Cyclosporine, tacrolimus and sirolimus retain their distinct toxicity profiles despite low doses in the Symphony study

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Abstract

Background. Reducing side effects of immunosuppressive regimens has become a priority in transplantation medicine because of the large number of patients and grafts that succumb to infection in the short term and cardiovascular disease in the long term. The Symphony study was a 12-month prospective, randomized, open-label, multi-centre, four parallel arm study that aimed to evaluate the safety and efficacy of low-dose immunosuppressive regimens compared with a standard-dose regimen in renal transplant recipients. This sub-analysis focuses on specific toxicities observed with the low-dose regimens.

Methods. Adult patients (n = 1645) scheduled to undergo renal transplantation received low-dose cyclosporine (CsA), tacrolimus (Tac) or sirolimus (SRL) in addition to daclizumab induction or standard-dose cyclosporine without induction. All patients received mycophenolate mofetil and corticosteroids. We evaluated the incidence of adverse events (AEs), tested specific group differences and assessed the relationship of selected AEs with drug levels.

Results. The four arms had similar incidences of AEs, but serious AEs were more common with low-dose SRL and led to more discontinuations. Infections were the most common AEs, with the highest incidence in the standard-dose CsA group, in particular, cytomegalovirus (CMV) infections. Low-dose Tac had the most reports of new-onset diabetes, leucopenia and diarrhoea. Low-dose SRL negatively influenced triglycerides, wound healing, lymphocoele and anaemia. We found only weak relationships between specific AEs and drug levels.

Conclusions. Despite the low doses, CsA, Tac and SRL retained distinct and different toxicity profiles. These findings may be of relevance for tailoring specific immunosuppressive regimens to patients with particular needs.

Keywords: calcineurin inhibitors; cyclosporine; drug toxicity; mycophenolate mofetil; sirolimus

Introduction

In renal transplantation, chronic immunosuppression is associated with considerable risks, in particular, related to infections and cardiovascular diseases, which are the predominant causes of death in those with a functioning graft [1]. Therefore, reducing toxicities has become a priority.

So far, most efforts have been focused on minimization of calcineurin inhibitors (CNIs). This drug class, which includes cyclosporine A (CsA) and tacrolimus (Tac), is associated with nephrotoxicity, hypertension and hyperlipidaemia [2–8]. Compared to CsA, Tac is asso-
associated with a higher incidence of new-onset diabetes mellitus after transplantation (NODAT) [9–11]. CNI avoidance regimens based on the anti-proliferative drug sirolimus (SRL) showed good efficacy, especially after early elimination of CNIs [12,13]. Among the distinctive adverse events (AEs) of SRL, hyperlipidaemia [14], leucopenia, development of lymphocele [15] and delayed wound healing [16] are frequent.

The working hypothesis in the Efficacy Limiting Toxicity Elimination (ELiTE)-Symphony study was that a regimen including daclizumab, mycophenolate mofetil (MMF) and steroids allowed for low doses of CsA, Tac or SRL to be used directly after transplantation with benefits in terms of efficacy and safety. As reported elsewhere [17], at 12 months post-transplant, the low-dose Tac arm had superior renal function, provided the best protection against acute rejection and led to the best graft survival. The two CsA regimens had intermediate outcomes, and patients randomized to low-dose SRL had the worst results.

Here, we focus on safety aspects of general relevance for transplant outcomes, such as infection or hypertension, and those known to be associated with CsA, Tac and SRL. Due to its repercussions on graft function, CNI nephrotoxicity is discussed in the context of the main study results [17]. The main questions include: Do the tested low-dose regimens reduce or eliminate toxicity? Is there a difference in toxicity between standard-dose and low-dose CsA? Are there any associations between drug exposure and AEs?

Materials and methods

Study design

The Symphony study [17] was a 12-month prospective, randomized, open-label, multi-centre, four parallel arm study in adult renal transplant recipients. Patients between 18 and 75 years of age, scheduled to receive a single-organ renal transplant were randomized pre-transplant in equal proportion to one of four groups: a control group, receiving standard-dose CsA (Stand-CsA; target trough level of 150–300 ng/mL for the first 3 months and 100–200 ng/mL thereafter), MMF and corticosteroids or daclizumab induction, MMF and corticosteroids in combination with low-dose CsA (Low-CsA; 50–100 ng/mL), low-dose Tac (Low-Tac; 3–7 ng/mL) or low-dose SRL (Low-SRL; 4–8 ng/mL). Daclizumab was dosed at 2 mg/kg before transplant followed by four doses of 1 mg/kg every 2 weeks. The recommended MMF dose was 2 g/day in all groups.

