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Donor-specific transfusions as a way of tolerance induction to living donor kidney transplant

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We aimed at evaluating the efficacy of the donor-specific transfusions (DST) in living kidney transplantations (Tx) performed in 1992–2000. Nineteen patients (pts) received DST 1–3 times with a two-week interval under CsA or AZA in the course of DST. One pt (5.3%) produced a donor-specific antibody after the first DST, one pt was not transplanted because of the donor's disease. Seventeen transplanted pts were compared with a non-DST group of 47 pts: 43 of them obtained grafts from relatives and 4 from spouses. The groups were comparable the recipients' age, the donors' age, the male / female ratio, HLA mismatches, time on dialysis, the number of re-transplantations, percentage of sensitized pts, the panel-reactive antibody (PRA ≥15–50%). The maintenance immunosuppressive therapy was the same in both groups. We did not reveal significant differences in actuarial graft survival, calculated by Kaplan–Meier, at 1, 5 and 10 years. In the DST group it was 100%, 93.8%, 87.1% and in the control group 91.5%, 87.3%, 70.5%, respectively. The group of DST contained significantly less pts that developed acute rejection episodes during the first year than the control group (11.8% of pts vs 46.8%, $\chi^2 = 6.5415$, $p < 0.02$).

The proportion of pts with an excellent graft function (serum creatinine <130 μmol/l) at 1 and 10 years in the DST group was 52.9%, 38.5% and in the control group 33.3% and 28.0%, respectively.

Our results demonstrate that in spite of an unavoidable low risk of sensitization, a beneficial effect of DST – a significantly lower incidence of acute rejection, tendency for a better graft survival and graft function and the absence of donor-specific antibodies within a long follow-up period – has been observed.

Key words: allograft rejection, donor-specific transfusion, graft function, graft survival rate, living kidney transplantation

INTRODUCTION

The induction of donor-specific tolerance with the minimum use of immunosuppressive drugs is one of the major aims in research transplantology [1]. Various strategies for approaching such a state of tolerance have been proposed: donor-specific blood transfusion, donor bone marrow infusion, simultaneous two-organ transplantation, administration of transplant acceptance-inducing cells (TAICs) [2–4]. Induction of transplantation tolerance, clinically defined as graft acceptance without functional impairment sustained for years in the absence of chronic immunosuppression, is widely regarded as a solution for two factors currently limiting long-term allograft survival, namely irreversible chronic rejection and side effects of standard immunosuppression [5]. Numerous experimental studies have been performed to understand the mechanisms of allograft tolerance. For example, one of the mechanisms that could operate is the release of inhibitory HLA molecules from the graft. Soluble HLA class I molecules of donor origin have been identified in the serum of pts with an allograft. They are endowed with the capacity to inhibit cell-mediated lympholysis by inducing the apoptosis of alloreactive CD8 cytotoxic T lymphocytes [6]. The donor whole blood transfusion to the recipient before Tx – the donor-specific transfusion – was introduced aiming at the inducement of unresponsiveness

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to allografts by the modulation of the immune system in pts until the cyclosporine era [2, 7].

Administration of DST in the cyclosporine era became abandoned in most centres. However, there are centres where the application of a pre-transplant DST to the recipient is still under investigation today. The recent publication of Marti et al. in the year 2006 showed that DST, even under modern immunosuppressive therapy (cyclosporine and monoclonal antibodies) improved the outcome of living kidney transplants [1]. In cases of a bad HLA-matched donor–recipient pair (spouses) it is desirable to induce tolerance to the graft in order to avoid aggressive immunosuppression. So, Claas et al. have shown that one HLA haplotype or DR-matched pre-transplant blood transfusions improve the kidney graft prognosis in humans [8].

However, the main complication of DST is the risk of sensitization to HLA antigens. The benefits are controversial and the long-term effects have not been well discussed. We aimed at evaluating the effect of DST on the outcome of kidney allografts during ten years and graft function in a long follow-up.

