Transplant Int (1990) 3: 70–72

TRANSPLANT
International

Cyclosporin A-induced tolerance is not amplified by the addition of a steroid therapy

S. M. L. Lim*, K. Kogure, and D. J. G. White

Department of Surgery, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK

Received October 26, 1989/Received after revision March 19, 1990/Accepted March 20, 1990

Abstract. The effect of adding steroids to a cyclosporin A (CyA) schedule designed to induce tolerance to heart allografts in rats was investigated. CyA treatment alone, at a dose of 15 mg/kg per day for 2 weeks, resulted in the successful induction of tolerance (graft survival > 100 days) in 70% of the rats. The inclusion of 5 mg/kg of steroids (Solumedrone), administered IM for 40 or 60 days, not only failed to improve this long-term survival (LTS) rate achieved with CyA alone but reduced it from 70% to 50% after 40 days of steroid treatment and to 30% after 60 days of steroid treatment. The administration of 5 mg/kg and 40 mg/kg of steroids during the "high-risk" period for graft rejection (days 30–50 or 40–60) was shown to delay but not prevent subsequent rejections from occurring. Steroid treatment alone (5 mg/kg per day) was found to be only weakly immunosuppressive. Thus, we have demonstrated that the addition of steroids to a CyA tolerizing schedule was detrimental to the induction of tolerance.

Key words: Tolerance, induced by cyclosporin A – Steroid therapy and tolerance – Rat, tolerance induction

The use of steroids in conjunction with cyclosporin A (CyA) in immunosuppressive schedules is common practice in clinical organ transplantation [4, 10, 11]. Whilst the efficacy of steroids in the reversal of rejection episodes [2, 16] is undisputed, there is little evidence to substantiate their inclusion in maintenance immunosuppressive schedules. Recent reports emerging from centres that have conducted control trials [6, 8, 15] of CyA monotherapy versus CyA combined with steroids have suggested that graft survival rates in both groups are comparable with a reduced patient morbidity in the CyA monotherapy group. There is, however, a widely-held, though unsubstantiated, belief that renal function may, in some kidney transplant recipients immunosuppressed with CyA, be improved by the addition of steroids. It has been demonstrated that steroids can both change the pharmacokinetics of CyA [12] and reduce its nephrotoxic component [11]. Thus, the precise immunological contribution of steroids to the prevention of graft rejection in combination therapies remains unresolved. While the success of current low-dose triple therapies is unquestionable, the presumed immunological value of steroids in such schedules is unproven and must be compared with the excellent survival results achieved by heart transplant groups that have used azathioprine and CyA alone as maintenance immunosuppressive schedules [1].

In this study we have addressed the issue of how the addition of steroids affects the induction of tolerance by CyA. We have previously shown in a particular rodent model [9] that a 2-week course of CyA at 15 mg/kg per day produces tolerance to auxiliary heart grafts in 70% of the cases and identified a "risk period" between days 40 and 60 during which this 30% graft rejection occurs. The analysis of this risk period and the criteria used to establish tolerance have been described in detail elsewhere [9]. This study was designed to establish whether a CyA/steroid schedule would change the frequency of graft loss from rejection and in any way influence the risk period seen with CyA therapy alone.

Materials and methods

DA(RT1a) donors (females) and PVG(RT1c) recipients (males) were used. Donors weighed between 100 and 150 g, recipients between 200 and 250 g. Both rat strains were obtained from Bantin and

* *Present address and address for offprint requests:* Department of Surgery, National University Hospital, 5 Lower Kent Ridge Road, Singapore 0511

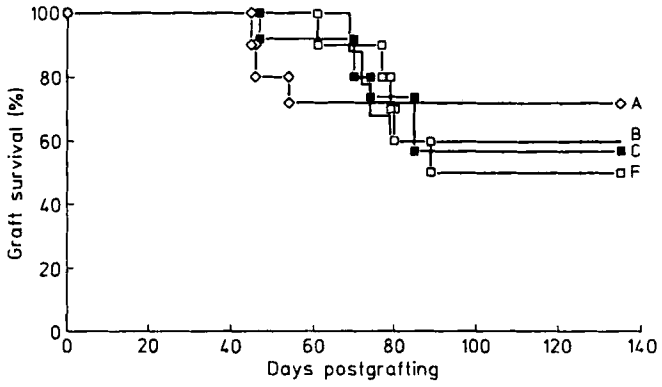

Fig. 1. Induction of tolerance by CyA/steroids during risk period. DA to PVG heart grafts. All groups (A, B, C, F) received CyA 15 mg/kg per day × 14 days. In addition, group B: steroids 5 mg/kg per day (days 30–50), group C: steroids 5 mg/kg per day (days 40–60), group F: steroids 40 mg/kg per day (days 40–60). Long- term heart graft survival (> 100 days) = 70% (A), 60% (B and C), 50% (F)