Patients were excluded if they had current or historic panel-reactive antibodies >20%, had positive cross-match, had a cold ischaemia time of the graft of >30 h or were receiving a graft from a non-heart-beating donor.

Toxicity assessment

The analysis of toxicity was based on reports of treatment-emergent AEs obtained from study sites and coded centrally based on the Medical Dictionary for Regulatory Activities (MedDRA) dictionary and on data from visits performed at baseline, at Week 1, 2, 4, 6 and 8, and at Month 3, 6, 9 and 12. AEs of special interest included failure to achieve primary closure of transplant surgical wound at 2 weeks, infection, hypercholesterolaemia, hypertriglyceridaemia, NODAT (defined as an AE of diabetes or hyperglycaemia after coding using MedDRA in a patient with no pre-transplant diabetes), lymphocele requiring intervention in the first 6 months post-transplant and diarrhoea.

Statistical methods

This exploratory analysis was conducted on the safety population (patients who received at least one dose of study medication) and the intention-to-treat (ITT) population (safety population patients who were transplanted). It employed descriptive methods and statistical tests with a significance level of 5% and no correction for multiplicity. The incidence of AEs, reported for patients until their final visit or premature withdrawal from the study, was evaluated descriptively using the chi-square or Fisher’s exact tests. For selected events, Kaplan–Meier estimates of cumulative incidences and comparisons with the log-rank test were performed.

The relationship between drug levels and selected AEs was assessed by comparing drug levels prior to the event with time-dependent averages of levels (first-degree robust local regression algorithm [18]) from patients without the specific event. The pre-event value was computed as the average exposure over an interval preceding the event which was chosen for each event based on assumptions regarding the pharmacodynamically relevant window. For diarrhoea and diabetes, we chose a window of 1 week (acute effect), for anaemia 4 weeks and for lymphocele the entire post-randomization period (chronic effect). We compared patients with or without the event with a Wilcoxon signed-rank test for the distance of the level prior to the event from the smoothing curve at the time of the event.

We also performed Cox regression analyses with the weekly drug level as a time-dependent explanatory variable (values in weeks without a measurement were obtained by linear interpolation).

Results

Baseline characteristics

As reported elsewhere [17], 1645 patients were enrolled, with 1602 and 1589 patients forming the safety and the ITT population, respectively. For 27 patients who received no treatment-group specific medication, no safety data are presented.

The majority of patients were Caucasian (93%) and male (65%) and the median age was 47 years. Most kidneys were obtained from deceased donors (64%) and 29% and 6% of donors were living related and unrelated, respectively. Treatment groups were well-balanced with respect to background characteristics.

Adverse events and premature withdrawals

The incidence of AEs was similar in the four study arms, but serious AEs (SAEs) were significantly more frequent in the Low-SRL group (Table 1).

The Low-SRL group had by far the highest rate of treatment failure (56%), which was in turn mostly a consequence of acute rejection. The Low-SRL group had clearly the highest number of AE-related withdrawals (P < 0.001; Figure 1).

According to the MedDRA system organ classes, the most common AEs were infections, in particular, urinary tract infections, followed by metabolism/nutrition disorders (including, e.g. hyperlipidaemia and diabetes) and gastrointestinal disorders (Table 1).

Table 2 shows incidence estimates and Figure 2 gives a visual impression of the relative risk of toxicities of special interest when each of the four treatment groups is compared to the rest of the patients.

During the first year, cancer developed in about 1% of the patients. The overall survival rate exceeded 96% with no significant between-group differences [17].

Drug exposure

Throughout the study, the average trough levels of the concentration-controlled immunosuppressants were at the upper limit of or slightly above the protocol-defined target levels. The working hypothesis in the Efficacy Limiting Toxicity Elimination (ELiTE)-Symphony study was that a regimen including daclizumab, mycophenolate mofetil (MMF) and steroids allowed for low doses of CsA, Tac or SRL to be used directly after transplantation with benefits in terms of efficacy and safety. As reported elsewhere [17], at 12 months post-transplant, the low-dose Tac arm had superior renal function, provided the best protection against acute rejection and led to the best graft survival. The two CsA regimens had intermediate outcomes, and patients randomized to low-dose SRL had the worst results.

