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Changes in the Hypothalamic–Pituitary–Gonadal Axis in Men After Cadaver Kidney Transplantation and Cyclosporine Therapy

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ABSTRACT: A variety of plasma androgens, estradiol, follicle-stimulating hormone, luteinizing hormone, prolactin, cortisol, and thyroid parameters were examined in 10 men followed serially before and after cadaver kidney transplantation. Before transplantation, plasma testosterone levels were below normal in 8 of the 10 men. Free testosterone, follicle-stimulating hormone, and luteinizing hormone were at the lower range of normal values, yet plasma estradiol levels were elevated 3-fold, and prolactin levels were also high. One month after transplantation, all hormones measured were suppressed, probably reflecting high-dose steroids and multiple-drug regimens used in the period following the operation. After 3 months, when other immunosuppressants were reduced and cyclosporine dosage was stabilized, plasma testosterone, androgens, follicle-stimulating hormone, and luteinizing hormone levels were restored toward normal. After 12 months, plasma testosterone levels exceeded pretransplant levels. Plasma estradiol and prolactin levels dramatically decreased after transplantation and remained in the normal range thereafter. These data indicate that abnormalities of plasma estradiol and prolactin levels observed in patients with end-stage renal disease are restored toward normal after cadaver kidney transplantation. Androgen levels that were suppressed in the period immediately after transplantation were restored to normal levels in the succeeding months despite chronic usage of cyclosporine, suggesting that cyclosporine, in currently used doses, does not prevent the restoration of the hypothalamic–pituitary–testicular axis.

Key words: Renal transplantation, hormonal changes, androgens, estrogen.

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Gonadal dysfunction in men with chronic renal failure has been well documented (Holdsworth et al, 1977, 1978; Van Kammen and Thijssen, 1978; Cowiden et al, 1981; Handelsman et al, 1984; Handelsman, 1985). Impaired spermatogenesis, loss of libido, and low plasma testosterone levels have been reported in men with chronic renal failure. Gonadal function frequently does not improve after dialysis, but renal transplantation often restores testicular function and fertility (Handelsman et al, 1980; Penn et al, 1980). Cyclosporine A (CSA) is now a widely used immunosuppressant drug for the long-term prevention of allograft rejection following cadaver kidney transplantation (CK-TSPL). We were concerned with reports that CSA appears to have a suppressive effect on the hypothalamic–pituitary–gonadal axis in animal studies (Rajfer et al, 1987; Seethalakshmi et al, 1987; Sikka et al, 1988a, 1988b). We tracked Leydig cell function in men who underwent CK-TSPL and who were maintained on chronic, immunosuppressant doses of CSA. The data assembled suggest that CSA does not interfere with the restoration of the pituitary–Leydig cell axis in men.

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Patients and Methods

Studies were carried out on 10 men with chronic renal failure before and after cadaver kidney transplantation (CK-TSPL). The underlying causes of renal failure in the men were chronic glomerulonephritis (6), diabetic glomerulosclerosis (3), and polycystic kidney disease (1). Mean age of patients was 32.1 years (range: 20 to 44) and average period of dialysis before CK-TSPL was 2.9 ± 0.4 years (SE), with a range of 0.5 to 10.0 years. A single, morning blood sample was taken before transplantation. This sample was drawn slowly over 10 minutes to minimize minute-to-minute variations in hormonal levels (Goldzieher, 1976). Following transplantation, whole blood samples were taken twice weekly to monitor CSA levels. Samples were centrifuged and plasma was stored at −20°C for hormonal determinations. All samples obtained from a given patient within the first month of transplantation were thawed and pooled before assay and constituted the individual patient's values reported as "1-month samples." Similarly, individual plasma samples obtained 1 to 3 months after transplantation were pooled and reported as "3-month samples." Three- to 6-month samples were similarly collected and pooled. Plasma was obtained from 8 of the 10 men during month 12. Each patient's plasma was pooled for this month

and assayed for testosterone. Similarly, plasma was obtained from two patients after 36 months.

