Serum 18-Hydroxycortisol in Primary Aldosteronism, Hypertension, and Normotensives

Lorena Mosso, Celso E. Gómez-Sánchez, Mark F. Foecking, Carlos Fardella

Abstract—This study reports the determination of plasma 18-hydroxycortisol (18-OHF) using a new and easy enzyme-linked immunosorbent assay (ELISA) method in primary aldosteronism and compares the values found in essential hypertensives and normotensive controls. In primary aldosteronism, we evaluated usefulness of plasma 18-OHF determination and the dexamethasone suppression test in the diagnosis of glucocorticoid-remediable aldosteronism using the genetic test as the gold standard. We studied 31 primary aldosteronism patients, 101 essential hypertensives, and 102 healthy normotensive controls. The plasma 18-OHF was measured using a biotin-avidin enzyme-linked assay by a new and purified polyclonal antibody. The 18-OHF value in primary aldosteronism was 6.3±8.05 nmol/L; this value is significantly higher than the value found in essential hypertensives and normotensive controls (2.81±1.42 and 2.70±1.41 nmol/L, respectively; P<0.0005). In primary aldosteronism, 4 of 31 patients had 18-OHF levels that were 10 times higher than the normal upper limit (2.983 nmol/L). The dexamethasone suppression test in primary aldosteronism patients was positive (serum aldosterone <4 ng/dL) in 13 of 31 cases. A chimeric CYP11B1/CYP11B2 gene was demonstrated in 4 primary aldosteronism patients, corresponding to the same cases that had higher level of 18-OHF. In conclusion, plasma 18-OHF determination by this ELISA method is reliable for detecting glucocorticoid-remediable aldosteronism, and it does so better than the dexamethasone suppression test. (Hypertension. 2001;38[part 2]:688-691.)

Key Words: 18-hydroxycortisol ■ hypertension, mineralocorticoid ■ glucocorticoids ■ aldosterone

The 18-hydroxycortisol (18-OHF) and 18-oxocortisol (18-oxoF) are secreted by the adrenal cortex.1,2 These hybrid C-18-oxygenated steroids are different from the other steroids produced in the adrenal cortex because they combine structural characteristics of the products secreted by the fasciculata and glomerulosa zones. Thus, they are hydroxylated at carbon 17, a feature of fasciculata steroids, and they are also oxidized at carbon 18, a characteristic of glomerulosa steroids.

The urinary excretion of 18-OHF and 18-oxoF is increased in patients with aldosterone-producing adenoma (APA) but not in patients with bilateral adrenal hyperplasia.3,4 Instead, the production of these hybrids is very high (10×) in patients with glucocorticoid-remediable aldosteronism.5 Glucocorticoid-remediable aldosteronism (GRA) is caused by an unequal crossover between the CYP11B1 and CYP11B2 genes that results in a chimeric CYP11B1/CYP11B2 gene that has an aldosterone synthase activity but is regulated by adrenocorticotropic hormone (ACTH) rather than angiotensin II.6,7 The expression of the chimeric gene in the zone fasciculate exposes the product of this gene to the alternative and more abundant substrate 11-deoxycortisol, which is converted into 18-OHF and 18-oxoF. In APA, a somatic mutation similar to GRA has not been demonstrated,8 but abnormal expression of steroidogenetic enzymes in the tumor has been previously described and could explain the overproduction of C-18-oxygenated steroids.9,10 Essential hypertensive patients with high levels of 18-OHF have been reported, but the meaning of this elevated excretion remains unclear.11,12

The biological activity of 18-OHF as glucocorticoid or mineralocorticoid is negligible, and 18-oxoF has only 1% of the salt-retaining activity of aldosterone and 3% of the glucocorticoid activity of cortisol.13 The possibility of effects other than stimulated transepithelial sodium transport, as a direct action on vasculature, has been raised but not probed.14 Thus, the low biological activity of these 18-oxygenated steroids probably indicates that they serve as markers rather than as part of the pathogenesis of hypertension.

The 18-OHF determination traditionally has been made by specific radioimmunoassays techniques in the urine or plasma; these methods were developed for investigation and have low clinical applicability.15–18 In the past few years, enzyme-linked immunoassays (ELISA) has been gaining popularity over radioimmunoassays because of the ever-
increasing difficulty of working with radioactive materials. The study reports an easy ELISA method for the plasma 18-OHF determination by a new purified polyclonal antibody and evaluates its capacity to discriminate GRA in patients with primary aldosteronism. We also compare these results with those found with the dexamethasone test, traditionally used as screening for GRA. Finally, we report the findings detected in essential hypertensive patients and the normal values found in healthy Chilean normotensive controls.

