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Inferior results with basic immunosuppression with sirolimus in kidney transplantation

J.M. van den Akker^{1*}, R.J. Hené², A.J. Hoitsma¹

¹Department of Internal Medicine, Division of Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, ²Department of Internal Medicine, Division of Nephrology, University Hospital Utrecht, Utrecht, the Netherlands, *corresponding author: tel.: +31 (0)24-361 43 64, fax: +31 (0)24-354 00 22, e-mail: a.hoitsma@NIER.umcn.nl

ABSTRACT

Background: The introduction of sirolimus has provided the opportunity to develop an immunosuppressive regimen without the nephrotoxic calcineurin inhibitors.

Methods: We conducted a first trial in 30 renal allograft recipients. Ten patients were followed prospectively and received sirolimus, to achieve a target blood level of 10 to 15 ng/ml, induction therapy with one dose of daclizumab, low-dose steroids and mycophenolate mofetil. We compared this group with a historical control group of 20 patients who received our standard treatment consisting of tacrolimus, low-dose steroids, and mycophenolate mofetil.

Results: After a mean follow-up of 15 weeks, seven patients developed an acute rejection in the sirolimus group (70%) compared with three patients in the tacrolimus group (15%) ($p < 0.01$).

Because of this unacceptable high rate of acute rejections we conducted a second prospective pilot study in nine patients. These patients received sirolimus in combination with two doses of daclizumab, high-dose steroids and mycophenolate mofetil. No rejections occurred under this immunosuppressive regimen; however, many immunosuppression-related adverse events were seen.

Conclusion: The present study demonstrates an unacceptably high rate of acute rejections (70%) in patients treated with sirolimus, daclizumab, mycophenolate mofetil and low-dose prednisolone. No rejections but many adverse events were seen when sirolimus was given in combination with high-dose steroids.

KEYWORDS

Calcineurin inhibitor, kidney transplantation, rejection, sirolimus, adverse events

INTRODUCTION

Immunosuppressive regimens including calcineurin inhibitors have greatly improved the results of kidney transplantations. Tacrolimus in combination with mycophenolate mofetil (MMF) and prednisolone decreased the number of acute rejection episodes within the first three months after transplantation to 15 to 20%. The incidence of graft failure from intractable acute rejections within one year after transplantation has dropped under the current regimen to below 5%. Therefore, tacrolimus combined with MMF and prednisolone is the standard regime in the first four months after transplantation in our centres. However, calcineurin inhibitors are nephrotoxic, which may eventually lead to loss of graft function. Long-term results are therefore disappointing. The introduction of sirolimus has provided the opportunity to develop an immunosuppressive regimen without nephrotoxic calcineurin inhibitors.¹

Obviously, removing calcineurin inhibitors from the immunosuppressive regime should not lead to a higher percentage of rejections. On the other hand, the additional amount of immunosuppression needed beside sirolimus to prevent acute rejection should not lead to an unacceptable amount of immunosuppression-related adverse events. Recently, Flechner *et al.*² demonstrated in kidney transplant recipients that treatment with sirolimus, prednisolone, MMF, and additional IL-2 receptor blocker (basiliximab) was accompanied with an acute rejection percentage of 6.4%. However, the additional immunosuppression given, high-doses of steroids and two induction therapies, is much more than we are used to giving in combination with tacrolimus.

The main purpose of our study was to investigate whether the nephrotoxicity that occurs under the current standard immunosuppressive regimen with tacrolimus, low-dose

steroids and MMF can be decreased by a regimen with sirolimus, daclizumab, low-dose steroids and MMF without an increased incidence of acute rejections.

MATERIALS AND METHODS

Patients

We included primary and secondary adult (aged above 18 years) renal allograft recipients in Nijmegen and Utrecht. Exclusion criteria consisted of HLA-identical living donor kidney; haemolytic uraemic syndrome as original renal disease; pregnancy or lactation; total white blood cell count $<3 \times 10^9/l$ or platelet count $<100 \times 10^9/l$ or haemoglobin level <5 mmol/l; current panel reactive antibodies (PRA) (last screening sample) $>85\%$; the use of non-registered medication during the last four weeks preceding transplantation and during the study, a renal allograft transplant as part of a multiorgan transplantation; or treatment with CYP3A4 inhibitors or inductors. All recipients had a negative visual complement dependent cytotoxicity crossmatch. Flow cytometry T-cell crossmatching did not take place.