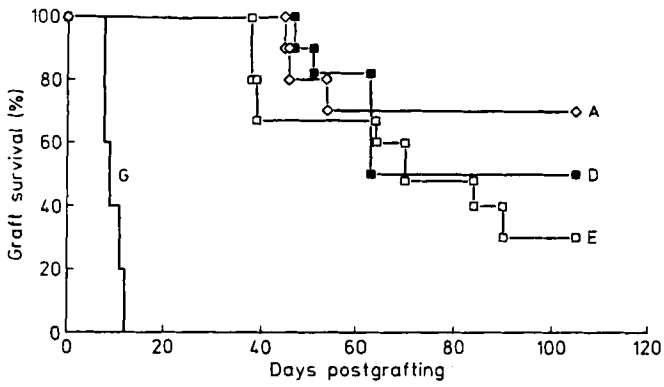

Fig. 2. Induction of tolerance by CyA/steroids throughout induction phase. DA to PVG heart grafts. Groups A, D, E received CyA 15 mg/kg per day × 14 days. In addition, group D: steroids 5 mg/kg per day × 40 days, group E: steroids 5 mg/kg per day × 60 days. Group G received steroids 5 mg/kg per day only. Long-term heart graft survival (> 100 days) = 70% (A), 50% (D), 30% (E), nil (G)

Kingman (Hull, UK). Accessory cervical heart transplantation was performed according to the technique described by Heron [7]. Rejection was diagnosed by the cessation of heartbeat and confirmed by histological examination. CyA (a gift from Jean Borel of Sandoz, Basel, Switzerland) was dissolved in olive oil at a concentration of 25 mg/ml. The steroid preparation used was Solumedrone (40 mg/ml; Upjohn, Crawley, W. Sussex, UK). It was administered IM at a dose of either 5 mg/kg per day or 40 mg/kg per day.

Experimental design

DA hearts were grafted into PVG rats on day 0. CyA was administered intramuscularly at 15 mg/kg per day for 14 days (days 0–13 inclusive). Previous extensive studies had shown that this schedule produces a 69% long-term survival (LTS) [14] and it was chosen because it was suitable for demonstrating a beneficial (> 69% LTS) or adverse (< 69% LTS) effect of the steroid treatment on tolerance induction. Methylprednisolone was administered intramuscularly at doses of 5 mg/kg per day in four different protocols to cover the risk period for CyA treatment (groups B and C), the induction phase of tolerance prior to the risk period (group D), and both the induction phase and the risk period (group E). Steroids were also used in large doses (40 mg/kg per day) in an attempt to prevent graft rejection during the risk period (group F). In addition, control groups receiving 15 mg/kg per day CyA only (group A), and 5 mg/kg per day steroids only (group G) were included. The exact timings of these various schedules are given in Table 1.

Statistical analysis was performed using the Spearman rank test.

Results

In the CyA-only control group (group A), tolerance to heart grafts was achieved in 70% of the cases. This was consistent with previous experimental findings. The addition of steroids to this CyA schedule (groups B–F) consistently reduced the number of grafts accepted long term (Table 2). Steroid treatment during the risk period (i.e. between days 40 and 60) both in a low dose (5 mg/kg per day; groups B and C) and a high dose (40 mg/kg per day; group F) postponed graft rejection to beyond the risk period (Fig. 1). However, in these groups (B, C, and F), graft loss due to rejection ultimately occurred. Furthermore, this steroid treatment during the risk period decreased the number of grafts going on to long-term survival compared with the group receiving CyA alone. Thus, graft survival decreased from 7/10 in the CyA-alone group to 6/10 in the CyA and steroid groups and 5/10 when the steroid was increased to 40 mg/kg (group F).