MATERIALS AND METHODS

Recipients of both the study and control groups underwent transplantation in 1992–2000. Transplants from living kidney donors comprised 32.2% (134 / 416) of all Tx performed in this period. This is a report of our experience with 19 pts who received DST. Following the first DST, one recipient (5.3%) developed a donor-specific antibody precluding Tx from living donation. One pt was not transplanted because of the donor's disease. The remaining 17 pts were grafted: 13 pts from relatives and 4 pts from spouses. These 17 transplanted pts (the study group) were compared with a group of 47 transplanted pts (the control group) without DST : 43 of them obtained a graft from relatives and 4 from spouses. These demographic data are listed in Table. The groups were comparable by the recipients' age (29.7 ± 15.9 vs 30.6 ± 9.6), the donors' age (44.1 ± 6.2 vs 45.6 ± 6.1), the male / female ratio (0.8 vs 0.8), HLA mismatches (3.1 ± 0.9 vs 3.1 ± 0.5), cold ischemia time (1.1 ± 0.3 vs 1.1 ± 0.1), the first Tx or re-transplantation, original disease, the panel-reactive antibody (PRA) level. There were small differences as regards the time on dialysis and the LRD / LURD ratio, but they were not significant.

DST (150–200 ml whole blood transfusions) from a potential living donor 1–3 times with a two-week interval was performed (6 pts received 3 DST, 7 pts 2 DST and 4 pts 1 DST). All the recipients received immunosuppressive coverage during the course of DST with AZA 50 mg/d or CyA 5 mg/kg/d. All pts of both groups were transplanted with a negative cross-match with donor's T and B lymphocytes. After Tx, the maintenance immunosuppression consisted of cyclosporine, azathioprine or mycophenolate mofetil, steroids for all the pts. One pt of the study group and two pts of the control group received induction therapy with monoclonal antibodies against the interleukin 2α receptor (basiliximab or daclizumab). Acute rejection episodes were treated with three methylprednisolone boluses of 500 mg. All the pts underwent Doppler ultrasound graft scan. A biopsy of the graft was required to confirm the diagnosis and to determine the histological rejection grade. Renal function was evaluated by the serum creatinine level according to the Collaborative Transplant Study (CTS) clinical grading scheme: serum creatinine <130 micromol/l – excellent graft function; serum creatinine 130–259 micromol/l – good graft function; serum creatinine 260–400 micromol/l – mediocre graft function,

Table. **Demographic and clinical data on study and control groups**

Parameter	Study group (DST^+)	Control group (DST^-)
Patients (n)	17	47
Recipient age (years, mean ± SD)	29.7 ± 15.9	30.6 ± 9.6
The male / female ratio	08	0.8
Donor age (years, mean ± SD)	44.1 ± 6.2	45.6 ± 6.1
Cold ischemia time (h, mean ± SD)	1.1 ± 0.3	1.1 ± 0.1
HLA mismatch (mean ± SD)	3.1 ± 0.9	3.1 ± 0.5
First transplant (%)	88.2	93.6
Re-transplant(%)	11.8	6.4
Sensitization ≥15%	23.5	29.2
Time on dialysis (months, mean ± SD)	14.6 ± 12.9	10.2 ± 7.1
Living related donation (%)	76.5	93.6
Living unrelated (spouses %)	23.5	6.4
Original disease: Diabetes (%)	5.8	8.5

Study group patients were treated with donor-specific transfusions (DST^+) and control group patients were not (DST^-). None of the parameters was significantly different in either of the groups.

and serum creatinine >400 micromol/l – poor graft function, but no chronic dialysis.

The presence of antibodies against donor HLA class I and class II antigens in the serum of all recipients was determined using complement-dependent cytotoxicity assays.

All the data are expressed as a mean ± SD. Statistical comparison of the values was performed using the Chi-squared test for categorical variables, and Student's t test was used for quantitative parameters. Statistical significance was defined as $p < 0.05$. Graft survival probabilities were calculated by the Kaplan–Meier method excluding non-immunological graft loss, and the significance of differences between the groups of pts was tested by the log-rank test. Tx was considered successful if the recipient remained alive without re-institution of permanent dialysis.