### Methods

#### Subjects

We studied 31 PA patients with primary aldosteronism (PA) detected as we described in a previous publication. In brief, we performed the determination of the serum aldosterone (SA)/plasma renin activity ratio in patients previously considered essential hypertension; we used a ratio > 2.5 for suspected PA and confirmed the diagnoses with a fludrocortisone suppression test. Computed tomography scan of the adrenal gland in PA patients showed a bilateral enlargement in 2 of 31 cases and was reported as normal in the other 29 cases. In all PA patients, a dexamethasone suppression test (DST) was performed (2 mg/d for 2 days) as previously described. We considered a test positive if SA was < 4 ng/dL after dexamethasone administration.

We also studied 101 other essential hypertensives and 102 normotensive healthy controls recruited at our center at Universidad Católica de Chile en Santiago. The 101 patients were considered hypertensive if their diastolic blood pressure was > 90 mm Hg and their systolic blood pressure was > 140 mm Hg on ≥ 3 occasions on different days and if they were not taking any antihypertensive medication. All individuals with clinical evidence of secondary hypertension, diabetes, or renal disease were excluded. The 102 normotensives had no family history of hypertension, at least in first-degree relatives. No patients were taking medications, including oral contraceptives. Their characteristics are described in Table 1. All the subjects were recruited at the Universidad Católica de Chile en Santiago. Informed consent was obtained from all participants according to the guidelines of the Declaration of Helsinki.

#### Protocol

SA was measured by radioimmunoassay with an antisemur from Diagnostic Products Corp. The plasma renin activity (PRA) was determined as previously described. The DST was considered positive if the PA levels were < 4 ng/dL, after the subject received 2 mg/d of dexamethasone for 2 days. A suppressed plasma cortisol (< 2.5 µg/dL) was used as an index of the dexamethasone suppression. A long extension PCR for chimeric gene was done in all idiopathic hyper-aldosteronism (IA) patients with a positive DST as was previously described.

### ELISA Procedure

The plasma 18-OHF was measured by a biotin-avidin enzyme-linked assay modified from one previously described. In summary, micrometer plates were coated with affinity-purified goat anti-rabbit IgG-Fc antibody (Jackson ImmunoResearch) at a concentration of 1 µg/200 µL per well in a carbonate buffer 0.1 mol/L, pH 9.4, overnight at 4°C. The plates were washed 5 times with PBS containing 0.1% Tween 80 and were either used immediately or stored at 4°C with plain PBS. The assay was performed by adding the standard produced as previously described or samples (extracted with 10 volumes of isopropanol) in 50 µL followed by 100 µL of a premixed solution of avidin-peroxidase (Vector Laboratories) and 18-OHF–biotin derivative. After mixing, 100 µL of the purified antibody was added. After an overnight incubation and washing, the plates were developed with substrate solution (tetrathionenbenzidine HCl 0.2% plus urea peroxide 0.01% in citrate buffer 0.1 mol/L, pH 4.0) for 1 hour and read at 450 nmol/L. The interassay variation was 4.6%; the intra-assay variation was 3.4%.

### Antibody Purification

The most important cross-reacting steroid of sufficient concentration in plasma is cortisol. Cortisol-3-carboxymethoxylamine was prepared as described previously and conjugated to propylamino-Controlled Pore Glass beads 1000 (100 micron beads) by the mixed anhydride technique in dimethylformamide. The beads were then washed extensively with dimethylformamide, sodium bicarbonate solution 1%, water, and finally PBS. One mL of anti-18-OHF-3-carboxymethoxylamine-turkey serum albumin antibody was then equilibrated with 2 mL of the beads for several hours. The beads in a 5-cm polyethylene column were then eluted with PBS and at decreasing pH. The aliquots, in 1-pH unit decreases, were collected and neutralized. Titters were then detected, and cross-reactivities were measured by comparing the displacement ability of increasing amounts of cross-reactants against the 18-OHF standard.

The initial antibody exhibited a cross-reactivity for cortisol of 0.16%; for corticosterone, 0.02%; and for 18-hydroxydeoxycorticosterone, 0.002%. The antibody that was not retained by the column exhibited a cross-reactivity for cortisol of < 0.002%; for corticosterone, 0.16%; for 18-hydroxydeoxycorticosterone, 0.03%; and for cortisone and tetrahydrocortisol, < 0.002%. The titer decreased from 1/100 k to approximately 1/2.5 k.

### Statistical Analysis

A 1-way ANOVA and Tukey’s pairwise comparisons were performed for compared variables between different groups.

### Results

We studied 31 PA patients, 101 essential hypertensive subjects, and 102 healthy normotensive subjects. As shown in Table 1, the PA patients had higher SA levels and SA/PRA ratios and lower PRA levels than did hypertensives and normotensives. The serum potassium levels were similar in all subjects studied. Also, there were no significant differences between PA patients and their controls in age, gender, serum creatinine, and sodium.

