Does kidney transplantation normalise cortisol metabolism in apparent mineralocorticoid excess syndrome?

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ABSTRACT. The syndrome of apparent mineralocorticoid syndrome (AME) results from defective 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2). This enzyme is co-expressed with the mineralocorticoid receptor (MR) in the kidney and converts cortisol to its inactive metabolite cortisone. Its deficiency allows the unmetabolized cortisol to bind to the MR inducing sodium retention, suppression of PRA and hypertension. Thus, the syndrome is a disorder of the kidney. We present here the first patient affected by AME cured by kidney transplantation. Formerly, she was considered to have a mild form of the syndrome (Type II), but progressively she developed renal failure which required dialysis and subsequent kidney transplantation. To test the ability of the transplanted kidney to normalise the patient's cortisol metabolism, we gave, in two different experiments, 25 and 50 mg/day of cortisone acetate or 15 and 30 mg/day of cortisol after inhibition of the endogenous cortisol by synthetic glucocorticoid (methylprednisolone and dexamethasone). The AME diagnostic urinary steroid ratios tetrahydrocortisol+5αtetrahydrocortisol/tetrahydrocortisone and cortisol/cortisone were measured by gas chromatography/mass spectrometry. Transplantation resulted in lowering blood pressure and in normalization of serum K and PRA. After administration of a physiological dose of cortisol (15 mg/day), the urinary free cortisol/cortisone ratio was corrected (in contrast to the A-ring reduced metabolites ratio), confirming that the new kidney had functional 11β-HSD2. This ratio was abnormally high when the supra-physiological dose of cortisol 30 mg/day was given. After cortisone administration, the tetrahydrocortisol+5αtetrahydrocortisol/tetrahydrocortisone ratio resulted normalised with both physiological and supra-physiological doses, confirming that the hepatic reductase activity is not affected. As expected, the urinary free cortisol/ cortisone ratio was normal with physiological, but increased after supra-physiological doses of cortisone. The described case indicates a normalisation of cortisol metabolism after kidney transplantation in AME patient and confirms the supposed pathophysiology of the syndrome. Moreover, it suggests a new therapeutic strategy in particularly vulnerable cohorts of patients inadequately responsive to drug therapy or with kidney failure.

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INTRODUCTION

Terminal cortisol metabolism in man is a summation principally of renal and hepatic transformation. The principal enzymatic players are hepatic 11β-hydroxysteroid dehydrogenase (now referred as 11β-HSD1) and a distinct renal enzyme recently characterized and named 11β-HSD2 (1, 2). Delineating the activities of the two principal enzymatic sites is a major problem since it is not possible in humans to test the individual response of the two organs to all precursors. The end-result of cortisol metabolism is a pattern of urinary steroids that reflects the cumulative metabolism of both organs. What is known is that hepatic 11β-HSD1 is principally reductive (from cortisone to cortisol) and renal 11β-HSD2 is oxidative (from cortisol to cortisone).

Defective 11β-HSD2 is the cause of the apparent mineralocorticoid excess syndrome (AME syndro-

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me) (3). Unmetabolized cortisol within the kidney strongly binds to the mineralocorticoid receptor (MR) in the distal tubule and collecting duct, inducing sodium retention. Cortisone, the product of 11β-HSD2 activity, is receptor inactive, allowing MR to be exclusively occupied by aldosterone (4). AME syndrome is thus a disorder of the kidney and we reasoned that it would be cured by transplantation. Three years ago a patient of ours was given a new kidney which resulted in normalization of blood pressure and the renin-angiotensin system function. Using exogenous cortisol and cortisone administration, the current study analyzes to what extent the transplanted kidney normalises overall cortisol metabolism in the patient. An abstract of the studies has been published as a letter to a journal editor (5).