RESULTS AND DISCUSSION

Despite the excellent one-year graft survival rates achieved by the introduction of new immunosuppressive drugs, only a slight improvement in long-term graft survival has been observed. In renal transplantation, approximately half of the graft losses are related to the death of a patient with a functioning graft, and most of the rest losses are associated with chronic allograft rejection [9, 10]. Prevention of graft rejection is the primary purpose of maintaining immunosuppression in solid organ transplantation. Inadequate (weak) immunosuppression can cause an acute rejection episode and, possibly, the loss of the graft. Over-immunosuppression can lead to an increased risk of infection, cancer and other complications such as nephrotoxicity, cardiovascular disease and gastrointestinal disturbances.

A positive effect of donor-specific blood transfusions as well as blood transfusions from blood donors has been shown in some retrospective studies [11, 12]. Thus, the clinical experience of other investigators and the good results obtained in the transfused recipients prompt us to choice such a strategy (DST).

The immunological mechanisms underlying the blood transfusion effect have never been fully elucidated. One of the oldest and best-studied approaches for establishing tolerance is chimerism. There are two types of chimerisms: macrochimerism and microchimerism. Macrochimerism occurs when bone marrow is transplanted in a conditioned recipient.

Microchimerism arises as a result of migration of passenger leukocytes from the transplanted allograft into a nonconditioned recipient [13]. Some reports have demonstrated that multiple organ transplantation from the same donors may facilitate the induction of immuno-tolerance [14, 15].

Among solid organ allografts, kidney is highly immunogenic and causes a strong immune response. A human liver allograft has a lower susceptibility to rejection than other organs. Nakamura et al. have shown that in case of liver–kidney transplantation from living-related donors, the incidence of acute rejection was low [16]. The pioneering work of Salvatierra showed that the transfusion of blood from a prospective donor – DST – led to a considerable improvement in the survival of one haplotype-mismatched graft under azathioprine-steroid therapy [2]. According to another author, this finding was extended to the recipients of two haplotype-mismatched grafts [3]. However, after the introduction of cyclosporine, the effect of DST on graft survival disappeared in some studies [17, 18] and an interest in DST decreased [19]. The main disadvantage of blood transfusions is the risk of sensitization to HLA antigens precluding transplantation [20]. There is some evidence that the administration of immunosuppressive drugs during the application of DST reduces the sensitization rate [21, 22]. In an attempt to reduce the incidence of sensitization, all of the potential recipients in our study group received immunosuppressive coverage in the course of DST. The sensitization rate was 5.3%. There is an unavoidable but low risk of sensitization after DST. However, it may be the way to preclude mismatched graft rejection. It is much less than some of the studies have reported [1, 7, 23]. Some of the studies demonstrated the beneficial effect of DST on short-term graft survival [1, 2]; other authors observed only a small benefit from DST, which disappeared within 8 years after transplantation [24]. We did not reveal significant differences in actuarial graft survival between the two groups at 1, 5 and 10 years. In the DST group it was 100%, 93.8%, 87.1% and in the control group 91.5%, 87.3%, 70.5%, respectively. However, our study showed that the graft survival rate after 10 years was by 16.6% higher in the DST group than in the non-DST one. These results indicate a trend toward a better outcome in the study group in the long-term follow-up. The pts' survival rate at 10 years was 94% in the DST group and 93% in the control group.

During the first year after Tx, 2 pts (11.8%) from the study group and 22 pts (46.8%) from the control group were treated for acute rejection (Fig. 1). These values were significantly different ($\chi^2 = 6.5415$, $p < 0.02$). Importantly, our data reflect the beneficial impact of DST on the reduction of rejection episodes: they were significantly less frequent than in the control group. Thus, DST have been shown to help reduce rejection and allow to reduce the intensity of immunosuppressive regimens. Moreover, it is well known that prevention of acute rejection is associated with the decreased risk of chronic allograft dysfunction [26, 27].

