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ORIGINAL ARTICLE

Short-term versus long-term induction therapy with antithymocyte globulin in orthotopic liver transplantation

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Summary

T-cell depletion is an essential aspect of clinical immunosuppression. The aim of the present study was to compare the efficacy of two dosage regimens in this setting. We retrospectively compared 246 patients (group 1) who received a 10-day antithymocyte globulin (ATG) induction protocol with 226 patients (group 2) who received a 3-day protocol. The 6-month rejection rate was 22.3% in group 1 and 12.7% in group 2 ($P = 0.03$). The sub-analysis showed a higher rejection rate in patients with cholestatic disease ($P = 0.01$), who were more numerous in group 1. This resulted in an overall difference between the groups. Rates of *de novo* malignancies and recurrent hepatocellular carcinoma were identical. Viral infection rates were 16% and 18%, respectively ($P > 0.5$). The rates of bacterial and fungal infection were also similar (37% vs. 42%, $P > 0.1$). However, infection and ATG administration are independent risk factors for survival. A lower rate of fatal infection was observed in group 2 ($P = 0.01$), while the 10-day ATG regimen had a detrimental effect on patients who had infection ($P < 0.0001$). Our results strongly support the application of 3-day ATG induction therapy regimen after orthotopic liver transplantation, as it is associated with the same rejection rate as long-term ATG induction therapy, without the negative survival effect of the latter due to lethal infection.

Introduction

Clinical immunosuppressive regimens used after solid organ transplantation are inevitably associated with lymphocyte depletion and selective T-cell depletion [1,2]. The use of antilymphocyte globulin and antithymocyte globulin (ATG) is not restricted to the treatment of severe acute rejection, but is also used in immunosuppressive induction therapy [3,4]. Further reasons for the use of ATG are its positive effects on regulatory T cells [5], the lower rate of cytomegalovirus (CMV) infection due to fewer acute rejection episodes [6], and reduction of ischemia/reperfusion injury [7]. The substances have been proved effective in several studies but their use is still controversially discussed. Particularly in orthotopic liver transplantation (OLT), the following issues are discussed: (i) whether the substance should be used in induction protocols and (ii) how long the antibodies should be administered in order to achieve the desired effect. The goal is to prevent acute rejection of the liver graft without generating life-threatening adverse events. Typical side effects include local or systemic infection and impaired primary organ function. In standard induction therapies, ATG is administered as a single shot for up to 10 days [8]. Various other time frames have been used, but have never been compared in a single-center or multi-center trial. The aim of the present study was to compare the potency and risk of a 3-day ATG induction protocol with a 10-day ATG induction protocol in a cyclosporine and steroid-based immunosuppressive regimen by analyzing a large number of consecutive liver-transplanted patients who received one of the two treatments at the transplan-

tation center in the University Hospital of Vienna, Austria.

Methods

In a retrospective investigation, 796 adults undergoing their first cadaveric OLT between 1990 and 2004 were reviewed. The patients' demographic data were collected from their medical reports. The diagnosis of primary liver disease had to be confirmed by histology. Patients who had been transplanted for acute hepatic failure, hepatocellular carcinoma (HCC) beyond the Milan criteria [9], malignancies other than HCC, and those who suffered from primary nonfunctioning of the graft or had participated in studies with immunosuppressive regimens excluding ATG or cyclosporine were excluded from further investigation. Finally, 473 patients were eligible for detailed analysis. After application of the exclusion criteria, the two groups contained nearly equal numbers of patients. Group 1 ($n = 247$) received 10-day ATG induction therapy and group 2 ($n = 226$) received 3-day ATG induction therapy ($n = 226$). The 10-day regimen was used from 1990 to 1997 and the 3-day regimen from 1998 to 2004. Thus, the decision in favor of one or the other immunosuppressive protocol was based on the time point of treatment. In all other respects, an identical transplantation procedure was used.

