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A Pilot Study of Calcineurin Inhibitors (CNIs) and Steroid Avoidance Immunosuppressive Protocol among Living Donor Kidney Transplant Recipients

Henry K. Oh, Philip Ding, and Nancy A. Satmary

Transplant Surgery, St. John Hospital and Medical Center, Detroit, Michigan, USA.

Calcineurin Inhibitors (CNIs) and Corticosteroids have been the main immunosuppressive agents in solid organ transplantation. Many studies have confirmed the positive impacts of withdrawal/avoidance of these agents, separately, on their side effect profiles. A pilot study was performed avoiding both agents among low-immunological-risk living donor kidney transplant recipients at a single center. Seventeen recipients were maintained on the double avoidance protocol during the study period beginning July 2002 through December 2003. Three rejection episodes occurred (out of ten) among related donor kidney recipients and six episodes (out of seven) among unrelated donor kidney recipients. Although most of the rejections were reversed with a short course of corticosteroids, the protocol was revised to exclude the unrelated donor kidney recipients. There were higher incidences of wound complications among recipients who received the initial loading dose of Sirolimus. Double avoidance of CNIs and corticosteroids is possible in living donor kidney transplant recipients with an acceptable incidence of rejection. Proper management of the side effects of Sirolimus could further minimize the incidence of rejection. A multi-center randomized study is recommended in order to recognize the benefits of avoiding CNIs and corticosteroids in renal transplant recipients.

Key Words: Calcineurin, immunosuppression, rejection, antiproliferative agents, wound dehiscence

INTRODUCTION

Calcineurin inhibitors (CNIs) and steroids have been the main drugs used for immunosuppression in solid organ transplantation for the past two decades since the first report by Dr. Starzle.[1] However, the use of these agents is associated with several major side effects. CNIs are associated with acute and chronic nephrotoxicity, which may lead to graft loss.[2-4] Steroids are associated with many unwanted side effects,[5-8] some of which may increase cardiovascular mortality of the recipients.[9] TOR inhibitor, Sirolimus and Anti-Purine Metabolite, Mycophenolic Acid Mofetil (MMF), both anti-proliferative agents, have shown to be very potent immunosuppressive agents without any nephrotoxicity. Herein, we report the use of unique immunosuppressive protocol that utilizes simultaneous avoidance of CNIs and corticosteroids among low immunological risk recipients as a pilot study at a single center.

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Reprint address: requests to Dr. Henry K. Oh, St. Transplant Surgery, St. John Hospital and Medical Center, 22101 Moross Rd., PB2, Suite 480, Detroit, MI 48236, Detroit, Michigan, USA. Tel: 313-343-3048, Fax: 313-343-7349, E-mail: henry.oh@stjohn.org

MATERIALS AND METHODS

Study Design

A pilot study trial was performed at St. John Hospital and Medical Center. Adult recipients of living related transplant (LRT) and living unrelated donor kidney transplant (LURT) were eligible for the study. Excluded were the recipients with panel reactive antibody over 50%, flow cytometry T or B cell positivity, BMI over 30, recipients who are on corticosteroids or other immunosuppressive medications at the time of transplantation, having a positive HCV Ab, and having lost a previous allograft from polyoma virus infection.

Endpoints

The primary endpoint was the incidence of a

first biopsy-proven acute rejection within the study periods. Other endpoints included patient and graft survival, wound complications, mouth ulcers, joint pain, and renal function measured by serum creatinine at three months and six months after transplantation. The effects of corticosteroid avoidance were studied by following the patient's weight, blood pressure, and the number of anti-hypertensive drugs used, at three months and six months, respectively after transplantation.