The patients who gave their informed consent were prospectively followed and treated with a calcineurin inhibitor free immunosuppressive protocol including sirolimus, daclizumab, MMF and low-dose steroids. This group was compared with a historical control group consisting of patients who met the same inclusion and exclusion criteria and had been treated directly before the start of the study with our standard immunosuppressive regimen including the calcineurin inhibitor tacrolimus, MMF and low-dose steroids.

The study was approved by both ethical committees of the participating centres and performed in accordance with the standards of the Declaration of Helsinki.

Immunosuppressive protocol and methods

First study

The patients in the calcineurin inhibitor free intervention group were treated with sirolimus at a loading dose of 15 mg prior to transplant surgery. As soon as a patient was capable of taking oral medication a second loading dose of 12 mg was given, followed by a daily dose of 6 mg, to achieve a target blood level of 10 to 15 ng/ml. The target trough level remained steady throughout the study.

The patients in the sirolimus treatment arm also received daclizumab during the transplant surgery intravenously at a dose of 1 mg/kg. At weekly intervals during the first ten weeks following transplantation, the coverage of IL-2 receptors was measured by flow cytometry.³ If free IL-2 receptors were detected on the lymphocytes (reappearance of CD3^{pos}CD25^{pos} lymphocytes) in the first four weeks, an extra dose of daclizumab at 1 mg/kg was given.

The steroid regimen in the sirolimus treatment arm consisted of 100 mg prednisolone intravenously on day 0 (day of transplantation); on day 1 to 5 prednisolone 4 times 25 mg orally/iv. From day 6 till week 17 the steroids were slowly reduced from the starting dose (determined by weight: >70 kg: 25 mg; 50 to 70 kg: 20 mg; <50 kg: 15 mg) to zero.

Patients in the historical control group were treated with tacrolimus at a dose of 0.2 mg/kg/day orally, divided over the morning and evening doses, to be started on day 1 or 2 after transplantation. The target blood level in the first 14 days was between 15 and 20 ng/ml, from week 3 to 7 between 10 and 15 ng/ml and starting from week 7 the trough level should be 6 to 10 ng/ml.

The steroid regimen in the tacrolimus treatment group consisted of 100 mg prednisolone intravenously on day 0 (day of transplantation); on day 1 and 2 prednisolone 25 mg four times orally/iv. From day 3 till week 17 the steroids were slowly reduced from the starting dose (determined by weight: >70 kg: 25 mg; 50 to 70 kg: 20 mg; <50 kg: 15 mg) to 0.1 mg/kg.

All patients were given MMF 750 mg twice daily from day 1 or 2 onwards. For patients with a body weight of ≥ 90 kg, the dose was 1000 mg twice daily. In case of leucopenia or abdominal complaints, the dose was lowered (the minimal dose is 250 mg twice daily).

All patients in whom a rejection was suspected underwent renal transplant biopsy, which were scored according the BANFF97 criteria.⁴ The primary study endpoints were the difference in renal function and the number of acute rejections between both treatment groups.

Second study

Because of the unacceptably high rate of acute rejections in the above-described patients treated with sirolimus (see results) we conducted a second prospective pilot study in nine patients. They received sirolimus and MMF following the same protocol as described above. Besides the daclizumab given during the transplant surgery, they received an additional dose of daclizumab 1 mg/kg ten days after transplantation. The steroid regimen consisted of 500 mg methylprednisolone intravenously on day 0 (day of transplantation) to 2, and then oral prednisolone from 120 mg to 30 mg by day 8, 27.5 mg by day 21, 25 mg by day 30, tapered by 2.5 mg each month to a maintenance of 7.5 mg daily.