All patients initially received a triple immunosuppressive regimen. Starting 6 h post-transplantation at the latest, rabbit antihuman thymocyte immunoglobulin (Thymoglobuline®; Sangstat, Lyon, France) was administered intravenously as a daily infusion over a 6-h period, at a dose of 2.5 mg/kg body weight. Prior to this infusion, all patients received 30 mg diphenhydramine hydrochloride (Dibondrin®; Montavit, Absam, Austria) and 2.5 g metamidazole (Novalgin®; Sanofi-Aventis, Frankfurt, Germany) to prevent allergy. This induction therapy was administered for 10 days in group 1 and for 3 days in group 2. Starting on the eighth postoperative day (group 1) and the second postoperative day (group 2), respectively, cyclosporine A (Sandimmun® or Neoral®; Novartis Pharma, Basel, Switzerland) was administered orally at a dose of 8 mg/kg/day in two doses. The dosage was adjusted to obtain a trough whole blood target level between 100 and 150 ng/ml on high-pressure liquid chromatography (150–200 ng/ml on fluorescence polarization immunoassay). Sandimmun® was used in 144 patients of group 1, whereas Neoral® was administered in the remaining 103 patients of group 1 and in all patients of group 2. As Sandimmun® was not available after 1995, all patients received Neoral® after this time. An intravenous bolus of 40 mg of dexamethasone was given intraoperatively and tapered to 4 mg by day 5. Twenty milligrams of prednisolone was administered orally for 3 months thereafter. The dosage was decreased by 5 g on a monthly basis until a dose of 5 mg/day was achieved. After 3–6 months, patients without cholestatic or autoimmune liver disease were usually withdrawn from steroids. Thus, only cyclosporine was used for long-term maintenance immunosuppression.

During the patients' hospital stay, a complete laboratory investigation was performed daily. Ultrasound investigations were also performed on a daily basis until the fifth postoperative day, and subsequently every second day during the patients' ICU stay. Primary graft function during the first five postoperative days was categorized according to transaminase levels and coagulation status as described elsewhere [10].

Patients were followed up at the outpatient department once a week during the first month after discharge, twice monthly during the second and third month, on a monthly basis during the next 3 months, and every 2 or 3 months thereafter, regardless of the duration of the observation period after transplantation. Patients were also free to visit the outpatient department at any time in the event of a particular problem. A complete laboratory investigation, as described earlier, was performed at each visit. No patient was lost to follow-up.

Acute rejection had to be proven by histological investigation during the patients' hospital stay as well as during follow-up. A fine-needle biopsy was performed in case of unexplained fever or persisting malaise, a rise in bilirubin and transaminase levels with a 10% or greater increase in liver parameters on more than two consecutive days, or an excessive increase between two controls. Rejection was categorized according to the Banff criteria [11]. Biopsies taken prior to the introduction of this classification were reviewed and re-classified by the pathologists

All results are expressed as mean ± SD. The chi-square, *t*-test, and the Cox model were used for statistical analysis. Patient survival curves and the absence of acute rejection episodes were calculated with the Kaplan–Meier method. The level of significance was set at $P < 0.05$.

Results

After exclusion of patients according to the above mentioned criteria, a nearly identical number of patients remained for each of the two periods of 7 years. The duration of follow-up was 29 ± 20 months (range, 1–73) in the treatment group and 81 ± 45 months (range, 1–181) in the historical control group ($P = 0.001$). No statistically significant differences were registered between the groups in terms of demographic data or primary organ function. The distribution of primary liver disease was

also similar. However, group 1 had a significantly higher rate of cholestatic disease (Table 1).

The overall 6-month rejection rate was 22.3% in group 1 and 12.7% in group 2 ($P = 0.03$; Fig. 1). The difference did not reach statistical significance in any of the Banff categories. The rates were 14% vs. 8% for grade I ($P = 0.08$), 8% vs. 4% for grade II ($P = 0.1$), and 0.8% vs. 0.9% for grade III ($P > 0.5$) in the 10-day ATG group and the 3-day ATG group, respectively. Sub-analysis with respect to the underlying disease revealed a significantly higher rejection rate in patients with cholestatic disease in either group: 36% in group 1 and 34% in group 2, respectively. For all other indications the rejection rate was a mere 16% ($P = 0.01$). Excluding patients with cholestatic disease, no statistically significant difference was observed for the cumulative frequency of acute rejection episodes at 6 months in the 3-day ATG and the 10-day ATG groups (14% vs. 19%; $P = 0.1$). Twenty-five percent of patients in each group with grade I acute rejection received no specific therapy because of the absence of clinical symptoms. Those who were symptomatic or had grade II rejection, regardless of clinical presentation, were given 100 mg dexamethasone intravenously once a day (43% in group 1 and 47% group 2; $P = 0.1$) or switched to a modified maintenance immunosuppressive regimen consisting of additional azathioprine or mycophenolate mofetil (32% in group 1 and 28% in group 2; $P > 0.1$). Patients suffering from grade III acute rejection were given horse anti-human thymocyte immunoglobulin for 7 days. Chronic rejection was observed in 2% ($n = 5$) of patients after 10-day ATG induction therapy and in 5% ($n = 12$) after 3-day ATG induction therapy; the difference was not statistically significant.