Here, we focus on safety aspects of general relevance for transplant outcomes, such as infection or hypertension, and those known to be associated with CsA, Tac and SRL. Due to its repercussions on graft function, CNI nephrotoxicity is discussed in the context of the main study results [17]. The main questions include: Do the tested low-dose regimens reduce or eliminate toxicity? Is there a difference in toxicity between standard-dose and low-dose CsA? Are there any associations between drug exposure and AEs?
Drug levels showed a large variability: for patients receiving 6 mg/day of Tac, levels exhibited an intra-patient coefficient of variation (CV) of 28% and an inter-patient CV of 42% in the first 2 months. For patients administered 3 mg/day of SRL, the CVs were 28% and 40%, respectively.

**Treatment-specific AEs and relationship with drug exposure**

**Cyclosporine.** The Stand-CsA group had the highest incidence of infections and significantly more serious infections and opportunistic infections than the other groups. The Stand-CsA group also had the highest CMV infection rate despite a slightly lower proportion of high-risk transplantations (positive donor into negative recipient). Compared to the Low-CsA group, the Stand-CsA group had a higher rate of infections, opportunistic infections and CMV infections, but these differences were not significant. Hypertension, as an AE, was also more common in the Stand-CsA group, although the difference with the other groups was not significant.

**Tacrolimus.** NODAT at 1 year occurred significantly more often with low-dose Tac than with other treatments. However, when considering patients who had documented anti-diabetic medication for at least 3 months or up to study conclusion, the percentages were much smaller and non-significant (Table 2). These effects on glucose tolerance were more common with low-dose Tac despite this group having the lowest average corticosteroid dose. The levels of fasting glucose and glycosylated haemoglobin were comparable among the groups over the study period (maximum between-group difference at any time point were <0.4 mmol/L and 0.5%, respectively). As Figure 3a shows, we could not find any relationship between NODAT and Tac levels or MMF dose prior to onset of the event. No significant relationship with diabetes could be identified in a Cox regression model including Tac levels and MMF dose ($P = 0.41$).

**Diarrhoea** was most common in the Low-Tac group (Table 2). Diarrhoea had only a minimal impact on premature withdrawal. In the week prior to the onset of diarrhoea, patients tended to have slightly higher Tac levels than patients without diarrhoea ($P = 0.057$; Figure 3b).

**Sirolimus.** Besides leading to more SAEs and premature withdrawals, low-dose SRL negatively influenced lipid metabolism (Tables 1 and 2).

At each post-baseline measurement time point, this group had the highest total cholesterol, LDL and triglyceride levels, and a consistently greater proportion of measurements considered significantly abnormal. However, at 3 months, when the difference to the other groups was the largest, no relationship between change in triglycerides level from baseline and SRL concentrations could be identified ($P = 0.46$).
Relative risk of experiencing one of the specific toxicities in each of the treatment groups in comparison to the rest of the patients [the relative risk is expressed by the estimate (square, with a size proportional to the number of affected patients) and 95% confidence interval (segment) of the odds ratios; these are computed at Month 12 based on Kaplan–Meier incidence estimates, except for lymphocele (at Month 6), delayed wound healing and delayed graft function (DGF) (both at Week 2)].
Delayed surgical wound healing at Week 2 was more common in the Low-SRL group than in the other groups (Table 2). Initial SRL levels (Week 1) did not seem to influence the occurrence of this event (P = 0.64 in a logistic regression model).

The Low-SRL group had the lowest frequency of delayed graft function (Table 2), but clearly had the largest proportion of cases with a duration exceeding 2 weeks (the assessment time point): 43% against 28%, 32% and 10% in Stand-CsA, Low-CsA and Low-Tac groups, respectively (P < 0.001 in an overall Fisher’s exact test including the four groups). As a result, at 2 weeks, Low-Tac was the group with the lowest number of patients who had not recovered from delayed graft function and the other three groups had approximately the same number of cases.

Lymphocele was most common in the Low-SRL group (Table 2). However, the average SRL level from transplantation to the date of the AE did not appear to predict the development of lymphocele (P = 0.32). Lymphocele occurred with approximately similar frequency in patients who had below- and above-average exposure to SRL prior to the event (Figure 3c).

Low-SRL was associated with a higher incidence of anaemia (Table 2), as well as lower haemoglobin values. During the entire treatment phase, the Low-SRL group had the lowest median haemoglobin, with a difference compared to the other groups generally slightly >0.5 g/dL. A significant effect of SRL levels or MMF dosages could not be identified using either the distance method (P = 0.52 and P = 0.99, respectively; Figure 3d) or Cox regres-
sion \((P = 0.30\) and \(P = 0.90\), respectively), which confirmed that the Low-SRL group had the highest risk of anaemia (relative risk 2.09 vs Stand-CsA group; \(P < 0.001\)).

