was administered orally twice daily at a dose of 0.05 mg/kg body weight (i.e. 0.10 mg/kg per day) and following an intraoperative steroid bolus of 5 mg/kg body weight; PRED was tapered gradually from 40 mg/day to 20 mg/day by day 5, and to 10 mg/day after 2 months. In the triple therapy group, tacrolimus was to be given twice daily at a dose of 0.03 mg/kg body weight (i.e. 0.06 mg/kg per day), and after an intraoperative steroid bolus of 5 mg/kg body weight, PRED was administered orally at 20 mg/day; it was subsequently tapered to 10 mg/day by the end of month 2. In addition, patients randomised to triple therapy received AZA at a dose of 1–2 mg/kg body weight; the dose was adjusted or AZA discontinued if leucopenia or thrombopenia were detected. Maintenance immunosuppression, including steroid reduction and/or withdrawal, was left to each individual centre to decide, although attempts were to be made to withdraw PRED medication from both treatment groups after 3 months.

Blood samples (2 ml) for the determination of tacrolimus whole blood trough concentrations were collected daily while the patients were hospitalised and subsequently at each outpatient visit. Whole blood concentrations were determined utilising a semi-automated microparticle enzyme immunoassay (MEIA), based on the Abbott IMx analyser. Although a trough concentration range of 5–20 ng/ml was believed to offer therapeutic levels of the drug, decisions to amend the tacrolimus dose were always based upon clinical needs.

Management of rejection episodes

Clinical and laboratory signs indicative of rejection (including fever, jaundice, pain, unpigmented watery bile from the T-tube, an increase in serum bilirubin levels, and rises in serum aminotransferase activity, alkaline phosphatase, gamma-glutamyl transferase, and prothrombin time) required subsequent histological confirmation by graft biopsy. Treatment of rejection entailed patients in both groups receiving either 200 mg PRED daily for 5 days or a 3-day PRED pulse of 500 mg per day. For recurrent or resistant rejection, a PRED recycle or administration of anti-CD3 monoclonal antibody (OKT 3, Orthoclone) or polyclonal antilymphocyte/thymocyte globulin (ALG/ATG) was to be utilised based on clinical judgement.

Concomitant treatment

Antiviral, -bacterial, and -fungal prophylaxes were administered to all patients according to the standard protocol of the participating centres. Microbial evaluation of various body fluids and orifices was performed routinely, and any clinically apparent infections were treated according to specific sensitivity testing with appropriate medication. Patients who underwent transplantation as a result

of hepatitis B-related liver failure received long-term postoperative prophylaxis with anti-HBs hyperimmunoglobulin [8, 11].

Statistical analyses

All significance tests were two-sided and carried out using the conventional 5% significance level. Patient and graft survival were both analysed using the Kaplan-Meier method. Survival times were compared between the treatment groups using the generalised Wilcoxon test. Differences in dose and resultant blood concentration data were assessed by means of the Wilcoxon two-sample test, as were laboratory data. Fisher's exact test was used to compare the respective incidences of rejection, infection, and adverse events for the two treatment groups. The normal ranges for functional data were defined according to the Oxford Textbook of Medicine.

Results

Patient characteristics

Sixty-six patients were randomised to receive dual therapy and a further 66 patients to triple therapy. Two patients did not receive the study drug and were excluded from the analysis; an additional two patients were misrandomised but were assessed according to the treatment that they received. Dual therapy, consisting of tacrolimus and PRED, was therefore administered to 68 primary OLT recipients, whilst tacrolimus, PRED, and AZA (the triple therapy regimen) were commenced as primary immunosuppressive therapy in 62 patients. The preoperative demographic characteristics, including primary diagnosis, age, sex, WHO performance score, and encephalopathy score, did not differ significantly between the two treatment groups, as demonstrated in Tables 1 and 2. HLA antigen mismatches between donor and recipient were disregarded.

