Steroids 127 (2017) 31–39

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Twenty-four hour urinary cortisol excretion and the metabolic syndrome in prednisolone-treated renal transplant recipients

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ARTICLE INFO

Keywords:
Prednisolone
HPA-axis
Urinary cortisol excretion
Metabolic syndrome
Kidney transplantation
Cardiovascular disease

ABSTRACT

Chronic prednisolone treatment in renal transplant recipients (RTR) causes metabolic abnormalities, which cluster in the metabolic syndrome (MS). It also suppresses the hypothalamic–pituitaryadrenal (HPA)-axis. We investigated whether HPA-axis suppression, as measured by 24 h urinary cortisol excretion, is associated with presence of the MS and its individual components, in outpatient RTR with a functioning graft for > 1 year. Urinary cortisol was measured in 24 h urine, using LC–MS/MS (LOQ 0.30 nmol/L). We included 563 RTR (age 51 ± 12 years; 54% male) at median 6.0 [IQR, 2.6–11.5] years post-transplantation. MS was present in 439/563 RTR (78%). Median 24 h urinary cortisol excretion was 2.0 [IQR, 0.9–5.1] nmol/24 h. Twenty-four hour urinary cortisol excretion was independently associated with MS presence (OR = 0.80 [95% CI, 0.66–0.98], P = 0.02). It was also independently associated with bodyweight (st.β = −0.11, P = 0.007), waist circumference (st.β = −0.10, P = 0.01), BMI (st.β = −0.14, P = 0.001), fasting triglycerides (st.β = −0.15, P = 0.001), diabetes (st.β = −0.12, P = 0.005), and number of antihypertensives used (st.β = −0.13, P = 0.003). Suppressed HPA-axis activity, as reflected by decreased 24 h urinary cortisol excretion, is associated with higher prevalence of MS and its individual components (i.e. central obesity, dyslipidemia, diabetes, hypertension) in prednisolone-treated RTR. Assessment of 24 h urinary cortisol excretion by LC–MS/MS may be a tool to monitor metabolic side-effects of prednisolone in RTR.

1. Introduction

Cardiovascular disease is the leading cause of mortality after kidney transplantation, with annual risk of cardiovascular incidents and mortality being 10–50-fold higher in renal transplant recipients (RTR) than in the general population [1,2]. In recent years, many conventional risk factors, such as central obesity, dyslipidemia, hypertension, and diabetes mellitus, have been identified to increase post-transplantation cardiovascular risk. Clustering of these risk factors in the metabolic syndrome occurs frequently in RTR [3–5]. In addition, several transplantation-related risk factors have been identified, such as decreased kidney function and use of immunosuppressive drugs [2,6].

Corticosteroids are known to cause a wide range of metabolic abnormalities, including weight gain, lipid derangement, new-onset diabetes mellitus after transplantation (NODAT), and hypertension [7,8]. Therefore, there has been a great effort to get rid of corticosteroids as part of maintenance immunosuppressive regimens after kidney transplantation [7–9]. Nevertheless, it has recently been concluded that corticosteroids have to remain part of the immunosuppressive regimen in order to maintain low acute rejection rates and optimal long-term graft survival [10,11]. Unfortunately, corticosteroid dosing regimens remain empiric to date, usually with fixed doses independent of either body size and/or steroid sensitivity [12].

The most often used corticosteroids in RTR are prednisone and prednisolone. Long-term treatment with these drugs is known to suppress the hypothalamic-pituitaryadrenal (HPA)-axis, leading to reduced endogenous cortisol production [13,14]. Twenty-four hour urinary cortisol excretion is often used to measure HPA-axis activity in patients with increased or normal endogenous cortisol production [15–17]. Twenty-four hour urinary cortisol excretion may, therefore, also be used to measure HPA-axis activity in patients with suppressed endogenous cortisol production, such as prednisolone-treated RTR.

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http://dx.doi.org/10.1016/j.steroids.2017.09.001
Received 6 June 2017; Received in revised form 3 September 2017; Accepted 5 September 2017
Available online 08 September 2017
0039-128X/

Measurement of urinary cortisol in prednisolone-treated patients remains a challenge to date, because of structural similarity of both compounds. Historically used immune-based assays were susceptible to cross-reactivity of exogenous steroids, leading to gross overestimation of urinary cortisol concentrations [18,19].

Using a highly sensitive and validated liquid chromatography-tandem mass spectrometry (LC–MS/MS) method, allows for accurate separation of urinary cortisol from prednisolone and for studying HPA-axis activity in prednisolone-treated RTR. Using such a method, we aimed to investigate whether the degree of HPA-axis suppression, as measured by 24 h urinary cortisol excretion, reflects the pharmacological effects of prednisolone, and thus is associated with the metabolic syndrome and its individual components, including central obesity, dyslipidemia, hypertension, and NODAT.

2. Materials and methods

2.1. Research design and subjects

In this cross-sectional study, we invited all adult stable RTR who visited our outpatient transplant clinic between August 2001 and July 2003 and had a functioning graft for at least 1 year. A total of 606 of 847 eligible RTR (72%) signed written informed consent. The group that did not sign informed consent was comparable with the group that signed consent. Samples for determination of urinary cortisol were available in 565 RTR (93%). Additional details of this study have been published previously [3,20–22]. The Institutional Review Board approved the study protocol (METc 2001/039), which adhered to the Declaration of Helsinki.