**Discussion**

The major finding of our analysis is that, despite low doses, CsA, Tac and SRL retained distinct toxicity profile components. The study design permits the direct comparison of CsA, Tac and SRL at low dosages and randomization stratified by centre eliminated a potential confounder for safety analyses. As the assessment and reporting of AEs are different among different sources, the comparison of the three drugs based on external controls (e.g. other study databases) would be problematic. Because multiple comparisons carry the risk of finding some spurious significances in the interpretation of the results, the data should be interpreted with caution, with a particular eye on the consistency across different analyses and the degree of significance.

For CsA, a comparison of the standard- and low-dose groups allows us to address the main hypothesis of the Symphony study: the superior efficacy of regimens with daclizumab induction providing stronger early immunosuppression to compensate for lower doses of another immunosuppressant. In terms of efficacy (renal function, acute rejection, graft loss), the Low-CsA group was systematically slightly better than Stand-CsA, but results did not reach statistical significance. However, the study was not powered for comparisons between these two groups. From the safety standpoint, we observed a systematically less severe AE profile in the Low-CsA group (e.g. infections, including opportunistic infections and CMV, and hypertension; see Figure 2). Consequently, the low-dose CsA regimen was a fruitful CNI-sparing strategy.

Previous studies have shown relationships between drug levels and specific toxicities. Lower CsA levels have been associated with less infections and less CsA-associated AEs [19,20]; lower Tac levels have been associated with better serum lipid profiles and glucose metabolism [21]; SRL has been associated with dose-dependent thrombocytopenia and leucopenia [22] and increased SRL doses have also been associated with hyperlipidaemia [23]. The exposure to the drugs of interest in these studies was, however, generally higher than those applied in our study and this may account for the fact that we detected only weak level–AE relationships. Assuming that this is true, genetic factors [24–26], comorbidity or interaction with co-medication are among the other possible explanations. One could speculate that additional reduction of drug levels would not reduce toxicity further but efficacy might not be maintained. However, these results can have several technical explanations. Firstly, exposure to the study drugs at low doses is quite variable over time. Secondly, the Symphony study was not designed to assess the correlation between drug levels and AEs: most levels were measured according to a fixed schedule rather than event-driven. Therefore, drug levels were only occasionally assessed at the onset of AEs. Thirdly, lack of statistical power cannot be excluded. However, the same methods identified significant relationships, for example, between SRL levels and the occurrence of acute rejections \((P = 0.015\) for the distance method and \(0.014\) for time-dependent Cox regression, a review of these data goes beyond the scope of this article).

An observation deserving a specific comment is the low incidence of delayed graft function in the Low-SRL group, which is in contrast with the literature [27]. However, in line with other reports [28], the duration of the graft function impairment was clearly extended in this group. In the Low-Tac group, it was reduced, so that, at 2 weeks, Low-SRL and the two CsA groups had approximately the same number of patients still with delayed graft function and a lower number of cases in the Low-Tac group.

The incidence estimate for NODAT and other assessments with a subjective component (those based on AE reports, such as infection, and delayed wound healing) may have been affected by reporting bias. Specifically regarding diabetes, our analysis has the limitations that diabetes was not diagnosed based on the American Diabetes Association (ADA) criteria [29] and that the reported AEs include a broad range of diagnoses. On the other hand, other toxicity measures, in particular, laboratory values, were completely objective. For anaemia and lipid disorders, AE reports and laboratory assessments were in agreement. This was not the case, however, for NODAT; but here, the insufficient sensitivity of the laboratory tests (glycaemia and glycosylated haemoglobin performed at the scheduled visits) and the confounding effect of anti-diabetic treatment make a comparison very challenging. From our data, no clear conclusion can be drawn about the extent of reporting bias for other types of toxicities.

The ELiTE-Symphony study demonstrated that a regimen including MMF 2 g/day, Tac at the designated doses, daclizumab induction and steroids has a superior efficacy over regimens containing CsA and SRL [17]. The results presented here indicate that the overall safety profiles of the studied regimens are comparable, but that certain specificities remain. These are plausibly related to individual factors such as patient disposition, interactions or possibly to transient drug level excursion. We argue that the overall superiority of the low-dose Tac regimen is not obscured by the emerging toxicities and that a further dose reduction could compromise the efficacy of the regimen.

The reported differences in the toxicity profiles may be of relevance for tailoring specific immunosuppressive regimens to patients with particular needs.

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