Assessing Systemic 11β-Hydroxysteroid Dehydrogenase With Serum Cortisone/Cortisol Ratios in Healthy Subjects and Patients With Diabetes Mellitus and Chronic Renal Failure

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11β-hydroxysteroid dehydrogenase (11β-HSD), an enzyme regulating mineralocorticoid like action of glucocorticoid, oxidizes active cortisol to inactive cortisone. Impaired activity of this enzyme is associated with apparent mineralocorticoid excess (AME) syndrome and is characterized by hypertension and hypokalemia. Recent investigations suggest the presence of hypertensive subjects with low activity of 11β-HSD. The blood concentration ratio of cortisone/cortisol reflects the overall conversion of cortisol to cortisone and may be an index to assess the systemic activity of 11β-HSD. We evaluated the peripheral blood concentration ratio of cortisone/cortisol as a possible marker to identify subjects with hypertension thought to represent impaired 11β-HSD activity. We compared this ratio in healthy subjects and patients with diabetes mellitus (DM) or chronic renal failure (CRF). Peripheral blood samples were collected from 69 healthy subjects, 44 DM, and 36 CRF patients in the morning (9:00 to 11:00 AM). Twenty-six DM patients (59%) and 32 CRF patients (89%) met the criteria for having hypertension. Serum cortisol and cortisone concentrations were determined by high performance liquid chromatography (HPLC). All values for serum cortisone and cortisol levels were within the normal range. Serum cortisone/cortisol ratio in the healthy subjects was distributed with a range of 0.113 to 0.494 (median, 0.243). Compared with healthy subjects, DM and CRF patients had significantly low ($P < .01$) serum cortisone/cortisol levels (median, 0.188 [range, 0.092 to 0.313] in DM and 0.088 [range, 0.031 to 0.140] in CRF). Bimodal distribution of cortisone/cortisol, found in DM patients with hypertension, represented high- and low-ratio groups around the border of the ratio 0.2. Kidney function, DM duration, and complications varied between the high- and low-ratio groups. The low ratio group (<0.2), whose 11β-HSD activity was considered low, had an increase in blood urea nitrogen (BUN) levels and experienced nephropathy, neuropathy, retinopathy, and prolonged DM duration when compared with the group with a ratio greater than 0.2. The data suggest that the serum cortisone/cortisol ratio reflects the change in 11β-HSD activity and is dependent kidney function. This is a possible marker to evaluate glucocorticoid excess hypertension observed in DM and CRF patients.

APPARENT MINERALOCORTICOID excess (AME) syndrome is characterized by hypertension and hypokalemia. It is caused by impaired activity of 11β-hydroxysteroid dehydrogenase (11β-HSD), an enzyme that converts active 11β-hydroxyl glucocorticoids, cortisol and corticosterone, to inactive 11-keto glucocorticoids, cortisone and dehydrocorticosterone, respectively.[1,2] In aldosterone target tissue, 11β-HSD blocks the access of cortisol to mineralocorticoid receptors (MCR) and allows specific binding of aldosterone.[1,2] A congenital defect of 11β-HSD or inhibition of the enzyme activity following administration of the strong inhibitors, glycyrrhetinic acid and its derivatives, leads to overactivation of MCR from cortisol access, resulting in sodium and water retention.[1]

Two isozymes of 11β-HSD, type 1 and 2, each with different tissue distribution are well known. The most active tissues for the former are liver, testis, and lung and for the latter, kidney, placenta, and colon.[1,2] Although the physiologic role of this differential distribution has not been established, the magnitude of the activities in each tissue is associated with the local action of glucocorticoids and affects the concentration ratio of 11-keto/11-hydroxy in peripheral blood and urine.[1,2]

It has been proposed that the patients with impaired activity of 11β-HSD can be found in several hypertensive disorders such as essential hypertension,[3] chronic renal failure (CRF),[2,4] diabetes mellitus (DM),[5] and liver disease secondary to alcohol excess.[6] Our interest focused on identifying patients with impaired 11β-HSD and characterizing their disease features. The serum concentration ratio of cortisone/cortisol might be one of the markers characterizing patients with impaired 11β-HSD activity. In the present study, we determined the serum concentration ratio of cortisone/cortisol in healthy subjects to establish the normal range. We then compared the ratio in healthy subjects with patients who had DM or CRF to evaluate the change in 11β-HSD activity due to the presence of these disease states.