Table 1 Indications for liver transplantation

Diagnosis	Dual therapy ($n = 68$)	Triple therapy ($n = 62$)
Posthepatitic cirrhosis	24	20
Alcoholic cirrhosis	8	9
Primary biliary cirrhosis	14	15
Primary sclerosing cholangitis	8	6
Metabolic disorders	3	4
Malignant disease	2	2
Fulminant hepatic failure	2	1
Miscellaneous	7	5

Table 2 Demographic data

Variable	Dual therapy ($n = 68$)	Triple therapy ($n = 62$)
Sex (male/female)	41/27	31/31
Age (median and range)	48 (20–69)	47 (20–65)
Cold ischaemia time (median and range)	11.3 (4.2–19)	10.7 (4 .3–18.7)
WHO performance score		
0	0	1
1	15	12
2	13	20
3	15	11
4	9	4
Not done	16	14
Encephalopathy		
0	36	38
1	12	13
2	7	8
3	10	1
4	2	0
Not done	1	2

Tacrolimus administration and blood levels

The median daily tacrolimus doses for both groups are depicted in Fig. 1. During the immediate postoperative period, the patients in the dual therapy group received approximately twice the tacrolimus dose as those in the triple therapy group. After 3 days, a gradual increase in the tacrolimus dose was observed in patients receiving triple therapy, i.e. from 0.059 mg/kg on day 3 to 0.092 mg/kg on day 14 to 0.118 mg/kg at week 4. In comparison, the dose for the dual therapy group remained stable at around 0.10 mg/kg during the first 2 weeks before reaching 0.11 mg/kg during week 4 (Fig. 1 a). After 6 months, the median daily tacrolimus doses had been reduced to 0.088 mg/kg in the dual therapy group and to 0.095 mg/kg in the triple therapy group ($P = 0.539$), whilst after 1 year, 0.063 mg/kg tacrolimus was administered to the dual therapy group and 0.077 mg/kg to the triple therapy group ($P = 0.193$). Four patients in the dual therapy group received tacrolimus per intravenous administration for 1–4 days and two patients receiving triple therapy were treated with intravenous tacrolimus for 1 and 5 days, respectively. All doses were below 0.06 mg/kg per day.

Evaluation of tacrolimus whole blood concentrations were determined daily during the first 2 weeks and twice or three times weekly thereafter; if dose adjustments were deemed necessary, these were initiated on the same day. Median tacrolimus concentrations above the level of detection of the assay were observed on day 1 for the dual therapy group (8.10 ng/ml) and on day 2 for the triple therapy group (6.65 ng/ml). While the median tacrolimus concentrations in the dual therapy group initially increased to 12.4 ng/ml before steadying around 9.5 ng/ml, levels in the triple therapy group increased slowly to around 8.0 ng/ml by day 14 (Fig. 1 b). After 1 month, the median whole blood concentrations

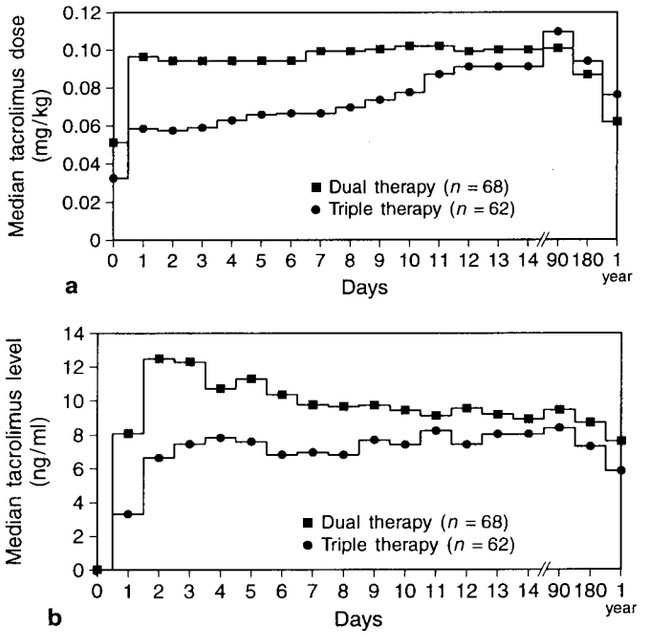

Fig. 1 **a** Postoperative course of median daily tacrolimus doses. Statistical differences were noted on days 1–8, inclusive. **b** Median tacrolimus whole blood trough concentrations. Significant differences were evident on days 1–12 inclusive, with the exception of day 11. Both measurements were taken following primary orthotopic liver transplantation and oral induction therapy with tacrolimus-based dual or triple therapy