Results

First study

Ten patients included in the sirolimus group were compared with 20 patients who were treated with tacrolimus. Patient characteristics are summarised in *table 1*. Apart from more older donors and an unfavourable donor type profile in the sirolimus group, no significant differences were found. After a mean follow-up of 15 weeks, seven patients

in the sirolimus group had developed an acute rejection (70%; 95% confidence interval 42 to 98%). This was significantly more than the 15% rejection rate in the control group ($p < 0.01$; Fisher's exact test). Characteristics of the rejection episodes that occurred in the sirolimus group are mentioned in *table 2*. In four patients the renal allograft function recovered after three pulses of solumedrol alone. Two patients required a second course of solumedrol and one patient required antithymocyte globulin (ATG) after the solumedrol treatment before renal function improved. All patients were converted to tacrolimus and returned to a stable allograft function, with a mean serum creatinine of 159 $\mu\text{mol/l}$ at one year after transplantation. Four rejection episodes occurred within two weeks after transplantation. One of them was not biopsy proven

because of the absence of renal tissue in the biopsy. In one of these patients there appeared to be no IL-2 receptor blockade because the patient did not receive any daclizumab by mistake. In all the patients who received daclizumab, the IL-2 receptor was fully blocked at two and three months after transplantation after one dose of daclizumab.

Three rejections occurred between 8 and 15 weeks after transplantation. In all these cases the trough sirolimus level appeared to be below the target range at the time of rejection. The mean sirolimus trough levels were within the target range in the different time periods (*table 3*), but 21% of the measurements were below target. This was comparable with 19% of the measurements below target in the tacrolimus treatment group.

Table 1. Demographics of the first study

| | Sirolimus (n=10) | Tacrolimus (n=20) | P |
|--|------------------|-------------------|------------|
| Recipients | | | |
| Gender (M:F) | 7:3 | 10:10 | NS |
| Age (years) (mean \pm sd) | 54 \pm 14 | 46 \pm 13 | NS |
| Age >65 years | 3 | 1 | NS |
| Donors | | | |
| Gender (M:F) | 2:8 | 8:12 | NS |
| Age (years) mean \pm sd) | 52 \pm 15 | 47 \pm 12 | NS |
| Age >65 years | 3 | 0 | 0.03 |
| Secondary transplant | 1 | 1 | NS |
| PRA = 0% | 10 | 18 | NS |
| >0 and <50% | 0 | 2 | |
| HLA mismatches (mean \pm sd) | 2.8 \pm 0.9 | 2.5 \pm 1.5 | NS |
| Donor type | | | |
| Low risk (HB+LR) | 3 | 14 | NS |
| High-risk (NHB+LUR) | 7 | 6 | $P < 0.05$ |

M = male; F = female; PRA = panel reactive antibodies; HLA = human leucocyte antigen; NHB = non-heart beating; HB = heart beating; LR = living related; LUR = living unrelated; NS = not significant.

Table 2. Sirolimus-treated patients with acute rejections in the first study (n=7)

| Rejections | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|----------------|--------|----------------|--------|--------|-----------------|--------|
| Week after KTx | 2 | 1 | 14 | 15 | 8 | 2 | 1 |
| Donor type | NHB | HB | LUR | LUR | LUR | LR | LR |
| HLA mismatches (A-B-Dr) | 1-0-0 | 0-2-1 | 1-1-1 | 1-1-2 | 0-1-2 | 1-1-1 | 1-1-1 |
| Sirolimus level at rejection (ng/ml) | 12 | 11 | 7.3 | 6.8 | 7.3 | 23 | 12 |
| IL-2R blockade at 3 months | No | Yes | Yes | Yes | Yes | Yes | Yes |
| Doses MMF at rejection (g/day) | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 | 2000 |
| Steroid dose at rejection (mg/day) | 20 | 25 | 2.5 | 2.5 | 7.5 | 22.5 | 25 |
| Banff score: | | | | | | | |
| First biopsy | IIa + ATN | Ib | Ib | IIa | Ib | No renal tissue | IIa |
| Second biopsy | IIa | | Ia | | | | |
| Therapy | 3g Sol (twice) | 3g Sol | 3g Sol (twice) | 3g Sol | 3g Sol | 3g Sol ATG | 3g Sol |
| Creatinine one year after transplantation | 230 | 168 | 130 | 160 | 147 | 126 | 150 |

KTx = kidney transplantation; NHB = non-heart beating; HB = heart beating; LUR = living unrelated; LR = living related; HLA = human leucocyte antigen; IL-2R blockade = interleukin 2 receptor blockade; MMF = mycophenolate mofetil; ATN = acute tubular necrosis; Sol = solumedrol; ATG = antithymocyte globulin.