METHODS

Patient

Born in 1960, patient FP is probably the oldest known individual with AME syndrome. The diagnosis of AME was made when she was 27 years old. At that time, her blood pressure was 170/100 mmHg and she was treated with nifedipine SR (20 mg tid). No pathological data were found at clinical examination. Plasma creatinine was 1.8 mg/dL (159 µmol/l). PRA and urinary aldosterone were suppressed (PRA<0.05 ng/ml/h, equivalent to <0.014 ng/l/sec; urinary aldosterone <3.0 µg/24 h, equivalent to <8.31 nmol/24 h), both under baseline conditions and after orthostatism stimulus, low salt diet (10 mmol of sodium/day), or furosemide 40 mg i.v. Initial studies seemed to rule out 11β HSD deficiency because the tetrahydrocortisol (THF)+5αtetrahydrocortisol (5αTHF)/tetrahydrocortisone (THE) ratio were within the normal range of the laboratory performing the assay (6). The disorder was considered due to a generalized deficiency of A-ring reduction and was termed AME Type II. However in later studies, the patient demonstrated a prolonged half-life for 11α^{3}H cortisol (7) and recent results from our laboratory showed mildly increased ratios of cortisol metabolites to cortisone metabolites. The (THF+5αTHF)/THE ratio and the ratio between the free urinary fraction of cortisol (UFF) and cortisone (UFE), both measured by gas chromatography/mass spectrometry (GC/MS), were increased. At this time (THF+5αTHF)/THE was 4.25 (normal 1.35±0.3) and UFF/UFE was 1.45 (normal 0.54±0.3). Serum K was 2.6 mmol/l. Latterly, diagnosis has been confirmed by molecular studies performed at Queen Elizabeth Hospital, Birmingham, UK, by Prof. Paul M. Stewart. The gene error is a point mutation in exon 5 of the 11β-HSD2, resulting in an amino acid substitution of arginine to cysteine at position 279 of the enzyme protein (R279C) (8).

The patient was treated with dexamethasone (0.5 mg tid for one month then 0.5 mg/day) which normalised serum K (3.8 to 4.5 mmol/l) and progressively improved control of blood pressure. Subsequently, it became necessary to add atenolol (100 mg/day) and enalapril (20 mg/day) for better control of the blood pressure values, but plasma creatinine gradually increased to 3.5 mg/dl. For three years the patient showed good response to treatment and kidney function improved (plasma creatinine 1.8 mg/dl).

In 1991 FP suddenly developed terminal renal failure and plasma creatinine rose to 13 mg/dl (1.15 mmol/l). Chronic hemodialysis was started at a rate of three times per week. In the years in which the patient had been subjected to hemodialysis, withdrawal of hypotensive drugs became necessary because of prolonged hypotension, and only dexamethasone 0.5 mg on the day prior to dialysis was administered. In May 1996 the patient underwent kidney transplantation and a cadaveric kidney was implanted by standard techniques. The patient's natural kidneys were not removed. Currently, blood pressure is normal in absence of any anti-hypertensive pharmacological treatment.

Study design

To test the ability of the transplanted kidney to normalise the patient's cortisol metabolism, the patient was referred to the Department of Nephrology. She was normotensive, with no anti-hypertensive or diuretic drugs administered. Plasma creatinine and serum K were in the normal range (1.1 mg/100 ml and 4.0 mmol/l), respectively. Normal post-transplantation immunosuppressant therapy was carried out by cyclophosphamide and methylprednisolone (9). The administration of methylprednisolone as immunosuppressant complicated the present investigation since it precludes measurement of true basal cortisol and cortisol metabolite ratios. Such ratios had to be measured either during suppression alone or suppression with exogenously administered cortisol.

Experiment 1: Cortisone acetate administration

Post-transplantation, the patient was taking 16 mg/day of methylprednisolone as immunosuppressant therapy which was able to suppress endogenous cortisol production as reflected by diminutive excretion of cortisol and cortisone metabolites.

Twenty-five and 50 mg/day of cortisone acetate were orally administered on three different days one day apart with administration at 8:00 am and 4:00

pm. Urine was collected for 24 hours following initial ingestion. Moreover three basal (but suppressed) 24-hours urinary collections were made before and between the days of drug administration. Serum K, PRA and blood pressure were monitored.