The evaluation of the functioning grafts showed that an excellent graft function (serum creatinine <130 μmol/l) at

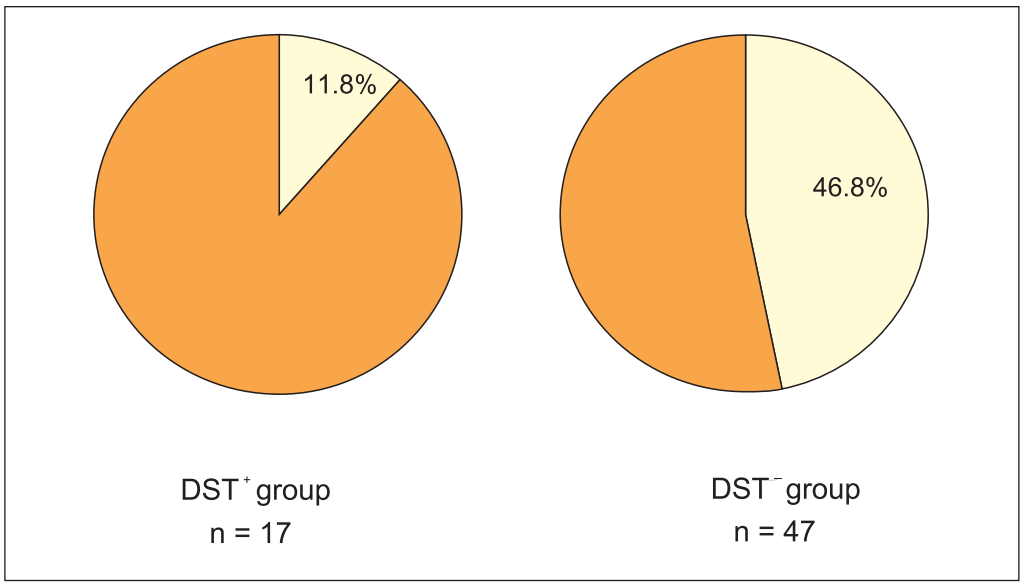

Fig. 1. Percentage of patients treated for allograft rejection during the first year after transplantation. Patients were treated or not with donor-specific transfusions (DST^+ and DST^- groups)

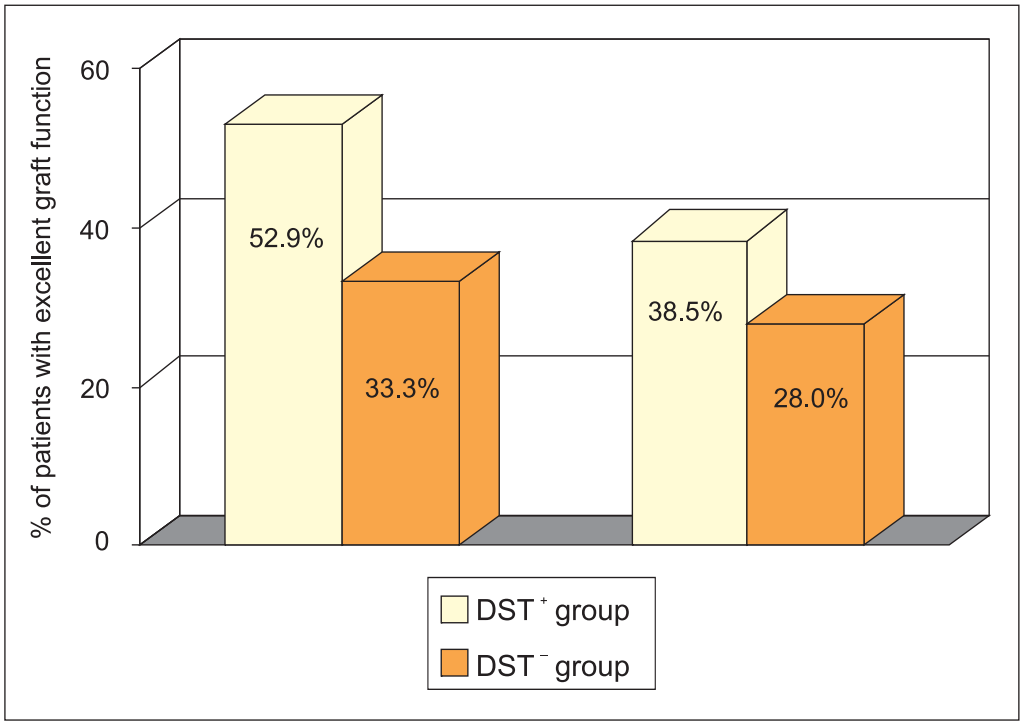

Fig. 2. Percentage of patients with excellent graft function (serum creatinine level range 89–130 µmol/l) one year and ten years after transplantation in both groups

1 and 10 years was in 52.9%, 38.5% in the DST group and 33.3%, 28%, respectively, in the control group (Fig. 2). The number of pts with an excellent renal graft function in the DST group after 10 years was by 10.5% higher than in the group without DST. These findings are in agreement with those from the other studies [25].

