Critical Evaluation of Plasma and Urinary Aldosterone Measurements by RIA for Clinical and Roution Purpose

Machiko UEDA, Makiko SUEHIRO, Akira KURODA, Tami YATABE
Taeko INABA, Hideo YAMADA and Masahiro IIO
Department of Nucl. Med. and Rad. Biology, Tokyo Metropolitan Geriatric Hospital

In order to evaluate a simple radioimmunoassay method for urinary and plasma aldosterone determination, critical comparison was made between a method with column chromatography (cyclohexan; benzen; methanol; 60: 40: 10) and that without purification. Using NEN antibody (aldosterone-18,21-dihemisuccinate) for the former method, plasma aldosterone was measured after CH_2Cl_2 extraction following column chromatogrphic purification and urinary aldosterone was mesured after chromatographic purification of CH_2Cl_2 extracted material from HCl hydrolysed urine specimen. For the latter method CIS kit (aldosterone-3-oxime antibody) was used as indicated in the instruction manual. Tritium labeled aldosterone was used as a tracer in both methods.

Satisfacory standard curves were obtained by both methods with B_0/T ratios of $41.6\pm1.71\%$ (NEN) and $43.4\pm2.81\%$ (CIS) respectively, showing rapid decline from 10 to 250 pg. Extraction ratios of plasma aldosterone were $51.5\pm6.91\%$ by chromatographic method and $84.06\pm6.12\%$ by CIS method. The latter extraction ratio was improved up to $98.9\pm18.42\%$ by three times dilution of plasma using distilled water before extraction. Urinary aldosterone was extracted almost completely by CIS method. However chromatographic method showed lower extraction ratio of $50.59\pm10.41\%$.

Specificity of antibodies compared using several steroids showed very high specificity in CIS antibody. Cross reactivity of cortisol was 0.01% by NEN antibody and $10^{-5}\%$ by CIS antibody. Cortisone showed 0.12% cross reactivity by NEN antibody and 0.001% by CIS antibody.

Within-assay variations were 7.59% by NEN kits and 9.29% by CIS kits. Between-assay variations were 26.3% by NEN and 15.7% by CIC kits.

In case of urinary aldosterone determination, both method showed excellent correlation with correlation coefficient of 0.944 ($p<0.001$). ($Y=0.865X-0.488$). Plasma aldosterone determinations were less well correlated ($r=0.751$, ($p<0.05$)) ($Y=0.677X+0.737$). Higher values were occasionally obtained by CIS method. (Y stands for NEN, and X for CIS)

However authors concluded the simple non-chromatographic radioimmunoassay of aldosterone could be used for routine examination.

A Simplified Method Measuring Plasma Aldosterone by Radioimmunoassay Using ^{125}-IAldosterone

Kazumi HARUYAMA, Katsuo NAKAJIMA, Soitsu FUKUCHI and Masaru SAITO
Third Department of Internal Medicine and Radioisotope Laboratory, Fukushima Medical College, Fukushima

A radioimmunoassay method was developed to measure plasma aldosterone levels.

Antibody was produced in rabbits by injecting aldosterone-oxime coupled with porcine gamma globulin.

Plasma aldosterone was measured simultaneously by four method described below.

The first was a simple method of extraction using trichloracetic acid, the second a method using methanol, the third a direct method without extraction and the fourth a method using paper chromatography.

^{3}H-aldosterone was used in the fourth method and ^{125}I-aldosterone in the others.

The antibody had a high specificity adequate to show almost zero water blank in all methods except the fourth.

The mean recovery of over 90%, adequate, precision, accuracy and sensitivity were obtained in the methods using ^{125}I-aldosterone.

Plasma aldosterone levels in normal subjects were the highest, 8.0±8.9 ng/100 m*l*, in the first method and lowest, 5.9±9.2 ng/100 m*l*, in the fourth method.

Correlative coefficient of these results was the best ($r=0.995$, $p<0.001$) between the second and the fourth methods, with a good relationship ($r=0.895$, $p<0.001$) between the third and the fourth methods.

Plasma aldosterone was high in primary aldosteronism, slightly high in secondary aldosteronism and normal range in Cushing's syndrome.

From these results, it is concluded that the second and the third methods were the most useful and reliable for measuring plasma aldosterone, and were superior in simplicity and there was no need to use a liquid scintillation counter.

Measurement of Plasma Dehydroepiandrosterone by Radioimmunoassay

Kazumi HARUYAMA, Katsuo NAKAJIMA, Soitsu FUKUCHI and Masaru SAITO
Third Department of Internal Medicine and Radioisotope Laboratory, Fukushima Medical College, Fukushima

A radioimmunoassay procedure was developed to measure dehydorepiandrosterone (DHEA) in human plasma.

Antibody was produced in rabbits by injecting DHEA-oxime coupled with porcine gamma globulin.

A mixture of 50 ul of plasma and 1,2 ^{3}H-DHEA was extracted with 1 m*l* of methanol and then incubated overnight at 4°C after adding the antibody.

Bound fraction was separated from free fraction by the saturated ammonium sulfate method.

Recovery of ^{3}H-DHEA was always over 90% using the above method.

The method showed adequate specificity, precision and accuracy.

Plasma DHEA was 0.18±0.10 μg/100 m*l* (N=19) in normal subjects; 0.21±0.14 μg/m*l* in males and 0.17±0.22 μg/100 m*l* in females. Normal levels of plasma DHEA were obtained in cases of normal- or high renin hypertension (0.25±0.15 μg/100 m*l*).

The mean level of 0.13±0.08 μg/100 m*l* was obtained in hypertensive patients with suppressed renin activity.

Plasma DHEA showed a normal level of 0.19±0.07 μg/100 m*l* in 4 patients with primary aldosteronism, a low level of 0.02 μg/100 m*l* in a case of Cushing's syndrome with adrenal adenoma, and 0.05±0.02 μg/100 m*l* in 2 patients with hypopituitarism.

An increase in plasma DHEA occurred with ACTH or Metopirone, and a decrease with dexamethasone.

Angiotensin II or furosemide on upright position induced no change in plasma DHEA.

It is concluded from these results, that DHEA secretion is not controlled by the renin-angiotensin system, but primarily by the anterior pituitary.