Long-Term Graft Survival with or without Donor-Specific Transfusion in Cyclosporine Era in One Haplo-Identical Living-Related Renal Transplant Recipients beyond the First Year: A 19-Year Experience

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SATOH, S., SUGIMURA, J., OMORI, S., SEINO, K. and FUJIZUKA, I. Long-Term Graft Survival with or without Donor-Specific Transfusion in Cyclosporine Era in One Haplo-Identical Living-Related Renal Transplant Recipients beyond the First Year: A 19-Year Experience. Tohoku J. Exp. Med., 2002, 197 (4), 201-207 — With improvement in immunosuppressive drugs, the beneficial role of donor-specific blood transfusion (DST) in the preconditioning of renal allograft recipients has been diminished. This retrospective study was conducted to investigate the influence of DST on long-term graft survival in successful one haplo-type-mismatched kidney transplantation in the cyclosporine (CsA) era at Iwate Medical University. Between August 1983 and October 1996, 52 one haplo-type-mismatched living related first renal transplants were performed. Fifty grafts survived beyond the first year after transplantation. These 50 patients were divided into two groups according to maintenance immunosuppression, 12 kidney graft recipients received azathioprine (AZA), prednisolone (PSL), CsA, and DST, and 38 recipients received AZA, PSL and CsA. Our DST protocol consisted of three transfusions of 30 ml of donor-specific Duffy-coat at 4-week intervals, without immunosuppressive coverage. In recipients receiving DST and CsA, the 5-, 10-, and 13-year graft survival rates were 100%, 83%, and 67%, respectively. In recipients without DST, the 5-, 10-, and 13-year graft survival rates were 95%, 74%, and 69%, respectively. There was no significant difference between the two groups in long-term graft survival. In conclusion, DST and CsA combination treatment in our protocol may not induce long standing donor-specific immunologic hyporesponsiveness. Other strategies are expected to induce immunotolerance. —— donor-specific transfusion; cyclosporine; long-term graft survival; one haplo-mismatch; living related kidney transplantation
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The introduction of cyclosporine (CsA) in the early 1980s for the prevention of acute and chronic rejection has increased the rate of graft survival at one year (Harihara et al. 2002). However, there has not been a noticeable improvement in long-term graft survival. Patient death with functioning graft and chronic allograft nephropathy (CAN) consisting of chronic rejection, recurrent kidney disease, and CsA nephropathy are the two major causes of renal allograft loss after the first year (Kreis and Ponticelli 2001). Among these causes of long-term graft failure, the most important and the most frequent cause of graft loss is chronic rejection (Merion et al. 1984). Modulation of the immune response to produce tolerance is needed to prevent chronic rejection.

Transfusion is suggested to induce tolerance due to microchimerism by the persistence of a small population of donor leukocytes (van Twuyver et al. 1991). The favorable effect of random blood transfusion on subsequent cadaver renal transplant was first reported by Opelz et al. (1973). It was confirmed that early graft survival of first cadaver renal transplant recipients with five or more random blood transfusions was higher than that of non-transfused recipients (Opelz and Terasaki 1978). Salvatierra et al. (1980) subsequently used directed donor-specific blood transfusions (DST) in one haplotype-mismatched living related transplant with a strong mixed lymphocyte culture (MLC) responsiveness and found that 1-year graft survival improved from 70 to 90%. It has subsequently been shown that a similar DST effect was observed even in two haplotype-mismatched, related and unrelated donor/recipient combinations (Sollinger et al. 1986). The beneficial effect of blood transfusion on early graft survival is strongly supported.

Although a beneficial effect of DST has been found in living donor renal transplantation, the advent of CsA appears to have decreased the role of DST in renal transplantation (Jayes and Levey 1989). With the introduction of CsA, early graft survival in nontransfused patients approximated that of transfused groups without the risk of recipient sensitization (Groth 1987; Opelz 1987). Despite improved early graft survival, longer term renal transplant recipients are at continued risk for graft loss due to CAN. Having achieved very good early results of renal transplantation, attention must now be focused on maintaining of long-term allograft function with the lowest risk to the recipient. Therefore, CsA-based therapy for one haplotype-mismatched living donor renal transplantation should again be considered.

Several studies have shown that combination treatment of CsA and DST reduced the number of acute rejection episodes and improved graft function and blood pressure control (Salvatierra et al. 1987; Veliddeoglu et al. 1992; Inoue et al. 1996). The use of DST and CsA in haploidentical living related transplant recipients may induce persistent donor-specific immunologic hyporesponse and improve graft survival. The effect of DST combined with the current immunsosuppressive therapy on long-term graft survival has not been fully elucidated. This retrospective study investigated long-term graft survival in successful one haplotype-mismatched living related first kidney transplantation with or without DST in the CsA era at Iwate Medical University.