These results are optimistic. Which of the two factors – DST as a stimulus to develop tolerance, or a relatively long immunosuppression before Tx in the course of DST – appears to be the main for these results is under discussion.

The incidence of the production of anti-donor HLA antibodies after Tx during 5–10 years was lower among pts

of the study group than in pts of the control group: nobody in the DST group within the long-term period (5 and more years) after Tx had produced a donor-specific antibodies (anti-HLA class I or class II antibodies). In the control group, a total of 7 pts (14.8%) developed donor-specific antibodies: against HLA class I antigens in 2 pts, against HLA class II antigens in 3 pts, and against both class I and class II antigens in 2 pts. Although these differences were not statistically significant ($\chi^2 = 2.8548$, $p > 0.05$), they provide some indirect evidence of transplant tolerance. According to the literature, now it is well recognized that anti-donor anti-HLA antibodies play an important role in the chronic allograft nephropathy [28, 29].

CONCLUSIONS

Thus, our comparatively small (one centre) study demonstrates that in spite of an unavoidable low risk of sensitization, a beneficial effect of the DST – a significantly lower incidence of acute rejection, a tendency of a better graft survival and function and the absence of donor-specific antibodies after Tx in the long follow-up – has been observed.

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References

1. Marti H-P, Henschkowski J, Laux G et al. Transplant Int 2006; 19(1): 19–26.
2. Salvatierra OJr, Vincenti F, Amend V et al. Deliberate Ann Surg 1980; 192: 543–52.
3. Brennan DC, Mohanakumar T, Flye MV. Am J Kidney Dis 1995; 26: 701–15.
4. Hutchinson JA, Brem-Exner BG, Riguelme P et al. Transpl Int 2008; 21(8): 742–54.
5. Martinez-Llordella M, Puig-Pey I, Orlando G et al. Am J Transplant 2007; 7: 309–19.
6. Zavazava N, Kronke M. Nature Med 1996; 2: 1005–10.
7. Anderson CB, Sicard GA, Etheredge EE. Surgery 1982; 92: 315–21.
8. Claas FHJ, de Koster HS, Lagaaij EL, van Rood JJ. Exp Nephrol 1993; 1: 134–8.
9. Fehr T, Sykes M. Transplant International 2008; 21(12): 1118–35.
10. Pascual M, Theruvath T, Kawai T et al. N Engl J Med 2002; 346: 580–90.
11. Opelz G, Terasaki P. N Engl J Med 1978; 299: 799–03.
12. Middleton D, Martin J, Douglas J et al. Transplantation 1994; 58: 845–8.
13. Thaunat O, Badet L, El-Jaafari A et al. Am J Transplant 2006; 6: 2238–42.
14. Perico N, Remuzzi G. Int J Clin Lab Res 1997; 27: 165–77.
15. Rasmussen A, Davies HF, Jamieson NV et al. Transplantation 1995; 59: 919–21.
16. Nakamura M, Fuchinoue S, Nakajima I et al. Nephrol Dial Transplant 2001; 16: 166–8.
17. Jayes RL, Levey AS. Kidney International 1989; 36: 485–96.
18. Kishimoto K, Yuan X, Auchincloss H et al. J Am Soc Nephrol 2004; 15: 2423–8.
19. Davies CB, Alexander JW, Cofer BR et al. Ann Surg 1992; 215: 618–26.
20. Rainienė T, Lapšytė V, Stanevičienė A et al. Medicina 2001; 37(5): 571–8.
21. Santiago SF, Fukuzavva M, Azuma T et al. Surgery Today 1994; 5: 429–34.
22. Amada N, Okazaki H, Sato T et al. Transplantation 2003; 75: 60–6.
23. Miura S, Okazaki H, Satoh T et al. Transplant Proc 2001; 33: 1221–3.
24. Otsuka M, Yuzawa K, Takada Y et al. Nephron 2001; 88: 144–8.
25. Potter D, Garovoy M, Hopper S et al. Pediatrics 1985; 76: 402–5.
26. Soulillou JP. Panel Discussion: Transplant Proc 1996; 28(6): 41–4.
27. Wood PJ, Roberts J, Yang CP et al. Transplantation 1996; 61(10): 1440–3.
28. Cardarelli F, Pascual M, Tolkoff-Rubin N et al. Transplant Int 2005; 18(5): 532–40.
29. Campos EF, Tedesco-Silva H, Machado PG et al. Am J Transplant 2006; 6: 2316–20.