2.2. Patient characteristics

The Groningen Renal Transplant Database contains information on all kidney transplantations performed at our center since 1968. Relevant transplantation-related characteristics were extracted from this database. Current medication was taken from the medical record. Standard immunosuppression regimen was as described previously [20]. In short, it consisted of 5–10 mg prednisolone daily in combination with either cyclosporine or tacrolimus, and/or either azathioprine or mycophenolate mofetil. Body mass index (BMI), waist circumference, and blood pressure (BP) were measured as described previously [20].

2.3. Study procedures

At the study visit, fasting blood samples and 24 h urine collections of the preceding day were taken. To ensure adequate 24 h urine collection, RTR were carefully instructed to start 24 h urine collection with emptying of the bladder, collect all subsequent urine through the next 24 h, and include the next morning's specimen on the day of their visit. Blood and urine samples were stored at −80 °C until assessment of biochemical measures for this study. Creatinine in plasma and urine were measured using a modified version of the Jaffé method (MEGA AU 510; Merck Diagnostics). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [23]. Glucose, insulin, hemoglobin A1c (HbA_{1c}), total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides, were measured as described previously [3,20–22].

2.4. Measurement of urinary cortisol

Urinary cortisol was measured by on-line solid phase extraction (SPE) coupled with liquid chromatography and tandem mass spectrometry (XLC-MS/MS). 250 μL of urine was mixed directly in an autosampler vial with an internal standard solution (cortisol-d4, Cambridge Isotope Laboratories (Andover, MA) and diluted with water to reach a final volume of 1 mL. Fifty μL of the sample was injected into the XLC-MS/MS system. Sample clean-up was performed by on-line SPE, using a Spark-Holland Symbiosis® system (Spark Holland, Emmen, the Netherlands), as described previously [24]. We used HySphere C18 HD SPE cartridges (Spark Holland, Emmen) for sample extraction and performed LC by use of a Phenomenex Luna Phenyl-Hexyl column (particle size 3 μm, 2.0 mm internal diameter by 150 mm; Waters). AJ0-4350 Security Guard Cartridges (4 × 2.0 internal diameter) were used as guards for the LC column. Detection was performed with a Quattro Premier® tandem mass spectrometer operated in positive electrospray ionization mode (Waters, Milford, MA). Cortisol and its deuterated internal standard were protonated to produce ions at the form $[M+H]^+$, with *m/z 363* and *m/z 367*, respectively. Upon collision-induced dissociation with Argon gas, these precursor ions produced characteristic product ions of *m/z* 121 for both cortisol and the deuterated internal standard. A multiple reaction monitoring mode was developed for the specific *m/z* transitions 363 → 121 and 367 → 121 (internal standard). For the aim of this study, in which high concentrations of prednisolone were present in urine, it was necessary to achieve complete chromatographic separation of cortisol and prednisolone. However, initial ionization lead to the same mass transitions for both cortisol and prednisolone. To accomplish complete separation of these molecules, an Agilent Zorbax SB-Phenyl column (particle size 1.8 μm, 2.1 mm internal diameter by 100 mm; Agilent Technologies, Santa Clara, CA) was added [25]. To minimize the effects of inter-assay variability on the data, all urine samples were analyzed in one lot. Intra- and inter-assay variation coefficients were 2.4% and 7.8% for the lower range and 1.4% and 3.8% for the higher range, respectively. Lower limit of quantitation was 0.30 nmol/L (assay linear range: 0.30–1419 nmol/L). Cortisol concentrations were stable when stored up to 7 days at 10 °C or 4 °C. At room temperature, it was stable up to 24 h. No changes in measured concentrations were observed in urine that had been subjected to 1, 2, or 3 freeze-thaw cycles. Twenty-four hour urinary cortisol excretion was calculated by multiplication of urinary cortisol concentration with urinary volume (in liters); urinary cortisol-to-creatinine ratio was calculated by dividing urinary cortisol concentration (in nmol) by urinary creatinine concentration (in mmol); body surface area (BSA)-corrected 24-h urinary cortisol excretion was calculated by dividing 24-h urinary cortisol excretion by BSA, which was calculated according to the formula of Dubois and Dubois ($BSA = (Weight^{0.425} \times Height^{0.725}) \times 0.007184$) [26].

2.5. Definitions

The metabolic syndrome was defined according to the Third Adult Treatment Panel of the U.S. National Cholesterol Education Plan (NCEP) definition [5]. This definition requires patients to have at least three of the five following criteria: (1) central obesity (waist circumference ≥102 cm for men and ≥88 cm for women); (2) serum triglycerides of ≥1.7 mmol/L, or specific treatment; (3) serum high-density lipoprotein (HDL) cholesterol < 1.03 mmol/L for males and < 1.29 mmol/L for females, or specific treatment; (4) systolic BP ≥130 and/or diastolic BP ≥85 mmHg, or treatment of previously diagnosed hypertension; (5) fasting plasma glucose ≥6.1 mmol/L, or previously diagnosed type 2 diabetes mellitus. For secondary analyses we used the International Diabetes Federation (IDF) definition [5]. The main difference between the NCEP definition and the IDF definition is that the former does not obligatory require central obesity to be present for diagnosis of the metabolic syndrome, whereas it is an obligatory requirement for the latter. Thus, the IDF definition requires patients to have central obesity and at least two of the four remaining criteria. In addition, the IDF defines central obesity as waist circumference ≥90 cm for men and ≥80 cm for women, and increased fasting plasma glucose as ≥5.6 mmol/L. Criteria for triglycerides, HDL cholesterol and blood pressure are the same for both definitions. New-onset diabetes