Immunosuppression

Basiliximab (20 mg.) was administered intravenously on the day of transplant and again on day four after transplantation for induction. Solumedrol (Methylprednisolone) was given intravenously at 500 mg. on day zero, 500 mg. on day one, and 250 mg. on day two. No oral prednisone was used. Mycophenolate Mofetil (MMF) was given at 1 gm. po B.I.D. starting the day of surgery. Sirolimus (initially loading dosage of 10 mg.) was started on the day of surgery and maintained at 4 mg per day. Trough levels were maintained at 10 - 15 ng. Rejection episodes were primarily treated with Methylprednisolone 250 mg. intravenously for three consecutive days and a short course of oral prednisone taper. In cases of a steroid-resistant rejection, depleting antibody therapy was given (either rabbit polyclonal antithymocyte globulin or a mouse anti-CD3 monoclonal antibody). In case of interruption of Sirolimus, Tacrolimus was administered achieving trough levels between 8 - 15 ng.

Assessments

If the initial transplant operation was complicated by technical difficulties or if the patient required re-exploration of the transplant wound within 48 hours of the transplant, Sirolimus was discontinued because of the fear of wound complications. A biopsy was performed in cases of deteriorating graft function without an obvious pre or post renal cause. No protocol biopsies were performed.

Statistical analyses

Results are given as means ± SD unless stated otherwise. Statistical analyses were performed on an intention to treat basis. In the primary analysis p-values and 95% confidence intervals were calculated using exact methods for the difference of proportions, and t-test for the difference of continuous variables. For all test a p-value less than 0.05 (two-sided) was considered significant.

RESULTS

Between July 2002 and January 2004, 38 patients were transplanted with living donor kidneys at our center. All kidneys were procured by a single general endoscopic surgeon using pure laparoscopic technique and transplanted by a single transplant surgeon. A total of 20 patients were included into the double avoidance protocol. However, three recipients were excluded because Sirolimus was discontinued after the loading dose to avoid wound complications. Thus, a total of 17 recipients were maintained on the protocol. Average age of the recipients was 49 years ranging from 28 to 69 years of age. There were eleven males and six females. There were 10 living related recipients (4 two-haplotype, 5 one-haplotype, and 1 zero-haplotype) and seven living unrelated recipients. Average age of the donor was 41 years ranging from 19 to 55 years of age. Mean initial hospitalization days were 4.5 ± 2.09 standard deviation (2 - 10 days). All rejection episodes were documented by biopsy. There were three rejection episodes in three patients among LRTs; Two borderline rejections by Banff criteria at 16 days and 5 months after transplant respectively and one Banff 1a rejection at day 86. One episode of borderline rejection was diagnosed in a patient in whom Sirolimus was discontinued due to joint pain and one 1a rejection was seen in a patient whose Sirolimus was temporarily replaced with Tacrolimus because of mouth ulcer. However, there was a higher incidence of rejections among LURTs. A total of six episodes of rejections were seen among five recipients. There was one borderline rejection in 47 days, then 1a rejection at seven months after transplantation in one patient. One patient suffered a Banff 1a rejection at day 57 when the MMF dose was lowered for the mouth ulcer. Two patients

developed Banff 1b rejection at days 30 and three months, respectively. One patient developed a Banff 2b rejection at day six without any evidence of prior sensitization or donor specific antibody formation, evidenced by negative T and B cell cross-match, zero panel reactive antibody levels prior to transplantation and a negative C4d staining of the biopsy specimen. The patient did not respond to OKT3 treatments and subsequently lost the graft.

Wound complications

Wound dehiscence was seen in five cases (5 out of 17) at 18 days to 46 days from the initial transplant operation. In all five cases, 10 mg of loading dose of Sirolimus was used.

Oral ulcers

Four recipients (4 out of 17) experienced oral ulcers at 36, 33, 82, and 57 days respectively while on Sirolimus treatment. The Sirolimus levels were all within our target values (less than 15 ng) when oral ulcers were diagnosed. However, Mycophenolic Acid (MPA) levels were higher than 4.0 ng in all cases. Sirolimus was replaced with Tacrolimus for a short period of time in three cases. However, we learned to manage the oral ulcers by just lowering the Rapamune dosage initially, then both Sirolimus and MMF dosages were decreased for persistent oral ulcers.