Clinical Protocol

Cyclosporine A and azathioprine therapy were initiated the day before surgery. Cyclosporine A was administered orally at a dose of 5 mg/kg; azathioprine was given orally at a dose of 4 mg/kg. After surgery, CSA was administered at the same daily dose to maintain plasma concentrations of 150 to 250 ng/ml. After surgery, azathioprine was given intravenously at a dose 3 mg/kg for 2 days and slowly tapered thereafter. Methylprednisolone was given intravenously at a dose of 500 mg during surgery and for 3 consecutive days thereafter. Prednisone was then administered orally at a dose of 30 mg twice daily for 4 days and tapered over the next few weeks to a final dose of 10 mg per day. During the study, one patient experienced a mild rejection reaction that was promptly treated with a short, intense course of immunosuppressants. Plasma hormone levels in this patient were no different than those of the other nine patients.

Hormone Assays

Testosterone was measured with a double-antibody procedure using a commercial radioimmunoassay kit (RSL, Los Angeles, CA). Intra-assay variation was 7.5%, interassay variation was 12.6%, sensitivity was 0.07 nmol/L. Free testosterone was determined by equilibrium dialysis (Chopra et al, 1972); Intra-assay and interassay variations were 4.5% and 6.5%; sensitivity was 0.69 pmol/L. Dehydroepiandrosterone sulfate (DHEAS) and estradiol were assayed by a direct solid-phase radioimmunoassay procedure on antibody-coated tubes with a commercial kit (Diagnostic Products Corporation, Los Angeles, CA). Androstenedione was measured using an accelerated double-antibody polyethylene glycol system with a commercial radioimmunoassay kit (Diagnostic Systems Laboratories Inc., Webster, TX). Plasma 3α-androstanediol glucuronide (3α-diol G) was measured using the method of Samojlik and coworkers (1984). Sex hormone-binding globulin (SHBG) was determined in plasma by an iodinated immunoradiometric assay based on ligand-coated tubes and three monoclonal antibodies, one iodine-125-labeled, the other two linked to a ligand (Diagnostic Products Corporation). Intra-assay and interassay variations were 3.8% and 6.2%; sensitivity was 0.04 nmol/L. Plasma cortisol, thyroxine, and triiodothyronine were measured using a magnetic solid-phase radioimmunoassay kit (CIBA Corning Diagnostic, Medfield, MA). Plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were determined using a double-antibody procedure radioimmunoassay kit (Serono Diagnostics, Randolph, MA). For FSH, intra-assay and interassay variations were 7.9% and 12.1%; sensitivity was 1.0 mIu/ml. For LH, intra-assay and interassay variations were 3.5% and 5.2%; sensitivity was 0.47 mIu/ml. Plasma prolactin was measured using an immunoradiometric assay magnetic solid-phase radioimmunoassay kit (Serono Diagnostics). Plasma creatinine was determined using a standard auto-analyzer method (Technicon, Tarrytown, NY).

Data Analysis

Statistical comparison between basal hormone values and levels 1, 3, and 6 months after CK-TSP was performed for all hormones using the repeated measures ANOVA (Abacus Concept, Inc., Berkeley, CA) This method provides an F test as well as the epsilon-adjusted Greenhouse-Geisser (conservative) and Hunyh-Feldt (liberal) estimates of probability. Data also were analyzed via Tukey boxplots and Wilk's Test for normality. By using adjusted Greenhouse-Geisser and Hunyh-Feldt estimates, probability levels were analyzed and were found to be significant in all cases. The repeated measures ANOVA indicated that there were significant changes over time and significant differences between months 1 and 6 and months 1 and 12. Results were compared at the $P < 0.05$ level of significance.

