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Impaired Prednisolone Sensitivities of the Endocrine System and Peripheral-blood Lymphocytes are Closely Related to Clinical Incidence in Renal Transplantation

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Abstract—Endocrine- and immune-responses to prednisolone and their relation to clinical incidence were assessed in 19 renal transplant recipients. All of the patients were treated with prednisolone and cyclosporin. Response of the hypothalamic-pituitary-adrenal (HPA) system to prednisolone was evaluated by measuring serum cortisol concentration. Cortisol concentration before transplantation was $126{\cdot}7 \pm 38{\cdot}6$ ng mL^{-1}, while it decreased to $4{\cdot}1 \pm 2{\cdot}5$ ng mL^{-1} within the period characterized by a cumulative dose of prednisolone from 300 to 700 mg. A statistically significant high incidence ($P < 0{\cdot}01$) of acute rejections was observed in low HPA responders; (mean cortisol concentration during prednisolone treatment exceeded 3·0 ng mL^{-1}), 6 of 12 with a low HPA response to prednisolone showed signs of rejection, while none of the 7 with a high HPA response showed signs of rejection. The concentrations of prednisolone suppressing the in-vitro response of pretransplant lymphocytes to concanavalin A by 50% (ID50) were determined. Lymphocytes from 8 patients were extremely insensitive (ID50 > 500 ng mL^{-1}), and 5 of the 8 showed signs of rejection. Lymphocytes from the other 11 patients showed high sensitivity (ID50 < 500 ng mL^{-1}), and only one of those showed signs of rejection. Thus, a significantly high incidence of rejection was observed in low lymphocyte-responders to prednisolone ($P < 0{\cdot}05$). The results suggest that an insensitive endocrine response to prednisolone correlates with an impaired lymphocyte response to the steroid, and that both of the indices are related to occurrence of rejection. Evaluation of these pharmacodynamic parameters in combination may serve as a guideline for successful immunosuppressive therapy in renal transplantation.

Prednisolone pharmacodynamics rather than pharmacokinetics (Gambertoglio et al 1980) have been suggested to be important for graft outcome in renal transplantation. Langhoff et al (1986) and Dumble et al (1986) reported that recipient lymphocyte sensitivity to methylprednisolone may affect cadaver kidney graft survival, and may serve as a guideline for the doses of methylprednisolone used (Dumble et al 1986; Langhoff et al 1986). Thus, intrinsic or acquired steroid resistance appears to be critical for successful renal transplantation. Recent experiments have shown that lymphocytes from depressive patients in whom HPA-suppression does not occur in the dexamethasone suppression test are resistant to the immunosuppressive effects of a standard dose of dexamethasone (Lowy et al 1984, 1985). These results suggest that lymphocyte sensitivity to steroids is parallel to the sensitivity of adrenocortical response to steroids. It is possible to consider that intrinsic or acquired resistance to steroids is associated with a generalized decrease in steroid receptor sensitivity or function. On the basis of these concepts, endogenous glucocorticoid monitoring of renal transplant patients undergoing immunosuppressive therapy with prednisolone might be a useful indicator to determine the efficacy of prednisolone on immune responses of patients and the incidence of acute allograft rejection.

The present study was designed to monitor simultaneously two pharmacodynamic parameters of prednisolone: (i) the post-transplant endogenous serum cortisol concentrations during the early days of transplantation (prednisolone cumulative dose from 300 to 700 mg) and (ii) pretransplant-lymphocyte sensitivity to prednisolone, in 19 consecutive renal transplant recipients. The relation of these parameters to clinical signs such as occurrence of acute allograft rejection is discussed.

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Materials and Methods

Patients

We studied 19 patients who had received renal transplantation since February, 1988, at the Hachioji Medical Center. Informed consent for this study was obtained from all patients. One received a second graft, and the others received primary grafts. Their mean age (±s.d.) at transplantation was $30{\cdot}4 \pm 7{\cdot}8$ years, 5 were female and 14 male; 8 received cadaver kidney grafts and 11 received grafts from living relatives. The kidney transplantations were carried out by conventional surgical techniques.