Graft and patient survival

During the study period two grafts were lost in LURT groups. One graft was lost due to severe vascular rejection and a transplant nephrectomy was performed on POD #15. One graft was lost due to non-compliance of the recipient who suffered a Banff 1b rejection at three months post transplant. The patient continued to develop chronic allograft nephropathy after she stopped all the immunosuppressive drugs and became dialysis-dependent at seven months after the transplant.

Blood pressure (BP)

Blood pressures were compared at three months and six months post-operatively.

Blood Pressure (± SD) — *mmHg*

	Mean Systolic BP ± SD	Mean Diastolic BP ± SD
Admission	135.7 ± 25.7	78.3 ± 16.9
3 months	123.2 ± 18.5	78.3 ± 10.9
6 months	134.3 ± 12.5	84.4 ± 7.8

Number of blood pressure medications used to treat hypertension decreased to lesser numbers.

Number of preoperative blood pressure medications used

Number of medications	Number of patients	Percentage of patients
0	2	11.8%
1	2	11.8%
2	8	47.0%
3	4	23.56%
4	1	5.9%

Number of postoperative blood pressure medications used

Number of medications	Number of patients	Percentage of patients
0	4	22.5%
1	8	47.1%
2	3	17.6%
3	2	11.8%

Body weight

Body weight changes were recorded at three months and six months postoperatively. There were no significant weight changes among the recipients during the followup period.

Weight (± SD) — *kg*

Admission	81.5 ± 19.5 ranging from 47-115
3 months	78.9 ± 17.7 ranging from 43-108
6 months	81.5 ± 18.0 ranging from 51-103

Cholesterol levels

Total cholesterol levels were recorded at the time of transplant and compared to post-operative months three and six. An increase in total cholesterol levels at three months and six months respectively were documented.

mg/dL

	Mean ± SD	Range
Admission	141.9 ± 52.7	75 - 266
3 months	233.0 ± 58.4	139 - 317
6 months	206.1 ± 39.4	157 - 250

Hemoglobulin levels

Hemoglobulin levels improved by post-operative month 6.

g/dL

	Mean ± SD	Range
Admission	11.8 ± 1.9	9.1 - 18.0
3 months	11.3 ± 2.1	7.4 - 15.1
6 months	13.1 ± 0.8	12.0 - 14.6

Kidney function measured by serum creatinine level

Postoperative creatinine level

mg/dL

	Mean + SD	Range
Discharge	2.7 + 2.3	0.8-8.3
1 month	1.4 + 0.6	0.7-3.6
6 months	1.6 + 0.5	0.9-3.0

DISCUSSION

Steroid withdrawal has been attempted in clinical renal transplant recipients in order to avoid unwanted side effects such as hypertension, post-transplant diabetes mellitus (PTDM), avascular necrosis of bones, cataract formations, fractures, weight gain, moon face, and mood swings, etc. It was estimated that more than $5000 per year per patient could be spent to treat these side effects.[10] The steroid withdrawal trials can be divided into three categories according to the timing: early withdrawals at about 90 days, late withdrawals after a year and complete avoidance. Meta-analysis of seven randomized studies involving 681 patients was reported by Dr. Hricik.[11] There was a significant increase in acute rejection episodes in short-term follow-up. Prospective multi-center randomized study in U.S. had to be stopped due to high rejection rates.[12] When induction agent was allowed, the acute rejection rate was not significantly higher at one year.[13] Late withdrawal of steroid was also associated with increased incidence of acute rejection, worsening of serum creatinine, and proteinuria.[14-16] Complete avoidance of steroids has been reported with acceptable range of acute rejection episodes and good graft outcomes when induction agent is used.[17,18] CNIs are associated with acute and chronic nephrotoxicities, which may lead to subsequent graft loss. Side effects profile of cyclosporine A includes hyperlipidemia, hyperuricemia, tremor, gingival hypertrophy, and hirsutism. The use of Tacrolimus is associated with increased incidence of PTDM, neurological symptoms, alopecia, etc. CNIs are also blamed for non-immunological causes for Chronic Allograft Nephropathy (CAN).[19] Thus, attempts to avoid CNI have been successful when induction agent plus MMF based immunosuppression protocols were used.[20-22] CNI avoidance was also successful when Sirolimus and MMF were used as an immunosuppressive protocol.[23,24] According to the pooled analysis, acute rejection rate was down to six% when Sirolimus and MMF were used with Basiliximab induction, and seven% with Thymoglobulin induction. However, steroids were used in all studies. Avoiding both corticosteroids and CNI will be of great benefit pending the acute rejection episode is controlled at the historic level (around 10%). Living donor recipients were studied as a pilot study. There was an increased incidence of acute rejection among LURT recipients compared to LRT recipients. The use of Sirolimus was complicated with development of wound complication (i.e. wound dehiscence and lymphocele formation) particularly when the loading dose of 10 mg was used (four out of seven patients). No wound complications were seen in patients who did not receive a loading dose. Oral ulcers developed in four cases (two in initial bolus cases) at 33, 36, 54,