Results

Serial plasma hormone levels before and after CK-TSPL are summarized and compared with normal adult male values in Table 1. Before transplantation, the average plasma testosterone level was 11.9 nmol/L, at the lower range of normal. However, 8 of the 10 patients exhibited testosterone levels that were in the hypogonadal range. Free testosterone levels were similarly low in six of the eight men examined in the basal state, although the mean level was 309 pmol/L. Follicle-stimulating hormone and LH levels were normal. The striking finding was that of elevated plasma estradiol levels (462 ± 71 pmol/L) and mildly elevated prolactin levels.

One month after CK-TSPL, plasma concentrations of most steroid hormones and SHBG were significantly suppressed compared to pretransplantation levels. However, after 3 months, most hormone levels began to return to pretransplantation levels. Six months after CK-TSPL, testosterone, free testosterone, 3α-diol G, SHBG, and cortisol returned to or exceeded baseline levels and were significantly higher than the levels 1 month after CK-TSPL. After 12 months, seven of the eight men exhibited plasma testosterone levels in the eugonadal range; mean testosterone level at that time was 19.5 ± 2.5 nmol/L (Fig 1). We examined plasma testosterone concentrations in two men 36 months after transplantation: values were 12.5 and 17.1 nmol/L, respectively. Plasma 3α-diol G levels were greatly suppressed in the first month after transplantation, dropping from 32 ± 3.7 to 16.7 ± 1.5 nmol/L. The recovery of this androgen metabolite after 6 months did not parallel that of testosterone. Androstenedione and DHEAS levels were greatly decreased in the first months after CK-TSPL, then both levels increased from their low points but remained on the low side of normal, probably reflecting continued adrenal suppression by exogenous steroids. An exception to this pattern of suppression followed by recovery was observed for estradiol, which was very high in the basal state (462 ± 71 pmol/L) and restored promptly to normal levels of 70.0 ± 6.2 pmol/L after CK-TSPL, and remained at normal levels thereafter. Serial values of plasma thyroxine and triiodothyronine studied 1, 3, and 6 months after CK-TSPL show a similar pattern of suppression in the postoperative

Table 1. *Plasma hormone profiles of 10 men with chronic renal failure before and after cadaver kidney transplant (mean ± SE)*

Hormone	Normal range	Before transplant	After transplant (mos) 1	3	6	12
Free testosterone (pmol/l)	190–400	309 ± 163	121 ± 42*	461 ± 101†	461 ± 101†	—
Androstenedione (nmol/l)	2.0–9.3	4.4 ± 0.5	1.1 ± 0.3*	1.6 ± 0.4	2.9 ± 0.6†	3.9 ± 0.5*
DHEAS (μmol/l)	2.2–15.2	5.8 ± 0.9	1.5 ± 0.3*	1.7 ± 0.4	2.5 ± 0.3†	—
Estradiol (pmol/l)	22–160	462 ± 71‡	187 ± 51*	93 ± 22†	70 ± 6.2†	—
SHBG (nmol/l)	10–73	34 ± 3.9	22 ± 3.2*	28 ± 2.8†	31 ± 3.7†	—
3α-androstanediol glucuronide (nmol/l)	2.4–43	32 ± 3.7	4.7 ± 1.5*	15.3 ± 1.6*†	28.0 ± 3.1†	—
Cortisol (nmol/l)	140–700	417 ± 77	208 ± 23*	295 ± 38†	482 ± 24†	—
Thyroxine (nmol/l)	57–161	88 ± 9.1	52 ± 4.8*	63 ± 6.2†	73 ± 6.9†	—
Triiodothyronine (nmol/l)	1.4–3.1	1.7 ± 0.2	1.1 ± 0.1*	1.5 ± 0.1†	1.7 ± 0.1†	—
FSH (iu/l)	2.0–25	2.0 ± 0.7	2.2 ± 0.8	6.2 ± 1.2*†	7.7 ± 1.8*†	—
LH (iu/l)	3.0–18	5.7 ± 1.9	5.2 ± 0.7	6.8 ± 1.4	10.4 ± 0.9*†	—
Prolactin (μg/l)	2.0–14	16.9 ± 1.9	8.5 ± 1.1*	10.7 ± 0.8	12.1 ± 1.0†	—

DHEAS = dehydroepiandrosterone sulfate; SHBG = sex hormone-binding globulin; FSH = follicle stimulating hormone; LH = luteinizing hormone; iu = international units.

