Rejection after simultaneous pancreas–kidney transplantation

Helmut Arbogast1, Jacques Malaise3, Wolf-Dieter Illner1, Anwar Tarabichi1, Christoph Dieterle2, Rüdiger Landgraf2, Walter Land1 and the Euro-SPK Study Group

1Department of Surgery, 2Department of Medicine, Diabetology Center, University of Munich, Munich, Germany and 3Department of Kidney and Pancreas Transplantation and Organ Procurement, Cliniques Universitaires St Luc, Université Catholique de Louvain, Brussels, Belgium

Abstract

Background. Simultaneous pancreas–kidney (SPK) transplantation is an accepted therapy for type 1 diabetic patients with end-stage renal disease. This study analyses the occurrence of rejection episodes in patients undergoing SPK.

Methods. The study population was obtained from 205 patients enrolled in the Euro-SPK 001 study and randomized to receive tacrolimus- (n = 103) or cyclosporin microemulsion (ME)-based (n = 102) immunosuppressive therapy. All patients received concomitant antibody induction therapy, mycophenolate mofetil and short-term corticosteroids.

Results. After 3 years of follow-up, rejection episodes occurred in 41 patients receiving tacrolimus and in 51 patients receiving cyclosporin-ME. The majority of first rejection episodes in both groups occurred during the first 6 months (93 and 90%, respectively) and in most cases were treated with corticosteroids alone (88 vs 90%). Actuarial rejection-free kidney and/or pancreas graft survival was similar for tacrolimus (54%) and cyclosporin-ME (44%). Human leukocyte antigen (HLA) compatibility (P = 0.003) and graft vessel extension (P = 0.000001) had a significant influence on rejection-free graft survival. Also, rejection influenced pancreas graft survival (P = 0.02) and pancreas graft loss due to rejection influenced patient survival (P = 0.01). In the intent-to-treat analysis of early rejection, significantly fewer tacrolimus- than cyclosporin-ME-treated patients had (i) more than one rejection episode (11 out of 40 vs 24 out of 47; P = 0.03); (ii) first moderate to severe rejection (one out of 40 vs 12 out of 47; P = 0.004); and (iii) refractory rejection (two out of 40 vs 10 out of 47; P = 0.03). Pancreas survival was lower in late rejectors (53%) than non-rejectors (86%; P = 0.002). Also, serum creatinine was highest in late rejectors.

Conclusion. Tacrolimus-based immunosuppressive therapy demonstrates significant advantages over cyclosporin-ME in terms of the severity of acute rejection in SPK transplant patients.

Keywords: cyclosporin microemulsion; kidney–pancreas transplantation; rejection; tacrolimus

Introduction

A pancreatic transplant is often performed at the same time as a kidney transplant [simultaneous pancreas–kidney (SPK) transplant] in uraemic patients with type 1 diabetes.

The present analysis of the Euro-SPK 001 study was undertaken to compare and analyse the occurrence, frequency and the severity of rejection episodes in patients receiving tacrolimus- or cyclosporin-microemulsion (ME)-based immunosuppressive regimens for up to 3 years after SPK transplantation.

Patients and methods

Study design

The overall objectives, methods and design of the Euro-SPK 001 study have been described in full previously [1] and also elsewhere in this supplement (Saudek et al.). A brief synopsis is provided below.

A total of 205 SPK transplant patients with end-stage type 1 diabetic nephropathy were recruited into the study from 10 centres in Europe and one centre in Israel. All patients received quadruple immunosuppressive therapy...
based on either tacrolimus \((n = 103)\) or cyclosporin-ME \((n = 102)\), given with mycophenolate mofetil (MMF), prednisone and rabbit anti-thymocyte globulin (rATG) induction therapy [1]. Corticosteroid therapy was progressively withdrawn from all patients between 3 and 6 months post-transplant. A graft vessel extension (GVE) was used in cases where the donor vein or artery was too short to be anastomosed directly to the recipient’s vessel.