The inclusion of steroids throughout the induction phase of CyA tolerance (days 0–40, group D) also produced a decrease in the number of grafts accepted in the long term compared with CyA-alone controls from 7/10 to 5/10 (Fig. 2). Steroid treatment during both the induction phase and the risk period (days 0–60; group E) resulted in the induction of tolerance in only 3/10 animals in this group. This reduction shows a statistically significant detriment to tolerance induction by the addition of steroids ($P < 0.01$). Histological examination of grafts lost in all the

Table 1. CyA/steroid treatment schedules used in the study

Group	CyA dose	CyA Rx days	Steroid dose	Steroid Rx days
A	15 mg/kg per day	0–13	–	–
B	15 mg/kg per day	0–13	5 mg/kg per day	30–50
C	15 mg/kg per day	0–13	5 mg/kg per day	40–60
D	15 mg/kg per day	0–13	5 mg/kg per day	0–40
E	15 mg/kg per day	0–13	5 mg/kg per day	0–60
F	15 mg/kg per day	0–13	40 mg/kg per day	40–60
G	nil	–	5 mg/kg per day	0–(60)[a]

[a] This group received 5 mg/kg steroids only from day 0 up until the time of established graft loss due to rejection

Table 2. The influence of steroid treatment on CyA-induced allograft tolerance in the rat

Groups	Survival times for heart grafts (days)	LTS
A (CyA only)	44, 45, 53, > 100 (× 7)	70%
B (30–50 days)	68, 71, 73, 78, > 100 (× 6)	60%
C (40–60 days)	46, 69, 73, 84, > 100 (× 6)	60%
D (0–40 days)	46, 50, 62, 62, 62, > 100 (× 5)	50%
E (0–60 days)	37, 37, 38, 63, 69, 83, 89, > 100 (× 3)	30%
F (40–60 days)[a]	60, 76, 78, 79, 88, > 100 (× 5)	50%
G(steroids only)	7, 7, 7, 7, 8, 8, 10, 10, 11, 11	0%

[a] Steroid dose 40 mg/kg per day

CyA/steroid groups confirmed the classic features of acute cellular rejection. Immunosuppression with steroids alone at a dose of 5 mg/kg per day failed to prolong graft survival beyond 11 days (Fig. 2).

Discussion

We have previously documented [9] that in this model the induction of allograft tolerance by CyA occurs between days 0 and 60. During this time a "risk period" for graft rejection can be identified between days 40 and 60 using a 2-week CyA schedule. The experiments described here were performed to determine the influence of steroids throughout the induction phase (days 0–40), at varying times during the risk period (days 30–50 and 40–60), and during both the induction phase and the risk period (days 0–60). The selection of a 5 mg/kg dose of steroids is based on studies of others in rodents where steroids were combined with azathioprine in immunosuppressive protocols [13]. The results reported here show that the inclusion of steroids in CyA tolerizing schedules not only failed to increase the yield of tolerant rats but consistently reduced the number of long-term survivors. Steroid treatment during the CyA risk period (days 30–50 and 40–60) postponed, but did not prevent, graft rejection. Following the cessation of steroids (after 60 days), acute graft rejection occurred. In the same way, steroid treatment throughout the induction phase of CyA tolerance (days 0–40) did not increase the yield of tolerant rats bearing heart grafts (Table 2). Rather, there was a reduction in the percentage of long-term survivors with this schedule also.

There is little experimental evidence that steroids can maintain prolonged graft survival. Thus, it is perhaps not surprising that our control group of steroids alone (5 mg/kg per day) showed an improvement of only 1 day (from a median of day 7 to a median of day 8) over controls, despite the fact that the dose chosen was a clinical equivalent of approximately 250 mg/day. Similar findings were reported by Dempster [5] in 1953. He showed that steroids alone were ineffective in prolonging the survival of kidney grafts in dogs. In rodents a weak immunosuppressive property for steroids has been eluded to by a requirement for azathioprine in combination with steroids in order to achieve a prolongation of cardiac allograft survival [13]. Indeed, Billingham and colleagues [3], using a rabbit skin graft model, showed that graft rejection, although delayed by cortisone treatment, still occurred. Thus, the failure of steroids to improve the rate of tolerance induction in our experiments is perhaps not a surprising observation. However, what does give rise to concern is the clear indication from our data that the addition of steroids to a CyA tolerizing regime is detrimental to the induction of tolerance. All those groups treated with steroids showed a lower frequency of long-term (i.e. tolerant) survivors, although in many of these groups this detriment was marginal and based on graft failure in only one or two additional animals. However, despite the small number of animals in each group ($n = 10$), that group of animals (group E) that was treated throughout both the induction and risk periods did show a statistically significant reduction in the number that progressed to long-term tolerance.