SUBJECTS AND METHODS

Subjects and Sample Collection

We recruited 44 DM and 36 CRF subjects from the outpatient clinic of Tokyo Medical University Hospital and 69 healthy subjects from Tokyo University of Pharmacy and Life Science. Informed consent was obtained from all volunteers, and the study was approved by the ethical committee of our University. Characteristics of the subjects are shown in Table 1. Healthy subjects did not take any medications for at least 4 weeks prior to the study. No abnormality was found by physical examination or biochemical testing. DM patients were currently receiving no medication (20 cases), oral hypoglycemics (9 cases), or insulin

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Table 1. Subject Profile of Healthy, DM, and CRF Groups

Subjects	Healthy (n = 69)	DM (n = 44)	CRF (n = 36)
Age	29.6 ± 12.1	57.2 ± 11.6*	61.6 ± 11.2*
Sex (male/female)	38/31	34/10	21/15
SBP (mm Hg)	114.9 ± 12.1	127.7 ± 15.1†	160.8 ± 21.2*§
DBP (mm Hg)	67.8 ± 9.8	77.6 ± 11.9†	83.9 ± 14.4*§
SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg	0	12*	30‡‖
Antihypertensive medication	0	14‡	27‡‖
Hypertensive/ normotensive	0/69	26/18‡	32/4‡‖

NOTE. Significant difference was observed in comparison with healthy subjects: *$P < .01$, †$P < .05$, ‡$P < .001$ or comparison with DM patients: §$P < .05$, ‖$P < .01$.

(15 cases) for blood sugar control. Twenty-six DM patients were defined as a hypertensive who had systolic blood pressure (SBP) greater than 140 mm Hg or diastolic blood pressure (DBP) greater than 90mm Hg (12 cases) or received 1 or 2 antihypertensive agents (14 cases) including diuretics, calcium antagonists, angiotensin-converting enzyme inhibitors, or β-antagonists. The DM patients had other complications: nephropathy (8 cases), neuropathy (14 cases), and retinopathy (16 cases). All CRF patients were undergoing hemodialysis 2 to 3 times per week with mean dialysis periods of 51.9 ± 7.9 months (mean ± SD). Thirty-two CRF patients were diagnosed as hypertensive according to the same criteria as indicated above. Peripheral blood samples were collected at 9:00 to 11:00 AM just after reading the blood pressure. Serum specimens were isolated and stored at −40°C until analysis.

Determination of Serum Cortisol and Cortisone

Simultaneous determination of serum cortisol and cortisone was conducted by using a combination of high performance liquid chromatography (HPLC) and rapid-flow fractionation (RFF) diatomaceous earth column extraction. Technical details have been described in our previous report.[7] Five hundred microliters of serum specimen spiked with 50 ng of dexamethasone, an internal standard, were subjected to the RFF column system using 7.0 mL of dichloromethane as the extraction solvent. Glucocorticoid fraction including cortisol, cortisone, and dexamethasone recovered with RFF was evaporated to dryness and then reconstituted with 20 μL of ethanol/dichloromethane (4/96; vol/vol) for HPLC injection. Our HPLC system was a U-880 series (Jusco, Tokyo, Japan) equipped with a conventional silica-gel column, LiChrosorb Si 60, 5 μm, 250 mm × 4 mm I.D. (Cica-Merck, Tokyo, Japan). The mobile phase solvent was a water/methanol/dichloromethane/n-hexane (0.1/6.0/30.0/63.9) at a flow rate of 1.5 mL/minute. Detection wavelength was set at 245 nm and sensitivities, 0.0025 to 0.005 aufs (absorbance unit full scale). The detection limit for both glucocorticoids was as low as 1 ng/mL, and the intra- and interday assay variations were less than 5%.