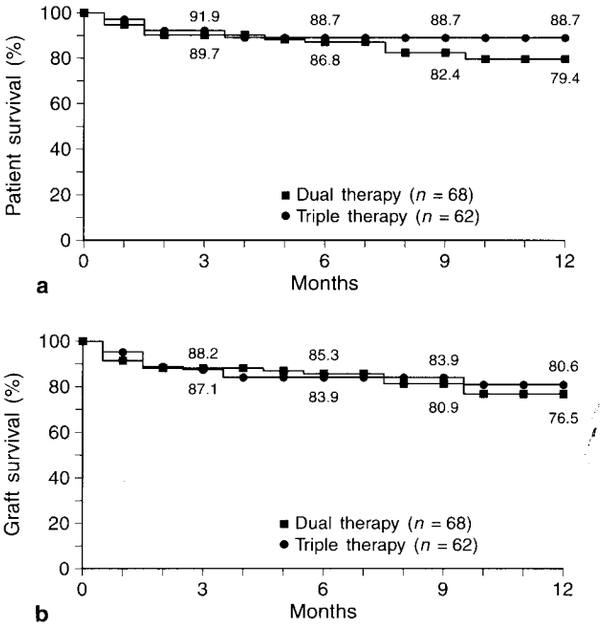

Fig. 2 **a,b** Kaplan-Meier estimates of: **a** patient and **b** graft survival following primary orthotopic liver transplantation and oral induction therapy with tacrolimus-based dual or triple therapy

were 8.50 ng/ml in the dual therapy group and 8.33 ng/ml in the triple therapy group ($P = 0.947$). Subsequently, median concentrations slowly decreased, reaching levels of 7.70 ng/ml and 5.90 ng/ml, respectively, in the dual and triple therapy treatment groups at 1-year post-transplant ($P = 0.012$). It was of interest to note that patients randomised to receive triple therapy were administered, on average, higher tacrolimus doses at 1 year but that this was associated with lower median blood levels than in the dual therapy group at the same time point.

Patient and graft survival

The Kaplan-Meier estimates of the 3-, 6-, and 12-month patient survival rates did not differ significantly between the two treatment groups. Figures of 89.7 %, 86.8 %, and 79.4 % were evident in the dual therapy group whereas triple therapy was associated with survival rates of 91.9 %, 88.7 %, and 88.7 %, respectively ($P = 0.194$ over the 12-month period). Similar results were observed in terms of graft survival; in the dual therapy group, 88.2 %, 85.3 %, and 76.5 % survival rates were determined compared with 87.1 %, 83.9 %, and 80.6 % for the triple therapy group ($P = 0.615$ over the 12-month period; Fig. 2).

During the first 3 months post-transplantation, seven patients (10.3 %) from the dual therapy group and five patients (8.1 %) receiving triple therapy died as a result of graft failure, infections, intractable rejection, or cardiovascular complications. Two of the seven fatalities in the dual therapy group occurred following retransplantation for initial nonfunction, one patient following a cardiac arrest on day 41 whilst the second patient experienced initial nonfunction of the second graft and died on day 10. In the latter post-transplantation course (i.e. > 3 months), a further seven patients (10.3 %) and two patients (3.2 %), respectively, from the dual and triple therapy groups died as a result of infections, disease recurrence, or cardiovascular/other complications (Table 3). Including the two patients with fatal outcome mentioned above, nine patients underwent retransplantation, four (5.9 %) of whom received dual therapy and five (8.1 %) triple therapy (Table 4).