Medication

All of the patients were treated with maintenance immunosuppressive therapy which consisted of prednisolone and cyclosporin A. Basically, the starting dose of prednisolone on the day of transplantation was 60 mg day^{-1}, and thereafter the dose was reduced gradually to 40–30 mg day^{-1} within two weeks. The starting dose of cyclosporin was 10–12 mg kg^{-1} day^{-1}, and the dose was reduced gradually thereafter. Dosage adjustments of cyclosporin were carried out by monitoring serum cyclosporin concentrations by RIA or HPLC. Acute rejection episodes were diagnosed by increases in serum creatinine concentrations of 0·2 mg dL^{-1} or more. Clinical signs of rejection such as urine decreases, graft

tenderness, graft enlargement, hyperthermia, weight gain, etc., supported the diagnosis. In some cases, this was further supplemented by allograft biopsy or renography. Acute rejection episodes were treated similarly in all patients. Intravenous pulses of 250–1000 mg methylprednisolone sodium hemisuccinate were given daily for 1–5 days.

Cortisol measurement

Blood was taken in the morning before administration of drugs. To measure cortisol concentrations, serum was removed after centrifugation of a sample at 1500 *g* for 5 min and frozen at −20°C until analysis. Serum cortisol concentration was measured by HPLC (Oka et al 1984, 1990; Kozaki et al 1989). In brief, 0·5 mL of serum sample with 5 or 50 ng of dexamethasone as an internal standard was extracted with an ethanol/dichloromethane mixture (1:99; v/v) using the rapid-flow fractionation system equipped with two diatomaceous earth columns (inner vol, 4 and 0·8 mL, respectively). The second column contained 50 μL of 50 mg mL^{-1} sodium hydroxide solution to eliminate acid components in the extract. The extract was dried, redissolved in 20 μL of a mixture of methanol/dichloromethane (2:98; v/v) and subsequently injected into the HPLC. Our HPLC system, a U/880 series (Jasco, Tokyo, Japan), consisted of a reciprocal pump, an ultraviolet absorbance detector, and a recorder. We used a conventional Hiber column (4 mm (i.d.) × 250 mm (Merck, Darmstadt, Germany), packed with 5 μm particles of LiChrosorb Si-60. The mobile-phase was a mixture of water/methanol/dichloromethane/n-hexane (0·1:6:30:63·9; v/v/v/v).

Lymphocyte culture

For assay of lymphocyte response to prednisolone, venous blood (10 mL) was taken, heparinized and then centrifuged at 1300 *g* for 15 min at room temperature (20°C). Mononuclear lymphocytes were separated by a Ficoll-Hypaque density gradient and rinsed two times with sterilized saline. The cells were suspended in an RPMI 1640 medium containing 100 000 int. units L^{-1} of penicillin, 100 mg L^{-1} of streptomycin and 10% foetal calf serum to a cell density of 1×10^6 cells mL^{-1}. Two hundred μL of this suspension were placed into each well of a microtitre plate with 96 flat-bottom wells. Concanavalin A was added to each well to a final concentration of 5·0 μg mL^{-1}. Subsequently, 4 μL of an ethanol solution containing prednisolone was added to a final concentration of 0·001, 0·01, 0·1, 1·0, or 10·0 μg mL^{-1}. Four μL of ethanol was added to a control well. The plate was incubated for 4 days in 5% CO_2 at 37°C. The cells were pulsed with 0·5 μCi/well of [^{3}H]thymidine for the last 16 h of incubation and then collected on glass fibre filter paper using a multiharvester device and dried. The radioactivity retained on the filter was further processed for liquid scintillation counting. The mean of the counts of a triplicate assay for each sample was determined. Agent concentration that would give 50% inhibition of lymphocyte mitosis (ID50) was determined from the dose response curve.

Materials

The chemicals were obtained from Wako Chemical Co. (Japan), except for steroid standards from Sigma Chemical Co. (USA). RPMI 1640 medium and foetal calf serum were obtained from GIBCO Laboratories (USA). Concanavalin A was from Seikagaku Kogyo Co. (Japan). [^{3}H]Thymidine was purchased from New England Nuclear Corporation (USA). Penicillin and streptomycin were obtained from Meiji Seika Pharm. Co. (Japan). All other reagents were of the best available grade.