Relapse of hepatitis C virus (HCV) infection was observed in 35% of patients of group 1 and in 51% of group 2, with no statistical significance between groups ($P = 0.1$). The rate of repeat OLT because of recurrence of HCV-induced cirrhosis was similar in the two groups (11.6% vs. 12.1%; $P > 0.5$). Repeat OLT was performed after a median period of 7 and 8 months, respectively, following primary OLT for post-hepatitis C cirrhosis (PHCC) ($P = 0.5$). Post-transplant lymphoproliferative disorder (PTLD) occurred in three patients (1.2%) after 10-day ATG induction therapy, leading to death after 12, 39, and 160 months, respectively, and did not occur at all after the 3-day regimen. Other *de novo* malignancies occurred at similar rates during the observation period: 3.5% ($n = 8$) in group 2 and 2.8% ($n = 7$) in group 1 ($P > 0.5$). Tumor recurrence after liver transplantation for HCC was a rare event and was only observed in 0.9% (group 2, $n = 2$) and 1.2% (group 1, $n = 3$; $P > 0.5$), respectively.

The overall rates of bacterial and fungal infection were 37% ($n = 84$) in group 2 and 42% ($n = 104$) in group 1; the difference was not statistically significant. The types of infection were also very similar (Table 2). The majority of

Table 1. Patient characteristics.

	3-day ATG ($n = 226$)	10-day ATG ($n = 247$)	*P*-value
Gender, M:F (%)	73:27	65:35	NS
Age of the recipient (years)	51.8 (±9)	50.1 (±10)	NS
Model for end-shape liver disease (MELD) at OLT	16.6 (±6.0)	17.2 (±5.1)	NS
Primary organ function (%)			
Good/fair	90.6	93.5	
Poor	8.4	6.5	NS
Underlying disease (%)			
Alcoholic cirrhosis	40	38	NS
Virus-induced cirrhosis	27	24	NS
Hepatocellular carcinoma	15	10	NS
Cholestatic disease	5	17	0.01
Other causes of cirrhosis	13	11	NS

ATG, antithymocyte globulin; OLT, orthotopic liver transplantation.

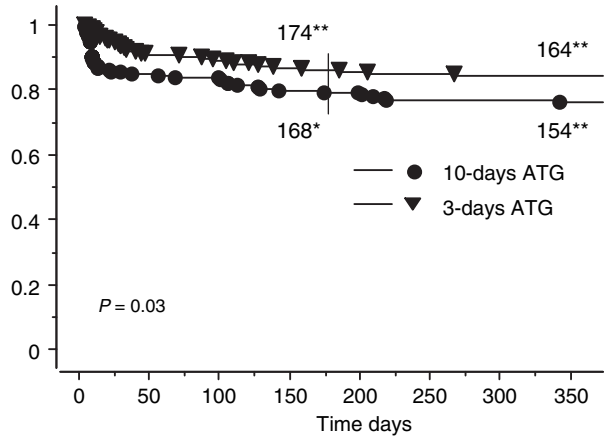

Figure 1 Probability of rejection-free survival at 1 year. Patients at risk at *6 and **12 months.

Table 2. Rate and type of bacterial and fungal infections after OLT.