Experiment 2: Cortisol administration

The therapeutic doses of methylprednisolone that the patient was taking when the second experiment started (6 mg/day) resulted in incomplete suppression of the ACTH-adrenal axis, so we included 3 mg/day of dexamethasone for three days before and during the experimental days to achieve adequate suppression.
Fifteen and 30 mg/day of cortisol were orally administered two days apart divided in two administrations at 8 am and 4 pm and urine collection was carried out. The 15 mg dose approximates the secretion rate for women (6-30 mg, mean 17 mg/day) and could be considered physiological. The 30 mg dose is probably supra-physiological. Serum K, PRA and blood pressure were monitored.

Analytical methodology

Steroids excreted in urine were measured by the specific GC/MS methods described by Shackleton (10) and Palermo *et al.* (11). The former reference describes measurement primarily of conjugated steroids (like THF, 5αTHF, THE etc.) while the other details a method for accurate measurement of urinary free cortisol and cortisone.
PRA was tested by a commercial RIA kit (RADIM, Pomezia, Italy). Plasma creatinine and serum K were measured in the laboratory of the Department of Nephrology, University of Sassari.

RESULTS

Blood pressure, potassium and PRA

The values obtained for serum K and PRA post-operatively were consistently normal, as was the blood pressure. Normal values have persisted in the three years since surgery and no changes were recorded after cortisol or cortisone administration at high doses (Table 1).

Basal steroid excretion and steroid excretion post-operatively

Tables 2 and 3 show urinary steroid concentrations and AME diagnostic ratios (cortisol metabolites/cortisone metabolites) under a variety of regimes. The first column represents pre-renal failure, pre-transplantation basal values for the patient. It can be seen that the ratio of "cortisol metabolites" to "cortisone metabolites" are in all cases raised. However, the elevated values were modest compared to most individuals with the disorder (12). The last column of the Table lists our laboratory's normal female excretions for the steroids of interest. Two values for cortisol and cortisone are given. Generally, a large proportion of cortisol and cortisone are excreted conjugated with glucuronic and sulfuric acids (11), frequently up to 80%. This fraction is measured after β-glucuronidase and sulfatase hydrolysis, and the measured steroids are referred to as urinary total cortisol (UTF) and urinary total cortisone (UTE). The portion of cortisol and cortisone excreted unconjugated are referred to as urinary free cortisol (UFF) urinary free cortisone (UFE).
The need of maintaining immunosuppression following transplantation necessitated methylprednisolone administration. Thus, post-operatively, uncompromised basal values for steroid excretion (and the AME diagnostic ratios) could not be obtained. Cortisol/cortisone metabolite ratios were determined on two occasions during methylprednisolone alone or methylprednisolone+dexamethasone suppression. Interestingly, in the latter case the (THF+5αTHF)/THE ratio remained at pre-operative levels. The α+β-cortol/α+β-cortolone ratios also remained moderately elevated.
The UFF/UFE ratio was unmeasurable because the excretion of the free steroids was below the detection limit of the method (3 μg/24 h).

Metabolism of exogenous cortisone post-operatively

Table 2 shows the excretion of cortisol metabolites after two different doses of cortisone acetate. THF+5αTHF/THE and the α+β-cortol/α+β-cortolone ratios were at the upper limit of normal. UFF/UFE was normal after the lower dose of the drug, but it

Table 1 - *Blood pressure, plasma renin activity and serum potassium.*

	Pre-transplant.	Suppressed (methylprednis.)	Suppressed (methylp.+Dxt)	After cortisol (30 mg)	After cortisone (50 mg)	Normal values
Mean blood pressure (mmHg)	135	92	90	90	90	<100
Plasma renin activity (ng/ml/h)	<0.005	1.7	1.5	1.38	1.42	1.0-2.5
Serum potassium (mmol/l)	2.6	4.1	4.1	3.8	3.9	3.5-5.1

Table 2 - Excretion of cortisol metabolites (μg/24 h) and ratios of excretions following cortisone administration.