Statistics

Statistical analysis was carried out with the two-tailed Student's *t*-test or Fisher's exact probability test. *P* values less than 0·05 were considered to be significant.

Results

The mean concentration of serum cortisol of the 19 patients before administration of immunosuppressive agents was 126·7 ± 38·6 ng mL^{-1}. The mean concentration of healthy subjects (age, 25·4 ± 4·3 years; n = 18) was 131·0 ± 34·8 ng mL^{-1}. Thus, cortisol serum concentrations of the patients were within the range of normal subjects ($P > 0·05$), suggesting that their adrenocortical function was not affected by chronic renal failure. Serum cortisol levels were monitored daily during the first 15 days after transplantation. Changes in mean serum-cortisol concentration after the operation are shown in Fig. 1. Within the first 5 days after transplantation, the mean cortisol concentration of the patients promptly decreased to 4·1 ± 2·5 ng mL^{-1}, and the level persisted for up to 60 days after grafting. Six of the patients showed acute rejection episodes 20 to 90 days after transplantation, while the other 13 did not show any signs of rejection. The mean cortisol concentrations in the recipients with rejection were almost always higher than those in the recipients without rejection during the first 15 days after transplantation (Fig. 1). A statistically significant difference ($P < 0·05$) was observed between the groups at day 7 after operation.

A high level (> 3 ng mL^{-1}) was observed in 12 patients during the time in which prednisolone cumulative dose

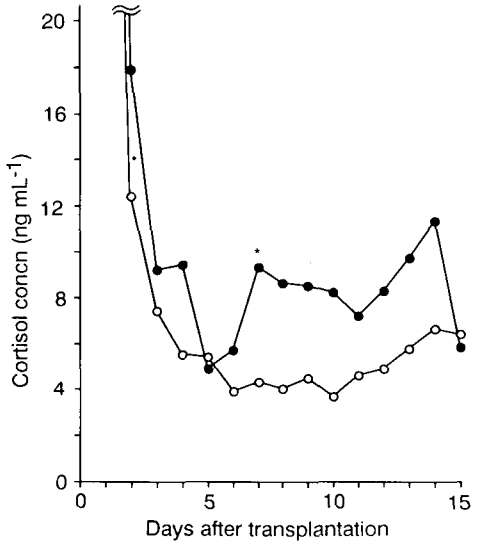

FIG. 1. Changes of serum cortisol concentrations after renal transplantation. ○ patients without rejection (n = 13); ● patients with rejection (n = 6). *$P < 0·05$.

Table 1. Incidence of acute rejection in low HPA-responders to prednisolone as assessed by mean serum cortisol concentration.

Patient group	With rejection	Without rejection	Total
Cortisol > 3 ng mL^{-1}*	6 (50·0%)**	6 (50·0%)	12
Cortisol < 3 ng mL^{-1}*	0 (0%)	7 (100%)	7

* Mean serum concentration (ng mL^{-1}) during cumulative prednisolone from 300 to 700 mg.
** Statistically significant high incidence of rejection was observed ($P < 0·01$).

increased from 300 to 700 mg (group I). Six (50·0%) of these, showed signs of serious rejection, and then received methylprednisolone pulse therapy. In the same period, lower serum cortisol (< 3 ng mL^{-1}) was observed in 7 patients (group II), none of which showed signs of rejection. A statistically significant high incidence of acute rejection episodes was observed in group I (Table 1, $P < 0·01$).

Lymphocytes from 8 patients were extremely insensitive to prednisolone (ID50 values > 500 ng mL^{-1}, Table 2). Five of the 8 lymphocyte-insensitive patients showed signs of acute rejection within 3 months after transplantation. In contrast, lymphocytes from the other 11 patients showed high sensitivities to prednisolone (ID50 < 500 ng mL^{-1}), and only one of those showed signs of acute rejection. A statistically significant high incidence of acute rejection episodes was observed in low lymphocyte-responders to prednisolone ($P < 0·05$).