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DONORUI SPECIFINĖS TRANSFUZIJOS – BŪDAS INDUKUOTI TOLERANCIJĄ PERSODINUS GYVO DONORO INKSTĄ

Santrauka

Mūsų darbo tikslas – persodinus inkstą iš gyvo donoro įvertinti donorui specifinių transfuzijų (DST) poveikį potransplantacinei eigai pirmaisiais metais po operacijos bei vėliau. Tiriamiems ligoniams inkstų transplantaciją (Tx) atlikome 1992–2000 m. Prieš Tx 19-ai tiriamosios grupės ligonių 1–3 kartus perpylėme būsimojo donoro kraują, darydami dviejų savaičių pertraukas ir kartu skirdami azatiopriną ar ciklosporiną viso DST kurso metu. Vienas ligonis (5,3 %) jau po pirmosios DST pradėjo gaminti donorui specifinius antikūnus, todėl potencialaus donoro inkstas nebuvo persodintas. Kitam ligoniui Tx neatlikome dėl donoro ligos. Tiriamoji

grupė (17 ligonių) palyginta su kontroline grupe (47 ligonių), kuriai neatlikome DST, bet persodinome gyvų donorų inkstus pagal daugelį parametrų: recipiento ir donoro amžių, vyrų / moterų santykį, žmogaus leukocitų antigenų (ŽLA) nesuderinamumą, gydymo dializėmis trukmę, pakartotinai transplantuotų ligonių skaičių, sensitizuotų ligonių santykinį skaičių (%) grupėje bei jų sensitizacijos lygį, nusakomą su leukocitų panėle reaguojančių antikūnų (PRA) procentais, kuris šiose grupėse siekia 15–50 %. Abiejų lyginamųjų grupių ligonių palaikomoji imunosupresija po Tx buvo vienoda.

Transplantato aktuarinį išlikimą apskaičiavome pagal Kaplan-Meierį metodą 1,5 ir 10 metų: DST grupėje buvo 100; 93,8 ir 87,1 %, kontrolinėje grupėje – 91,5; 87,3 ir 70,5 % atitinkamai. DST grupėje pirmaisiais metais po Tx ūmaus atmetimo epizodai išsivystė mažesniam ligonių skaičiui nei kontrolinėje, skirtumas ženklus (11,8 % vs 46,8%; $\chi = 6,5415$; $p < 0,02$). Tiriamoje ligonių grupėje po vienerių metų transplantatas labai gerai funkcionavo (ligonių kraujo serume kreatino koncentracija <130 μmol/l) 52,9 % ligonių, po 10 metų – 38,5 %, kontrolinėje grupėje – 33,3 % ir 28,0 % atitinkamai. Be to, po Tx DST grupės ligoniai negamino donorui specifinių antikūnų (0 % vs 14,8 % kontrolinėje grupėje).

Taigi, nepaisant palyginti nedidelės, nors ir neišvengiamos rizikos būti sensitizuotam, DST poveikis yra teigiamas – mažiau ūmaus atmetimo epizodų per pirmuosius metus po Tx, inksto transplantatas ilgiau funkcionuoja, yra didesnė tikimybė ir vėliau išsaugoti jo labai gerą funkciją.

Raktažodžiai: alografto atmetimas, donorui specifinės transfuzijos, gyvo donoro inkstų transplantacija, transplantato išgyvenimas, transplantato funkcija