**Patients and Methods**

Between August 1983 and October 1996, 52 one-haploidentical living related first renal transplants with CsA were performed Iwate Medical University. Two patients died of infectious acute liver dysfunction 5 and 7 months after transplantation. The remaining 50 grafts survived beyond the first year after
transplantation. These 50 subjects were analyzed as successful transplant recipients in the present study.

The 50 patients were divided into two groups according to the maintenance immunosuppression. In the first period (between August 1983 and February 1988), 12 kidney graft recipients received azathioprine (AZA), prednisolone (PSL), CsA, and DST (Group A), in the second period (between March 1988 and October 1996), 38 recipients received AZA, PSL, and CsA (Group B).

According to the DST protocol at Sendai Shakhijoken Hospital (Okazaki et al. 1989), our DST protocol consisted of three transfusions of 30 ml of donor-specific buffy-coat separated from 200 ml whole blood at 4-week intervals, without immunosuppressive coverage. All patients receiving DST were fully informed about the aims and risks of DST, and consented to our protocol.

Oral administration of AZA (1-2 mg/kg) was started 2 days prior to transplantation. Methylprednisolone (MP) administration was started on the day of transplantation, at a dose of 500 mg/day, and was reduced to a maintenance dose of PSL 10 mg/day 3 to 6 months later. Oral administration of CsA, 6 to 8 mg/kg per day, was started 2 days prior to transplantation, and drip infusion of CsA, 3 mg/kg, was administered on the day of transplantation. CsA administration was then adjusted to maintain the CsA trough level in whole blood at 200 ng/ml for 5 weeks after transplantation, then reduced to a maintenance level of 80-100 ng/ml.

All of patients were followed at our hospital at least once a month after transplantation.

Results were expressed as means±s.d. Data from two groups were compared using chi-square test and unpaired Student’s t-test, where appropriate. Graft survival curves were drawn according the Kaplan-Meier method, and a difference between curves was analyzed by generalized Wilcoxon test. Significance was defined as p<0.05.

**RESULTS**

Two of 12 patients with DST (16.7%) developed antibodies against the blood donors. Both converted to a negative T and B cell crossmatch by double filtration plasmapheresis. In all patients with DST, a kidney from the DST donor was successfully transplanted. There were no other serious complications of DST, such as graft vs. host disease or infectious hepatitis.

Clinical characteristics of the two groups are shown in Table 1. There were no significant differences in recipient age or the incidence of hypertension. The donor age of Group A was younger than that of Group B. The incidence of acute rejection episode within one year after transplantation was slightly lower in Group A compared with that of Group B.

<table>
<thead>
<tr>
<th>Table 1. Clinical characteristics of one-haploidentical living related first renal transplant recipients</th>
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<td><strong>Group</strong></td>
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<tr>
<td>Recipient age (years)</td>
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<tr>
<td>Donor age (years)</td>
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<tr>
<td>Incidence of AR (%)</td>
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<td>Hypertension (%)</td>
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Values are expressed as means±s.d.

DST, donor specific transfusion; AZA, azathioprine; PSL, prednisolone; CsA, cyclosporine; AR, acute rejection. *p<0.05 vs. group B.
B, but there was no significant difference.

The effect of DST on graft survival was evaluated by comparing DST-treated recipients (Group A) to DST-nontreated recipients (Group B) (Fig. 1). In Group A, the 3-, 5-, 10-, 11-, and 13-year graft survival rates were 100%, 100%, 83%, 75%, and 67%, respectively. In Group B, the 3-, 5-, 10-, 11-, and 13-year graft survival rates were 100%, 95%, 74%, 69%, and 60%, respectively. There were no significant differences between the two groups from 3 to 13 years after transplantation.

Fifteen of 50 patients had lost their allografts at the time of analysis (June, 2002): 50% (6/12) and 24% (9/38) of patients in Groups A and B, respectively (Table 2). Two of 6 recipients (33%) in Group A died with a functioning graft of hepatocellular carcinoma at 15 years and breast cancer at 14 years after transplantation. One of 9 (11%) recipients in Group B died of cirrhosis of the liver at 7 years after transplantation with a functioning graft. The remaining 4 of 6 patients in Group A and 8 of 9 in Group B lost their graft due to CAN (Table 2).