Table 3. Sirolimus and tacrolimus trough levels in the first and second study

| | 0-14 days | 2-7 weeks | 7 weeks-3 months |
|----------------------------------|------------|------------|------------------|
| Tacrolimus trough level (ng/ml): | | | |
| Target | 15-20 | 10-15 | 5-10 |
| Actually reached (mean ± SEM) | 16.4 ± 0.9 | 12.3 ± 0.4 | 9.3 ± 0.3 |
| Sirolimus trough level (ng/ml): | | | |
| Target | 10-15 | 10-15 | 10-15 |
| Actually reached (mean ± SEM): | | | |
| - First study | 13.4 ± 1.1 | 14.9 ± 1.0 | 11.8 ± 1.0 |
| - Second study | 10.8 ± 0.8 | 15.6 ± 0.9 | 13.1 ± 0.9 |

SEM = standard error of the mean.

One sirolimus-treated patient had a serious wound-healing problem.

Two of the three rejections in the tacrolimus group occurred within one week after transplantation. The third rejection occurred after 11 weeks. All of the patients required ATG after the course of solumedrol. One of them died as a consequence of this therapy.

Second study

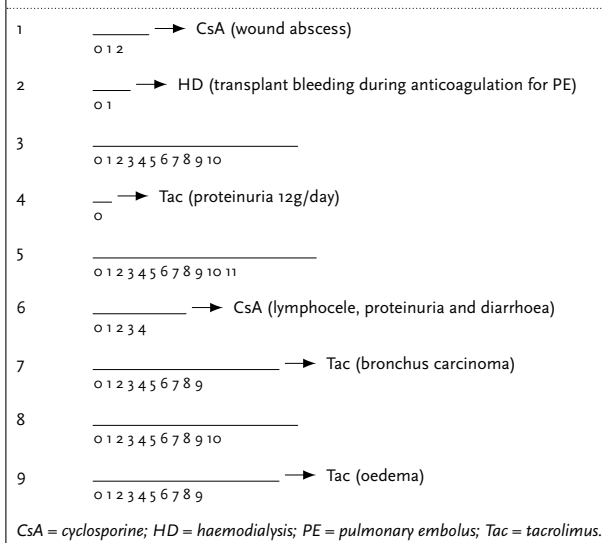
No acute rejections occurred in the second sirolimus treatment group (n=9) with high-dose additional immunosuppression after a mean follow-up of ten months. On the contrary, many serious adverse events were seen in this group, as summarised in table 4. Six patients (67%) suffered delayed wound healing, with a secondary wound infection in three of them. Operative abscess drainage was necessary in one of them. Four patients (44%) developed a lymphocele requiring drainage. In one patient a secondary infection developed in the lymphocele. One patient developed a pulmonary embolus and thereafter during anticoagulation therapy a bleeding in the transplant. After insertion of a vena cava filter, a vena cava inferior syndrome occurred and because of continuous

bleeding in the kidney transplant a transplantectomy was performed and haemodialysis was restarted. Three patients (33%) developed proteinuria after transplantation. One of them is the above-described patient with pulmonary embolus. Another patient developed proteinuria of 12 g/day one week after transplantation. A kidney biopsy showed tubulointerstitial damage without glomerular damage. The proteinuria disappeared within one month after switching to tacrolimus. The third patient with proteinuria developed proteinuria till 1.5 g/day, which also disappeared after switching to cyclosporine. Three patients developed diarrhoea (33%), two of them requiring hospitalisation. Three patients could be maintained on the sirolimus regimen during the mean follow-up period of ten months. The other six patients were switched to another immunosuppressive regimen because of severe complications. The time till the switch of immunosuppression and the main reason for switching is shown in figure 1. Two patients were switched to cyclosporine (after two and four months), three patients were switched to tacrolimus (one after one week and two after nine months), and one patient restarted haemodialysis after nephrectomy (seven weeks after transplantation).