Statistics

Data were presented as mean ± SD or as median and range. Analysis of variance for the multiple comparison between the groups was performed by the Student-Newman-Keuls test. Unpaired Student's or Welch's *t* test was used to compare the mean values between the 2 DM groups. The Fisher's exact probability test was used to compare the occurrence of hypertension and complications between the subject groups. Correlation coefficient between the 2 determinants was examined by Pearson's correlation coefficient test. The *P* values less than .05 were considered to be significant.

RESULTS

Subject characteristics are presented in Table 1. Healthy subjects differed from the DM and CRF groups with respect to age and occurrence of hypertension. The mean SBP and mean DBP in DM and CRF groups were significantly higher than those in the healthy group ($P < .01$), even though the 14 (32%) DM and 27 (75%) CRF patients had been receiving antihypertensive medication, respectively. Occurrence of hypertension was 59.1% in DM and 88.9% in CRF.

All measurements for serum cortisol and cortisone were in the normal range. However, the mean cortisone concentration in the CRF and DM groups was significantly lower than those of the healthy group ($P < .01$) (Table 2). A marked decrease of cortisone concentration was observed in the CRF group. The measured levels were 30% and 40% of those in the healthy and DM groups, respectively. The mean cortisone/cortisol concentration ratio was highest in the healthy subjects and progressively decreased in the patients with DM and CRF. The decrease corresponded with the increased incidence of hypertension. The difference in the ratios between healthy and the DM and/or CRF groups was statistically significant ($P < .01$).

The frequency distribution for the cortisone/coirtisol ratio was compared in 3 groups (Fig 1). The proportion of the histogram for the DM and CRF groups shifted to a lower ratio when compared with the healthy group. The medians (range) of the ratio were 0.243 (0.113 to 0.494) for healthy, 0.188 (0.092 to 0.313) for DM, and 0.088 (0.031 to 0.140) for CRF. Hypertensive subjects in the DM group were divided into 2 subpopulations, high- and low-ratio groups at the border of 0.2 line (Fig 1). The medians (range) for the high- and low-ratio groups were 0.263 (0.201 to 0.303) and 0.150 (0.092 to 0.193), respectively.

To characterize the hypertension with low ratio, we compared the clinical events and biochemical data between high- and low-ratio groups (Table 3). A significant difference was observed in body mass index (BMI), disease duration, liver and kidney function, and complications between the groups. The high-ratio group had higher BMI and serum aminotrasferase ($P < .05$), which indicated obesity and mild liver dysfunction owing to the fatty liver. The low-ratio group had long disease duration, higher blood urea nitrogen (BUN) levels and having DM-related complications, nephropathy, neuropathy and retinopathy.

DISCUSSION

The serum cortisone/cortisol ratio in healthy subjects ranged from 0.113 to 0.494 (median, 0.243). This may correspond to

Table 2. Serum Concentration of Cortisol and Cortisone and Concentration Ratio of Cortisone/Cortisol in Healthy, DM, and CRF Groups

	Healthy (n = 69)	DM (n = 44)	CRF (n = 36)
Cortisol (ng/mL)	110.2 ± 46.8	109.2 ± 15.8	95.5 ± 29.8
Cortisone (ng/mL)	26.2 ± 8.4	20.9 ± 8.0*	7.9 ± 2.8*†
Cortisone/cortisol	0.253 ± 0.076	0.202 ± 0.059*	0.086 ± 0.029*†

NOTE. Significant difference was observed in comparison with healthy subjects: *$P < .01$ or comparison with DM patients: †$P < .01$.