Table 3 summarizes HPA-response to prednisolone assessed by mean serum cortisol concentration during the relevant prednisolone treatment, lymphocyte-sensitivity to prednisolone (ID50), and actual rejection episodes in the 19 patients. Based on the data described in Table 1, the patients who showed mean cortisol concentration during the prednisolone treatment period > 3 ng mL^{-1} were considered to be a high-risk group. Similarly, the patients who showed IC50 values > 500 ng mL^{-1} were also considered to be a high-risk group on the basis of the data described in Table 2. Acute rejection episodes were actually observed in 6 of the 19 patients examined. Retrospectively, all of the 5 patients who were deemed high-risk subjects on the basis of both cortisol- and lymphocyte-criteria showed signs of rejection, whereas, 13 of the 14 patients who had only one or none of the risk factors remained free from evidence of rejection. A statistically significant high incidence ($P < 0·01$) of acute rejection episodes was observed in patients who showed both HPA- and lymphocyte-resistance to prednisolone (Table 4).

There were no statistically significant differences in recipient sex, age, ratio of living-related donation, HLA-A, B, and DR mismatch numbers, transplantation history, or pretransplant cortisol concentration, between 'with rejection' and 'without rejection' groups, whereas statistically significant differences could be observed in post-transplant serum cortisol and pre-transplant ID50 between the groups (Table 5).

Table 2. Incidence of acute rejection in low lymphocyte-responders to prednisolone (Pred).

Patient group	With rejection	Without rejection	Total
Pred ID50 > 500 ng mL^{-1}	5 (62·5%)*	3 (37·5%)	8
Pred ID50 < 500 ng mL^{-1}	1 (9·0%)	10 (91·0%)	11

* Statistically significant high incidence of rejection was observed ($P < 0·05$).

Table 3. Correlation between HPA- and lymphocyte-sensitivities to prednisolone and occurrence of acute rejection.

Case	Serum mean cortisol concn (ng mL^{-1})	Lymphocyte response to prednisolone (ID50 ng mL^{-1})	Acute rejection episode
1	4·2 ± 0·9 o	> 1000 o	+
2	12·3 ± 8·5 o	> 1000 o	+
3	3·6 ± 3·0 o	> 1000 o	+
4	4·4 ± 1·5 o	> 1000 o	+
5	5·9 ± 2·4 o	> 1000 o	+
6	3·0 ± 0·9 o	60	+
7	1·2 ± 1·0	86	−
8	2·0 ± 0·9	56	−
9	2·4 ± 1·3	> 1000 o	−
10	6·7 ± 1·7 o	36	−
11	7·1 ± 1·8 o	24	−
12	3·0 ± 0·8 o	32	−
13	2·8 ± 1·2	> 1000 o	−
14	2·7 ± 0·9	> 1000 o	−
15	2·5 ± 0·9	12	−
16	3·5 ± 0·9 o	75	−
17	3·5 ± 1·6 o	14	−
18	2·9 ± 0·4	5	−
19	3·8 ± 1·6 o	16	−

o Low HPA- or lymphocyte-responders.

Table 4. Incidence of acute rejection in patients who showed low HPA- and lymphocyte-response to prednisolone.

Patient group	Number of patients with or without actual rejection episode (%)		Total
	with	without	
Cortisol > 3 ng mL^{-1} and ID50 > 500 ng mL^{-1}	5 (100%)*	0 (0%)	5
Cortisol < 3 ng mL^{-1} and/or ID50 < 500 ng mL^{-1}	1 (7·1%)	13 (92·9%)	14

* Statistically significant high incidence of rejection was observed ($P < 0·01$).

Discussion

Our data strongly suggest that HPA sensitivity to prednisolone correlates with immune response to the steroid in renal transplant recipients. Moreover, the data indicate that low responders to prednisolone in terms of their HPA- or lymphocyte-sensitivity might result in the occurrence of acute allograft rejection. Thus, these biological markers, in combination, may be useful as predictive indices of acute rejection episodes.

Our patients were treated with a combination of prednisolone and cyclosporin therapy, and the effects of cyclosporin on graft outcome could not be neglected. However, the incidence of extremely-low lymphocyte-responders to cyclosporin (ID50 > 500 ng mL^{-1}) in patients with chronic renal failure was quite low (3·8%), whereas the incidence of extremely-low lymphocyte-responders to prednisolone (ID50 > 500 ng mL^{-1}) in such patients was significantly high

Table 5. Data on renal transplant patients with and without rejection during the first three months after transplantation.