	3-day ATG (226)	10-day ATG (247)	*P*-value
Urinary tract infection	7.5 (17)	4.9 (12)	NS
Wound infection	6.2 (14)	8.0 (20)	NS
Intraabdominal infection	5.3 (12)	6.5 (16)	NS
Esophagitis	1.8 (4)	1.6 (4)	NS
Enteritis	1.3 (3)	2.0 (5)	NS
Cholangitis	2.2 (5)	3.6 (9)	NS
Pneumonia	5.8 (13)	4.8 (12)	NS
Sepsis	5.3 (12)	9.3 (23)	NS
Others	1.8 (4)	1.2 (3)	NS
Total	37.1 (84)	42.1 (104)	NS

OLT, orthotopic liver transplantation; ATG, antithymocyte globulin. Values denote % (*n*).

Table 3. Rate and type of viral infections after OLT.

	3-day ATG (226)	10-day ATG (247)	*P*-value
Cytomegalovirus	8.0 (18)	11.3 (28)	NS
Herpes zoster	1.8 (4)	2.4 (6)	NS
Herpes simplex	5.3 (12)	2.8 (7)	NS
Others	0.5 (1)	1.2 (3)	NS
Total	15.5 (35)	17.8 (44)	NS

OLT, orthotopic liver transplantation; ATG, antithymocyte globulin. Values denote % (*n*).

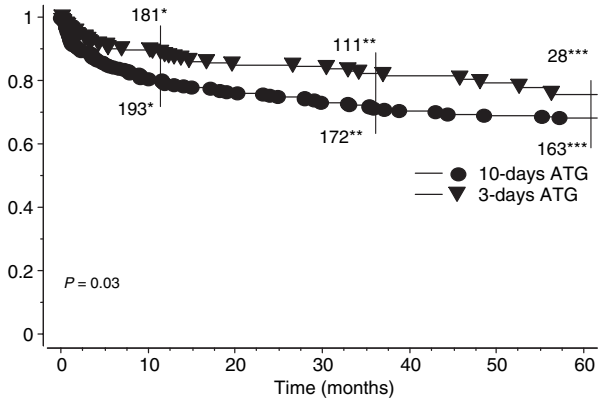

Figure 2 Five-year patient survival. Patients at risk at *1, **3, and ***5 years.

viral infections were caused by CMV, which occurred at a nearly equal rate, followed by herpes simplex infections (Table 3).

Long-term follow-up showed a significant difference in patient survival in favor of group 2 (Fig. 2). The causes of death at 1 year revealed a significantly lower rate of fatal infection in group 2 (5.8%, $n = 13$) compared with controls (14.6%, $n = 36$; $P = 0.01$). Thus, 15.5% of infections in group 2 and as many as 34.6% of infections in group 2 proved lethal ($P = 0.01$). In the latter group, 69% of all deaths that occurred during the first 12 months after transplantation were related to sepsis. Multivariate analysis for the entire observation period disclosed infection ($P < 0.001$) and 10-day ATG administration ($P < 0.03$), but not rejection ($P = 0.13$), as risk factors. A combination of both risk factors had a detrimental effect on survival within the first year after transplantation while long-term ATG dosing alone did not impair survival (Table 4).

Discussion

As the purpose of the present investigation was to compare a post-transplant therapeutic regimen with a historical control group, the evaluation was performed after a period of more than 5 years for each group. Further reasons for using this extended time frame were: (i) to collect an adequate number of cases and (ii) to even out transient imbalances in a transplantation program of long duration. We excluded indications that might have biased the analysis or necessitated a longer or shorter observation time, such as that in cases of transplantation for hepatic malignancies [12]. Based on these criteria, our database was qualified to answer the issues under investigation.

Table 4. Risk* of death within 12 months after OLT.

Risk factors	Odds ratio	95% CI	*P*-value
10-day ATG	1.0672	0.4384–2.5980	NS
Infection	3.4412	1.5053–7.8666	<0.001
10-day ATG + infection	8.7377	4.1058–18.5949	<0.0001

*Risk of death compared with patients without any risk factor, i.e. 3-day ATG and no infection.