**Rejection episodes**

The primary end-point of the study was the incidence of biopsy-proven acute rejection at months 6 and 12 post-transplant. A biopsy was taken in all cases of suspected kidney or pancreas graft rejection. Local histopathologists undertook biopsy analyses, and rejection was graded according to the Banff 97 criteria [2] for the kidney and the Drachenberg scale [3] for the pancreas. The selection (corticosteroids, monoclonal or polyclonal antibodies) and dosage of anti-rejection therapy were based on routine practice at each individual centre. Responses to the treatment were classified as corticosteroid-sensitive, corticosteroid-resistant, antibody-sensitive, antibody-resistant or refractory rejection.

The intention was to perform a kidney or pancreatic biopsy in all cases prior to initiating anti-rejection therapy. However, in some cases, this was not adhered to. For the purpose of analysis, such cases were considered to be clinical rejections.

**Acute tubular necrosis**

Acute tubular necrosis (ATN) was defined as a non-functional kidney for a maximum of 1 month post-transplant, requiring at least one episode of dialysis during the first post-transplant week and not associated with a rejection episode.

**Delayed kidney graft function**

If the creatinine level did not decrease from 50% within the first 7 post-operative days, it was considered to be a delayed graft function (DGF). This poor function of the transplanted graft required a late dialysis or no dialysis at all.

**Statistical analysis**

\(\chi^2\) and Fisher’s exact tests were used to compare categorical variables, and the Mann–Whitney U-test was used to compare continuous variables. For univariate analyses, survival rates were obtained using the Kaplan–Meier method and differences between groups were compared by using the log-rank test. The Cox regression analysis was used for multivariate analyses. \(P < 0.05\) was considered statistically significant. Mean values were expressed with SDs.

**Results**

**Demographic data**

In total, 205 patients (127 male and 78 female) between 18 and 55 years of age were enrolled in the study; 103 were assigned pre-operatively to receive tacrolimus and 102 to receive cyclosporin-ME. The two treatment groups were comparable at baseline with respect to age, gender and sensitization [panel-reactive antibody (PRA) < 5%]. Significantly more patients assigned to treatment with tacrolimus (91%) than with cyclosporin-ME (81%) were dialysis dependent prior to transplantation \((P < 0.05)\). However, the two groups were comparable with respect to surgical technique, type of exocrine drainage of the pancreas, type of venous drainage and the use of GVE.

**In-study patients: first clinical or biopsy-proven acute rejection episodes**

After 3 years of follow-up, 41 patients in the tacrolimus group and 51 in the cyclosporin-ME group experienced at least one rejection episode while in the study. As shown in Table 1, the first rejection episode occurred during the first 6 months for 38 out of 41 (93%) patients in the tacrolimus group and for 46 out of 51 (90%) patients in the cyclosporin-ME group.

The 41 patients in the tacrolimus group had confirmed first acute rejection episodes in 24 kidney biopsies, in two pancreas biopsies and in one SPK

<table>
<thead>
<tr>
<th>Table 1. Overview of first acute rejection episodes and time of onset</th>
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<tbody>
<tr>
<td><strong>Acute rejection</strong></td>
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<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td><strong>Biopsy-proven</strong></td>
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<tr>
<td>Kidney</td>
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<tr>
<td>Pancreas</td>
</tr>
<tr>
<td>SPK</td>
</tr>
<tr>
<td><strong>Clinically proven</strong></td>
</tr>
<tr>
<td>Kidney</td>
</tr>
<tr>
<td>Pancreas</td>
</tr>
</tbody>
</table>

The table shows the number of acute rejection episodes and time of onset in months that occurred in tacrolimus- and cyclosporin microemulsion-treated in-study and out-of-study patients. SPK = simultaneous pancreas-kidney.
biopsy. The other 14 patients had clinically proven episodes of kidney rejection. Of the 51 patients in the cyclosporin-ME group who had first acute rejection episodes, 34 were biopsy-confirmed kidney rejection, two were biopsy-confirmed pancreas rejection, 13 were clinically confirmed kidney rejection and two were clinically confirmed pancreas rejection (Table 1).