Graft function

To evaluate graft function, transaminase levels and cholestasis parameters were compared between the two treatment groups. Median serum AST levels (normal range 5–35 U/l) reached peaks of around 500 U/l on day 1 before decreasing consistently during the first

Table 3 Causes of death following primary OLT

Death from	Dual therapy ($n = 68$)	Triple therapy ($n = 62$)	p value
Graft failure[a]	3 (4.4 %)	0	0.246
Rejection	1 (1.5 %)	0	> 0.999
Infection	4 (5.9 %)	3 (4.8 %)	> 0.999
Disease recurrence	1 (1.5 %)	1 (1.6 %)	> 0.999
Cardiovascular and other[b]	5 (7.4 %)	3 (4.8 %)	0.720
Total	14 (25.1 %)	7 (11.2 %)	0.162

[a] One patient died as a result of primary nonfunction following retransplantation
[b] One patient (dual therapy group) died from a cardiac arrest following retransplantation

Table 4 Causes for retransplantation and outcome after re-OLT

Reason for re-OLT	Dual therapy ($n = 68$)	Outcome	Triple therapy ($n = 62$)	Outcome
Graft failure	3 (4.4 %)	2 Died[a], 1 Well	1 (1.6 %)	Well
Arterial thrombosis	1 (1.5 %)	Well	1 (1.6 %)	Well
Intractable rejection	–	–	1 (1.6 %)	Well
Recurrence of disease	–	–	2 (3.2 %)	Well
Total	4 (5.9 %)	2 Died, 2 Well	5 (8.1 %)	All well

[a] Reasons for fatal outcome in these patients were primary nonfunction of the second graft and cardiac arrest, respectively

2 weeks and reaching levels of 30.0 U/l for the dual therapy group and 29.5 U/l for the triple therapy group after 1 month. Median AST levels were within the normal range at both 6 months and 1 year.

A similar pattern was evident for serum ALT levels (normal range 5–35 U/l). Following an early postoperative rise reaching medians of 520 U/l and 439 U/l for the dual and triple therapy groups, respectively, on day 2 ($P = 0.961$), levels declined congruently to reach median values of 46.5 U/l in the dual therapy group and 29.0 U/l in the triple therapy group at 1 month ($P = 0.028$). The subsequent ALT courses were uneventful and median values at both 6 months and 1 year were again within the normal range.

Evaluation of the serum bilirubin levels (normal range 3–17 μmol/l) indicated that, after an initial 40 % decrease during the immediate postoperative period, levels rose again, reaching median values of 97 μmol/l on day 6 in the dual therapy group and 123 μmol/l in the triple therapy group on day 8. This corresponded with the closure of the T-tube, and median bilirubin levels subsequently fell consistently, with values of 20.5 μmol/l and 22.1 μmol/l, respectively, being recorded for the dual and triple therapy treatment groups at 1 month. Higher median bilirubin concentrations were observed for the triple therapy group between days 6 and 11, but these did not reach statistical significance. At 6 months and 1 year, median bilirubin levels were within the normal range for both treatment groups.

In addition to serum bilirubin, median alkaline phosphatase (AP; normal range 30–300 U/l) and gamma-GT (normal range 7–51 U/l) levels were also followed closely. Median AP levels remained within the normal range during the entire study period whilst median gamma-GT levels rose following closure of the T-tube as seen for bilirubin (data not shown), but declined steadily to reach normal values at 6 months and 1 year.

Rejection episodes

During the 1st year post-transplantation, 24 patients (35.3 %) receiving dual therapy experienced rejection episodes necessitating bolus steroid therapy. In a further 3 cases (4.4 %), antibody treatment (ATG/ALG or OKT3) was necessary, and despite maximal antirejection therapy, 1 patient (1.5 %) developed chronic rejection (not necessitating retransplantation during the study period) and 1 patient (1.5 %) died from intractable rejection on day 60. In the triple therapy group, we observed 27 patients (43.5 %) with rejection episodes requiring steroid boluses for successful treatment and a further 3 cases (4.8 %) where additional antibody therapy was administered. One patient with a diagnosis of irreversible rejection was successfully retransplanted on POD 82 (Table 5).

Infectious complications

Infections were observed in nearly all patients (94.0 % from the dual therapy group and 96.8 % from the triple therapy group) and no significant differences were apparent between the two treatment groups (Table 6). Classification of the infectious complications into those occurring early (< 1 month after OLT) and late (> 1 month after OLT) also failed to yield any significant differences (data not shown). Besides the seven patients who died as a result of infections (Table 3), all other episodes were successfully treated by immediately commencing therapy upon suspicion and subsequently instituting a specific antibiotic regimen following sensitivity testing.