	With rejection	Without rejection
n	6	13
Age, years (mean ± s.d.)	30·0 ± 7·8	30·6 ± 8·1
Related/cadaveric graft, no.	3/6	5/13
HLA-AB mismatches, mean no.	1·8	2·9
HLA-DR mismatches, mean no.	0·5	0·8
Cortisol concn, ng mL^{-1} (mean ± s.d.)		
Pre-transplant	129·3 ± 34·8	125·5 ± 41·5
Post-transplant	5·6 ± 3·1	3·4 ± 1·5[a]
Lymphocyte response to prednisolone (ID50)	843·3 ± 383·8	258·2 ± 423·6[b]

[a] Significantly different from 'with rejection' patients ($P < 0·05$).
[b] Significantly different from 'with rejection' patients ($P < 0·02$).

(38·2%). This prednisolone-resistant population (38·2%) in chronic renal failure is similar to the incidence of rejection (31·6%) in the patients after renal transplantation. In addition, a statistically significant high incidence of acute rejection was observed in low lymphocyte-responders to prednisolone in this and our previous studies (Kozaki et al 1989). These observations suggest that insufficiency of immunosuppression, which results in acute allograft rejection, may occur mainly because of resistance to prednisolone rather than resistance to cyclosporin. The relationship between lymphocyte-sensitivity to cyclosporin and the incidence of rejection episodes after renal transplantation remains a problem, but the study should also be carried out on recipients undergoing cyclosporin monotherapy.

HPA-sensitivity during the treatment with prednisolone cumulating from 300 to 700 mg, as assessed by serum cortisol concentration, was suggested to be critical for the graft outcome (Kozaki et al 1989; Oka et al 1990). This period corresponds roughly to three to 12 days after transplantation. There is a well-established negative feedback mechanism (Haynes & Murad 1985) involving suppression of the HPA axis during long-term or high-dose administration of prednisolone. A dull response of the HPA axis to prednisolone may result from impaired negative-feedback mechanisms via steroid receptors. HPA resistance as indicated by zero suppression with dexamethasone is reported to be associated with a decreased sensitivity of lymphocyte to steroid-induced immunosuppression (Lowy et al 1984, 1985).

Neither HLA-AB nor DR mismatch numbers between donors and recipients significantly affected graft outcomes in the present study. This suggests that, if an immunologically appropriate, graft has been donated, the pharmacodynamic factors might be the most important criteria for the prognosis of graft outcome. Up until now, no significant relationship had been demonstrated between the plasma concentration of prednisolone and its therapeutic efficacy (Gambertoglio et al 1980). Furthermore, the biological effects cannot be derived directly from the measured concentrations of prednisolone in patients with the nephrotic syndrome for several reasons (Moothy et al 1976; Rosner et al 1982; Frey & Frey 1985). Therefore, prednisolone pharmacodynamics, such as those described in this study and those reported by others (Dumble et al 1983, 1986; Lowy et al 1984, 1985; Langhoff et al 1986), should be evaluated widely in renal transplant recipients and in patients with chronic renal failure awaiting renal transplantation.

There have been a number of approaches to establish biochemical (White et al 1981; Edwards et al 1983; Bourbouz et al 1985; Uchida et al 1985; Lente et al 1986; Krishna et al 1987; Lin et al 1989) and cytological (Bogman et al 1989; Segasothy et al 1989) markers for prediction of acute renal allograft rejection. Most of these, however, become positive as a result of immunological or inflammatory destruction of the graft caused by rejection. On the contrary, our approach is based on the intrinsic sensitivity of the patient to prednisolone and it could be possible to determine high-risk patients before operation. Thus, the glucocorticoid-sensitivity test may give some valuable information for individual dosage adjustments of prednisolone before operation. High responders to glucocorticoid may be able to receive reduced starting doses of prednisolone which may prevent side effects, while low responders should be treated with higher doses or other immunosuppressants to which their peripheral blood lymphocytes are susceptible.

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