82 days, respectively. In all three cases except for one, the Sirolimus was temporarily discontinued. The management of oral ulcers has not been well established in the literature. The Sirolimus dosage was decreased by one half for one week and if no response, MMF dosage was reduced. The MPA levels were higher than 4 ng. in all cases when the oral ulcers were discovered. Whether reducing MMF dose is beneficial in healing mouth ulcers is unknown. Non-therapeutic levels of immunosuppressive drugs can be blamed for most of the acute rejection episodes among the study group. Two episodes of acute rejections seen in one-haplotype LRT recipients, Sirolimus was discontinued in both patients; one for a wound complication and one for joint pain, at 86 days and five months after transplant, respectively. Both cases responded to steroid treatments and are currently back on Sirolimus and MMF without corticosteroids. Among the LURTs, one patient was overtly non-compliant, one patient was on a lower dosage of Sirolimus for mouth ulcer, and one patient's MMF dosage was reduced due to GI symptoms. However, one patient developed a Banff 2b vascular rejection on POD #6 without any evidence of prior sensitization, donor specific antibody formation, or inadequate drug levels. The patient required OKT3 monoclonal antibody treatment without success and went into irreversible rejection. Because of the higher incidence of acute rejections seen in LURT group, it was decided to treat this group as deceased donor recipients, utilizing Thymoglobulin induction instead of Basiliximab, maintenance therapy of Sirolimus and low dose Tacrolimus for three months followed by conversion to MMF and Sirolimus. Although, the numbers of patients enrolled in this study are limited, better control of blood pressure with lesser blood pressure medications seem to be the trend, and the body weight did not change significantly from pre-operative values. Renal functions measured by serum creatinine levels were stable at three and six months post-transplantation. Because Sirolimus and MMF are both anti-proliferative agents, anemia was prevalent during the first few months. However, Hemoglobulin reached 13.1 ± 0.8 gms by six months post-transplant. Total cholesterol levels were significantly elevated at three months from the pre-operative values however it trended down ward at six months.

CONCLUSION

This pilot study has shown that it is feasible to avoid both CNIs and corticosteroids in some selected recipients without the higher incidence of acute rejections when treated with Basiliximab induction followed by maintenance immunosuppressive protocol using Sirolimus and MMF. However, maintaining the therapeutic levels of both agents seems to be important to minimize the incidence of acute rejections. Avoiding the initial loading dose of Sirolimus to minimize the incidence of wound complications is recommended. It is even possible to delay the introduction of Sirolimus by few days for the wound healing process is well underway if the recipient's immune system is well suppressed with the use of more potent induction agent such as Thymoglobulin. Not well- understood side effects such as mouth ulcers, joint pains and interstitial pneumonias and the managements of these need further studies. Long- term benefits from avoiding both CNIs and corticosteroids require larger scale multi-center randomized studies.

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