Table 5 Acute rejection

Treatment	Dual therapy ($n = 68$)	Triple therapy ($n = 62$)	p value
Acute rejection	24 (35.3 %)	27 (43.5 %)	0.372
Steroid-resistant rejection	3 (4.4 %)	3 (4.8 %)	> 0.999
Ongoing rejection	2 (2.9 %)	1 (1.6 %)	> 0.999
Total	29 (42.6 %)	31 (50.0 %)	0.482

Table 6 Infectious complications

Infectious complication	Dual therapy ($n = 68$)	Triple therapy ($n = 62$)	p value
Urinary tract infection	14 (20.6 %)	13 (21.0 %)	> 0.999
Cholangitis	16 (23.5 %)	25 (40.3 %)	0.058
CMV	10 (14.7 %)	8 (12.9 %)	0.805
Pneumonia	13 (19.1 %)	14 (22.6 %)	0.669
Sepsis	13 (19.1 %)	11 (17.7 %)	> 0.999

Table 7 Serious adverse events / adverse events leading to dose reduction

Adverse events	Dual therapy ($n = 68$)	Triple therapy ($n = 62$)	p value
Haemodialysis	12 (17.7 %)	5 (8.1 %)	0.124
New-onset diabetes mellitus	2 (2.9 %)	3 (4.8 %)	0.669
Tremor	9 (13.2 %)	6 (9.7 %)	0.591
Headache	10 (14.7 %)	3 (4.8 %)	0.081
Aphasia	1 (1.5 %)	1 (1.6 %)	> 0.999

Serious adverse events / adverse events leading to tacrolimus dose reduction

Impairment of renal function

Twelve patients (17.6 %) receiving dual therapy and five patients (8.1 %) receiving triple therapy required haemodialysis during the 12-month treatment period ($P = 0.124$; Table 7). However, at the end of the study period, no patient required haemodialysis; median serum creatinine concentrations of 112.0 μmol/l in the dual therapy group and 97.2 μmol/l in the triple therapy group were within the normal range. Serum urea levels, with medians of 7.0 mmol/l and 6.2 mmol/l, respectively, for patients receiving dual and triple therapy, also fell within the normal range at 1 year.

Hyperglycaemia

Hyperglycaemia was reported in 16 patients (23.5 %) from the dual therapy group and 11 patients (17.7 %) from the triple therapy group ($P = 0.517$). A diagnosis of diabetes mellitus was made in 11 (16.2 %) and 5 patients (8.1 %), respectively, from the dual and triple therapy groups ($P = 0.189$); tacrolimus dose reduction was necessary in 2 patients (2.9 %) in the dual therapy group and in 1 patient (1.6 %) receiving triple therapy. In the majority of cases, the diabetes resolved and, at the end of the study period, two patients (2.9 %) receiving dual therapy and three patients (4.8 %) receiving triple therapy were diagnosed with new-onset diabetes mellitus ($P = 0.669$; Table 7). Furthermore, normal median serum glucose levels of 6.5 mmol/l and 5.6 mmol/l were evident for the dual- and triple therapy groups at the end of month 12.

Neurological complications

Minor neurological complications, such as headache, dysesthesia, dizziness, and confusion, were relatively common during the 1st postoperative weeks in both treatment groups. In ten patients (14.7 %) receiving dual therapy and three patients (4.8 %) receiving triple therapy, severe headaches led to a tacrolimus dose reduction ($P = 0.081$). Furthermore, nine patients (13.2 %) from the dual therapy group and six patients (8.1 %) from the triple therapy group developed tremor, and one patient from each group (dual therapy 1.5 %; triple therapy 1.6 %) presented with aphasia that necessitated tacrolimus dose reduction (Table 7).

Requirement of maintenance immunosuppression

After the initial few months post-OLT, steroid doses appeared stable at either 5 or 10 mg/day in those patients receiving steroid therapy. However, only 55 patients (80.9 %) from the dual therapy group remained on steroid medication at 3 months; this number was further reduced to 39 patients (57.4 %) by 6 months and to 24 patients (35.3 %) at the end of 1 year. In the triple therapy group, 53 patients (85.5 %) continued to receive steroids at 3 months, 40 patients (64.5 %) at 6 months, and 32 patients (51.6 %) at 1 year ($P = 0.03$). In terms of AZA usage, 45 patients (72.6 %) from the triple therapy group received AZA at 3 months, 38 (61.3 %) at 6 months, and 26 (41.9 %) at 1 year. In three patients (4.4 %) in the dual therapy group, AZA was added to the tacrolimus/PRED regimen based upon clinical needs (with two of these patients commencing AZA therapy more than 6 months after primary OLT).