The significant difference in the duration of follow-up between the two groups is self-explanatory in terms of method. The higher rate of cholestatic disease at the beginning of the observation period is due to the condition of patients suffering from primary biliary cirrhosis, who were placed on a waiting list when OLT became standard treatment for end-stage liver disease. This aspect was taken into account in the analysis. In contrast to some publications that reported significantly higher liver enzyme levels in patients receiving ATG, initial graft function was nearly identical and normally distributed in our two study groups [13]. This factor also was of no consequence for the classification of initial graft function. It proves that the harvesting or preservation technique was not significantly altered during the observation period. All other patient characteristics were similar and did not influence the results.

In respect of overall freedom from acute rejection, patients who received ATG for a shorter period of time achieved a significantly better outcome. However, this difference was registered cumulatively for all grades of rejection; no statistically significant difference was registered for any of the subgroups. In the study group as well as the control group, our results are similar or even slightly better than those reported in other publications [14–16]. Further analysis with respect to the underlying disease strongly suggests that both immunosuppressive regimens have the same effect in preventing acute rejection and the same lack of efficacy in cases of cholestatic disease. Significantly higher rejection rates were observed in patients with cholestatic disease. This explains the differences in the overall results, which confirm our previously published data in respect of disease-related rejection rates [17,18].

The treatment strategy for acute rejection in OLT has been consistent over the years and throughout several studies [19,20]. The majority of grade I rejections cause practically no symptoms. Treatment in this setting is deemed unnecessary, particularly because mild rejection episodes have no negative impact on the long-term outcome of OLT. Strategies for the treatment of moderate rejection differ in the various transplant centers. A 3-day steroid bolus therapy and/or switching to a triple-drug regimen are common and widely accepted procedures that were often used equally in both groups. Therefore, the type of rejection therapy had no impact on the general outcome. The rate of chronic rejection was also similar in the study group and the control group.

As several studies have clearly shown a strong correlation between the use of immunosuppressive antibodies and cancer [21,22], we analyzed the rate of tumor recurrence and *de novo* malignancies. The published rate of PTLD in large series is approximately 1% [23], which coincided with our findings. Other *de novo* malignancies were observed in 3.5% and 2.8% of patients, respectively. The estimated rate of 1% per year [24] was registered in patients who had 3 days of induction therapy, but was not observed in those who received the 10-day ATG dosing. The difference may have been due to underreporting in controls. Awareness of potential malignancies was much lower 15 years ago than it is today. However, malignancies were no major cause of death during the observation period. Recurrence of HCC after OLT is mainly influenced by the pretransplant tumor stage [9,25]. As all patients met the Milan criteria, recurrence rates were as low as 0.9% and 1.2%, respectively.

The infection rates in group 1 were comparable with those in group 2. The number also concurs with previously published data [16,26,27]. However, the clinical course, rather than the frequency or type of infection, is the decisive aspect of infectious diseases after OLT. In group 1, the prolonged use of ATG led to a severe reduction of patient survival. The rate of lethal infection was significantly higher in controls (34.6% vs. 15.5%). Besides, the number of deaths ($n = 36$ vs. $n = 13$) and the ratio of the causes of death (69% vs. 46%) were evidently in favor of patients treated with the 3-day ATG regimen. This difference was the main factor underlying the benefit of short-term induction therapy. The 1-year survival rate clearly shows that infection *per se* is a highly significant risk factor. In addition, administering ATG for 10 days had a further detrimental effect on the results. Our analysis of other potential factors revealed that acute rejection had no impact on the 1-year survival rate ($P = 0.13$). Initial graft function, a further strong predictor of the outcome [28,29], was nearly identical in both groups and could not account for the difference in survival curves.

In conclusion, our data showed that short-term administration of ATG possesses the same immunosuppressive potency as a long-term regimen. The differences in rejection rates are strongly correlated to the underlying disease and independent of the number of administered doses of ATG. Furthermore, we registered a nearly identical rate of side effects in both groups. However, the clinical course of infectious complications was much more severe in patients receiving 10-day induction therapy and was associated with a significantly lower survival rate. Notwithstanding general advancements in medicine and changes in the treatment of complications over a long observation period such as that used in the present study, our results clearly support 3-day ATG induction therapy after OLT, as it offers the same immunosuppressive benefit of low rejection rates without the negative survival effect of long-term induction therapy.

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