In the majority of patients, the first rejection episodes were treated with corticosteroids (88% in the tacrolimus group and 90% in the cyclosporin-ME group). However, some cases were treated directly with OKT3 (7 vs 8%, respectively) or ATG (5 vs 0%). In addition, there was one case of pancreas graft loss due to untreated rejection in the cyclosporin-ME group.

**In-study patients: all clinical and biopsy-proven acute rejection episodes**

As shown in Table 2, 41 patients experienced 59 rejection episodes in the tacrolimus group; 30 presented with one episode of rejection; six patients had two episodes; three had three episodes; and two had four episodes. In the cyclosporin-ME group, there were 73 episodes of rejection in 51 patients: 34 patients had one episode; 13 had two episodes; three had three episodes; and one had four episodes. There was no statistically significant difference between tacrolimus (11 patients) and cyclosporin-ME (17 patients) in terms of the incidence of recurrent (more than one) rejection. The frequency of clinical and biopsy-proven acute rejection while in the study was 1.44 episodes per patient with tacrolimus and 1.43 episodes per patient with cyclosporin-ME (Table 2).

Rejection episodes were treated mainly with corticosteroids (81% in the tacrolimus group and 75% in the cyclosporin-ME group). Some patients were treated with OKT3 (15 vs 19%, respectively) or ATG (3 vs 0%). One case of antibody treatment was not specified and one pancreas was lost before treatment in the cyclosporin-ME group. Two patients in the cyclosporin-ME group received only temporary insulin during pancreatic rejection.

**In-study patients: first biopsy-proven acute rejection episodes**

Twenty-seven patients receiving tacrolimus and 36 patients receiving cyclosporin-ME had a primary biopsy-proven acute rejection, whereas four patients treated with tacrolimus and three receiving cyclosporin-ME had their first biopsy-proven acute rejection after a clinical rejection.

The severity of the first biopsy-proven episode was borderline or mild for 30 out of 31 patients receiving tacrolimus. This included two episodes occurring at 6–12 months and one episode occurring after 1 year. Only one episode in this group was graded as moderate to severe. In contrast, severity was borderline or mild for 28 out of 39 cases in the cyclosporin-ME group (including one episode occurring at 6–12 months and one occurring at 2–3 years) and moderate or severe for 11 episodes (including one occurring after 1 year).

There were no statistical differences between the two groups regarding the first biopsy-proven acute rejection episode at 6 and 12 months (28 and 30 tacrolimus-treated patients compared with 36 and 37 cyclosporin-ME-treated patients). However, the number of patients with a first moderate or severe acute rejection episode was significantly higher with cyclosporin-ME (11 out of 39 patients) than with tacrolimus (one out of 31 patients; $P=0.009$).

**In-study patients: all biopsy-proven acute rejection episodes**

During the first 3 years post-transplant, 15 out of 38 biopsy-proven acute rejection episodes were classified as borderline in the tacrolimus group compared with 17 out of 56 in the cyclosporin-ME group. In addition, 22 episodes in the tacrolimus group were classified as mild and one as moderate. In the cyclosporin-ME group, 22 episodes were classified as mild, 13 as moderate and four as severe. As with first biopsy-proven acute rejection, the occurrence of all moderate to severe biopsy-proven acute rejection was significantly lower with tacrolimus than with cyclosporin-ME (one out of 38 vs 17 out of 56, respectively; $P=0.001$).

**Graft survival (intent-to-treat)**

At 3 years post-transplant, five patients in each group experienced chronic kidney rejection. An additional patient in the tacrolimus group and two others in the cyclosporin-ME group had chronic pancreas rejection and received insulin.