After 1 year, 36.2 % of the study population (dual therapy 37 patients; triple therapy 10 patients) received tacrolimus monotherapy, and 35.4 % (29 vs 17 patients, respectively) were treated with tacrolimus and PRED.

In 10.0 % of the cases (2 vs 11 patients), tacrolimus and AZA were administered in combination, and in the remaining 18.4 % of patients (0 vs 24 patients), triple therapy was given.

Discussion

Orthotopic liver transplantation as a life-saving therapeutic procedure for patients with end-stage liver disease was established as a standardised clinical method as the result of an international consensus conference in 1983. Subsequently, multiple studies were undertaken to improve organ preservation, surgical technique, and immunosuppressive therapy, and the results have broadened the indications for OLT. Even with over 5000 OLTs successfully performed each year worldwide, graft rejection remains a major cause of morbidity and mortality [20].

The powerful new immunosuppressant tacrolimus has been used successfully in clinical organ transplantation since 1989 [13]. The results from the recent multicentre trials conducted in Europe and the United States underlined the superior efficacy of tacrolimus in comparison with optimal, centre-specific, cyclosporin-based therapy [3, 14]. Given the perception that initial high-dose intravenous tacrolimus therapy (when administered over a 4-h period) resulted in an increased number of adverse events, a randomised multicentre trial was conducted to evaluate the efficacy and safety of oral tacrolimus for induction immunosuppression following OLT. The evaluation of two different immunosuppressive regimens, both based upon the administration of initial oral tacrolimus therapy, failed to yield significant differences in either patient or graft survival rates at 1 year; furthermore, no differences were apparent with the patient and graft survival rates previously reported in the European multicentre trial [3]. Tacrolimus, administered solely via the oral route, can therefore be considered to be as effective as treatment with optimal, centre-specific, cyclosporin-based regimens and tacrolimus-based induction therapy commencing with intravenous administration [3, 14]. Furthermore, the early postoperative toxicity observed in conjunction with intravenous therapy during the European trial was not detected in the present study; however, this may, in part, have been related to a lack of appropriate therapeutic drug monitoring in the initial phase of the European study [3].

The results of the present trial were achieved despite the poor oral absorption characteristics previously reported for tacrolimus (C_{max} of 0.4–5.6 ng/ml occurring between 0.5 and 8 h (t_{max}) following a single oral dose of 0.15 mg/kg [6, 21]). A subsequent follow-up study by Venkataramanan and his colleagues [22] showed an absolute oral bioavailability ranging from 5 % to 67 % in patients with differing hepatic function, and they subsequently emphasised the influence of graft function rather than absorptive capacity on bioavailabilty. The results of the determination of tacrolimus trough concentrations in this study indicated that a median blood level at the lower end of the target range (5–20 ng/ml) was detectable on day 1 in the dual therapy group and on day 2 in the triple therapy group. Whilst tacrolimus doses were lower than those administered in the past, 24-h blood levels were higher than previously reported [21]. The respective starting doses in the dual and triple therapy groups (0.10 mg/kg per day vs 0.06 mg/kg per day) were markedly different and yet, 2 weeks post-transplant, similar median doses of approximately 0.10 mg/kg per day were recorded in both groups. When compared with the oral dosages administered following the intravenous induction periods of the European and American multicentre trials [3, 14], the tacrolimus administered in both of our groups was considerably less. These results underline the clinical impression that tacrolimus should be administered based upon clinical judgement and that low oral doses are indeed effective in providing therapeutic blood trough levels. In addition, blood level measurements appear to be useful for maintaining tacrolimus concentrations within the target range. However, decisions regarding dose adjustments and/or the institution of additional immunosuppressive therapy should not be based solely upon tacrolimus blood levels. Furthermore, since side effects were not evident in many patients receiving high tacrolimus doses and/or with elevated blood levels whilst, in contrast, a number of patients developed severe adverse events with lower tacrolimus doses and/or blood levels (data not shown), additional studies should be performed to characterise the diagnostic and therapeutic significance of tacrolimus blood level determinations.