Although steroids have been successfully used in organ transplantation for three decades, the mechanism of action of these drugs is complex and their role, if any, in the induction of tolerance has yet to be defined. The reduction reported here in the number of tolerant rats in the steroid-treated groups may indicate that steroids can actively suppress the tolerizing mechanism. The organizational commitment and logistics needed to treat large numbers of rats with steroids daily over many months makes the expansion of these experiments a most unattractive prospect. However, in view of the widespread clinical use of steroids in transplantation, the possibility that the use of steroids is detrimental to long-term tolerance induction warrants further investigation.

References

1. Banner NR, Fitzgerald M, Khaghani D, Yacoub MH (1987) Cardiac transplantation at Harefield Hospital. In: Terasaki PI (ed) Clinical transplants. UCLA Tissue Typing Laboratory, Los Angeles, pp 17–27
2. Bell PRJ, Briggs JD, Calman KC, Paton AM, Wood RFM, Macpherson SG, Kyle K (1971) Reversal of acute clinical and experimental organ rejection using large doses of intravenous prednisolone. Lancet I: 876
3. Billingham RE, Krohn PL, Medawar PB (1951) Effect of cortisone on survival of skin homografts in rabbits. Brit Med J 1: 1157
4. Calne RY, White DJG, Evans DB, Wright C (1982) Three years' experience with cyclosporin A in clinical cadaveric kidney transplantation. In: White DJG (ed) Cyclosporin A. Proceedings of an international conference on cyclosporin A. Elsevier, New York, pp 347–353
5. Dempster WJ (1953) The effects of cortisone on the homotransplanted kidney. Arch Int Pharmacodyn Ther 95: 253
6. Griffin PJA, Ross WB, Williams JD, Salaman JR (1987) Low-dose cyclosporine monotherapy in renal transplantation. Transplant Proc 19 [Suppl 5]: 3685
7. Heron I (1971) A technique for accessory cervical heart transplantation in rabbits and rats. Acta Pathol Microbiol Immunol Scand [A] 79: 366
8. Johnson RWG, Wise MH, Bakran A, Short C, Dyer P, Mallick NP, Gokal R (1985) A four-year prospective study of cyclosporine in cadaver renal transplantation. Transplant Today 8: 1197
9. Lim SML, White DJG, Calne RY (1987) Identifying a susceptible period following cyclosporine A-induced tolerance of heart grafts in the rat. Transplant Proc 19 [Suppl 5]: 4218
10. Morris PJ, Chan L, French ME, Ting A (1982) Low dose oral prednisolone in renal transplantation. Lancet II: 525
11. Nott D, Griffin PJA, Salaman JR (1985) Low dose steroids do not augment cyclosporine immunosuppression but do diminish cyclosporine nephrotoxicity. Transplant Proc 17: 1289
12. Ost L (1984) Effects of cyclosporine on prednisolone metabolism. Lancet I: 451
13. Salaman JR (1983) Influence of steroid dosage on the survival of cardiac allografts in the rat. Transplantation 35: 510
14. Starzl TE, Hakala TR, Rosenthal JT, Iwatsuki S, Shaw BW Jr (1983) The Colorado-Pittsburgh cadaveric renal transplantation study with cyclosporine. Transplant Proc 15 [Suppl 1]: 2459
15. Stiller C (1983) The requirements for maintenance steroids in cyclosporine-treated renal transplant recipients. Transplant Proc 15 [Suppl 1]: 2490
16. Turcotte JG, Feduska NJ, Carpenter EW, McDonald FD, Bacon GE (1972) Rejection crises in human renal transplant recipients. Arch Surg 105: 230