* Value significantly different from basal value at $P < 0.05$.

† Value significantly different from 1 month value at $P < 0.05$.

‡ Basal value significantly different from normals at $P < 0.05$.

period with a gradual return toward normal levels after 6 months.

Basal values of FSH and LH were in the low normal range, whereas prolactin was elevated (16.9 ± 1.9 μg/L). Little change in FSH and LH was noted in the first months after transplantation; however, 6 months after CK-TSPL, values of plasma FSH increased 3.5-fold and LH rose 2-fold ($P < 0.05$) By contrast, post-CK-TSPL plasma prolactin levels came down to 12.1 ± 1.0 μg/L after 6 months.

During the 12-month period after CK-TSPL, blood concentrations of CSA remained stable at 150 to 250 ng/ml. Plasma levels of creatinine were elevated (680 ± 140 μmol/L) before transplantation, but were greatly lowered to 172 ± 30 1 month after CK-TSPL, and averaged 162 ± 16 after 6 months. In 5 of the 10 men, creatinine values before transplantation were very high; in these men basal levels of LH and FSH were most suppressed (not shown).

Discussion

Gonadal dysfunction is evident in men with chronic renal failure. Previous studies have demonstrated impaired spermatogenesis with histologic evidence of spermatogenic arrest, along with testicular atrophy, and loss of libido and potency (Handelsman et al, 1984; Handelsman, 1985). The endocrine features of uremic hypogonadism include decreased plasma concentrations of total and free testosterone, normal sex hormone-binding globulin, concentration and elevated levels of luteinizing hormone, follicle-stimulating hormone, and prolactin (Holdsworth et al 1977, 1978; Van Kammen et al, 1978; Cowiden et al 1981; Handelsman, 1985). Our patients did not demonstrate elevated FSH and LH levels in the pretransplant dialysis phase of their chronic renal disease. Gonadal function usually does not improve with dialysis, but renal transplantation often restores testicular function and fertility (Holdsworth et al, 1978; Handelsman et al, 1980). Immunosuppressive therapy with azathioprine and prednisone used for transplantation does not appear to cause gonadal toxicity or teratogenicity in the offspring of male transplant recipients (Penn et al, 1980).

In recent years, CSA has been widely used in combination with azathioprine and prednisone to prevent allograft rejection. Unfortunately, this drug has been reported to have suppressive effects on the hypothalamic–pituitary–gonadal axis in animal studies (Rafjer et al, 1987; See-

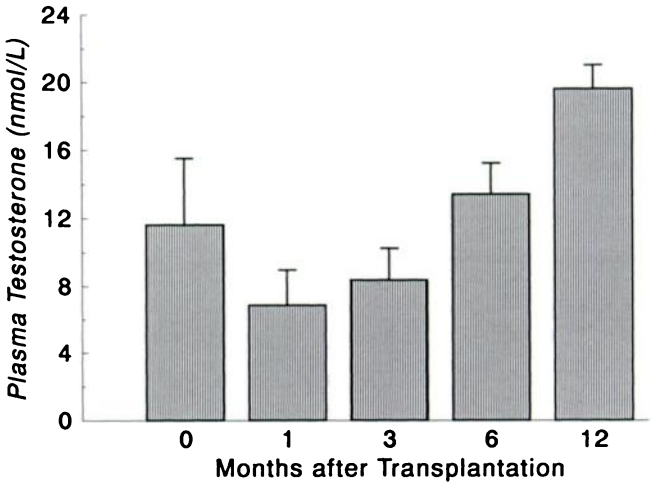

FIG. 1. Serial plasma testosterone levels before and after successful cadaver kidney transplantation in 10 men. Values are presented as the mean ± SE.

thalakshmi et al, 1987; Sikka et al, 1988a). Little is known about the effects of CSA on human gonadal function, although hirsutism is a well known side effect in women (Handelsman et al, 1984; Schmidt et al, 1986). In the current study we were anxious to determine if CSA interferes with the restoration of the pituitary–Leydig cell axis after renal allograft transplantation. We serially followed plasma levels of a variety of hormones before and after CK-TSPL.