Table 4. Adverse events in sirolimus-treated patients (second study)

| Patient no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|--|---|---|---|---|---|---|---|---|---|
| Acute rejection | - | - | - | - | - | - | - | - | - |
| Graft loss | - | + | - | - | - | - | - | - | - |
| Surgical complications | | | | | | | | | |
| - Delayed wound healing | + | + | + | - | + | + | + | - | - |
| - Haematoma | + | - | - | - | - | - | - | - | - |
| - Wound abscess/infection | + | + | - | - | + | - | - | - | - |
| - Lymphocele | - | - | + | - | + | + | + | - | - |
| Hypercholesterolaemia (>6 mmol/l) | - | - | - | - | + | + | + | + | - |
| Hyperglycaemia (fasting glucose >7 mmol/l) | + | + | - | - | - | + | - | - | - |
| Pulmonary embolus | - | + | - | - | - | - | - | - | - |
| Proteinuria (>1g/day) | - | + | - | + | - | + | - | - | - |
| Candidiasis (oral) | + | - | - | - | - | - | - | - | - |
| Diarrhoea | - | - | + | - | + | + | - | - | - |

Figure 1. Time frame (in months) for sirolimus-treated patients: reason for switch of immunosuppression (second study)



DISCUSSION

The use of calcineurin inhibitors has resulted in improved graft survival following kidney transplantation. However, this is associated with acute and chronic nephrotoxicity and may be an important contributor to the development of chronic transplant nephropathy and chronic graft loss.⁵ Calcineurin inhibitor nephrotoxicity is becoming increasingly prevalent, and is virtually universal by ten years after transplantation and progressive despite mild to moderate reductions in calcineurin doses.⁶ The introduction of sirolimus has provided the opportunity to develop an immunosuppressive regimen without nephrotoxic calcineurin inhibitors. Recently, Flechner *et al.*² demonstrated in kidney transplant recipients that treatment with sirolimus, prednisolone, MMF and additional IL-2 receptor blocker (basiliximab) was accompanied with an acute rejection percentage of 6.4 vs 16.6% in the control arm (cyclosporine, prednisolone, MMF and IL-2 receptor blocker). At 12 months their sirolimus-treated patients enjoyed significantly better creatinine clearances than their cyclosporine-treated patients (81.1 and 61.1 ml/min, respectively). However, the additional amount of immunosuppression given beside sirolimus is very high. In our first study we achieved a rejection percentage of 70% in the sirolimus group compared with a 15% rejection rate in the tacrolimus group ($p < 0.01$) within a mean follow-up of 15 weeks. Because of this unacceptably high rejection rate we ended the study prematurely and switched the patients to the standard immunosuppressive regimen including tacrolimus. To date, none of the patients have lost their grafts in the mean follow-up of 18 months. This

high percentage of rejections cannot be explained by the fact that only patients with a high rejection risk were included in the sirolimus group. All rejections occurred in patients who underwent a first kidney transplantation with a PRA of 0% and there were no significant differences in the number of HLA mismatches and number of non-heart beating donors between the groups. However, when we divided the donors into a low-risk group (heart beating and living related donors) and a high-risk group (non-heart beating and living unrelated donors) significantly more patients with an unfavourable donor type were found in the sirolimus-treated patients. Although this can be partly responsible for the bad outcome in the sirolimus group we do not think this can totally explain the very high rejection rate of 70%.