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**Table 2. Summary of all acute rejection episodes occurring while patients were in the study**

<table>
<thead>
<tr>
<th>Acute rejection</th>
<th>Tacrolimus ($n=41$)</th>
<th>Cyclosporin-ME ($n=51$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsy-proven</td>
<td>35</td>
<td>51</td>
</tr>
<tr>
<td>Clinically proven</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>64</td>
</tr>
<tr>
<td>Pancreatic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsy-proven</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Clinically proven</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>SPK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsy-proven</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Clinically proven</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Biopsy-proven rejections</td>
<td>38</td>
<td>56</td>
</tr>
<tr>
<td>Clinically proven rejections</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>Total rejections</td>
<td>59</td>
<td>73</td>
</tr>
<tr>
<td>Episodes per patient</td>
<td>1.44</td>
<td>1.43</td>
</tr>
</tbody>
</table>

SPK = simultaneous pancreas-kidney.
The actuarial rejection-free kidney and/or pancreas graft survival rate was similar in the two treatment groups, with respective values for tacrolimus at 6 months 1, 2 and 3 years of 61, 57, 54 and 54%. The corresponding values for the cyclosporin-ME group were 50, 47, 45 and 44%.

Univariate and multivariate analyses indicated that human leukocyte antigen (HLA) compatibility (both \( P = 0.003 \)) and the requirement for a venous or arterial GVE for the pancreas (univariate, \( P = 0.000001 \); and multivariate, \( P = 0.0005 \)) had a significant effect on rejection-free kidney and/or pancreas graft survival in the entire study population (Figures 1 and 2). In addition, rejection was found to influence pancreas graft survival \( (P = 0.01) \), and pancreas graft loss due to rejection appeared to influence patient survival \( (P = 0.02) \).

Intent-to-treat analysis at 3 years' follow-up
To analyse the impact of acute rejection on follow-up, the data were divided into three subgroups.

(i) Those patients with no episodes of rejection during the 3-year post-transplant follow-up (tacrolimus, \( n = 58 \); cyclosporin-ME, \( n = 45 \)). This subgroup included three patients in each group who had died, lost both organs or were lost to follow-up before 3 years without having any rejection episodes.

(ii) Those patients with an early (occurring during the first 3 months post-transplant) first rejection episode (tacrolimus, \( n = 40 \); cyclosporin-ME, \( n = 47 \)).

(iii) Those patients experiencing late (occurring >3 months post-transplant) first rejection episodes (tacrolimus, \( n = 5 \); cyclosporin-ME, \( n = 10 \)).

Rejection episodes
Although the difference in total number of first early rejection episodes in each treatment group was not statistically significant, there were significantly fewer moderate or severe rejections in the tacrolimus group than in the cyclosporin-ME group (one out of 40 vs 12 out of 47, respectively; \( P = 0.004 \)) (Table 3). Also, there were significantly fewer refractory rejection episodes associated with tacrolimus than with cyclosporin-ME (two out of 40 vs 10 out of 47; \( P = 0.03 \)). Interestingly, the occurrence of refractory rejection was more frequent among late rejectors (six out of 15, 40%) than among early rejectors (12 out of 87, 14%; \( P = 0.01 \)).

A summary of all rejection episodes by subgroup is shown in Table 4. Among tacrolimus-treated patients with early rejection, 11 out of 40 (28%) experienced more than one episode during the first 3 years post-transplant compared with 24 out of 47 (51%) in the cyclosporin-ME group \( (P = 0.03) \). Nine rejection episodes occurred in four patients >3 months post-transplant in the tacrolimus group compared with 10 episodes in eight patients receiving cyclosporin-ME. No difference regarding rejection after 1 year post-transplant could be observed: four episodes in three patients treated with tacrolimus and three episodes in three patients treated with cyclosporin-ME.