Overall, the incidence of rejection episodes, especially those requiring steroid bolus therapy or antibody treatment, was not higher in this study than in the earlier multicentre trials [3, 14]. We observed fewer rejection episodes than in the American study and a comparable incidence to that of the European multicentre trial. In addition, the incidence of steroid-resistant rejection was particularly low (under 5 % in both treatment groups). Furthermore, only two cases of refractory rejection and one case of chronic rejection were diagnosed during the study period, again a much reduced incidence than would typically be expected with cyclosporin-based immunosuppression [3, 14]. The outcome in terms of patient and graft survival for patients with diagnoses of refractory and/or chronic rejection (the immunosuppressive treatment failures) are, in general, poor. The marked reduction in their incidence is certainly beneficial, and the efficacy of low-dose oral tacrolimus for immunosuppressive induction therapy after OLT is, therefore, apparent from the present study.

Looking carefully at the two treatment regimens, we detected a trend towards a higher incidence of rejection in the triple therapy group but a similar number of infections in the two groups. This led us to consider that an initial fixed regimen of 0.06 mg/kg might possibly be insufficient for tacrolimus-based induction after OLT; this was supported by the increasing course of tacrolimus administration in the triple therapy group, where similar median doses to those administered to the dual therapy group were reached 2 weeks post-transplantation. However, the increased incidence of rejection in the triple therapy group may also have resulted, in part, from the lower initial steroid doses administered to this treatment group.

Another important observation underlining the immunosuppressive efficacy of tacrolimus was the high proportion of patients (> 50 %) initially randomised to dual therapy receiving successful treatment with tacrolimus monotherapy 1 year post-OLT. Although the patient survival rate in the dual therapy group was numerically lower than that of the triple therapy group, the deaths reported after the end of month 3 (the point at which steroid therapy was targeted for withdrawal) were not attributable to underimmunosuppression. We might therefore wish to speculate whether steroid therapy could have been successfully withdrawn from further patients as those individuals who received steroids for prolonged periods were prescribed low doses of 5 or 10 mg/day and the results from the triple therapy group indicated that low-dose induction steroid therapy was successful when administered in conjunction with tacrolimus as the base immunosuppressant. Furthermore, AZA was also withdrawn from over 40 % of the patients receiving triple therapy by the end of the 1st year; this caused us to contemplate whether long-term AZA was indeed necessary in conjunction with tacrolimus-based immunosuppression. However, a randomised trial would perhaps be necessary to evaluate the long-term safety of tacrolimus monotherapy as maintenance immunosuppression. Future studies will presumably also evaluate whether combination therapy with tacrolimus and a new immunosuppressive agent, such as an interleukin-2 receptor antibody or mycophenolate mofetil, would further increase the immunosuppressive efficacy.

The incidence of infectious complications in this study was similar to that reported in the earlier multicentre trials whilst the number of serious side effects necessitating dose reduction was decreased [3, 5, 14]. Tacrolimus dose reduction appeared to be appropriate action in most of these cases and resulted in the successful resolution of the events in question; prolonged toxicity was observed only rarely. Overall, the triple therapy regimen, incorporating AZA, was associated with a significant reduction in the incidence of serious adverse events or adverse events leading to dose reduction, although none of the individual events themselves was reported significantly more frequently in the dual therapy group. Disorders of glucose metabolism were transient in nature in both treatment groups with an overall point-prevalence of new-onset diabetes mellitus at the end of the 12-month treatment period (< 4.0 %) much reduced when compared with earlier data.

In summary, tacrolimus-based immunosuppressive induction therapy may be commenced orally at 0.06–0.10 mg/kg per day, but doses at the lower end of this range may subsequently need to be increased to maintain an effective level of immunosuppression. Tacrolimus dosing should subsequently be adjusted according to graft function and the incidence and nature of any adverse events, in conjunction with therapeutic drug monitoring. Oral administration of tacrolimus and PRED alone, or in combination with AZA, constitutes a successful therapeutic regimen following OLT and is associated with high levels of efficacy and limited toxicity.

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