	Pre-transplant. (basal)	Post-transplant. (suppressed)	Cortisone (25 mg)	Cortisone (50 mg)	Normal values (mean and range)
THF	656	91	2034	5383	1076 (458-1907)
α-THF	433	ND	1604	568	713 (142-1589)
THE	255	ND	2077	3235	2126 (727-3815)
α-cortol	167	ND	352	352	223 (122-365)
β-cortol	155	ND	230	400	342 (124-690)
α-cortolone	255	ND	718	737	910 (467-1564)
β-cortolone	89	ND	259	638	480 (216-814)
UTF	73	ND	122	297	74 (25-115)
UTE	53	ND	204	233	143 (49-215)
Total	2136	<100	7600	11843	6683 (3649-8099)
UFF	42	ND	29	74	23 (8-61)
UFE	29	ND	100	66	49 (21-107)
UTF/UTE	1.38	ND	0.6	1.27	0.50 (0.35-0.70)
UFF/UFE	1.45	ND	0.29	1.12	0.53 (0.33-0.67)
(THF+αTHF)/THE	4.27	ND	1.78	1.82	0.88 (0.55-1.5)
Cortols/cortolones	0.93	ND	0.59	1.28	0.41 (0.27-0.41)

was higher than normal after 50 mg. The UTF/UTE result was normal with 25 mg and increased after 50 mg.

Metabolism of exogenous cortisol post-operatively

The excretions of metabolites following 15 mg and 30 mg administrations of cortisol are listed in Table 3. Following the 15 mg administration, the ratios of A-ring reduced steroids did not differ markedly from pre-operative and post-operative methylprednisolone/dexamethasone suppressed values. This was true both for the THF+5αTHF/THE ratio and the α+β-cortol/α+β-cortolone ratio. The UTF/UTE ratio remained elevated following 15 mg cortisol administration, but significantly, the UFF/UFE ratio was normalised, mimicking the value found in unaffected individuals. The 30 mg cortisol dose resulted in THF+5αTHF/THE, UTF/UTE and UFF/UFE being considerably higher than values found pre-operatively.

DISCUSSION

Before discussing whether renal transplantation normalises exogenous cortisol and cortisone metabo-

Table 3 - *Excretion of cortisol metabolites (μg/24 h) and ratios of excretion following cortisol administration.*

	Pre-transplant. (basal)	Post-transplant. (suppressed)	Cortisol (15 mg)	Cortisol (30 mg)	Normal values (mean and range)
THF	656	274	2962	4664	1076 (458-1907)
α-THF	433	113	1960	2653	713 (142-1589)
THE	255	108	1467	1971	2126 (727-3815)
α-cortol	167	42	455	728	223 (122-365)
β-cortol	155	32	412	552	342 (124-690)
α-cortolone	255	68	544	748	910 (467-1564)
β-cortolone	89	20	190	246	480 (216-814)
UTF	73	9	210	728	74 (25-115)
UTE	53	8.5	157	368	143 (49-215)
Total	2136	674	8357	12659	6683 (3649-8099)
UFF	42	ND	38	119	23 (8-61)
UFE	29	ND	62	101	49 (21-107)
UFF/UFE	1.38	1.05	1.33	1.98	0.50 (0.35-0.70)
UFF/UFE	1.45	ND	0.61	1.1	0.53 (0.33-0.67)
(THF+αTHF)/THE	4.27	3.59	3.80	3.86	0.88 (0.55-1.5)
Cortols/cortolones	0.93	0.84	0.61	1.28	0.41 (0.27-0.41)

lism in the AME patient, it is important to know whether the doses of the two drugs were physiological and equivalent. Our two cortisol doses (15 and 30 mg) were chosen to correspond to a typical normal cortisol secretion for an adult female (range 6 - 30 mg/24 h, mean 17 mg/24 h). The high cortisol dose was twice the low one. Previous experience had shown that because of a relatively poor absorption, much more cortisone acetate has to be given to give an equivalent "absorbed" amount of corticosteroid (about 1.7 times) (13). Thus, our low cortisone acetate dose was 25 mg and high dose 50 mg/day.