**DISCUSSION**

The ultimate goal in transplantation is a modulation of the immune response to produce tolerance without immunosuppression. To date, only a state of pseudo tolerance for the allograft has been achieved through the use of potent pharmacological and biological manipulation. Despite this manipulation to prevent acute rejection, chronic rejection can eventually result in graft failure (Burke et al. 1994). CsA has improved the overall results of kidney transplantation compared to the pre-CsA era, but chronic rejection remains a major problem (Merion et al. 1984; Tilney et al. 1991; Slaton et al. 1994; Tanabe et al. 1998). Thus, different strategies, such as DST and donor bone marrow infusion, have been sought to induce tolerance and prevent acute and chronic rejection (Brennan et al. 1995).

The mechanism of the DST effect is un-

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**Table 2. Number of graft loss and patients death**

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<tr>
<th>Group</th>
<th>A</th>
<th>B</th>
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<tr>
<td>DST + CsA (n = 12)</td>
<td>CsA (n = 38)</td>
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<tr>
<td>Overall graft loss</td>
<td>6 (50%)</td>
<td>9 (24%)</td>
</tr>
<tr>
<td>Death with functioning graft</td>
<td>2 (33%)</td>
<td>1 (11%)</td>
</tr>
<tr>
<td>Graft loss due to CAN</td>
<td>4 (67%)</td>
<td>8 (89%)</td>
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CAN, chronic allograft nephropathy.
clear. The following have been suggested: clonal deletion or inactivation, induction of suppressor cells, and induction of blocking alloantibodies and/or anti-idiotypic antibodies. Some or all of these mechanisms are likely to act concurrently to induce a more favorable immune modulation in recipients (Kong et al. 1995). However, DST sensitizes some potential recipients to donor blood antigens, thereby precluding transplantation from those donors. The risk of sensitization is 10 to 30% (Sollinger et al. 1986; Jayes and Levey 1989). Although the sensitization rate in our study was 16.7%, fortunately there was no patient who was canceled transplantation. CsA immunosuppression, in contrast, does not sensitize potential donors and provides all recipients with the benefits of a living-related transplantation. Since the introduction of CsA, many centers including our hospital have abandoned DST in living related transplants.

The widespread use of CsA resulted in a remarkable increase in overall graft survival after living related and cadaver renal allograft transplants. By 1990, the benefits of DST had been reduced to 2–3% in one haplotype-mismatched living related kidney recipients (Cicciairelli 1990). However, the induction of immunotolerance has not been achieved with CsA. Several studies suggested that DST could induce a reduction in donor-specific allorreactivity, allowing a decrease in the requirements for nonspecific immunosuppression over the long term (Reed et al. 1991; Salvatierra et al. 1991). The beneficial effects of various DST protocols on short- and long-term graft survival are still being reported (Okazaki et al. 1997; Miura et al. 1998; Alexander et al. 1999; Christiaans et al. 1999; Reinsmoen et al. 1999).

Improved graft survival has been shown for CsA-treated living-related renal transplantation when combined with DST (Cheigh et al. 1991; Velidedeoglu et al. 1992; Inoue et al. 1996; Sharma et al. 1997). Sharma et al. (1997) suggested that DST and CsA given 24 hours before living related renal transplantation is effective in improving graft function and reducing the number of acute rejection episodes which could have a beneficial effect on long-term graft survival. Preoperative (24 hours before transplant) use of DST in haplo-identical living related transplant recipients on CsA appears to have several advantages: (1) it induces persistent donor-specific immunologic hyporesponsiveness and improved graft survival; (2) there is a reduced risk of sensitization due to DST, and (3) there is a decreased risk of transfusion-related infectious disease (Flye et al. 1995; Sharma et al. 1997). Furthermore, Okazaki et al. (1995) reported that the addition of 15-deoxyspergualin to an initial immunosuppressive regimen improved long-term graft survival of recipients who received DST.

Our DST protocol consisted of three transfusions of 30 ml of buffy-coat at 4-week intervals without immunosuppressive coverage. DST in our study was not performed immediately before transplantation. We failed to find significant differences in long-term graft survival between patients with DST and without DST in our protocol. Although the number of the patients with DST in this study is too small to evaluate graft survival, the result resembled that in other reports (Inoue et al. 1996; Christiaans et al. 1999). Salvatierra et al. (1980) suggested that one role of DST is to segregate high responders from low responders to the specific blood donor. Induction of tolerance may not be achieved solely by selection of donors with low response.

In conclusion, we did not observe significant differences in long-term graft survival in patients receiving DST for CsA-treated living-related renal transplant compared to that of patients without DST. This combination treatment in our protocol may not induce persistent donor-specific immunologic hyporesponsiveness. Other immunological strategies or DST protocol are expected to induce im-
munotolerance.

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