Four of the seven rejections in the sirolimus group occurred within two weeks after transplantation. One of these rejection episodes occurred in a patient who did not receive any daclizumab by mistake. In all other patients the IL-2 receptor was fully blocked at two and three months after transplantation by one infusion of daclizumab during transplant surgery. Three of the seven rejections occurred between 8 and 15 weeks after transplantation. These three rejections occurred when the prednisolone was reduced to below 10 mg/day, in accordance with the protocol. All patients used at least 1500 mg MMF during the study period. At the time of rejection the sirolimus levels appeared to be lower than the target level in all three of them. The sirolimus levels were below target in 21% of all measured levels in the sirolimus group, but were never measured below 6.8 ng/ml. In the tacrolimus group 19% of all measured levels were below the target level. Some fluctuation in (sirolimus) trough levels is inevitable, but we must conclude that this seems immediately catastrophic in our low immunosuppressive regimen of the sirolimus group. There have been reports of calcineurin inhibitor free therapy, even without using antibody induction, that describe lower rates of acute rejection than we found. Kreis *et al.*⁷ using sirolimus, MMF and steroids reported an acute rejection rate of 27.5% one year after transplantation and Groth *et al.*⁸ using sirolimus, azathioprine and steroids reported an acute rejection rate of 41% at one year. In comparison with our protocol the target trough sirolimus level amounted to 30 ng/ml for the first two months in both studies and they started with 500 mg of methylprednisolone tapered to a maintenance dose of 10 mg daily. In the Symphony trial standard immunosuppression with normal dose cyclosporine (target trough level 150 to 300 ng/ml) was compared with three regimens with low doses of either cyclosporine, tacrolimus or sirolimus in combination with MMF, daclizumab and corticosteroids in 1645 *de-novo* renal transplant patients. The rate of biopsy-proven acute rejections with low-dose sirolimus (target trough level 4

to 8 ng/ml) at one year (35%) was higher than in the other groups (15 to 25%). The conclusion of this study was that the room for increasing sirolimus immunosuppression should be evaluated against the specific sirolimus toxicity profile.^{9,10} Contrary to our study, Flechner *et al.* started with 500 mg methylprednisolone intravenously on day 0 to 2, and then oral prednisolone from 120 mg to 30 mg by day 8, and thereafter slowly tapered to a maintenance dose of 7.5 mg daily at eight months. Their mean trough sirolimus levels appeared to be 13.2 ± 7.9 ng/ml at one month after transplantation and 11.2 ± 5.8 ng/ml at three months after transplantation. They also gave a higher dose of MMF of 1 g twice daily instead of the 750 mg twice daily in our study and they used two gifts of basiliximab. These differences might explain the high rejection rate we found.

To prove this supposition we conducted a second prospective trial in nine patients. This protocol differed from the first by an additional dosage of daclizumab 1 mg/kg at ten days after transplantation and higher doses of MMF and steroids according to the Flechner protocol. No acute rejections occurred under this treatment regimen. On the contrary many serious adverse events were seen, likely to be related to the combination of sirolimus and high-dose steroids. These findings are in accordance with Dean *et al.*⁹ using sirolimus, six gifts of antithymocyte globulin induction, MMF, and prednisone. They achieved an acute rejection rate of 9% at one year, but a wound complication rate of 35% in comparison with 10% in the tacrolimus control group. These adverse events and the interventions needed to treat them might also lead to a decline in renal function. This takes away the advantage of sirolimus, no nephrotoxicity, in the first place. However, the number of treated patients in our study is too small to compare renal function under the different regimens. In the Symphony trial where renal function was determined at 12 months they showed that low-dose tacrolimus was significantly superior to low-dose sirolimus with respect to glomerular filtration rate.¹¹ The results from our study showed that in order to replace a calcineurin inhibitor by sirolimus aiming to avoid calcineurin nephrotoxicity, higher additional immunosuppression is needed to prevent an unacceptable rejection rate. Because of the immunosuppression-related adverse events we experienced under such a regimen we do not think there should be a place for a sirolimus-based regimen without calcineurin inhibitor in the direct post-transplant period.

CONCLUSION

The present study demonstrates an unacceptably high rate of acute rejections (70%) in patients treated with sirolimus, daclizumab, MMF and low-dose prednisolone in the first months after transplantation and no rejections but many adverse events when sirolimus was combined with two times induction therapy and high-dose prednisolone.

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