The total cortisol metabolite excretion values affirm that we chose the correct and equivalent dose of cortisone acetate (Tables 2, 3). On the day of low dose administration, the excretion of cortisol and cortisone metabolites was 8357 and 7600 µg/day, respectively, figures we consider comparable. That the total metabolite excretion does not match the doses is not surprising. The absorption of both steroids is not quantitative, particularly of cortisone acetate; the steroid metabolites were only measured on the days of administration whereas they are also excreted on the day immediately following; and finally, our panel of steroids includes important individual metabolites but leaves out many quantitatively less important ones. The quantitative excretion of metabolites following the high doses of cortisol and cortisone was also comparable at 12658 and 11843 µg/day, respectively. Confirmation that the low doses were in the physiological range also comes from the UFF and UFE levels following steroid administration. These levels were well within the normal range for natural hormone excretion of female adults. In contrast, the high doses (cortisol 30 and cortisone 50 mg/day) gave values well above the normal range.

Administration of cortisol post-operatively did not give the expected universal correction of urinary steroid metabolite ratios. It was probably naive to expect that it would, due to the complexity of cortisol metabolic processes as a whole. For one thing, only a functioning kidney 11β-HSD2 had been provided; the defective enzyme was still present in its other sites (e.g., intestine and colon) and the original kidneys remained *in situ*. The second major player, hepatic, but almost universal, 11β-HSD1 was unchanged by the operation. It would be expected to favor 11-oxo-steroid reduction as usual. Another complication is the presence of the immunosuppressive corticosteroid methylprednisolone which may or not affect 11β-HSD activity. One indication of the new kidney's normal 11β-HSD2 function was noted. The UFF/UFE ratio following 15 mg cortisol administration was completely normal, as previously mentioned, and the measured amount of free cortisol and cortisone were identical to those expected in normal individuals without supplementation. We believe that this indicates that at a cortisol dose that mimics normal physiological synthesis, the fraction of administered cortisol which escapes hepatic first-pass metabolism (A-ring reduction and conjugation) and reaches the kidney is normally oxidized and excreted. This was not the case following the higher cortisol dose (30 mg/day), since UFF/UFE remained elevated and the excreted amounts of the free steroids were well above normal. At this dose we are probably witnessing substrate saturation as elevated F/E ratios result when supra-physiological levels of cortisol are given to normal individuals or in the case of hypercortisolism due to the ectopic ACTH form of Cushing's disease (14, 15). Interestingly, the expressed "R279C" enzyme showed activity appropriate for substrate overload. Unlike other 11β-HSD2 mutations, it had normal Km but attentuated Vmax (8). This latter feature could explain the low cortisol concentrations that can cause such enzyme saturation.

In contrast to cortisol, administration of physiological doses of cortisone after total suppression of the endogenous cortisol, resulted in normalization of both the (THF+αTHF)/THE and UFF/UFE ratios. That these would be a difference relative to cortisol metabolism is not surprising since some administered cortisone must be A-ring reduced in the liver prior to being C-11 reduced. The higher cortisone acetate dose again gave elevated (THF+αTHF)/THE and UFF/UFE ratios as expected in situations of excess of corticosteroid.

Thus, our finding suggests that the saturated (hepatic) metabolite ratio remains elevated after transplantation and following exogenous cortisol administration, but is normalised following cortisone acetate administration. In both cases the key ratio, UFF/UFE, was normal with low dose administration so it can be safely reported that the new kidney was functioning at mineralocorticoid receptor protection. While we've made our best attempt to define the overall cortisol metabolism of our patient post-operatively, caution must be exercised in reviewing the data because the patient remained on immunosuppressant corticoid throughout the study.

In its most definitive form AME is a rare but life-threatening disease. Hypertension-associated diseases of kidney, eyes, brain and heart are usually present at the time of diagnosis, both in affected children and adults. Although our patient was considered to have a mild form of the disorder because her F-metabolite/E-metabolite ratios were not greatly increased, her clinical condition had reached

a critical situation in spite of classical therapeutic treatment. Her kidney failure required immediate dialysis and subsequent kidney transplantation. The ill effects of AME in our patient have been completely cured. At the time of writing more than three years have passed since surgery and there has been no recurrence of hypertension and serum K and PRA remain normal.
The described case confirms the supposed pathophysiology of the syndrome (3) and suggests a new treatment strategy in a selected cohort of patients such as drug-unresponsive children and in patients with end-stage kidney failure.

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