Among patients with early rejection, corticosteroid withdrawal resulted in three rejection episodes in a patient receiving tacrolimus and one rejection episode in the cyclosporin-ME group. In the group of late rejectors, there were two rejection episodes resulting from corticosteroid withdrawal in the tacrolimus group and three in the cyclosporin-ME group. In addition, there were five episodes of rejection in the tacrolimus group and 10 in the cyclosporin-ME group among patients still receiving corticosteroids.

Pre-transplant factors had no influence on the occurrence of rejection episodes after transplantation: no differences were found between the three rejection subgroups regarding requirement for dialysis, duration.
of dialysis, duration of the diabetes, maximum PRA, requirement for transfusion, pregnancy prior to trans-plant or total and DR HLA mismatches. Likewise, no differences were found between the subgroups with respect to the type of venous or exocrine drainage of the pancreas or the pancreatic and kidney ischaemic times.

The only surgery-related factor that had an influence on the occurrence of rejection episodes after transplantation was GVE. The number of patients who had a venous or arterial GVE for the pancreatic vascular anastomosis was significantly lower among non-rejectors (40%) than among early rejectors (75%; \( P \approx 0.000001 \)).

Post-transplant factors did not influence the occurrence of rejection episodes after transplantation. No differences were found between subgroups regarding the number of patients who had ATN or DGF.

Graft survival, patient survival and study withdrawal

As shown in Table 4, when the first rejection episode occurred >3 months post-transplant, it resulted in a significantly higher rate of graft loss (40%) than when it occurred before 3 months (10%; \( P = 0.003 \)).

There appeared to be no differences between the three subgroups regarding patient and kidney survival (Table 5). In contrast, pancreas survival was significantly higher in non-rejectors (86%) than in late rejectors (53%; \( P = 0.002 \)). In addition, significantly fewer non-rejectors were withdrawn from the study during the first 3 years post-transplant (29%) than early (66%; \( P < 0.001 \)) or late rejectors (67%; \( P = 0.007 \)).

Biochemical parameters

At 6 months, fasting glucose was significantly higher among early rejectors (96 ± 18 mg/dl) than among non-rejectors (94 ± 36 mg/dl; \( P = 0.03 \)). However, there were no differences in fasting blood glucose between the subgroups at 3 years. Based on WHO criteria for diabetes classification [4], there were more patients with impaired glucose function (glycaemia >110 mg/dl) at 3 years among the late rejectors (38%) than among the early (7%; \( P < 0.05 \)) and non-rejector (7%; \( P = 0.03 \)) subgroups.
Serum creatinine at 6 months was significantly higher in the early (1.5 ± 0.5 mg/dl; \( P = 0.03 \)) and late rejector subgroups (1.9 ± 1.0 mg/dl; \( P = 0.04 \)) than in non-rejectors (1.3 ± 0.3 mg/dl). At 3 years post-transplant, serum creatinine remained significantly higher in the late rejectors (2.3 ± 0.7 mg/dl) than in non-rejectors (1.4 ± 0.3 mg/dl; \( P < 0.001 \)) and also in comparison with early rejectors (1.6 ± 0.9 mg/dl; \( P = 0.0002 \)). The number of patients with elevations in serum creatinine of >0.25 mg/dl between 6 months and 3 years post-transplant was higher among late rejectors (75%) than among early rejectors (19%, \( P = 0.0003 \)) and non-rejectors (29%, \( P = 0.003 \)).

### Discussion

Long-term allograft function in SPK transplantation has been shown to be dependent on the occurrence of acute rejection episodes [5–10]. The severity and the number of such episodes significantly impact the half-life of the kidney transplant. Several publications have confirmed the superiority of immunosuppressive regimens containing MMF in reducing the number of acute rejection episodes in patients undergoing SPK transplantation [11–13]. Some authors report a decrease in kidney dysfunction and reduced severity of rejection episodes when tacrolimus is used as a primary immunosuppressant in comparison with cyclosporin [7,14–16]. However, most of these studies are too small to provide conclusive evidence.