The 18-OHF value in PA patients was 6.3 ± 8.05 nmol/L; this value is significantly higher than the one found in essential hypertensives and normotensive controls (2.81 ± 1.42 and 2.70 ± 1.41 nmol/L, respectively; P < 0.0005). In essential hypertensive patients, all 18-OHF levels were in the normal range; in this group we did not find any differences in plasma 18-OHF levels between low- and normal-renin hypertensives (low renin, 2.28 ± 1.17 nmol/L, n = 19, versus normal renin, 2.88 ± 1.44 nmol/L, n = 82). In PA...
patients, however, 4 of 31 had 18-OHF levels that were 10 times higher than the normal upper limit (2.983 nmol/L with 95% confidence interval). Indeed, if we excluded these 4 patients (patients 1, 5, 18, and 27 in Table 2) the mean of 18-OHF levels in the resting 27 IHA was 3.414 nmol/L, and this was not significantly different from that of hypertensives or controls in ANOVA 1-way analysis ($P=0.089$).

All 31 PA patients responded to the DST test, with a decrease in SA from 17.66 ng/dL to 6.14 ng/dL. Plasma cortisol suppression after dexamethasone ($<2.5\, \mu g/dL$) was found in all patients as an index of ACTH suppression. The suppression of SA to $<4$ ng/dL was found in 13 IHA patients; to $<2$ ng/dL, in 5 patients (Table 2).

A chimeric CYP11B1/CYP11B2 gene was demonstrated in 4 PA patients. These 4 patients were the ones that had very high 18-OHF levels, and all were positives for DST (patients 1, 5, 18, and 27 in Table 2). The other 9 patients who had a positive DST and normal or minor elevations of 18-OHF were negative for genetic testing (Table 2).

**Discussion**

The results of this study demonstrate that a newly modified ELISA for plasma 18-OHF is a reliable an easy method to diagnose GRA, even better than the traditional DST when the genetic test is used as a gold standard.

The 18-OHF showed a 100% concordance with the genetic test in identifying GRA if values $>10$ nmol/L are used (corresponding to the lower 99% confidence interval in the 4 GRA patients). Instead, the DST had a high frequency (29%) of false-positive with 4 ng/dL as the cutoff for the aldosterone levels. The reason of the high frequency of false-positive is unknown, but an ACTH-dependency in some types of IHA cannot be discarded.

The false-positive in the DST had been suggested by Mulatero et al., who recently showed that several patients with a primary hyperaldosteronism and negative genetic test had PA suppressed by dexamethasone to the same degree as patients with GRA. The prevalence of GRA is unknown, but the diagnosis is clinically relevant because it is potentially dangerous and has specific treatment using a synthetic glucocorticoid such as dexamethasone to suppress ACTH, resulting in remission of the disease.

In our sample of PA, we only studied idiopathic PA because it is the more prevalent cause of hyperaldosteronism and is the clinical form of GRA presentation. Rarely, patients with GRA can present with APAs. In APA, the level of 18-OHF may be higher than that in idiopathic PA, but GRA is easily distinguished from APA because 18-OHF levels are much higher than those in APA. We measured 18-OHF by our method in 2 APA patients and found 18-OHF levels of 4.2 and 5.6 nmol/L, respectively (data not shown).

The 18-OHF level in normotensives and essential hypertensives was very similar, and no differences were detected when compared with normal- or low-renin patients. These findings suggest that screening determination for 18-OHF is not justified in essential hypertensives. We suspect that previously reported hypertensives with high levels of 18-OHF may have undiagnosed GRA; most of them had low renin.

The advantage of performing plasma 18-OHF by ELISA method is that radioimmunoassays techniques use radioactive reagents that are not available. The ELISA method is common in many general clinical chemistry laboratories, and it has a perfect correlation with the genetic test as described. The long-extension PCR technique is a simple test, but a specialized laboratory is necessary for molecular biology techniques. Another advantage is that the ELISA method is cheaper than long-extension PCR. An additional advantage of performing plasma 18-OHF is that it helps in the follow-up of the treatment of GRA patients, as dexamethasone treatment results in normalization of the 18-OHF levels in GRA patients.

In conclusion, plasma 18-OHF by this ELISA method is an easy, cheaper, and better form for GRA diagnoses in idio-

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**TABLE 2. Results of DST, Plasma 18-OHF, and Genetic Test for Chimeric CYP11B1/CYP11B2 Gene in PA**

<table>
<thead>
<tr>
<th>No.</th>
<th>SA, ng/dL Before Dx</th>
<th>18-OHF, nmol/L</th>
<th>Genetic Test</th>
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<tr>
<td>1</td>
<td>41.0</td>
<td>19.40*</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>37.3</td>
<td>2.10</td>
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</tr>
<tr>
<td>3</td>
<td>28.6</td>
<td>6.14</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>26.7</td>
<td>2.99</td>
<td>–</td>
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<tr>
<td>5</td>
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<td>+</td>
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<tr>
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<td>9.0</td>
<td>2.97</td>
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</table>

Dx indicates dexamethasone.

*Abnormal results.
pathic PA and may overcome the high incidence of false-positives in DSTs.

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References

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