The 10 men in our study were all of reproductive age and exhibited the common causes of modern-day chronic renal failure. Plasma testosterone and free testosterone levels were borderline to low in the basal (chronic dialysis) state, and our patients exhibited increased plasma estradiol and prolactin levels in the basal state, possibly accounting for the suppression of FSH and LH levels. Of interest, high levels of FSH and LH that had been observed previously in men with chronic renal disease (Holdsworth et al, 1977; Cowiden et al, 1981; Handelsman, 1985) were not present in our patients.

In the first weeks following renal transplantation, multidrug regimens were used to support the patient and to prevent allograft rejection. High doses of steroids were used in combination with azathioprine and CSA. It thus was not surprising that 1 month after CK-TSPL all of the sex hormones, adrenal cortical hormones, and thyroid and pituitary hormones were suppressed. During the next months, when allograft survival was a reality and doses of supportive drugs were tapered, it was reassuring to determine that testosterone and free testosterone levels began to return to basal values and then exceeded the pretransplantation levels after 12 months. Many clinical and hormonal changes were occurring during the period after transplantation that could explain the changes in plasma testosterone levels. First, prolactin and estradiol levels promptly returned to normal after CK-TSPL, eliminating any suppressive effect these hormones might have had on Leydig cell function. Second, nutritional factors improved, as did a "state of well-being." These factors could have restored gonadotropin secretion, with secondary Leydig cell response. Decreasing use of prednisone, azathioprine, and other supportive pharmacologic agents in the months after CK-TSPL also could have removed any suppressive effects of these agents on the hypothalamic–Leydig cell axis. Of great interest to us was the fact that these hormonal changes took place despite continued administration of CSA. This observation strongly suggests that CSA, in clinically used doses that achieve blood levels of 150 to 250 ng/ml, does not suppress the hypothalamic–pituitary–Leydig cell axis in men.

Our studies demonstrated very high plasma estradiol levels in all 10 men before to CK-TSPL. Plasma estradiol levels promptly returned to normal male levels after CK-TSPL and remained there in the succeeding months. The mechanism of elevated plasma estradiol levels in men with chronic renal disease currently is not known. Similarly, basal plasma prolactin levels were above the normal range in our patients while on dialysis (Schernthaner et al, 1979). These values decreased after transplantation, but demonstrated some secondary elevations over the ensuing months, although not to pretransplant levels.

In order fully to assess the hypothalamic–pituitary–gonadal axis before and after transplantation in our patients, it would have been desirable to examine sperm counts and motility at various times. Unfortunately, these determinations were not made in our study. Thus, our conclusions are limited to the hypothalamic–pituitary–Leydig cell axis, although recent studies by Haberman and colleagues (1991) suggest that CSA does not impair spermatogenesis and fertility after CK-TSPL.

Finally, one might question whether the steroidogenic axis may have been partially suppressed by CSA, and that androgen and pituitary hormone levels would have rebounded more quickly or to higher levels were it not for the mildly suppressive effect of CSA. At present, we cannot clinically test this hypothesis since CSA represents the most effective immunosuppressant therapy following CK-TSPL, and it is not possible to withhold its use. Perhaps in the future other therapies might be available that would enable us to carry a cohort of CK-TSPL recipients through the postoperative period without CSA so that we could make this determination. For the present we remain with the comforting data that CSA usage is associated with rebound of the hypothalamic–pituitary–Leydig cell axis toward normal, although we cannot exclude partial blunting of the axis by this agent.

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