The data presented here, obtained from a relatively large cohort of 205 patients recruited in the Euro-SPK 001 study, confirm the findings of these smaller studies. After 3 years of follow-up, our analysis shows that clinical and biopsy-proven rejection tends to occur more frequently among patients receiving immunosuppression with cyclosporin-ME than in those treated with tacrolimus. However, the difference did not reach significance at a 95% confidence level. Likewise, the number of patients with recurrent rejection episodes was similar in the two treatment groups.

A high proportion of biopsy-proven rejection episodes were classified as being of borderline to mild severity, and the majority of all rejection episodes were successfully treated by corticosteroid therapy alone and did not require antibody treatment. These findings are in accordance with the literature, which shows that only a minority of rejection episodes in SPK transplantation require treatment with antisera [8,17].

Analysis of first biopsy-proven rejection episodes indicated a similar incidence in both treatment groups. However, in accordance with previous findings, there was a significantly lower incidence of first moderate to severe rejection episodes with tacrolimus than with cyclosporin-ME [14,16,18]. These findings were confirmed by the data on all biopsy-proven moderate to severe rejection episodes.

The onset of the first rejection episode is known to be an important prognostic factor. In our study, a poor outcome was associated with late rejection episodes, mainly affecting pancreatic graft survival. This decreased from 86% in immunologically sound patients to 53% in patients with late rejection episodes, although there was no change in kidney and patient survival. This not only underlines the necessity for a sufficient immunosuppressive induction treatment, but also the importance of frequent follow-up and sufficient maintenance treatment [19].

There appeared to be a higher incidence of acute rejection episodes within our population than in previously reported US studies [14,15,18,20], even though the proportion of patients of African origin in our study was virtually non-existent. However, relatively poor HLA matching in the European trial could have an influence on this phenomenon.

Additionally, pancreatic biopsies are not as commonly performed in Europe as in some US centres, so a significant number of rejection episodes in our study were based on clinical diagnosis alone. However, the most likely reason for the higher rejection rates in the Euro-SPK 001 study is the inclusion of all borderline-classified rejection episodes into the count. This explanation is supported further by the significantly higher percentage of corticosteroid-resistant rejection episodes reported by most US studies. In these centres, the rejection rate is typically between 7 and 25%, and 82–100% of rejections are classified as corticosteroid resistant [9,14,18,21].

Surprisingly, in our study, the higher diabetogenic potency of tacrolimus, which has been described in the literature [22,23], did not result in significant differences...
between treatment groups in terms of fasting blood glucose levels. Impaired glucose function, however, was more probable in the case of late rejection episodes. Late rejection also had a significant negative influence on kidney function. However, the relatively short 3-year follow-up period may be insufficient in terms of demonstrating a significant effect of late rejection on kidney survival [24,25].

Besides the predicted influence of HLA incompatibility on rejection, one of the striking results of this multicentre study was the high probability of a rejection episode in cases where GVE was performed. Since none of the centres propagated venous extensions, most patients in the GVE group received an arterial Y-graft. This resulted in a >50% relative risk increase of rejection, which was highly significant. Whether this phenomenon is due to a longer ischaemic time during back table preparations in the case of GVE or is due to other factors cannot be answered by the underlying data. However, based on our findings, we recommend omitting GVE in both vascular anastomoses of the pancreas, whenever possible.

In pancreas transplantation, one of the main concerns remains the optimization of immunosuppressive treatment, since rejection is still one of the parameters significantly influencing pancreas graft survival, and pancreas loss through rejection has a significant influence on patient survival. The findings from this study suggest that tacrolimus-based immunosuppressive therapy shows advantages over cyclosporin-ME in terms of preventing severe acute rejection in SPK recipients.

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Conflict of interest statement. None declared.

References