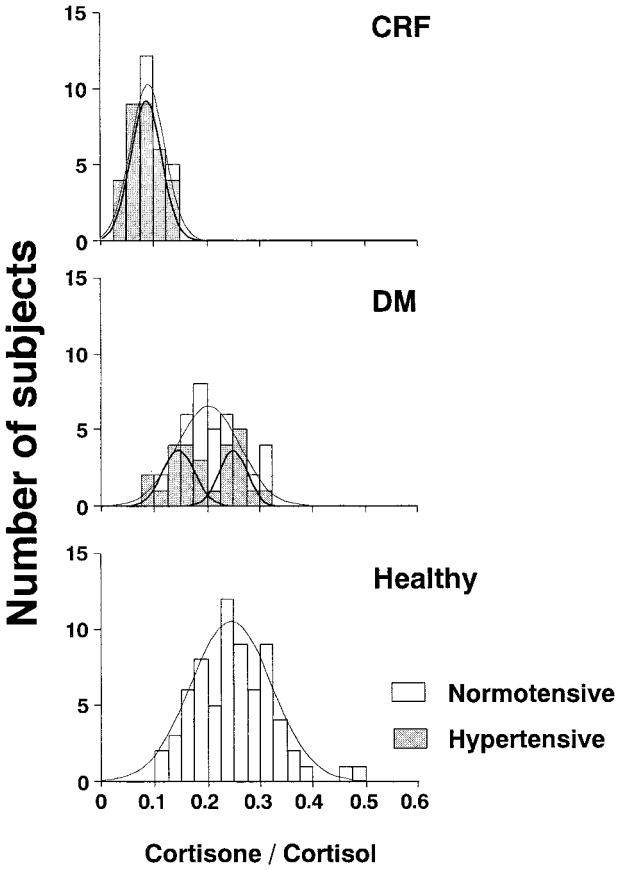

Fig 1. Histogram of concentration ratio of cortisone/cortisol in healthy subjects, DM, and CRF patients.

the individual difference in activities for 11β-HSD type 1 and type 2, which is expressed among the different tissues. The serum cortisone/cortisol ratio decreased with the increasing occurrence of hypertension. It was considered that the ratio might represent the local action of cortisol on aldosterone target tissues such as the vascular endothelium and the distal nephron, which regulate blood pressure. We assumed that a low-cortisone/cortisol ratio associated with decreased activity of 11β-HSD type 2 supports the relevance that intracellular cortisol-enhanced MCR activation results in sodium retention even though the circulating cortisol was maintained at normal levels.

This hypothesis is supported by the change in the cortisone/cortisol ratios of the patients suffering DM and CRF, which decreased depending on the kidney dysfunction and occurrence of hypertension. Despite a decrease in the mean cortisol concentration, CRF patients demonstrated a 60% reduction in the cortisone/cortisol ratio compared with the healthy group. This observation suggests that type 2 activity of kidney 11β-HSD, which catalyzes the preferable conversion of cortisol to cortisone, is markedly impaired in CRF. Similar results have been reported by the investigation of Vogt et al.[4] They observed that the urinary excretion rate of the cortisone metabolite, tetrahydrocortisone (THE), decreased in nephrotic syndrome, suggesting downregulation of 11β-HSD in this patients' population.[4]

Similar observations were found in the DM group when the 2 hypertensive subpopulations as characterized by the cortisone/cortisol ratio were compared. The subpopulation with low-cortisone/cortisol ratio had mild kidney dysfunction with elevated BUN levels. These observations again supported the idea that the serum cortisone/cortisol ratio was decreased by kidney dysfunction in DM patients, which leads to impaired activity of 11β-HSD type 2 found mainly distributed in the distal tubules of the nephron.[5] The low-ratio group also had long disease duration and other complications including nephropathy, neuropathy, and retinopathy. Because substantial exposure of high glucose has been known to inhibit 11β-HSD present in vascular cells,[5,8] a low-cortisone/cortisol ratio may also display the systemic microvascular function, which plays an important role in developing DM-related complications. On the other hand, the high-cortisone/cortisol ratio group may have developed hypertension for other reasons. This subpopulation had obesity and mild liver dysfunction, which may be associated with fatty liver. This observation is not inconsistent with the current finding of Stewart et al,[9] in which 11β-HSD type 1 catalyzing preferable conversion of cortisone to cortisol was impaired in obese subjects. Hyperinsulinemia may arise as a cause of hypertension in this population group.

The decrease in serum cortisone/cortisol can be assigned to hypertension with impaired activity of 11β-HSD type 2 present mainly in the kidney. Angiotensin-converting enzyme inhibitors (ACEIs) can be used for this type of hypertension if the patients' kidney function and potassium levels are maintained within a normal range, as ACEIs have been known to activate

Table 3. Comparison of Biochemical Data and Complications Between Low- and High-Ratio Groups of Concentration Ratio of Cortisone/Cortisol

Subjects	Low (n = 14)	High (n = 12)
Age (yr)	55.6 ± 12.7	48.7 ± 13.0
Sex (male/female)	10/4	9/3
BMI (kg/m^2)*	23.1 ± 3.1	28.1 ± 6.3
SBP (mm Hg)	130.9 ± 14.9	139.1 ± 38.1
DBP (mm Hg)	77.6 ± 13.7	85.5 ± 11.2
Serum cortisol (ng/mL)	118.7 ± 53.7	108.5 ± 38.1
Serum cortisone (ng/mL)*	16.5 ± 7.0	26.6 ± 8.4
Cortisone/cortisol*	0.145 ± 0.031	0.249 ± 0.026
Fasting blood glucose (mg/dL)	177.1 ± 54.9	165.4 ± 56.8
Hemoglobin A_{1C} (%)	9.7 ± 2.6	8.9 ± 2.5
Serum cholesterol (mg/dL)	224.4 ± 41.6	229.3 ± 43.5
Serum creatinine (mg/dL)	1.07 ± 1.08	0.70 ± 0.17
BUN (mg/dL)*	19.7 ± 10.0	13.5 ± 2.9
Serum aspartate aminotransferase (U/L)*	21.3 ± 7.9	46.7 ± 38.8
Serum alanine aminotransferase (U/L)*	20.7 ± 9.9	56.3 ± 54.0
Serum sodium (mEq/L)	139.7 ± 1.4	139.4 ± 2.4
Serum potassium (mEq/L)	4.1 ± 0.5	4.3 ± 0.3
Serum chloride (mEq/L)	102.9 ± 3.6	102.4 ± 2.6
Disease duration (> 10 years)*	11	2
Nephropathy	4	0
Neuropathy	4	0
Retinopathy†	7	0

NOTE. Significant difference was observed between 2 groups: *$P <$ .05; †$P <$ .01.

renal 11β-HSD by inhibiting the production of angiotensin II, an inhibitor of 11β-HSD type 2, and via unknown mechanisms.[10-12] Dullaart et al[11] reported that a change in the ratio of tetrahydrocortisol (THF)/tetrahydrocortisone (THE), another marker of 11β-HSD responsible for improving blood pressure after administration of ACEI in insulin-dependent DM patients. The serum cortisone/cortisol ratio, as well as urinary THE/THF ratio, may be used as a marker evaluating the efficacy of ACEIs in DM patients with hypertension, although further clinical investigations are required.

Gene mutation in 11β-HSD type 2 has been examined in healthy subjects, renal transplant patients, DM patients, and in patients with essential hypertension.[13] The prevalence of gene mutation in exon 3 at codon 178 was higher in renal transplant patients (18.0%) than in the healthy controls (8.6%), but was lower in patients with DM (4.0%) or essential hypertension (4.8%). These observations partly support our present finding that CRF patients produced low-cortisone/cortisol ratio compared with healthy subjects. However, the subpopulation with low cortisone/cortisol as observed in the DM group is not explained by the type 2 gene mutation alone. Several factors, such as 11β-HSD type 1 activity, endogenous regulators of 11β-HSD, inhibitors and promoters, and other cortisol metabolic enzymes may be involved in the change in serum cortisone/cortisol ratio of DM patients. Because type 1 activity[5] or glucose,[14] insulin,[15] and dehydroepiandrosterone sulfate,[16] which act as the inhibitor or promoter for 11β-HSD, are dramatically changed in DM disease state, as well as type 2 activity,[17] phenotyping enzyme activity measuring serum cortisone/cortisol or urinary metabolites ratio has some advantages in assessing the 11β-HSD over genotyping.

In conclusion, the serum cortisone/cortisol ratio is decreased in CRF and DM patients and can be used as a reference to assess intracellular activity of cortisol and to identify hypertension caused by impaired activity of 11β-HSD. Changes in the ratio associated with disease stage or antihypertensive and hypoglycemic medications should be evaluated in future clinical investigations.

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